

ISSN: 1939-0211



Journal of
**Dietary
Supplements**

Editorial board

Editor-in-Chief

Taylor C. Wallace – George Mason University (twallac9@gmu.edu)

Associate Editors

Oliver Chen – Biofortis (oliver.chen@tufts.edu)

Roger Clemens – University of Southern California (clemens@usc.edu)

Chris DAdamo – University of Maryland (CDAdamo@som.umaryland.edu)

Termeh Feinberg – Yale University School of Medicine (Termeh.Feinberg@yale.edu)

Nana Gletsu-Miller – Purdue University (ngletsum@purdue.edu)

Douglas MacKay – Council for Responsible Nutrition (dmackay@crnusa.org)

Diane McKay – Tufts University (diane.mckay@tufts.edu)

Peter Pressman – The Daedalus Institute (drpressvm2@gmail.com)

Howard Sesso – Brigham and Women's Hospital (hsesso@hsph.harvard.edu)

Andrew Shao – Global Nutrition Solutions, LLC (drashao@aol.com)

Barbara C. Sorkin – National Institutes of Health (sorkinb@mail.nih.gov)

Susan Whiting – University of Saskatchewan (sjw084@mail.usask.ca)

Volume 15 No. 5, 2018

Article

Article

Evaluation of the Protective Role of *Glycine max* Seed Extract (Soybean Oil) in Drug-Induced Nephrotoxicity in Experimental Rats

Anand Ramasamy MPharm, Nandhakumar Jothivel PhD, Saibal Das MD, A. Swapna MPharm, Alice Padmini Albert MTech, Preeti Barnwal MSc & Dinesh Babu PhD

Pages: 583-595

Published online: 28 Sep 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(527 KB\)](#)

Article

Effects of n-3 Polyunsaturated Fatty Acid Supplementation on Serum Leptin Levels, Appetite Sensations, and Intake of Energy and Macronutrients in Obese People: A Randomized Clinical Trial

L. Payahoo, A. Ostadrahimi, N. Farrin & Y. Khaje-Bishak

Pages: 596-605

Published online: 05 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(411 KB\)](#)

Articles

Article

***Curcuma longa* (Turmeric) for Prevention of Capecitabine-Induced Hand-Foot Syndrome: A Pilot Study**

Vanessa Armenio Scontre MD, Janine Capobiango Martins MD, Claudia Vaz de Melo SetteMD, Haila Mutti MD, Daniel Cubero MD, Fernando Fonseca PhD & Auro Del Giglio MD

Pages: 606-612

Published online: 02 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)| [PDF \(390 KB\)](#)

Article

Solvent Extraction and Gas Chromatography–Mass Spectrometry Analysis of *Annona squamosa* L. Seeds for Determination of Bioactives, Fatty Acid/Fatty Oil Composition, and Antioxidant Activity

Mohammad Zahid M. Pharm, Muhammad Arif PhD, Md. Akhlaquer Rahman PhD, Kuldeep Singh PhD & Mohd Mujahid PhD

Pages: 613-623

Published online: 02 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)| [PDF \(527 KB\)](#)

Article

The Protective Effect of Hydroalcoholic Extract of *Rosa canina* (Dog Rose) Fruit on Liver Function and Structure in Streptozotocin-Induced Diabetes in Rats

Mohsen Taghizadeh PhD, Ali Akbar Rashidi MSc, Ali Akbar Taherian PhD, Zarichehr Vakili PhD & Mehdi Mehran MSc

Pages: 624-635

Published online: 02 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(627 KB\)](#)

Article

Factors Associated with Vitamin D Testing, Deficiency, Intake, and Supplementation in Patients with Chronic Pain

Manasi Gaikwad PhD, Simon Vanlint PhD, G. Lorimer Moseley PhD, Murthy N. Mittinty PhD & Nigel Stocks PhD

Pages: 636-648

Published online: 02 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(441 KB\)](#)

Article

Effects of Treatment with *Nauclea latifolia* Root Decoction on Sexual Behavior and Reproductive Functions in Male Rabbits

Quadri Kunle Alabi MSc, Olaoluwa Sesan Olukiran MSc, Modinat Adebukola Adefisayo MSc & Benson Akinloye Fadeyi MSc

Pages: 649-664

Published online: 31 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(791 KB\)](#)

Article

Estrogenic Activities of Food Supplements and Beers as Assessed by a Yeast Bioreporter Assay

Iyekhoetin Matthew Omoruyi BSc, MSc, PhD & Raimo Pohjanvirta DVM, PhD

Pages: 665-672

Published online: 31 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(408 KB\)](#)

|[Supplemental](#)

Article

Purified Dry *Paullinia cupana* (PC-18) Extract for Chemotherapy-Induced Fatigue: Results of Two Double-Blind Randomized Clinical Trials

Claudia Vaz de Melo Sette MD, Bárbara Bonaparte Ribas de Alcântara MD, Jean Henri Maselli Schoueri (Medical Student) , Felipe Melo Cruz PhD, Daniel de Iracema Gomes Cubero PhD, Luiz Francisco Pianowski PhD, Luke Joseph Peppone PhD, Dr Fernando Fonseca PhD & Professor Auro del Giglio PhD

Pages: 673-683

Published online: 30 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(515 KB\)](#)

Article

Augmented Reversal of Cisplatin-Induced Delayed Gastric Emptying by Amla (*Emblica Officinalis*) Fruit Extract in Sprague-Dawley Rats

Asad Ahmad, Mohammad Khushtar, Ranjan Kumar, Badruddeen, Ambreena Riyaz, Mohammad Irfan Khan & Md. Azizur Rahman

Pages: 684-691

Published online: 08 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(386 KB\)](#)

Article

Are the Level of Knowledge and Practices of Pregnant Women Regarding Folic Acid Supplementation Still Inadequate? A Cross-Sectional Study in a Middle Eastern Urban Setting

Antoine Aoun, Lara Faddoul, Flora El Jabbour, Nada El Osta, Sani Hlais & Lana El Osta

Pages: 692-703

Published online: 13 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(374 KB\)](#)

Article

Panchakola Reduces Oxidative Stress in MCF-7 Breast Cancer and HEK293 Cells

Tooba Naz Shamsi PhD student, Romana Parveen PhD student & Sadaf Fatima Assistant Professor

Pages: 704-714

Published online: 16 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(557 KB\)](#)

Article

Feeding Vitamin C during Neonatal and Juvenile Growth Improves Learning and Memory of Rats

Mahmoud Hosseini Professor, Farimah Beheshti Ph.D., Farzaneh Sohrabi Ph.D Student, Farzaneh Vafae Ph.D Student, Mohammad Naser Shafei Associate Professor & Hamid Reza Sadeghnia Associate Professor

Pages: 715-727

Published online: 27 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(759 KB\)](#)

Article

Article

The Effect of Phytosterol-Rich Fraction from Palm Fatty Acid Distillate on Blood Serum Lipid Profile of Dyslipidemia Rats

Kgs Ahmadi PhD, Huda Oktafa MD & Teti Estiasih PhD

Pages: 728-739

Published online: 16 Jan 2018

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(591 KB\)](#)

Review

Review

L-Carnitine and Potential Protective Effects Against Ischemia-Reperfusion Injury in Noncardiac Organs: From Experimental Data to Potential Clinical Applications

Azadeh Moghaddas & Simin Dashti-Khavidaki

Pages: 740-756

Published online: 20 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(464 KB\)](#)

Review

New Findings on Biological Actions and Clinical Applications of Royal Jelly: A Review

Mozafar Khazaei PhD, Atefe Ansarian MD & Elham Ghanbari MSc

Pages: 757-775

Published online: 13 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(600 KB\)](#)

Review

Effect of Probiotic Supplementation on CD4 Cell Count in HIV-Infected Patients: A Systematic Review and Meta-analysis

Asma Kazemi PhD student, Kurosh Djafarian PhD, John R. Speakman PhD, Parviz Sabour PhD, Sepideh Soltani PhD student & Sakineh Shab-Bidar PhD

Pages: 776-788

Published online: 29 Nov 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(626 KB\)](#)

Commentary

Article commentary

Adulteration and Counterfeiting of Online Nutraceutical Formulations in the United States: Time for Intervention?

Mohamed Ismail Nounou BPharm, MPSc, PhD, Yamin Ko PharmD Candidate, Nada A. Helal BPharm, MPSc & Jeremy F. Boltz PharmD Candidate

Pages: 789-804

Published online: 11 Oct 2017

[Abstract](#)|[Full Text](#)|[References](#)|[PDF \(503 KB\)](#)

ARTICLE



The Effect of Phytosterol-Rich Fraction from Palm Fatty Acid Distillate on Blood Serum Lipid Profile of Dyslipidemia Rats

Kgs Ahmadi, PhD ^a, Huda Oktafa, MD^b, and Teti Estiasih, PhD ^b

^aDepartment of Agroindustry Technology, Faculty of Agriculture, Tribhuwana Tunggal University, Jl. Telaga Warna, Tlogomas, Malang, Jawa Timur, Indonesia; ^bDepartment of Food Science and Technology, Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, Malang, Jawa Timur, Indonesia

ABSTRACT

Phytosterol-rich fraction (PRF) obtained from palm fatty acid distillate (PFAD) was investigated for its effect on blood serum lipid profile of dyslipidemia rats. Dyslipidemia was induced by force feeding cholesterol to five groups of rats; one group was a control or normal group. Cholesterol force-fed groups were treated with 0, 10, 20, 30, and 40 mg PRF/kg/day for 4 weeks. All groups of rats were fed standard diet. A normal group was fed standard diet without PRF treatment and cholesterol force feeding. Lipid profile was measured every week (0, 1, 2, 3, and 4). Four-week treatment resulted in significant blood serum lipid profile improvement. PRF improved blood serum lipid profile by decreasing total cholesterol, triglyceride, and low density lipoprotein (LDL) cholesterol level and increasing high density lipoprotein (HDL) cholesterol level. The doses of PRF significantly affected blood serum lipid profile as well as duration of PRF treatment. PRF inhibited cholesterol absorption, which delayed blood serum total cholesterol rise. Cholesterol absorption inhibition was also indicated by higher fecal cholesterol concentration after PRF feeding. These results indicate the beneficial effect of PRF in the treatment of dyslipidemia.



KEYWORDS

deodorization; distillate; dyslipidemia; lipid profile; palm oil; phytosterols

Introduction

Palm fatty acid distillate (PFAD) is a by-product of the physical refining of crude palm oil in the deodorization step. In general, PFAD contains 80% to 90% of free fatty acid, some unsaponifiable components, neutral lipid, glycerides, and other high-molecular-weight compounds. For the past decade, PFAD has been used as a source of vitamin E, squalene, and phytosterols for pharmaceutical and cosmetic industries (Gapor, 2010). Palm oil phytosterols are composed of campesterol (13%), β sitosterol (60%), and stigmasterol (24%) (Loganathan et al., 2009). Phytosterol composition did not change during the degumming and bleaching process in palm oil refining (Puah et al., 2004). Goh and Gee (1985) reported that squalene was the main component of PFAD hydrocarbons, with other minor components.

Phytosterols are a triterpene that have similar molecular structure to cholesterol, with four steroid rings, and are classified as 3β -hydroxyl; they frequently have double bonds at 5 and 6 positions. Phytosterols stabilize the phospholipid bilayer in the cell membrane (Fernandes and Cabral, 2007). Phytosterols can reduce total cholesterol as well as LDL cholesterol

CONTACT Teti Estiasih  teties@yahoo.co.id or teties@ub.ac.id  Department of Food Science and Technology, Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, Malang, Jawa Timur, Indonesia 65145.

(Jones et al., 2000; Lichtenstein and Deckelbaum, 2001; Yang et al., 2004; Nguyen et al., 2009). The hypocholesterolemic effect of phytosterols is related to cholesterol absorption inhibition. Phytosterol supplementation at a dose of 2 g/day effectively reduced cholesterol absorption in digestive tracts, thus reducing plasma total cholesterol to 10%, reducing LDL cholesterol to 10%–15%, and preventing HDL cholesterol decrease, without causing malabsorption (Jones et al., 2003). Quilez et al. (2003) also indicated that phytosterol intake of 1.5–3.0 g/day decreased LDL cholesterol 8%–15%. The safety of phytosterols, phytostanols, and ester phytosterols has been declared by the U.S. Food and Drug Administration and the European Union Scientific Committee (Kritchevsky and Chen, 2005).

This research aimed to study the effect of PRF in blood serum lipid profile as well as the suitable dose for lipid profile improvement. The mechanism of blood serum lipid profile improvement was studied through cholesterol absorption inhibition and fecal cholesterol concentration analysis.

Materials and methods

Materials

PFAD was obtained from a palm oil refinery (PT Salim Ivomas Pratama) at Surabaya, East Java, Indonesia. Standard diet was referred to AIN-93M (Reeves et al., 1993) and Wistar male rats (*Rattus norvegicus*) had body weight of ~200 g. Pure cholesterol (Sigma Co., Singapore) was used to induce dyslipidemia. Blood serum lipid profile was analyzed using Diasys-Diagnostic Systems (GmbH and Co., Holzheim, Germany). Other chemical reagents were used for PRF preparation (technical grade) and analysis (pro-analysis grade).

Phytosterol-rich fraction preparation

Phytosterol-rich fraction (PRF) was prepared according to the method of Wardhana (2011) by low temperature solvent crystallization. PRF was obtained from PFAD by saponification followed by solvent crystallization. Saponification used potassium hydroxide (KOH) in ethanolic solution, and the unsaponifiable matter was extracted by hexane. After evaporation of hexane, unsaponifiable matter was crystallized at low temperature (-10°C) by using hexane as a solvent, and the white crystal formed was taken for further purification. The crystal fraction was dissolved in hexane and recrystallized to obtain more pure crystal. This crystal was expressed as phytosterol-rich fraction (PRF).

The PRF obtained and the original PFAD were analyzed for phytosterol concentration and composition. Phytosterol analysis followed the method of Khatoon et al. (2010). About 1 mg of unsaponifiable matter of PFAD was added to ethanol 1 ml and subsequently filtered; 20 μl of sample solution was injected into high-performance liquid chromatography (Shimadzu LC20AT) with column VPODS (C18) 250 \times 4.6 mm and UV vis detector (Shimadzu SPD20A) at wavelength of 206 nm. The mobile phase was methanol:water (99:1 v:v) with a flow rate of 1 ml/min. Identification and quantification were conducted using phytosterols (stigmasterol, β sitosterol, campesterol, Sigma Co) standard that was injected into HPLC separately. PRF and PFAD were also analyzed for yield (%), free fatty acids (AOCS, 1990), peroxide value (Hills and Thiel, 1946), and *p*-anisidin value (ISO, 2016).

Bioassay for lipid profile changes

Bioassay was performed using six groups of six rats in each group, except three rats per group were used for cholesterol absorption inhibition test. Rats were adapted to the environment and

standard diet for a week and subsequently induced by cholesterol force feeding (1% based on standard diet or 200 mg per rat) to obtain dyslipidemia condition. The normal group as a control was not force fed cholesterol. All groups had free access to diet (*ad libitum*). Dyslipidemia condition (blood serum total cholesterol of >200 mg/dL; hypercholesterolemia) was achieved after one week of cholesterol administration.

Rats were divided into six groups as follows: five groups of dyslipidemia rats were fed AIN-93M standard diet and force fed PRF 0, 10, 20, 30, and 40 mg/kg/day diluted in 2 ml of palm oil; the normal group was fed AIN-93M without PRF force feeding. The test was conducted for four weeks. Body weight of rats and blood serum lipid profiles were measured every week after the rats were fasting for 16 hours. Blood was taken from the rats retro-orbitally, and the blood was then centrifuged at 4,000 rpm for 15 min to separate serum and plasma. The upper layer was taken and analyzed for total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol by enzymatic colorimetric test (CHOD-PAP) and triglyceride was measured by enzymatic colorimetric test (GPO-PAP).

The colorimetric method of total cholesterol analysis was carried out according to Allain et al. (1974), based on the formation of D4 cholestenone. Cholesterol was produced from cholesterol ester by cholesterol esterase. Cholesterol was converted into D4 cholestenone by cholesterol oxidase and released hydrogen peroxide. Hydrogen peroxide reacted with 4-aminophenazone and 4-chlorophenol that catalyzed by peroxidase and formed color complex; the absorbance was measured at 500 nm.

The colorimetric method of HDL cholesterol measurement was based on the method of Seguchi et al. (1995). The apoB-containing lipoproteins in the specimen react with a blocking reagent that renders them nonreactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB-containing lipoproteins are thus effectively excluded from the assay and only HDL cholesterol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2} , which forms complexes with apoB-containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL cholesterol measurement. Hydrogen peroxide liberated from a series of enzymatic reactions of HDL cholesterol was reacted with 5-aminophenazone and N-ethyl-N-(3-methylphenyl)-N'-succinyl ethylenediamine that was catalyzed by peroxidase to form complex color that was measured at 600 nm. LDL cholesterol was calculated by diminishing total cholesterol with HDL cholesterol and triglycerides (Nigam, 2011) with the following equation:

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \left[\text{triglycerides}/5 \right]$$

The principle of triglyceride analysis in the sample is that triglycerides are hydrolyzed by lipase into glycerol and free fatty acids. Glycerol is then phosphorylated by glycerol kinase in the presence of ATP and Mg^{+2} ions. Glycerol-3-P is oxidized by glycerol-3-phosphate oxidase (GPO) in the presence of oxygen into dihydroxyacetone phosphate and hydrogen peroxide. A colored product is produced after reaction of hydrogen peroxide with 4-aminoantipyrine and phenol-derivative in the presence of the peroxidase. Absorbance was measured at 500 nm (Bucolo and David, 1973).

Fecal cholesterol analysis

Fecal cholesterol analysis was performed from feces of rats after four weeks of treatment or at the end of bioassay for lipid profile changes. Fecal cholesterol analysis was conducted by the Lieberman-Burchard method (Plummer, 1977).

Cholesterol absorption inhibition test

The cholesterol absorption inhibition test was conducted in six groups of rats with 3 rats per group. The treatment of PRF was in accordance with bioassay for lipid profile changes; five groups of rats were treated with PRF 0, 10, 20, 30, and 40 mg/kg body weight, respectively. All groups were normal rats and were force fed 80 mg cholesterol/kg body weight simultaneously with PRF force feeding. Blood was taken retro-orbitally at hours 0, 1, 2, 3, and 4 after cholesterol and PRF feeding and then analyzed for blood serum total cholesterol.

Data analysis

Data were stated as mean \pm standard deviation from the average of six rats per group (three rats per group for the cholesterol absorption inhibition test). This experiment used a nested design, and the data were analyzed for analysis of variance ($\alpha = .05$) and least significant difference test if there was a significant effect of doses. Statistical analysis was performed using Minitab 7.0.

Results and discussion

PFAD and PRF characteristics

Data in Table 1 show that PFAD had high free fatty acids of 87.83%; PRF had free fatty acids of 14.40%. Free fatty acids of PRF were an impurity that ideally should be eliminated during low-temperature solvent crystallization in PRF preparation. Both PFAD and PRF revealed low oxidation levels, indicated by peroxide value as an indicator of primary oxidation and anisidine value as an indicator of secondary oxidation. The *p*-anisidine value of PRF was higher than that of PFAD, possibly due to accumulation of low molecular secondary oxidation products in PRF. The peroxide value of PFAD was higher than that of PRF because peroxides are primary oxidation products that usually have high molecular weight and dissolve in solvent during low-temperature solvent crystallization; thus, they did not accumulate well in PRF.

PRF had high phytosterol content of 18.31% compared to PFAD, which only contained phytosterols of 0.75%. Other components in PRF are not known yet. The study by Moreau et al. (2009) showed that phytosterol content of crude corn fiber oil was 8.79%, refined corn fiber oil was 2.40%, and refined corn kernel oil was 1.11%; commercial corn oil had phytosterol content of 0.84%. Jiang and Wang (2005) reported that by-products of cereal contained phytosterols of 9.35 mg/g oil. Phytosterol content of some types of oil varies depending on their plant sources: mustard (64 mg/g), corn (23 mg/g), soybean (9 mg/g), rapeseed (5 mg/g),

Table 1. Characteristics of palm fatty acid distillate and phytosterol-rich fraction.

Characteristic	PFAD	PRF
Free fatty acids (%)	87.83	14.40
Peroxide value (meq/kg)	1.53	0.22
<i>p</i> -anisidine value	6.91	12.75
Phytosterols (%)	0.75	18.31
β -sitosterol (%)	0.39	10.68
Stigmasterol (%)	0.18	4.95
Campesterol (%)	0.17	2.69
Yield (%)	—	0.06

PFAD = palm fatty acid distillate; PRF = phytosterol-rich fraction.

and coconut (0.8 mg/g) (Sabir et al., 2003). In general, vegetable oils contain phytosterols of 1–5 g/kg, except rice bran oil, which has phytosterols of 30 g/kg (Hoed et al., 2006).

Predominant phytosterols of PFAD and PRF were β -sitosterol and stigmasterol, respectively. This difference presumably was due to different solubility and melting point of each phytosterol during low-temperature solvent crystallization in PRF preparation. The β -sitosterol content of PRF (Table 1) was higher than that of other phytosterol sources such as sunflower oil (1,232–1,474 ppm) and olive oil (1,472–1,678 ppm) (Than et al., 2006). Phytosterol concentration in soybean oil deodorizer distillate was 6%–10%; PFAD had phytosterols of 0.04%–0.75% (Khatoun et al., 2010; Estiasih et al., 2013).

The yield of PRF based on PFAD was 0.06%, which indicated only 600 mg of PRF was obtained from 1 kg of PFAD. The low yield of PRF was suggested due to low unsaponifiable matter of PFAD, indicated by high free fatty acids of 87.83%. Benites et al. (2009) reported that free fatty acids in distillate deodorizer of soybean oil was 53%; Estiasih et al. (2013) reported that PFADs from several palm oil refineries had free fatty acids of 85%–93%.

Lipid profile changes

Lipid profile changes after PRF feeding are shown in Table 2. The decrease of total cholesterol, triglyceride, and LDL cholesterol, and the increase of HDL cholesterol levels were the highest in a PRF dose of 40 mg/kg/day (Figure 1): 131.56 mg/dL; 75.20 mg/dL; 107.79 mg/dL; 57.42 mg/dL, respectively. Higher PRF doses resulted in greater decreases of blood serum total

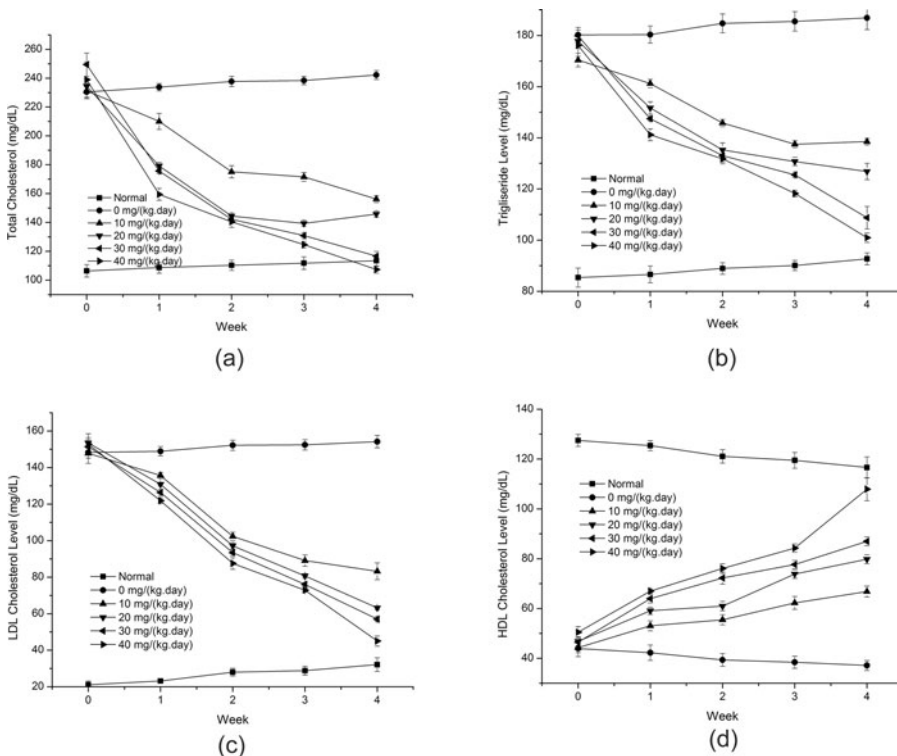


Figure 1. Blood serum lipid profile changes of group of rats treated by various doses of phytosterol-rich fraction (PRF) for 4 weeks. (a) Total cholesterol; (b) triglyceride; (c) low-density lipoprotein (LDL) cholesterol; (d) high-density lipoprotein (HDL) cholesterol.

Table 2. Lipid profile changes of dyslipidemia rats during 4 weeks of PRF feeding at various doses.

Cholesterol total (mg/dL)						
Week	Normal	Hypercholesterolemia group + PRF (mg/kg/day)				
		0	10	20	30	40
0	106.40 ± 4.31 b	230.43 ± 3.60 c	231.20 ± 5.46 a	234.50 ± 5.08 a	249.42 ± 8.07 a	238.95 ± 12.34 a
1	108.69 ± 3.85 ab	233.78 ± 2.55 c	210.04 ± 5.64 b	179.15 ± 2.44 b	175.68 ± 2.04 b	159.46 ± 4.11 b
2	110.34 ± 3.59 a	237.74 ± 3.56 b	175.10 ± 4.22 c	144.44 ± 2.03 c	142.15 ± 2.54 c	140.42 ± 3.83 c
3	111.83 ± 4.34 a	238.36 ± 2.95 ab	171.57 ± 3.08 c	139.31 ± 2.20 d	130.92 ± 2.02 d	124.62 ± 2.28 d
4	113.45 ± 3.88 a	242.23 ± 3.12 a	156.44 ± 2.19 d	145.83 ± 2.19 c	116.67 ± 3.27 e	107.39 ± 2.50 e
Triglyceride (mg/dL)						
Week	Normal	Hypercholesterolemia group + PRF (mg/kg/day)				
		0	10	20	30	40
0	85.37 ± 3.70 d	180.19 ± 2.99 b	170.37 ± 2.64 a	177.78 ± 2.90 a	179.81 ± 2.29 a	176.11 ± 4.29 a
1	86.58 ± 3.31 cd	180.33 ± 3.36 b	161.21 ± 1.63 b	151.65 ± 2.43 b	147.43 ± 0.95 b	141.18 ± 2.33 b
2	88.93 ± 2.29 bc	184.69 ± 3.68 ab	145.76 ± 1.41 c	135.24 ± 2.79 c	133.03 ± 2.51 c	131.73 ± 1.95 c
3	90.07 ± 1.95 ab	185.48 ± 3.77 a	137.50 ± 1.34 d	130.70 ± 1.63 d	125.55 ± 1.10 d	118.20 ± 1.26 d
4	92.70 ± 2.31 a	186.86 ± 4.65 a	138.50 ± 1.25 d	126.82 ± 3.17 e	108.76 ± 4.42 e	100.91 ± 1.82 e
LDL cholesterol (mg/dL)						
Week	Normal	Hypercholesterolemia group + PRF (mg/kg/day)				
		0	10	20	30	40
0	21.09 ± 2.07 c	148.28 ± 3.08 c	147.81 ± 5.56 a	153.44 ± 2.95 a	151.41 ± 3.83 a	152.81 ± 5.72 a
1	23.14 ± 1.28 c	148.91 ± 2.55 bc	135.71 ± 1.64 b	130.90 ± 1.06 b	126.40 ± 1.64 b	121.89 ± 1.55 b
2	27.97 ± 2.13 b	152.19 ± 2.77 ab	102.34 ± 2.25 c	97.19 ± 1.65 c	93.44 ± 1.65 c	87.50 ± 3.19 c
3	28.82 ± 2.41 b	152.49 ± 2.85 a	89.10 ± 3.09 d	80.69 ± 1.30 d	76.01 ± 1.14 d	72.90 ± 1.14 d
4	32.14 ± 3.81 a	154.19 ± 3.45 a	83.23 ± 4.62 e	63.20 ± 1.06 e	56.99 ± 1.38 e	45.03 ± 2.80 e
HDL cholesterol (mg/dL)						
Week	Normal	Hypercholesterolemia Group + PRF (mg/kg/day)				
		0	10	20	30	40
0	127.52 ± 2.39 a	43.96 ± 3.31 a	44.30 ± 1.98 d	46.81 ± 1.77 d	46.48 ± 1.85 e	50.50 ± 2.22 e
1	125.42 ± 2.01 a	42.26 ± 3.08 ab	53.03 ± 2.01 c	59.09 ± 1.49 c	63.97 ± 1.74 d	67.00 ± 0.87 d
2	121.10 ± 2.68 b	39.37 ± 2.57 bc	55.48 ± 2.07 c	60.96 ± 1.91 c	72.26 ± 2.51 c	76.08 ± 1.92 c
3	119.51 ± 3.18 bc	38.41 ± 2.42 c	62.25 ± 2.59 b	73.84 ± 1.75 b	77.65 ± 1.66 b	84.27 ± 1.66 b
4	116.67 ± 4.22 c	37.13 ± 1.97 c	66.83 ± 2.18 a	79.79 ± 1.80 a	86.96 ± 1.82 a	107.92 ± 4.62 a

Data in the same column that have the same letter are not significantly different at $\alpha = .05$. PRF = phytosterol-rich fraction; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

cholesterol, triglyceride, and LDL cholesterol levels. The higher PRF doses made the higher HDL cholesterol level rise (Figure 1d). After four weeks of PRF feeding of 40 mg/kg/day, the lipid profile of dyslipidemia rats was approximately normal. Meanwhile, dyslipidemia rats fed only AIN-93M without PRF treatment still showed dyslipidemia condition, indicated by high total cholesterol, LDL cholesterol, and triglyceride levels and low HDL cholesterol levels. PRF doses significantly ($\alpha = .05$) affected the lipid profiles of dyslipidemia rats, as did duration of PRF treatment (Figure 1).

Figure 1 shows that lipid profiles tended to improve every week, and it was suggested that longer PRF treatment would result in greater lipid profile improvement. The trend of lipid profile changes was almost linear with an increase for HDL cholesterol and decreases for total cholesterol, triglyceride, and LDL cholesterol. However, there is a variation of R^2 (0.73–0.98, data not shown) between duration of treatment and blood lipid level. Duration of treatment affected lipid profile changes significantly ($\alpha = .05$). The highest linear correlation

with duration of treatment was found in a PRF dose of 40 mg/kg/day for all blood lipid. This means the change of lipid profile at high dose was time dependent. After four weeks of treatment, the condition of approximately normal was found in PRF dose of 40 mg/kg/day. It is highly possible that lower doses would result in lipid profiles similar to normal in longer PRF treatment (more than four weeks).

Phytosterols are well known to effectively inhibit cholesterol absorption from food and bile acid in the digestive tract by several mechanisms: cholesterol replacement in micelles, decreasing cholesterol solubility in digestive tract, and reducing cholesteryl ester synthesis in intestine. Phytosterols have high hydrophobicity and low solubility, but have higher affinity to micelles than cholesterol. Therefore, phytosterols have the ability to replace cholesterol from intraluminal micelles; thus, cholesterol was discarded into feces (Ikeda et al., 1989; Ling and Jones, 1995). In the intestine, phytosterols solubilize in the micelle form, and this micelle interacts with brush border and is transferred into enterocyte. Phytosterols are incorporated into chylomicron and secreted in lymph through the biliary system (John et al., 2007). Phytosterols can reduce cholesterol absorption 30%–50% (Jones et al., 2000). Co-crystallization of phytosterols and cholesterol in the gastrointestinal tract reduces cholesterol uptake due to lower solubility of crystal compared to cholesterol alone (Garti et al., 2006). The study by Ling and Jones (1995) showed that β -sitosterol increased bile acid micelles affinity in high concentration of sterols. Phytosterols reduce cholesteryl ester synthesis in enterocyte through inhibition of acyl-coenzyme A cholesterol acyltransferase (ACAT) activity (Ikeda et al., 1989). Therefore, ACAT activity inhibition increases intracellular free cholesterol concentration, which reduces intestinal cholesterol uptake (Krauss et al., 2000).

According to Mel'nikov et al. (2003), phytosterols have a similar structure to cholesterol. Furthermore, Berry (2004) stated that phytosterols reduce cholesterol absorption because they compete with cholesterol and phytosterols show higher affinity (Normén et al., 2000). Cholesterol and phytosterol competition occurs in the digestive tract in the esterification stage, or phytosterols partially inhibit lipoprotein binding to cholesterol for transportation from intestinal mucosa. This inhibition increases fecal cholesterol secretion. The study by Chen et al. (2010) showed that phytosterol intake of 2 g/kg feed for five weeks reduced hepatic cholesterol 40%, cholesterol in the aorta 31%, and renal cholesterol 19%.

PRF doses significantly affected blood serum total cholesterol level reduction (Figure 1a). According to Hendriks et al. (1999) and Volpe et al. (2001), a phytosterol dose of 0.8 g/day did not significantly reduce serum total cholesterol and LDL cholesterol levels. The suggested dose was 2–3 g/day (FDA, 2000; Lichtenstein and Deckelbaum, 2001), but doses more than 3 g/day have a slight effect in cholesterol reduction (Scientific Committee on Food of the EU, 2003). Figure 1b shows that PRF feeding effectively decreased blood serum triglyceride level. According to Lamarch et al. (1999), increasing plasma triglyceride is strongly related to decreasing size of predominant LDL. Bile acid secretion due to phytosterol intake affected blood serum triglyceride. Bile acid influences triglyceride absorption, and presumably bile acid secretion is higher in the existence of phytosterols in the intestine.

PRF decreased blood serum LDL cholesterol level even in low dose (Figure 1c). As previously explained, this decrease was related to cholesterol absorption and ACAT activity inhibition. This finding is in accordance to that reported by Turley and Dietschy (2003) that phytosterol intake reduced LDL cholesterol due to cholesterol synthesis and absorption inhibition. Cohen (2008) stated that phytosterols also inhibit bile cholesterol reabsorption in the colon. Phytosterols have a similar structure to cholesterol but are more hydrophobic; therefore, they can replace cholesterol from micelles. Phytosterols enter enterocytes and will

be resecreted into lumen more efficiently. Kamal-Eldin and Moazzami (2009) revealed that intake of phytosterols or phytostanols 1–2 g/day reduced LDL cholesterol level 10%–20%.

Blood serum HDL cholesterol increase in dyslipidemia rats fed PRF was related to reduction in LDL cholesterol level (Figure 1d). Increasing PRF doses enhanced HDL cholesterol level. HDL has a role in cholesterol transportation from the bloodstream to the liver. Cholesterol is not soluble in blood; thus, this compound is transported in the form of lipoprotein such as HDL cholesterol. Excessive cholesterol in the liver will be eliminated through bile acid secretion. Cholesterol transportation into the liver is important to maintain cholesterol balance (Cohen, 2008). LDL cholesterol level reduction decreased cholesterol transportation from the liver to the blood resulting in HDL cholesterol level increase. From the liver, cholesterol is transported into the gall bladder to be converted into bile acid and then secreted into feces.

Cholesterol absorption inhibition and fecal cholesterol concentration

In this experiment, PRF (dosages 0–40 mg/kg body weight) and cholesterol (80 mg/kg body weight) were concomitantly force fed to five groups of rats. The aim of this treatment was to learn the effect of phytosterol feeding on cholesterol absorption inhibition. PRF delayed the increase of blood serum total cholesterol level at hours 1 and 2 after feeding (Figure 2). At PRF dosage of 0 mg/kg body weight, 2 hours after feeding, blood serum total cholesterol level remained constant. At PRF dosage of 10 mg/kg body weight, blood serum total cholesterol level decreased slightly at hour 4. However, total cholesterol level decreased 2 hours after feeding at higher doses of PRF (dose 30 and 40 mg/kg body weight). The sharp increase of total cholesterol level after feeding was found in PRF dosage of 0 mg/kg. This phenomenon indicated that PRF had the ability to delay blood serum total cholesterol level rise after cholesterol ingestion. Increasing the dosage of PRF caused decreasing blood serum total cholesterol level rise, meaning the higher the PRF dose, the lower the blood serum total cholesterol level rise after cholesterol ingestion.

These facts show that PRF inhibited cholesterol absorption even though cholesterol and phytosterols were ingested concomitantly. This finding indicates that formulating PRF

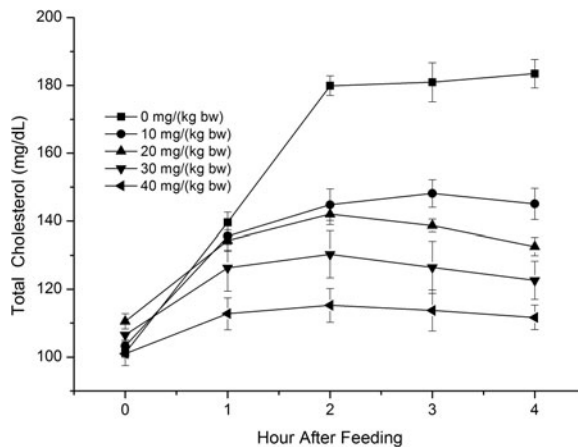


Figure 2. Blood serum total cholesterol level rise after feeding as affected by doses of phytosterol-rich fraction (PRF).

into cholesterol-containing food products might inhibit cholesterol absorption. Moreover, phytosterol-enriched food products can be formulated to inhibit cholesterol absorption, even more for cholesterol-containing products because phytosterols prevent cholesterol absorption in the intestine (Tasan et al., 2006). The human study by Shin et al. (2005) showed that phytosterols administered as phytosterol-emulsifier vehicle significantly reduced cholesterol absorption by 23.3% at a dose of 300 mg and by 32.0% at a dose of 500 mg compared with the placebo, indicating micellar phytosterols reduced cholesterol absorption effectively at very low doses. Phytosterols used in that study were formulated in nanoparticles and thus can be readily dispersed in water, providing a flexible delivery system for various food products such as beverages, confections, and cooking oil.

Inhibition of cholesterol absorption was also proved by measuring fecal cholesterol concentration after four weeks of PRF feeding. Figure 3 shows that PRF inhibited cholesterol absorption as indicated by the fact that fecal cholesterol concentration of rats treated with PRF was higher than that of the normal group (without PRF feeding). The increase in PRF doses resulted in increasing fecal cholesterol concentration, indicating that the ability of PRF to inhibit cholesterol absorption also increased. This finding was in accordance with the result of the cholesterol absorption inhibition test. Increasing fecal cholesterol was related to the ability of phytosterols to inhibit cholesterol absorption in the intestine; thus, cholesterol accumulated in the caecal digesta and was secreted into feces. High fecal cholesterol concentration indicated low cholesterol absorption in the digestive tract (Lupton and Turner, 2000).

The findings of this research show that PRF effectively improved lipid profile in dyslipidemia condition. Further research is needed to prove the effect of PRF on humans. The best PRF dosage from this study was 40 mg/kg body weight); this dosage should be converted if it is used for clinical study in humans. According to Shin et al. (2010), the significance of dose conversion is based on body surface area and starting dose estimation for clinical trials. The body surface area seems to have good correlation among species with several parameters, including oxygen utilization, caloric expenditure, basal metabolism, blood volume, and circulating plasma protein.

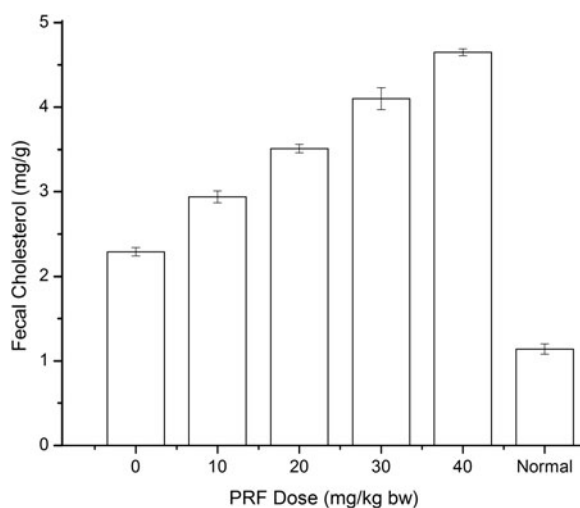


Figure 3. Fecal cholesterol of rats treated by various doses of phytosterol-rich fraction (PRF) after 4 weeks of PRF feeding.

Conclusion

PRF improved blood serum lipid profile by decreasing total cholesterol, triglyceride, and LDL cholesterol levels and increasing HDL cholesterol level. The doses of PRF significantly affected blood serum lipid profile as did duration of PRF treatment. Rats fed PRF at a dose of 40 mg/kg/day reached a blood serum lipid profile similar to that of the normal group. PRF inhibited cholesterol absorption, which delayed blood serum total cholesterol rise. This was indicated by the increase of blood serum total cholesterol level after ingestion of cholesterol and PRF concomitantly. Blood serum total cholesterol level after cholesterol ingestion was affected by PRF dose. Higher doses of PRF were more effective in inhibiting blood serum total cholesterol rise. Cholesterol absorption inhibition was also indicated by fecal cholesterol concentration after PRF feeding. PRF doses affected fecal cholesterol concentration, and higher fecal concentrations were found in higher PRF doses. PRF may be of use in phytosterol-enriched foods for cholesterol-containing products.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding

The authors thank the Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia for funding this research through Penelitian Hibah Bersaing with contract no. 0062/SP2H/PP/K7/KL/II/2012, February 9, 2012.

About the authors

Kgs Ahmadi, PhD, Department of Agroindustrial Technology, Faculty of Agriculture, Tribhuwana Tunggal University, Malang, Indonesia. Interest in the research of lipid technology and bioactive compound separation.

Huda Oktafa, MD, Health Department, State Polytechnic of Jember. Research interest in food nutrition and sensory.

Teti Estiasih, PhD, Department of Food Science and Technology, Agricultural Technology Faculty, Brawijaya University, Malang, Indonesia. Interest in lipid chemistry & technology, and bioactive compounds.

ORCID

Kgs Ahmadi  <http://orcid.org/0000-0002-8483-4314>

Teti Estiasih  <http://orcid.org/0000-0001-6638-1086>

References

- Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem*. 20:470–475. PMID:4818200
- AOCS. 1990. Official Methods and Recommended Practices of the American Oil Chemistry Society. 4th ed. Champaign (IL): Broadmaker Drive.
- Benites CI, Concha VOC, Reis SMPM, de Oliveira AC. 2009. Physicochemical characterization of soybean oil deodorizer distillate. *Chem Eng Transact*. 17:903–908.
- Berry D. 2004. Opportunities for dairy foods to load up better-for-your ingredients. *Dairy Food*. April:36–41.

- Bucolo G, David H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem.* 19 (5):476–482. PMID:4703655
- Chen Q, Gruber H, Swist E, Coville K, Pakenham C, Ratnayake WMN. 2010. Dietary phytosterols and phytosterols decrease cholesterol levels but increase blood pressure in WKY inbred rats in the absence of salt-loading. *Nutr & Metab.* 7:1–9. doi:10.1186/1743-7075-7-11.
- Cohen DE. 2008. Balancing cholesterol synthesis and absorption in the gastrointestinal tract. *J Clin Lipidol.* 2:S1–S3. doi:10.1016/j.jacl.2008.01.004. PMID:19343078
- Estiasih T, Ahmadi K, Widyaningsih T, Maligan JM, Mubarak AZ, Zubaidah E, Muchlisiyyah J, Puspitasari R. 2013. Bioactive compounds of palm fatty acid distillate (PFAD) from several palm oil refineries. *Adv J Food Sci Technol.* 5:1153–1159. doi:10.19026/ajfst.5.3074.
- FDA (Food and Drug Administration). 2000. Food labeling: Health claims; plant sterol/stanol esters and coronary heart disease. *FDA Fed. Reg.* 65:54685–54739.
- Fernandes P, Cabral JMS. 2007. Phytosterols: applications and recovery methods. a Review. *J Elsv.* 98:2335–2350.
- Gapor AB. 2010. Production and utilisation of palm fatty acid distillate (PFAD). *Lipid Technol.* 22: 11–13. doi:10.1002/lite.200900070.
- Garti N, Avrahami M, Aserin A. 2006. Improved solubilization of celecoxib in U-type nonionic microemulsions and their structural transitions with progressive aqueous dilution. *J Colloid Interface Sci.* 229:352–365. doi:10.1016/j.jcis.2006.01.060.
- Goh SH, Gee PT. 1985. Noncarotenoids hydrocarbon in palm oil and palm fatty acid distillate. *J Am Oil Chem Soc.* 63:226–230. doi:10.1007/BF02546143.
- Hendriks HF, Weststrate JA, van Vliet T, Meijer GW. 1999. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normo cholesterolaemic and mildly hypercholesterolaemic subject. *J Eur Clin Nutr.* 53:319–327. doi:10.1038/sj.ejcn.1600728.
- Hills GL, Thiel CC. 1946. The ferric thiocyanate method of estimating peroxide in the fat of butter, milk, and dried milk. *J. Dairy Res.* 14:340. doi:10.1017/S0022029900004799.
- Hoed VV, Depaemelaere D, Ayala VJ, Santiwattana P. 2006. Influence of chemical refining on the major and minor component of rice bran oil. *J Am Oil Chem Soc.* 83:315–321. doi:10.1007/s11746-006-1206-y.
- Ikeda I, Tanabe Y, Sugano M. 1989. Effects of sitosterol and sitostanol on micellar solubility of cholesterol. *J Nutr Sci Vitaminol.* 35:361–369. doi:10.3177/jnsv.35.361. PMID:2585153
- ISO. 2016. Animal and vegetable fats and oils – Determination of anisidine value. ISO 6885 2016. International Organization for Standardization. <https://www.iso.org>.
- Jiang Y, Wang T. 2005. Phytosterols in cereal by product. *J Am Oil Chem Soc.* 86:439–444. doi:10.1007/s11746-005-1090-5.
- John S, Sorokin AV, Thompson PD. 2007. Phytosterols and vascular disease. *Curr Opin Lipidol.* 18: 35–40. doi:10.1097/MOL.0b013e328011e9e3. PMID:17218830
- Jones PJ, Raeini-Sarjaz M, Ntanos, Vanstone CA, Feng JY, Parsons WE. 2000. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytosterol esters. *J Lipid Res.* 41:697–705. PMID:10787430
- Jones PJ, Racini-Sarjaz M, Ntanos FY, Vanstone CA, Feng JY, Paarsons WE. 2003. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytosterol esters. *J Lipid Res.* 41:297–705.
- Kamal-Eldin A, Moazzami A. 2009. Plant sterols and stanols as cholesterol-lowering ingredients in functional foods. *Recent Pat Food Nutr Agric.* 1:1–14. doi:10.2174/2212798410901010001. PMID:20653521
- Khatoun S, Rajan RGR, Krishna AGG. 2010. Physicochemical characteristics and composition of Indian soybean oil deodorizer distillate and the recovery of phytosterols. *J Am Oil Chem Soc.* 87:321–326. doi:10.1007/s11746-009-1499-8.
- Krauss RM, Eckel RH, Howard B. 2000. AHA dietary guidelines. *Circulation.* 102:2284–2299. doi:10.1161/01.CIR.102.18.2284. PMID:11056107
- Kritchevsky D, Chen SC. 2005. Phytosterols-health benefits and potential concerns: a review. *J Nutr Res.* 25:413–428. doi:10.1016/j.nutres.2005.02.003.
- Lamarche B, Lemieux I, Despres JP. 1999. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. *Diabetes Metab.* 25:199–211. PMID:10499189

- Lichtenstein AH, Deckelbaum RJ. 2001. Stanol/sterol ester-containing foods and blood cholesterol levels. *Circulation*. 103:1177. doi:10.1161/01.CIR.103.8.1177. PMID:11222485
- Ling WH, Jones PJ. 1995. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci*. 57:195–206. doi:10.1016/0024-3205(95)00263-6. PMID:7596226
- Loganathan R, Selvaduray KR, Radhakrishnan A, Nesaretnam K. 2009. Palm oil rich in health promoting phytonutrients. *Palm Oil Development*. 50:16–25
- Lupton JR, Turner D. 2000. *Dietary fiber, biochemical and physiological aspect of human nutrition*. London (UK): WB Saunders Company.
- Mel'nikov SM, Jack WM, ten Hoorn S, Berstrand B. 2003. Can cholesterol absorption be reduced by phytosterols and phytostanols via a cocrystallization mechanism. *Chem Phys Lipids*. 13:1321–1330.
- Moreau RA, Lampi AM, Kevin BH. 2009. Fatty acid, phytosterol, and polyamine conjugate profiles of edible oils extracted from corn germ, corn fiber, and corn kernels. *J Am Oil Chem Soc*. 86:1209–1214. doi:10.1007/s11746-009-1456-6.
- Nguyen DV, Drover VA, Knopfel M, Dhanasekaran P, Hauser H, Phillips MC. 2009. Influence of class β scavenger receptors on cholesterol flux across the brush border membrane and intestinal absorption. *J Lipid Res*. 50:2235–2244. doi:10.1194/jlr.M900036-JLR200. PMID:19454765
- Nigam PK. 2011. Serum lipid profile: fasting or non-fasting? *Ind J Clin Biochem*. 26:96–97. doi:10.1007/s12291-010-0095-x.
- Normén L, Dutta P, Agot L, Anderson H. 2000. Soy sterol esters and β -sitostanol ester as inhibitors of cholesterol absorption in human small bowel. *Am J Clin Nutr*. 71:908–913. PMID:10731496
- Plummer DT. 1977. *An Introduction to Practical Biochemistry*. New York (NY): McGraw Hill Companies.
- Puah CW, May CY, Ahngan M, Hock CC. 2004. Degumming and bleaching: Effect on selected constituents of palm oil. *J Oil Palm Res*. 16:57–63.
- Quilez J, Garcia LP, Salas SJ. 2003. Potential uses and benefits of phytosterols in diet: Present situation and future directions. *J Clin Nutr*. 22:343–351. doi:10.1016/S0261-5614(03)00060-8.
- Reeves PG, Nilson FH, Fahey GC. 1993. Purified diet for laboratory rodents: Final report of the american institute of nutrition Ad Hoc writing committee on the reformulation of AIN-76 a Rodent Diet. *J Nutr*. 123:1939–1951. PMID:8229312
- Sabir SM, Hayat I, Gardezi DA. 2003. Estimation of sterols in edible fats and oils. *Pak J Nutr*. 2:178–181. doi:10.3923/pjn.2003.178.181.
- Scientific Committee on Food of the EU. 2003. Opinion on application for approval of a variety of plant sterol-enriched foods. [http://Europa.eu.int/comm/food/fs/sc/out174_en.pdf], accessed on 5/18 2007.
- Seguchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N. 1995. Direct measurement of high density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated α -cyclodextrin. *Clin Chem*. 41:717–723. PMID:7729051
- Shin JW, Seol IC, Son CG. 2010. Interpretation of animal dose and human equivalent dose for drug development. *J Korean Orient Med*. 31(3):1–7.
- Shin MJ, Lee JH, Jang Y, Lee-Kim YC, Park E, Kim KM, Chung BC, Chung N. 2005. Micellar phytosterols effectively reduce cholesterol absorption at low doses. *Ann Nutr Metab*. 49:346–351 doi:10.1159/000087880. PMID:16127299
- Tasan M, Bilgin B, Geçgel U, Demirci AS. 2006. Phytosterols as functional food ingredients. *Journal of Tekirdag Agricultural Faculty*. 3:153–159.
- Than TT, Vergnes MF, Kaloustian J, El-Moselhy TF, Amiot-Carlin MJ, Portugal H. 2006. Effect of storage and heating on phytosterol concentration in vegetable oils determined by GC/MS. *J Sci Food Agric*. 86:220–225. doi:10.1002/jsfa.2322.
- Turley SD, Dietschy JM. 2003. The intestinal absorption of biliary and dietary cholesterol as a drug target for lowering the plasma cholesterol level. *Prev. Cardiol*. 6:29–33. doi:10.1111/j.1520-037X.2003.01691.x. PMID:12624559
- Volpe R, Niittynen L, Korpela R, Sirtori CC, Bucci A, Fraonen, Pazzucconi F. 2001. Effect of yoghurt enriched with plant sterol on serum lipids in patients with moderate hypercholesterolemia. *Br J Nutr*. 86:233–239. doi:10.1079/BJN2001395. PMID:11502237
- Wardhana T. 2011. *Low temperature solvent recrystallization in phytosterols rich fraction preparation from palm fatty acid distillate*. Research Report. Malang (Indonesia): Brawijaya University.
- Yang C, Yu L, Li W, Xu F, Cohen JC, Hoobs HH. 2004. Disruption of cholesterol homeostasis by plant sterol. *J Clin Invest*. 114:813–822. doi:10.1172/JCI22186. PMID:15372105