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The Effect on Growth and Lipid Profile in Rats Fed Microwave Heated Corn Oil

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Abstract: The domestic use of microwave heating is common. Therefore we believe that the study of microwave heated edible oils using animal models is appropriate and may help in understanding its effect on human nutrition. Currently there is growing interest to investigate the effect of microwave heating on the oxidative stability of food, as it is used more frequently than ever before. Effect of microwave heating of corn oil for 1 h and 40 min and 3 h and 20 min was investigated by assessing its fatty acid composition and peroxide value. The amount of polyunsaturated fatty acid decreased with the increase in the peroxide values of the microwave treated oil indicates decreased stability. The microwave heated oils (4% v/v) were fed to male albino rats by supplementing to the diet, to investigate whether or not they have some deleterious effect. The microwave treated oil did not affect the body weight gain as well as lipid level in rats. More over the rate of *in vitro* RBC hemolysis and tissue TBARS remained unaffected after ingestion of diet supplemented with microwave treated oil. These findings indicate that microwave treatment had no harmful consequences in our experimental condition.

Key words: Microwave, lipids, oxidative stability, rats

INTRODUCTION

Microwave heating has now become increasingly popular to the processing of foods and food products. There has been tremendous increase in the use of microwave energy in the food industry, in catering services and in domestic field because of its speed, convenience and efficiency compared to conventional heating method (Finot and Merabet, 1993). There are numerous advantages of microwave treatment than by autoclaving (Zhang *et al*., 2009; Ezzohra *et al*., 2009; Chen *et al*., 2010). It has also been reported by Ramezanzadeh *et al*. (2000) that the rice bran heated in microwave and packed in zipper top bags can stay without any adverse effect for 16 weeks at 4-5°C. Microwave irradiation is known to induce and enhance various chemical reactions in food components (Finot *et al*., 1968; Jahnen *et al*., 1990; Lubec *et al*., 1989; Almeck *et al*., 2006). Lubec *et al*. (1989) has reported the formation of stereo isomers of D-proline and cis-hydroxyproline in baby formula heated in microwave oven. Many workers have studied the effect of microwave heating on the stability of fatty acids in various meats (Janicki and Appledorf, 1974; Mai *et al*., 1980) and also the autooxidation of unsaturated fatty acids (Wong *et al*., 1991). Stability of vitamins have also been studied by many workers during microwave cooking of foods (Yoshida *et al*., 1992; Yoshida and Kajimoto, 1989). Villamiel and co-workers (1996) have observed the isomerization of lactose during microwave heating of cow's milk samples. They have also observed higher Millard's reaction and protein denaturation as compared to conventionally heated milk as a result of an extra accelerating effect of microwave heating.

Thus it appears from the large number of studies that microwave heating does not impair the nutritional value of foods very differently from that of the conventional cooking conditions. Though formation of toxic substances have also been observed (Miller *et al*., 1989) during microwave cooking, but very little is known about their toxicological effects in animals. Therefore the present study was designed to examine if there is any adverse effect of microwave treated oils on the growth and the tissue lipid profile in the rats.

MATERIALS AND METHODS

Thiobarbutaric Acid (TBA), chloroform and methyl alcohol were obtained from BDH chemical Ltd. Poole (England). 1,1,3,3 - tetraethoxypropane was obtained from Sigma, (St. Louis, Mo. USA). Kits for the estimation of triacylglycerol and cholesterol were purchased from bioMerix, France. Corn oil was obtained from local super markets. Other chemicals used were of highest grade and purity.

Microwave heating of oil: Commercial grade fresh corn oil samples (15 g) were placed in a 25 ml glass bottle (internal diameter 30 mm) and sealed with polyethylene film. The oil samples were simultaneously heated in the oven for 1 h and 40 min and 3 h and 20 min for 5 and 10 days, respectively. Microwave heating was carried out at

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2450 MHZ, in an oven model R-9H20 (M)/(W) Sharp Corp. Osaka, Japan. After heating the oils were allowed to cool at room temperature and stored at -25°C under nitrogen in sealed bottles for further chemical analysis.

Animals and diets: Male wistar albino rats obtained from Animal Care Center of King Saud University and housed as approved by the Committee of the Research Center of the College of Applied Medical Sciences, King Saud University, Riyadh, weighing about 93 g at the start of the experiment. They were kept in a room maintained at 23±2°C on 12 h light-dark cycle. The rats were randomly divided into 3 groups of 6 animals each. The animals were grouped into control A and test B1 and B2. Control group A received basal (commercial rats chow diet in pellet form provided by Grain Silos, Riyadh, Saudi Arabia) diet only. Test group B1 was given basal diet plus 4% microwave heated corn oil (PV 30.8). Group B2 was given basal diet plus 4% microwave heated corn oil (PV 17.8). Food and drinking water was provided *ad libitum*. Animals were weighed every second day.

Measurement of peroxide value: Quantitative changes in the peroxide value of the microwave treated and fresh oils were determined by the AOCS official methods (1980).

Sample collection: After 5 weeks of feeding period, rats were fasted overnight and anesthesized with diethyl ether (BDH Chemicals, Poole, UK). Rats were sacrificed by exsanguination via heart puncture. Blood was collected in chilled heparinized vacutainer tubes (Bectin Dickinson Co.; Rutherford NJ). A small portion of whole blood was immediately processed for hemolysis. Liver and kidney were excised washed in ice cold saline, dried and weighed. The tissue was immediately frozen at -70°C until analyzed.

RBC hemolysis: *In vitro*, RBC hemolysis was measured according to the method of Draper and Csallany (1969) as modified by Buckingham (1985). Heparinized blood (0.5 ml) was mixed gentle and centrifuged at 2000 rpm for 15 min (Model RT 6000D and T6000D, Sorvell Table Top Centrifuge, Dupont Co.; Wilmington, USA) to separate plasma and red blood cell. The separated RBC's were washed thrice with 0.9% saline. The cells were then suspended in 0.1 M PBS containing 0.15 M NaCl at the concentration of 0.5% v/v at 37°C for 20 h and absorbance was read at 540 nm against PBS. The rate of percent hemolysis was calculated by using completely hemolyzed sample of RBCs in distilled water.

Assay of lipid peroxide: The content of lipid peroxide was measured according to the method of Uchiyama and Mishara (1978). The following reagents were added to 0.5 ml of the tissue homogenate (10% w/v); 3 ml of 1% phosphoric acid and 1 ml of 0.6% aqueous solution of thiobarbituric acid. The mixture was heated in boiling water bath for 45 min and the product of thiobarbituric acid was extracted into 4 ml of n-butanol. The product of lipid peroxide was calculated from absorbance at 532 nm and 520 nm using 1,1,3,3 tetraethoxypropane as standard. The results were expressed as nmole MDA/g wet tissue.

Lipid analysis: Lipids from the tissue were extracted by the method of Folch *et al.* (1957) and sonicated (Fisher, Sonic Dismembrator, Model 150) in saline. Plasma and tissue lipids were estimated spectrophotometrically using commercial enzymatic kits.

Fatty acid analysis: To perform fatty acid analyses, samples of fresh and microwave treated oils were converted to methyl esters by transmethylation (Ahmed *et al.*, 1986). Fatty acid methyl esters were analyzed by gas chromatography using gas chromatograph (Hewlett Packard, Model HP 5048A) as described earlier (Al Kanhal, 1997).

Statistical analysis of data: Standard deviation of the means were calculated and data were subjected to student's t test for significance. Value were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Studies have been performed to investigate the toxicological safety of microwave treated foods (Miller *et al.*, 1989). However, there is a paucity of studies on the effect of microwave heated oil on the growth and lipid profile *in vivo*. The present study is an effort in this direction to study the effect of microwave heating. Corn oil was heated in microwave for 1.4 and 3.2 h. The fatty acid composition and peroxide values of corn oil samples before and after microwave treatment are shown in Table 1.

A significant increase in the peroxide value, of microwave treated corn oil (1.4 and 3.2 h) was observed (Table 1) which indicates a decreased stability and increased rancidity of the oil during microwave heating. Yoshida and co-workers (1990) have also reported an increased peroxide value during microwave treatment of linseed, soybean, corn, olive and palm oil. Table 1 presents the fatty acid composition of microwave treated and fresh corn oil. Major fatty acid seen in the samples were palmitic, oleic and linoleic acid. Saturated fatty acids (16:0, 18:0) and the oleic acid content as well as the ratio of saturated and unsaturated fatty acids did not change significantly during microwave treatment as compared to fresh oil. However an appreciable changes were observed in the linoleic acid content of control and microwave treated corn oil. The linoleic and linolenic

Table 1: Fatty acid composition and peroxide values of fresh and microwave heated corn oil

	Corn oil		
	Fresh	Microwave heated 1.4 h	Microwave heated 3.2 h
	0.8 (PV meq/kg)	30.8 (PV meq/kg)	17.8 (PV meq/kg)
Fatty acid	Wt (%)		
14:0	0.1	0.1	0.1
16:0	11.5	12.0	12.2
18:0	2.0	2.6	02.9
18:1	26.0	24.9	25.2
18:2	59.0	57.7	57.0
18:3	0.9	0.5	0.2
Others	0.1	1.9	2.0

Each value is the average of 4 determinations

Table 2: Body and liver weights of rats fed fresh and microwave heated corn oil

	Body weight (g)		
	Initial	Final	Liver weight (g)
Group A (Fresh corn oil)	92.7±10.3[a]	279.9±27.3[a]	8.51±0.79[a]
Group B1 (Microwave heated corn oil; PV 30.8)	93.3±8.3[a]	280.2±60.9[a]	10.22±1.35[c]
Group B2 (Microwave heated corn oil; PV 17.8)	93.4±9.2[a]	277.8±00.3[a]	10.05±1.18[b]

Values are mean±SD, n = 6. Values with different superscript in the same column are significantly different at p<0.05

Table 3: Tissue and plasma lipids of rats fed fresh and microwave treated corn oil

	Liver			Plasma	
	Total lipids (mg/g)	Triacylglycerol (mg/g)	Cholesterol (mg/g)	Triacylglycerol (mg/dl)	Cholesterol (mg/dl)
Group A (Fresh corn oil)	76.15±5.25[a]	1.80±0.6[a]	2.25±0.8[a]	43.05±8.9[a]	58.27±7.7[a]
Group B1 (Microwave heated corn oil; PV 30.8)	80.60±5.3[a]	2.03±0.8[a]	2.40±0.41[a]	46.40±7.35[a]	61.40±7.3[a]
Group B2 (Microwave heated corn oil; PV 17.8)	81.17±3.59[a]	2.40±0.78[a]	2.32±0.58[a]	49.30±11.1[a]	61.90±7.6[a]

Values are mean±SD, n = 6. Values with different superscript in the same column are significantly different at p<0.05

Table 4: Tissue TBARS (nmole MDA/g wet tissue) and rate of RBC hemolysis of rats fed fresh and microwave heated corn oil

	Kidney	Heart	Liver	Hemolysis (%)
Group A (Fresh corn oil)	335.0±25.09[a]	198.30±12.9[a]	330.0±27.5[a]	5.0±1.9[a]
Group B1 (Microwave heated corn oil; PV 30.8)	351.7±39.2[a]	216.67±40.3[a]	335.0±31.5[a]	5.8±1.9[a]
Group B2 (Microwave heated corn oil; PV 17.8)	347.3±31.5[a]	211.60±34.3[a]	318.3±30.5[a]	7.7±2.49[a]

Values are mean±SD, n = 6. Values with different superscript in the same column are significantly different at p<0.05

acid content decreased from 59.0-57.7% and 0.9-0.2% respectively after microwave treatment. The same pattern was also observed by Yoshida *et al.* (1990). These reductions in unsaturated fatty acid result from oxidation that occurs during microwave treatment.

In our study rats showed steady growth during first 5 weeks of feeding period of diet supplemented with microwave treated and fresh corn oil. There was no change in the final body weight of different groups (Table 2). Many workers reported the reduction of growth in the rats after feeding with diet containing oxidized oils (Yoshida and Kajimoto, 1989; Brosting *et al.*, 1994; Corcos Benede *et al.*, 1987). However in our study oxidized oil fed rats did not show marked reduction in body weight gain. This could possibly be due to the oils used in our study were moderately oxidized compared to those used in other studies and hence did not produce general toxic effect in the rats. In addition, Eder *et al.* (2002) observed a slight reduction in body weight by feeding 10% thermally oxidized oil with low vitamin E concentration. However the decrease in body weight was not apparent after feeding 5% oxidized oil (Eder *et al.*, 2002). The liver weight of microwave treated oil fed group was significantly high compared to the rats fed on fresh corn oil supplemented diet (Table 2). Although Kim *et al.* (2001) observed different organ weight among groups which tend to increase by feeding microwave heated fat. The RBCs membrane has been used as a model for oxidative damage to biomembrane as well as an index of vitamin E status (Yoshida and Kajimoto, 1989). Hemolysis is also a typical type of membrane damage induced by various lipids. Rats fed microwave heated oil did not show a significant increase in hemolysis as compared to controls. This might be due to low ability of the oil to produce oxidative stress (Table 4).

This study identifies no critical differences in hepatic and plasma cholesterol level between rats fed microwave

heated corn oil and fresh corn oil (Table 3). In contrast Liu and Lee (1998) observed an increase plasma cholesterol level in Oxidized Frying Oil (OFO) fed rats. No significant differences were observed in hepatic total lipids and triacylglycerol levels between microwave treated and fresh corn oil fed rats (Table 3).

It is well known that the ingestion of autoxidized oil appeared to promote *in vivo* lipid peroxidation because of decrease of essential fatty acids and increased peroxide values and carbonyl values in lipids. However, in our study, feeding rats with microwave treated corn oil did not affect the tissues (kidney, heart and liver) TBARS in rats (Table 4). These findings further reiterate that the oxidized oil used in our study is not exerting toxic effects in rats.

The results of the present study indicate decreased oxidative stability and linoleic acid content of microwave treated corn oil. Microwave treatment did produce some acceleration in the oxidation of oil, negatively affecting free acidity, peroxide value and oxidative stability. However microwave heated dietary oil resulted in no significant changes in plasma and liver lipid metabolism in rats. Moreover, *in vitro* RBC hemolysis and tissue TBARS remained unaffected by microwave heated oils. Accordingly use of microwave for 1.4 and 3.2 h seems to be of no harm since there appears to be no harmful effect on tissue lipids of rats. It has been reported earlier by Liu and Huang (1995) that heating of oil at high temperature for a short period of time leads to the faster deterioration of oil. Further investigation is now needed to see the effect of high temperature for a shorter period of time on microwave heated oil and its impact on lipid metabolism.

REFERENCES

Ahmed, F., H. Schiller and K.D. Mukherjee, 1986. Lipids containing isoricinoleoyl (9-hydroxy-cis-12-octadecenoyl) moieties in seeds of *Wrightia* species. Lipids, 21: 486-490.

Al Kanhal, M.A., 1997. Lipid analysis of *Coffea arabica* Linn. beans and their possible hyper-cholesterolemic effects. Int. J. Food Sci. Nutr., 48: 135-139.

Almeck, K.A.D., T. Clergé, K. César, F. Jacques and P. Michel, 2006. Changes in Canarium schweinfurthii Engl. Oil quality during microwave heating. Eur. J. Lipid Sci. Technol., 108: 429-433.

Brosting, C.F., R.M. Engberg, K. Kakobsen, S.K. Jensen and J.O. Andersen, 1994. Inclusion of oxidized fish oil in mink diets: The influence on nutrient digestibility and fatty acid accumulation in tissues. J. Anim. Physiol. Anim. Nutr., 72: 132-145.

Buckingham, K.M., 1985. Effect of dietary polyunsaturated/saturated fatty acids ratio and dietary vitamin E on lipid peroxidation in rats. J. Nutr., 115: 1425-1435.

Chen, Y., X. Xueming, X. Baocai, J. Zhengyu, L. Ruixin, B. Mahanad and Y. Na, 2010. Microwave-assisted biosynthesis of glycerol monolaurate in reverse microemulsion system: Key parameters and mechanism. Eur. Food Res. Technol., 231: 719-726.

Corcos Benede, H.P., M. D'Aquino, M. DiFelice, V. Gentili, B. Tagliamonte and G. Tomassi, 1987. Effect of a fraction of thermally oxidized soya bean oil on growing rats. Nutr. Rep. Int., 36: 387-401.

Draper, H.H. and A.S.A. Csallany, 1969. Simplified hemolysis test for vitamin deficiency. J. Nutr., 98: 390-394.

Eder, K., P. Skufa and C. Brandsch, 2002. Thermally oxidized dietary fats increase plasma thyroxine concentration in rats irrespective of the vitamin E and selenium supply. J. Nutr., 132: 1275-1281.

Ezzohra, N., T. Valeri, H. Hakima, B. Es-Seddik, C. Farid and D. Olivier, 2009. Microwave assisted water extraction of green tea polyphenols. Ibid., 20: 408-415.

Finot, P.A., J. Bricout, R. Viani and J. Mauron, 1968. Identification of new lysine derivative obtained upon acid hydrolysis of heated milk. Experientia., 24: 1097-1099.

Finot, P.A. and M. Merabet, 1993. Nutritional and safety aspects of microwaves. History and critical evaluation of reported studies. Int. J. Food Sci. Nutr., 44 (Suppliment 1): S65-S75.

Folch, J., M. Lees and S.G.H. Sloane, 1957. A simple method of isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Jahnen, E.G.E., R.R. Lentz, P.H. Pesheck and P.H. Sackett, 1990. Hydrolysis of adenosine triphosphate by conventional or microwave heating. J. Org. Chem., 55: 3406-3409.

Janicki, L.J. and H. Appledorf, 1974. Effect of broiling, grill frying and microwave cooking on moisture, some lipid components and total fatty acids of ground beef. J. Food Sci., 39: 715-717.

Kim, E., S.M. Jeon and M.S. Choi, 2001. Effect of γ-irradiated fats on plasma lipid concentrations and hepatic cholesterol metabolism in rats. Ann. Nutr. Metab., 45: 152-158.

Liu, J.F. and Y.W. Lee, 1998. Vitamin C supplementation restores the impaired vitamin E status of guinea pigs fed oxidized frying oil. J. Nutr., 128: 116-122.

Liu, J.F. and C.J. Huang, 1995. Tissue α-tocopherol retention in male rats is compromised by feeding diets containing oxidized frying oil. J. Nutr., 125: 3071-3080.

Lubec, G., C. Wolf and B. Bartosch, 1989. Amino acid isomerization and microwave exposure. Lancet., 334: 1392-1393.

Mai, J., H. Tsai, G. Ambruster, P. Chu and J.E. Kinsella, 1980. Effect of microwave cooking on food fatty acids: No evidence of chemical alteration or isomerization. J. Food Sci., 45: 1753-1755.

Miller, B.J., S.M. Billedeau and D.W. Miller, 1989. Formation of N-nitrosomines in mirowaved verses skillet-fried bacon containing nitrite. Food Chem. Toxicol., 27: 295-299.

Official and Tentative Methods of the American Oil Chemists Society, 3rd Edn., 1980, Edited by R.O. Walker, AOCS, Champaigne, IL.

Ramezanzadeh, F.M., R.M. Rao, W. Prinyawiwatkul, W.E. Marshall and M. Wind Hauser, 2000. Effect of microwave heat, packings and storage temperature on fatty acid and proximate composition in rice bran. J. Agric. Food Chem., 48: 464-467.

Uchiyama, M. and M. Mishara, 1978. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Ann. Biochem., 86: 271-278.

Villamiel, M., N. Corzo, I. Martinez-Castro and A. Olano, 1996. Chemical changes during microwave traetment of milk. Food Chem., 56: 385-388.

Wong, J.W., H.C.H. Yeo and T. Shibamoto, 1991. Determination of malondealdehyde and formaldehyde formed from fatty acid ethyl esters upon microwave and thermal heating. J. Agric. Food Chem., 39: 2260-2262.

Yoshida, H., N. Hirooka and G. Kajimoto, 1990. Microwave energy effects on quality of some seed oils. J. Food Sci., 55: 1412-1416.

Yoshida, H. and G. Kajimoto, 1989. Effect of dietary vitamin E on the toxicity of autoxidized oil to rats. Ann. Nutr. Metab., 33: 153-161.

Yoshida, H., K. Izumi and K. Goro, 1992. Effect of microwave energy on the relative stability of vitamin E in animal fat. J. Sci. Food Agric., 58: 531-534.

Zhang, F., Y. Yi, S. Ping and G. Zhenku, 2009. Microwave-assited extraction of rutin and quersetin from the stalks of Euonymus alatus (Thunb) Sieb. Phytochem. Anal., 20: 33-37.