Effect of Coconut Water Extract on the Growth and Serum Biochemical Values of Rats Fed Fish Oil Diet

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ABSTRACT

To justify the safety of ethanolic extract of coconut water (CWE), a study with rats was conducted. Wistar rats fed fish oil diet and rats fed the standard diet of AIN-93G groups were orally administrated 0.8 ml CWE/rat twice per day for 14 days. During experimental period, the behavior and growth of rats were monitored. At the end of experiment, the rats were anaesthetized and the internal organs were taken and measured for each weight. Blood was taken from the hearth and then analyzed for serum biochemical values and TBARS. The results, no adverse effects of CWE administration were observed as evaluated by growth pattern, food intake and feces appearance. Serum biochemical values and weight of internal organs of CWE receiving rats were comparable to those of untreated control. Serum TBARS value of rats fed fish oil was significantly higher than that of those fed standard diet, however, oral administration of CWE did not significantly decrease serum TBARS.

INTRODUCTION

Coconut (*Cocos nucifera* Linn.) is one of the main crops in Indonesia. Coconut plays an important role in the economy and social life of this country, where its status ranks second after rice (Persley, 1992). Although the main utilization of coconut is related to coconut milk and cooking oil, thus, only the matured coconut meat is fully used for the purpose at present, many other uses of coconut for human food are common.

The young coconut water is well consumed by the people in Indonesia and in other coconut producing countries as a traditional refreshing drink. The tender nut is regarded as a delicious and nutritious natural drink (Fernandez, 1988, Puet et al., 1992). It is reported that the water from unripe coconut is sterile, pyrogenic free, and there was no evidence of an *in vitro* or *in vivo* hemolysis of canine and human blood (Eiseman, 1954). In man, intravenous injection caused no undesirable effect (Brito and Dreis, 1944[a,b]), and green coconut water has been tried for therapeutic uses with gratifying results (Mojumdar, 1951; Carpenter *et al.*, 1964). However, on the nutritional point of view the young coconut used as a human food has not yet received broad and intense study (Jayalekshmy *et al.*, 1986). Therefore, research leading to diversification of the product derived from coconut especially for food use, and to explore its functional properties, is required to realize the potential of the crop.

Previous paper reported that ethanolic extract of the coconut water (CWE) had antioxidant activity in the assay using emulsified aqueous system of linoleic acid and β-carotene, and oral administration of CWE gave antioxidative effect as indicated by suppressing the increase of TBARS value in liver of rats fed fish oil diet (Santoso, 1996). The objective of the present study was to evaluate the toxicological effect of oral administration of the coconut water extract on the growth and on the serum biochemical values of rats. The antioxidative effect of the extract on lowering serum TBARS of rats fed fish oil diet was also evaluated.

MATERIAL AND METHODS

Preparation of the coconut water extract

Preparation of the coconut water extract (CWE) was performed as described by (Santoso, 1996). Principally, a 1000 ml of the coconut water from freshly split fruits

(the fruits were purchased at a fruit shop in Tokyo, Japan) was freeze-dried. The freeze-dried material was then extracted with approximately 500 ml of 99.5% ethanol (Wako Pure Chem. Indust., Ltd.) by stirring for 15 minutes at room temperature. After filtration (Whatman No. 2), the filtrate was evaporated on a rotary evaporator at 60°C until all ethanol evaporated, and the residue (34 g) was then filled up to 100 ml with water.

Animals

Male Wistar rats five weeks old were purchased from Tokyo Animal Experimental Co., Ltd. (Tokyo). After one week of adaptation, the rats were divided into four groups and used for this experiment, the schematic of experimental design is shown in Table 1.

Table 1. **Design of animal experiment**

Group (No. of rat*)	Treatment
FOW (8)	Fish oil diet + water
FOC (8)	Fish oil diet + CWE**
AINW (5)	AIN-93G diet + water
AINC (5)	AIN-93G diet + CWE

* Male Wistar rats, six weeks old, were maintained under experimental condition for 14 days

** CWE, coconut water extract (340 mg/ml). Water or CWE was orally administrated 0.8 ml/rat twice per day started at 10.00 a.m. and 5.00 p.m.

Diet and treatment

Groups FOW and FOC (eight rats/group) were fed fish oil diet that the composition basically followed that of the standard diet of AIN-93G (Reeves et al., 1993) by replacing soybean oil with fish oil and by omitting TBHQ, groups AINW and AINC were fed AIN-93G standard diet. The composition of the diets is shown in Table 2. Fatty acid composition of fish oil (San Omega EPA-18, Nihon Yushi Co. Ltd.) used for this experiment was confirmed to contain a high PUFAs. The rats were maintained individually in cages in a room with temperature of 22 ± 1°C and a humidity of 55 ± 5% with a 12 diurnal system. Feed and drinking water were provided *ad libitium*.

The rats of groups FOC and AINC were orally administrated by 0.8 ml of CWE/rat twice per day, those of group FOW and AINW were by water in the same amount as a control treatment. Oral administration was done using a syringe started at 10.00 a.m. and 5.00 p.m. daily. The rats were sacrificed after 14 days of experimental period.

Table 2. **Diet composition**

Ingredient	AIN-93G#	Fish Oil Diet
	g/kg diet	*g/kg diet*
Cornstarch	397.486	397.500
Casein (≥ 85% protein)	200.000	200.000
Dextrinized cornstarch	132.000	132.000
Sucrose	100.000	100.000
Soybean oil*	70.000	–
Fish oil**	–	70.000
Fiber	50.000	50.000
Mineral mix (AIN-95G-MX)	35.000	35.000
Vitamin mix (AIN-93G-VX)	10.000	10.000
L-Cystine	3.000	3.000
Choline bitartrate	2.500	2.500
Tert-butylhydroquinone	0.014	–

\# Reeves et al. (1993)

* Wako Pure Chem. Indust., Ltd.

** San Omega EPA-18, Nihon Yushi Co. Ltd.

At the end of experiment the rats were anaesthetized under Nembutal (70 µl/100g body weight of rat). After opening of the abdominal cavity, each internal organ of rats was taken and measured for the weight. Blood was taken from the heart for serum biochemical analysis and TBARS value. The preparation of serum was conducted by centrifugation at 4000 rpm for 15 minutes at 0 ~ 4°C (Alexander et al, 1985).

Performance of rats

The performance of rats during experimental period was examined by the pattern of daily body weight, food intake, and appearance of the feces. The weight of each internal organ and serum biochemical composition were examined for abnormality.

Serum biochemical values

Biochemical analysis of serum was performed for total protein, albumin/globulin (A/G), blood urea nitrogen (BUN), creatinine, total cholesterol, HDL-cholesterol,triglyceride (TG), total lipid, non-esterified fatty

acids (NEFA), glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), and γ-glutamyl transpeptidase (γ-GTP). This was conducted at Center for Biochemical Research of Houken Kagaka Kenkyujō, Tokyo, Japan.

TBARS determination

Thiobarbituric reactive substances (TBARS) in serum was determined according to Yagi method (Yagi, 1984). The standard procedure of Yagi is a as follows, Using a pipette for determination of blood cells, 0.05 mL of blood is taken. The blood is put into 1.0 mL of physiological saline in a centrifuge tube, and shaken gently. After centrifugation at 3000 rpm for 10 minutes, 0.5 mL of the supernatant is transferred to another tube. In the case of serum, 20 μL of the specimen is taken. To this solution, 4.0 mL of N/12 H_2SO_4 is added and the mixture is shaken gently. Then, 0.5 mL of 10% phosphotungstic acid is added and mixed. After standing at room temperature for 5 min., the mixture is centrifuged at 3000 rpm for 10 min. The supernatant is discarded, and the sediment is mixed with 2.0 mL of N/12 H_2SO_4 and 0.3 mL of 10% phosphotungstic acid. The mixture was centrifuged at 3000 rpm for 10 min. The sediment is suspended in 4.0 mL of distiled water, and 1.0 mL of TBA reagent (a mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid) is added. The reaction mixture is heated for 60 min at 95°C in an oil bath. After cooling with tap water, 5.0 mL of n-butanol is added and the mixture is shaken vigorously. After centrifugation at 3000 rpm for 15 min, the n-butanol layer is taken for fluorometric measurement at 553 nm with 515 nm excitation. Taking the fluorescence intensity of the standard solution, which is obtained by reacting 0.5 nmol of tetramethoxypropane with TBA by step 7 – 9, as *F* and that of the sample as *f*, the lipid peroxide level (*Lp*, that is equal TBARS value) can be expressed in terms of malonaldehyde:

$$\text{Serum Lp} = 0.5 \times \frac{f}{F} \times \frac{1.0}{0.02} = \frac{f}{F} \times 25 \text{ (nmol/mL of serum)}$$

Statistical analysis

Data are presented as mean ± SD. Data were analyzed by one way analysis of variance (ANOVA), and *Student's* t-test was used to estimate the significant differences between groups. Differences with $P < 0.05$ were considered statistically different.

RESULTS AND DISCUSSION

Behavior and growth of rats

The pattern of daily intake of rats is shown in Figure 1. Figure 1-A shows daily food intake of rats fed fish oil diet and treated with coconut water extract, and Figure 1-B shows that of rats fed standard diet of AIN 93G. No difference was observed of the pattern of daily food intake of rats orally administrated by 0.8 CWE (340 mg/ml)/rat twice per day for 14 days on both of rat groups, although the average of daily food intake of rats fed fish oil was significantly lower that of those fed standard diet (Santoso, 1996). The phenomenon of the effect of fish oil diet in lowering average daily food intake is in agreement with the investigation of Saito & Nakatsugawa (1994).

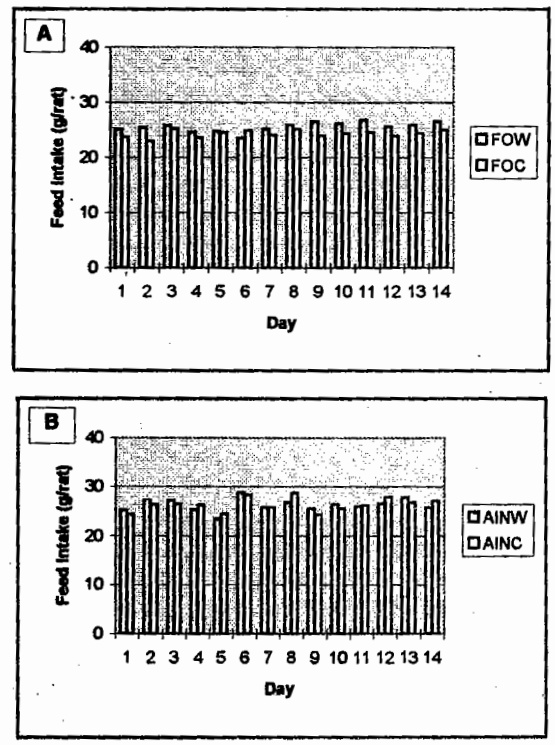

Figure 1. **Daily feed intake of rat. (A) Group FOC and FOW were fed fish oil diet, (B) Group AINC and AINW were fed AIN-93G standard diet for 14 days.**
Group FOC and AINC were orally admnistrated by 0.8 ml CWE/rat twice per day, FOW and AINW were by water at the same amount as a control treatment.

Table 3. **Weight of internal organs of rats**

Group	Liver		Kidney		Adrenal		Spleen		Pancreas		Lung		Stomach		S. Intestine		Colon		Caecum		Testis	
	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW	g	g/100gBW
FOW	17.97± 0.85	5.508± 0.86a	2.541± 0.29	0.731± 0.04^{ab}	0.057± 0.02	0.018± 0.004^{a}	1.016± 0.158	0.296± 0.05^{a}	0.718± 0.219	0.223± 0.004^{a}	1.389± 0.118	0.409± 0.08^{ab}	1.509± 0.085	0.44± 0.06^{ab}	5.047± 0.502	1.471± 0.27^{a}	1.133± 0.135	0.327± 0.05^{a}	0.925± 0.232	0.268± 0.07^{a}	3.017± 0.279	0.874± 0.11^{a}
FOC	17.02± 1.05	4.886± 0.4^{b}	2.336± 0.225	0.681± 0.04^{a}	0.054± 0.005	0.019± 0.01^{a}	0.963± 0.141	0.281± 0.04^{a}	0.758± 0.151	0.206± 0.07^{a}	1.382± 0.165	0.403± 0.04^{ab}	1.369± 0.105	0.399± 0.02^{a}	4.603± 1.485	1.348± 0.22^{a}	1.025± 0.086	0.298± 0.02^{a}	0.831± 0.137	0.241± 0.47^{a}	2.996± 0.401	0.90± 0.04^{a}
AINW	18.31± 2.51	5.152± 0.46^{a}	2.558± 0.258	0.72± 0.05^{ab}	0.066± 0.011	0.018± 0.004^{a}	1.019± 0.169	0.278± 0.03^{a}	0.958± 0.09	0.272± 0.04^{b}	1.569± 0.137	0.414± 0.04^{b}	1.672± 0.1	0.472± 0.05^{b}	5.182± 0.921	1.46± 0.25^{a}	0.998± 0.268	0.28± 0.06^{a}	0.832± 0.143	0.218± 0.02^{a}	3.256± 0.282	0.914± 0.02^{a}
AINC	18.66± 3.92	5.204± 0.68^{a}	2.832± 0.341	0.794± 0.09^{b}	0.062± 0.015	0.018± 0.004^{a}	1.00± 0.1	0.282± 0.01^{a}	0.94± 0.12	0.264± 0.03^{b}	1.322± 0.093	0.372± 0.03^{a}	1.552± 0.183	0.436± 0.02^{b}	4.948± 0.772	1.382± 0.12^{a}	1.068± 0.165	0.30± 0.03^{a}	0.862± 0.211	0.242± 0.04^{a}	3.23± 0.182	0.906± 0.04^{a}

* Rats of group FOW and FOC were fed fish oil diet, those of group AINW and AINC were AIN-93G standard diet for 14 days. Group FOC and AINC were orally administrated by 0.8 ml CWE/rat twice per day, group FOW and AINW were by water at the same amount.

\# g/100 body weight of rat.

Each value represents mean ± SD, means within the same column without a common superscript letter are significantly different at $P < 0.05$

The pattern of the increase of body weight of rats during experimental period is shown in Figure 2. Oral administration of 0.8 CWE (340 mg/ml) rat twice per day for 14 days resulted in no significant differences on the growth pattern of rat groups. Oral administration CWE did not change the pattern of daily food intake.

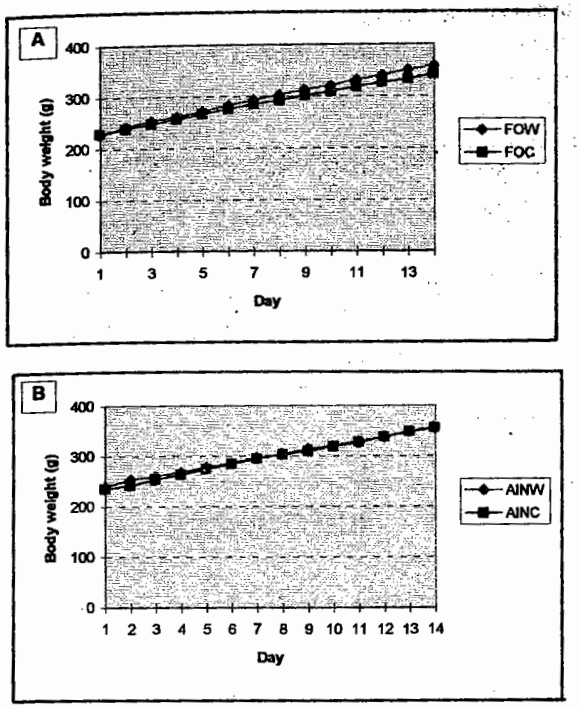

Figure 2. **Growth curve of rats. (A) Group FOC dan FOW were fed fish oil diet. (B) group AINC and AINW were fed AIN-93G standard diet for 14 days.**
Group FOC and AINC were orally administrated by 0.8 ml CWE/rat twice per day, FOW and AINW were by water at the same amount as a control treatment.

During experimental period, the behavior of rats was visually monitored. No abnormality was observed on the behavior of rats in all groups. The feces was in a normal appearance, and there was no diarrhea phenomenon in all experimental rats.

Weight of internal organs

Table 3 shows the weight of internal organs of experimental rats. The organs measured were liver, kidney, adrenal, spleen, pancreas, lung, stomach, small intestine, colon, caecum, and testis. Expressed on gram organ per 100 gram body weight, there was no significant differences between weight of each organ of rats fed fish oil diet and the corresponding organ of rats fed AIN-93G standard diet. Oral administration of 0.8 ml CWE/rat twice perday for 14 days did not influence the weight of internal organs, including liver, of rats fed fish oil died and those fed the standard diet of AIN-93G.

Biochemical values of serum

Biochemical values of rat serum was shown in Table 4. Among the values, the concentrations of serum glutamate -oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) are important values to detect the severity of liver damage and the toxic effect of a substance (Lehninger, 1985). There are no significant differences in concentration of GOT nor GPT in serum of rats orally administration by 0.8 ml CWE (340 mg/ml)/rat twice perday for 14 days compared to the untreatead groups (between FOW and FOC groups, and between AINW and AINC groups). Therefore, CWE can be considered to have no toxic effect.

Table 4. **Biochemical and TBARS values of rat serum**

Constituent		RAT Group			
		FOW	FOC	AINW	AINC
Total Protein	g/dl	5.62±0.1^{ab}	5.54±0.16^{ab}	5.84±0.18^{c}	5.66±0.20^{bc}
A/G		1.76±0.15	1.59±0.10	1.76±0.13	1.76±0.23
B.U.N	mg/dl	17.3±1.83	14.90±1.60	19.40±1.14	17.2±1.92
Creatinine	mg/dl	0.31±0.03	0.34±0.05	0.34±0.06	0.32±0.05
Uric acid	mg/dl	0.51±0.23	0.53±0.13	0.60±0.16	0.56±0.09
T-Cho	mg/dl	70.78±10.65^{ab}	67.9±8.81^{a}	97.2±17.43^{c}	84.8±8.26^{bc}
HDL-Cho	mg/dl	52±10.88^{a}	50.40±5.66^{a}	66±15^{b}	60.2±7.8^{ab}
T.G	mg/dl	59.3±15.08^{a}	68.9±21.08^{a}	128.8±33.3^{b}	117±33.74^{b}
T.L	mg/dl	305.2±32.67^{a}	296.40±46.82^{a}	474.8±58.41^{b}	421.6±42.11^{b}
NEFA	mEg/l	0.31±0.14^{a}	0.36±0.17^{a}	0.56±0.47^{a}	0.54±0.63^{a}
GOT	IU/l	78.5±6.02^{a}	79.89±8.60^{ab}	73.2±3.11^{a}	78.40±7.02^{a}
GPT	IU/l	18.25±1.73^{ab}	20.70±4.83^{abc}	16.80±1.92^{a}	17.20±2.59^{ab}
γ-G.T.P	IU/l	1.3±0.46^{a}	1.10±0.32^{a}	1.60±0.89^{a}	1.20±0.45^{a}
TBARS	**nmolMDA/ml**	**58.14+8.00^{a}**	**47.36+23.22^{a}**	**11.44+1.83^{b}**	**5.48+0.55^{b}**
Vit. E	mg/dl	1.058±0.14^{a}	0.944±0.148^{a}	1.686±0.228^{b}	1.478±0.103^{c}

* Rats of group FOW and FOC were fed fish oil diet, those of group AINW and AINC were AIN-93G standard diet for 14 days. Group FOC and AINC were orally administrated by 0.8 ml CWE/rat twice per day, group FOW and AINW were by water at the same amount.

** Each value represents mean ± SD, means within the same column without a common superscript letter are significantly different at $P < 0.05$.

The other main constituents of serum are total cholesterol, HDL-cholesterol, triglycerides and total lipid. The concentration of these constituents is significantly lower in rats fed fish oil diet than that in rats fed standard diet fed IAN-93G standard diet. The hypolipidemic and hypocholesterolemic effects of fish oil has been associated

to its considerably high content of n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (20:6 n-3, DHA) (Nardini *et al.*, 1995; Nalbone *et al.*, 1989; Kobatake *et al.*, 1983; Meydani *et al.*, 1990; Saito & Nakatsugawa, 1993). Administration of 0.8 ml CWE (340 mg/ml)/rat twice perday for 14 days did not influence the concentrations of total cholesterol, HDL-cholesterol, triglyceride and total lipid (Table 4).

TBARS value in rat serum

Table 4 also shows the effect of oral administration of CWE on the degree of lipid peroxidation in rat serum that indicated by thiobarbituric acid reactive substances (TBARS) value. Fish oil diet significantly increased serum TBARS during 14 days of experimental period. This result was consistent with some investigations (Meydani *et al.*, 1990; Nardini *et al.*, 1993; Saito & Nakatsugawa, 1993) that fish oil diet enchanced lipid peroxidation in serum of rats. Oral administration 0.8 mL CWE/rat twice per day for 14 days tended to suppress the increase of TBARS in serum of rats fed fish oil diet, although no significant difference was observed between serum TBARS value of FOW and FOC groups. On the serum α-tocopherol, no significant difference was observed on the TBARS value in serum of rats receiving administration of CWE and those untreated control. Oral administration of 0.8 mL CWE/rat twice per day for 14 days did not influence serum α-tocopherol.

The young coconut is commonly consumed as a natural refreshing drink in the growing areas. In the natural state coconut water or meat is sterile (Fernandez, 1988) and the use of coconut as food is notable for its reported lack of antinutritional factors (Padua-Resurreccion & Banzon, 1979). The coconut water ethanolic extract was found to have antioxidant activity in the bioassay using rats fed fish oil diet as indicated by suppressing the increase of liver TBARS (Santoso, 1996). It has been confirmed in the present study that no toxicological effect has been observed on rats orally administrated by coconut water extract. No adverse effects of CWE administration were observed as evaluated by growth pattern, food intake and feces appearance. Serum biochemical values and weight of internal organs of CWE receiving rats were comparable to those of untreated control. Natural products that possess antioxidant activity have been consumed for centuries and assumed to be safe for human use (Wanasundara & Shahidi, 1994).

The results of this study can also provide us with some basic understanding of the functional properties of a tropical fruit. At present, coconuts may be not considered particularly nutritious by some, since they do not contain large amounts for specific vitamins. However, their content of natural antioxidants (Santoso, 1996) represents a major beneficial property which may improve their market value in the future. Coconut is an exotic fruit whose use was once restricted to people living in limited areas, however, recently exotic fruits have become quite a common food in non-exotic country.

ACKNOWLEDGEMENTS

The study was financially supported by the Hitachi Scholarship Foundation, Tokyo, Japan. The technical assistance of Ms. M. Kyan and Mr. T. Aragaki was highly appreciated.

REFERENCES

Alexander, R.R., Griffith, J.M. and Wilkinson, M.L., 1985. *Basic Biochemical Methods*. John Wiley & Sons, Inc., USA.

Brito, J.C and Dreiss, G. 1944a. Trials with intravenous coconut water in therapeutic. Biol Abst. *18*, 19371.

Brito, J.C. and Dreiss, G. 1944b. The use of intravenous coconut water in therapeutic. Biol. Abst. *18*, 6423.

Carpenter, C.C.J., Mondal, A., Mitra, P.P. and Mondal, H. 1964. Green coconut water: A readily available source of potassium for the cholera patient. Bull. Cal. School Trop. Med. *12*, 20–21.

Eiseman, B. 1954. Intravenous infusion of coconut water. Chem. Abst. *48*: 5441.

Fernandez, W.L. 1988. Microbial examination of mature coconut fruit. Phil. Agric. *71* (1), 13 – 20.

Jayalekshmy, A., Arumughan, C., Narayanan, C.S. and Mathew, A.G., 1986. Changes in the chemical composition of coconut water during maturation. J. Food Sci. Tech. Vol. *23*: 203 – 207.

Kobatake, Y., Hirahara, F., Innami, S. and Nishide, E. 1983. Dietary effect of ω-3 type polyunsaturated fatty acids on serum and liver lipid levels in rats. J. Nutr. Sci. Vitaminol. *29*, 11 – 21.

Lehninger, A.L. 1982. *Principles of Biochemistry*. Worth Publ. Inc., New York.

Meydani, M., Natiello, F., Goldin, B., Free, N., Woods, M., Schaefer, E., Blumberg, J.B. and Gorbach, S.L. 1991. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. J. Nutr. *121*, 484 – 491.

Mojumdar, N.G. 1951. Intravenous use of green coconut water in pediatric practice. J. Indian Med. Assoc. *20*, 211 – 212.

Nalbone, G., Leonardi, J., Termine, E., Portugal, H., Lechene, P., Pauli, A-M. and Lafont, H. 1989. Effect of fish oil, corn oil and

lard diets on lipid peroxidation status and glutathione peroxidase activities in rat heart. Lipids 24, 179 – 186.

Nardini, M., D'Aquino, M., Tomassi, G., Gentili, V., Di Felice, M. and Scaccini, C. 1995. Dietary fish oil enhances plasma and LDL oxidative modification in rats. J. Nutr. Biochem. 6, 474 – 480.

Padua-Resurreccion, A.B. and Banzon, J.A. 1979. Fatty acid composition of the oil from progressively maturing bunches of coconuts. Phil. J. Coco. Stud. *IV* (3): 1 – 15.

Persley, G.J., 1992. *Replanting the Tree of Life, Towards an International Agenda for Coconut Palm Research.* CAB International. Wallingford, UK.

Pue, A.G., Rivu, W., Sundarrao, K., Kaluwin, K., and Singh, K., 1992. Preliminary studies on changes in coconut water during maturation of the fruit. Science in New Guinea. *18* (2): 81 – 84.

Reeves, P.G. Nielsen, F.H. and Fahey Jr., G.C. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J. Nutr. *123*, 1939 – 1951.

Saito, M. and Nakatsugawa, K. 1994. Increased susceptibility of liver to lipid peroxidation after ingestion of a high fish oil diet. Intern. J. Vit. Nutr. Res. 64, 144 – 151.

Santoso, U. 1996. Antioxidative effect of coconut (Cocos nucifera L.) water extract on TBARS value in liver of rats fed fish oil diet. Indonesian Food and Nutrition Progress, 3 (2): 42 – 50.

Wanasundara, U.N. and Shahidi, F., 1994. Stabilization of canola oil with flavonoids. Food Chem. 50: 393 – 396.