

# Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation

K.G. Nevin and T. Rajamohan\*

*Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram 695 581, India*

Received 23 June 2003; received in revised form 17 December 2003; accepted 30 April 2004

Available online 25 June 2004

## Abstract

**Objectives:** The present study was conducted to investigate the effect of consumption of virgin coconut oil (VCO) on various lipid parameters in comparison with copra oil (CO). In addition, the preventive effect of polyphenol fraction (PF) from test oils on copper induced oxidation of LDL and carbonyl formation was also studied.

**Design and methods:** After 45 days of oil feeding to Sprague–Dawley rats, several lipid parameters and lipoprotein levels were determined. PF was isolated from the oils and its effect on in vitro LDL oxidation was assessed.

**Results:** VCO obtained by wet process has a beneficial effect in lowering lipid components compared to CO. It reduced total cholesterol, triglycerides, phospholipids, LDL, and VLDL cholesterol levels and increased HDL cholesterol in serum and tissues. The PF of virgin coconut oil was also found to be capable of preventing in vitro LDL oxidation with reduced carbonyl formation.

**Conclusion:** The results demonstrated the potential beneficiary effect of virgin coconut oil in lowering lipid levels in serum and tissues and LDL oxidation by physiological oxidants. This property of VCO may be attributed to the biologically active polyphenol components present in the oil.

© 2004 The Canadian Society of Clinical Chemists. All rights reserved.

*Keywords:* Virgin coconut oil; Cholesterol; Polyphenols; LDL oxidation; PF; HDL

## Introduction

A number of components of the diet have been shown to influence plasma lipid and lipoprotein concentration. For example, replacing saturated fatty acids with polyunsaturated fatty acids (PUFA) is considered to lower low-density lipoprotein (LDL) concentration and may promote a modest lowering in HDL concentration [1,2]. The mechanism underlying such a beneficial effect is still under debate [3,4]. There are reports indicating that in addition to PUFA content several unsaponifiable components like vitamin E and polyphenols may also play a beneficial role in reducing the cholesterol level and lipid peroxidation [5]. As per epidemiological and clinical studies, elevated levels of plasma cholesterol associated with circulating oxidized LDL are correlated with coronary heart disease (CHD) [6,7]. The inverse correlation between high-density lipoprotein (HDL) cholesterol and CHD has also been known for several decades [8,9]. In this context, any dietary oil that

lowers LDL cholesterol and elevates HDL cholesterol is considered to have health benefits.

Coconut oil is believed to elevate blood cholesterol since it contains mostly saturated fatty acids. Usually coconut oil is obtained by dry process from copra, which is exposed to very high temperatures or sunlight for several days until most of the moisture is removed. Exposure to sunlight or high temperatures may inactivate the minor components like tocopherols, tocotrienols, and polyphenols [10]. On the other hand, virgin coconut oil (VCO) extracted by wet process directly from coconut milk under a controlled temperature may have more beneficial effects than copra oil (CO) since it retains most of the unsaponifiable components.

The primary purpose of the present study was to determine the comparative influence of the consumption of VCO with CO on lipid parameters (total cholesterol, triglycerides, phospholipids, and lipoproteins) in serum and tissues. In addition, we determined whether the polyphenol fraction (PF) from VCO has any beneficial effect over the PF from other oils in preventing in vitro  $\text{Cu}^{2+}$  induced LDL oxidation and carbonyl formation.

\* Corresponding author.

*E-mail address:* [trmohan@sancharnet.in](mailto:trmohan@sancharnet.in) (T. Rajamohan).

## Methods

### Preparation of virgin coconut oil

The solid endosperm of mature coconut (West coast tall variety) was crushed, made into a viscous slurry and squeezed through cheese cloth to obtain coconut milk which was refrigerated for 48 h. After 48 h, the milk was subjected to mild heating (50°C) in a thermostat oven. The obtained virgin oil was filtered through cheesecloth and was used for the present study.

### Animal experiments

Male Sprague–Dawley rats (100–130 g) bred in the department animal house were used for the study. The animals were housed individually in polypropylene cages in a room maintained at  $25 \pm 1^\circ\text{C}$  with a 12-h light and 12-h dark cycle. The experimental groups were as follows: Group I (Control)—ground nut oil (GNO), Group II—CO (8%), Group III—VCO (8%). Oils were fed along with a semi synthetic diet for 45 days (Table 1). Gain in body weight was recorded weekly. After 45 days, animals were fasted overnight and sacrificed by sodium pentathone injection; blood and tissues were collected for various estimations.

### Lipid analysis

Total lipids were extracted using chloroform/methanol (2:1) as described by Folch et al. [11]. Five hundred-milligram tissue/n ml serum was homogenized with chloroform/methanol (2:1), filtered and washed with chloroform/methanol at least three times. Calcium chloride, (0.02%), was added to the mixture (20% of the total volume of the extract), mixed vigorously and allowed to stand for a few minutes. The upper layer was washed three times with 5-ml chloroform/methanol/calcium chloride (3:48:48 v/v). The washed upper layer was evaporated to dryness and the residue was redissolved in a known volume of chloroform. From this, aliquots were used for lipid analysis. Total cholesterol was estimated as described by Abell et al. [12]. Triglycerides and phospholipids were estimated by the method of Zilversmith and Davis and Van Handel and Zilversmith respectively [13].

Table 1  
Formulation of synthetic diet

Diet <sup>a</sup>	Group I	Group II	Group III
Corn starch	71	71	71
Casein	16	16	16
Virgin coconut oil	–	–	8
Copra oil	–	8	–
Ground nut oil	8	–	–
Salt mixture	4	4	4
Vitamin mixture	1	1	1

<sup>a</sup> g/100 g weight.

### HDL-cholesterol assay

Two milliliters of serum was thoroughly mixed with 0.2 ml of heparin–manganese solution (final concentration 1.4 mg/ml and 0.092 M respectively). Samples were incubated for at least 10 min at room temperature and centrifuged at  $1500 \times g$  for 30 min. Clear supernatant was removed manually for the assays [14]. HDL-C was measured by the method described by Abell [12]. LDL and VLDL cholesterol levels were calculated using the standard Friedewald equation [15].

### Total polyphenol content

PF from the test oils was extracted according to the method described by Vazquez Roncero et al. [16]. Ten-gram oil was dissolved in 50-ml hexane and extracted three times with 20-ml portions of 60% methanol successively. The vacuum-dried final residue obtained from the combined extract was dissolved in a known volume of methanol. The total polyphenol content of this solution was estimated using Folin-Ciocalteu reagent [17].

### In vitro LDL oxidation studies

A volume of 3 ml plasma was centrifuged at  $d = 1.006$  kg/l in an ultracentrifuge (Sorvall Ultra 80) at 40,000 rpm using a T-865 rotor at 14°C for 10 h [18]. After ultracentrifugation floating VLDL and chylomicrons were removed and LDL was separated by precipitation from the solution [19]. Forty microliters of 4% phosphotungstic acid in 1 M NaOH was added, stirred and 10  $\mu\text{l}$  of 2 M  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  was added and centrifuged at  $1500 \times g$  for 30 min at 4°C. The supernatant was discarded and the precipitated LDL was redissolved in 0.4 ml 0.5 M  $\text{Na}_2\text{CO}_3$ , kept in ice overnight and dialyzed against three changes of PBS for 12 h.

Isolated LDL (200  $\mu\text{g}$  protein/ml) was preincubated with 50  $\mu\text{g}$  PF from the respective oils for 30 min. LDL oxidation was initiated by adding 1.7 mM copper sulphate to the reaction mixture [20]. The thiobarbituric acid reactive substance (TBARS) was determined after 6 h by the procedure described by Wills [21].

### Determination of carbonyl content

Aliquots from the  $\text{Cu}^{2+}$  oxidized samples treated with PF from the three oils were tested for carbonyl formation. To the samples, 0.5 ml of 2, 4-dinitrophenyl hydrazine (10 mM in 2N HCl) was added and was allowed to react for 60 min at room temperature with vortexing every 15 min. After the reaction was complete, 20% TCA (0.5 ml) was added and the samples were centrifuged. The precipitate was washed with ethanol/ethyl acetate (1:1), dissolved in 0.6 ml of guanidine (6 M in phosphate buffer, adjusted to pH 2.3 with trifluoroacetic acid) and incubated at 37°C for

15–20 min. Results were expressed as the percentage of inhibition in carbonyl formation relative to the control using the absorbance at 390 nm [22].

#### Statistical analysis

Results were statistically analyzed by one-way ANOVA (SPSS 10) using Duncan's variance.

## Results

#### Effect on lipid levels

In the present study no significant change in body weight of animals from the three groups was observed. The concentration of cholesterol in serum, liver, and heart of the VCO-treated group was significantly lower compared to CO fed and control animals. Triglycerides in serum and tissues were significantly lower in VCO-treated animals compared to CO and control animals and phospholipid levels also showed the same pattern among the three groups (Table 2). HDL cholesterol in VCO fed animals was increased while LDL cholesterol levels were significantly decreased, compared to the other two groups (Fig. 1).

#### Preventive effect on *in vitro* LDL oxidation

In *in vitro* oxidation studies (Fig. 3), the PF of VCO was found to be more beneficial than the PF of CO and GNO in preventing the copper-induced oxidation of LDL as indicated by the low TBARS formation and reduced carbonyl formation (Fig. 4).

## Discussion

Experiments with VCO could decrease total cholesterol, triglycerides, and phospholipids in serum and tissues as well as increase HDL cholesterol with a corresponding decrease in LDL cholesterol. This may be due to the quality difference of the two oils, particularly the higher polyphenol content of VCO (80 mg/100 gm oil) compared to CO (64.4 mg/100 gm oil) (Fig. 2), as well as minor constituents including vitamin E. The wet extraction done in the dark and at a controlled temperature may be responsible for retaining the biological activities of the minor components in the oil.

In this context, it is important to remember that  $\alpha$ -tocopherol concentration is greatly affected by the storage conditions of the oil including temperature and light exposure [23]. Studies have also shown that  $\alpha$ -tocopherol has a synergetic effect in association with some phenolic compounds having significant antioxidant activity [24,25] (Fig. 3). Although the fatty acid composition of coconut oil is well established, relatively little is known about the minor constituents and their biological effects [26]. Experiments have also shown that the diet based on GNO is atherogenic [27,28] (Fig. 4).

In the present study, feeding of VCO resulted in decreased concentrations of all the lipids tested (cholesterol, triglycerides, and phospholipids) when compared with CO. An explanation for the lower lipid levels in serum and tissues (liver, heart, and kidney) accompanying VCO feeding may be due to the relative rate of synthesis and catabolism of these lipids. It is possible that the minor and/or unsaponifiable components in VCO may be influencing the rate of synthesis and oxidation of fatty acids in the liver. In the previous study (unpublished data), HMG CoA reductase, the rate limiting enzyme in cholesterol

Table 2  
Levels of total cholesterol, triglyceride, and phospholipids in VCO, CO, and GNO fed rats

Parameters	Serum	Liver	Heart	Kidney
<i>Cholesterol<sup>a</sup></i>				
Group I	91.59 ± 0.01	311.24 ± 0.016	221.84 ± 0.018	658.74 ± 0.017
Group II	90.43 ± 0.016 <sup>b</sup>	257.23 ± 0.014 <sup>b</sup>	219.72 ± 0.015 <sup>b</sup>	654.52 ± 0.013
Group III	75.72 ± 0.015 <sup>b,c</sup>	217.52 ± 0.009 <sup>b,c</sup>	168.25 ± 0.008 <sup>b,c</sup>	661.46 ± 0.012
<i>Triglycerides<sup>a</sup></i>				
Group I	13.97 ± 0.01	180.32 ± 0.03	59.88 ± 0.02	76.16 ± 0.30
Group II	11.59 ± 0.16 <sup>b</sup>	187.37 ± 0.02 <sup>b</sup>	50.01 ± 0.02 <sup>b</sup>	61.16 ± 0.01 <sup>b</sup>
Group III	7.98 ± 0.02 <sup>b,c</sup>	154.18 ± 0.02 <sup>b,c</sup>	38.44 ± 0.35 <sup>b,c</sup>	42.06 ± 0.01 <sup>b,c</sup>
<i>Phospholipids<sup>a</sup></i>				
Group I	127.11 ± 0.02	2243.20 ± 0.02	2148.75 ± 0.15	1842.20 ± 0.16
Group II	126.01 ± 0.01 <sup>b</sup>	2122.68 ± 0.02	2034.25 ± 0.20 <sup>b</sup>	1562.75 ± 0.02 <sup>b</sup>
Group III	92.41 ± 0.01 <sup>b,c</sup>	1844.55 ± 0.01	1829.13 ± 0.01 <sup>b,c</sup>	1141.42 ± 0.01 <sup>b,c</sup>

Values are mean of six rats ± SEM,  $P < 0.05$ .

VCO—virgin coconut oil, CO—copra oil, GNO—ground nut oil.

<sup>a</sup> Expressed as mg/100 g tissue or mg/dl.

<sup>b</sup> Compared to group I.

<sup>c</sup> Compared to group II.

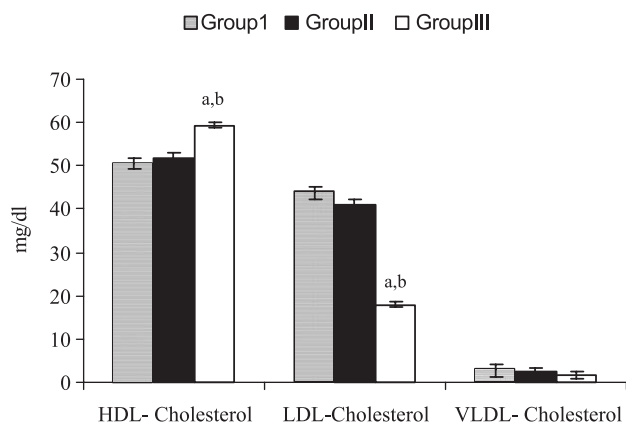


Fig. 1. Levels of serum lipoprotein cholesterol in VCO, CO, and GNO fed animals (Mean  $\pm$  SEM). <sup>a</sup>Compared to Group 1,  $P < 0.001$ . <sup>b</sup>Compared to Group 2,  $P < 0.001$ .

biosynthesis, showed decreased activity. In addition, the activity of lipogenic enzyme, glucose-6-phosphatase dehydrogenase, showed decreased lipogenesis. This enzyme provides NADPH for fatty acid synthesis. These observations correlate with the decrease in triglycerides and phospholipids in liver and other tissues.

The HDL cholesterol levels in VCO-treated animals were found to be higher compared to the other group, an indication of a beneficiary effect of the oil. The protective effect of HDL is most widely attributed to its key role in mediating the reverse cholesterol transport from the peripheral tissues to the liver for reutilization [29]. Saturated fatty acids increase the HDL cholesterol concentration, which has been associated with increased Lecithin cholesterol acyl transferase (LCAT) [30]. HDL particles also enhance the inhibition of LDL oxidation, prevention of monocyte adhesion, inhibition of endothelial dysfunction, and apoptosis [31].

Oxidation of LDL lipids is a risk factor for atherosclerosis and coronary heart disease [32,33]. It is currently believed that lipid peroxidation is involved in the oxidative modification of low-density lipoprotein [34,35]. The anti-

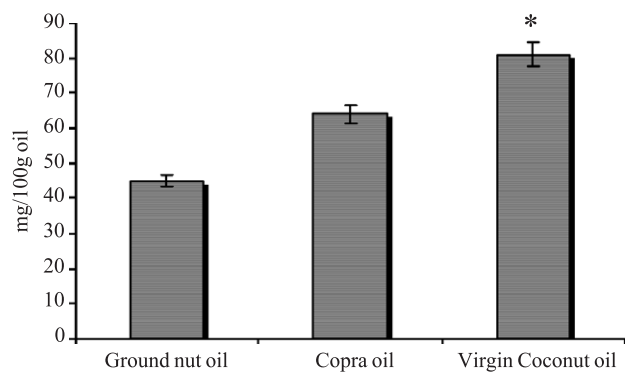


Fig. 2. Total polyphenol content of virgin coconut oil (VCO), copra oil (CO) and ground nut oil (GNO). Values are mean of three estimations. \*Statistically significant compared to copra oil ( $P < 0.001$ ).

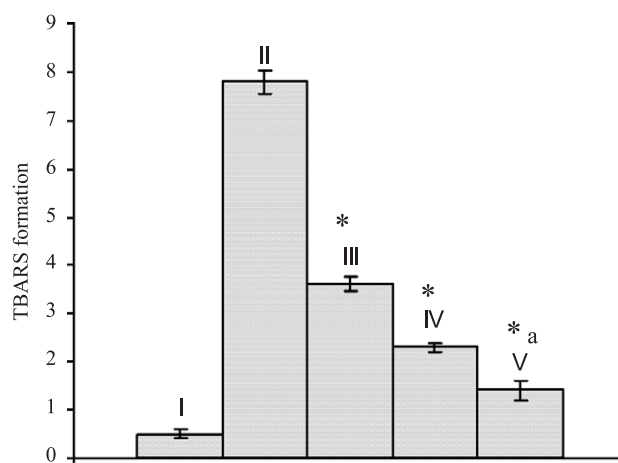


Fig. 3. Effect of PF from VCO, CO and GNO on in vitro  $\text{Cu}^{2+}$  induced LDL oxidation. I—LDL, II—LDL + 1.7 mM  $\text{CUSO}_4$ , III—LDL + 1.7 mM  $\text{CUSO}_4$  + 50  $\mu$ g GNO Polyphenols, IV—LDL + 1.7 mM  $\text{CUSO}_4$  + 50  $\mu$ g CO polyphenols, V—LDL + 1.7 mM  $\text{CUSO}_4$  + 50  $\mu$ g VCO polyphenols. Values are mean of three estimations. \*Significant compared to Group II,  $P < 0.05$ ; <sup>a</sup>significant compared to Group IV,  $P < 0.05$ . TBARS formation is measured as nmol of MDA formed/mg protein.

oxidative properties of the polyphenol fraction separated from VCO, CO, and GNO on LDL oxidation as measured by the production of TBARS showed that the polyphenol fraction from VCO has a significant advantage over other oils in preventing the oxidation of LDL. Several studies have revealed the antioxidant activity of polyphenolic substances, especially from red wine and olive oils in oxidation of LDL [36–38]. These polyphenolic compounds might trap reactive oxygen species in aqueous components such as plasma and interstitial fluid of the arterial wall thereby inhibiting LDL oxidation and showing anti-atherogenic activity [39]. In addition, these compounds are capable of reversing cholesterol transport and reducing intestinal ab-

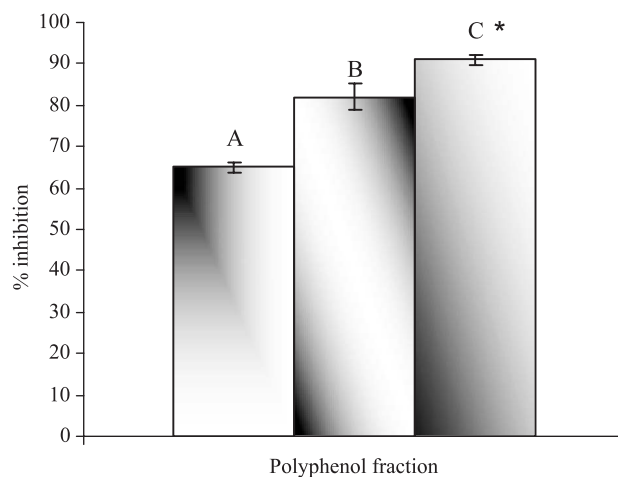


Fig. 4. Effect of VCO, CO and GNO in inhibition of carbonyl formation during in vitro  $\text{Cu}^{2+}$  induced LDL oxidation. (A) Polyphenol fraction from ground nut oil (GNO), (B) polyphenol fraction from copra oil (CO), (C) polyphenol fraction from virgin coconut oil (VCO). Values are mean of three estimations. \*Significant compared to PF of copra oil,  $P < 0.05$ .



sorption of cholesterol [40]. The cholesterol lowering activity of VCO may be partly attributed to this process.

Another oxidative modification of LDL is carbonyl formation. Increase in the formation of carbonyl residues is due to the modification of the protein part of LDL [41]. The property of preventing carbonyl formation of LDL by the polyphenol fraction of VCO may be due to the highly active polyphenols and the presence of tocopherols in its active form.

To conclude, our preliminary data strongly suggest that virgin coconut oil with its high polyphenol content was capable of maintaining the normal levels of cholesterol and other lipid parameters in tissues and serum and also increased the concentration of HDL cholesterol in rats. The antioxidant activity of the PF of VCO against copper-induced LDL oxidation confirms the high potential of this oil in protecting LDL against oxidative stress induced by physiological oxidants.

## References

- [1] Richard W, Henderson GR, Heaster V, Wagner WD, McMahan MR. Influence of dietary fats and an oral contraceptive on plasma lipids, high density lipoproteins, gall stones and atherosclerosis in African Green monkeys. *Atherosclerosis* 1980;37:103–21.
- [2] Wardlaw GM, Snook JT. Effect of diets high in butter corn oil or high oleic acid sunflower oil on serum lipids and apolipoproteins in men. *Am J Clin Nutr* 1990;51:815–21.
- [3] Chiiin JPF. Marine oils and cardiovascular reactivity. *Prostaglandins, Leukotrienes Essent Fatty Acids* 1994;50:211–22.
- [4] Schurgers LJ, Vermeer C. Corn oil induced decrease in arterial thrombosis tendency may be related to altered plasma vitamin K transport. *J Lipid Res* 2001;42:1120–4.
- [5] Khor HT, Raajeswari R, Muralidharan G. The role of unsaponifiable components in lipidimic property of olive oil. *Malays J Nutr* 1998;4:73–80.
- [6] Jun-Jun W, Xiao-Zhuan L, Yi-Yi Z, Lu-Yan L. Correlation between susceptibility of LDL subfractions to in vitro oxidation and in vivo oxidized LDL. *Clin Biochem* 2000;33:71–3.
- [7] Orth-Gomer K, Mittleman MA, Schenck-Gustafson K. Lipoprotein (a) as a determinant of coronary heart disease in young women. *Circulation* 1997;95:329–34.
- [8] Goldbourt U, Yaari S, Medalie JH. Isolated HDL cholesterol as a risk factor for coronary heart disease mortality; a 21-year follow-up of 8000 men. *Arterioscler, Thromb, Vasc Biol* 1997;17:107–13.
- [9] Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease; the Framingham study. *Am J Med* 1977;62:707–14.
- [10] Wyatt CJ, Carballido SP, Mendez RO.  $\alpha$ -Tocopherol content of selected foods in Mexican diet. *J Agric Food Chem* 1998;46:4657–61.
- [11] Folch J, Lees M, Saloane GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497–509.
- [12] Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J Biol Chem* 1952;195:357–66.
- [13] Menon PVG, Kurup PA. Dietary cholesterol metabolism—Effect of fiber rich polysaccharide from black gram on cholesterol metabolism in rats fed normal and atherogenic diet. *Biomedicine* 1976;2:248–51.
- [14] Warnick RC, Albers JT. A comprehensive evaluation of heparin manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 1978;19:65–76.
- [15] Friedwald WT, Levy RI, Fredrickson DS. Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [16] Vazquez Roncero A, Janer del valle L, Janer del valle C. Determination of olive oil total polyphenols. *Grassa Aceites* 1973;24:350–5.
- [17] Gutfinger T. Polyphenols in olive oil. *J Am Oil Chem Soc* 1981; 1:966–8.
- [18] Bairaktari E, Elisaf M, Tzallas C, et al. Evaluation of five methods for determining low density lipoprotein cholesterol (LDL-C) in hemodialysis patients. *Clin Biochem* 2002;34:593–602.
- [19] Alder L, Hill JS, Frohlich J. Precipitation of apo B containing lipoproteins. *Clin Biochem* 2000;33:187–90.
- [20] Puhl H, Waeg G, Esterbauer H. Methods to determine oxidation of low density lipoprotein. *Methods Enzymol* 1994;233:425–44.
- [21] Wills ED. Lipid peroxide formation in microsomes. *Biochem J* 1969;113:315–24.
- [22] Levine RL, Garland D, Oliver CN, et al. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;186:464–80.
- [23] Pirisi FM, Angioni A, Bandino G, et al. Photolysis of  $\alpha$ -tocopherol in olive oils and model systems. *J Agric Food Chem* 1998;46: 4529–33.
- [24] Servili M, Baldioli M, Miniati E, Montedoro GF. Antioxidant activity of new phenolic compounds extracted from virgin olive oil stability measured by rancimat. *J Agric Food Chem* 1999;47:4150–5.
- [25] Galli C, Petroni A, Visioli F. Natural antioxidants, with special reference to those in olive oil and cell protection. *Eur J Pharm Sci* 1994;2:67–8.
- [26] Pehwich DJ, Gomes AV, Barnes JA. Fatty acid composition and possible health effects of coconut constituents. *West Indian J Med* 2000;49:128–9.
- [27] Augusti KT, Aswathy N, Lekha SP, et al. Beneficial effects of garlic (*Allium sativum* Linn) on rats fed with diets containing cholesterol and either of the oil seeds, coconuts or ground nuts. *Indian J Exp Biol* 2001;39:661–7.
- [28] Elaine B, Feldman MD. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *J Nutr* 2002;132:1062S–101S.
- [29] Eckarstein V, Nofer JR, Assmann G. High density lipoproteins and atherosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler, Thromb, Vasc Biol* 2002;21:13–27.
- [30] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A metaanalysis of 27 trials. *Arterioscler Thromb* 1992;12:911–9.
- [31] Nofer J, Kehrel B, Foker M, et al. HDL and atherosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 2002;161:1–16.
- [32] Diaz MN, Frei B, Vita JA, Keane JF. Antioxidants and atherosclerotic heart disease. *N Engl J Med* 1997;337:408–16.
- [33] Dillon SA, Burmi RS, Lowe GM, Billington D, Rahmani K. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoproteins. *Life Sci* 2003;21:1583–94.
- [34] Beaudoux JL, Peynet J, Guillausseau A, Legrand A, Delattre J, Ropussel F. Susceptibility to copper induced oxidation of low density lipoproteins from healthy subjects and diabetic patients is not related to their vitamin E content. *Ann Biol Clin* 1993;51:494–503.
- [35] Cominacini L, Garbin U, Cenci B, et al. Predisposition to LDL oxidation during copper catalyzed oxidative modification and its relation to  $\alpha$ -tocopherol content in humans. *Clin Chim Acta* 1991; 204:57–68.
- [36] Frankel EN, Kamer J, German JB, Kinsella JE. Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *Lancet* 1993;341(1):454–7.
- [37] Covas MI, Fito M, Lamuela-Raventos RM, Sebastia N, De la Torre-Boronat C, Marrugat RM. Virgin olive oil phenolic compounds: binding to human low density lipoproteins(LDL) and effect on LDL oxidation. *Int J Clin Pharmacol Res* 2000;341:49–54.
- [38] Andrikopoulos NK, Kaliora AC, Assimopoulou AN, Papageorgiou VP.

- Inhibitory activity of minor polyphenolic and non-polyphenolic constituents of olive oil against in vitro low density lipoprotein oxidation. *J Med Food* 2002;5:1–7.
- [39] Tebib K, Bitri L, Beasancon P, Rouanet JM. Polymeric grape seed tannins prevent plasma cholesterol changes in high cholesterol fed rats. *Food Chem* 1994;49:403–6.
- [40] Tebib K, Beasancon P, Rouanet JM. Dietary grape seed tannins effect lipoproteins lipoprotein lipase and tissue lipids in rats fed hypercholesterolemic diets. *J Nutr* 1994;124:2451–7.
- [41] Teresa CPD, Conceicao LS, Leonor MA. The apo protein is the preferential target for peroxynitrite-induced LDL damage protection by dietary phenolic acids. *Free Radical Res* 2002;36:531–43.