UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORY PEPTIDES FROM RED TILAPIA PROTEIN HYDROLYSATES

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By

MARYAM SHAMLOO

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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To My Parents

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

PRODUCTION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORY PEPTIDES FROM RED TILAPIA PROTEIN HYDROLYSATES

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Chairman : Professor Jamilah bt. Bakar, PhD

Faculty : Food Science and Technology

Fish proteins are considered as valuable nutrient and a good source of many bioactive peptides such as angiotensin converting enzyme (ACE) inhibitory peptides. Very few reports are available on the ACE inhibitory peptides in freshwater fish hydrolysates. Therefore, this study was carried out with the objective to produce tilapia protein hydrolysates by commercial proteases, named Alcalase, Flavourzyme and Protamex, investigating the ACE (Angiotensin Converting Enzyme) inhibitory activity, the radical scavenging ability and identifing the best enzyme to produce the highest bioactivity; optimizing the production of ACE inhibitory peptides using response surface methodology (RSM); and to fractionate the ACE inhibitory peptides using ultrafiltration membranes. The ACE inhibitory activities were determined using an in *vitro* method and the IC_{50} (peptide concentration which reduced ACE inhibitory by 50%) was calculated. The result indicated that Alcalase was the best enzyme to

produce tilapia hydrolysates since it had the highest ACE inhibitory activity when compared to Protamex and Flavourzyme. A central composite design (CCD) involving 16 cube points, 8 axial points and 7 center points was employed to study the effect of temperature, time, pH and enzyme-substrate ratio on Alcalase hydrolytic activity. The combined level of 55.8 ˚C, 259.99 min, pH 7.5 and enzyme-substrate ratio of 3.58 % (w/w) was predicted to provide the most desirable bioactivity, which produce high ACE inhibitory activity in tilapia hydrolysates. The coefficient of determination value (R^2) was 0.883 for the experimental data, which indicated a satisfactory adjustment of the reduced response models. The time, temperature and enzyme-substrate ratio of the hydrolysis had significant ($p < 0.01$) effects on the ACE inhibitory activity in tilapia hydrolysates. The most desirable hydrolysates were fractionated using three different molecular weight cut-off membranes (10 kDa, 5 kDa and 2 kDa). Four fractions (> 10 kDa, 10-5 kDa, 5-2 kDa and < 2 kDa) obtained had the ACE inhibitory activity, however, the fraction with molecular weight of < 2 kDa, appeared to have a significantly ($p < 0.05$) lower IC_{50} compared to the unfractionated hydrolysate, and the other fractions.

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

PENGHASILAN PEPTIDA PERENCAT ENZIM PENUKAR ANGIOTENSIN DARIPADA PROTEIN HIDROLISAT TILAPIA MERAH

Oleh

MARYAM SHAMLOO

Disember 2010

Pengerusi : Profesor Jamilah bt. Bakar, PhD

Fakulti : Sains dan Teknologi Makanan

Protein ikan dianggap sebagai nutrien yang berharga dan sumber peptida bioaktif yang baik seperti peptida perencat enzim penukar angiotensin (ACE). Laporan mengenai peptida perencat ACE daripada hidrolisat ikan air tawar sangat sedikit. Oleh sebab itu, kajian ini di jalankan dengan objektif untuk menghasilkan hidrolisat protein tilapia menggunakan proteasa homersil, iaitu Alcalase, Flavourzyme dan Protamex, mengkaji aktiviti perencat ACE, aktiviti pemeranghapan bilasan radikal bebas dan mengenalpasti jenis enzim yang dapat menghasilkan bioaktiviti tertinggi, mengoptimumkan penghasilan peptida perencat ACE berdasarkan kaedah permukaan respons (RSM), dan mengfraksinasikan peptida perencat ACE dengan menggunakan membran ultrafiltrasi. Kaedah *in vitro* digunakan untuk menentukan aktiviti perencatan ACE dan pengiraan nilai IC_{50} (kepekatan peptida untuk mengurangkan perencat ACE sebanyak 50%). Keputusan menunjukkan bahawa Alcalase merupakan

enzim yang terbaik untuk menghasilkan hidrolisat tilapia dengan aktiviti perencat ACE yang tertinggi. Reka bentuk komposit memusat (CCD) yang melibatkan 16 titik kubus, 8 titik paksi dan 7 titik pusat telah digunakan untuk mengkaji kesan suhu, masa, pH dan nisbah enzim-substrat terhadap aktivitis hidrolisat Alcalase. Gabungan suhu, masa, pH dan nisbah enzim-substrat pada 55.8 ˚ C, 259.99 minit, 7.5 dan 3.58 % (berat/berat) masing-masing telah ramalkan dapat menghasilkan aktiviti perencat ACE yang tinggi. Nilai koefisien hubung-kait yang tinggi (R^2= 0.883) menunjukkan bahawa model regresi yang dihasilkan menerangkan variasi data dengan memuaskan. Masa, suhu dan nisbah enzim-substrat terhadap aktiviti hidrolysis telah menunjukkan kesan yang ketara (p <0.01) ACE keatas aktiviti perencatan pada hidrolisat tilapia yang dihasilkan. Hidrolisat telah di ultrafiltrasi dengan menggunakan tiga jenis membrane deugan cut-off berat molekul yang berbeza (10 kDa, 5 kDa dan 2 kDa). Keempat fraksi (> 10 kDa, 10-5 kDa, 5-2 kDa dan <2 kDa) yang diperolehi telah menunjukkan aktiviti perencatan ACE, walau bagaimarapun fraksi dengan berat molekul <2 kDa nampaknya telah menunjukkan nilai IC_{50} yang lebih rendah (p < 0.05) dari fraksi yang lain.

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I certify that an Examination Committee has met on 23/12/2010 to conduct the final examination of Maryam Shamloo on her Master of Science thesis entitled "Production of Angiotensin Converting Enzyme Inhibitory Peptides From Red Tilapia (*Oreochromis Niloticus*) Hydrolysates" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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

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DECLARATION

I declare that the thesis is my original work except for quotations 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.

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MARYAM SHAMLOO

Date: 23 December 2010

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