UNIVERSITI PUTRA MALAYSIA

BIOTRANSFORMATION OF FERULIC ACID, ANTIOXIDANT ACTIVITY, AND CHOLESTEROL GENE REGULATION OF RICE BRAN BYPRODUCTS

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By

HADIZA ALTINE ADAMU

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Chairman : Professor Maznah Ismail, PhD

Faculty : Institute of Bioscience

An astronomical proportion of lignocellulosic waste such as husks, bran, bagasse, cobs, lint and fibre are produced via paper-pulp, timber, forestry and agricultural industries. The said "waste" can be transformed into numerous value added products such as biofuels, chemicals, cheap energy sources for fermentation, improved animal feed and human nutrients. In this study, the potential of *Lactobacillus farciminis* 29644, *Lactobacillus reuteri* 23272 and *Lactobacillus fermentum* 11976 to biotransform ferulic acid via submerged fermentation and solid state fermentation of rice bran to other phenolic compounds, their antioxidant activities and ability to regulate the genes involved in cholesterol metabolism were investigated. Ferulic acid is an important bioactive constituent of agro-industrial wastes such as brans, husks, cobs, bagasse, fiber and lint. Biotransformation started after 5 h incubation of *L. farciminis* 29644 with ferulic acid in Man Rogosa and Sharpe (MRS) broth at 37 °C under 5% CO_2 and production rate was at its peak after 48 h. The impact of initial concentrations of ferulic acid and bacteria on the production of biotransformed products in particular 4-vinyl-guaiacol (4VG) was studied. Biotransformation via

solid state fermentation (SSF) using rice bran (RB) as substrate was equally determined over a 24 h period. Identification and quantification of biotransformed products was by high performance liquid chromatography (HPLC). Subsequently the antioxidant capacity of the biotransformed product from submerged and SSF along with the ability of the byproduct from SSF of rice bran to regulate the genes involved in cholesterol metabolism was investigated. The results indicated that the production of 4VG via submerged fermentation was significantly affected by the initial concentration of ferulic acid, and empirically 15, 25, 35 and 50 mg/l of ferulic acid yielded 3.34, 6.84, 8.93 and 10.26 mg/l of 4VG, respectively. Under SSF, the biotransformed products after 24 h were considered the most significant. The production of 4VG and vanillin were at their highest after 1h at 29.0 and 7.1 mg/l respectively while ferulic acid and vanillic acid were at 17h at 19.6 and 9.6 mg/l respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2´-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity of the byproduct from submerged fermentation showed that the higher the ferulic acid concentration the better the activity as was observed with 25, 35 and 50 mg/l of ferulic acid. The same trend was equally observed for the byproduct from SSF. As for the ability of the SSF byproduct to regulate genes involved in cholesterol metabolism, 5µg/ml of byproduct up regulated LDLR, 5 and 20µg/ml of byproduct down regulated ApoB100, 20µg/ml of byproduct down regulated HMG-CoAR, and 40µg/ml of byproduct up regulated ApoA1. The findings are a milestone towards economical high yielding means of biotransforming some common agro-industrial wastes to a value added product such as nutraceuticals.

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

BIOTRANSFORMASI ASID FERULIC, AKTIVITI ANTIOKSIDAN, DAN KAWALATUR GEN KOLESTEROL OLEH HASIL SAMPINGAN

Oleh

HADIZA ALTINE ADAMU

Disember 2012

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Kebanyakan sisa lignoselulosik seperti sekam, dedak, hampas tebu, tongkol, lin dan serat adalah hasil daripada industri pulpa kertas, pembalakan, perhutanan dan pertanian. "Sisa" tersebut boleh ditransformasikan kepada bahan api bio, bahan kimia, sumber tenaga murah bagi proses penapaian, makanan haiwan dan manusia serta pelbagai produk lain yang mempunyai nilai tambah. Dalam kajian ini, potensi *Lactobacillus farciminis* ATCC 29644 *Lactobacillus reuteri* 23272 and *Lactobacillus fermentum* 11976 bagi proses biotransformasi asid ferulik melalui penapaian tenggelam dan penapaian dedak beras dalam keadaan pepejal kepada sebatian fenol yang lain, aktiviti antioksidan serta keupayaan untuk mengawalatur gen yang terlibat dalam metabolisme kolesterol telah diselidik. Asid ferulik adalah sebatian bioaktif utama dalam sisa buangan industri pertanian seperti dedak, sekam, tongkol, hampas tebu, serat dan lin. Proses biotransformasi asid ferulik bermula selepas lima jam inkubasi *L. farciminis* dengan acid ferulik dalam media 'Man Rogosa and Sharpe' (MRS) pada 37 °C dan 5% CO_2. Kadar penghasilan mencapai kemuncaknya selepas 48 jam. Kesan konsentrasi awal asid ferulik dan bakteria dalam pengeluaran produk biotransformasi terutamanya 4-vinyl-guaiacol (4VG) turut dikaji. Proses

biotransformasi melalui penapaian dalam keadaan pepejal (SSF) dengan menggunakan dedak beras (RB) sebagai substrat telah diselidik sepanjang tempoh 24 jam. Proses identifikasi dan kuantifikasi produk biotransformasi telah dijalankan dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Sementara itu, kapasiti antioksidan serta keupayaan produk biotransformasi penapaian tenggelam dan produk sampingan SSF dalam mengawalatur gen yang terlibat dalam metabolisme kolesterol juga dikaji. Hasil kajian menunjukkan produksi 4VG melalui penapaian tenggelam dipengaruhi dengan signifikan oleh konsentrasi awal asid ferulik, di mana 15, 25, 35 dan 50 mg/l asid ferulik telah menghasilkan 3.34, 6.84, 8.93 dan 10.26 mg/l 4VG masing-masing. Di bawah keadaan SSF, produk biotransformasi pada jam ke-24 adalah yang paling signifikan. Produksi 4VG dan vanillin adalah paling tinggi selepas 1 jam, mencapai 29.0 dan 7.1 mg/l masing-masing, sementara asid ferulik dan asid vanilik mencapai tahap maksimum selepas 17 jam, dengan produksi sebanyak 19.6 dan 9.6 mg/l masing-masing. Aktiviti memerangkap radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan 2,2´-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) oleh produk sampingan penapaian tenggelam menunjukkan aktiviti yang lebih tinggi dengan konsentrasi asid ferulik yang lebih pekat, seperti yang diperhatikan dengan 25, 35 dan 50 mg/l asid ferulik. Gaya yang sama turut diperhatikan dengan produk sampingan SSF. Produk sampingan SSF turut berupaya mengawalatur gen yang terlibat dalam metabolisme kolesterol. 5µg/ml produk sampingan meningkatkan regulasi LDLR, 5 and 20µg/ml produk sampingan mengurangkan regulasi ApoB100, 20µg/ml produk sampingan mengurangkan regulasi HMG-CoAR, dan 40µg/ml produk sampingan meningkatkan regulasi ApoA1. Penemuan dalam kajian ini membantu mewujudkan cara yang

berhasil tinggi dan berekonomikal dalam proses biotransformasi sisa buangan industri pertanian kepada produk bernilai tambah seperti nutraseutikal.

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I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Hadiza Altine Adamu on her Master of Science thesis entitled **"Biotransformation of ferulic acid, antioxidant activity and cholesterol gene regulation of rice bran byproducts"** is in accordance with the Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the Master of Science.

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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 and citations which have been duly acknowledged. I also declare that it has not been previously and is not currently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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HADIZA A. ADAMU

Date: 18 December 2012

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