

Apple peels and pulp as a source of bioactive compounds and their influence on digestibility and lipid profile in normal and atherogenic rats

HANNA LEONTOWICZ, MARIA LEONTOWICZ, SHELA GORINSTEIN*,
OLGA MARTIN-BELLOSO**, SIMON TRAKHTENBERG***

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Poland

*Hebrew University of Jerusalem, School of Pharmacy, Jerusalem, Israel

**Department of Food Technology, University of Lleida, Lleida, Spain

***Institute of Cardiology, Kaplan Medical Center, Rehovot, Israel

Leontowicz H., Leontowicz M., Gorinstein S., Martin-Bellosa O., Trakhtenberg S.

Apple peels and pulp as a source of bioactive compounds and their influence on digestibility and lipid profiles in normal and atherogenic rats

Summary

Golden delicious apples were separated for peels and pulp, dietary fibre content and some bioactive compounds were determined. Rats were fed a semipurified or LSM diet with or without the addition of cholesterol and 5% of apple peels or pulp, feed, and digestibility and lipid profiles in plasma were estimated.

Apple peels were a better source of dietary fibre and bioactive compounds that had an influence on its TRAP value than apple pulp. Freeze-dried apple peels and pulp decreased protein digestibility but significantly affected the plasma lipid profile, expressed by a lowering of total cholesterol and LDL-C fraction contents only in rats fed a diet with cholesterol were noted. The high content of biologically active compounds in apples makes it preferable for dietary prevention of atherosclerosis and other diseases.

Keywords: apple peels and pulp, bioactive compounds

Traditional fruits (especially apples) and vegetables have many healthy properties. The positive influence of these natural products is attributed to their bioactive compounds: dietary fibre and antioxidants, mainly phenolic compounds, flavonoids, phenolic acids (3, 5, 7, 12, 13).

As it has been shown, diets rich in dietary fibre and other bioactive substances have decreased the risk of civilisation diseases (coronary atherosclerosis, obesity and cancer) (16). It is well known that phenolic compounds possess antioxidant properties and prevent the oxidation of low density lipoprotein cholesterol (LDL-C) (20).

The amount of these biologically active compounds can vary in different parts of fruits and may influence lipid metabolism and antioxidant activity in humans in a different way. The aim of this study was to compare the amount of some bioactive compounds in peels and pulp obtained from apples and to evaluate the influence of these products on the digestibility and plasma lipid profile in rats fed different diets with or without cholesterol.

Material and methods

In vitro study. Apples (*Malus domestica* Borkh var. Golden delicious) were washed in distilled water and peeled in order to separate peels and pulp. Peels and pulp were stored in -18°C , then cut into small pieces (2 mm), freeze-dried, ground up and placed in plastic bags of N_2 atmosphere until the beginning of the experiments in animals. Dietary fibre, total polyphenols, flavonoids, phenolic acids and total radical-trapping antioxidative

potential (TRAP) in fresh material (peels and pulp) were determined. The content of total dietary fiber and also its soluble and insoluble fraction according to Prosky et al. (15), total polyphenols (after extraction with ethanol) using Folin-Ciocalteu reagent (17), total colorimetrically (4), phenolic acids according to Garcia-Sanches et al. (6) and TRAP (in methanolic extract) according to Slavikova et al. (18) were determined.

In vivo study. Two experiments, lasting 21 days each, on male Wistar rats with an initial body weight of 85-95 g were performed. In the first experiment animals were divided into three groups: the control (C) receiving a semipurified diet containing 5% of cellulose and experimental groups with 5% of apple peels (PE) or apple pulp (PU) (instead of cellulose). In the second experiment rats were also divided into three groups: the control (C) which was fed the standard diet (LSM), experimental groups with 1% of cholesterol (CCH) without supplementation and 5% of apple pulp supplementation (CCHPU). Rats were housed in individual plastic cages at temperature $24 \pm 2^{\circ}\text{C}$ with a 12 h light : dark cycle. Water and feed were provided *ad libitum*, food intake and body gain were monitored.

The apparent digestibility of dry matter and protein in the third week of each experiment were estimated. For this purpose faeces were collected for five days and dry matter (105°C) and nitrogen (Kjeltec-Tecator) were determined.

Rats were anaesthetised and blood samples were taken from the left ventricle of the heart for plasma lipid profile determination. The following were calculated: total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL-C) according Randox enzymatic kit reagents were determined, low density lipoproteins (LDL-C) according to Tsi et al. (19).

Statistical evaluation. Data were analyzed statistically and variance analysis method (ANOVA) was performed. Duncan multiple range test to determine the differences between groups was used. Differences between the means at the level of $P \leq 0.05$ were considered as significant.

Results and discussion

Bioactive components (dietary fibre, antioxidants) are a very important ingredients of a healthy diet. As it was shown in tab. 1, the amount of total dietary fibre was higher in apple peels than in apple pulp, a similar relation for soluble and insoluble dietary fibre fractions was found and also in relation to other fruits (13). Dietary fibre proves different physiological effects: SDF fraction became viscous after being mixed with water, high viscosity delay gastric emptying and slower transport digesta along the small intestine and also influences of lower rate of glucose, lipid or sterols absorption (11). Insoluble fiber fraction influences the transit of digesta and intestinal regulation, protects against diverticulosis, constipation and cancer in the gut. High dietary fiber-containing diets are associated with the prevention and treatment of different diseases, so health organizations have recommended ingestion of 30-45 g dietary fibre daily.

Characteristics of some phenolic components included in apple peels and pulp is presented in tab. 2. The contents of phenolic acids were higher in fresh apple peels than in pulp (peeled apples); caffeic, p-coumaric and ferulic acids in pulp amounted about 70.4, 76.7 and 83.6% of its contents in peels, respectively. Total polyphenols in fresh peels amounted to 107 mg/100 g, and in pulp was lower by over 36%. The content of flavonoids was 45 vs 14 mg/100 g for peels and pulp (over 69% less), respectively. The amount of dietary fibre and other bioactive substances in an apple is higher than in other traditional fruits (peach, pear) (7) and higher in peels than in peeled fruits (2, 13, 14). It is known that phenolic compounds possess antioxidant properties and prevent oxidation of low density lipoprotein cholesterol (5), and their consumption is inversely related to coronary arteriosclerosis and stroke (10).

The higher content of the bioactive compounds in apple peels had an influence on total radical-trapping antioxidant potential value which was over two fold higher in fresh peels than in pulp (6.91 vs 3.21) (tab. 2). As it was shown in our previous paper there is a high correlation between total polyphenols and TRAP values in fruits (7, 13), which could have a positive influence on plasma antioxidant capacity and lipids in laboratory animals.

In our study the effect of two different rat diets semisynthetic and LSM, supplemented with 5% of apples peels and pulp was evaluated on performance. The 5% addition of freeze-dried apple peels or pulp to the semisynthetic diet for rats (experiment 1) did not significantly affect food intake, body weight gain or feed efficiency ratio (FER) (data not shown). Dry matter digestibility was similar in all rats and amounted to 92.5% on average. However protein digestibility was significantly lower in groups PE and PU (an average 83.2%) than in group C (85.5%) that was fed a semipurified diet (tab. 3).

Another reaction in atherogenic rats that received a diet with a similar amount of apple pulp was conducted (tab. 4). The supplementation of standard cholesterol diet (LSM) with 5% of apple pulp decreased ($P \leq 0.05$) feed intake by

Tab. 1. Dietary fibre and its fractions (g/kg⁻¹) in fresh apple peels and pulp

Item	Apple peels	Apple pulp
Total dietary fiber (TDF)	28.7	21.8
Insoluble fraction (IDF)	17.9	13.0
Soluble fraction (SDF)	10.8	8.9

Tab. 2. Content of phenolic acids (mg/kg⁻¹), polyphenols (mg GAEs/100 g), flavonoids (mg/100 g of catechin equivalent) and TRAP (mmol/ml⁻¹) in fresh apple peels and pulp

Item	Apple peels	Apple pulp
Phenolic acids:		
Ferulic	134	112
p-coumaric	524	369
Caffeic	2599	1994
Polyphenols	107	68
Flavonoids	45	14
TRAP	6.91	3.21

Tab. 3. The influence of freeze-dried apple peels and pulp on the apparent digestibility (%) of dry matter and protein in rats (n = 5)

Item	Dry matter digestibility	Crude protein digestibility
Group C	91.2 ^a	85.5 ^b
Group PE	93.4 ^a	83.0 ^a
Group PU	92.8 ^a	83.4 ^a

Explanation: a-b – means in columns tagged with the different letter differ at $P \leq 0.05$

Tab. 4. The influence of freeze-dried apple pulp on performance and the apparent digestibility (%) of dry matter and protein, and nitrogen retention in rats fed LSM diets supplemented with cholesterol (n = 5)

Item	Control LSM	CCH	CCHPU
Feed intake, g/day	18.8 ^b	18.3 ^b	16.1 ^a
Body gain, g/day	5.2 ^b	4.9 ^b	4.3 ^a
FER, g/g	3.62 ^a	3.73 ^b	3.74 ^b
Digestibility, %:			
Dry matter	74.2 ^c	68.5 ^b	63.5 ^a
Protein	71.5 ^c	65.4 ^b	56.7 ^c
N-retention, g/day	0.42 ^c	0.24 ^b	0.14 ^a

Explanation: a-c – means in columns tagged with the different letter differ at $P \leq 0.05$

(12.0%) and body gain by (12.2%). Dry matter and protein digestibility were also lower in group CCHPU than in CCH (7.3 and 13.3%, respectively). The diminishing of dry matter and protein digestibility in atherogenic rats obtaining different sources of dietary fibre (high methoxylated citrus pectin, apple pomace, potato fibre „Povex”, sugar beet pulp) was also presented by Galak et al. (8). As it is shown in tab. 4, nitrogen retention was diminished by 41.7% in rats from CCHPU in comparison to the CCh group. These results indicated that apples possess slimming properties and

Tab. 5. The influence of freeze-dried apple peels and pulp on plasma lipid profile (mmol/L) in rats fed semipurified diets

Item	Group C (semipurified diet)	Group PE	Group PU
TC	2.84 ± 0.29	2.57 ± 0.24	2.66 ± 0.21
HDL-C	1.62 ± 0.07	1.59 ± 0.06	1.55 ± 0.09
LDL-C	1.21 ± 0.23	0.98 ± 0.18	1.14 ± 0.21
TG	0.69 ± 0.05	0.72 ± 0.04	0.70 ± 0.04
HDL-C/TC	0.57	0.61	0.58
AI*	1.33	1.62	1.36

Explanation: AI* Atherogenic index = HDL-C/TC-HDL-C

Tab. 6. The influence of freeze-dried apple pulp on plasma lipid profile (mmol/L) in rats fed the LSM diet

Item	Group C (LSM diet)	Group CCH	Group CCHPU
TC	2.14 ^a	2.89 ^c	2.53 ^b
HDL-C	1.29 ^a	1.25 ^a	1.40 ^a
LDL-C	0.89 ^a	1.60 ^c	1.13 ^b
TG	1.37 ^a	1.45 ^a	1.46 ^a
HDL-C/TC	0.60 ^c	0.43 ^a	0.55 ^b
AI*	1.52 ^c	0.76 ^a	1.24 ^b

Explanation: as in tab. 3.

can be use in connection with obesity in human with high cholesterol levels as well as in animals. It can also be pointed out that apple pulp and cholesterol positively influence calcification of bones, but a high level of cholesterol reduces ($P \leq 0.05$) bioavailability and/or storage of magnesium (9).

Products obtained from apples influenced the lipid profile of rats fed the semipurified diet or atherogenic diet (LSM) in a different way (tab. 5 and 6). In normal rats apple peels and pulp (sources of dietary fibre and antioxidants) slightly changed the lipid profile in plasma: total cholesterol, its fractions of low density lipoprotein (LDL) and high density lipoprotein (HDL), as well as triglycerides (TG) (tab. 5). As it is presented in tab. 6, total cholesterol and its fraction LDL significantly decreased only when the 1% cholesterol diet was supplemented with 5% of apple pulp. Apple peels and pulp (also isolated from peach and pear), had a hypolipidemic effect in rats fed atherogenic diets in our previous investigations (13, 14). Our results also agree with data presented by others (1) who had demonstrated that lyophilized apple counteracts the development of hypercholesterolemia.

Based on plasma TC and HDL-C contents the atherogenic index in rats' plasma was calculated. The highest value of this index (the best) was stated in a group fed the semipurified diet with apple peels, rich in bioactive compounds and also in rats fed the LSM cholesterol diet with the apple pulp addition.

Conclusion

In conclusion we can say that apple peels have a higher content of dietary fibre, as well as other bioactive compounds (polyphenols, phenolic acids, flavonoids) and TRAP

value, than apple pulp and can influence the antioxidant capacity in rats.

The inclusion of 5% freeze-dried apple peels or pulp in the semipurified diet without cholesterol decreased protein digestibility but did not significantly affect the plasma lipid profile in rats. The inclusion of 5% freeze-dried apple pulp in the standard LSM diet with 1% of cholesterol diminish performance indices as well as apparent digestibility, but significantly improved the lipid profil. These products may to be used in individual consumption, especially in patients with a high plasma cholesterol level, in obesity and in the prevention of cardiovascular and other diseases. It seems that the products can be suitable for industrial processing.

References

1. Aprikian O., Buserrolles J., Manach C., Mazur A., Morand C., Davicco M. J., Besson C., Rayssiguier Y., Remesy C., Demigne C.: Lyophilized apple counteracts the development of hypercholesterolemia, oxidative stress, and renal dysfunction in obese Zucker rats. *J. Nutr.* 2002, 132, 1969-1976.
2. Belitz H. D., Grosch W.: *Fruits*. In Food Chemistry. Belitz H. D., Grosch W. (ed.), Springer, Berlin 1999, 764-777.
3. Cao G., Booth S. L., Sadowski J. A., Prior R. L.: Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr.* 1998, 68, 1081-1087.
4. Eberhardt M. V., Lee C. Y., Liu R. H.: Antioxidant activity of fresh apples. *Nature* 2000, 405, 903-904.
5. Frankel E. N., Waterhouse A. L., Kinsella J. E.: Inhibition of human LDL-C oxidation by resveratrol. *Lancet* 1993, 341, 1103-1104.
6. Garcia-Sanches F., Carnero C., Heredis A.: Fluorometric determination of p-coumaric acid in beer. *J. Agric. Food Chem.* 1988, 36, 80-82.
7. Gorinstein S., Martin-Belloso O., Lojek A., Ciz M., Soliva-Fortuny R., Park Y. S., Caspi A., Libman I., Trakhtenberg S.: Comparative content of some phytochemicals in Spanish apples, peaches and pears. *J. Sci. Food Agric.* 2002, 82, 1166-1170.
8. Gralak M. A., Leontowicz M., Morawiec M., Bartnikowska W., Kulasek G.: Comparison of the influence of dietary fibre sources with different proportions of soluble and insoluble fibre on Ca, Mg, Fe, Zn, Mn and Cu apparent absorption in rats. *Arch. Anim. Nutr.* 1996, 49, 293-299.
9. Gralak M. A., Leontowicz H., Leontowicz M., Stryczek A., Czerwiński J., Gorinstein S., Dębski B.: Effect of apple pulp on calcium, magnesium and manganese content in organs of rats fed atherogenic diets. *Pol. J. Food Nutr. Sci.* 2006, 15/56, S12, 55-58.
10. Hertog M. G., Kromhout D., Aravanis C., Blackburn H., Buzina R., Finanza F., Giampaoli S., Jansen A., Menotti A., Nedeljkovic S., Pekkarinen M., Simic Bs., Toshima H., Feskens E. J., Hollman P. C., Catan M. B.: Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* 1995, 155, 381-386.
11. Klont R.: Fiber in the new millennium. *World Food Ingred (April/May)* 2000, 52-59.
12. Leontowicz M., Gorinstein S., Bartnikowska W., Leontowicz H., Kulasek G., Trakhtenberg S.: Sugar beet pulp and apple pomace dietary fibres improve lipid metabolism in rats fed cholesterol. *Food Chem.* 2001, 72, 73-78.
13. Leontowicz H., Gorinstein S., Lojek A., Leontowicz M., Ciz M., Soliva-Fortuny R., Park Y. S., Yung S. T., Trakhtenberg S., Martin-Belloso O.: Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. *J. Nutr. Biochem.* 2002, 13, 603-610.
14. Leontowicz M., Gorinstein S., Leontowicz H., Krzemiński R., Lojek A., Katrich E., Ciz M., Martin-Belloso O., Soliva-Fortuny R., Haruenkit R., Trakhtenberg S.: Apple and pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol containing diets. *J. Agric. Food Chem.* 2003, 51, 5780-5785.
15. Prosky L., Asp N. G., Schwaizer T., De Vries J. W., Fruda I.: Determination of insoluble and soluble dietary fiber in food and food products: collaborative study. *J. AOAC Int.* 1992, 75, 360-367.
16. Rimm E. B., Ascherio A., Giovannucci E., Spiegelman D., Stampfer M., Willett W.: Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *J. Am. Med. Assoc.* 1996, 275, 447-451.
17. Singleton V. L., Orthofer R., Lamuela-Raventos R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, Oxidants and Antioxidants. Part A* 1999, 299, 152-178.
18. Slavikova H., Lojek A., Hamar J., Duskova M., Kubala L., Vondracek J., Ciz M.: Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. *Free Radical Biol. Med.* 1998, 25, 9-18.
19. Tsi D., Das N. P., Tan B. K. H.: Effects of aqueous celery (*Apium graveolens*) extract on lipid parameters of rats fed a high fat diet. *Planta Med.* 1995, 61, 18-21.
20. Witztum J. L., Steinberg D.: Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* 1991, 88, 1785-1792.

Author's address: prof. dr hab. Hanna Leontowicz, Nowoursynowska 159, 02-776 Warsaw; e-mail: Hanna_Leontowicz@sggw.pl