

Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats

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Abstract

The aim of this study was to compare some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. The content of total polyphenols (g/100g) was 0.23 ± 0.03 ; 0.22 ± 0.03 and 0.68 ± 0.1 in peeled fruits and 0.48 ± 0.04 , 0.47 ± 0.04 and 1.2 ± 0.12 in peels of peaches, pears and apples, respectively. Caffeic, p-coumaric and ferulic acids and the total radical-trapping antioxidative potential (TRAP) values in peeled apples and their peels were significantly higher than in peaches and pears, respectively. Contrary, no significant differences in the content of dietary fiber among the studied fruits were found. The content of all studied indices in peels was significantly higher than peeled fruits ($p < 0.05$). A good correlation between the total polyphenols and the TRAP values was found in all fruits. Diets supplemented with apples and to a less extent with peaches and pears have improved lipid metabolism and increased the plasma antioxidant potential especially in rats fed with added cholesterol. The highest content of biologically active compounds and the best results in the experiment on rats makes apple preferable for dietary prevention of atherosclerosis and other diseases. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Fruits; Dietary fiber; Polyphenols; Rats; Lipids; Antioxidative potential

1. Introduction

It is widely accepted that fruits and vegetables have many healthful properties [1]. Epidemiological and clinical investigations demonstrate significant decrease in morbidity and mortality from cardiovascular and other diseases among fruits and vegetables consumers [2,3]. The positive influence of these natural products is attributed to their dietary fiber and antioxidants, especially to phenolic compounds

[4,5]. It was shown that high dietary fiber diets are associated with the prevention of the dangerous coronary atherosclerosis and other diseases [1,5]. Therefore, high dietary fiber formulated food products are currently being developed [6,7]. It is an established fact that phenolic compounds possess antioxidant properties and prevent oxidation of low density lipoprotein cholesterol [4]. As a consequence, consumption of these natural antioxidants is inversely related to coronary atherosclerosis and stroke [3,8]. A high amount of biologically active compounds were found in tropical and subtropical fruits [9]. However, most of the consumers are very conservative and do not use these fruits. Therefore, the aim of this investigation was to find among traditional fruits the most biologically active for prevention of diseases. In the first stage of this investigation the contents of dietary

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fiber, total polyphenols and some essential phenolic acids were determined and compared with TRAP. In the second stage the influence of fruits on lipid metabolism and plasma antioxidant activity on rats fed with and without added cholesterol was compared. Peels of fruits is one of the major sources of natural antioxidants [10,11]. Some authors even proposed to use the by-products of juice extraction industry as natural source of dietary fiber and antioxidants [11]. Therefore, the peeled fruits and peels were studied separately.

As far as we know, there are not such comprehensive investigations of traditional fruits, which include experiments on laboratory animals.

2. Materials and methods

2.1. Fruits

Israeli peaches (*Prinus persica*), pears (*Pyrus communis*) and apples (*Malus sylvestris*) were used. They were of the same ripeness and purchased from the same farmer.

2.2. Samples

32 of each selected at random fruits were washed in distilled water, separated into peeled fruits and peels. Samples of 1 kg were obtained for each part of the three fruits. Peeled fruits and peels were studied separately. For the experiment on the rats, whole fruits were frozen under liquid nitrogen and then lyophilized. These lyophilized fruits were used as supplement to the diets.

2.3. Laboratory tests for fruits

The content of dietary fiber was determined according to Prosky et al. [12].

Total polyphenols were extracted with 95% ethanol and the content was determined according to Folin-Ciocalteu method and measured at 675nm [13].

Phenolic acids were estimated according to García-Sánchez et al. [14], with our modifications and changes in the extraction procedure of samples, using a combination of methanol, petroleum ether and ethyl acetate [15]. Fluorescence emission was measured with model FP-770, Jasco spectrofluorometer (Japan Spectroscopic Co., Ltd., Hachioji City, Japan) at an excitation (λ_{ex}) and emission (λ_{em}) wavelengths suitable for each of determined phenolic acids.

2.4. Total radical-trapping antioxidative potential (TRAP)

0.5 g of each peeled fruits and peels were separately ground and shaken at room temperature with 2 ml of methanol. After centrifugation at 2800 g, the methanolic extract was used for TRAP analyses. Peroxyl radicals, produced at a constant rate by thermal decomposition of 2,2-azo-bis-2-

amidinopropane hydrochloride (ABAP-Polyscience, Warington, PA), were monitored by luminol-enhanced CL. The reaction was initiated by mixing 475 μ l phosphate buffered saline, 50 μ l 10 mM luminol in 100 mM borate buffer (pH 10.0), and 50 μ l ABAP. This mixture was incubated (37°C) in the temperature controlled sample carousel of the luminometer BioOrbit 1251 (BioOrbit, Finland) for 15 min. During this period of time a steady state of the chemiluminescence (CL) signal was reached. Then 20 μ l of methanolic extract were added directly into the cuvette and the samples were measured for another period of time (τ) until a 50% recovery of the original steady state CL signal. 8.0 nM Trolox (Aldrich Chemical Co., Milwaukee, WI), a water soluble analogue of tocopherol, was used as a reference inhibitor instead of sample. The stoichiometric factor of trolox (the number of peroxyl radicals trapped per added molecule of antioxidant) is 2.0. The TRAP value for sample measured was obtained from the equation: $TRAP = 2.0 \cdot [trolox] \cdot \tau_{sample} / f \cdot \tau_{trolox}$, where f is the dilution of sample measured. The results obtained are expressed as nmol of peroxyl radicals trapped by 1 ml of sample. Solvents were verified to have negligible TRAP [16].

2.5. Rats

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 = 80) with a mean weight of 120 g. They were divided into 8 groups of 10 and housed in stainless steel metabolic cages.

2.6. Diets

During 4 weeks of the experiment the rats of eight groups were fed different diets (Table 1). The rats of the Control group were fed the basal diet (BD) only. The BD for the other 7 groups was supplemented with: 10% of whole dry apples (Apple group), 10% of whole dry pears (Pear group), 10% of whole dry peaches (Peach group), 1% of nonoxidized cholesterol (NOC) of analytical grade (Chol group), 1% of NOC and 10% of whole dry apples (Apple/Chol group), 1% of NOC and 10% of whole dry pears (Pear/Chol group) and 1% of NOC and 10% of whole dry peaches (Peach/Chol group). Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St Louis, MO. The dietary cholesterol was checked according to the HPLC method of Ansari et al [17] and was found not to contain cholesterol oxides. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Several prior experiments on laboratory animals and human studies have shown that cellulose has not significant hypocholesterolemic effects [10,18]. Therefore, in Chol and Control groups cellulose was used as a control fiber. The diets contained as percentage of energy 66% of carbohydrates, 25% of protein and 9% of fat. The

Table 1
The composition of the diets (%)

Components	Ap	P-r	P-ch	Control	Ap/Chol	P-r/Chol	P-ch/Chol	Chol
Corn starch	58	58	58	58	57	57	57	57
Casein	16	16	16	16	16	16	16	16
Soy bean oil	11	11	11	11	11	11	11	11
Dry apple	10	—	—	—	10	—	—	—
Dry pear	—	10	—	—	—	10	—	—
Dry peach	—	—	10	—	—	—	10	—
Cellulose	—	—	—	10	—	—	—	10
NOC	—	—	—	—	1	1	1	1
Mineral mixture	4	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1	1

Abbreviations: Ap, apple diet group; Ap/Chol, apple/Chol diet group; Chol, Cholesterol diet group; NOC, Nonoxidized cholesterol; P-ch, Peach diet group; P-ch/Chol, Peach/Chol diet group; P-r, Pear diet group; P-r/Chol, Pear/Chol diet group.

calculated energy of the diets was from 394.5 to 400.4 kcal/100g and this difference was statistically not significant.

All rats were fed ad libitum once a day and the intake of the diet was monitored daily. The diets were given once a day at 10 am. All rats had unrestricted access to drinking water. The body gains every monitored weekly. Before the experiment the blood samples were taken 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 total cholesterol.

2.7. Laboratory tests for rats

Two time points were used in this experiment: before and after 28 days of feeding. At these time points a wide range of laboratory tests was performed.

2.8. Lipids

Total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), total phospholipids (TPH), HDL-phospholipids (HDL-PH), triglycerides (TG) and TC in liver were determined as previously described [10].

2.9. Total radical-trapping antioxidative potential of plasma of rats (TRAP)

Also this test was performed as previously described [19].

2.10. Lipid peroxidation (MDA assay)

The concentration of MDA (thiobarbituric acid reactive substances), an index of lipid peroxidation, was determined spectrophotometrically [20]. The procedure was described previously and has been adapted for plasma samples. The

reaction mixture consisting of 1% thiobarbituric acid (Sigma, USA) in 10% trichloroacetic acid (Sigma, USA) in the ratio of 1:2 (v/v) was added to the samples. After incubation and cooling, 4 ml of *n*-butanol were added. 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 (Sigma, USA) 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.

2.11. Statistics

The results are expressed as means \pm SD of five measurements. To compare several groups analysis of variance (ANOVA) was used. 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. Dietary fiber

The ranges of the total, insoluble and soluble dietary fiber content (g/100 g fresh fruit) were 2.6–3.3 and 1.9–2.7, 1.5–2.1 and 1.1–1.6, 0.9–1.3 and 0.7–1.1 for peels and peeled fruits, respectively. According to the statistical evaluation (ANOVA) there were no significant differences among the studied fruits.

Total polyphenols in peeled apples and peels were significantly higher than in peaches and pears, respectively (Figure 1).

The contents of phenolic acids (mg/100g fresh fruits) were: a) caffeic-19.8 \pm 1.9; 72.3 \pm 9.2; 214.3 \pm 19.2 and 25.1 \pm 2.3; 88.1 \pm 9.5; 265.1 \pm 23.0 b) *p*-coumaric – 3.9 \pm 0.4; 12.1 \pm 1.1; 38.2 \pm 3.6 and 5.2 \pm 0.5; 15.7 \pm 1.4; 53.1 \pm 4.9 c) ferulic – 1.2 \pm 0.1; 4.2 \pm 0.5; 12.2 \pm 1.0 and 1.5 \pm 0.1; 4.9 \pm 0.6; 14.9 \pm 1.3 d) TRAP – 1.54

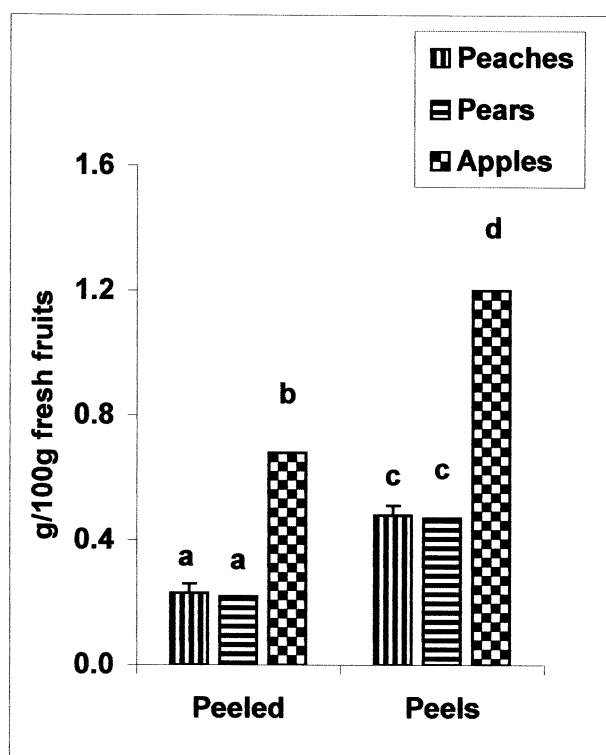


Fig. 1. Total polyphenols in peeled peaches, pears and apples and their peels. Means \pm SD (vertical lines). Bars with different letters are significantly different ($P < 0.05$).

± 0.2 ; 1.61 ± 0.2 ; 3.2 ± 0.3 and 2.7 ± 0.2 ; 2.9 ± 0.2 ; 6.9 ± 0.6 for peeled pears, peaches and apples and peels, respectively. The highest content of phenolic acids and TRAP was in peeled apples and peels and the lowest—in peeled pears and peels, respectively.

The results have shown that the contents of dietary fiber, total polyphenols, phenolic acids and the TRAP values in peels of apples, peaches and pears were significantly higher than in the peeled fruits ($p < 0.05$).

A very good correlation was observed between the TRAP and the total polyphenols ($R^2 = 0.9203$). In contrast, a poor correlation was observed between the TRAP values and the content of the total dietary fiber ($R^2 = 0.3267$). When the TRAP values were correlated with individual phenolic acids, the best correlation was found between *p*-coumaric acid and TRAP ($R^2 = 0.7767$), less good—between TRAP and caffeic acid ($R^2 = 0.5609$) and no correlation—between TRAP and ferulic acid ($R^2 = 0.0253$).

The addition of apples, pears and peaches or cholesterol to the diets did not affect food intake, body weight gain or feed efficiencies (data not shown). At baseline, the eight diet groups did not differ from one another in plasma lipid concentration (data not shown). Apples, pears and peaches supplemented diets (Table 2) in groups fed cholesterol significantly hindered the rise of plasma lipids: a) TC — 2.97 vs. 3.69 mmol/L, — 20%, 3.01 vs. 3.69 mmol/L, — 18.4%, and 3.05 vs. 3.69 mmol/L, — 17.3%, respectively; b) LDL-C — 1.36 vs. 2.02 mmol/L, — 32.6%, 1.39 vs. 2.02 mmol/L, — 31.1%, and 1.40 vs. 2.02 mmol/L, — 30.7%, respectively;

Table 2

Plasma lipids and total cholesterol concentration in liver of rats fed diets with and without 1% Cholesterol (Chol) and with and without 10% of apples, pears and peaches

Plasma lipids							Liver
Diet	TC	LDL-C	HDL-C	TG	TPH	HDL-PH	TC
			mmol/L				$\mu\text{mol/g}$
Control	2.86 ± 0.15^c	1.23 ± 0.05^c	1.62 ± 0.07	0.69 ± 0.04^b	1.76 ± 0.08^a	1.05 ± 0.06^a	5.86 ± 0.24^c
Chol	3.69 ± 0.21^a	2.02 ± 0.12^a	1.61 ± 0.07	0.88 ± 0.05^a	1.74 ± 0.08^a	0.63 ± 0.04^c	24.3 ± 0.31^a
Apple	2.74 ± 0.14^c	1.12 ± 0.05^c	1.60 ± 0.07	0.68 ± 0.04^b	1.71 ± 0.07^a	1.03 ± 0.06^a	5.81 ± 0.24^c
Apple/Chol	2.97 ± 0.17^b	1.36 ± 0.05^b	1.59 ± 0.07	0.73 ± 0.05^b	1.34 ± 0.06^b	0.79 ± 0.04^b	17.1 ± 0.25^b
Pear	2.81 ± 0.15^c	1.19 ± 0.05^c	1.61 ± 0.07	0.70 ± 0.05^a	1.72 ± 0.08^a	1.04 ± 0.06^a	5.74 ± 0.24^c
Pear/Chol	3.01 ± 0.18^b	1.39 ± 0.05^b	1.60 ± 0.07	0.75 ± 0.05^b	1.37 ± 0.06^b	0.75 ± 0.04^b	17.9 ± 0.25^b
Peach	2.81 ± 0.15^c	1.19 ± 0.05^c	1.61 ± 0.07	0.70 ± 0.05^a	1.72 ± 0.08^a	1.04 ± 0.06^a	5.74 ± 0.24^c
Peach/Chol	3.05 ± 0.18^b	1.40 ± 0.05^b	1.60 ± 0.07	0.79 ± 0.05^b	1.41 ± 0.06^b	0.71 ± 0.04^b	18.7 ± 0.25^b
2-way ANOVA							
(P-value)							
Apple	NS	NS	NS	NS	NS	NS	NS
Pear	NS	NS	NS	NS	NS	NS	NS
Peach	NS	NS	NS	NS	NS	NS	NS
Chol	<0.001	<0.001	NS	<0.001	NS	<0.001	<0.0005
Apple+chol	<0.001	<0.001	NS	<0.001	<0.005	<0.025	<0.0005
Pear+Chol	<0.05	<0.05	NS	<0.05	<0.01	<0.05	<0.0005
Peach+chol	<0.05	<0.05	NS	<0.05	<0.01	<0.05	<0.0005

^a Values are means \pm SD, $n = 10$.

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

^c Abbreviations used: Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; HDL-PH, HDL phospholipids; LDL-C, LDL cholesterol; NS, not significant ($P \geq 0.05$); TC, total cholesterol; TG, triglycerides; TPH, total phospholipids.

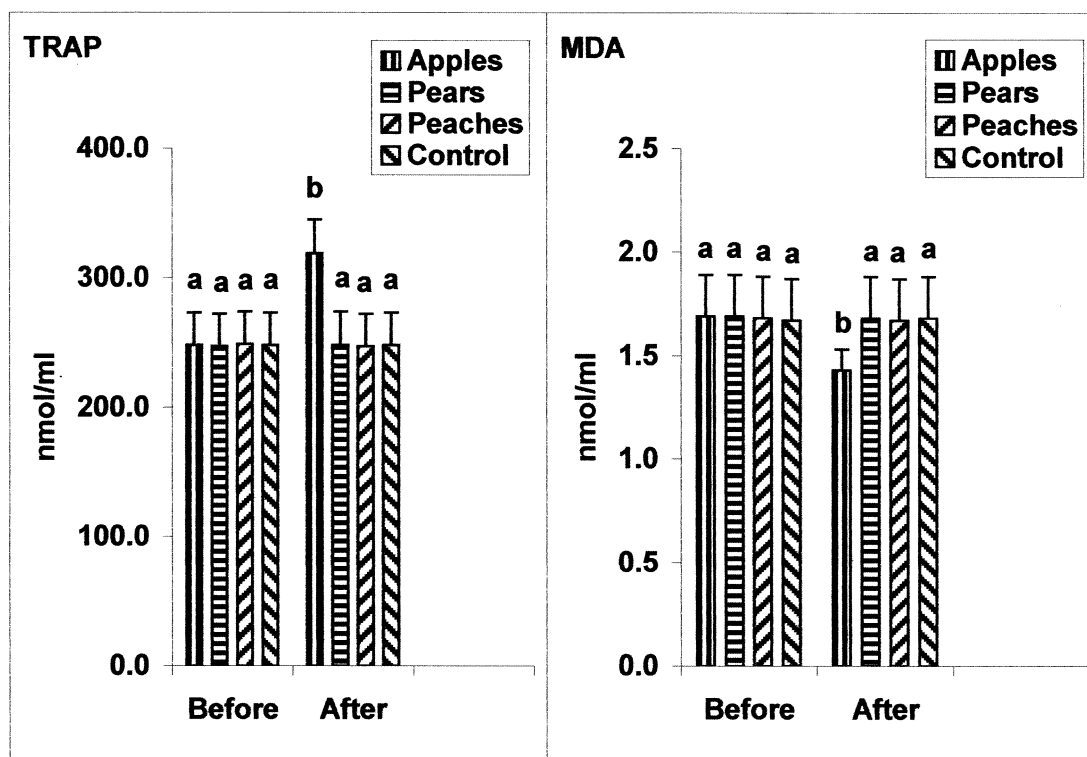


Fig. 2. Total radical-trapping antioxidative potential (TRAP) and lipid peroxidation (MDA) in rats fed without cholesterol. $M \pm SD$ (vertical lines). Bars with different letters are significant different ($p < 0.05$).

c) TG-0.73 vs. 0.88 mmol/L, - 17%, 0.75 vs. 0.88 mmol/L, - 14.8%, and 0.79 vs. 0.88 mmol/L, - 13.3%, respectively. The same diets significantly decrease the levels of HDL-PH (0.79 vs. 0.63 mmol/L, - 25.3%, $P < 0.025$, 0.75 vs. 0.63 mmol/L, - 19%, $P < 0.05$, and 0.71 vs. 0.63 mmol/L, - 17.7%, $P < 0.05$, respectively) and TPH (1.34 vs. 1.74 mmol/L, - 23%, $P < 0.005$, 1.37 vs. 1.74 mmol/L, - 21.3%, $P < 0.01$ and 1.41 vs. 1.74 mmol/L, - 19%, $P < 0.01$, respectively).

Liver weight was ± 4.37 g in all groups. Liver TC concentration in rats of the Chol group was 4.17 times higher than in the Control group. Liver TC concentrations in rats of the Apple/Chol, Pear/Chol and Peach/Chol groups were significantly higher than in the Control group (2.9, 3.05 and 3.21 times, respectively) and significantly less than in the Chol group ($P < 0.005$ in all three cases). These results show that apples, pears and peaches supplemented diet hindered an increase in the liver TC concentration in rats fed dietary cholesterol (17.1 vs. 24.3 $\mu\text{mol/g}$, - 29.6%, 17.9 vs. 24.3 $\mu\text{mol/g}$, - 26.3%, and 18.7 vs. 24.3 $\mu\text{mol/g}$, - 24.3%, for Apple/Chol, Pear/Chol and Peach/Chol groups, respectively).

A significant increase in the plasma antioxidant activity in the Apple group was found: an increase in the TRAP value (Figure 2) and a decrease in MDA value (Figure 2).

In contrast, a significant decrease in the plasma antioxidant activity in rats fed with added cholesterol (Apple/Chol, Pear/Chol, Peach/Chol and Chol diet groups) was regis-

tered: a significant decrease in the TRAP value (Figure 3) and a significant increase in MDA value (Figure 3). However, the decrease in the plasma antioxidant activity was significantly less than in the group of rats fed without added fruits (Chol group). These results were expected: also Tsai and Chen [21] and Uysal [22] have observed that cholesterol supplemented diet decreases the blood antioxidant activity.

4. Discussion

The main aim of this report as in previous ones [9,10, 23–26] was to find the relationship between the bioactive compounds of natural products and their influence on the lipid metabolism and antioxidant status of laboratory animals or humans.

The results of this investigation showed that the content of dietary fiber was high in all fruits without significant differences. Therefore, from the point of view of dietary fiber of these traditional fruits are comparable. It is known that the content of the studied compounds is influenced by some conditions, which include region, climate and ripeness [27]. Thus, it could be expected that these results would be different from the results of others. However, the results are in accordance with other reports [6,28–30].

Some authors claim that dietary fiber possesses antioxidant properties [31,32]. In our previous investigations *in*

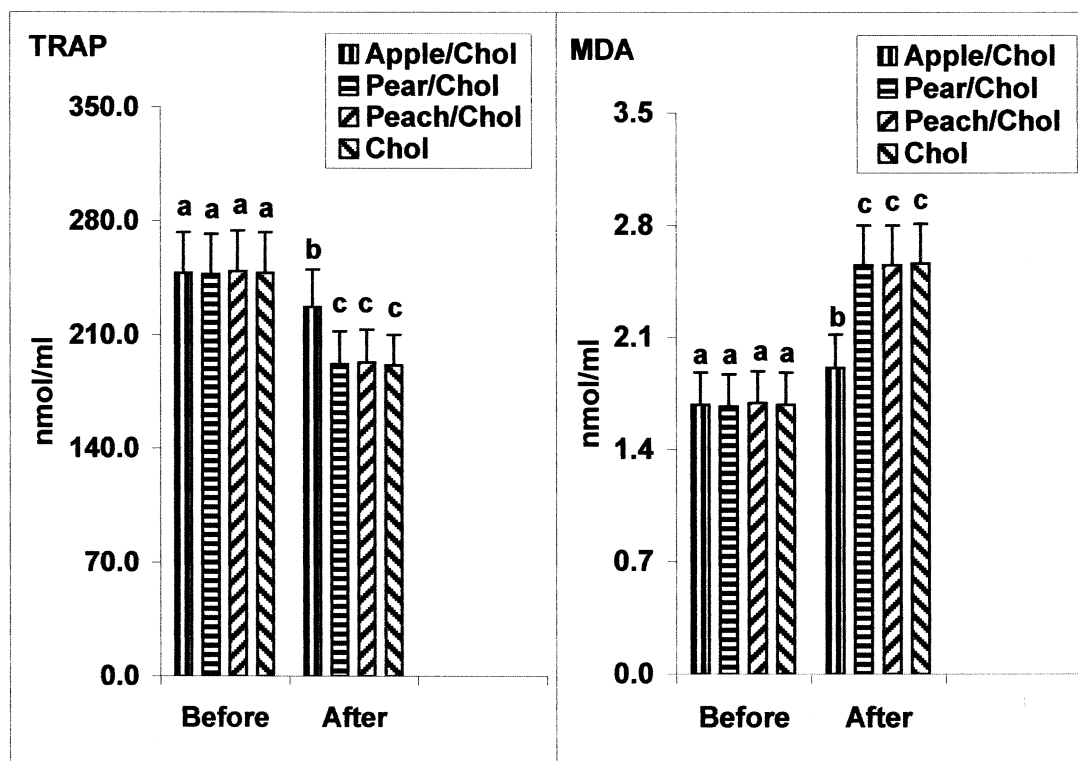


Fig. 3. TRAP and MDA in rats fed with added cholesterol. $M \pm SD$ (vertical lines). Bars with different letters are significant different ($p < 0.05$).

vitro it was found that dietary fiber exercises antioxidant effect [26]. Therefore, it was important to investigate the correlation between dietary fiber and its antioxidant capacity (TRAP). The correlation was very poor ($R^2 = 0.3267$). Therefore, this investigation *in vivo* as our previous *in vitro* did not support the claims that dietary fiber possesses antioxidant properties [31,32].

Dietary fiber diets are associated with the prevention and treatment of some diverse diseases such as diverticular and coronary heart diseases [1]. Thus, health organizations recommended the ingestion of 30–45 g of dietary fiber per day [33]. Dietary fiber has different physiological effects. Soluble dietary fiber became viscous when mixed with water. The viscosity is associated with delayed gastric emptying, altered mixing in the intestinal contents and slower transit of digesta along the small intestine and thus, with a slower rate of glucose, lipid or sterol absorption along a greater length of the small intestine [34]. The insoluble part is related to both water absorption and intestinal regulation, whereas the soluble fraction may influence the lipid metabolism in decreasing the levels of LDL-C and is associated with the reduction of cholesterol in blood [35,36]. The above-mentioned support the opinion that dietary fiber is a very important component of a healthy diet.

Polyphenols, caffeic, *p*-coumaric and ferulic acids and TRAP values in peeled apples and peels were significantly higher than in peaches and pears, respectively and higher than in peeled fruits. Also these results are in accordance with other investigations [37].

As was mentioned, some authors have proposed to use the by-products of juice extraction industry as natural source of dietary fiber and antioxidants [11]. Therefore, in this investigation the peeled fruits and peels were studied separately. It was found *in vitro* studies that peels have a high content of bioactive compounds and can be used by individuals and for industrial processing. Therefore, in the experiment on laboratory animals whole fruits were used.

This investigation has shown that apples positively influence the plasma antioxidant potential in both groups of rats: fed with and without added cholesterol. These results could be predictable. Also Abu-Amsa et al [38] have suggested that the higher the total polyphenol content the greater is its antioxidant activity.

The phenolic acids in the investigated fruits were in the following order: caffeic > *p*-coumaric > ferulic. It is known that antioxidant activity of phenolic acids is generally governed by their chemical structures. Their activity increases with the number of hydroxyl groups. Therefore, our results are of particular interest regarding the amount of caffeic acid found in apples and are in agreement with others [39].

Apples and to a less extent pear and peach supplemented diet significantly hindered a rise of plasma and liver lipids in rats fed cholesterol and the decrease in HDL-PH. According to recent investigation the level of HDL-PH is even more important than the level of HDL-C [40]. These authors claim that the amphiphilic properties of HDL-PH are essential for removal and transport of hydrophobic cholesterol.

We have found that all studied fruits in rats fed the basal diet without cholesterol did not affect the lipid levels. Also others have demonstrated that hypolipidemic effect of fruits and vegetables is evident when they are added to diets of rats fed cholesterol [41,42].

In conclusion, we were able to show that:

1. There are no significant differences in dietary fiber in studied fruits and the content is significantly higher than in peels.
2. Polyphenols, phenolic acids and TRAP values in peeled apples and their peels are significantly higher than in peaches and pears, respectively, and are higher in peels than in peeled fruits.
3. The antioxidant potential of dietary fiber in traditional fruits is questionable. The antioxidant properties of these fruits can be attributed to the content of the phenolic compounds. The degree of the antioxidant potential depends upon the level of total polyphenols.
4. Apples and to a less extent pears and peaches supplemented diet exercise hypocholesterolemic effect in rats fed cholesterol and positively influence the plasma antioxidant potential in rats fed diets with and without added cholesterol.
5. The high contents of dietary fiber, polyphenols and phenolic acids and TRAP values, hypolipidemic and antioxidant properties of apples makes this fruit preferable for dietary prevention of cardiovascular and other diseases.
6. The peels of traditional fruits are rich in dietary fiber and suitable for individual consumption and industrial processing.

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