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Original Article

The effect of consuming oxidized oil supplemented with fiber on lipid profiles in rat model*

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Abstract

BACKGROUND: This study was conducted to evaluate the effects of consuming thermally oxidized oil supplemented with pectin on liver glutathione peroxidase activity, serum malondialdehyde and lipid profiles in male Sprague-Dawley rats.

METHODS: Fifty growing male Sprague-Dawley rats were randomly divided into different groups. The diets differed only in their fat and pectin content. The diets had fresh sunflower oil or thermally oxidized sunflower oil. The diets were supplemented with pectin in the amount of 50 g/kg diet or not supplemented. Thus, there were four experimental groups: "fresh oil", "oxidized oil", "fresh oil + pectin", "oxidized oil + pectin". Study duration was 42 days. Non parametric, Kruskal-Wallis and Mann-Whitney tests were used to evaluate mean values of variables in groups.

RESULTS: In oil consumption, peroxide, p- Anisidine, thiobarbituric acid, free fatty acid values and total polar compounds increased but iodine value was decreased**.** In the oxidized oil group compared to the fresh oil group, total cholesterol, high density lipoprotein cholesterol and malondialdehyde increased ($p < 0.05$). Serum malondialdehyde was decreased in the "oxidized oil + pectin" group compared to the oxidized oil alone (2.82 ± 0.51 vs. 3.61 ± 0.72 nmol/ml; $p < 0.05$)**.** Total cholesterol decreased in both groups containing pectin compared to their respective diets without supplementation (70.10 ± 10.75 vs. 81.20 ± 13.10 mg/dl; $p < 0.05$).

CONCLUSIONS: Pectin consumption could decrease serum malondialdehyde and cholesterol in the diet that contains oxidized oil. Pectin supplementation could decrease the detrimental effects of thermally oxidized oil.

KEYWORDS: Thermally Oxidized Oil, Pectin, Malondialdehyde, Lipid Profile, Lipid Peroxides.

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During recent decades, many studies have shown the role of dietary factors in the prevalence of cardiovascular diseases.[1] Excess amounts of fat intake contribute to coronary heart diseases and even other chronic diseases.[2,3] Deep fat frying is a widely used procedure for meal preparation.[4] During deep fat frying, oil is heated at high temperature (up to 190˚C) for a long time in the presence of air. Under these conditions both oxida-

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tion and thermally breakdown of the oil may occur. These products have toxic effects, and may cause different adverse effects.[5,6]

High concentration of total polar compounds in thermally oxidized oil might induce damage in different parts of body.[7] Therefore, monitoring of these compounds in heated oils is necessary.[8] Several compounds in the thermally oxidized oils induce oxidation of polyunsaturated fatty acids (PUFAs) and increase oxidative stress.[9] Feeding thermally oxidized oil increases the level of reactive substances such as thiobarbituric acid (TBARS) which is an indicator of lipid peroxidation[10] and lipid peroxidation may be an important factor in the etiology of atherosclerosis.[10] Thermally oxidized sunflower oil ingestion affects on the intestinal antioxidant enzyme activity and gene expression in male Wistar rats[9] and even might provide endothelial dysfunction.[11]

Increasing oxidized oil consumption by increasing the amount of fast food intake as a part of industrial life style is dramatically increased in the recent years.[12] It has been reported that viscous fiber such as pectin could improve serum lipid profiles.[13-16] Previous studies[13,17] showed that high cholesterol diet supplemented with pectin could decrease serum cholesterol in animals. To our knowledge, no studies so far have reported the effect of diet containing thermally oxidized oil and pectin on the cardiovascular risks. Therefore, this study was conducted to determine the effect of pectin supplementation on the adverse effect of thermally oxidized oil in a rat model.

Methods

Oil procedure: Sunflower oil was purchased from Narges Shiraz oil factory, Shiraz, Iran. Half of the fresh sunflower oil was stored at 15˚C in darkness, while the other part was heated at 180˚C for 48 hours in 1 liter beakers in the oven and stored at -20˚C.[6] Each of these oils was included at 10% (w/w) in their corresponding diets. Chemical and physical analytical tests were performed in duplicate on both fresh and thermally oxidized sunflower oil. Free fatty acids (FFA), acid value (AC), peroxide value (POV), Iodine value (IV) (Wijs method), thiobarbituric Acid (TBA) (direct method), p-anisidine value (PAV), total polar compound (TPC) and color (lovibond method) was determined as the chemical and physical tests.[18] American oil chemists society (AOCS) method were used for all tests.[18] This study was the results of a MS thesis which was supported by Shiraz University and Shiraz University of Medical Science (grant No 2582).

Animal and diets: Fifty growing male Sprague-Dawely rats initially weighting approximately 80 g were supplied by animal house of Shiraz University of Medical Sciences, Iran. The rats were randomly divided by spiral method. After adaptation period of 7 days, they were transfer to different groups of ten rats. The rats were kept in individual stainless steel cages in a room maintained at 21-23˚C and 50-60% relative humidity with 12 hours light-12 hours dark cycle. They had free access to food and water during 42 days experimental period. Purified diets were prepared according to the criteria of American Institute of Nutrition, 1993 (AIN-93).[19] Diets were prepared in pellet forms two times during the experimental period and stored at 4˚C until being used. All vitamin and mineral mixture were supplied by department of food science, Shiraz University. Their ingredients separately milled by a mixer (Moulinex) and precisely weighted to the needed amounts. Finally they were mixed for 3 minute in an industrial mixer to result in completely homogenized mixture and kept at 4˚C until being included in the diets. In order to convert the resulted powder into pellets, distilled water was added to the powder with the ratio of 2:6, then the diets were passed through a meat grinder and produced strings were cut into 1.5-2 cm pellet, and place into steel trays to be dried at 30˚C for 24 hours in an oven. All the ingredients are mentioned in table 1. Four experimental diets were prepared. These diets differed in their kind of fat content consisting whether fresh or thermally oxidized sunflower oil, and in pectin content of ingredients which was whether non-pectin supplemented or pectin supplemented at 50 g/kg diets. Therefore, we had four groups:

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Table 1. Ingredients of experimental diets containing thermally oxidized or fresh sunflower oil with or without pectin

Ingredients	Amount in diets*(g/kg diet)*	
	Fresh or Oxidized oil groups	**Fresh or Oxidized oil +Pectin groups**
Cornstarch[1]	503	453
Caseine[2]	200	200
Sugar	100	100
Fat	10	10
Fiber[3]	50	50
DL-methionine[4]	2	2
Pectin[5]	-	50
Mineral mixture[6]	10	10
Vitamin mixture[7]	35	35

[1]Cornstarch was supplied by Glucosan Company (Ghazvin, Iran).
[2]Casein was supplied by Caseinate Company Iran.
[3]Microcrystalline cellulose was supplied by India (RC-591, NO 2351).
[4]DL-methione was supplied Merck Germany.
[5]Pectin was supplied by (Sigma Saint Louis USA, NO 9135).
[6]Mineral mixture composition (g/kg diet): Calcium Carbonate: 357, Potassium phosphate, monobasic: 196, Potassium citrate, tri-potassium, monohydrate: 70.78, Sodium chloride: 74, Potassium sulfate: 46.60, Magnesium oxide: 24, Ferric citrate: 6.06, Zinc carbonate: 1.65, Manganese carbonate: 0.63, Cupric carbonate: 0.3, Potassium iodate: 0.01, Ammonium paramolybdate, 4 hydrate: 0.00795, Sodium selenite: 0.022, Sodium meta-silicate, 9 hydrate: 1.45, Lithium chloride : 0.0174, Boric acid: 0.0815, Sodium fluoride: 0.0635, Ammonium vanadate: 0.0066, Powdered sucrose: 217.899.
[7]Vitamin mixture composition (g/kg diet): Nicotinic acid: 3, Ca Pantothenate: 1.6, Pyridoxine-HCl: 0.7, Thiamine-HCl: 0.6, Riboflavin: 0.6, Folic acid: 0.2, D-Biotin: 0.02, B_{12} (cyanocobalamin): 2.5, E (tocopheryl acetate) (500 IU/g): 15, A (all-trans-retinylpalmitate) (500,000 IU/g): 0.8, D3 (cholecalciferol) (400,000 IU/g): 0.250, K (phylloquinone): 0.075, Powdered sucrose: 974.655.

"Fresh oil" (fresh oil group), "Fresh oil supplemented with pectin"(fresh oil + pectin group), "Oxidized oil supplemented with pectin" (oxidized oil + pectin group), "Oxidized oil" (Oxidized oil group). The dietary intake of rats was assessed by measuring the given foods at the beginning of each week and then measuring the remained foods at the end of the week.

Analytical determination-Food intake: Food intake was measured weekly. Food intake was weighted for each rat at the beginning of every week.[6] Food intake had to be expressed by the weight of dry material (DM).[6] The six final weekly DM food intakes of each rat were added to each other and divided by the number of days of food consumption, thus the food intake results were expressed as food intake (g/day). In addition to food intake, food efficiency ratio and protein efficiency ratio were calculated.[20]

Body weight: All rats were weighted weekly by a digital scale

Liver weight: The liver of each rat was weighted by a digital scale after being excised on the day of sample collection. Hepatosomatic index[21] was also calculated as another variable of liver deterioration.

Sample collection: 42 days after being maintained on these diets,[6] the animals were anesthetized with diethyl ether and their blood were collected by cardiac puncture with syringes into tubes, and serums were immediately isolated by centrifugation at 2500gr, 4˚C for 15min and liquated into microcentrifuge tubes which were for assaying lipid profile and MDA levels. Serum and livers were stored at -70˚C until performing the biochemical tests.

Biochemical parameters: The Cayman GPx assay kit, catalog number 703102, was used to assay liver GPx activity.[22] Total protein of all supernatant were determined by biuret assay using Pars Azmoon kit reagent.[23]

Serum lipid profile: Serum total and high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were determined by an autoanalyzer (Technicon, RA-100) system using MAN commercial kits. Very low density lipoprotein

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cholesterol (VLDL-C) was calculated as TG/6, and low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula.[24] These tests were performed in the clinical laboratory of Namazi hospital, Shiraz University, Iran.

Serum MDA: 250 ml of serum sample was placed in a tube and 500 ml TCA 10% (Merck) was added to it, after being vortexes and left stable for 10 min, it was centrifuged at 2200×g for 15min at 4˚C. Then 500 ml of the supernatant and standards [1,1,3,3-tetramethoxy propane (Sigma)] were placed into new tubes and an equal volume of TBA 0.67% (Merck) was added to them. The tubes were incubated in a boiling bath for 10min. The absorption of these samples was read by a Techno Specgene (Cambridge, UK) Spectrophotometer at 532 nm.[25]

Statistical analysis: Non parametric Kruskal-Wallis and Mann-Whitney tests were used to evaluate mean value of groups, including:"Fresh group" and "Fresh + Pectin group ", "fresh oil group" and "oxidized oil group", "Oxidized oil group" and "Oxidized oil + Pectin group", "Oxidized oil + Pectin group" and "Fresh oil + Pectin group". Differences were considered statistically significant at $p < 0.05$. $SPSS_{11.5}$ software was used for statistical analysis.

Results

Physical and chemical characteristics of oil: The physical and chemical characteristics of the fresh and oxidized oil are listed in table 2. TPC, FFA, AV, POV, TBARS and p-anisidine value increased from 1%, 0.04%, 0.08%, 0.75%, 0.01%, 9.84% in fresh oil to 31.50%, 0.1%, 0.21%, 1.70%, 0.18%, 107.05% in thermally oxidized oil and IV decreased from 129.44% to 106.60%, respectively.

Food, protein, fat and polar intake and weight gain: No significant difference were observed between food, protein and fat intake and weight gain between oxidized and fresh oil groups. Oxidized group consumed significantly higher amount of polar material intake than those given the fresh oil ($p \leq 0.001$). In the groups containing pectin, diarrhea was observed only during the first week of study. After one week, all the rats got used to the mentioned diets. So, diarrhea did not continue. Diarrhea was seen only in 2 rats during the first week in the fresh+ pectin and only in one rat in the oxidized+ pectin group. Food and protein efficiency ratios were not significantly different among the mentioned groups ($p > 0.05$) (Table 3).

Serum lipid profile: Serum total cholesterol (TC) concentration were significantly higher in oxidized oil group compared to fresh oil and in oxidized oil + pectin groups ($p < 0.05$). Serum TC concentration was significantly lower in fresh oil + pectin group compared to oxidized oil + pectin and fresh oil groups ($p < 0.05$). Serum HDL-C level was significantly higher in oxidized oil compared to the fresh oil group ($p < 0.05$). Serum VLDL-C, TG and LDL did not vary among 4 groups (Table 3).

Table 2. Physical and chemical characteristics of fresh and oxidized sunflower oils used in the experimental diets

Characteristic	Type of sun flower oil					
	Fresh			Oxidized		
	Yellow	Red	Blue	Yellow	Red	Blue
Free fatty acid value (%)	0.04 ± 0.00[1]			0.10 ± 0.00		
Acid value (mg KOH/g)	0.08 ± 0.00			0.21 ± 0.00		
Peroxide value (meq/1000g)	0.75 ± 0.07			1.70 ± 0.14		
Iodine value	129.44 ± 11.96			106.60 ± 7.18		
Thiobarbituric acid value	0.01 ± 0.00			0.18 ± 0.00		
ρ-Anisidine value	9.84 ± 1.31			107.05 ± 1.48		
Total polar compounds (%)	1.00 ± 0.00			31.50 ± 2.12		

[1]Values are presented as means ± SD of duplicate measurements.

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Table 3. Comparison of food component intakes and efficiency ratios, weight measurements, among the four groups of rats receiving the experimental diets during 42 days

Variables	Type of Diets[1]				
	Fresh	Fresh + pectin	Oxidized + pectin	Oxidized	P-values
Food intake (g/d)	11.81 ± 1.53	11.63 ± 1.65	11.30 ± 1.35	12.95 ± 1.32	0.11
Fat intake (g/d)	1.18 ± 0.15	1.16 ± 0.16	1.13 ± 0.13	1.30 ± 0.13	0.11
Protein intake (g/d)	2.37 ± 0.32	2.35 ± 0.33	2.28 ± 0.27	2.61 ± 0.27	0.13
Polar material intake (g/d)	0.01 ± 0.00 [a]	0.04 ± 0.01	0.35 ± 0.04	0.40 ± 0.04	0.001
Food efficiency ratio[3]	0.31 ± 0/04	0.25 ± 0.05	0.30 ± 0.05	0.29 ± 0.03	0.06
Protein efficiency ratio[4]	1.55 ± 0.22	1.32 ± 0.22	1.52 ± 0.25	1.40 ± 0.20	0.06
Total weight gain (g)	148.22 ± 25.58	124.80 ± 27.83	137.90 ± 25.81	146.50 ± 28.23	0.11

[1]Taype of diets were included: "fresh oil", "fresh oil supplemented with pectin", "oxidized oil supplemented with pectin", "oxidized oil".
[2]values are Mean ± SEM for groups of 10 animals.
[3] Food efficiency ratio: (100 × Total weight gain(g))/(Food Intake(g))
[4] Protein efficiency ratio: (100 × Total weight gain(g))/Protein Intake(g))

Liver GPx activity and hepatosomatic Index: As it is indicated in table 4, liver GPx activity and hepatosomatic index did not significantly changed when thermally oxidized oil was consumed compared to fresh oil or oxidized oil supplemented with pectin.

Serum MDA: At the end of study, the serum MDA concentration in oxidized oil group was significantly higher than in fresh oil (p = 0.01) and oxidized oil + pectin groups (p = 0.01). Also, Pectin supplemented fresh oil reduced the serum MDA more than pectin supplemented oxidized oil significantly (p = 0.01) (Table 4).

Table 4. Comparison of serum lipid profile, serum malondialdehyde, liver glutathione peroxidase activity and, hepatosomatic Index among the four groups of the rats receiving the experimental diets on day 42

Variables	Type of Diets[1]				
	Fresh	Fresh + petin	Oxidized + pectin	Oxidized	P-value
TG (mg/dl)	82.22 ± 30.38	79.80 ± 19.58	88.40 ± 20.95	93.30 ± 33.28	0.05
HDL-C (mg/dl)	36.55 ± 12.00	38.30 ± 22.00	40.00 ± 8.41	43.50 ± 7.38	0.01
LDL-C (mg/dl)	15.61 ± 7.13	8.14 ± 6.30	14.03 ± 3.60	17.10 ± 10.21	0.1
VLDL-C (mg/dl)	14.95 ± 5.52	14.51 ± 3.56	16.07 ± 3.81	20.60 ± 6.05	0.05
TC (mg/dl)	67.11 ± 8.68	58.10 ± 5.85	70.10 ± 10.75	81.20 ± 13.10	0.04
MDA (nmol/ml)	2.57 ± 1.03	2.29 ± 0.55	2.82 ± 0.51	3.61 ± 0.72	0.01
Liver GPx Activity (nmol/min/mgpro)	119.25 ± 74.96	125.39 ± 59.41	88.54 ± 55.21	124.88 ± 89.75	0.69
Hepatosomatic Index[3]	4.98 ± 0.42	5.82 ± 1.58	5.36 ± 0.53	5.13 ± 0.36	0.36

[1]Taype of diets were included:"fresh oil", "fresh oil supplemented with pectin", "oxidized oil supplemented with pectin", "oxidized oil".
[2]Values are Mean ± SEM for groups of 10 animals.
[3]Hepatosomatic index: (Liver weight×100)/body weight

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Discussion

The results of the present study revealed that rats consumed thermally oxidized oil had significantly higher concentration of serum cholesterol, MDA, HDL-C compared to fresh oil group. However, serum cholesterol and MDA were significantly lower in rats fed diet containing oxidized oil supplemented with pectin. Heated sunflower oil differed from the untreated oil by its high content in secondary lipid peroxidation products (carbonyl compounds). Previous studies reported the effects of oxidized oil consumption on the lipid profiles of rats.[26-27] The design of the mentioned previous studies was experimental. However, there is not any study for reducing the detrimental effects of oxidized oils.

The oxidized oil in our study did not cause liver enlargement. This result is in contradiction with data reported by Sanchez-muniz et al.[26] They indicated that oxidized oils induced an increase hepatosomatic index. However, lower POV content as well as shorter period of experiment time may explain such discrepancy. The lack of significant increase in liver GPx activity in rats fed oxidized oil compared tofresh oil group may be explained by two theories: Inactivation of GPx due to exposure of cells to MDA or generation of peroxide radicals and increase GPx activity due to increasing POV in oxidized oil. The lack of significant increase in GPx activity in oxidized group in the present study may be a response to the high level of MDA production in body and relatively low level of POV in thermally oxidized oil compared to the fresh oil groups.[29] However, Hayam et al.[30] explained decrease liver GPx activity by possible liver damages after consumption of oxidized oil. Findings of present study was also in contrast with the results of the study by Ammouche et al.,[21] and Ringseis et al.[31] who explained the significant increase in GPx activity by the large quantities of toxic peroxide radical products which stimulate GPx activity.

It is unknown whether increase in plasma TBARS or MDA is caused by the ingestion of oxidized oil (exogenous source) or is related to in vivo peroxidation (endogenous source). However, hydroperoxide might be mostly converted to aldehyde compounds by gastric fluid before absorption.[32] In the present study TBARS was slightly increased in thermally oxidized oil. This result is in line with previous experiment.[26] They suggested that ingestion of oxidized oil causes in vivo secondary lipid peroxidation. Significant difference in serum MDA level between the pectin supplemented groups compared to their respective non supplemented groups were observed. It might be due to the positive effect of pectin on decreasing the oxidative stress. Although some physiologic properties are detected for pectin in this regard, the exact role of dietary pectin in the etiology of oxidative stress is not clearly understood.[13] Adverse effects of such oils were partly reversed when the diet including the heated oil was supplemented with pectin.

Plasma cholesterol concentration is a risk factor for coronary heart disease. In the present study plasma cholesterol increased following feeding a diet containing oxidized oil diet. Some previous studies also indicated the same results.[25,26] However there are some studies, reporting reducing plasma cholesterol concentration in animals fed oxidized oil.[22] Enhanced HMG-COA reductase activity in the liver might be responsible for increased concentration of plasma cholesterol.[23,26,27] The increase in HDL-C may be a consequence of the low intake of linoleic acid content of oxidized oil or may be a protection mechanism against the oxidative stress caused by the diet containing oxidized oil and a mechanism to avoid oxidative changes in other lipoprotein such as LDL.[33] Total cholesterol in pectin supplemented groups compared to non supplemented groups was reduced in the present study which might be due to SCFA formation or lowering HMG-COA reductase activity or lowering hepatic cholesterol biosynthesis.[34,35]

Several factors such as the amount of oil intake, the period of experimental study, the level of secondary and primary oxidation products can be attributed to the different results in different studies.[36]

In the present study, the influence of pectin

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supplementation could be interesting. Primary mechanism of the pectin action for reducing LDL-C is via the absorption of cholesterol and bile acids. Shortening of the transit time through the intestine with increase suppression of fat absorption may participate in the mechanism of the inhibitory action of pectin.[37] Pectin lowers the reabsorption of bile acids. Therefore, hepatic conversion of cholesterol into bile acids increases, which finally can lead to increased LDL uptake by the liver.[38] Pectin also increases the microbial population of the colon, which could, in turn, increase the amount of microbial protein available to the microbiota for fermentation. These products can be fermented to phenols, indole, and biogenic amines. So, ammonia will be increased. Pectin increases producing the short chain fatty acids and exerts its beneficial effects by these compounds to some extent.[39] Pectin also may have antioxidant properties as it may scavenge peroxy radicals.[39] All these proposed mechanisms could show the role of pectin in reducing the oxidation status.

Rather than simply assessing the concentrations of lipid profiles, it was better to test the oxidizability of LDL in the different groups in the present study. As it was not available in the present study as a limitation of the study, we considered MDA concentration for assessing the oxidation level. Therefore, we assumed that we can consider the oxidation level by considering the MDA. Furthermore, since heating sunflower oil mainly affects the p-anisidine index, it was better to apply this test to the serum and to LDL particles to check the possible accumulation of secondary lipid peroxidation products in the circulation. However, the limited budget of the research did not allow us to do more tests.

Current and future developments:

This study demonstrated the harmful effect of an unbalanced diet containing thermally oxidized oil intake on lipid profiles parameters and MDA concentration. Consumption of oxidized oil supplemented with soluble fibers such as pectin, could be associated with decreased serum cholesterol concentration. Therefore, consumption of oxidized oils from fast-food should be markedly limited and more research is necessary to be done on the effect of oxidized oil supplemented with pectin on hepatic and kidney enzyme activity. Diet supplemented with fiber could reduce the oxidative stress and also could reduce the harmful effects of oxidized oil. Previous studies showed that high fiber diets could reduce the oxidative stress[40] and the present study showed that adding pectin to a diet involved oxidized oil could reduce the detrimental effects of such oils.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

SS, JJ, AAO, RR, NK, AR, NT conducted the study and drafted the paper. LA helped in preparing and editing the manuscript.

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