UNIVERSITI PUTRA MALAYSIA

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

BAHAREH HOSSEINPOURSARMADI

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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

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By

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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
(r²=0.762), α-amylase inhibition (r²=0.766) as well as insulinotropic effect, at pH 5.2, (r²=0.940). A significant (P<0.01) and moderate correlation was observed between protein content and ACE inhibition (r²=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

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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 yang terhasil pada pH 5.2 meningkat dengan penambahan kepekatan. Namun begitu, autolisate yang 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 (r²=0.827), keupayaan untuk menghalang pelunturan β-karotena (r²=0.762), perencatan α-amilase (r²=0.766) dan kesan insulinotropik pada pH 5.2, (r²=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 dieksplotisasikan kepada makanan berfungsi atau digunakan sebagai sumber neutraseutikal.
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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:

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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
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