UNIVERSITI PUTRA MALAYSIA

ANTIOXIDATIVE, ANTIHYPERTENSIVE, AND ANTIDIABETIC ACTIVITIES OF COCOA AUTOLYSATES USING *IN VITRO* MODELS

BAHAREH HOSSEINPOURSARMADI

FPSK(m) 2011 36

ANTIOXIDATIVE, ANTIHYPERTENSIVE, AND ANTIDIABETIC ACTIVITIES OF COCOA AUTOLYSATES USING *IN VITRO* MODELS

BAHAREH HOSSEINPOURSARMADI

Master of Science
UNIVERITI PUTRA MALAYSIA

JANUARY 2011

This dissertation is dedicated

To my parents that words alone cannot express the thanks I owe them, for their guidance, never-ending support and unconditional love,

To my dearest husband for his continued love, support and patience through my study,

To my beloved brother & sister for their bright spirit and love,

To my respectful supervisor, his guidance and motivations encourage me to keep going ahead

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ANTIOXIDATIVE, ANTIHYPERTENSIVE, AND ANTIDIABETIC ACTIVITIES OF COCOA AUTOLYSATES USING *IN VITRO* MODELS

By

BAHAREH HOSSEINPOURSARMADI

JANUARY 2011

Chairman : Assoc. Prof. Amin Ismail, PhD

Faculty : Medicine and Health Sciences

This study investigated antioxidant, antihypertensive and antidiabetic activities of cocoa (*Theobroma cacao L.*) autolysates using *in vitro* methods. After removal of cocoa fat, alkaloids and polyphenols, the remaining proteinous powder was prepared and autolyzed at pH 3.5 and 5.2. Antioxidant capacity was assayed using two different methods namely, ferric reducing/antioxidant potential (FRAP) and β-carotene bleaching assays. The antihypertensive potential of cocoa autolysates was measured using angiotensin converting enzyme (ACE) inhibitory effects. α-Amylase, α-glucosidase inhibition and insulinotropic activities of autolysates were measured to assess potential antidiabetic activities. Qualitative and quantitative tests were applied to assure that the results from the aforementioned assays were not due to the polyphenols of cocoa

autolysates. In addition, amino acid compositions of autolysates and their protein content were determined by HPLC following Pico-Tag and Kjeldah methods, respectively. At similar concentrations (10 mg/ml), autolysates of UIT 1 produced at pH 3.5 exhibited the highest reducing power (723 μM) and ACE inhibition activity (75%). However, autolysates of PBC 140 generated at pH 5.2 showed the highest antioxidant activity (54%) based on β-carotene bleaching assay. In the case of antihyperglycemic properties, autolysate of UIT produced at pH 3.5 had the highest ability (68%) to inhibit α-amylase. No α-glucosidase inhibition activity was observed from autolysates. Autolysates produced under pH 3.5 caused the highest amount of insulin secretion in the concentration of 0.62 mg/ml, although the difference was not significant. The reducing power, antioxidant, ACE inhibitory and α-amylase inhibitory activities of all autolysates as well as insulinotropic properties of autolysates produced at pH 5.2 was enhanced by increasing their concentration. However, autolysates produced at pH 3.5 showed maximum potential insulinotropic activity at 0.62 mg/ml and then decreased. No polyphenols could be detected from cocoa autolysates. Based on amino acids composition, slight differences were detected between autolysates, and as it was found, they were rich in hydrophobic amino acids. There was a significant ($P<0.01$) and high correlation between protein content and reducing power ($r^2=0.827$), ability to suppress β-carotene bleaching

(r^2=0.762), α-amylase inhibition (r^2=0.766) as well as insulinotropic effect, at pH 5.2, (r^2=0.940). A significant (P<0.01) and moderate correlation was observed between protein content and ACE inhibition (r^2=0.649). It can be indicated that among other useful substances of cocoa, its peptides and amino acids could contribute to its health-promoting properties. Furthermore, these bioactive substances can be exploited into functional foods or used as a source of nutraceuticals.

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

AKTIVITI ANTIOKSIDAN, ANTIHIPERTENSIF DAN ANTIDIABETIK DARI AUTOLISATE KOKO MENGGUNAKAN MODEL *IN VITRO*

Oleh

BAHAREH HOSSEINPOURSARMADI

Janiari 2011

Pengerusi : Prof. Madya Amin Ismail, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kajian ini mengkaji aktiviti antioksidan, antihipertensif dan antidiabetik menggunakan kaedah *in vitro* autolisate koko (*Theobroma cacao* L.). Selepas penyingkiran lemak koko, alkaloid dan polifenol, serbuk berprotein disediakan dan diautolisis pada pH 3.5 dan 5.2. Kapasiti antioksidan ditentukan dengan menggunakan dua kaedah iaitu penurunan ion ferum/potensi antioksidan (FRAP) dan pelunturan β-karotena. Potensi antihipertensif oleh autolisate koko diukur dengan menggunakan kesan rencatan ACE. Perencatan aktiviti α-amilase dan α-glukosidase dan insulinotropik oleh autolisate ditentukan untuk menilai kesan potensi antidiabetik. Ujian kualitatif dan kuantitatif dijalankan untuk memastikan keputusan yang diperoleh daripada kaedah yang dinyatakan bukan disebabkan oleh polifenol di dalam autolisate koko. Selain itu, komposisi asid amino dan kandungan protein ditentukan dengan

menggunakan HPLC, kaedah Pico-Tag dan Kjeldah. Pada kepekatan yang sama, autolisate koko UIT 1 yang terhasil pada pH 3.5 menunjukkan potensi antioksidan (723 μM) dan aktiviti perencatan ACE (75%) tertinggi. Walau bagaimanapun, autolisate koko PBC 140 yang dihasilkan pada pH 5.2 menunjukkan aktiviti antioksidan tertinggi (54%) dengan kaedah pelunturan β-karotena. Bagi ciri antihiperglisemik, autolisate koko UIT yang terhasil pada pH 3.5 mempunyai keupayaan tertinggi (68%) untuk merencat α-amilase. Tiada rencatan aktiviti α-glukosidase daripada autolisate ini. Autolisate koko yang terhasil pada pH 3.5 menyebabkan perembesan jumlah insulin tertinggi pada kepekatan 0.62 mg/ml. Potensi, antioksidan, aktiviti perencatan ACE dan α-amilase dan juga ciri-ciri insulinotropik autolisate koko terhasil pada pH 5.2 meningkat dengan penambahan kepekatan. Namun begitu, autolisate terhasil pada pH 3.5 menunjukkan aktiviti insulinotropik yang maksimum (0.62 mg/ml) dan kemudian menurun. Tiada polifenol dapat dikesan daripada autolisate koko. Berdasarkan komposisi asid amino, sedikit perbezaan antara autolisate tersebut, dan seperti mana yang didapati, ia kaya dengan asid amino hidrofobik. Terdapat korelasi signifikan ($P<0.01$) di antara kandungan protein dan potensi antioksidan ($r^2=0.827$), keupayaan untuk menghalang pelunturan β-karotena ($r^2=0.762$), perencatan α-amilase ($r^2=0.766$) dan kesan insulinotropik pada pH 5.2, ($r^2=0.940$). Korelasi signifikan yang sederhana didapati di antara

kandungan protein dan perencatan ACE (r^2=0.649). Ini menunjukkan selain komponen bernilai dalam koko, peptida dan asid amino boleh menyumbang kepada promosi kesihatan. Tambahan lagi, komponen bioaktif ini boleh dieksploitasikan kepada makanan berfungsi atau digunakan sebagai sumber neutraseutikal.

ACKNOWLEDGEMENTS

The thesis owes its existence to all those who rendered me their kind assistance in different ways in its completion.

Special thanks go to Associate Professor Dr. Amin Ismail, the chairman of the supervisory committee, who was always there to support and guide me all through the way. I would also like to extend my thanks to Associate Professor Dr. Muhajir Hamid for providing crucial advice.

My sincere gratitude is dedicated to my husband, who constantly gave me the emotional support when I needed it most. I would like to thank my friends Abbe Maleki Mohd Jalil, Kong Kin Weng, Beh Joo Ee among others who assisted me in this research.

I certify that an Examination Committee has met on 24 of Jan 2011 to conduct the final examination of Bahareh Hosseinpoursarmadi on her Master of Science thesis entitled "*In vitro* Antidiabetic and Antihypertensive Effects of Cocoa Autolysates" in accordance with university Pertanian Malaysia (Higher degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master of Science. Members of the Examination Committee are as follows:

HASANAH MOHD. GHAZALI, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of university Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of supervisory committee were as follows:

Amin Ismail, PhD
Associate Professor
Faculty of Medicine and Health Sciences
(Chairman) Universiti Putra Malaysia

Muhajir Hamid, PhD:
Associate Professor
Faculty of Biotechnology and Bimolecular Sciences
(Member) Universiti Putra Malaysia

HASANAH MOHD HAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

(Signature)

BAHAREH HOSSEINPOURSARMADI

Date 24. January. 2011

TABLE OF CONTENT

		Page
		iii
ABSTRACT		vi
ABSTRAK		ix
ACKNOWLEGEMENTS		x
APPROVAL		xii
DECLARATION		xiv
LIST OF TABLES		xv
LIST OF FIGURES		xvi
LIST OF ABBREVIATIONS		xvii
CHAPTER		
1	**INTRODUCTION**	1
	1.1 Background	1
	1.2 Statements of problem	6
	1.3 Significant of study	7
	1.4 Objectives	9
2	**LITERATURE REVIEW**	10
	2.1 Bioactive peptides	10
	2.1.1 Enzymatic production of bioactive peptides	11
	2.1.2 Bioavailability of bioactive peptides	14
	2.1.3 Safety concerns of bioactive peptide	18
	2.2 Health effects of bioactive peptides	19
	2.2.1 Peptides with antioxidative activities	19
	2.2.2 Peptides with antihypertensive activities	32
	2.2.3 Antidiabetic peptides	36
	2.2.4 Other functions of peptides	43
	2.3 Cocoa	46
	2.3.1 History	46
	2.3.2 Cocoa peptides and amino acids from the perspective of food sciences	49

3	**METHODOLOGY**		50
	3.1	Material	50
	3.2	Preparation of sample	51
		3.2.1 Sample collection	51
		3.2.2 Extraction of fat and alkaloids	51
		3.2.3 Preparation of acetone-dry powder (AcDP)	52
		3.2.4 Preparation of cocoa autolysate	53
	3.3	Preparation of sample extract	56
	3.4	Determination of amino acid composition	56
	3.5	Determination of protein content	57
	3.6	Determination of antioxidant capacity	58
		3.6.1 Ferric reducing/antioxidant power (FRAP)	58
		3.6.2 β-carotene-linoleate bleaching assay	58
	3.7	Angiotensin converting enzyme inhibition assay	59
	3.8	Determination of antidiabetic activity	60
		3.8.1 α-Amylase inhibition assay	60
		3.8.2 α-Glucosidase inhibition assay	62
		3.8.3 Determination of insulin-releasing activity	63
	3.9	Statistictical analysis	65
4	**RESULTS AND DISCUSSION**		66
	4.1	Preparation of cocoa autolysates	66
	4.2	Amino acid composition of cocoa autolysates	79
	4.3	Atioxidant capacity	73
		4.3.1 Ferric reducing/antioxidant power (FRAP)	74
		4.3.2 β-carotene bleaching inhibition activity	79
	4.4	Angiotensin Converting Enzyme inhibition	84
	4.5	Inhibition of digestive enzymes activities	88
	4.6	Isulinotroic properties of cocoa autolyates	93
5	**CONCLUSION AND RECOMMENDATION**		98
	5.1	Conclusion	98
	5.2	Recommendation	100
REFERENCES			101
APPENDICES			123
BIODATA OF STUDENT			139
LIST OF PUBLICATIONS			140