



UNIVERSITI PUTRA MALAYSIA

**CALCIUM ABSORPTION AND BIOAVAILABILITY OF ISOFLAVONES
FROM TEMPEH COMPARED TO MILK AMONG POSTMENOPAUSAL
MALAY WOMEN**

HASNAH HARON

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POSTMENOPAUSAL MALAY WOMEN**

By

HASNAH HARON

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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September 2009

Chairman : Associate Professor Amin Ismail, PhD

Faculty : Medicine and Health Sciences

The main objective of this study was to determine the absorption of calcium from tempeh compared to milk and apparent bioavailability of isoflavone in urinary excretions of postmenopausal Malay women consuming tempeh. There were three phases in this study. In phase one, food analyses on tempeh showed every 100 g of fried tempeh contained $41.8 \pm 5.1\%$ moisture, $18.6 \pm 1.2\%$ crude protein, $18.8 \pm 7.2\%$ crude fat, $19.9 \pm 3.4\%$ total carbohydrate, $0.8 \pm 0.2\%$ total ash, 63.3 ± 2.7 mg Ca, 34.57 ± 11.07 mg daidzein (DA) and 30.50 ± 11.41 mg genestein (GE), based on wet weight. Deep frying tempeh in batter for 30 minutes decreased 45% of the total isoflavones in fried tempeh (113 ± 41 mg) compared to the raw one (205 ± 56 mg). Raw tempeh contained the highest total amount of DA (25.64 ± 5.65 mg) and GE (28.41 ± 9.15 mg) compared to other studied local soy products.



In phase two, health screening was conducted to select healthy subjects for clinical trial in phase three. Ethical approval was obtained from the ethical committee of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) prior to the study. A total of 125 postmenopausal Malay women screened from five locations in suburban of Kuala Lumpur and 42 of them met the inclusion criteria and were qualified to take part in the clinical trial. The mean age of the subjects was 59 ± 4 years and they were on average 10 ± 7 years postmenopausal. Average weight, height and body mass index (BMI) for these subjects were 63.7 ± 10.1 kg, 1.5 ± 0.1 m and 28.1 ± 4.2 kg/m². Majority (46%) of the women was overweight while 31% were obese and two percent were underweight.

Two-thirds of them have been taking medication for chronic diseases like hypertension (27%), diabetes mellitus (9%), heart disease (1%) and combination of the three chronic diseases (19%). Average values for fasting serum lipid for these subjects were 5.97 ± 1.23 mmol/L of total cholesterol (TC), 1.40 ± 0.33 mmol/L of high density lipoprotein cholesterol (HDL), 3.84 ± 1.02 mmol/L of low density lipoprotein cholesterol (LDL) and 1.77 ± 0.96 mmol/L of triglyceride (TG). About 74% of subjects were hypercholesterolemic and 58% were hypertriglyceridemic. Based on the calcaneal measurement, 37% of the subjects were osteopenic while 6% were osteoporotic.

Based on the dietary history questionnaire, average calcium intake of the subjects was 505 ± 263 mg/d when. Their main source of calcium was obtained from vegetables (37%), dairy products (32%), meat and seafood (17%), cereal (7%), fruits (5%) and beverages (2%). Using the semi food frequency questionnaire (SFFQ) for soy products,



the average estimated isoflavone intake for these subjects was 25 ± 15 mg/d. The most frequently consumed soy products for this population was tempeh (25%), fujook (17.4%), homemade soy bean drink (11.2%), unfried tofu (10.3%), fried tofu (8.9%), tofufah (8.4%), soft tofu (7.4%), boxed soy bean drink (7.2%) and egg tofu (4.3%).

In phase three, 21 healthy postmenopausal Malay women volunteered to take part in the clinical trial for calcium absorption and apparent bioavailability study. The study was carried out at the in patient clinical trial ward of UKMMC. Only 20 subjects completed the study since one subject was excluded for not completing the urine collection. The mean age of these subjects was 57 ± 3 years and they were on average 9 ± 5 years postmenopausal. Average weight, height and BMI for these subjects were 63 ± 11 kg, 1.5 ± 0.1 m and 27 ± 4 kg/m². Majority (55%) of these women was overweight while 20% were obese. Measurement of bone mineral density (BMD) using dual energy absorptiometry (DXA), indicated that 50% of the subjects was osteopenic, 35% were normal and 15% were osteoporotic. Body weight was significantly correlated to the BMD of the total body ($r = 0.457$, $p = 0.037$) and neck ($r = 0.507$, $p = 0.019$).

Based on 3-day food records, 20 postmenopausal Malay women have average low calcium intake of 426 ± 122 mg/d, and 30% of them have reported of not taking any milk. Their mean values for parathyroid hormone (PTH), serum 25-hydroxyvitamin D (25(OH)D), urinary deoxypyridinoline (DPD) and serum alkaline phosphatase (BAP) were : 59.5 ± 21.6 pg/ml, 11.1 ± 4.1 ng/ml, 11.1 ± 1.8 nmol/mmol and 37.1 ± 8.3 U/L, respectively. The majority (95%) subjects had serum 25(OH)D less than 20 ng/ml, which

are indicative of vitamin D insufficiency. Correlation analysis showed a significant inverse association between serum 25(OH)D and BMI ($r = -0.388$, $p = 0.045$). About 30% of the subjects had secondary hyperparathyroidism with PTH concentrations exceed 65 pg/ml.

Fractional calcium absorption from tempeh was compared to that observed from milk, using a dual stable isotope approach in a randomized cross-over design. Subjects consumed the same calcium load (130-150 mg Ca) from either milk or tempeh with a one-month washout period between each test meal. ^{42}Ca (0.036 mg/kg) was administered intravenously to subjects prior to oral administration of ^{44}Ca (0.272 mg/kg) in milk. All urine from subjects was collected for 24 h post-dosing in 8 h pools. Average percent calcium absorption from tempeh ($36.9 \pm 10.4\%$) was not significantly different ($p > 0.05$) from that observed from milk ($34.3 \pm 8.4\%$). Estimated calcium balance (V_{Bal}) from taking tempeh (108 ± 63 mg/d) was significantly higher ($p < 0.05$) compared to milk (71 ± 64 mg/d).

Apparent bioavailability of isoflavones was determined from the urinary isoflavone excretions following ingestion of 240 g tempeh (160 mg isoflavones) and milk. Tempeh consumption for day one was carried out at the clinical trial ward and the same three-8h urine pool collected for calcium absorption study at the ward was used for isoflavone study. Tempeh consumption and 24 h urine collection for day two and three was carried out at subjects' home. DA, GE, equol (EQ) and flavone (FLA) standards eluted at mean retention time of 16.8 ± 0.1 , 20.6 ± 0.1 , 21.1 ± 0.1 , 25.4 ± 0.1 min, respectively. An

average excretion of $3.51 \pm 0.62 \mu\text{mol/h}$ DA and $2.79 \pm 0.35 \mu\text{mol/h}$ GE were detected after consumption of milk. DA ($47.06 \pm 4.18 \mu\text{mol/h}$), GE ($33.27 \pm 3.71 \mu\text{mol/h}$) and EQ ($24.35 \pm 4.34 \mu\text{mol/h}$) were detected in three-8 h urine pool, following tempeh consumption (Day 1). There was a significant correlation ($r = 0.453$, $p = 0.045$) between percent calcium absorption and total isoflavone excretion in 9-16 h urine pool. Urinary isoflavone excretions following ingestion of tempeh (Day 1) were significantly higher ($p < 0.05$) compared to that of the milk.

The average amount of total isoflavones consumed in three days of tempeh consumption was $154.83 \pm 1.82 \text{ mg}$ per day. Total isoflavones excreted in each day one, two and three of tempeh consumption were as follows: 104.68 ± 9.21 , 32.64 ± 3.18 and $30.25 \pm 3.99 \mu\text{mol/day}$, respectively. The average isoflavone excreted from three days of tempeh consumption were $26.16 \pm 2.64 \mu\text{mol/h}$ DA, $16.64 \pm 1.98 \mu\text{mol/h}$ GE and $13.06 \pm 1.79 \mu\text{mol/h}$ EQ. Almost all subjects excreted EQ following three days of tempeh consumption. There was only one subject (5%) that can be classified as equol producer based on ratio of equol produced to daidzein consumed > 0.2 . Isoflavone intake of the 20 subjects was estimated to be $26 \pm 13 \text{ mg}$ per day, ranging from 6 - 58 mg. Based on SFFQ for soy products, the frequently consumed local soy products were consisted of tempeh (19.6%), fujook (16.5%), firm tofu (13.4%), fried firm tofu (11.3%), tofufah (10.3%), homemade SB drink (10.3%), boxed SB drink (7.2%), soft tofu (6.2%) and egg tofu (5.2%)

In conclusion, this sample of postmenopausal Malay women has low calcium intake that achieved only 40 - 50% of the Malaysian RNI. Low intake of calcium among these subjects may be due to their predominantly non-milk based diet where 30-40% of them do not take any milk. Calcium bioavailability from tempeh provided similar amounts of absorbed calcium to that obtained from a glass of milk. These findings indicated that tempeh may have the potential to contribute significantly to the calcium needs of these postmenopausal Malay women who were at risk of low bone mass and were insufficient of vitamin D. Increased incorporation of tempeh, the affordable and available plant sources of calcium and isoflavones may contribute significantly to the calcium needs of this high-risk population and also help to reduce the abnormal serum lipid levels in majority of these subjects.



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**PENYERAPAN KALSIUM DAN BIOAVAILABILITI ISOFLAVON DARI
TEMPEH BERBANDING SUSU DI KALANGAN
WANITA MELAYU MENOPAUS**

Oleh

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Objektif utama kajian ini adalah untuk menentukan penyerapan kalsium dari tempeh berbanding susu dan bioavailabiliti ketara isoflavon melalui urin wanita Melayu menopaus yang memakan tempeh. Terdapat tiga fasa dalam kajian ini. Dalam fasa pertama, analisis makanan ke atas tempeh menunjukkan setiap 100 g tempeh goreng mengandungi $41.8 \pm 5.1\%$ air, $18.6 \pm 1.2\%$ protein kasar, $18.8 \pm 7.2\%$ lemak kasar, $19.9 \pm 3.4\%$ karbohidrat total, $0.8 \pm 0.2\%$ abu total, 63.3 ± 2.7 mg kalsium, 34.57 ± 11.07 mg daidzein (DA) dan 30.50 ± 11.41 mg genestein (GE), berdasarkan berat basah. Penggorengan tempeh bersalut tepung dalam minyak yang banyak (terendam) selama 30 minit telah mengurangkan kandungan isoflavon sebanyak 45% dalam tempeh goreng (113 ± 41 mg) berbanding tempeh mentah (205 ± 56 mg). Tempeh mentah mengandungi jumlah isoflavon total DA (25.64 ± 5.65 mg) dan GE (28.41 ± 9.15 mg) yang tertinggi berbanding produk kacang soya tempatan lain yang dikaji.



Dalam fasa kedua, penyaringan kesihatan telah dijalankan untuk memilih subjek yang menepati kriteria yang telah ditetapkan untuk percubaan klinikal dalam fasa ketiga. Kelulusan etika telah diperolehi dari jawatankuasa etika dari Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM) sebelum menjalankan kajian. Sejumlah 125 orang wanita Melayu menopause yang telah disaring dari lima lokasi di pinggir bandar Kuala Lumpur. Terdapat 42 orang dari mereka telah menepati kriteria yang telah ditetapkan dan layak untuk mengambil bahagian dalam kajian klinikal. Subjek yang telah disaring mempunyai purata umur 59 ± 4 tahun ketika mula menopause dan telah pun menopause selama 10 ± 7 tahun. Purata berat badan, ketinggian dan jisim tubuh badan (BMI) para subjek adalah 63.7 ± 10.1 kg, 1.5 ± 0.1 m and 28.1 ± 4.2 kg/m². Kebanyakan subjek (46%) mempunyai berat badan berlebihan manakala 31% adalah obes dan dua peratus mempunyai kekurangan berat badan.

Dua pertiga dari subjek telah melaporkan yang mereka menerima rawatan dan mengambil ubatan untuk penyakit seperti hipertensi (27%), diabetes mellitus (9%), penyakit jantung (1%) dan gabungan dari tiga jenis penyakit kronik ini (19%). Nilai purata lipid serum ketika subjek berpuasa adalah 5.97 ± 1.23 mmol/L kolesterol total (TC), 1.40 ± 0.33 mmol/L kolesterol lipoprotein berketumpatan tinggi (HDL), 3.84 ± 1.02 mmol/L kolesterol lipoprotein berketumpatan rendah (LDL) dan 1.77 ± 0.96 mmol/L trigliserida (TG). Hampir 74% dari subjek mengalami hiperkolesterolemik manakala 58% mengalami hipertrigliseridemi. Terdapat 37% dari subjek mengalami osteopenia manakala sebanyak 6% mengalami osteoporosis berdasarkan pengukuran tulang pada bahagian tumit.

Purata pengambilan kalsium bagi subjek adalah 505 ± 263 mg/hari, berdasarkan soal-selidik sejarah pengambilan makanan (DHQ). Sumber utama kalsium untuk kumpulan ini diperolehi dari sayur - sayuran (37%), produk tenusu (32%), daging dan makanan laut (17%), bijirin (7%), buah-buahan (5%) dan minuman (2%). Purata anggaran pengambilan isoflavon para subjek adalah 25 ± 15 mg/hari, berdasarkan soal selidik (SFFQ) untuk produk kacang soya. Produk soya yang sering diambil oleh populasi ini adalah tempeh (25%), fujook (17.4%), minuman kacang soya yang disediakan di rumah (11.2%), tahu tak digoreng (10.3%), tahu digoreng (8.9%), tofufah (8.4%), tahu lembut (7.4%), minuman kacang soya dalam kotak (7.2%) dan tahu telur (4.3%).

Dalam fasa ketiga, terdapat 21 orang wanita menopause Melayu yang sihat yang telah mengambil bahagian secara sukarela untuk kajian klinikal penyerapan kalsium dan bioavailabiliti isoflavon secara ketara. Kajian ini dijalankan di wad percubaan klinikal PPUKM. Di akhir kajian ini, hanya 20 subjek yang dapat menghabiskan kajian ini kerana seorang subjek telah dikeluarkan disebabkan pengumpulan urিনnya yang tidak lengkap. Purata umur 20 subjek ini adalah 57 ± 3 tahun dan purata jangkamasa menopause adalah 9 ± 5 tahun. Purata berat badan, ketinggian dan BMI subjek kajian adalah 63 ± 11 kg, 1.5 ± 0.1 m dan 27 ± 4 kg/m². Sebahagian besar (55%) wanita menopause ini mengalami berat badan berlebihan manakala 20% adalah obes. Pengukuran ketumpatan tulang (BMD) subjek menggunakan 'dual energy absorptiometry' (DXA) menunjukkan 50% dari subjek mengalami osteopenia manakala 35% adalah normal dan 15% mengalami osteoporosis. Berat badan adalah berkait secara signifikan kepada BMD keseluruhan badan ($r = 0.457$, $p = 0.037$) dan bahagian leher ($r = 0.507$, $p = 0.019$).

Kesemua 20 wanita Melayu menopause mempunyai purata pengambilan kalsium yang rendah iaitu 426 ± 122 mg/hari berdasarkan rekod pengambilan makanan tiga hari dan 30% dari subjek ini didapati tidak meminum susu. Nilai purata untuk hormon paratiroid (PTH), serum 25-hidroksivitamin D (25(OH)D), deoksipiridinolin urin (DPD) dan serum alkaline phosphatase (BAP) adalah seperti berikut : 59.5 ± 21.6 pg/ml, 11.1 ± 4.1 ng/ml, 11.1 ± 1.8 nmol/mmol dan 37.1 ± 8.3 U/L. Kebanyakan subjek (95%) mempunyai serum 25(OH)D kurang dari 20 ng/ml dan menunjukkan subjek kekurangan vitamin D. Analisis korelasi menunjukkan perkaitan signifikan yang berlawanan antara serum 25(OH)D dan BMI ($r = -0.388$, $p = 0.045$). Hampir 30% dari subjek mengalami 'secondary hyperparathyroidism' dengan kepekatan PTH mereka melebihi 65 pg/ml.

Fractions kalsium yang diserap dari tempeh berbanding susu telah ditentukan melalui pendekatan dwi isotop stabil, menggunakan rekabentuk rawak silang. Subjek mengambil amaun kalsium (130-150 g) yang sama dari tempeh atau susu, di mana jarak masa pengambilan di antara kedua ujian makanan ini adalah selama sebulan. ^{42}Ca (0.036 mg/kg) telah diberikan kepada subjek secara intravena sebelum pengambilan ^{44}Ca (0.272 mg/kg) secara oral. Kesemua urin dari subjek dikumpulkan pada setiap lapan jam, selama 24 jam iaitu selepas pengambilan dos isotop yang terakhir. Purata peratus penyerapan kalsium dari tempeh ($36.9 \pm 10.4\%$) adalah tidak berbeza secara signifikan ($p > 0.05$) berbanding susu ($34.3 \pm 8.4\%$). Anggaran kalsium imbalan (V_{Bal}) dari pengambilan tempeh (108 ± 63 mg/d) adalah lebih tinggi secara signifikan ($p < 0.05$) berbanding susu (71 ± 64 mg/d).



Penentuan bioavailabiliti isoflavon ketara ditentukan berdasarkan kepekatan isoflavon dalam urin selepas pengambilan 240 g tempeh (160 mg isoflavon) dan susu. Pengambilan tempeh untuk hari pertama telah dijalankan di wad percubaan klinikal yang menggunakan takungan urin tiga-8 h yang sama untuk kajian kalsium. Pengambilan tempeh dan pengumpulan urin 24 jam untuk hari kedua serta ketiga telah dijalankan di rumah para subjek. Piawai DA, GE, equol (EQ) dan flavone (FLA) dikesan pada purata masa retensi masing – masing, iaitu 16.8 ± 0.1 , 20.6 ± 0.1 , 21.1 ± 0.1 and 25.4 ± 0.1 min. Purata pengeluaran isoflavon dalam urin selepas pengambilan susu adalah sebanyak 3.51 ± 0.62 $\mu\text{mol DA/h}$ dan 2.79 ± 0.35 $\mu\text{mol/h GE}$. Purata pengeluaran isoflavon sebanyak 47.06 ± 4.18 $\mu\text{mol/h DA}$, 33.27 ± 3.71 $\mu\text{mol/h GE}$ dan 24.35 ± 4.34 $\mu\text{mol/h EQ}$ telah dikesan dalam takungan urin tiga-8 h selepas memakan tempeh (Hari pertama). Terdapat perkaitan signifikan ($r = 0.453$, $p = 0.045$) antara peratus penyerapan kalsium dan kepekatan isoflavon total dalam takungan 9-16 jam. Kepekatan isoflavon dalam urin subjek selepas pengambilan tempeh (Hari 1) adalah lebih tinggi secara signifikan ($p < 0.05$) berbanding dengan susu.

Purata amaun isoflavon total yang diambil semasa pengambilan tempeh selama tiga hari adalah 154.83 ± 1.82 mg/hari. Isoflavon total yang didapati dalam urin selepas pengambilan tempeh pada hari pertama, kedua dan ketiga adalah seperti berikut : 104.68 ± 9.21 , 32.64 ± 3.18 and 30.25 ± 3.99 $\mu\text{mol/hari}$. Purata isoflavon dikeluarkan melalui urin selepas pengambilan tempeh selama tiga hari adalah seperti berikut : 26.16 ± 2.64 $\mu\text{mol/h DA}$, 16.64 ± 1.98 $\mu\text{mol/h GE}$ dan 13.06 ± 1.79 $\mu\text{mol/h EQ}$. Hampir kesemua subjek boleh menghasilkan EQ selepas pengambilan tempeh selama tiga hari. Terdapat

seorang subjek (5%) yang boleh dikelaskan sebagai pengeluar EQ berdasarkan ratio equol terhasil kepada DA yang diambil >0.2 . Pengambilan isoflavon oleh 20 subjek telah dianggarkan sebagai 26 ± 13 mg/hari, dengan julat pengambilan sebanyak 6 - 58 mg isoflavon setiap hari. Produk kacang soya yang sering diambil adalah tempeh (19.6%), fujoek (16.5%), tahu tak digoreng (13.4%), tahu digoreng (11.3%), tofufah (10.3%), minuman kacang soya yang dijual di pasar malam (10.3%), minuman kacang soya dalam kotak (7.2%), tahu lembut (6.2%) dan tahu telur (5.2%).

Kesimpulannya, sampel wanita Melayu menopause ini mempunyai pengambilan kalsium yang rendah iaitu hanya mencapai 40-50% dari RNI Malaysia. Kesemua subjek mempunyai pengambilan kalsium yang rendah kerana pengambilan diet mereka yang sejumlah besarnya tidak berasaskan susu. Sebanyak 30-40% dari subjek tidak mengambil susu langsung. Bioavailabiliti kalsium dari tempeh telah memberikan penyerapan amaun kalsium yang setanding dari segelas susu. Hasil kajian ini menunjukkan tempeh berpotensi untuk menyumbang kepada keperluan kalsium wanita Melayu menopause yang kebanyakannya mempunyai ketumpatan tulang yang rendah dan kekurangan vitamin D. Penambahan pengambilan tempeh yang merupakan sumber kalsium serta isoflavon, murah dan mudah didapati akan menyumbang secara signifikan kepada keperluan kalsium populasi ini. Ia juga mungkin akan mengurangkan kandungan lipid yang tidaknormal dalam serum kebanyakan subjek ini.

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I certify that a Thesis Examination Committee has met on 3 September 2009 to conduct the final examination of Hasnah binti Haron on her thesis entitled “ Calcium Absorption and Bioavailability of Isoflavones from Tempeh Compared to Milk Among Postmenopausal Malay Women ” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15



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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

HASNAH BT. HARON

Date :

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