

## Apple and Pear Peel and Pulp and Their Influence on Plasma Lipids and Antioxidant Potentials in Rats Fed Cholesterol-Containing Diets

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The aim of this study was to assess the bioactive compounds of apple and pear peel and pulp in vitro and their influence on plasma lipids and antioxidant potentials in vivo. The antioxidant potentials measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH),  $\beta$ -carotene bleaching ( $\beta$ -carotene), and nitric oxide inhibition radical scavenging (NO) tests in apple peel and pulp were significantly higher than in pear peel and pulp, respectively. The ethanol extract of apple peels showed the strongest inhibition of lipid peroxidation as a function of its concentration and was comparable to the antioxidant activity of butylated hydroxyanisole. The pear pulp extract had the weakest antioxidant ability, whereas other extracts such as apple pulp and pear peel were nearly equal. The antioxidant activities comprised contributions from polyphenols, phenolic acids, and flavonoids and correlated well with polyphenols and flavonoids. The correlation coefficients between polyphenols and antioxidant activities by DPPH,  $\beta$ -carotene, and NO were as follows: 0.9207, 0.9350, and 0.9453. Contrarily, the correlation coefficient between the content of dietary fiber and the antioxidant activities test was low. The content of all studied indices in apple and pear peel was significantly higher than in peeled fruits ( $p < 0.05$ ). Diets supplemented with fruit peels exercised a significantly higher positive influence on plasma lipid levels and on plasma antioxidant capacity of rats than diets with fruit pulps.

**KEYWORDS:** Apple; pear; peel; pulp; bioactive compounds; rats

### INTRODUCTION

Diets rich in fruits and vegetables can prevent various diseases (1–3). Our investigations have shown that fruits are a natural source of dietary fiber, trace elements, and antioxidant compounds (4, 5) and that diets rich in fruits positively influence plasma lipid levels and antioxidant capacities in experiments on laboratory animals (6, 7). Recently, we have investigated whole traditional fruits and their influence on lipid metabolism in rats (7). Some authors have demonstrated that peels of fruits

possess a higher content of bioactive substances than peeled fruits (8, 9). However, the fear of pesticides prevents most fruit-consuming people to eat whole fruits. Therefore, we decided in this investigation to study separately the bioactive compounds in apple and pear peel and pulp and their influence on plasma lipids and antioxidant potential in rats fed cholesterol-containing diets.

In the past, we have determined the total antioxidant potential of different fruits by the TRAP (6, 7). However, the TRAP test is a relatively unspecific marker of the free radical scavenging activity, particularly in fruits (4). Therefore, in this investigation, three other methods were used as follows: (i) antioxidant assay using the  $\beta$ -carotene (10), (ii) radical scavenging activity (RSA) using the DPPH method (DPPH) (10), and (iii) NO test (11, 12). As far as we know, there are no such investigations of apple and pear peel and pulp that also include experiments on laboratory animals.

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## MATERIALS AND METHODS

**Materials.** DPPH;  $\beta$ -carotene; Greiss reagent; 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox); sodium nitroprusside; BHA; caffeic, ferulic, and *p*-coumaric acids; and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO).

**Fruits and Samples Preparation.** In this study, Spanish apples (*Malus domestica* Borkh var Golden Delicious) and pears (*Pyrus communis* L. var. Blanquilla) were used, which were purchased at the peak of the harvest time (the time of the optimal maturity). The samples were prepared as previously described (7).

**Methods.** The content of dietary fiber was determined according to Prosky et al. (13).

**Fruit Extracts.** Apples (541 g of pulp and 95 g of peel) and pears (656.5 g of pulp and 109 g of peel) were pitted, cut into small pieces, and homogenized with 385 and 115 mL of 90% aqueous EtOH for apples and 468 and 132 mL of 90% aqueous EtOH for pears. After the extract was filtered, another portion of the same amount of aqueous EtOH was added to the residue. This procedure was repeated five times. The combined extract was evaporated under vacuum to remove EtOH. The obtained extract was used for the experiment (14).

**Determination of Polyphenols, Phenolic Acids, and Total Flavonoids.** Total polyphenols were measured at 765 nm using Folin-Ciocalteu reagent with gallic acid as the standard (15). The results are given in mg/100 g FW of GAE. Determination of phenolic acids was done according to the method of García-Sánchez et al. (16) with our modifications (7).

Total flavonoids were determined colorimetrically. A 0.25 mL amount of the EtOH extracts of apple and pear peel and pulp was diluted with 1.25 mL of distilled water. Then, 75  $\mu$ L of a 5% NaNO<sub>2</sub> solution was added to the mixture followed by 150  $\mu$ L of a 10% AlCl<sub>3</sub>·6H<sub>2</sub>O solution. Half a milliliter of 1 M NaOH was added after 5 min. The total was made up to 2.5 mL with distilled water. The absorbance was measured against the prepared blank at 510 nm in comparison with standards prepared similarly with known (+)-catechin concentrations. The results are expressed as milligrams of catechin equivalents (17, 18).

**Antioxidant Potentials of Fruits. A. Antioxidant Assay Using the  $\beta$ -Carotene.**  $\beta$ -Carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) was mixed. Chloroform was removed at 40 °C under vacuum. The resulting mixture was diluted with 10 mL of water. To this emulsion was added 40 mL of oxygenated water. Four milliliter aliquots of the emulsion were added to 0.2 mL of the EtOH extracts of apple and pear peel and pulp (50 and 100 ppm). The synthetic antioxidant BHA in EtOH was used for comparative purposes. A control containing 0.2 mL of EtOH and 4 mL of the above emulsion was prepared. The absorbance at 470 nm was taken at 50 °C at zero time ( $t = 0$ ). Measurement of absorbance was continued for 180 min at an interval of 15 min. A mixture prepared as above without  $\beta$ -carotene served as the blank. The AA of the extracts was evaluated in terms of bleaching of the  $\beta$ -carotene:  $AA = 100 [1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$ , where  $A_0$  and  $A_0^\circ$  are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and  $A_t$  and  $A_t^\circ$  are the absorbances measured in the test sample and control, respectively, after incubation for 180 min (10).

**B. RSA Using the DPPH Method (DPPH).** Different concentrations (50 and 100  $\mu$ L equivalent to 50 and 100 ppm) of the EtOH extracts of apple and pear peel and pulp and BHA (25 and 50 ppm) were taken. The volume was adjusted to 100  $\mu$ L by adding EtOH. Five milliliters of a 0.1 mM ethanolic solution of DPPH was added. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract, and EtOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. The RSA was expressed as the inhibition percentage: % RSA = (control OD - sample OD/control OD)  $\times$  100 (10).

**C. NO.** Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. A 0.5 mL amount of the sample (at various concentrations) was diluted with 0.5 mL of 10 mM sodium nitroprusside solution and incubated at 25 °C for 150 min. At the end of the incubation, 1 mL of Greiss reagent was added to each sample of

Table 1. Dietary Fiber Content in Apple and Pear Peel and Pulp<sup>a</sup>

samples	total	soluble	insoluble
apple peel	28.9 $\pm$ 0.1 <sup>a</sup>	10.9 $\pm$ 1.1 <sup>a</sup>	18.0 $\pm$ 1.6 <sup>a</sup>
apple pulp	22.2 $\pm$ 2.1 <sup>b</sup>	8.9 $\pm$ 0.7 <sup>b</sup>	13.3 $\pm$ 1.2 <sup>b</sup>
pear peel	28.1 $\pm$ 3.1 <sup>a</sup>	10.2 $\pm$ 1.1 <sup>a</sup>	17.9 $\pm$ 1.6 <sup>a</sup>
pear pulp	21.3 $\pm$ 2.1 <sup>b</sup>	8.4 $\pm$ 0.6 <sup>b</sup>	12.9 $\pm$ 1.2 <sup>b</sup>

<sup>a</sup> g/kg FW; values are means  $\pm$  SD of five measurements. Means in columns without letters in common differ significantly ( $p < 0.05$ ).

Table 2. Content of Phenolic Acids in Apple and Pear Peel and Pulp<sup>a</sup>

samples	ferulic acid	<i>p</i> -coumaric	caffeic acid
apple peel	136.7 $\pm$ 9.8 <sup>a</sup>	523.1 $\pm$ 43 <sup>a</sup>	2597 $\pm$ 43 <sup>a</sup>
apple pulp	119.8 $\pm$ 8.9 <sup>b</sup>	367.9 $\pm$ 31 <sup>b</sup>	1993 $\pm$ 81 <sup>b</sup>
pear peel	49.1 $\pm$ 4.4 <sup>c</sup>	150.9 $\pm$ 10 <sup>c</sup>	877 $\pm$ 52 <sup>c</sup>
pear pulp	21.3 $\pm$ 2.1 <sup>d</sup>	111.7 $\pm$ 9.1 <sup>d</sup>	701 $\pm$ 50 <sup>d</sup>

<sup>a</sup> mg/kg FW; values are means  $\pm$  SD of five measurements. Means in columns without letters in common differ significantly ( $p < 0.05$ ).

apple and pear peel and pulp and absorbance was read at 542 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Results were expressed as percentage nitrite production with respect to control values (sample: 0  $\mu$ L) (11, 12).

**Rats and Diets.** The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences (Jablonna, Poland) provided male Wistar rats ( $n = 40$ ) with an initial mean weight of 120 g. They were divided into five groups of eight and named Chol, Chol/Apulp, Chol/Apeel, Chol/Ppulp, and Chol/Ppeel.

During the 4 weeks of the experiment, the rats of all five groups were fed different diets as follows: (i) Chol group, BD and 1% of NOC of analytical grade; (ii) Chol/Apulp, BD, 10% of apple pulp, and 1% of NOC; (iii) Chol/Apeel, BD, 10% of apple peel, and 1% of NOC; (iv) Chol/Ppulp, BD, 10% of pear pulp, and 1% of NOC; and (v) Chol/Ppeel, BD, 10% of pear peel, and 1% of NOC.

The diets were prepared, and the rats were fed as previously described (7). Before the experiment, blood samples were taken from the tail vein. At the end of the experiment, the rats were anaesthetized 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.

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. TC, HDL-C, LDL-C, TPH, TG, TC in liver, TRAP, and MDA tests were determined as previously described (19).

## RESULTS

**Dietary Fiber.** The contents of total, soluble, and insoluble dietary fibers in apples and pears were without significant differences (Table 1). Insoluble dietary fiber was significantly higher than the soluble one. Total, soluble, and insoluble dietary fiber in peels of both apples and pears were significantly higher than in pulps of fruits ( $P < 0.05$  in all cases).

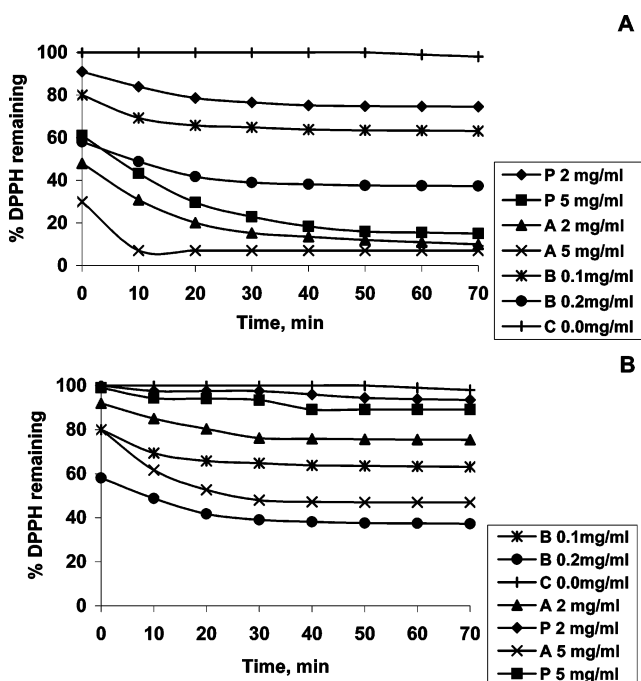
**Polyphenols, Phenolic Acids, and Total Flavonoids.** The content of caffeic acid (Table 2) was significantly higher than that of both *p*-coumaric and ferulic acids ( $P < 0.05$  and  $P < 0.001$ , respectively). The contents of all studied acids were significantly higher in peels of both apples and pears than in peeled fruits ( $P < 0.05$  in all cases).

The results for the flavonoids and total polyphenols in peeled fruits and their peels are summarized in Table 3. As can be seen, the highest content (mg/100 g FW) of flavonoids (45.0  $\pm$  3.9) and polyphenols (107.4  $\pm$  9.6) was found in apple peel ( $P < 0.05$ ).

**Table 3.** Flavonoids, Polyphenols, and Total Antioxidant Potential in Apple and Pear Peel and Pulp<sup>a</sup>

samples	flavonoids <sup>b</sup>	polyphenols <sup>c</sup>	DPPH (% RSA)	NO AA (%)	$\beta$ -carotene AA (%)
apple peel	45.0 $\pm$ 3.9 <sup>a</sup>	107.4 $\pm$ 9.6 <sup>a</sup>	87.9 $\pm$ 7.6 <sup>a</sup>	82.2 $\pm$ 7.5 <sup>a</sup>	88.6 $\pm$ 7.7 <sup>a</sup>
apple pulp	14.1 $\pm$ 1.3 <sup>b</sup>	61.7 $\pm$ 5.1 <sup>b</sup>	69.1 $\pm$ 5.3 <sup>b</sup>	62.1 $\pm$ 5.1 <sup>b</sup>	72.3 $\pm$ 6.1 <sup>b</sup>
pear peel	19.8 $\pm$ 1.7 <sup>c</sup>	65.0 $\pm$ 5.3 <sup>b</sup>	53.6 $\pm$ 5.0 <sup>c</sup>	66.0 $\pm$ 5.3 <sup>b</sup>	65.3 $\pm$ 5.2 <sup>c</sup>
pear pulp	7.6 $\pm$ 0.5 <sup>d</sup>	13.0 $\pm$ 1.1 <sup>c</sup>	9.9 $\pm$ 0.6 <sup>d</sup>	20.7 $\pm$ 2.1 <sup>c</sup>	24.5 $\pm$ 2.2 <sup>d</sup>

<sup>a</sup> Values are means  $\pm$  SD of five measurements. Means in columns without letters in common differ significantly ( $p < 0.05$ ). <sup>b</sup> mg/100 g FW of catechin equivalents. <sup>c</sup> mg/100 g – mg of GAEs/100 g FW.



**Figure 1.** Reaction kinetics of apple and pear peel and pulp extracts with DPPH radical. The DPPH radical concentration was 100  $\mu$ M in all reaction mixtures. (A) Reaction kinetics of apple and pear peel extracts at 0, 2, and 5 mg/mL. A, apple; B, BHA at 0.1 and 0.2 mg/mL; and P, pear. (B) Reaction kinetics of apple and pear pulp extracts at 0, 2, and 5 mg/mL. A, apple; B, BHA at 0.1 and 0.2 mg/mL; and P, pear.

**Antioxidant Potential of Fruits.** The three tests used show that the antioxidant potentials are significantly higher in apple peel (Table 3). Two concentrations of extracts from apple and pear peel and pulp were examined and compared for their free radical properties by DPPH. The time and concentration effects of the extracts were used. The results of the reaction kinetics showed that the extracts of fruit peel and pulp differed in their capacities to inhibit DPPH. The apple peel extract showed the greatest activity to quench DPPH radicals (Figure 1A), and the pear pulp extracts showed the smallest activity (Figure 1B). As can be seen, the apple peel extract at the level of 5 mg/mL quenched 98% of DPPH radicals. In contrast, the apple pulp extract at the same concentration quenched only 54.4% of DPPH radicals. Pear pulp extract at levels of 2 and 5 mg/mL quenched only 6.5 and 10.8% of DPPH radicals, respectively. The control and these curves were very close to each other. BHA was used to compare the different levels of concentration of fruit extracts.

A very good correlation was observed between the antioxidant potentials determined by DPPH, NO,  $\beta$ -carotene (Figure 2A–C), and the total polyphenols ( $R^2 = 0.9207$ , 0.9453, and 0.9350). Flavonoids showed lower correlation coefficients (Figure 2A–C) than the total polyphenols ( $R^2 = 0.6325$ , 0.6588, and 0.6327).

The best correlation was found with the NO method; therefore, dietary fiber and individual phenolic acids were correlated with the values of the NO scavenging test. In contrast, a poor correlation was observed between the NO values and the content of the total dietary fiber ( $R^2 = 0.2937$ ). When the NO values were correlated with individual phenolic acids (Figure 2D), the best correlation was found between *p*-coumaric acid and NO ( $R^2 = 0.5760$ ), and a smaller correlation was found between NO and both caffeic acid and ferulic acids ( $R^2 = 0.5184$ ).

The addition of apple and pear peel and/or pulp and cholesterol to the diets did not differently affect food intake, body weight gain, or feed efficiencies (data not shown). Apple and pear peels and pulps supplemented diets in rats fed added cholesterol significantly hindered the rise of plasma lipids vs Chol group: (i) TC: 2.91, 2.99, 3.11, and 3.17 vs 3.71 mmol/L and  $-21.6$ ,  $-19.4$ ,  $-16.2$ , and  $-14.6\%$ , respectively; (ii) LDL-C: 1.30, 1.34, 1.43, and 1.46 vs 2.01 mmol/L and  $-35.3$ ,  $-33.3$ ,  $-18.9$ , and  $-17.4\%$ , respectively; (iii) TG: 0.73, 0.76, 0.81, and 0.83 vs 0.89 mmol/L and  $-18.0$ ,  $-14.6$ ,  $-9.0$ , and  $-6.8\%$ , respectively; (iv) TPH: 1.30, 1.37, 1.39, and 1.44 vs 1.78 mmol/L and  $-27.0$ ,  $-23.0$ ,  $-22.0$ , and  $-19.2\%$ , respectively; and (v) TC in liver: 16.7, 17.8, 18.1, and 18.4 vs 24.4  $\mu$ mol/g and  $-31.6$ ,  $-27.0$ ,  $-25.8$ , and  $-24.6\%$ , respectively.

These results show that apple and pear peels and to a lesser degree apple and pear pulps supplemented diets hindered an increase in the plasma lipids and in liver TC concentration in rats fed dietary cholesterol. A significant decrease in the plasma AA in rats fed added cholesterol (Chol, Chol/Apple, Chol/Pulp, and Chol/Pear diet groups) was registered: a significant decrease in the TRAP value (Figure 3) and a significant increase in the MDA value (Figure 4). However, the decrease in the plasma AA was significantly less, particularly in the Chol/Apple group than in the Chol group fed without added fruits. The above-mentioned results show that among used parts of fruits, apple peels possess the highest biological activity.

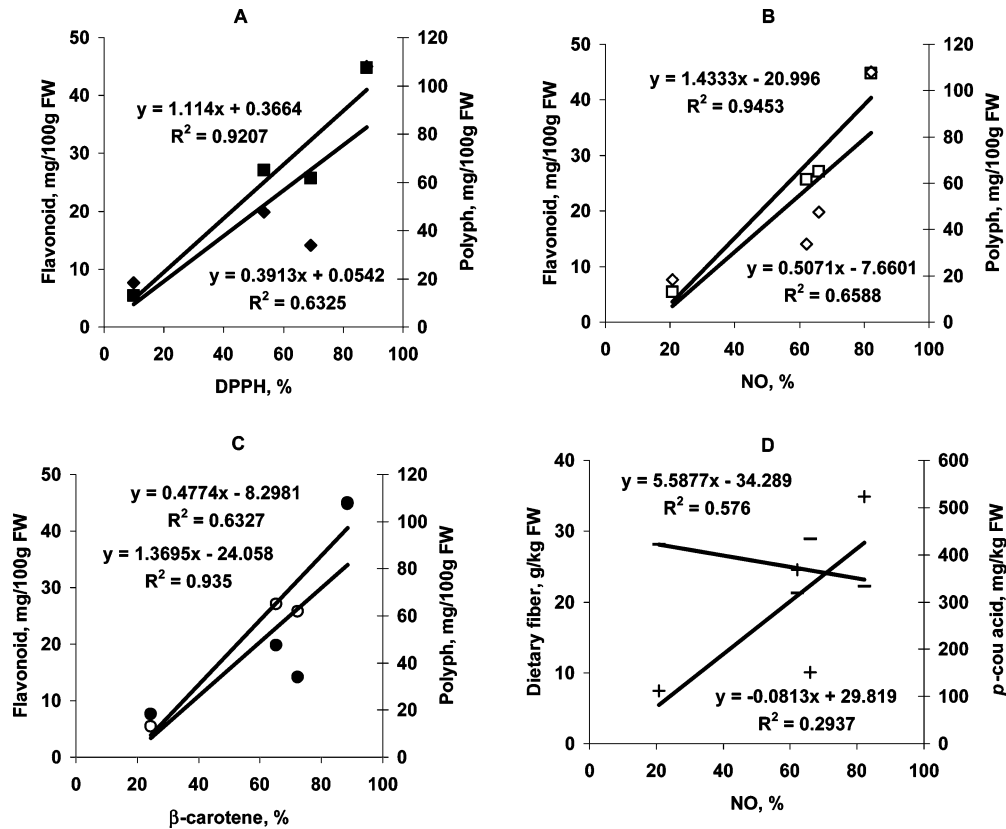
## DISCUSSION

Fruit- and vegetable-containing diets positively influence human health, and this fact is directly connected to their bioactive compounds (20, 21). Some authors have shown that fruit peels possess a higher content of these compounds than fruit pulps (8, 9). However, because of the fear of pesticides, most fruit-consuming people use peeled fruits. As was mentioned in the Introduction, to underline the importance of using whole fruit, we decided to assess Spanish apples and pears in vitro and in vivo. Dietary fiber, phenolic acids, flavonoids, total polyphenols, and the antioxidant potential of peeled fruits and their peels were studied separately. The bioactive potential of fruit peel and pulp was then assessed in vivo.

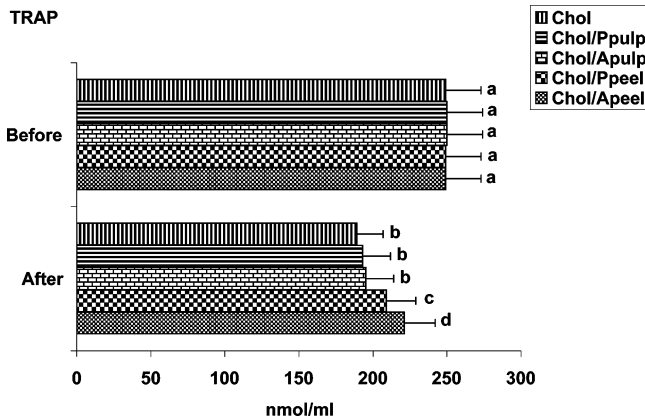
In our recent investigations, we have studied the total antioxidant potential of different fruits using the TRAP test (6, 7). There are many methods for total antioxidant determination, and every one has its limitations (4, 22). Some antioxidant assay methods give different AA trends (23). Therefore, in this investigation, the free radical scavenging properties of the studied fruits were determined by three different more specific tests ( $\beta$ -carotene, DPPH, and NO).

The results of our investigation in vitro showed that the contents of dietary fiber, phenolic acids, flavonoids, and total polyphenols are significantly higher in apple and pear peels than in pulp, respectively. The obtained results support conclusions of others and our previous data (8, 9).

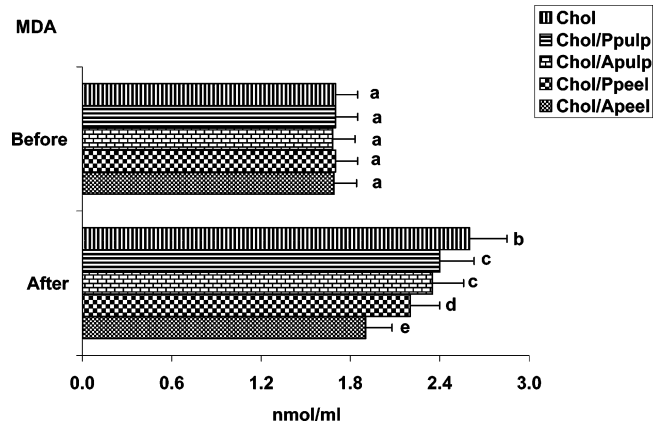
The results of the determination of the antioxidant potential of fruits peel and pulp by all three tests showed the same



**Figure 2.** Relationship, calculated by linear regression analysis, for apple and pear extracts between (A) DPPH scavenging effect (%),  $X$  to flavonoids (mg/100 g FW,  $Y_1$ ) (◆) and DPPH scavenging effect (%),  $X$  to polyphenols (mg/100 g FW,  $Y_2$ ) (■). (B) NO (%),  $X$  to flavonoids (mg/100 g FW,  $Y_1$ ) (<) and NO (%),  $X$  to polyphenols (mg/100 g FW,  $Y_2$ ) (□). (C)  $\beta$ -Carotene bleaching effect (%),  $X$  to flavonoids (mg/100 g FW,  $Y_1$ ) (●) and  $\beta$ -carotene bleaching effect (%),  $X$  to polyphenols (mg/100 g FW,  $Y_2$ ) (○). (D) NO (%),  $X$  to total dietary fibers (g/kg FW,  $Y_1$ ) (—) and NO (%),  $X$  to  $p$ -coumaric acid (mg/kg FW,  $Y_2$ ) (+). Abbreviations:  $p$ -cou,  $p$ -coumaric acid; polyph, polyphenols.



**Figure 3.** Significant decrease in the plasma AA in rats fed with added cholesterol; significant decrease in the TRAP values. Mean  $\pm$  SD (horizontal lines). Bars with different letters are significantly different ( $p < 0.05$ ); however, the decrease in the plasma AA is significantly less in the group of rats fed added fruits, particularly apple peels.



**Figure 4.** Significant decrease in the plasma AA in rats fed with added cholesterol; an increase in MDA values. Mean  $\pm$  SD (horizontal lines). Bars with different letters are significant different ( $p < 0.05$ ); however, the decrease in the plasma AA is significantly less in the group of rats fed added fruits, particularly apple peels.

trends: the antioxidant potential is significantly higher in peels than in apple and pear pulp, respectively. Therefore, we cannot support conclusions of others (23), who claim that antioxidant assay methods give different AA trends.

The results of the present investigation demonstrate that peels of apples and pears, peeled apples, and peeled pears supplemented diets in rats fed added cholesterol significantly hindered the rise of plasma lipids. These data agree with that of others, who have found that lyophilized apple counteracts the development of hypercholesterolemia (24).

Some authors claim that there is no correlation between the total phenolic content and the radical scavenging capacity (25). The results obtained by us did not support these claims. Our data are in accordance with the data of others, who have shown that high total polyphenol contents increase AA and there is a linear correlation between phenolic content and AA (26, 27).

We found that apple and pear peels contain high amounts of dietary fiber. High dietary fiber-containing diets are associated with the prevention and treatment of different diseases (28). Thus, health organizations recommended the ingestion of 30–

45 g per day (29), and high dietary fiber formulated food products are currently being developed (30, 31). However, in peels of the studied fruits, there is also a high concentration of very active antioxidant compounds. Therefore, a proper technology must be used to keep intact the above-mentioned compounds in the high dietary fiber formulated food products.

In conclusion, (i) apple and pear peels have a significantly higher content of all of the studied bioactive compounds than pulps. (ii) Apple and pear peel supplemented diets have a significantly higher positive effect on plasma lipids and plasma antioxidant capacity of laboratory animals. (iii) Apple and pear peels have to be used in individual consumption and for industrial processing.

#### ABBREVIATIONS USED

AA, antioxidant activity; BD, basal diet;  $\beta$ -carotene,  $\beta$ -carotene linoleate model system; BHA, butylated hydroxyanisole; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EtOH, ethanol; FW, fresh weight; GAE, gallic acid equivalent; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MDA, malondialdehyde lipid peroxidation test; NO, scavenging activity against nitric oxide; NOC, nonoxidized cholesterol; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids; TRAP, total radical-trapping antioxidative potential.

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