POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 1, pp. 73-78

GRAPEVINE LEAVES AS A SOURCE OF NATURAL ANTIOXIDANTS

Ryszard Amarowicz[1], Olga Narolewska[1, 2], Magdalena Karamać[1], Agnieszka Kosińska[1], Stanisław Weidner[2]

[1]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Olsztyn;
[2]Chair of Biochemistry, Department of Biology, University of Warmia and Mazury, Olsztyn

Keywords: grapevine leaves, antioxidant activity, phenolic compounds, phenolic acids, HPLC

The crude extracts of phenolic compounds were obtained from grapevine leaves using 80% acetone and 80% methanol (v/v). The content of total phenolic and condensed tannins was determined using Folin & Ciocalteau's phenol reagent, vanillin/HCl reagent, and protein precipitation method. The antioxidant properties of the extracts were investigated using the total antioxidant activity (TAA), DPPH radical scavenging activity and reducing power. The content of individual phenolic acids was determined using the HPLC method.

The content of total phenolics in grapevine leaves and their extracts determined in this study was high (257 mg/g acetone extract and 232 mg/g methanolic extract). The content of condensed tannins in acetone extract was higher than in the methanolic one. Antiradical activity of both extracts against DPPH radical and reducing power were similar and strong. The acetone and methanolic extracts exhibited TAA of 1.37 and 1.44 mmol Trolox/g, respectively. *Vitis vinifera* leaves extracts were observed to contain gallic, caffeic, and *p*-coumaric acid. Gallic acid was a dominant phenolic acid. The majority of phenolic acids were found in the form of esters.

INTRODUCTION

Wine grapes contain biologically active compounds (BACs) with antioxidant activities that have significant effects on the prevention of cardio-vascular diseases by reduction of atherosclerosis development, on decreased incidence of cancer, and favourable influence on chronic inflammatory diseases and on the protection against metabolic disease by improving mitochondrial function. BACs reduce the susceptibility of LDL to oxidation, which is important for reduction of atherosclerosis development, and increase of serum antioxidant capacity [Cooper *et al*., 2004]. Proanthocyanidins from red wine might trap reactive oxygen species in aqueous series such as plasma and interstitial fluid of the arterial wall, thereby inhibiting oxidation of LDL and showing and antiatherosclerosis activity [Yamakoshi *et al*., 1999]. Xia *et al*. [1998] concluded their research that dietary supplementation with polyphenols from grape may exert partial protection on oxidative insults such as those elicited by chronic ethanol ingestion. The inhibition of LDL oxidation by phenolic extracts from different types of fresh grapes has already been investigated [Meyer *et al*., 1997]. The relative LDL antioxidant activity correlated highly with the level of catechins, total phenolics, and hydroxybenzoates.

The extract obtained from grape seed powder exhibited strong oxygen radical absorbance capacities (ORAC) [Yilman & Toledo, 2006]. The antiradical activity against DPPH radical of the phenolic fractions separated from the extracts of winemaking waste solids was reported [Cruz *et al*., 2004]. Application of hydrolytic process improved antiradical properties.

The literature data reported that plant leaves are a source of phenolic compounds exhibiting antioxidant activity [Nishino & Yoshida, 2002; Ito *et al*., 2002; Amakura *et al*., 2002; Siddhuraju & Becker, 2003; Naczk *et al*., 2003; Amaral *et al*., 2005; Pari *et al*., 2007]. Therefore we decided to investigate antioxidant activity of grapevine leaves extracts and to characterize phenolic constituents present in this material.

MATERIALS AND METHODS

Plant material

Leaves of *Vitis vinifera* (cultivare Chasselas rose) were collected from the private garden in Olsztyn.

Chemicals

All solvents used were of analytical grade. Methanol, acetone, ethanol, acetonitrile, acetic acid, potassium ferricyanide and trichloroacetic acid were acquired from the P.O.Ch. Company (Gliwice, Poland). Folin & Ciocalteau's reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), gallic acid, caffeic acid, *p*-coumaric acid, (+) catechin, Hide – Remazol Brillant Blue R were obtained from Sigma-Aldrich (Poznań, Poland).

Author's address for correspondence: doc. Ryszard Amarowicz, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn, Poland, tel.: (48 89) 523 46 27, fax: (48 89) 524 01 24; e-mail: amaro@pan.olsztyn.pl

Dry matter

The content of dry matter in leaves was determined by drying at 105°C for 24 h.

Extraction

Phenolic compounds were extracted from leaves using 80% (v/v) acetone or 80% (v/v) methanol at a solid to solvent ratio of 1:8 (w/v), at 70°C, for 15 min [Amarowicz *et al.*, 1995]. Extraction was carried out in dark-coloured flasks using a shaking water bath. The extraction was repeated twice more, supernatants combined and acetone evaporated under vacuum at 40°C in a rotary evaporator; the remaining water solution was lyophilized.

Total phenolics

The content of total phenolic compounds in extracts was estimated using the Folin & Ciocalteau's phenol reagent [Naczk & Shahidi, 1989]. (+)-Catechin was used as a standard.

UV spectra

UV spectra of the crude extracts dissolved in methanol were recorded using a Beckman DU 7500 diode array spectrophotometer.

Condensed tannins

The content of condensed tannins in the extracts and leaves was determined using the modified vanillin method assay [Price *et al*., 1978] and expressed as absorbance units at 500 nm per 1 g of extract (A_{500}/g extract), per 1 g of leaves dry matter (A_{500}/g d.m.), and per 1 g of leaves fresh matter (A_{500}/g f.m.). Tannins in the extracts were also determined using a protein precipitation method with Hide – Remazol Brillant Blue R, as described by Wang & Goodman [1999]. The results were presented as a % of precipitated protein *versus* content of the extract.

Total antioxidant activity/capacity (TAA/TAC)

The total antioxidant activity of the extracts and total antioxidant capacity of leaves were determined according to the Trolox equivalent antioxidant activity (TEAC) assay described by Re *et al*. [1999]. TAA and TAC were expressed as mmol Trolox equivalent/g of extract, mmol Trolox equivalent/g d.m., and mmol Trolox equivalent/g f.m., respectively.

Scavenging of DPPH radical

Scavenging effect of phenolics from the crude extracts was monitored as described by Amarowicz *et al.* [2002]. A 0.1 mL methanolic solution containing 0.5 to 2.5 mg of extract was mixed with 2 mL of methanol and then added to a methanolic solution of DPPH (1 mmol/L 0.25 mL). The mixture was vortexed for 1 min, then left to stand at room temperature for 20 min and the absorbance of this solution was subsequently read at 517 nm.

Reducing power

Reducing power of the crude extracts was determined as described by Oyaizu [1986]. The suspension of the extract was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Following this, 2.5 mL of 10% (w/v) trichloroacetic acid was added and the mixture was then centrifuged at 1750 × g for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) $FeCl_3$; the absorbance of the mixture was read at 700 nm.

Separation of phenolic acids from extract

Separation of phenolic acids (free, esterified, and glycosided) was carried out according to Amarowicz & Weidner [2001]. The samples obtained in this way were injected onto an HPLC column.

HPLC analysis of phenolic acids

Phenolic acids were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCL 10A system controller and SPD-M 10A photo-diode array detector. Phenolic acids separation was done by a prepacked Luna 100 RP-18 column (4 × 250 mm, 5 μm; Phenomenex). The mobile phase water-acetonitrile-acetic acid (88:10:2; v/v/v) [Amarowicz & Weidner, 2001] was delivered at a rate of 1 mL/min. The detection was monitored at 280 and 320 nm.

RESULTS AND DISCUSSION

The content of total phenolics in the extracts of grapevine leaves depended on the solvent used for extraction (Table 1). The use of 80% acetone resulted in the higher content of total phenolics (257 mg/g) than 80% methanol (232 mg/g). The same tendency was observed for results expressed in relation of leaves dry and fresh matter.

The UV spectra were similar for both extracts (Figure 1). The maxima were noted for 336 nm (acetone extract) and 340 nm (methanolic extract). The strong absorption bands at 330-340 nm can be caused by the presence of hydroxycinnamic acid derivatives and flavanols in the extracts [Amarowicz & Weidner, 2001; Mabry *et al.*, 1970]. The presence of flavanols in grapevine leaves was reported before by Amarowicz *et al.* [2007].

The yield of condensed tannins extraction was higher for 80% acetone (167 A_{500}/g) than 80% methanol (142 A_{500}/g) (Ta-

TABLE 1. Content of total phenolics in the extracts and grapevine leaves.

Solvent used for extraction	Extracts (mg/g extract)	Leaves	
		(mg/g d.m.)	(mg/g f.m.)
80% acetone	257.0±2.8	72.8±0.8	22.8±0.3
80% methanol	232.0±6.9	63.3±1.9	19.8±0.6

TABLE 2. Content of condensed tannins in the extracts and grapevine leaves.

Solvent used for extraction	Extracts (A_{500}/g extract)	Leaves	
		(A_{500}/g d.m.)	(A_{500}/g f.m.)
80% acetone	167.0±1.1	72.8±0.8	22.8±0.3
80% methanol	142.0±0.5	63.3±1.9	19.8±0.6

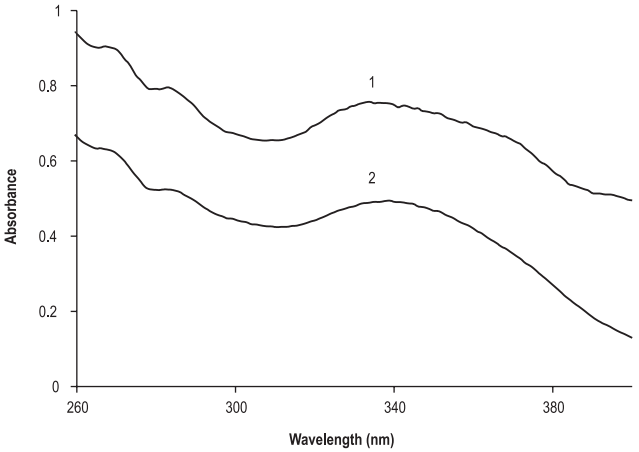

FIGURE 1. UV spectra of crude extracts from grapevine leaves (1 – acetone extract, 2 – methanolic extract).

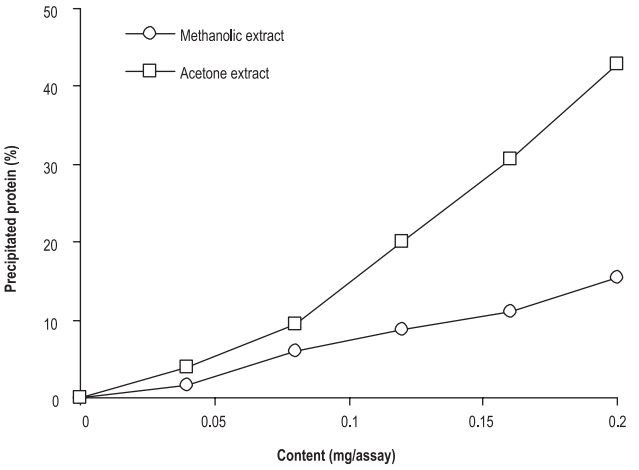

FIGURE 2. Precipitation of protein by tannins present in the extracts from grapevine leaves.

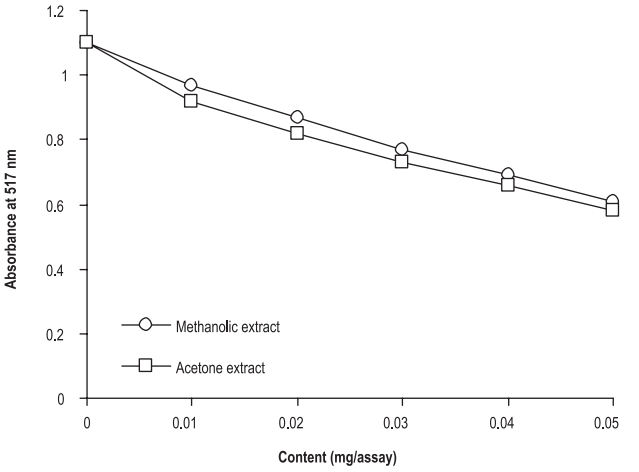

FIGURE 3. Scavenging effect of crude extracts from grapevine leaves on the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), as measured by changes in absorbance at 517 nm.

TABLE 3. Total antioxidant activity (TAA) of the extracts and total antioxidant capacity (TAC) of grapevine leaves.

Solvent used for extraction	TAA (mmol Trolox/g extract)	Leaves	
		TAC (mmol Trolox/g d.m.)	TAC (mmol Trolox/g f.m.)
80% acetone	1.37 ± 0.07	0.39 ± 0.02	0.12 ± 0.01
80% methanol	1.44 ± 0.07	0.39 ± 0.02	0.12 ± 0.01

ble 2). The results recalculated to leaves dry and fresh matter were also higher when 80% acetone was used for extraction. The higher content of condensed tannins in the acetone crude extract was confirmed by the results of protein precipitation assay (Figure 2). The ability to precipitate Hide – Remazol Brilliant Blue R. by acetone extract was higher than that of methanolic extract.

The higher extractionability of polyphenolics from grapevine leaves by 80% acetone than 80% methanol is in accordance with literature data. According to Amarowicz *et al.* [1995], 80% acetone ensured the most complete extraction of phenolic compounds from lentil seeds, especially of flavonols and tannins. The high capability of acetone-water system to extract phenolic compounds from plant material was confirmed by Troszyńska *et al.* [1993], Naczk *et al.* [2005], Rocha-Guzmán *et al.* [2007], and Pegg *et al*. [2007].

The content of total phenolics in grapevine leaves and their extracts determined in this study is high. Durmaz *et al*. [2007] reported the content of total phenolics in some edible leaves in range from 0.25 to 14.22 mg/g f.m. The highest amounts were found in the leaves of mulberry (*Morus alba*), quince (*Cydonia oblonga*), and cherry (*Prunus avium*). The content of total phenolics in the extract of *Moringa oleifera* and *Scoparia dulcis* leaves were 118 and 88 mg/g, respectively [Pari *et al*., 2007]. Leafy vegetables such as red lettuce, rough lettuce, red and white cabbage were charcterised by the total phenolic content of 1.70, 0.53, 0.40, and 1.78 mg/g f.m. Hassimoto *et al.* [2005]. Ciska *et al*. [2005] reported that the content of total phenolic in white cabbage extract was only 5.72 mg/g. In the study of Velioglu *et al.* [1998] the content of total phenolics in the extracts of 28 plant products ranged from 2.13 mg/g to 105.48 mg/g. It is worth emphasizing that the content of total

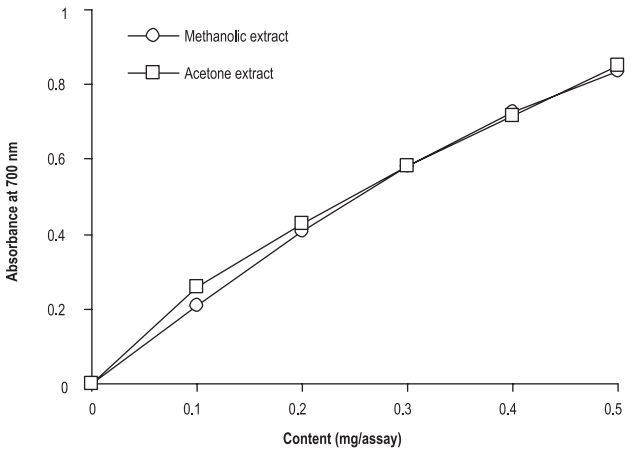

FIGURE. 4. Reducing power of crude extracts from grapevine leaves.

phenolics in the extracts of *Vitis vinifera* leaves can be compared with those of the seeds of *Vitis riparia* and *Vitis amurensis* [Wróbel *et al.*, 2005; Weidner *et al.*, 2007].

Total antioxidant activity of the grapevine leaves extracts and total antioxidant capacity of leaves (Table 3) were high. Ciska *et al*. [2005] reported that the TAA of the extract of white cabbage was only 0.025 mmol Trolox/g. The TAA of the leguminous seeds extract ranged from 0.30 mmol Trolox/g (pea) to 1.76 mmol Trolox/g (adzuki bean) [Amarowicz *et al.*, 2004]. The extracts of *Moringa oleifera* and *Moringa oleifera* leaves were characterised by TAA of 0.636 mmol Trolox/g and 0.432 mmol Trolox/g, respectively [Pari *et al*., 2007]. Total antioxidant capacity of some edible leaves was several time lower than results reported in this study and range from 0.004 to 0.015 mmol Trolox /g f.m. Durmaz *et al.* [2007]. The TAC of muscadine grapes leaves was higher than reported in this study and ranged from 0.16 to 0.30 mmol Trolox/g f.m. [Pastrana-Bonila *et al.*, 2003].

The antiradical activity and reduction power of the *Vitis vinifera* leaves extract was not dependent on the solvent used for extraction (Figures 3 and 4). Similar observation was noted by Naczk et al. [2003] for the ethanolic and acetone extracts of blueberry leaves. The results of both assays were much higher than those reported for the extracts of food product such as white cabbage [Ciska *et al*., 2005], pea [Amarowicz & Troszyńska, 2003], almonds [Amarowicz *et al*., 2005], and

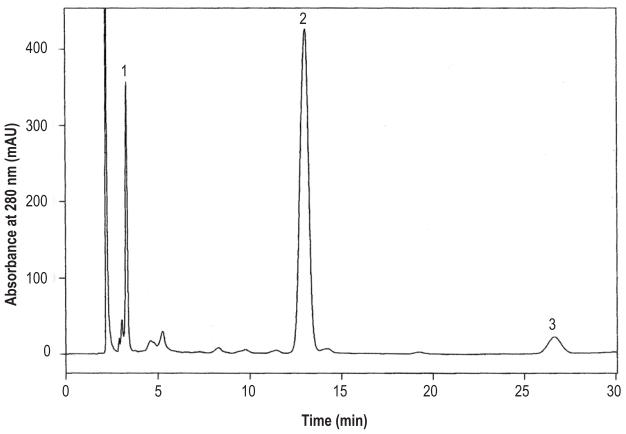

FIGURE 5. HPLC chromatogram of phenolic acids from methanolic crude extract after alkaline hydrolysis (detection at 280 nm); 1 – gallic acid, 2 – caffeic acid, 3 – *p*-coumaric acid.

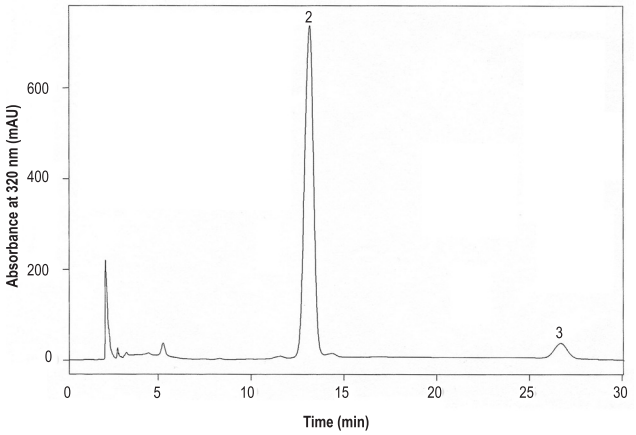

FIGURE 6. HPLC chromatogram of phenolic acids from methanolic crude extract after alkaline hydrolysis (detection at 320 nm); 2 – caffeic acid, 3 – *p*-coumaric acid.

TABLE 4. Content of phenolic acids in the extracts and grapevine leaves.

Phenolic acid	Solvent used for extraction	Form of phenolic acid	Extracts (μg/g extract)	Leaves	
				(μg/g d.m.)	(μg/g f.m.)
Gallic	80% acetone	F	-	-	-
		E	2829 ± 36	802 ± 16	251 ± 5
		G	777 ± 38	220 ± 11	69 ± 3
		T	3606 ± 36	1022 ± 20	320 ± 6
	80% methanol	F	-	-	-
		E	1637 ± 32	447 ± 8	140± 2
		G	1282 ± 64	350 ± 17	109 ± 5
		T	2919 ± 64	797 ± 15	249± 5
Caffeic	80% acetone	F	26 ± 1	7	2
		E	453 ± 13	129 ± 4	40 ± 1
		G	11	3	1
		T	491 ± 14	139 ± 4	43 ± 1
	80% methanol	F	29 ± 1	7	2
		E	526 ± 15	143 ± 4	45 ± 1
		G	19 ± 1	5	2
		T	574 ± 16	155 ± 5	49 ± 1
p-Coumaric	80% acetone	F	-	-	-
		E	32 ± 1	9	3
		G	4	1	trace
		T	36 ± 1	10	3
	80% methanol	F	-	-	-
		E	49 ± 1	13 ± 1	4
		G	3	1	trace
		T	52 ± 1	14 ± 1	4

F – free phenolic acid; E – phenolic acid liberated from esters; G – phenolic acid liberated from glycosides; T – total.

cereals [Amarowicz *et al*., 2002; Karamać *et al*., 2002, 2004].

The content of gallic, caffeic, and *p*-coumaric acid in *Vitis vinifera* leaves extracts was determined using an HPLC method. Gallic acid was a dominant phenolic acid (3606 μg/g extract; 1022 μg/g leaves d.m.; 320 μg/g leaves f.m. – 80% acetone was used for extraction) (Table 4). In the analysed material caffeic acid was found in the form of free, esterified, and glycoside constituent. Gallic and *p*-coumaric acids were determined only as compounds liberated from esters and glycosides (Figures 5 and 6). The majority of phenolic acids was found as esters. The presence of the same phenolic acids in *Vitis amurensis* seeds germinated under osmotic stress was reported by Weidner *et al*. [2007]. In the cited work, the content of the mentioned phenolic acids was lower when compared with results of this work. Tartare esters of caffeic and *p*-coumaric acids were detected in grapevine leaves extract by Kolb & Pfündel [2005]. The content of gallic acid in muscadine grapes leaves ranged from 61 to 187 μg/g f.m. [Pastrana-Bonila *et al.*, 2003].

CONCLUSION

The analytical findings show that grapevine leaves which are waste materials from wine grape cultivation are a rich source of natural phenolic antioxidants. Therefore they might be used as functional ingredients for processing into nutraceuticals and health foods in the food industry.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support of the Polish Ministry of Science and Higher Education for 2006-2008 (COST/19/2005).

REFERENCES

1. Amakura Y., Umino Y., Tsuji S., Ito H., Hatano T., Yoshida T., Tonogai Y., Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. Food Chem., 2002, 77, 47-56.
2. Amaral J.S., Ferreres F., Andrade P.B., Valentao P., Pinheiro C., Santos A., Seabra R., Phenolic profile of hazelnuts (*Corylus avellena* L.) leaves cultivars grown in Portugal. Nat. Prod. Res., 2005, 19, 157-163.
3. Amarowicz R., Piskuła M., Honke J., Rudnicka B., Troszyńska A., Kozłowska H., Extraction of phenolic compounds from lentil seeds (*Lens culinaris*) with various solvents. Pol. J. Food Nutr. Sci., 1995, 45, 53-62.
4. Amarowicz R., Weidner S., Content of phenolic acids in rye caryopses determined using HPLC-DAD method. Czech J. Food Sci., 2001, 19, 201-203.
5. Amarowicz R., Karamać M., Weidner S., Abe S., Shahidi F., Antioxidant activity of wheat caryopses and embryos extracts. J. Food Lipids, 2002, 9, 201-210.
6. Amarowicz R., Troszyńska A., Antioxidant activity of extract of pea and its fractions of low molecular phenolics and tannins. Pol. J. Food Nutr. Sci., 2003, 53 SI 1, 10-15.
7. Amarowicz R., Troszyńska A., Baryłko-Pikielna N., Shahidi F., Extracts of polyphenolics from legume seeds – correlation between their total antioxidant activity, total phenolics content, tannins content and astringency. J. Food Lipids, 2004, 11, 278-286.
8. Amarowicz R., Troszyńska A., Shahidi F., Antioxidant activity of almond extract. J. Food Lipids, 2005, 11, 278-286.
9. Amarowicz R., Narolewska O., Karamać M., Kosińska A., Weidner S., Grape vine leaves as a source of flavonoids – potential antioxidants of food. Proceeding of the conference "Safety Food and Control", 28-29 March 2007, Nitra (Slovakia), pp. 203-206.
10. Cooper A., Chopra M., Thurnham D.I., Wine polyphenols and promotion of cardiac health. Nutr. Res. Rev., 2004, 17, 111-129.
11. Ciska E., Karamać M., Kosińska A., Antioxidant activity of extracts of white cabbage and sauerkraut. Pol. J. Food Nutr. Sci., 2005, 55, 367-373.
12. Cruz J.M., Dominguez H., Parajo J.C., Assessment of the production of antioxidants from winmaking waste solids. J. Agric. Food Chem., 2004, 52, 5612-5620.
13. Durmaz G., Cam M., Kutlu T., Antioxidant activities of some edible leaves. Book of Abstracts of the 2nd International Congress on Food and Nutrition, 24-26 October 2007, Istanbul, Turkey, p. 34.
14. Hassimoto N.M.A., Genovese M.I., Lajolo F.M., Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J. Agric. Food Chem., 2005, 53, 2928-2935.
15. Ito H., Kobayashi E., Li S.-H., Hatano T., Suita D., Kubo N., Shimura S., Itoh Y., Tokuda H., Nishino H., Yoshida T., Antitumor activity of compounds isolated from leaves of *Eriobotrya japonica.* J. Agric. Food Chem., 2002, 50, 2400-2403.
16. Karamać M., Amarowicz R., Weidner S., Abe S., Shahidi F., Antioxidant activity of rye caryopses and embryos extracts. Czech J. Food Sci., 2002, 20, 209-214.
17. Karamać M., Amarowicz R., Weidner S., Abe S., Shahidi F., Antioxidant activity of triticale caryopses and embryos extracts. Food Sci. Biotechnol., 2004, 13, 421-424.
18. Kolb C.A., Pfündel E.E., Origin of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant Cell Environ., 2005, 25, 580-590.
19. Mabry T.J., Markham K.R., Thomas M.B., The Systematic Identification of Flavonoids. 1970, Springer-Verlag, New York-Heidelberg-Berlin, pp. 41-164.
20. Meyer A.S., Yo O.-S., Pearson D.A., Waterhouse A.L., Frankel E.N., Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). J. Agric. Food Chem., 1997, 45, 1638-1643.
21. Naczk M., Shahidi F., The effect of methanol – ammonia – water treatment on the content of phenolic acids of canola. Food Chem., 1989, 31, 159-164.
22. Naczk M., Amarowicz R., Zadernowski R., Pegg R., Shahidi F., Antioxidant capacity of crude phenolic extracts from wild blueberry leaves. Pol. J. Food Nutr. Sci., 2003, 53 SI 1, 166- 169.
23. Naczk M., Amarowicz R., Zadernowski R., Shahidi F., Antioxidant capacity of phenolics from canola hulls as affected by different solvents. ACS Symposium Series, 2005, 909, 57-66.
24. Nishino H., Yoshida T., Antitumor activity of compounds isolated from leaves of *Eriobotrya japonica.* J. Agric. Food Chem., 2002, 50, 2400-2403.
25. Oyaizu M., Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 1986, 44, 307-315.

26. Pari L., Karamać M., Kosińska A., Rybarczyk A., Amarowicz R., Antioxidant activity of the crude extracts of drumstick tree (*Moringa oleifera* Lam.) and sweet broomweed (*Scoparia dulcis* L.) leaves. Pol. J. Food Nutr. Sci., 2007, 57, 203-208.
27. Pastrana-Bonila E., Akoh C.C., Sellappan S., Krewer G., Phenolic content and antioxidant capacity of muscadine grapes. J.Agric. Food Chem., 2003, 51, 5497-5503.
28. Pegg R.B., Amarowicz R., Nacz. M., Shahidi F., PCHOTOCHEM for determination of antioxidant capacity of plant extracts. ACS Symposium Series, 2007, 956, 140-158.
29. Price M.L, Van Scoyoc S., Butler L.G., A critical evaluation of the vanillic reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 1978, 26, 1214-1218.
30. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med., 1999, 26, 1231-1237.
31. Rocha-Guzmán N.E., Herzon A., Gonzáles-Laredo R.F., Ibarra-Pérez F.J., Zambrano-Galván G., Gallegos-Infante J.J., Antioxidant and antimutagenic activity of phenolic compounds in three different color groups of common bean cultivars (*Phaseolus vulgaric*). Food Chem., 2007, 103, 521-527.
32. Siddhuraju P., Becker K., Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem., 2003, 51, 2144-2155.
33. Troszyńska A., Honke J., Kozłowska H., Polyphenolic compounds in faba bean (*Vicia faba*) seed coat. 1993, *in*: Recent Advances of Research in Antinutrional Factors in Legume Seeds (eds. A.F.B. Van der Poel, J. Huisman, H.S. Saini). Wageningen Press, Wageningen, pp. 91-94.
34. Velioglu Y.S., Mazza G., Gao L., Oomah B.D., Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem., 1998, 46, 4113-4117.
35. Wang W., Goodman M.T., Antioxidant property of dietary phenolic agents in a human LDL-oxidation *ex vivo* model: Interaction of protein binding activity. Nutr. Res., 1999, 19, 191-202.
36. Weidner S., Karamać M., Amarowicz R., Szypulska E., Gołgowska A., Changes in composition of phenolic compounds and antioxidant properties of *Vitis amurensis* seeds germinated under osmotic stress. Acta Physiol. Plant., 2007, 29, 283-290.
37. Wróbel M., Karamać M., Amarowicz R., Frączek E., Weidner S., Metabolism of phenolic compounds in *Vitis riparia* seeds during stratification and during germination under optimal and low temperature stress conditions. Acta Physiol. Plant., 2005, 27, 313-320.
38. Xia J., Allenbrand B., Sun G.Y., Dietary supplementation of grape polyphenols and chronic ethanol administration on LDL oxidation and platelet function in rats. Life Sci., 1998, 63, 383-390.
39. Yamakoshi J., Katoka S., Koga T., Ariga T., Proanthocyanidin-rich extract from grape seed attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. Arteriosclerosis, 1999, 142, 139-149.
40. Yilman Y., Toledo R.T., Heath effect of functional grape seed constituents. Trends Food Sci. Technol., 2006, 15, 422-433.

Received, revised and accepted December 2007.

LIŚCIE WINOROŚLI JAKO ŹRÓDŁO NATURALNYCH PRZECIWUTLENIACZY

Ryszard Amarowicz[1], Olga Narolewska[1, 2], Magdalena Karamać[1], Agnieszka Kosińska[1], Stanisław Weidner[2]

[1]Instytut Rozrodu Zwierząt i Badań Żywności Polskiej Akademii Nauk, Olsztyn; [2]Katedra Biochemii, Wydział Biologii, Uniwersytet Warmińsko-Mazurski w Olsztynie

Ekstrakt surowy związków fenolowych otrzymano z liści winorośli stosując 80% aceton i 80% metanol (v/v). Całkowitą zawartość fenoli oznaczono odczynnikiem fenolowym Folina i Ciocalteau, skondensowane taniny z odczynnikiem wanilina/HCl oraz poprzez precypitację białka. Właściwości przeciwutleniające ekstraktu badano stosując metodę całkowitej aktywności przeciwutleniającej (TAA), wymiatanie wolnego rodnika DPPH oraz zdolność redukcyjną.

Zawartość fenoli ogółem w ekstraktach była wysoka i wynosiła 257 mg/g (ekstrakt acetonowy) oraz 232 mg/g (ekstrakt metanolowy). Zawartość skondensowanych tanin w ekstrakcie acetonowym była wyższa niż w metanolowym. Aktywność przeciwrodnikowa wobec rodnika DPPH, i zdolność redukcyjna były wysokie i nie zależały od składu ekstrakcyjnego. Ekstrakt acetonowy i metanolowy wykazywały TAA równe 1,37 i 1,44 mmol Trolox/ g. W ekstraktach z liści *Vitis vinifera* stwierdzono obecność kwasu galusowego, kawowego oraz *p*-kumarowego – dominował kwas galusowy. Większość fenolokwasów występowała w postaci estrów.