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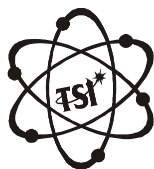

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Dietary effect of mustard (*Brassica nigra* L) protein hydrolysate on blood and tissue lipids and lipid peroxidation in hypercholesterolemic rats

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ABSTRACT

Dietary protein is one of the most primary energy containing micronutrient consumed on a routine basis in humans. The influence of dietary mustard protein hydrolysate (MPH, protein content 88.5%), produced from dehulled, defatted mustard meal on blood and tissue lipid profile and lipid peroxidation has been assessed in hypercholesterolemic rats. To evaluate their hypocholesterolemic and antioxidative activity *in vivo,* we fed 18% MPH with 2% cholesterol in comparison with casein to rats for 28 days. There was no significant difference in growth rate and food efficiency ratio between the two groups during the four weeks experimental period. The total cholesterol, TAG and LDL cholesterol level were decreased at 21%, 38.4% and 31.6% level respectively in the experimental group than the control casein group. There was a significant lowering of LDL and EM lipid peroxidation in the experimental group (MPH containing cholesterol) than the corresponding control casein group. Liver lipid profile of the experimental group was also improved than the corresponding control group. Therefore, our results indicate that MPH can be used in various food formulations as hypocholesteromic and antioxidative component.

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KEYWORDS

Lipid peroxidation;
Protein hydrolysate;
Mustard (*Brassica nigra* L).

INTRODUCTION

Mustard is grown extensively in tropical and subtropical areas as an important oilseed crop and ranking fifth with respect to oil production after soybean, cotton seed, peanut and sunflower. It is one of the important oil seed crops in India. It is widely grown in northern part of the country and has shown promise both under normal as well as late sown conditions. Mustard seed meal is rich source of edible protein (about 45-55%) with balanced amino acid composition. The presence of sulphur containing amino acids is an added advantage for mustard meals. The undesired components of seed proteins can be eliminated to a large extent by producing protein isolate and the protein utilization can be further improved by producing protein hydrolysate[1-3].

Diet content of proteins[4] is important for atherosclerosis development and diet manipulations can retard or accelerate the progression of this pathology. Dietary protein has positive link with the plasma cho-

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lesterol concentration and metabolism of polyunsaturated fatty acids in the liver. It also affects the lipid peroxidation and distribution of fatty acids among liver and different lipoprotein classes. Soy protein has been extensively studied and has been found to have an effective weight reducer[5] and also have hypocholester olemic effect compared with animal proteins such as casein[6].

The presence of anti nutritional factors such as glucosinolates, phenols was a drawback for the utilization of mustard meal for human consumption. The fibre content in the defatted meal should be eliminated to enrich the final product in protein. Defatted rapeseeds are used to produce protein isolates of good quality by various workers[2-3].

It has been demonstrated that the nutritional value of mustard meal was similar to canola meal if the mustard meal was first treated with ammonia to reduce the glucosionale content[7-8]. The nutritional value of low glucosinolate cultivars of mustard (*Brassica juneca*) meal was examined on broiler chicken[9]. It was concluded that the nutritional value of low glucosinolate mustard meal was equal or superior to that of canola meal samples. In an animal experiment Sen and Bhattacharyya[10] observed that mustard meal (prepared by isopropanol extraction) was equally effective as casein with respect to serum cholesterol, triglyceride, and other lipid profiles.

In Indian context, mustard protein can play an important role due to availability and favorable nutrient composition. Functional properties of protein can be further improved by producing mustard protein hydrolysate(MPH). Due to presence of short peptides MPH is better absorbed in the digestive tract at higher rate. The presence of sulphur containing amino acids is an added advantage for mustard protein. The mustard protein hydrolysate(MPH) of improved functional properties can be utilized in various food formulations. There is not much report published on the dietary effects of MPH. To obtain a better understanding of the effects of dietary MPH on lipid metabolism in rats we compared the effects of diets based on MPH and casein supplemented with 2% cholesterol on growth rate, food efficiency ratio, plasma and erythrocyte membrane(EM) lipid profile, plasma, LDL, EM, and liver lipid profile and peroxidations in rats.

MATERIALS AND METHODS

Preparation of mustard protein hydrolysate (MPH)

For preparation of MPH, dehulled mustard meal was milled and passed through an 80-mesh screen(80% passed through) and mixed with water(1:10, w/v). The pH was adjusted to 11.0 with 1N NaOH solution, stirred for 1h at 50-55^{0}C, and then centrifuged at 4000×g for 10min(to remove the insoluble portion). The supernatant liquid was separated; the pH adjusted to 3.5 with 1N HCl (at isoelectric point) and was hydrolyzed with 5%(v/w) viscozyme.. The hydrolysis reaction was carried out at 37^{0}C for 30min with constant stirring. After hydrolysis the product was lyophilized (EYELA, Japan) and stored in a refrigerator at –20^{0}C.

Analysis of mustard protein hydrolysate (MPH)

Determination of protein content by Kjeldal method

A known quantity of the sample, containing (about 0.25-1.0g sample) was taken in a 800mL long necked flask to which 16.7gm K_2SO_4, 0.6gm TiO_2, 0.01g $CuSO_4$ and 20mL of conc. H_2SO_4 were added. The solution was carefully heated above 300^{0}C until it become clear green in color or almost colorless. It was cooled and 100mL of distilled water was carefully added, 50mL of NaOH solution having sp. gravity 1.84 was added through the side tube of the distillation apparatus filled with a stopcock. Then, the flask was immediately distilled, by passing steam through it. The distillate was collected in 25 mL of 0.5(N) H_2SO_4 taken in a receiving flask and the excess acid was titrated with 0.25 (N) NaOH using methyl red as indicator. The same process was repeated without addition of sample. The % nitrogen was calculated and the % protein of the sample was obtained by multiplying % N_2 by 6.25.

Amino acid analysis of MPH

To a dried sample of standard amino acid mixture (amino acids std. Sol. AA-S-18) or the protein hydrolysates (2-200μg) 1ml of 1M sodium borate buffer(pH-9.0) containing 0.02% of sodium azide and 0.8μL of diethyl ethoxy methylene malonate was added. The reaction was carried out at 50^{0}C for 50min with vigorous shaking. The resulting mixture was cooled to room temperature and 15μL was injected into Waters HPLC system. Separations were attained with a 300×3.9mm

i.d. reversed phase column(Novapack C18) using a binary gradient system. The solvents used were(A) water containing 25mM sodium acetate and 0.02% sodium azide(pH 6.0) and (B) acetonitrile. The solvent was delivered at a flow rate of 0.9mL/min as follows: time 0.0-3.3 min, linear gradient from A/B(91:9) to A/B(86:14); 3.0-13.0min, elution with A/B(86:14); 13.0-30.0 min, linear gradient from A/B(86:14) to A/B(69:31); 30.0-35.0min, elution with A/B(69:31). The column temperature was maintained at 18°C with a temp controller.

Feeding experiment

Male albino rats of Charles Foster strain(selected for the authenticity of the strain) were housed in individual cages. The work was done under the supervision of the Animal Ethical Committee of the Department of Chemical Technology, University of Calcutta. For 7d, the rats received a semi synthetic diet(18% casein, 20% soybean oil, 4% salt mixture, 3% cellulose and 55% cornstarch). After this adoption period, the animals were divided into four groups(average body weight 80-90g), each consisting of six animals and fed different diets for 28 d. The two groups of rats received a diet containing 18% MPH(group MPH-C) or casein(group CAS-C), 20% soybean oil, 4% salt mixture[11], 3% cellulose and 55% cornstarch(TABLE 1) supplemented with 2% cholesterol as hypercholesterolemic diet.

Rats were maintained on the above diets ad libitum for 4 weeks. The amount of daily diet consumed by each rat and weekly body weight gain were noted. Rats were fasted overnight for 12h and then sacrificed under anesthesia; blood was collected, and liver, heart, and brain were immediately excised, blotted, and stored at deep freeze temperature(-40^{0}C) for analysis.

Lipid analysis

According to the standard methods, the lipid components such as total cholesterol[12], and high-density lipoprotein (HDL)-cholesterol[13] and TAG[14] of plasma were analyzed using enzymatic kits supplied by Ranbaxy Diagnostics Ltd. (New Delhi, India).

Lipoprotein oxidation susceptibility (LOS) test

A 500µL plasma sample was treated with 50µL of a solution containing 0.2mM dextran sulphate(MW 50,000, Genzyme, Cambridge, MA) and 0.5M $MgCl_2$.

TABLE 1: Composition of experimental diets of two dietary groups

Ingredients (g/kg)	CAS-C	MPH-C
Casein*	180	-
Mustard protein hydrolysate (MPH)#	-	180
Soybean oil$	549	549
Starch	30	30
Cellulose	1.0	1.0
Vitamin mixture**	40	40
Mineral mixture¶	20	20
Cholesterol§	20	20

CAS-C, 18% casein + 2% cholesterol; MPH-C, 18% mustard protein hydrolysate + 2% cholesterol; *Casein 95.7% protein, #Mustard protein hydrolysate (protein content 88.5%), $Refined soybean oil provided the following fatty acids(% total fatty acids-16:0, 10.8;18:0,3.8;18:1,22.1;18:2,55.9;18:3,7.4), **Two multivitamin capsules of 500 mg (vitamin A I.P. 10,000 units, Thiamine mononitrate I.P. 5mg, Vit B I.P. 5mg, Calcium pantothenate USP 5mg, Niacinamide I.P. 50mg, Ascorbic acid I.P. 400 units, Cholecalciferol USP 15 units, Menadione I.P. 9.1mg, Folic acid I.P. 1mg, Vitamin E USP 0.1 mg) per kg of diet, ¶Composition of salt mixture No.12 (in g): NaCl, 292.5; KH_2PO_4, 816.6; MgSO4, 120.3; $CaCO_3$, 800.8; $FeSO_4.7H_2O$, 56.6; KCl, 1.66; $MnSO_4.2H_2O$, 9.35; $ZnCl_2$, 0.5452; CuSO4.5H2O, 0.9988; $CoCl_2.6H_2O$, 0.0476), $Cholesterol was purchased from E. Mark (India) Pvt. Ltd.

$6H_2O$ to precipitate the apo B-containing lipoproteins (LDL and VLDL) according to Bachorik and Albers[16]. After centrifugation at 3000×g at 20^{0}C for 10min, the supernatant was removed, and 1mL of 6% bovine serum albumin and another 50µL of the dextran sulfate magnesium solution were added. The solution was briefly vortexed and recentrifuged as above to wash away any HDL or residual serum proteins(except, of course, albumin). The supernatant was removed and washed precipitate(containing LDL and VLDL) was dissolved in 2.5µL of 4% NaCl. A volume of redissolved precipitate containing 100µg of non-HDL cholesterol was combined with sufficient 4% NaCl to give a total volume of 500µL(app. a 1:5 dilution). 50µL of a 0.5mM $CuCl_2$. $2H_2O$ solution was added(final copper concentration was 46µM), and then the samples were incubated at 37^{0}C in a shaking water bath for 3 h. Next TBARS was measured by adding 2mL of the TBARS reagent(26mM TBA in 0.25N HCl containing 15.03 g TCA in 100mL) to each tube. The mixture was heated at 100^{0}C in a water bath for 15min. After removing and cooling the tubes, 2.5mL n-butanol was added, the tubes were vortexed and then centrifuged for 15min at 3000 rpm at room temperature. The pink upper layer was removed and the optical density was determined in a spectrophotometer at 532 nm according to the method described by Phelps and Harris[17].

TABLE 2 : Chemical composition of casein and mustard protein hydrolysate

Amino acids	Casein	Mustard protein hydrolysate
Ash (%)	3.24± 0.25	2.11 ± 0.34
Fiber (%)	0.13± 0.14	3.23 ± 0.15
Protein (%)	95.7± 0.34	88.50 ± 0.53
Amino acids (g/100 g protein)		
Isoleucine	4.7	4.0
Leucine	9.5	7.5
Lysine	7.8	5.6
Phenylalanine + tyrosine	10.2	9.3
Cystine	0.8	3.2
Methionine	2.5	2.3
Threonine	4.4	5.0
Valine	6.4	4.6
Tryptophan	1.4	1.5
Histidine	2.5	3.2
Arginine	3.8	7.0
Aspartic acid	11.1	7.2
Glutamic acid +Glutamine	21.1	19.2
Serine	4.1	5.0
Proline	-	4.8
Glycine	4.5	5.6
Alanine	4.8	4.9

Preparation and oxidative sensitivity of EM ghost[18]

After plasma separation, the red blood cells(RBC) were washed three times by centrifugation at 3000×g for 10min with three volume of a cooled isotonic solution containing 0.15M NaCl and 10^{-5}M EDTA. RBC was hemolized using hypotonic solution and centrifuged at 20,000×g for 40min in a cold centrifuge at 4^0C. The supernatant was removed carefully with a Pasteur pipette. The process was repeated two more times. After the last wash step, the supernatant was removed as much as possible and the loosely packed milky-looking membrane pellet was re-suspended at the bottom of the tube using 0.89% NaCl solution. Concentrated membrane solution was taken in 2 mL screw cap vial and stored at –40^0C.

A modification of the 2-thiobarbituric acid test[19] was used to measure the lipid peroxides. A 0.5mL aliquot of the red blood corpuscle membrane suspension was mixed with 1.0mL of 10% trichloroacetic acid and 2.0mL of 0.67% of 2-thiobarbituric acids. The mixture was heated at 95^0C for 15min, cooled, and centrifuged. The absorbance of the supernatant was measured at 534 nm in a spectrophotometer(Shimadzu, Tokyo, Japan), and the amount of malonedialdehyde(MDA) formed was calculated by taking the extinction coefficient of MDA to be $1.56\times10^5 M^{-1}cm^{-1}$.

Liver tissue lipid extraction and lipid profile assay

Total lipids were extracted from an aliquot of tissue homogenate by the method of Bligh and Dyer[20]. Lipid profile was determined by using enzymatic kit as discussed earlier.

Assay of protein

EM protein was estimated by the method of Lowry et al[21].

Total phospholipid content in tissue lipid

Phospholipid content in tissue lipid was measured by the method of Chen et al.[22].

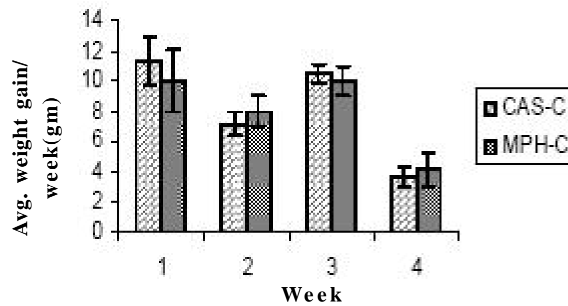
Statistical analysis

The data were expressed as mean ± standard error of mean(SEM). A one-way ANOVA was also used for statistical analysis between groups. F ratio of one-way ANOVA is significant when p value <0.05. Tukey's multiple range method[23] was used for comparison. The statistical program was MINITAB release 13.31 (MINITAB, State College, PA).

RESULTS AND DISCUSSION

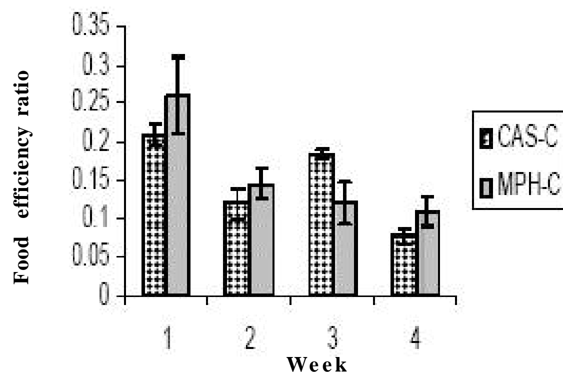
The chemical compositions along with amino acid profile of mustard protein hydrolysate (MPH) and casein were given in TABLE 2. The protein content in MPH and casein was 88.5% and 95.7% respectively. MPH content 3.23 % fibre where as casein content 0.13% fibre. Cystine and arginine content was higher and lysine content was lower in MPH than that of casein. Other amino acids in MPH were more or less similar in comparison with casein. Figure 1 and 2 represent growth patterns and food efficiency ratio (FER) of rats fed casein and MPH respectively. There was no significant difference in growth rate and FER between the two groups during the four weeks experimental period.

TABLE 3 lists the plasma total protein, total cholesterol, HDL-cholesterol, LDL cholesterol, and TAG level of rats raised on casein containing 2% cholesterol (CAS-C group), and MPH containing 2% cholesterol (MPH-C group). The total cholesterol, TAG level and LDL-cholesterol level were significantly reduced in the MPH-C group than the corresponding control CAS-C group. The total cholesterol level was decreased about



CAS-C, 18% casein+2% cholesterol; MPH-C, 18% mustard protein hydrolysate +2% cholesterol; Values are mean ± SEM, n=6

Figure 1 : Growth response of rats fed casein and mustard protein hydrolysate (MPH) of two groups at different time period.



CAS-C, 18% casein+2% cholesterol; MPH-C, 18% mustard protein hydrolysate +2% cholesterol; values are mean ±SEM, n=6

Figure 2 : Food efficiency ratio of rats fed casein and mustard protein hydrolysate(MPH) of two groups at different time period

21% than the corresponding control casein group. Where as, TAG and LDL cholesterol level was decreased at 38.4 and 31.6% level respectively. The plasma protein content and HDL-cholesterol level was more or less similar in control and experimental groups.

TABLE 4 : Plasma lipoprotein and erythrocyte membrane lipid peroxidation of two dietary groups

Groups	EM lipid peroxidation n mole of MDA/mg of protein	LDL-peroxidation (n mole of MDA/mg of non-HDL cholesterol)
CAS-C	6.3± 0.71	10.6 ± 0.61
MPH-C	3.56 ± 0.48 [a]	5.93 ± 0.74 [a]

Values are mean ± SEM, n=6; CAS-C, 18% casein +2% cholesterol; MPH-C, 18% mustard protein isolate +2% cholesterol, [a]MPH-C vs CAS-C($p<0.001$)

The hypocholesterolemic effect of vegetable protein is well documented. We have no direct explanation for the mechanism of cholesterol lowering effect of MPH. It was the amino acids or the non-protein components e.g. isoflavons that was responsible for the hypocholesterlemic effect as observed by others[24-25]. The dietary proteins with low ratios of methionine/glycine and lysine/arginine have hypocholesterolemic effect[26-27]. In MPH lysine to arginine ratio was 0.8 and methionine to glysine ratio was 0.4 and corresponding values in casein was 2.0 and 0.6 respectively. During the production of protein isolates and respective hydrolysates the isoflavones were more or less eliminated. Probably, the hypocholesterolemic effect was mainly due to the amino acid composition as well as the small size of the peptide molecules in hydrolysates.

TABLE 4 showed the plasma LDL peroxidation and extent of lipid peroxidation in EM ghost of rats. There was significant lowering(44%) of LOS in the experimental group (MPH-C) than the corresponding control(CAS-C) group. EM lipid peroxidation in the MPH-C group significantly decreased(43.5%) than the control CAS-C group.

Lipid peroxides have attracted much attention due to their deleterious effects related to aging and atherosclerosis. The antioxidative effect of protein hydroly-

TABLE 3 : Plasma protein and plasma lipid profile of rats of two dietary groups

Groups	Total Protein (g/dL)	Total Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
CAS-C	7.52± 0.22	181.4±2.62	83.84 ± 3.11b[a]	11.3±0.88	161.15±2.11[a]
MPH-C	7.56± 0.08	143.01 ± 2.22 [a]	51.66 ± 3.55 [a]	13.13±1.97	110.27± 2.66 [a]

Values are mean ± SEM, n=6; CAS-C, 18% casein +2% cholesterol; MPH-C, 18% mustard protein isolate + 2% cholesterol, [a]MPH-C vs CAS-C ($p<0.001$)

TABLE 5: Liver weight and liver lipid profile of two dietary groups

Groups	Weight of liver (gm)/100g rat	Total lipid (gm/g of tissue)	Total cholesterol (mg/g of tissue)	Phospholipid (mg/g of tissue)	TAG (mg/g of tissue)
CAS-C	5.67± 0.31	0.197±0.012	11.16±0.79	10.96± 0.72	187.32±7.56
MPH-C	4.18 ±0.23[a]	0.128±0.01[a]	7.78±0.82[b]	11.21± 0.84	99.41±7.91[c]

Values are mean ± SEM, n=6; CAS-C, 18% casein +2% cholesterol; MPH-C, 18% mustard protein isolate + 2% cholesterol; [a]MPH-C vs CAS-C ($p<0.01$), [b]MPH-C vs CAS-C ($p<0.02$), [c]MPH-C vs CAS-C ($p<0.001$)

sates containing short peptides is already been reported[28-29]. Previous research on di- or tripeptides in oil or metal catalysed lyposomal suspensions indicated that it was the chelation of prooxidative metal ions and termination of free radical chain reaction by the presence of antioxidant peptides either through the specific amino acid residue side chain groups or through the specific peptide structure[30-31]. But the detail mechanism by which protein hydrolysates is working as antioxidants is not clear in the present work. Only it supports the others observations.

Liver weight, total lipid, total cholesterol, phospholipids and TAG of the two dietary groups of rats were presented in TABLE 5. The weight and lipid content of liver tissue were significantly decreased in the cholesterol fed experimental group than the control group. There was a significant decrease in liver lipid cholesterol and triacylglycerol content in MPH-C group than the CAS-C group. Aoyama et al[5] have also observed the decrease in liver weight when soy protein hydrolysate was fed to rats in comparison with casein. This may be due to the hydrophobic nature of the short peptides present in mustard protein hydrolysates.

In conclusion, the present result indicated that mustard protein hydrolysate reduce plasma lipid profiles, LDL and EM lipid peroxidations and liver lipid profiles, suggesting its beneficial effect. Therefore, MPH may be recommended as a nitrogen source in various food formulations as hypocholesteromic and antioxidative component.

REFERENCES

[1] J.Vioque et al.; J.Am.Oil.Chem.Soc., **76**, 819-823 **(1999)**.
[2] J.Vioque, R.Sanchez-Vioque, A.Clemente, J. Pedroche, F.Millan; J.Am.Oil.Chem.Soc., **77**, 447-450 **(2000)**.
[3] K.Bandyopadhyay, S.Ghosh; J.Agri.Food Chem., **50**, 6854-6857 **(2002)**.
[4] A.C.Beynen, E.N.W.Winnubst, C.E.West; Z. Tierphysiol Tierernahr Futtermittelkd., **49**, 49-53 **(1983)**.
[5] T.Aoyama, K.Fukui, K.Takamatsu, Y.Hashimoto, T.Yamamoto; Nutrition., **16**, 349-354 **(2000)**.
[6] N.R.T Damasceno, M.A.Gilund, H.Goto, C.T.S. Dias, F.S.Okawabata, D.S.P.Abdalla; Ann.Nutri. Metab., **45**, 38-46 **(2001)**.
[7] J.M.Bell, M.O.Keth, J.A.Blake, D.I.McGregor; Can.J.Animal.Sci., **64**, 1023-1033 **(1984)**.
[8] R.Blair; Poultry Sci., **63**, 754-759 **(1984)**.
[9] R.W.Newkirk, H.L.Classen, R.T.Tyler; Poultry Sci., **76**, 1272-1277 **(1997)**.
[10] M.Sen,, D.K.Bhattacharyya; European J.Lipid Sc.And Technol, **102**, 727-733 **(2000)**.
[11] J.H.Joanes, C.A.Foster; J.Nutr., **24**, 245-256 **(1942)**.
[12] C.C.Allain, L.S.Poon, C.S.G.Chan, W.Richmond, P.C.Fu; Clin.Chem., **20**, 470-475 **(1974)**.
[13] G.R.Warnick, T.Nguyen, A.Albers; Clin.Chem., **31**, 217-222 **(1985)**.
[14] G.Bucolo, M.David; Clin.Chem., **19**, 476-482 **(1973)**.
[15] E.D.Wills; 'Evaluation of lipid peroxidation in lipids, biological membranes.In biological toxicology: A practical approach', K.Snell, B.Mullock (Ed); IRL Press: Oxford Washington DC, 127-151 **(1987)**.
[16] P.S.Bachorik, J.J.Albers; Methods Enzymol., **129**, 78-100 **(1986)**.
[17] S.Phelps, W.S.Harris; Lipids., **28**, 475-477 **(1993)**.
[18] H.G.Rose, M.J.Oklander; Lipid Res., **6**, 428-431 **(1965)**.
[19] N.R.Brownlee, J.J.Huttner, R.V.Panganamala, D.G.Cornwell; J.Lipid Res., **18**, 635-644 **(1977)**.
[20] E.H.Bligh, W.J.Dyer; J.Biochem.Physiol., **37**, 911-916 **(1959)**.
[21] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265-275 **(1951)**.
[22] P.S.Chen, T.Y.Toribara, H.Warner; Anal.Chem., **28**, 1756-1758 **(1956)**.
[23] S.Pearson, H.O.Hartley; Cambridge, **1**, **(1966)**.
[24] M.Sugano, N.Ishiwaki, L.Ratio; Ann.Nutr.Metab., **28**, 192-199 **(1984)**.
[25] S.Madani, J.M.Frenoux, J.Prost, J.Belleville; Nutrition., **20**, 554-563 **(2004)**.
[26] D.Kritchevsky, S.A.Tepper, S.K.Czarnecki, D.M. Klurfeld; Atherosclerosis., **41**, 429-431 **(1982)**.
[27] T.Morita, A.Oh-hashi, K.Takai, M.Ikai, S.Kasaoka, S.Kiriyama; J.Nutr., **127**, 470-477 **(1997)**.
[28] K.Koba, K.Wakamatsu, K.Obata, M.Sugano; Lipids, **28**, 457-464 **(1993)**.
[29] S.Sakanaka, Y.Tachibana, N.Ishihara, L.R.Juneja; Food.Chem., **86**, 99-103 **(2004)**.
[30] B.J.Lee, D.G.Henricks, D.P.Cornforth; J.Food.Sci., **63**, 394-398 **(1998)**.
[31] W.K.M.Chan, E.A.Decker, J.B.Lee, D.A. Butter field; J.Agric.Food.Chem., **42**, 1407-1410 **(1994)**.