UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF Mangifera pajang KORT. FRUIT FIBRES AND POLYSACCHARIDES AND THEIR ROLES IN SURVIVAL AND HYPOCHOLESTEROLEMIC EFFECTS OF BIFIDOBACTERIA

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By

SADEQ HASAN AL-SHERAGI MUGAHED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April 2012

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DEDICATION

First and foremost, I am sincerely grateful to God for sending this opportunity to continue my studies in a field that I am interested a lot, also my gratitude to our Prophet Mohammed (Peace be upon him).

This thesis is dedicated to my loving parents, for giving me life and love. My best and honest friend Mr. Fouad Abdulrahman Hassan for his encouragement and enormous support throughout different stage of my study. Also for my wife for her love, continuing encouragement, patience and support all these years. To those who fill my life with happiness, joys, hopes, wishes and aspiration also bring the meaning and importance to my life my son Zaid and my daughter Malak. It is also dedicated to my sisters and brothers and the rest of my family who provided the support that helped me to make it through the tough times.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

CHARACTERIZATION OF *Mangifera pajang* KORT. FRUIT FIBRES AND POLYSACCHARIDES AND THEIR ROLES IN SURVIVAL AND HYPOCHOLESTEROLEMIC EFFECTS OF BIFIDOBACTERIA

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SADEQ HASAN AL-SHERAGI MUGAHED

April 2012

Chairman: Professor Amin Ismail, PhD

Faculty: Medicine and Health Sciences

A dried high fiber product from bambangan (*Mangifera pajang* Kort.) fruit pulp was prepared and evaluated for proximate composition, functional properties, and soluble and insoluble dietary fiber composition. The glucose dialysis retardation index of *M. pajang* pulp dietary fiber (60) was approximately double than that of cellulose fiber (31.7). These characteristics indicate that *M. pajang* pulp is a rich source of dietary fiber (87.6 g/ 100g *M. pajang* pulp fibres) and has physico-chemical properties which make it suitable as a potential food additive in various food products and/or dietetic, low-calorie high-fiber foods to enhance their nutraceutical properties.

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Polysaccharides were isolated from the fibres pulp of *M. pajang* Kort . using hot water. Neutral and acidic polysaccharides were separated with DEAE-Cellulose anion-exchange chromatography. Size exclusion chromatography analysis showed the average molecular weight (MW) of the neutral *M. pajang* polysaccharides (F1) was (7.1 kDa), and those of three acidic polysaccharides (F2, F3 and F4) were approximately 12.6, 24 and 9.4 KDa, respectively. The monosaccharide compositions of these polysaccharides were determined using high performance liquid chromatography. F1 contained erythrose, rhamnose, arabinose, mannose, fructose and glucose (5, 7, 21, 42, 4 and 21 mg/100mg fraction respectively), F2 consisted of rhamnose, xylose and arabinose (33, 7 and 51 mg/100mg fraction respectively), F3 consisted of fructose (14 mg/100mg fraction) and glucose (72 mg/100mg fraction), and F4 comprised arabinose, mannose, fructose and glucose (32, 36, 2 and 10 mg/100mg fraction respectively). Results derived from Fourier transform infrared spectroscopy and the monosaccharide composition analysis suggested that the fibres pulp of *M. pajang* fruit consisted of heteropolysaccharide and belongs to –type of the pyran group. Additionally, this crude polysaccharide and its fractions showed strong prebiotic properties.

The viability and activity of *Bifidobacterium pseudocatenlatum* G4 and *Bifidobacterium longum* BB536 were studied in yoghurt containing 0.75% (w/v) of *M. pajang* fibres polysaccharide (MPFP). Growth of probiotics, their proteolytic activities, the development of short chain fatty acids, pH and viscosity of yoghurts were determined during storage at 4°C for 28 d. *B. longum* BB536 and *B. pseudocatenulatum* G4 showed better growth around 8.3 and 8.5 log CFU/mL respectively, in the presence of prebiotics.

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The activity of *B. longum* BB536 and *B. pseudocatenulatum* G4 significantly improved thereby the production of propionic acid (8.2mg/ mL) and proteolytic activity (0.97) of probiotic organisms significantly increased. The viscosity of probiotic yoghurts significantly ($p < 0.05$) increased (1.78 mPa·s) when MPFP was added.

The effect of yoghurt supplemented with *B. pseudocatenulatum* G4 or *B. longum* BB536 in the presence of MPFP on plasma lipids, lipid peroxidation and the faecal excretion of bile acids was determined in rats fed on a cholesterol-enriched diet. Results showed a significant ($p < 0.05$) increased in levels of plasma total cholesterol (TC) in positive control (PC) group (7.01 mmol/L) as compared to the negative control (NC) group (2.4 mmol/L), low-density lipoprotein cholesterol (LDL-C) was 4.56 mmol/L in PC and 0.97 mmol/L in NC also plasma malondialdehyde (MDA) levels were 3.98 nmol/L in PC and 1.72 nmol/L in NC after 8 weeks.

Atherosclerotic index, triglyceride (TG), and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine significant ($p < 0.05$) increased in positive control (PC) group as compared to the negative control (NC) group after 4 and 8 weeks. However, the groups fed on a cholesterol-enriched diet supplemented with yoghurt containing *B. pseudocatenulatum* G4 or *B. longum* BB536 with or without MPFP and inulin as a prebiotics had significantly lower levels of plasma TC, LDL, very low-density lipoprotein cholesterol (VLDL-C), atherosclerotic index, TG, MDA and the activities of ALT, AST, urea and creatinine than the positive control (PC) group at week 4 and 8 of the treatment. The levels of TC, LDL-C and MDA in group consumed yoghurt containing *B. pseudocatenulatum* G4 with MPFP were 2.42

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mmol/L, 0.84 mmol/L and 1.29 nmol/L respectively. In addition, the faecal excretions of bile acids were markedly promoted in yoghurt containing *B. pseudocatenulatum* G4 or *B. longum* BB536 groups with or without MPFP or inulin compared PC and NC groups. The concentration of cholic acid in PC and NC was 28.67 and 28.79 mg/g faeces and significantly ($p < 0.05$) increased in group consumed *B. pseudocatenulatum* G4 with MPFP 34.64 mg/g faeces. It is noteworthy from the data that the presence of MPFP and inulin were more effective. The effect of MPFP on the survival activity and hypocholesterolaemic effects of bifidobacteria was consistent with that of inulin (a commercial prebiotic) thus MPFP could be added to fermented dairy instead of inulin.

M. pajang polysaccharides significantly stimulated the growth of *B. longum* BB536 and *B. pseudocatenulatum* G4; however no effect on the growth of *Salmonella choleraesuis* JCM 6977 and *Escherichia coli* ATCC 35922. *B. longum* BB536 and *B. pseudocatenulatum* G4 had a positive effect on the lipid profile and bile acids of the rats.

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PENCIRIAN FIBER DAN POLISAKARIDA BUAH *Mangifera pajang* DAN PERANANNYA TERHADAP PERTUMBUHAN BIFIDOBAKTERIA SERTA KESAN HIPOKOLESTEROLEMIK

Oleh

SADEQ HASAN AL-SHERAGI MUGAHED

April 2012

Pengerusi: Profesor Amin Ismail, PhD

Fakulti: Perubatan dan Sains Kesihatan

Satu produk berserat tinggi yang dikeringkan telah disediakan daripada pulpa buah bambangan (*Mangifera pajang* Kort.) dan ia dikaji untuk mengetahui komposisi proksimat, ciri-ciri berfungsi, dan komposisi serat pemakanan larut dan tidak larut air. Serat pemakanan pulpa *M. pajang* mempunyai indeks perencatan dialisis glukosa (60) yang hampir dua kali ganda berbanding dengan serat selulosa (31.7). Ciri ini menunjukkan bahawa pulpa *M. pajang* adalah sumber kaya serat pemakanan (87.6 g/ 100 g pulpa) dan mempunyai ciri-ciri fizikokimia yang menjadikan ia sesuai sebagai bahan penambah makanan dengan potensi penggunaan dalam pelbagai produk makanan dan/atau dietetik, makanan yang tinggi serat dan berkalori rendah untuk meningkatkan ciri nutraseutikal produk.

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Polisakarida diekstrak daripada pulpa berserat *M. pajang* dengan menggunakan air panas. Pecahan polisakarida berciri neutral dan asid diasingkan dengan kromatografi penukaran-ion DEAE-selulosa. Analisis kromatografi penyisihan saiz menunjukkan bahawa purata berat molekul (MW) pecahan polisakarida neutral (F1) *M. pajang* adalah 7.1 kDa, serta lebih kurang 12.6, 24 dan 9.4 kDa untuk tiga pecahan asid polisakarida (F2, F3 dan F4). Komposisi monosakarida pecahan polisakarida tersebut ditentukan dengan kromatografi cecair berprestasi tinggi. F1 mengandungi eritrosa, ramnosa, arabinosa, mannosa, fruktosa dan glukosa (dengan masing-masing sebanyak 5, 7, 21, 42, 4 dan 21 mg/100mg), F2 mengandungi ramnosa, xylosa and arabinosa (dengan masing-masing sebanyak 33, 7 dan 51 mg/100mg), F3 mengandungi fruktosa (14 mg/100mg) dan glukosa (72 mg/100mg), dan F4 mengandungi arabinosa, mannosa, fruktosa dan glukosa (dengan masing-masing sebanyak 32, 36, 2 dan 10 mg/100mg). Keputusan diperoleh daripada spektroskopi inframerah jelmaan fourier dan analisis komposisi monosakarida mencadangkan bahawa pulpa berserat *M. pajang* terdiri daripada heteropolisakarida dan merupakan jenis dari kumpulan piran. Tambahan pula, kandungan polisakarida kasar dan pecahannya menunjukkan ciri prebiotik yang kukuh.

Viabiliti dan aktiviti *Bifidobacterium pseudocatenlatum* G4 dan *B. longum* BB536 telah dikaji dalam yogurt yang mengandungi 0.75% (berat/kandungan) polisakarida berserat *M. pajang* (MPFP). Pertumbuhan probiotik, aktiviti proteolitik, penghasilan asid lemak berantaian pendek, pH and kelikatan yogurt ditentukan sepanjang penyimpanan pada suhu 4°C selama 28 hari. *B. longum* BB536 dan *B. pseudocatenulatum* G4 telah menunjukkan pertumbuhan yang lebih baik iaitu masing-masing 8.3 dan 8.5 log CFU/mL, dengan kehadiran prebiotik. Aktiviti *B. longum* BB536 dan *B.*

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pseudocatenulatum G4 meningkat secara signifikan di mana penghasilan asid propionik (8.2mg/ mL) dan aktiviti proteolitik organisma probiotik (0.97) meningkat secara signifikan. Kelikatan yogurt probiotik meningkat secara signifikan (1.78 mPa·s, $p < 0.05$) apabila MPFP ditambah.

Kesan suplementasi yogurt yang mengandungi *B. pseudocatenulatum* G4 atau *B. longum* BB536 dengan kehadiran MPFP ke atas lipid plasma, pengoksidaan lipid dan perkumuhan asid hempedu dalam najis dikaji dalam tikus yang diberi diet kaya kolesterol. Keputusan menunjukkan peningkatan yang signifikan ($p < 0.05$) bagi paras jumlah kolesterol plasma (TC) dalam kumpulan kontrol positif (PC) iaitu 7.01 mmol/L berbanding kumpulan kontrol negatif (NC) iaitu 2.4 mmol/L, lipoprotein berketumpatan rendah (LDL) ialah 4.56 mmol/L (PC) dan 0.97 mmol/L (NC) dan malondialdehid plasma (MDA) ialah 3.98 nmol/L (PC) dan 1.72 nmol/L (NC) selepas 8 minggu.

Indeks atherosklerotik, trigeliserida (TG) dan aktiviti *alanine aminotransferase* (ALT), *aspartate aminotransferase* (AST), urea dan kreatinin meningkat secara signifikan ($p < 0.05$) dalam kumpulan kawalan positif (PC) berbanding dengan kumpulan kawalan negatif (NC) selepas minggu ke-4 dan ke-8. Walau bagaimanapun, kumpulan yang diberi diet kaya kolesterol dengan suplementasi yogurt yang mengandungi *B. pseudocatenulatum G4* atau *B. longum* BB536 dengan atau tanpa MPFP dan inulin sebagai prebiotik mempunyai paras TC, LDL, lipoprotein-kolesterol berketumpatan sangat rendah (VLDL-C), indeks atherosklerotik, TG, MDA serta aktiviti ALT, AST, urea dan kreatinin yang secara signifikan lebih rendah berbanding dengan kumpulan kawalan positif (PC) pada minggu ke-4 dan ke-8. Paras TC, LDL-C dan MDA bagi

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kumpulan yang mengambil yoghurt dengan *B. pseudocatenulatum* G4 dan MPFP ialah masing-masing 2.42 mmol/L, 0.84 mmol/L dan 1.29 nmol/L. Tambahan pula, perkumuhan asid hempedu dalam najis meningkat ketara dalam kumpulan yang menerima suplementasi yogurt yang mengandungi *B. pseudocatenulatum* G4 atau *B. longum* BB536 dengan atau tanpa MPFP atau inulin berbanding dengan kumpulan PC dan NC. Kepekatan asid kolik bagi PC dan NC masing-masing ialah 28.67 dan 28.79 mg/g najis dan ia meningkat secara siginifikan ($p < 0.05$) bagi kumpulan yang mengambil *B. pseudocatenulatum* G4 dengan MPFP 34.64 mg/g najis. Data juga menunjukkan bahawa kehadiran MPFP dan inulin memberi kesan yang lebih baik. Kesan MPFP ke atas aktiviti pertumbuhan dan kesan hipokolesterolemik oleh bifidobakteria adalah konsisten dengan inulin (prebiotik komersial) maka MPFP boleh ditambah ke dalam susu yang ditapai berbanding inulin.

Polisakarida *M. pajang* merangsangkan pertumbuhan *Bifidobacterium longum* BB536 dan *B. pseudocatenulatum* G4 secara signifikan; tetapi tiada kesan ke atas pertumbuhan *Salmonella choleraesuis* JCM 6977 dan *Escherichia coli* ATCC 35922. *B. longum* BB536 dan *B. pseudocatenulatum* G4 mempunyai kesan positif ke atas profil lipid dan asid hempedu tikus.

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I certify that a Thesis Examination Committee has met on (23-04-2011) to conduct the final examination of SADEQ HASAN AL-SHERAGI MUGAHED on his thesis entitled, "Characterization, Prebiotic Properties of *Mangifera pajang* Fruit Fibrous, Polysaccharides and Their Roles on Survival, Hypocholesterolemic Effects of Bifidobacteria" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy degree.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment was of the requirement for the degree of Doctor of Philosophy. 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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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