Note

Preparation of a Lemon Flavonoid Aglycone and its Suppressive Effect on the Susceptibility of LDL to Oxidation Following Human Ingestion

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Lemon flavonoid (LF) prepared from lemon peel predominantly contains eriocitrin as an antioxidant. It is indicated to have low bioavailability compared with lemon flavonoid aglycone (LFA), which predominantly contains eriodictyol. This study attempted to prepare LFA which has high bioavailability, using enzymes that are commonly used in the citrus industry, such as cellulase, naringinase, hesperidinase, and pectinase. LFA containing the highest amount of eriodictyol (19.4%) was prepared with naringinase, a debittering enzyme for citrus juice. Ten male normolipidemic subjects ingested LFA (3.7 g) after an overnight fast, and low-density lipoprotein (LDL) was prepared from 0–4 h plasma after intake of LFA. The LDL oxidizability was measured with lag time of the conjugated diene formation induced by an oxidative inducer. LDL in 0.5 h plasma after ingestion of LFA was shown to have a significantly longer lag time for oxidation than that before ingestion ($P < 0.05$). LFA was suggested to have the resistance effect of LDL to oxidation *ex vivo*. Eriodictyol, homoeriodictyol, and hesperetin were not detected in plasma by HPLC analysis, but they were detected in plasma treated with β -glucuronidase and sulfatase. The flavonoids were suggested to be glucuro- and/or sulfo-conjugates and to be metabolites in plasma after ingestion of LFA.

Keywords: aglycone, eriodictyol, lemon flavonoid, LDL oxidation, naringinase

Introduction

Flavonoids are widely present in plant foods such as fruits, vegetables, nuts, and seeds. Flavonoids in citrus fruit have been reported to have antioxidative activity, with anti-hypertension, and antihypercholesterolemia properties (Middleton and Kandaswami 1994). Lemon has been reported to contain flavanone glycoside such as eriocitrin (eriodictyol 7-*O*- β -rutinose) (Kawai *et al.*, 1999). Lemon flavonoid (LF), which is prepared from lemon peel, has been reported to predominantly contain eriocitrin and to have a suppressive effect on oxidative stress in diabetic rats (Miyake *et al.*, 1998) and lipid-lowering effect in rats on high-fat and high-cholesterol diet (Miyake *et al.*, 2006a). However, LF has been shown to have low bioavailability in tests of single-dose oral ingestion

in humans. Meanwhile, a lemon flavonoid aglycone (LFA), which is prepared from LF by β -glucosidase for regent, was shown to have higher bioavailability than LF (Miyake *et al.*, 2006b). In this study, we prepared LFA from LF, using various enzymes that are commonly used in the citrus industry for citrus processing, such as cellulase, naringinase, hesperidinase, and pectinase.

Studies have indicated that intake of flavonoids is associated with a reduced risk of coronary heart disease (Hertog *et al.*, 1997; Sies *et al.*, 2005). The oxidative modification of LDL by free radicals is believed to be a key early event in the pathogenesis of atherosclerosis (Goldstein *et al.*, 1979; Sies *et al.*, 2005). The rapid uptake of oxidatively-modified LDL via a scavenger receptor leads to the formation of foam cells, and oxidized LDL also has a number of other atherogenic properties. Several dietary antioxidants have been shown to inhibit the oxidative modification of LDL (Frankel

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et al., 1993; Takahashi *et al.*, 2005; Baba *et al.*, 2007). The resistance effect of LDL oxidation in plasma has been associated with prevention of cardiovascular disease. Furthermore, we examined LFA for resistant effect on LDL (low-density lipoprotein) oxidation in plasma after ingestion of the LFA by human subjects.

Materials and Methods

Enzymes Hesperidinase (85 units/g) and naringinase (150 units/g) were obtained from Mitsubishi Tanabe Pharma Co., Osaka, Japan. Cellulase (Amano T; 30,000 units/g) and pectinase (Amano PL; 50,000 units/ml) were obtained from Amano Enzyme Inc., Nagoya, Japan. They are used in citrus fruit squeezing factories and are food additives used in Japan. β -Glucuronidase was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Sulfatase (type H-5) was obtained from Sigma Chemical Co., USA.

Reagents Eriocitrin, *C*-diglucosylapigenin, and *C*-diglucosyldiosmetin were prepared from lemon peel using the reported method (Miyake *et al.*, 2007). Other flavonoids of eriodictyol, hesperetin, hesperidin, and homoeriodictyol were obtained from Funakoshi Co. Ltd., Tokyo, Japan. Other reagents used in this study were of analytical or HPLC grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Change of flavonoids in LF by enzyme treatment LF was prepared from lemon peel using the reported method (Miyake *et al.*, 2006b). The solution (5 mL) prepared with 10 mg/mL of LF, 1 mg/mL of each enzyme (cellulase, hesperidinase, naringinase, pectinase) and 50 mM of sodium acetate-HCl buffer solution (pH 3.5) at final concentration was incubated at 37°C for 3 h. The reaction was carried out at pH 3.5, because enzymes have been used in citrus juice of pH 3-4 in citrus factories. The solution (0.5 mL) treated by each enzyme was added with ethanol of equal volume, and the mixed solution was centrifuged at $20,627 \times g$ for 10 min after stirring vigorously. The flavonoid content of the supernatant was determined by HPLC (900 series, JASCO Co., Ltd., Tokyo, Japan) using a YMC-ODS column (YMC-Pack Φ 4.6 $\times$ 150 mm, S-5 μ m, YMC Co., Ltd., Kyoto, Japan), UV detection of 280 nm, mobile solvents of methanol and water containing 5% acetic acid, a flow rate of 1 mL/min, and a column temperature of 40°C. In the mobile phase condition, the concentration of methanol was changed from 10% to 90% over 15 min, and 100% methanol was eluted for 5 min. The retention times of *C*-diglucosylapigenin, *C*-diglucosyldiosmetin, eriocitrin, hesperidin, eriodictyol, and hesperetin were 7.90, 8.43, 9.09, 10.60, 11.73, and 13.48 min, respectively. The content of flavonoids is shown as mean $\pm$ SD (μ g/mg of LF or LFA, $n=3$).

Preparation of LFA LFA for the intake sample was pre-

pared from LF by treatment with naringinase. Naringinase at a final concentration of 0.1% was added to 100 mM sodium phosphate buffer solution (pH 3.5) containing 2% LF, and the solution was incubated at 37°C for 3 h. It was applied to a reversed-phase resin (Amberlite XAD-2). The resin was washed with water to remove carbohydrates and enzymes, and then the resin was eluted with pure ethanol. The eluate was evaporated under reduced pressure and freeze-dried to obtain powder. LFA 3.7 g was prepared from 4.5 g LF.

Assay for susceptibility of LDL oxidation after ingestion of LFA Ten healthy male volunteers from 32 to 39 years of age (mean $\pm$ SD, 35.7 ± 2.7) participated in the ingestion test. This study was carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983, and approved by the Ethics Committee of Ochanomizu University. The procedures were fully explained to all the volunteers in advance, and all gave their signed informed consent before participating. The subjects ingested powder of lemon flavonoid aglycone (3.7 g), wrapped with oblate, with 500 mL water over a period of less than 5 min at the study site in the morning after an overnight fast. The baseline blood sample was obtained 10-20 min before administration. Blood samples of 10 mL were taken from subjects, and collected into tubes containing EDTA, at 0.5, 1, 2, and 4 h after intake of the sample. Plasma samples were immediately prepared by centrifugation at $2,000 \times g$ for 10 min at 4°C. The LDL was separated by single-spin density gradient ultracentrifugation ($417,000 \times g$, 40 min, 4°C) using a TLA-100.4 fixed angle-rotor (Beckman Instrument Inc., CA). The LDL protein concentration was determined using a Micro BCA Protein Assay Kit (Pierce Laboratories, Inc., Rockford, IL). Before the start of oxidation experiments, the LDL samples were diluted with PBS to give a final concentration of 70 μ g/mL LDL protein. The LDL oxidizability was measured according to reported methods (Hirano *et al.*, 1997). The prepared LDL samples were oxidized by 400 mM of an oxidative inducer, 2,2'-azobis-4-methoxy-2,4-dimethylvaleronitrile. The kinetics of LDL oxidation were obtained by monitoring the absorbance of conjugated dienes at 234 nm with a Beckman Model DU 650 spectrophotometer (Beckman Coulter, Inc., CA) at 4 min intervals at 37°C. The lag time of lipid peroxidation is defined as the time interval between the initiation and the intercept of two tangents drawn to the lag and propagation phase of the absorbance curve at 234 nm, and was expressed in minutes.

Determination of flavonoids in plasma after ingestion of LFA Eriodictyol, hesperetin, and homoeriodictyol in plasma were analyzed by the previously described method (Miyake *et al.*, 2006b). For the determination of free type, the plasma sample (0.5 mL) was applied to a DISPO COLUMN C18H050 (Toyo Roshi Ltd., Tokyo, Japan) and the methanol

elute solution was evaporated to dryness. For the determination of glucuro- and/or sulfo-conjugated type, the plasma sample (0.5 mL) was treated with β -glucuronidase (5.4×10^2 units/mL) and sulfatase (0.2×10^2 units/mL) for 60 min at 37°C. The mixtures were applied to a DISPO COLUMN C18H050, and the methanol elute solution was evaporated to dryness. The dried samples were dissolved in 50 μ L of methanol, and eriodictyol, hesperetin, and homoeriodictyol in the solution were analyzed by HPLC as previously described (Miyake *et al.*, 2006b). The concentration values of flavonoids in the plasma were represented as mean $\pm$ SE (μ g/mL of plasma, n=10).

Statistical Analysis Each data value is presented as the mean $\pm$ SE (n=10) in the test of LFA ingestion. Statistical analysis comparing each period was carried out using the Wilcoxon signed rank sum test with StatView J 5.0 software (Abacus Concepts Inc, USA).

Results and Discussion

Preparation of LFA from LF by enzyme Lemon fruit has been reported to contain flavonoids of flavanone glycosides such as eriocitrin and hesperidin (hesperetin 7-O- β -rutinose) and such flavone glucosides as 6,8-C-diglucosylapigenin and 6,8-C-diglucosyldiosmetin, as shown in Fig. 1 (Kawai *et al.*, 1999; Miyake *et al.*, 2007). LF, which is prepared from lemon peel and predominantly contains eriocitrin, has been reported to have low bioavailability when orally ingested by humans (Miyake *et al.*, 2006b), although it exhibited bioactivities such as a suppressive effect on oxidative stress in experimental animals (Miyake *et al.*, 1998, 2006a). An oral

intake test in rats has shown that eriocitrin is absorbed after it is converted to eriodictyol of an aglycone by intestinal bacteria (Miyake *et al.*, 2000). LFA containing 14.7% eriodictyol has been reported to be prepared from LF by β -glucosidase (G4511, from almonds, Sigma Chemical Co., USA) as a reagent, and has been shown to have higher bioavailability than LF in oral ingestion tests in humans (Sakurai, *et al.*, 2003; Miyake *et al.*, 2006b). ILFA, which predominantly contains eriodictyol, has been suggested to be absorbed earlier, more easily, and in greater amounts than LF because of the aglycone. In this study, we attempted to prepare LFA from LF using cellulase, hesperidinase, naringinase, and pectinase, which are enzymes commonly used in the citrus industry and food additives. Cellulase and pectinase are used for the clarification of squeezed citrus juice and for the treatment of by-products such as hydrolysis of peel waste, which is produced in citrus fruit squeezing factories (Wilkins *et al.*, 2007). Naringinase is used as a debittering enzyme, to improve the bitter taste caused by high naringin content in grapefruit juice and bitter orange juice (Barm and Solomons 1965; Puri and Banerjee 2000). It can suppress the bitter taste, because the bitter naringin is converted to naringenin 7-O- β -glucoside or naringenin of no taste. Hesperidinase has been used to prevent the generation of white turbidity in orange juice and the syrup of canned orange fruit caused by high concentrations of hesperidin (Walton 1984). It is converted from hesperidin, with low solubility, to hesperetin 7-O- β -glucoside, which is easy to dissolve in water compared to hesperidin (Sanchez *et al.*, 1987). Cellulase and pectinase have been reported for enzymatic hydrolysis of flavonoid glycosides in bergamot

Fig. 1. Structural formula of lemon flavonoids and metabolites of LFA.

(Mandalari *et al.*, 2006; 2007). Naringinase and hesperidinase have been reported for preparation of aglycones from flavonoid glycosides such as isoliquirtin and kaempferol glycoside (Sato, *et al.*, 2007; Park *et al.*, 2006). Enzymes having pervasive substrate specificity have been reported for preparation of aglycones from flavonoid glycosides.

Figure 2 show the flavonoid content in the LFA treated by each enzyme: cellulase, hesperidinase, naringinase, and pectinase. LF prepared from lemon peel was shown to contain flavonoid glycosides such as *C*-diglucosylapigenin, *C*-diglucosyldiosmetin, eriocitrin, narirutin, and hesperidin. LF contained the highest amount of eriocitrin in flavonoids and did not contain aglycones of flavonoid glycosides. The highest amounts of eriodictyol (19.4%) were produced in LFA by naringinase. Naringinase was shown to be suitable for preparation of LFA from LF, containing abundant eriodictyol, and was not only able to convert from naringin to naringenin but also from eriocitrin to eriodictyol. *C*-Diglucosylapigenin and *C*-diglucosyldiosmetin remained after treatment with each enzyme. These were thought not to be converted by the hydrolysis reaction of their enzymes because of the *C*-glucoside bonds between flavonoid and sugar chain (Fig. 1). These results showed that LFA can be produced effectively from LF by naringinase, which is an enzyme used to reduce the bitterness citrus juice.

Suppressive effect of susceptibility of LDL oxidation after intake of LFA LF has been reported to have suppressive effect of oxidative stress by measurement of oxidative stress

marker in diabetic rats (Miyake *et al.*, 1998). Moreover, the resistance effect of oxidation in whole plasma after ingestion of LA in rat has been reported (Miyake *et al.*, 2000). The resistance effect of LDL oxidation in plasma has been associated with prevention of cardiovascular disease (Goldstein *et al.*, 1979; Sies *et al.*, 2005). Furthermore, we examined LF for resistance effect on LDL oxidation in plasma after ingestion of the LF by human subjects in the preliminary test (three subjects). LDL in plasma (0–4 h) after ingestion of LF (4.5 g) containing 30% eriocitrin was prepared and examined for suppressive effect of the susceptibility of LDL oxidation according to the lag time method. Lag time of the LDL oxidation after ingestion of LF was 57.6 min (0 h plasma), 58.2 min (0.5 h plasma), 58.2 min (1.0 h plasma), 56.4 min (2.0 h plasma), and 58.4 min (4.0 h plasma). The suppressive effect on the susceptibility of LDL to oxidation in the test of ingestion LF in human was not shown. We thought that the effect was not shown because LF has low absorption. Therefore, LFA prepared with naringinase was examined for its suppressive effect on the susceptibility of LDL oxidation after oral ingestion by the ten subjects in this study. The lag time of LDL oxidation on 0.5 h after ingestion of LFA (3.7 g), is equivalent with that of LF (4.5 g), and was shown to be significantly longer than that before ingestion as shown in Fig. 3 ($P < 0.05$). The results demonstrate that LFA has a suppressive effect on the susceptibility of LDL oxidation after oral ingestion by humans and suggest that it has an antioxidative activity *ex vivo*.

Fig. 2. Contents of flavonoids in LFA prepared from LF by each enzyme.

LF solution was prepared with 10 mg/mL of LF and 50 mM of sodium acetate-HCl buffer solution (pH 3.5) at final concentration. * Enzyme was not added to the solution. # Each enzyme at a final concentration of 1 mg/mL was added to the LF solution. The solutions were incubated at 37°C for 3 h. Flavonoids in the solutions were determined by HPLC as described in Materials and Methods. Values denote mean $\pm$ SD ($n=3$).

Metabolites in plasma after intake of LFA Eriodictyol, hesperetin, and homoeriodictyol had been reported to exist as metabolites in plasma after oral ingestion of eriocitrin or LF, as shown in Fig. 1 (Miyake *et al.*, 2000; Miyake *et al.*, 2006). HPLC was employed in an attempt to detect the flavonoids in plasma after the oral ingestion of LFA by humans. They were not detected in plasma after ingestion of LFA. However, they were detected in plasma after treatment by β -glucuronidase and sulfatase and determined by HPLC (Fig. 4). Therefore, they are thought to exist in plasma as glucuro- and/or sulfo-conjugates and to be metabolites of LFA. The resistance effect of LDL oxidation was presumed

Fig. 3. Lag time of LDL oxidation in plasma after ingestion of LFA. *Value is shown to be significant $P < 0.05$ to 0 h. Values are shown as mean $\pm$ SE (n=10).

to be related to metabolites of LFA in plasma after the ingestion of LFA, although research will have to be conducted for relation with the effect. Eriodictyol had been suggested to be metabolized by methylation and conjugation with glucuronic acid and/or sulfonic acid after it was absorbed from the intestine (Miyake *et al.*, 2000; 2006). Hesperetin has been reported to exhibit low activity in the assay using measuring of LDL lag time *in vitro* in comparison with eriodictyol (Miyake *et al.*, 1997). Antioxidative activity of flavonoids has been reported to be related to the number of hydroxyl groups and the existence of an o-hydroxy catechol group (Woodman *et al.*, 2005). Eriodictyol conjugates of glucuronic acid and/or sulfonic acid were presumed to relate with the activity, although the activity of metabolite conjugates requires future research.

In this study, LFA, which predominantly contains eriodictyol, was prepared effectively from LF using *n*-naringinase, a debittering enzyme in general use in the citrus industry. LFA was shown to have a suppressive effect on the susceptibility of LDL to oxidation on plasma after oral ingestion by humans. Eriodictyol, hesperetin, and homoeriodictyol were suggested to exist in plasma as glucuro- and/or sulfo-conjugates, suggesting involvement in the efficacy.

References

- Baba, S., Osakabe, N., Kato, Y., Natsume, M., Yasuda, A., Kido, T., Fukuda, K., Muto, Y. and Kondo, K. (2007) Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma

Fig. 4. The contents of eriodictyol, hesperetin, and homoeriodictyol in plasma after the oral ingestion of LFA by humans. The plasma sample was treated with β -glucuronidase and sulfatase according to the methods and materials. The mixtures were applied to a DISPO COLUMN C18H050 and the methanol elute solution was analyzed by HPLC. The concentration values of flavonoids in the plasma were represented as mean $\pm$ SE (μ g/mL of plasma, n=10).

- HDL-cholesterol concentrations in humans. *Am. J. Clin. Nutr.*, **85**, 709-717.
- Barm, B. and Solomons, G.L. (1965) Production of the enzyme naringinase by *Aspergillus niger*. *Appl. Microbiol.*, **13**, 842-845.
- Frankel, E.N., Kanner, J., German, J.B., Parks, E. and Kinsella, J.E. (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, **341**, 454-457.
- Goldstein, J.L., Brown, M.S., Krieger, M., Anderson, R.G. and Mintz, B. (1979) Demonstration of low density lipoprotein receptors in mouse teratocarcinoma stem cells and description of a method for producing receptor-deficient mutant mice. *Proc. Natl. Acad. Sci. USA*, **76**, 2843-2847.
- Hirano, R., Sasamoto, W., Matsumoto, A., Itakura, H., Igarashi, O. and Kondo, K. (2001) Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *J. Nutr. Sci. Vitaminol.*, **47**, 357-362.
- Hertog, M.G., Feskens, E.J. and Kromhout, D. (1997) Antioxidant flavonols and coronary heart disease risk. *Lancet*, **349**, 699.
- Kawai, S., Tomono, Y., Katase, E., Ogawa, K. and Yano, M. (1999) Quantitation of flavonoid constituents in citrus fruits. *J. Agric. Food Chem.*, **47**, 3565-3571.
- Mandalari, G., Bennett, R.N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C.B., Gasson, M.J. and Narbad, A. (2007) Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *J. Appl. Microbiol.*, **103**, 2056-2064.
- Mandalari, G., Bennett, R.N., Kirby, A.R., Lo Curto, R.B., Bisignano, G., Waldron, K.W. and Faulds, C.B. (2006) Enzymatic hydrolysis of flavonoids and pectic oligosaccharides from bergamot (*Citrus bergamia* Risso) peel. *J. Agric. Food Chem.*, **54**, 8307-8313.
- Middleton, E. and Kandaswami, C. (1994) Potential health promoting properties of citrus flavonoids. *Food Technol.*, **48**, 115-119.
- Miyake, Y., Mochizuki, M., Okada, M., Hiramitsu, M., Morimitsu, Y. and Osawa, T. (2007) Isolation of antioxidative phenolic glucosides from lemon juice and their suppressive effect on the expression of blood adhesion molecules. *Biosci. Biotechnol. Biochem.*, **71**, 1911-1919.
- Miyake, Y., Sakurai, C., Usuda, M., Fukumoto, S., Hiramitsu, M., Sakaida, K., Osawa, T. and Kondo, K. (2006b) Difference in plasma metabolite concentration after ingestion of lemon flavonoids and their aglycones in humans. *J. Nutr. Sci. Vitaminol.*, **52**, 54-60.
- Miyake, Y., Shimoi, K., Kumazawa, S., Yamamoto, K., Kinae, N. and Osawa, T. (2000) Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats. *J. Agric. Food Chem.*, **48**, 3217-3224.
- Miyake, Y., Suzuki, E., Ohya, S., Fukumoto, S., Hiramitsu, M., Sakaida, K., Osawa, T. and Furuichi, Y. (2006a) Lipid-lowering effect of eriocitrin, the main flavonoid in lemon fruit, in rats on a high-fat and high-cholesterol diet. *J. Food Sci.*, **71**, S633-637.
- Miyake, Y., Yamamoto, K., Morimitsu, Y. and Osawa, T. (1997) Isolation of C-glucosylflavone from lemon peel and antioxidative activity of flavonoid compounds in lemon fruit. *J. Agric. Food Chem.*, **45**, 4619-4623.
- Miyake, Y., Yamamoto, K., Tsujihara, N. and Osawa, T. (1998) Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids*, **33**, 689-695.
- Park, J.S., Rho, H.S., Kim, D.H. and Chang, I.S. (2006) Enzymatic preparation of kaempferol from green tea seed and its antioxidant activity. *J. Agric. Food Chem.*, **54**, 2951-2956.
- Puri, M. and Banerjee, U.C. (2000) Production, purification, and characterization of the debittering enzyme naringinase. *Biotechnol. Adv.*, **18**, 207-217.
- Sakurai, T., Utsu, H., Tago, M., Usuda, M., Tani, M., Tanaka, M., Okumura, M., Kido, T., Nghiem, N.T., Hiramitsu, M., Miyake, Y., Sakaida, K., Kiyose, T., Suzuki, E. and Kondo, K. (2003) Research for susceptibility of human LDL to oxidation following lemon flavonoids. *J. Jpn. Mibyou Sys. Associ.*, **9**, 239-242. (in Japanese)
- Sanchez, M.A., Romero, C., Manjon, A. and Iborra, J.L. (1987) Activity of soluble and immobilized hesperidinase on insoluble hesperidin. *Biotechnol. Lett.*, **9**, 871-874.
- Sato, Y., He, J.X., Nagai, H., Tani, T. and Akao, T. (2007) Isoliquiritigenin, one of the antispasmodic principles of Glycyrrhiza uralensis roots, acts in the lower part of intestine. *Biol. Pharm. Bull.*, **30**, 145-149.
- Sies, H., Stahl, W. and Sevanian, A. (2005) Nutritional, dietary and postprandial oxidative stress. *J. Nutr.*, **135**, 969-972.
- Takahashi, R., Ohmori, R., Kiyose, C., Momiyama, Y., Ohsuzu, F. and Kondo, K. (2005) Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. *J. Agric. Food Chem.*, **53**, 4578-4582.
- Walton, B. Sinclair. (1984) Some soluble and insoluble constituents of citrus fruits. In "Specialty by-products." ed. by W. B. Sinclair. Division of Agriculture and Natural Resources, University of California, Oakland, CA, pp. 775-781.
- Wilkins, M.R., Widmer, W.W., Grohmann, K. and Cameron, R.G. (2007) Hydrolysis of grapefruit peel waste with cellulase and pectinase enzymes. *Bioresour. Technol.*, **98**, 1596-1601.
- Woodman, O.L., Meeker, W.F. and Boujaoude, M. (2005) Vasorelaxant and antioxidant activity of flavonols and flavones: structure-activity relationships. *J. Cardiovasc. Pharmacol.*, **46**, 302-309.