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Seed oils improve lipid metabolism and increase antioxidant potential in rats fed diets containing cholesterol

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Abstract

The goal of this investigation was to find the most valuable among four often-used seed oils for atherosclerosis preventing diets. Fatty acids, sterols, antioxidant compounds, stability and total radical-trapping antioxidative potential (TRAP) in sunflower, sunflower high oleic, rapeseed and grapeseed oils were determined. The highest stability and the highest TRAP (3.8 Rancimat 120°C, hours and 324 nmol/ml) and the lowest stability and the lowest TRAP (2.4 Rancimat 120°C, hours and 201 nmol/ml) were in rapeseed and sunflower oils, respectively. The effect of these two seed oils on

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lipid metabolism and antioxidant activity was investigated on 60 (divided in six diet groups of 10) male Wistar rats adapted to cholesterol-free or 1% cholesterol diets. The control group (Control) consumed basal diet (BD) only. To the BD were added 10g/100g rapeseed (Rapeseed group) or sunflower (Sunflower group) oils, 1 g/100 g cholesterol (Chol group) or both (Chol/Rapeseed group) and (Chol/Sunflower group). The experiment lasted 4 weeks. In the Chol/Rapeseed and Chol/Sunflower vs. Chol group, the oil supplemented diets significantly ($P < 0.05$) hindered the rise in plasma lipids due to dietary cholesterol. Rapeseed and to less degree sunflower oils have increased the plasma antioxidant activity in rats fed BD without cholesterol (an increase in TRAP: 20.8% and 16.0% and a decrease in MDA: 22.0% and 14.9%, respectively). In the rats of Chol/Rapeseed and Chol/Sunflower vs. Chol diet group the added oils significantly hindered the decrease in the plasma antioxidant activity (TRAP: 21.7% and 16.3% and MDA: 26.2% and 21.5%, respectively). Therefore, rapeseed and to less degree sunflower oils possess hypolipidemic and antioxidant properties. It is more evident when these oils are added to the diets of rats fed cholesterol. In conclusion, rapeseed oil has high organoleptic properties and the highest antioxidant capacity. Its influence on plasma lipid levels and antioxidant potential is significantly higher than of oils with relatively low antioxidant capacity. The above-mentioned properties make rapeseed oil preferable choice for atherosclerosis preventing diets. © 2003 Elsevier Science Inc. All rights reserved.

Keywords: Seed oils; Fatty acids; Sterols; Antioxidants; Rats; Lipoproteins; Antioxidant capacity

1. Introduction

Despite the successes in prevention of atherosclerosis, coronary artery disease (CAD) is still responsible for one of every three cases of death [1]. It was shown that in every atherosclerosis lesion of patients and experimental animals there are cholesterol plaques [2,3]. The plaques' lipids are derived from plasma oxidized low-density lipoprotein cholesterol [4]. Thus, prevention of atherosclerosis is a fight against low-density lipoprotein (LDL-C) oxidation [5]. It is known that nutritional antioxidants and, especially phenolic substances, prevent lipid peroxidation [6]. In the last years some authors have emphasized the role of Mediterranean diet in prevention of some diseases including atherosclerosis [7,8]. These authors attributed the positive influence of such diets to their low saturated and high monounsaturated fatty acids content. Diets low in saturated fatty acids and high in monounsaturated fatty acids are effective in controlling blood lipid levels [9]. When a typical diet high in saturated fat is replaced with a southern Mediterranean-type diet, plasma cholesterol levels were decreased [7]. Experiments *in vitro* and on laboratory animals have demonstrated that LDL-C oxidation is inhibited by olive oil constituents [10,11]. Investigations of hyperlipidemic subjects have demonstrated that dietary oils affect lipid peroxidation and antioxidant levels, and lead to favorable changes in the lipid status [10,12].

The results of these investigations arouse well-founded interest in vegetable oils. Most authors claim that olive oils are preferable [10,11]. However, recently published investigation of Cabrini et al. 2001 [13] demonstrated that the highest total radical-trapping antioxidant parameters (TRAP) were in sunflower oil, whereas the TRAP values of olive oils were very low. These data are very reliable: extra virgin olive and seed oils were taken from a local oil mill, produced in the same month. Therefore, we decided to investigate four widely used

seed oils (sunflower, sunflower high oleic, rapeseed and grapeseed). As was stated, the goal of this investigation was to find the most valuable among often used seed oils for atherosclerosis preventing diets. Therefore, from the four *in vitro* investigated seed oils, we decided to use two (with high and low antioxidant potentials) for experiment *in vivo* on rats fed cholesterol-containing and cholesterol-free diets.

2. Methods and materials

2.1. Chemicals

All reagents were of analytical grade. Deionized and distilled water was used throughout. All used chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Samples

All samples of the refined sunflower, sunflower high oleic, rapeseed and grapeseed oils were purchased in supermarkets.

2.3. Determination of the fatty acids

Fatty acids were determined following the analytical methods described in Regulations EEC/2568/91 and C/1429/92 of European Union Commission (EC 1991 Commission regulation EC No 2568/91 July 11, 1991. Official EC Journal L 8121. 10. 1991) [14].

2.4. Determination of sterols

The content of sterols was done according to the Regulations EEC/2568/91 and C/1429/92 of European Union Commission, as described by Gutiérrez, Varona, & Albi, 2000 [15]. The results of the determination of fatty acids and of individual sterols are in %.

2.5. Determination of phenolic compounds

All phenolic compounds were isolated by extraction of a solution of oil in hexane with water-methanol 60:40 for three times. Folin-Ciocalteu reagent and sodium molybdate 5% in ethanol 50% reagent, respectively, were added to suitable aliquots of the combined extracts. The absorption of the solution at 725 nm (phenolic compounds) and 370 nm (orthodiphenolic compounds) was measured on a spectrophotometer (Hewlett-Packard 8450 A UV/vis). Results are given as mg/kg of caffeic acid [16].

2.6. Determination of tocopherols

Tocopherols were evaluated following the IUPAC Standard Method (IUPAC, 1992).

2.7. Determination of the stability of oils

Stability was evaluated by measuring the oxidation induction time, with the use of the Rancimat apparatus (Metrohm CH 9100). A flow of air (10 L/h) was bubbled through the oil heated at 100°C, and the volatile compounds were collected in cold water, increasing the water conductivity. The time to reach a fixed level was recorded [17].

2.8. Determination of the total radical-trapping antioxidative potential (TRAP)

The extracts were obtained in an organic solvent. 0.5 ml of oil sample was mixed with 2 ml of acetone and shaken for 1 hour. The extracts were separated from oils in a freezer (–25°C, 1 hour).

The principle of the method was described previously [18,19]. The results obtained are expressed as nmol of peroxy radicals trapped by 1 ml of acetonic extract. Acetone was verified to have negligible TRAP.

2.9. Malondialdehyde (MDA) assay

The concentration of MDA, an index of lipid peroxidation, was determined spectrophotometrically [18]. The reaction mixture consisting of 1% thiobarbituric acid in 10% trichloroacetic acid in the ratio of 1:2 (v/v) was added to the samples. The samples were incubated in a water bath (100°C) for 20 min. After being cooled, 4 ml of n-butanol were added and the mixture was shaken vigorously. The samples were centrifuged (10 min, 2000 g) and the absorbance of upper layer was measured spectrophotometrically at 532 nm. 1,1,3,3-tetraethoxypropane in the final concentration of 0.1 μ M was used as a standard. Lipid peroxidation was expressed in nmol of thiobarbituric acid reactive substances per 1 ml of the sample.

2.10. Rats and diets

The experiment lasted 4 wks [20,21]. The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland) provided male Wistar rats ($n = 60$) with a mean weight of 120 g. They were housed individually in stainless steel metabolic cages and were divided into 6 groups of 10.

Six groups were fed basal diet (BD), which included wheat starch, casein, cellulose, mineral and vitamin mixtures. Control (Control) group was fed BD and control peanut oil in concentration of 10 g/100 g. The other five groups were named Rapeseed, Sunflower, Chol, Chol/Rapeseed and Chol/Sunflower. To BD of these groups were added 10 g/100 g rapeseed (Rapeseed) or sunflower (Sunflower) oils, 1 g/100 g nonoxidized cholesterol and control peanut oil in concentration of 10 g/100 g (Chol), or both (Chol/Rapeseed) and (Chol/Sunflower). As TRAP test has shown, that the antioxidant capacity of oil peanut oil was minimal. The cholesterol batches were mixed carefully with the basal diet (1:99) just before the diets were offered to the rats. The dietary cholesterol was checked according to the HPLC method and was found not to contain cholesterol oxides. The exact compositions of the diets are presented in Table 1.

Table 1
Composition of the diets (g/kg diet)

Ingredients	Control	Chol	Rapeseed	Chol/Rapeseed	Sunflower	Chol/Sunflower
Wheat starch	693	683	693	683	693	683
Casein	150	150	150	150	150	150
Peanut oil	100	100	0	0	0	0
Rapeseed oil	0	0	100	100	0	0
Sunflower oil	0	0	0	0	100	100
Cholesterol	0	10	0	10	0	10
Cellulose	10	10	10	10	10	10
Vitamin mixt*	10	10	10	10	10	10
Mineral mixt	37	37	37	37	37	37

* Abbreviation: mixt, mixture.

The diets contained, as percentage of energy, 61% carbohydrates, 26% of fat and 13% protein. The calculated energy values of all diets were not significantly different.

All rats consumed food ad libitum once a day beginning at 10⁰⁰ h. They had unrestricted access to drinking water. Diet intake was monitored daily. Before the experiment the blood samples were drawn from the tail vein. At the end of the experiment the rats were anesthetized using diethyl ether. Blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. After anesthesia, the abdomen was opened to take samples of the liver for determination of TC. The weight gain of the rats was recorded on a weekly basis. Two time points were used in this experiment: before and after 4 wks of feeding. At these points wide range of laboratory tests was performed. Total plasma (TC) and liver cholesterol was determined with Randox kit reagents No Cat. CH 280, Appl No 7. [22], HDL-cholesterol (HDL-C)—according to Izawa et al. [23], LDL-cholesterol (LDL-C)—using Friedewald et al. method [24], triglycerides (TG)—with Randox kit reagents No Cat. 1697, Appl No 8. [25] and total phospholipids (TPH)—with ANALCO kit reagents No Cat. A-161 [26].

2.11. Statistical analysis

The results are expressed as means \pm SD of five measurements. To compare several groups analysis of variance (ANOVA) was used. Where it was appropriate, data by 2-way ANOVA were tested. Spearman correlation coefficient (R) and p-value were used to show correlations and their significance. P values of < 0.05 were adopted as statistically significant.

3. Results

3.1. In vitro

3.1.1. Fatty acids

The amounts of fatty acids content in all oil samples are summarized in the Table 2. As was expected, oleic acid is a dominant fatty acid in sunflower high oleic oil (81%), whereas

Table 2
Content of fatty acids in the studied seed oils* (in %)

Oils	Myristic 14:0	Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Sunflower	0.06 ± 0.005	6.0 ± 0.61	0.12 ± 0.012	3.80 ± 0.390	30.0 ± 3.20	54.0 ± 5.51	0.07 ± 0.008
Sunflower HO**	0.04 ± 0.004	3.7 ± 0.35	0.09 ± 0.010	3.40 ± 0.350	81.0 ± 8.20	10.0 ± 1.10	0.07 ± 0.008
Rapeseed	0.10 ± 0.010	5.0 ± 0.49	0.20 ± 0.020	2.00 ± 0.210	57.0 ± 5.80	22.0 ± 2.31	10.00 ± 1.100
Grapeseed	0.04 ± 0.004	6.8 ± 0.69	0.10 ± 0.010	4.20 ± 0.430	17.5 ± 1.80	69.5 ± 7.10	0.30 ± 0.030

* Values are means ± SD of five measurements.

**Abbreviation: HO, high oleic.

the highest content of linoleic acid is in rapeseed oil (69.5%). Also in sunflower oil the content of linoleic acid is relatively high (54%), but significantly lower than in rapeseed oil ($p < 0.05$).

3.1.2. Sterols

According to some publications, cholesterol also occurs in small amount in plants [27]. Table 3 summarized the results of the individual sterols. As can be seen, β -sitosterol dominates the sterol fraction, and in all samples exceeds 60% (in rapeseed oil its content is 82%). Two among 4 studied oils (rapeseed and grapeseed oils) are cholesterol-free.

3.1.3. Stability, antioxidant compounds and TRAP

Antioxidant compounds, stability and TRAP in the studied oils are summarized in Table 4. The highest stability and the highest TRAP (3.8 Rancimat 120°C, hours and 324 nmol/ml) and the lowest stability and the lowest TRAP (2.4 Rancimat 120°C, hours and 201 nmol/ml) were in rapeseed and sunflower oils, respectively. Only the content of polyphenols is higher in rapeseed oil. Therefore, it could be suggested that the content of polyphenols plays dominant role in the antioxidant potential of seed oils. The high correlation between TRAP and total phenols confirms this suggestion ($R^2 = 0.9739$).

The kinetics of the studied oils is reflected in Fig. 1. It is obvious from the picture that there are significant differences among samples labeled in graph as Rapeseed and Sunf (Sunflower) oils. Sunflower has the lowest antioxidative activity in comparison with the other samples.

Table 3
Content of sterols in some seed oils* (in %)

Oils	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β -sitosterol	Δ -7-stimasterol
Sunflower	0.6 ± 0.04	0	10.4 ± 0.9	11.3 ± 0.9	62.0 ± 6.1	15.7 ± 2.1
Sunflower HO**	0.6 ± 0.04	0	10.0 ± 0.9	11.2 ± 0.9	62.2 ± 6.1	16.0 ± 2.2
Rapeseed	0	10.0 ± 0.9	27.0 ± 2.3	0	63.0 ± 6.2	0
Grapeseed	0	0	10.0 ± 0.9	8.0 ± 0.8	82.0 ± 8.3	0

* Values are means ± SD of five measurements.

**Abbreviation: HO, high oleic.

Table 4

Some antioxidant compounds (mg/kg), stability (Rancimat 120°C, hours) and TRAP (nmol/ml) in studied seed oils*

Oils	α -tocopherols	β -tocopherols	γ -tocopherols	Polyphenols	Stability	TRAP
Sunflower	529 \pm 23.2	19 \pm 1.3	15 \pm 1.1	1.73 \pm 0.2	2.4 \pm 0.3	201 \pm 11.2
Sunflower HO**	517 \pm 19.2	18 \pm 1.2	14 \pm 1.1	2.60 \pm 0.2	3.7 \pm 0.3	319 \pm 12.9
Rapeseed	313 \pm 21.2	16 \pm 1.0	33 \pm 3.2	2.80 \pm 0.2	3.8 \pm 0.3	324 \pm 13.2
Grapeseed	169 \pm 10.2	16 \pm 1.0	32 \pm 3.2	2.45 \pm 0.2	3.2 \pm 0.3	290 \pm 11.0

* Values are means \pm SD of five measurements.

**Abbreviation: HO, high oleic.

3.2. In vivo

Addition of oils or/and cholesterol to the diets did not affect food intake, body weight gain or feed efficiency (data not shown). At baseline, the six groups did not differ from one another in plasma lipid concentration (data not shown). The statistically evaluated results (ANOVA) of the changes in plasma lipid concentration after trial are summarized in the Table 5. The oil supplemented diet significantly hindered the rise in plasma lipids due to dietary cholesterol ($P < 0.05$): TC (3.60 vs. 4.82 mmol/L; -25.5% and 3.75 vs. 4.82 mmol/L; -22.2%); LDL-C (1.93 vs. 3.20 mmol/L; -40% and 2.11 vs. 3.20 mmol/L; -34%); TG (0.71 vs. 0.88 mmol/L; -19.3% and 0.73 vs. 0.88 mmol/L; -17.0%), and TC in liver (30.7 vs. 48.3 μ mol/g; -36.4% and 31.1 vs. 48.3 μ mol/g; -35.6%) for the Chol/Rapeseed and Chol/Sunflower groups vs. the Chol group, respectively (Table 5). Liver weight was 4.35 ± 0.4 g in all 6 groups. After 4 weeks of feeding the liver TC concentration in the rats of Chol/Rapeseed, Chol/Sunflower and Chol diet groups was 30.7 ± 0.25 , $31.1 \pm$

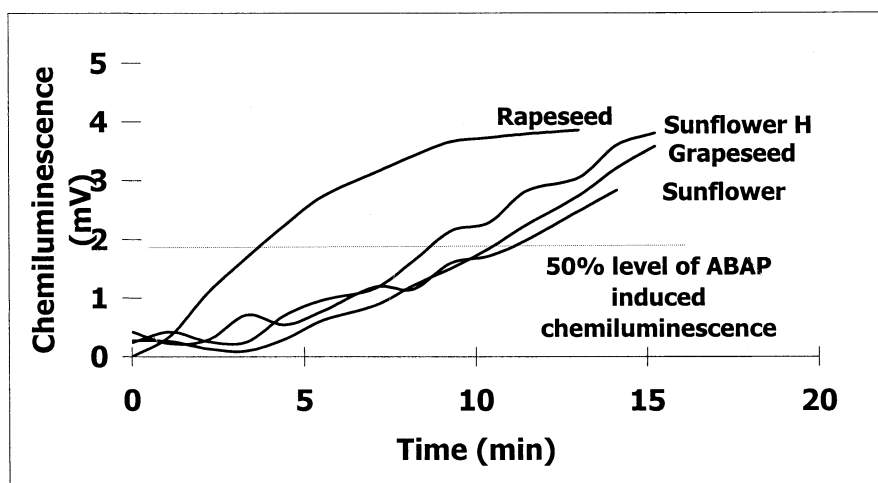


Fig. 1. Kinetics of ABAP-induced, luminol-enhanced chemiluminescence in the presence of sunflower, sunflower high oleic, rapeseed and grapeseed oils acetonic extracts as well as of the reference antioxidant Trolox. Dashed line represents control ABAP-induced CL.

Table 5

Plasma lipids and total cholesterol concentration in liver of rats fed diets with and without 1% cholesterol and with and without rapeseed and sunflower oils

Diets	Plasma lipids					Liver
	TC	LDL-C	HDL-C	TG	TPH	TC
	mmol/L					$\mu\text{mol/g}$
Control	2.84 \pm 0.15 ^c	1.22 \pm 0.05 ^c	1.62 \pm 0.07 ^a	0.69 \pm 0.04 ^b	1.76 \pm 0.08 ^a	5.86 \pm 0.24 ^c
Chol	4.82 \pm 0.21 ^a	3.20 \pm 0.12 ^a	1.61 \pm 0.07 ^a	0.88 \pm 0.05 ^a	1.74 \pm 0.08 ^a	48.3 \pm 0.31 ^a
Sunflower	2.85 \pm 0.15 ^c	1.21 \pm 0.05 ^c	1.63 \pm 0.07 ^a	0.71 \pm 0.05 ^b	1.72 \pm 0.08 ^a	5.78 \pm 0.24 ^c
Chol/Sunfl	3.75 \pm 0.18 ^b	2.11 \pm 0.05 ^b	1.63 \pm 0.07 ^a	0.73 \pm 0.05 ^b	1.37 \pm 0.06 ^b	31.1 \pm 0.25 ^b
Rapeseed	2.81 \pm 0.15 ^c	1.19 \pm 0.05 ^c	1.61 \pm 0.07 ^a	0.70 \pm 0.05 ^b	1.72 \pm 0.08 ^a	5.73 \pm 0.24 ^c
Chol/Rapes	3.60 \pm 0.18 ^b	1.93 \pm 0.05 ^b	1.66 \pm 0.07 ^a	0.71 \pm 0.05 ^b	1.31 \pm 0.06 ^b	30.7 \pm 0.25 ^b

2-way ANOVA (P-value)						
Sunflower	NS	NS	NS	NS	NS	NS
Rapeseed	NS	NS	NS	NS	NS	NS
Chol	<0.001	<0.001	NS	<0.001	NS	<0.0005
Chol/Sunfl	<0.050	<0.050	NS	<0.050	<0.010	<0.0005
Chol/Rapes	<0.050	<0.050	NS	<0.050	<0.010	<0.0005

*Values are means \pm SD, n = 10.

**Means in columns without letters in common differ significantly ($P < 0.05$).

***Abbreviations: Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NS, not significant; Rapes, Rapeseed oil; Sunfl, Sunflower oil; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids.

0.25 and 48.3 \pm 0.31 $\mu\text{mol/g}$, 5.24, 5.3 and 8.24 times higher than in Control group, respectively. The liver TC concentration in the Chol group was 57.3% and 55.3% higher than in Chol/Rapeseed and Chol/Sunflower, respectively ($p < 0.001$ in both cases). Therefore, oil supplemented diets significantly hindered the rise of TC in liver due to dietary cholesterol.

Rapeseed and sunflower oils in rats fed basal diet without cholesterol did not affect the lipid variables measured.

Rapeseed and to a less degree sunflower oil has increased the plasma antioxidant activity in rats fed basal diet without cholesterol: an increase in TRAP values (299.9 vs. 248.2; -20.8% and 287.9 vs. 248.2 nmol/ml; -16.0% , Fig. 2) and a decrease in MDA values (1.31 vs. 1.68; -22% and 1.43 vs. 1.68 nmol/ml; -14.9% , Fig. 3) for Rapeseed and Sunflower groups vs. Control group, respectively.

After four weeks of feeding a decrease was registered in plasma antioxidant activity in the Chol/Rapeseed, Chol/Sunflower and Chol groups. These results corresponded with the observations that cholesterol supplemented diet leads to a decrease in blood antioxidant activity [28,29]. However, the diets supplemented with rapeseed and to a less degree with sunflower oil significantly hindered the decrease of the plasma antioxidant activity in rats fed added cholesterol: decrease in TRAP 232.5 vs. 191.1, -21.7% and 222.2 vs. 191.1, nmol/ml, -16.3% (Fig. 4), an increase in MDA 1.89 vs. 2.56, -26.2% and 2.01 vs. 2.56 nmol/ml, -21.5% (Fig. 5) for the Chol/Rapeseed and Chol/Sunflower vs. the Chol group, respectively.

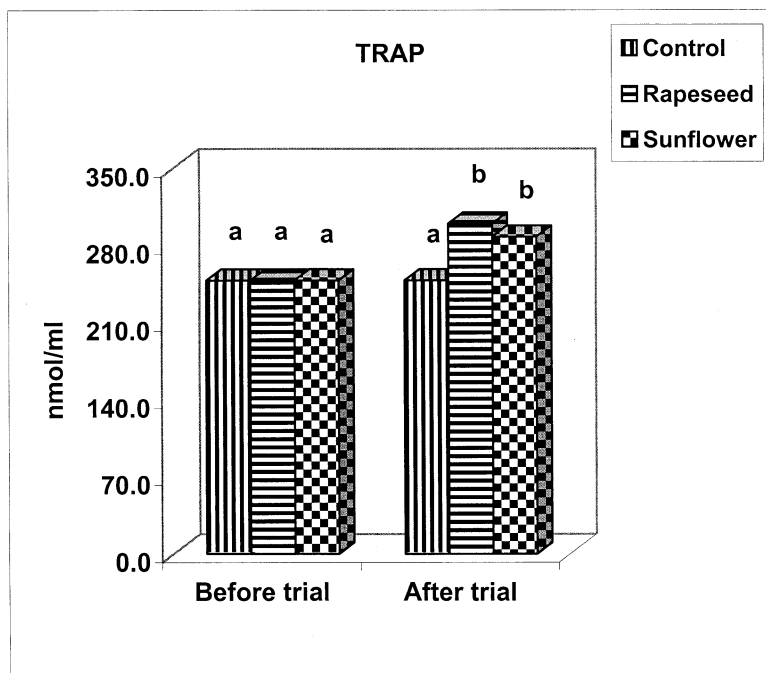


Fig. 2. Total radical-trapping antioxidative potential (TRAP) in rats fed without added cholesterol. Means (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

These results demonstrate that rapeseed and to a less degree sunflower oils positively affect plasma antioxidant activity of rats fed both cholesterol-containing and cholesterol-free diets.

4. Discussion

The role of Mediterranean diet in prevention of atherosclerosis and other diseases were proven [7,8,30]. Epidemiological studies, experiments on laboratory animals and clinical investigations have shown that such diets, which include vegetable oils, improve plasma lipid status and plasma antioxidant activity [12,30,31]. These and other investigators attribute the positive influence of Mediterranean diet to low content of saturated and high content of monounsaturated fatty acids in vegetable oils [7,8,30].

During the last 15 years, our international team of cardiologists, biochemists and dietitians has been studying various kinds of nutritional products in order to improve diets for patients suffering from coronary atherosclerosis [19–22,32–34].

Most authors claim that olive oils are preferable [10,11]. However, recently published paper demonstrated that sunflower oil has high TRAP values [13]. Therefore, in the present investigation we tried to assess the bioactive components of the often used seed oils and to find the most valuable among them for atherosclerosis prevention diets.

The seed oils are widely used. They are known for their preferable organoleptic properties.

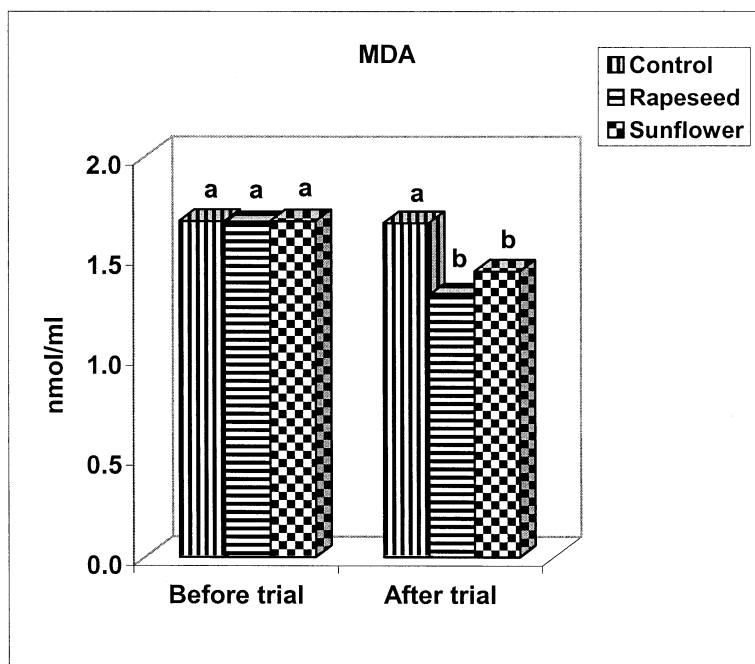


Fig. 3. Malondialdehyde lipid peroxidation test (MDA) in rats fed without added cholesterol. Means (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

Do they possess other positive qualities, which justified the wide use of these oils? To answer this question, fatty acid, sterols, and antioxidant compounds of 4 seed oils were studied and compared with their stability and total radical-trapping antioxidative potential (TRAP).

The quality of the oils in plant seeds is affected by soil conditions, ripeness of fruits or seeds and the length of storage. Therefore, it was expected that the results of our investigation could be different of the data of others. However, the results of the fatty acids and sterols investigation are in accordance with others [27]. The high content of β -sitosterol in seed oils is an additional advantage of these vegetable oils [35].

We have found that sunflower oils possess the highest content of α -tocopherols. These results are in accordance with the data of others [36,37]. However, the highest content of polyphenols was found in rapeseed oil. Also stability and TRAP were higher in rapeseed oil. These results are consistent with the data of other investigators, who have found that antioxidant activity of oils is related to contents of total phenols [38].

The results of our investigation *in vitro* show: rapeseed oil has high organoleptic properties and the highest antioxidant capacity among the studied seed oils. The above-mentioned properties could make this oil a preferable supplement to atherosclerosis prevention diets. Our investigation supports the suggestion of others that seed oils (rapeseed and sunflower) could replace oils and fats rich in polyunsaturated fatty acids in a lipid-lowering diet [12,31]. However, these suggestions have to be proven in experiment *in vivo*. Therefore, we decided to use two oils with high and low antioxidant

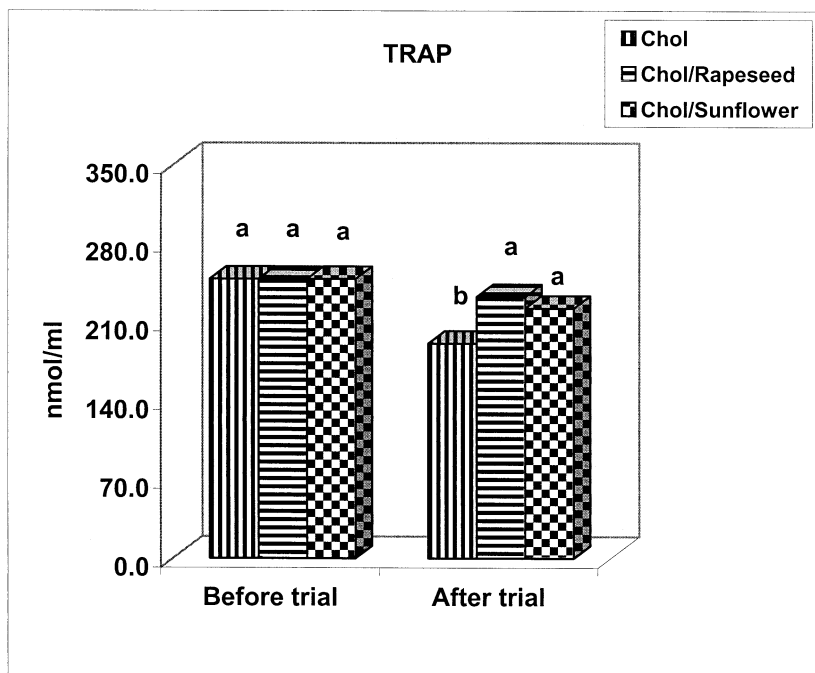


Fig. 4. Total radical-trapping antioxidative potential (TRAP) in rats fed with added cholesterol. Means (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

potentials (rapeseed and sunflower, respectively) in experiment on rats fed cholesterol-containing and cholesterol-free diets.

After four weeks of trial we have found that rapeseed and to a less degree sunflower oils positive affect lipid metabolism in rats fed cholesterol-containing diet: the TC, LDL-C, TG and TPH concentrations in the Chol/Rapeseed and Chol/Sunflower diet groups were significantly lower than in Chol diet group. These results were predictable. It was expected that oil supplemented diets, which contain high concentration of antioxidant components will positively influence lipid metabolism.

It must be emphasized that the improvement in lipid metabolism was observed only in the groups of rats fed cholesterol-containing diet. These results are consistent with those obtained by others [12,39]. These authors have demonstrated that lipid-lowering natural products are effective only in cases of hyperlipidemia both in experiments on laboratory animals and in investigations of humans. Our previous experience is in accordance with these studies [20,21,40].

Rapeseed and to a less degree sunflower oils have exerted marked antioxidant effect in both groups of rats, fed cholesterol-containing and cholesterol-free diets. Also these results are in correspondence with the results of others [10,13].

Therefore, the results of the investigation *in vivo* support suggestions that oil with higher antioxidant capacity (rapeseed) is biologically more active than oil with lower antioxidant capacity (sunflower).

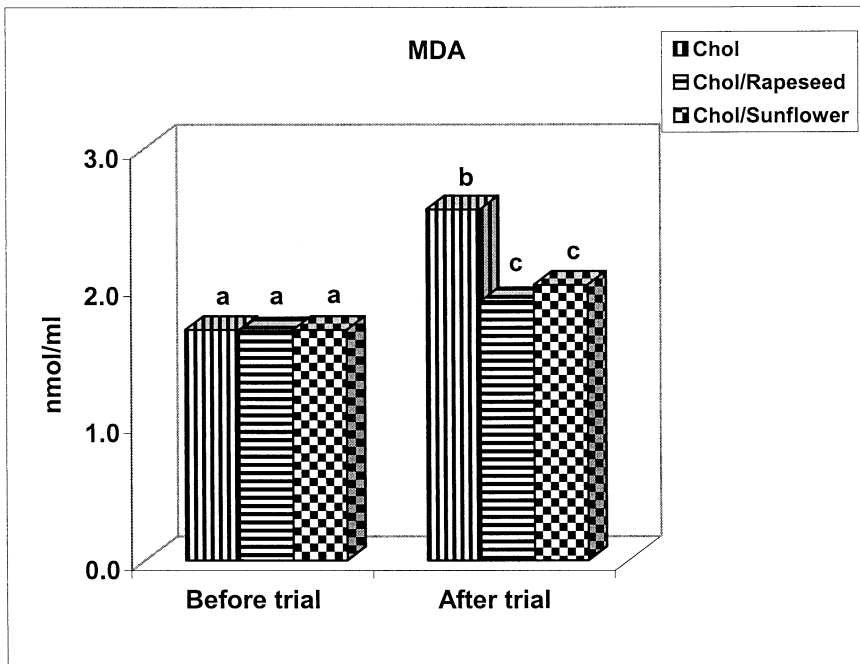


Fig. 5. Malondialdehyde lipid peroxidation test (MDA) in rats fed with added cholesterol. Means (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

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