Biomarkers, 2011; 16(5): 445–452

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.590228

informa
healthcare

RESEARCH ARTICLE

The effects of *Bifidobacteria* on the lipid profile and oxidative stress biomarkers of male rats fed thermally oxidized soybean oil

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Abstract

Over the years, there has been concern about the changes taking place in heated oils and the effects on individuals consuming them. The present study investigated the effects of a diet containing thermally oxidized soybean oil (TO) or TO supplemented with probiotic *Bifidobacteria* (TO+Pro) on the serum lipid profile and oxidative stress biomarkers of male rats. The data showed several indicators of oil deterioration after thermal processing, including high levels of % free fatty acid (FFA; 15-fold), acid value (AV; 14-fold), peroxide value (8-fold), *p*-anisidine value (AnV; 39-fold), total oxidation value (TOTOX; 19-fold), thiobarbituric acid–reactive substances (TBARS) value (8.5-fold), and *trans*-FA (TFA) isomers (2.5-fold) compared to the control. The rats that were fed a diet containing TO showed a significant ($p < 0.05$) decrease in body weight gain, food efficiency values, and liver weight. Furthermore, the total cholesterol and low-density lipoprotein (LDL) levels were increased, while the high-density lipoprotein (HDL) level was decreased in blood serum samples. High levels of TBARS, superoxide dismutase (SOD), and glutathione reductase (GR) activities were also detected in the livers, kidneys, testes, and brains of rats. Interestingly, a diet containing TO+Pro restored all biological parameters to their control values. The present data suggested that *Bifidobacteria* may ameliorate the serum lipid profile and oxidative stress biomarkers that are generated in animals that are fed a TO diet.

Keywords: Food safety, thermally oxidized oil, probiotic *Bifidobacteria*

Introduction

Repeated frying in edible oil is a common practice throughout the world that leads to several physical and chemical changes in the oils as a result of oxidation, pyrolysis, polymerization, hydrolysis, and isomerization reactions (Fritsch 1981; Tyagi & Vasishtha 1996; Gertz & Kochhar 2001). Although repeated frying in edible oil seems to reduce the cost of food preparation, a total of 211 decomposed products have been identified in thermally abused oils. Most of these products are oxidative materials produced from triglyceride degradation, which not only affect the quality of fried foods but are also of much concern to human health, particularly when frying oil is thermally oxidized over a long period of time at high temperature (Cortesi & Privett 1971; Chang et al. 1977; Totani 2006).

A number of studies have been undertaken to assess the hazardous effects of thermally oxidized oil on experimental animals (Aladedunye & Przybylski 2009). Previous studies have indicated that the amounts of lipid peroxide and polar compounds are increased with time during oil thermal processing due to oxidation reactions (Totani et al. 2008). The induction of hepatic cytochrome P-450 monooxygenase (CYP) activity in rats that were fed thermally oxidized oil suggested that substances formed during thermal processing need to be metabolized by CYPs in the liver. CYPs are the major enzymes involved in drug metabolism and bioactivation of toxic

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(Received 17 March 2011; revised 17 May 2011; accepted 18 May 2011)

chemicals in the liver (Huang et al. 1988). It has also been reported that the ingestion of thermally oxidized oil alters eicosanoid metabolism, increases oxidative stress parameters such as plasma thiobarbituric acid–reactive substances (TBARS) and nitric oxide contents, and decreases plasma total antioxidant capacity in rats (Yen et al. 2010). Much concern has been focused on the amount of *trans*-fatty acids (TFAs) formed in thermally oxidized oil as a result of isomerization reactions during the frying process (Stender & Dyerberg 2004). Public concern about TFA amounts in daily food intake has grown in recent years, primarily due to its hazardous effects on plasma lipoproteins. TFAs increase low-density lipoprotein (LDL) levels, decrease high-density lipoprotein (HDL) levels and contribute to an increase in the LDL/HDL ratio, which is considered to be an important risk indicator for the development of cardiovascular diseases (Ascherio & Willett 1997; Hunter 2005; Hunter et al. 2010).

Bifidobacteria is a bacterium that normally inhabits a healthy bowel in most mammals, including humans. It can be found in fermented foods or taken as a supplement because the oral administration of these probiotic bacteria is safe. *Bifidobacteria* is considered to be an important probiotic used in the food industry for a range of beneficial health effects related to the relief and treatment of many digestive disorders. These beneficial bacteria thrive on plant fiber, and they may assist digestion, have a protective anticancer effect, and reduce inflammation. Indeed, they have been shown to significantly reduce serum cholesterol levels in male rats (Gomes & Malcata 1999). *Bifidobacteria* also have the ability to modulate the intestinal microbiota of rats and the bifidobacterial population levels in human colon (Salazar et al. 2011). Other studies have indicated that *Bifidobacteria* possess strong antioxidant activity and a strong ability to inhibit plasma lipid peroxidation (Lin & Chang 2000).

In practice, soybean oil is predominantly used in the preparation of most popular fried foods in Egypt as well as many other countries because it is cheap, healthy, and has a high smoke point. Soybean oil is free from cholesterol, rich in antioxidants, and contains high levels of unsaturated and essential FAs, which play important roles in maintaining good health. Although soybean oil has a good nutritional profile, it has less oxidative stability than other edible oils. Repeated heating causes thermal oxidation and changes the oil composition (Mounts et al. 1988).

This study aimed to evaluate the serum lipid profile and oxidative stress status in male rats that were fed thermally oxidized soybean oil (TO) with or without probiotic *Bifidobacteria* (TO±Pro) for 12 weeks. The physicochemical characteristics of soybean oil before and after the thermal processing were also determined by monitoring FA composition, peroxide value (PV), anisidine value (AnV), total oxidation value (TOTOX), free FA (FFA) content, acid value (AV), and TBARS levels.

Materials and methods

Oil thermal treatment

Commercially refined fresh soybean oil (control) was purchased from a local market in Alexandria, Egypt, in April 2009. TO was prepared in a glass conical flask filled with 2 L of oil and heated for five cycles of 20-min thermal treatment at 185°C and 2-h cooling in an electric oven/day for 5 days. At the end of each day, the oven was shut off, and the oil was left to cool overnight. Both control and TO samples were stored at –20°C for oil quality analysis.

Physical and chemical parameters of oil quality

Official methods were used to characterize the quality of the control soybean oil and TO (AOAC 1984).

FA composition

FAs were methylated following the AOCS Official Method (Ce 1–62). The resulting FA methyl esters (FAMEs) were analyzed on a GC Ultra gas chromatograph (Thermo Electron Corporation, Rodano, Italy) using a fused silica capillary column (100 m × 0.25 mm × 0.25 mm). Hydrogen was used as the carrier gas with a flow rate of 1.5 mL min^{-1}. The column temperature was programmed from 70 to 160°C at 25°C min^{-1} and held for 30 min and then further programmed to 210°C at 3°C min^{-1}. The starting and final temperatures were held for 5 and 30 min, respectively. The detector temperature was set at 250°C. The FAME samples, 5 μL, were injected with an AS 3000 autosampler. FAs were identified by comparison of retention time with authentic standards. The results are expressed in the relative percentage of each FAME, calculated by internal normalization of the chromatographic peak areas (Radwan 1978).

Infrared spectroscopic determination of total TFAs

The level of TFAs was assessed in the control and TO according to International Organization for Standardization method 15304. A thin film of the control or TO (50 μL) was pressed down on the zinc selenide crystal surface of the attenuated total reflection—Fourier transform infrared (FTIR) spectroscope. A 128-scan single-beam FTIR spectrum at 4 cm^{-1} resolution was collected for each sample and saved. Triolein (C18:1, 9-*cis*) and trielaidin (C18:1, 9-*trans*) were used as standards to design the calibration set. For each of the calibration standards and the oil samples, the absorbance spectrum was displayed in the expanded wave number range of 1050–900 cm^{-1}. The area under the 966 cm^{-1} band between the 990 and 945 cm^{-1} limit was electronically integrated. A calibration curve was created by performing a linear regression analysis of the area under the 966 cm^{-1} band versus the amount of trielaidin (as percent of total fat) in the calibration standards. Using the slope and intercept of the linear regression equation generated for the calibration standards, the *trans* level (as percent of total fat) for each test sample was calculated by substituting the *trans* band integrated area into the equation: % *trans* = (area – intercept)/slope.

AV

AV was determined using AOAC method (3a-36). Oil (5 g) was weighed, and 50 mL of hot neutral alcohol was added with a few drops of phenolphthalein. The mixture was shaken vigorously and titrated with 0.05 M alcoholic potassium hydroxide solution with constant shaking until the pink color remained permanently. The percent of FFAs was calculated from the following equation: AV = % FFAs (as oleic) × 1.99.

Peroxide value

The peroxide value (POV), expressed in milliequivalents of active oxygen per kilogram (mEq O_2/kg), was determined as follows. A mixture of soybean oil and chloroform/acetic acid 2:3 (v/v) was left to react in the dark with a saturated potassium iodine solution, and the free iodine titrated with a sodium thiosulfate standard solution (AOAC method: cd8-53).

p-Anisidine test

p-AnV were determined on a Beckman DU spectrophotometer. Absorption readings were made at 350 nm in 1-cm cells. The analytical reagent is 1 mL of 0.25% anisidine in glacial acetic acid; it was allowed to react for 10 min at room temperature in the dark. The AnV is defined as 100 times the absorbance measured in a 1-cm cell of a solution resulting from the reaction of 1 g fat with 100 mL mixture of isooctane solvent and reagent (AOAC method: Cd 18-90).

TOTOX

The total oxidant value (TOTOX) was used to describe total oxidation to which oil has been exposed. It is calculated using the following equation: TOTOX = 2PV + AnV.

TBARS

The amounts of TBARS were determined according to AOAC method (Cd 19-90). Oil (0.1 mL) was mixed with water (0.9 mL) and TBA reagent (2.0 mL, 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (1000*g*) for 15 min. The absorbance was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane.

Experimental protocol of animal studies

A total of 24 male Sprague-Dawley weanling rats (21 days old) weighing 60 ± 4 g were obtained from the College of Pharmacy's Animal House, Alexandria, Egypt. The animals were randomly divided equally into four groups. For 12 weeks, the rats were given diets containing 18% protein and 10% of either fresh soybean oil control, TO, TO+Pro, or *Bifidobacteria* BB-12 only (Pro). The vitamin and mineral contents of the diets were adjusted as a standard diet that meets the nutrient requirements for growing rats. *Bifidobacteria* BB-12 were produced as freeze-dried powder by CHR HANSEN Company and kindly donated to our laboratory by Prof. Mona Massoud of the Agriculture Research Center in Alexandria, Egypt. The level of *Bifidobacteria* was 0.07 g/100 g body weight. Fresh preparation of *Bifidobacteria* dissolved in distilled water was prepared daily and administrated as a 1-mL single dose by gavage feeding. All animals were kept in stainless-steel cages at a room temperature of 27 ± 2 °C and were quarantined for 2 weeks before the introduction of the test diets. All of the animals were allowed food and water *ad libitum* throughout the experiment. Food intake and body weight gains were determined weekly. The animal care local committee approved the design of the experiment, and the protocol conformed to the guidelines of the National Institutes of Health for animal care and handling.

Blood and tissue preparation

After 12 weeks, the rats were fasted overnight then ether-anesthetized and sacrificed by cervical decapitation. Blood samples were collected from the jugular vein in chilled nonheparinized tubes. Serum samples were obtained by centrifugation at 860*g* for 20 min at 4°C and stored at −20°C until assayed. Liver, kidney, testes, and brain were immediately removed; weighed, and washed using chilled saline solution. Tissues were minced and homogenized separately (10% w/v) in ice-cold 1.15% KCl 0.01 M potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000*g* for 20 min at 4°C, and the resultant supernatant was stored at −70°C until used for various enzyme assays.

Serum lipid profile assays

Serum was assayed for total lipids (TL) using the method of Knight et al. (1972) and total cholesterol by the method of Carr et al. (1993). HDL and LDL levels were determined according to the methods of Warnick et al. (1983) and Bergmenyer (1985), respectively.

Oxidative stress measurements

Serum, liver, kidney, testes, and brain samples were assayed for glutathione reductase (GR; EC 1.6.4.2) activity according to Mannervik and Carlberg (1985). TBARS were measured in plasma, liver, kidney, testes, and brain at 532 nm using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA) and an extinction coefficient of 156,000 M^{-1} cm^{-1} as described by Tappel and Zalkin (1959). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to Nishikimi et al. (1972).

Statistical analysis

The collected data were recorded, analyzed, and expressed as the means ± SE (standard error). The significance of the differences among experimental groups was tested by analysis of variance or paired and unpaired Student's *t*-tests, as appropriate. A p value of 0.05 was considered statistically significant.

Results

Chemical characteristics of soybean oil

The quality of the control and TO is shown in Table 1. Several indicators of oil quality deterioration became clear in TO, which possessed a higher FFA value (% FFA) (15-fold), AV (14-fold), peroxide value (8-fold), *p*-AnV (39-fold), TOTOX value (19-fold), and TBARS value (8.5-fold) than the control sample. The peroxide value (PV) represents the levels of peroxide and other similar oxidation products in oil. The *p*-AnV primarily measures the level of secondary oxidation products, namely α- and β-unsaturated aldehydes. Consequently, PV in conjunction with AnV can be used to map past and future degradation profiles presented as a TOTOX value, which is useful for quantifying oxygen-directed oil degradation. In addition, the FFA value as an indication of the degree of triacylglycerol hydrolysis is considered to be a good indicator of oil quality. The data presented in Table 2 shows that palmitic (16:0), oleic (18:1), and linoleic [18:2 (*n*-6)] acids are the major FAs in both control and TO. Due to the loss of polyunsaturated FAs by oxidation during thermal treatment of the oil, TO had lower proportion of linoleic acids (–17.38%) and linolenic acid (–53.95%) than control whereas higher proportions of saturated FAs (SFA) (+26.32%) and oleic acid (+25.91%) were also detected in TO than the control. The total contribution of TFA isomers in TO increased (2.5-fold) from 2.4 to 5.9%

Table 1. Fatty acid composition of fresh (control) and thermally oxidized (TO) soybean oil.

Fatty acid%	Symbol	Control[a]	TO[a]	% Change
Myristic	C14:0	0.18 ± 0.02	0.44 ± 0.05*	+59.23
Palmitic	C16:0	11.07 ± 2.6	14.90 ± 0.1.4*	+25.70
Stearic	C18:0	3.98 ± 1.1	5.33 ± 1.3*	+25.33
Oleic	C18:1	23.08 ± 2.7	31.15 ± 0.9*	+25.91
Linoleic	C18:2 (*n*-6)	54.09 ± 3.1	44.70 ± 3.0*	–17.38
Linolenic	C18:3 (*n*-3)	7.60 ± 0.9	3.50 ± 0.6*	–53.95
SFA		15.23 ± 1.62	20.67 ± 1.77*	+26.32
MUFA		23.08 ± 2.7	31.15 ± 0.9*	+25.91
PUFA		61.69 ± 2.1	48.17 ± 3.2*	–21.92
SFA/UFA		0.18 ± 0.03	0.26 ± 0.04*	+30.77
TFA		2.4 ± 0.51	5.9 ± 1.54*	+59.32

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, *trans*-fatty acids; UFA, unsaturated fatty acids.

[a]All values are relative percentage averages of triplicate analyses ± SE.

*Significantly different from fresh soybean oil, $p < 0.05$.

Table 2. Chemical characteristics of fresh (control) and thermally oxidized (TO) soybean oil.

Parameter	Control	TO
Free fatty acid content (%)	0.12 ± 0.02	1.85 ± 0.09*
Acid value (g KOH/kg)	0.26 ± 0.03	3.64 ± 0.28*
Peroxide value (meq/kg)	1.89 ± 0.39	14.00 ± 3.12*
p-Anisidine value	2.23 ± 0.75	87.41 ± 2.76*
TOTOX value	6.01 ± 1.67	115.41 ± 4.32*
TBARS (mg MDA/kg)	0.14 ± 0.04	1.19 ± 0.28*

TBARS, thiobarbituric acid–reactive substance; TOTOX, total oxidant value.

[a]All values are averages of triplicate analyses ± SE.

*Significantly different from control, $p < 0.05$.

Body weight gain and food efficiency

The inclusion of TO in the diet impaired to some extent the growth of the animals (Figure 1). A significant decrease ($p < 0.05$) in body weight gain was observed in the TO group (145.66 ± 10.4 g), which was significantly ($p < 0.05$) restored in TO+Pro group (169.67 ± 13.86 g), compared to control group (188.67 ± 11.89 g). No significant differences in body weight gain were detected between the Pro (197.33 ± 2.06 g) and control groups. The average food intake was 16.6 ± 1.5 g/day/rat in all of the dietary experimental groups, and the food efficiency values were 11.37 ± 1.19, 8.78 ± 1.04, 11.89 ± 0.26, and 10.22 ± 1.39 for the control, TO, Pro, and TO+Pro groups, respectively. Rats that were fed TO or Pro diet had significantly ($p < 0.05$) higher liver weights compared with rats that were fed the control diet (Figure 2). In contrast, rats that were fed TO+Pro had liver weights similar to the normal weight observed in the control group. No significant

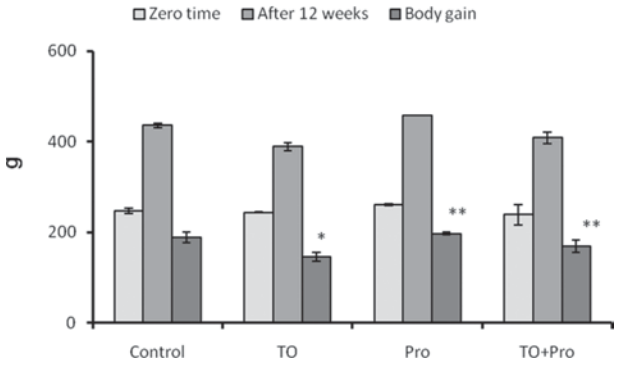

Figure 1. Weights of animals fed a diet containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ±SD (n = 6). *Significantly different from control oil, **significantly different from TO at $p > 0.05$.

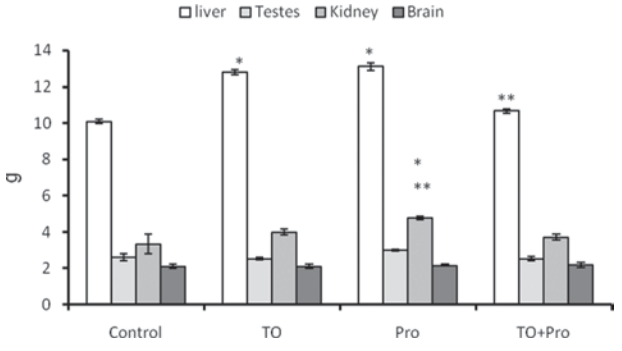

Figure 2. Organ weights of animals fed a diet containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ± SD ($n = 6$). *Significantly different from control oil, **significantly different from thermally oxidized oil at $p < 0.05$.

changes were detected in the testes or brain weights of the groups, whereas a significant increase ($p < 0.05$) was observed in the kidney weight of the Pro group compared to the control group.

Serum lipid profile

Figure 3 demonstrates the effects of the control, TO, Pro, and TO+Pro diets on rats' serum lipid profiles. No significant differences were detected in the TL amounts among the tested groups. Elevated values of total serum cholesterol and LDL levels and reduced HDL levels were measured in animals that were fed a TO diet when compared to the control diet. The total cholesterol, LDL, and HDL levels did not show significant ($p < 0.05$) differences between the control, Pro, and TO+Pro groups. The HDL/LDL ratios were 0.4, 0.2, 0.36, and 0.37 for the control, TO, Pro, and TO+Pro groups, respectively.

Oxidative stress parameters

Figure 4 shows that the diets containing TO caused significant ($p < 0.05$) increases in the TBARS levels in liver (53.42 ± 1.8 MDA nm/g tissue), kidney (55.12 ± 7.2 MDA nm/g tissue), testes (36.49 ± 1.12 MDA nm/g tissue), and brain (15.11 ± 1.9 MDA nm/g tissue) compared to the control group. The supplementation of probiotic *Bifidobacteria* to the TO diet in the Pro+TO animals restored the TBARS levels in liver (46.85 ± 1.6 MDA nm/g tissue) and testes (15.71 ± 1.6 MDA nm/g tissue) but reduced it in kidney (37.12 ± 5.1 MDA nm/g tissue) and brain (2.71 ± 0.17 MDA nm/g tissue) compared with the control diet. Figure 5 illustrates that animals fed TO possessed high levels of SOD activities in liver (30.36 ± 0.65 U/mg protein) and brain (3.17 ± 0.55 U/mg protein) compared with the control diet. The addition of probiotic *Bifidobacteria* to the TO diet restored the SOD levels in liver (17.22 ± 0.48 U/mg protein) and brain (1.44 ± 0.16 U/mg protein). Figure 6 shows that the TO diet caused significant ($p > 0.05$) increases in GR activities in liver (57.21 ± 2.42 U/g protein) and kidney (72.78 ± 1.08 U/g protein) compared with corresponding values in the control group, whereas the addition of probiotic *Bifidobacteria* to the TO diet decreased the GR

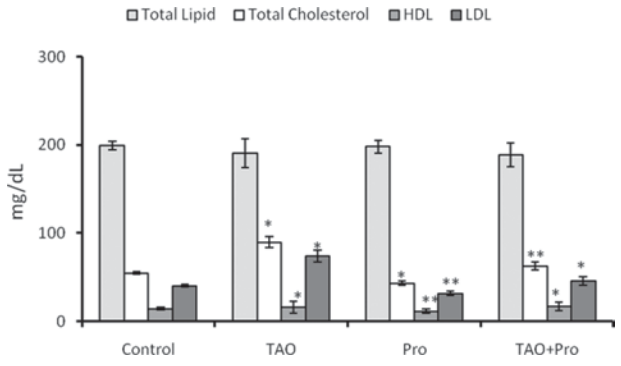

Figure 3. Serum total lipids, total cholesterol, HDL, and LDL of animals fed diets containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ± SD ($n = 6$). *Significantly different from control oil, **significantly different from oxidized abused oil at $p < 0.05$. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

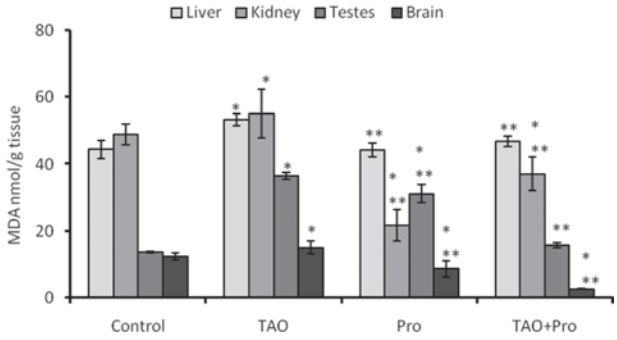

Figure 4. Liver, brain, kidney, and testes TBARS of animals fed diets containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ± SD ($n = 6$). *Significantly different from control oil, **significantly different from TO at $p < 0.05$.

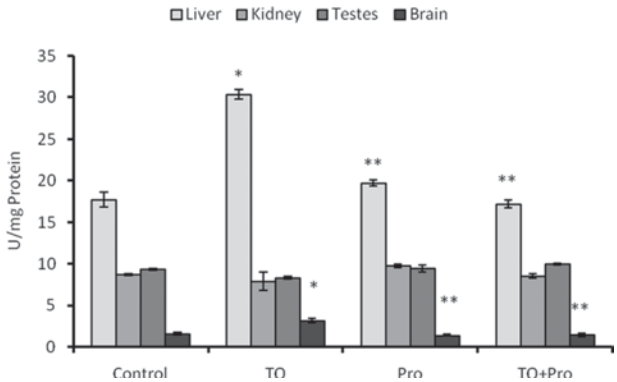

Figure 5. Superoxide dismutase (SOD) activity in liver, brain, kidney, and testes of animals fed diets containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ± SD ($n = 6$). *Significantly different from control oil, **significantly different from TO at $p < 0.05$.

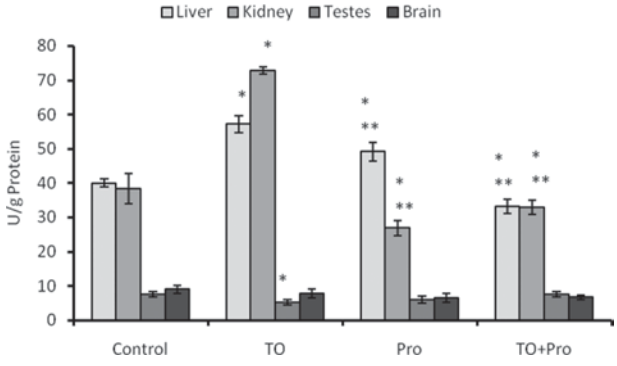

Figure 6. Glutathione reductase (GR) activity in liver, brain, kidney, and testes of animals fed diets containing fresh soybean oil (control), thermally oxidized soybean oil (TO), probiotic *Bifidobacteria* (Pro), or TO with Pro (TO+Pro) for 12 weeks. Values are expressed as the means ± SD ($n = 6$). *Significantly different from control oil, **significantly different from thermally oxidized oil at $p < 0.05$.

activities in liver (33.80 ± 2.04 U/g protein) and kidney (32.94 ± 2.17 U/g protein) to levels similar to the control group.

Discussion

The ingestion of thermally oxidized oil affects several physiological functions in rats such as poor appetite (Clark & Serbia 1991), growth retardation (Akiya et al. 1973), hepatic and renal lesions (Alexander 1978), metabolism of serum lipoprotein and liver lipids (Eder et al. 2004), and even death (Kaunitz 1967; Pax et al. 1992). The present study demonstrated that the final body weight, weight gain, and food efficiency of rats in the TO group were lower than in the control group, although the difference in food intake between all groups was insignificant. In addition, a significant increase in liver weight was reported in TO rats compared with the control rats. Similar results were obtained when growing male Wistar rats were fed a diet containing 7% heated soybean oil. Significant reductions in the final body weight, weight gain, and food efficiency were reported even though food intake did not differ from the control group (Burenjargal & Totani 2008). Heated soybean oil was reported to accelerate gastrointestinal tract content transfer so that the contents of the colon were actively excreted, resulting in remarkable weight loss even though the amounts of food ingested were the same. This study showed that the weight loss–promoting effects of heated soybean oil were not attributable to the inhibition of digestive enzyme activities but is probably due to insufficient nutrient absorption because of the rapid transfer of gastrointestinal content. Interestingly, our data showed that the TO+Pro diet restored the body weight gain, food efficiency and liver weight values to their normal levels, suggesting that this probiotic *Bifidobacteria* was involved in regulating gastrointestinal movements by improving the efficiency of bowel movements.

Dietary fat intake is positively correlated with serum total cholesterol value and morbidity from coronary heart disease (Kris-Etherton and Yu 1997). Specifically, the FA composition of food is associated more with variations in the plasma total cholesterol concentration than the amount of fat consumed. The general picture shows that SFAs increase the plasma total cholesterol level and thus increase the risk of coronary heart disease, while unsaturated FAs have the opposite effect (de Lorgeril et al. 1999; GISSI 1999). Compared with control oil, data presented in Table 1 show that the ratio between SFAs/unsaturated FAs and the percentage of TFA content in TO have been increased by 30.77 and 59.32%, respectively, due to the thermal oxidation processing. In addition, Figure 2 shows that the TO diet not only increased the total serum cholesterol level but also reduced the HDL/LDL ratio compared with the control diet. Thus, it is our view that the high concentrations of serum total cholesterol and LDL observed in the TO group may be explained by the high ratio of SFAs/unsaturated FAs and the high content of TFAs in TO diet. Recent epidemiological studies indicate that TFAs may increase coronary heart disease risk by raising the serum cholesterol level and reducing the particle size of LDL-cholesterol (Mensink et al. 2003), each of which may further raise the risk of coronary heart disease. Katan et al. (1995) found that LDL-cholesterol is increased by about 1.5 mg/dL and HDL cholesterol is decreased by approximately 0.5 mg/dL for every additional percentage of TFAs in the diet. Therefore, therapeutic lifestyle changes including dietary interventions, in particular a reduction of saturated fat and cholesterol, are established as a first line therapy to reduce LDL-cholesterol. A change in dietary habits, such as eating fermented products containing lactic acid bacteria, can reduce cholesterol. Since the early study of Mann and Spoerry (1974), the cholesterol-lowering potential of lactic acid bacteria such as *Lactobacillus* and Bifidobacterium has been commonly studied *in vitro* and *in vivo* in both experimental and animal subjects (Klaver et al. 1993; Tahri et al. 1996; Xiao et al. 2003). Interestingly, our data showed that the diet that containing TO+Pro had a better positive impact than TO diet on the serum lipid profile by reducing the total cholesterol level and increasing the HDL/LDL ratio to levels similar to the corresponding levels in the control group. A previous study reported that probiotic *Bifidobacteria* significantly reduced serum cholesterol in rats and provided evidence that sonication-killed cells of *Bifidobacteria* have a great potential to be used as a cholesterol-lowering agent (Shin et al. 2010). Cholesterol reduction by lactic acid bacteria can be explained by five mechanisms: (a) fermentation products of lactic acid bacteria inhibit cholesterol synthesis enzymes and thus reduce cholesterol production; (b) the bacteria facilitate the elimination of cholesterol in feces; (c) the bacteria inhibit the absorption of cholesterol back into the body by binding with cholesterol; (d) the bacteria interfere with the recycling of bile salt, a metabolic product of cholesterol, and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in low serum cholesterol level; and (e) assimilation of lactic acid (Do et al. 2009).

The data presented in Figures 4–6 show that the TO diet affects the oxidative status in terms of elevated levels of TBARS in liver, kidney, testes, and brain, of SOD activities in liver and brain, and of GR activities in liver and kidney compared to control levels. Oxidative stress reported in the TO group was reduced in animals that were fed Pro+TO, which were supported by the antioxidant effects of the *Bifidobacteria* species (Picard et al. 2005). Animals that were fed a diet containing deep frying oil exhibited induced CYP-450 activity in the liver (Lamboni & Perkins 1996; Pandey et al. 2006). Reactive oxygen species generated as intermediates during the metabolic activation of different xenobiotics by CYP-450 enzymes may play an important role in the oxidative status (Bernhardt 1996). They may also be responsible for lipid peroxidation

processes and eventually result in DNA strand breakage (Cavalieri & Rogan 1995). *Bifidobacteria* species possess excellent antioxidant activity by inhibiting linoleic acid peroxidation, scavenging α,α-diphenyl-β-picrylhydrazyl free radicals and then protecting plasma lipids from oxidation (Lin & Chang, 2000). Recently, Xiao et al. (2011) reported the first proteomic information about the antioxidant response of *Bifidobacteria* to oxygen stress through the involvement of oxidative stress–protective proteins and DNA oxidative damage–protective proteins in the defense strategy of these probiotic bacteria.

In conclusion, our data suggest the involvement of probiotic *Bifidobacteria* in the modifications of the serum lipid profile and oxidative stress biomarkers generated in animals that were fed TO.

Declaration of interest

The author declares that there are no conflicts of interest.

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