Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2014, Vol. 64, No. 2, pp. 91–99
DOI: 10.2478/v10222-012-0097-x
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

A Comprehensive Study on Antioxidant Properties of Crude Extracts from Fruits of *Berberis vulgaris* L., *Cornus mas* L. and *Mahonia aquifolium* Nutt.

*Katarzyna Pyrkosz-Biardzka[1], Alicja Z. Kucharska[2], Anna Sokół-Łętowska[2], Paulina Strugała[1], Janina Gabrielska[1,3]**

[1]Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, 50–375 Wroclaw, Norwida 25, Poland
[2]Department of Fruit, Vegetable and Cereal Technology, Wroclaw University of Environmental and Life Sciences, Chełmańskiego 37/41, 51–300 Wrocław, Poland

Key words: plant extracts, lipid peroxidation, antioxidative protection, antiradical properties, partition coefficient

The antioxidant capacity of methanolic crude extracts of *Berberis vulgaris* L., *Cornus mas* L. and *Mahonia aquifolium* Nutt. was tested with the thiobarbituric acid reactive substances formation assay, the ferric reducing power (FRAP) and 2,2-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging assay. The content of antioxidant components in the extracts, their partition coefficient on 1-octanol:water and affinity to liposome membranes were determined as well. The results show that the IC_{50} parameter connected with the antioxidant activity on phosphatidylcholine liposome membrane decreased as follows: *B. vulgaris* (0.14±0.01 mg/mL) > *M. aquifolium* (0.34±0.03 mg/mL) > *C. mas* (1.13±0.01 mg/mL) for AAPH-induced oxidation and *M. aquifolium* (0.29±0.03 mg/mL) > *C. mas* (1.24±0.07 mg/mL) > *B. vulgaris* (1.50±0.05 mg/mL) for Fe(II)/ascorbic acid-induced oxidation, and *M. aquifolium* (2.35±0.10 mg/mL) > *B. vulgaris* (2.69±0.04 mg/mL) > *C. mas* (6.17±0.06 mg/mL) for UVC irradiation. All the extracts exhibited the ability to quench DPPH• and to reduce Fe(III) ions to Fe(II) *via* redox reaction. The content of active components in the extracts, the partition coefficient and extracts affinity to membranes correlated well with their antioxidant activities.

This study has shown that fruits of *B. vulgaris*, *M. aquifolium* and *C. mas*, from which the extracts were obtained, are attractive for consumption and can potentially be used in production of new processed fruit.

INTRODUCTION

Free radicals ubiquitous in the environment affect a human organism, resulting, under oxidative stress-induced damage, in many diseases [Halliwell & Gutteridge, 1984; Gutteridge, 1993]. It is thus necessary to find the so-called exogenous antioxidants that can support the organism in its struggle against the actions of free radicals [Jacob & Burri, 1996]. It would be best if a well-balanced and differentiated diet supplied appropriate amounts of such substances. Polyphenols contained in *e.g.* fruits and vegetables constitute a large group of health-boosting substances whose activity depends to a large extent on their antioxidant action [Liu, 2003, 2004]. Producers of processed fruit are eager to attract consumers with new and attractive products. In recent years, increasing attention has been paid by producers to lesser known fruits, such as cornelian cherry (*Cornus mas* L.), barberry (*Berberis vulgaris* L.) and mahonia (*Mahonia aquifolium* Nutt.) which possess unusual flavour, and are rich in antioxidants and natural colorants. Moreover, fruits and leaves of these plants are known for their biological activity, such as *e.g.* anti-histaminic, anti-inflammatory, anti-malarial, hepatoprotective and vasodilatory [Ji *et al*., 2000; Seeram *et al*., 2002; Fatehi *et al*., 2005; Minaiyan *et al*., 2011]. The antioxidant activity of *Berberis vulgaris* and *Mahonia aquifolium* has been poorly known until now [Račková *et al*., 2007; Koncić *et al.*, 2010]. It was found that *Berberis vulgaris* possesses some radical-scavenging and antioxidant activities, as determined by the scavenging effect on DPPH•radical, ferric reducing power (FRAP) and β-carotene-linoleic acid model systems. Fruits of *Cornus mas* L. are known as the European and Asiatic cornelian cherry and are used in Europe as a raw material in the food industry [Vareed *et al*., 2006; Tural & Koca, 2008]. The fruits are very good for fresh consumption and very valuable for processing to produce syrups, juices, jam and other traditional products [Rop *et al*., 2010]. Popović *et al.* [2012] investigated the antioxidant activity of 10 genotypes of cornelian cherry from Vojvodina province (in Serbia) by different methods, including DPPH•, •NO, $O_2^{\bullet-}$ antiradical power, reducing power (FRAP) and lipid peroxidation. These studies and those of other authors [Tural & Koca, 2008; Yilmaz *et al.*, 2009] have indicated a high antioxidative potential of cornelian cherry fruits. There are no research papers on

* Corresponding Author: Tel. +48 0 71 320 5294; Fax: +48 0 71 320 5167; E-mail: janina.gabrielska@up.wroc.pl (Janina Gabrielska)

the antioxidant activity of fruit extracts and their affinity to the lipid membrane tested on liposomes as a lipid model of the cell membrane. Reports of their antioxidant activity and antiradical properties suggested to investigate the affinity of the extracts to the organic phase and the liposome membrane. The present *in vitro* investigation is aimed at answering the question if the fruit extracts are effective antioxidants with respect to oxidized lipids and whether they are free radical scavengers in redox processes, in view of their potential application in the food industry to protect food lipids against rancidity. Polyphenols were extracted from fruits with an 80% methanol solution, as literature data indicate methanol to be commonly used in such cases, with its extraction efficiency being comparable with that by ethanol, or a bit lower [Turkmena *et al.*, 2006; Wang *et al.*, 2008].

The aim of this study was to comprehensively investigate the antioxidant properties of methanolic crude extracts of *B. vulgaris* L., *M. aquifolium* Nutt. and *C. mas* L. fruit from different sites in Poland, by employing various *in vitro* assays, *i.e.* DPPH$^{\bullet}$, FRAP, and TBARS test for lipid peroxidation in model lipid membranes, in order to establish the usefulness of the fruit extracts for the functional food industry. Additional analyses were carried out for the content of total polyphenols, anthocyanins and flavonoids with identification of main components in the extracts as well as their ability to incorporate into the octanol:water interface and the phosphatidylcholine liposome membrane.

MATERIALS AND METHODS

Materials

2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) of 97% purity, *N,N*-dimethylformamide (DMF), iron (II) chloride tetrahydrate ($FeCl_2 \times 4H_2O$) of 99% purity, 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), 1-octanol, rutin hydrate of 85% purity, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), gallic acid, iron (III) chloride anhydrous ($FeCl_3$) of 97% purity, 2-thiobarbituric acid (TBA) 98% and aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). L-Ascorbic acid, acetic acid, sodium acetate trihydrate, sodium carbonate anhydrous, Folin-Ciocaltaeu's phenol reagent and chloroform were obtained from POCH Gliwice (Gliwice, Poland). The fluorescence probe 3-*p*-(6-phenyl)-1,3,5--hexatrienyl (DPH) was obtained from Molecular Probes (Eugene, OR, USA). Methanol and tris(hydroxymethyl)aminomethane (Tris:HCl) were obtained from "Chempur" Piekary Śląskie (Piekary Śląskie, Poland). Trichloroacetic acid (TCA) and hydrochloric acid (HCl) 36% were obtained from "Stanlab" P.P.H. Lublin (Lublin, Poland).

Plant extracts

Fruits of *B. vulgaris* L. were collected around Wroclaw, *M. aquifolium* Nutt. fruits were obtained from the Research Experimental Station in Piastów, whereas fruits of *C. mas* L. were obtained from the Arboretum and Institute of Physiography in Bolestraszyce. Fruits were harvested in September 2007.

Preparation of 80% methanol extracts

Extracts were prepared at the Department of Fruit, Vegetables and Cereals Technology. To 5.00 g of fruit pulp (*B. vulgaris* L., *C. mas* L., *M. aquifolium* Nutt.) 10 mL of 80% methanol acidified with hydrochloric acid were added. Samples were sonicated (INTERSONIC IS-3) for 10 min. Afterwards, the extracts were filtered on Schott's funnel under vacuum. Precipitates were collected into 50 mL flasks.

Preparation of phosphatidylcholine

Phosphatidylcholine was extracted from hen egg yolk at the Department of Chemistry, Wrocław University of Environmental and Life Sciences, according to the procedure as in the paper by Gładkowski *et al.* [2009]. Briefly, to 30 g of dried egg yolk 50 mL of acetone was added. The sample was shaken for 15 min and centrifuged. The operation was repeated three times. Afterwards, the precipitate was extracted three times in 50 mL of methanol and then centrifuged. The obtained residue was filtered; next the ooze was evaporated and dissolved in 35 mL of hexane with the addition of 150 mL of frosted acetone. After centrifugation the precipitate was separated in a chromatographic column and, as a result, pure phospholipids were obtained (phosphatidylethanolamine, phosphatidylocholine and cholesterol).

Liposome preparation for lipid peroxidation

Lecithin was dissolved in chloroform (10 mg/mL) and dried under an N_2 stream for one minute and under vacuum for another 30 min. Dried lipids were resuspended in Tris-HCl buffer (pH 7.4). The preparations were sonicated for 5 min using a 20 kHz sonicator. During sonication the samples were thermostated at 0–2°C.

Antioxidant capacity assay of the extracts

Lipid peroxidation in egg phospholipid liposomes was induced by ultraviolet radiation from a bactericidal lamp (λ=253.7 nm) at 3.0 mW/cm^2 intensity (UVC) or by incubation with 10 mmol/L AAPH [2,2′-azobis (2-methylpropionamidine) dihydrochloride] at 37°C; or with 10 μmol/L of an Fe(II)/ascorbic acid mixture. The accumulation of phospholipid peroxidation products was estimated by determination of 2-thiobarbituric acid reactive substances (TBARS) in the incubation medium [Buege & Aust, 1978]. The measure of thiobarbituric acid reactive substances gives the concentration of malondialdehyde (MDA) and related compounds generated from cyclic peroxides or endoperoxides. A pink chromophore that can be detected at 535 nm was formed as a consequence of their reaction with thiobarbituric acid at a high temperature. A sample without antioxidant addition served as the control. The percentage of phosphatidylocholine (PC) liposome oxidation induction or inhibition was calculated from the formula:

$$\% \, INHIBITION = \{(1 - \Delta A_A) / \Delta A_0\} \times 100\%$$

where: ΔA_A – absorption increase (at λ=535 nm) after 30 min exposure (or 60 min of incubation with AAPH, or with a mixture Fe(II)/ascorbic acid with an antioxidant added), ΔA_0 – absorption increase (at λ=535 nm) after 30 min of light ex-

posure of PC liposomes (or 60 min incubation with AAPH or with Fe(II)/ascorbic acid mixture) without antioxidant added. The IC_{50} value was determined from the dependency of the percentage of oxidation inhibition after 30 min of oxidation induction or 60 min of incubation with AAPH or with Fe (II)/ascorbic acid on the concentration of the extracts studied. This allowed calculation of the value of $x = IC_{50}$ was calculated for y = 50%. IC_{50} denotes the antioxidant concentration (in mg of fresh fruit/mL) causing 50% inhibition of liposome oxidation after 30 min of UV irradiation or after 60 min of incubation with AAPH with Fe(II)/ascorbic acid mixture. The antioxidant activity of the extracts was compared with the antioxidant activity of the dissolvable-in--water tocopherol analogue – Trolox – and expressed in TEAC (Trolox Equivalent Antioxidant Capacity, in mmol TE/100 g FW). All determinations were performed in six replicates (n=6).

Free-radical scavenging assay

The effect of the studied extracts on reduction of free DPPH• radicals was measured spectrophotometrically, as described previously by Brand-Williams *et al.* [1995]. Briefly, a DPPH• methanol solution with absorption of approximately 0.9 was mixed with a proper amount of extract or Trolox solution and immediately placed in a spectrophotometer. As a control, the absorption of DPPH• radicals (without addition of an extract) was measured at time t=0. The % reduction of DPPH• in the sample after 15 min incubation with an antioxidant (of fixed concentration) was determined using the formula:

$$\%\ REDUCTION = \{(\Delta A_C - \Delta A_A)/\Delta A_C\} \times 100\%$$

where: ΔA_C is a change of absorbance at λ=517 nm, after 15 min in the absence of an antioxidant; and ΔA_A is a change in absorbance at λ=517 nm, after 15 min of the probe with the antioxidant at t=15 min. The results were expressed in mmol TE/100 g FM (TEAA). All determinations were performed in six replicates (n=6).

Determination of ferric reducing/antioxidant power (FRAP)

The total antioxidant potential of the sample was determined using a ferric reducing ability of plasma (FRAP) assay presented by Benzie & Strain [1996]. Three hundred μL of freshly prepared FRAP reagent (0.3 mol/L acetate buffer pH 3.6; 10 mmol/L TPTZ in 40 mol/L HCl, 20 mmol/L $FeCl_3$) were warmed to 37°C and a 10 μL sample was then added and vortexed. The absorption of the blue complex Fe (II)-TPZT was measured at 595 nm after 10 min. The results were expressed by the FRAP in μmol Trolox per 1 g of fresh mass (μmol TE/1 g FM). A standard curve was plotted using different concentrations of Trolox. The solutions were used on the day of preparation. All determinations were performed in six replicates (n=6).

Determination of total phenolic content

In brief, 100 μL of methanolic solution of each properly diluted extract and 200 μL of Folin-Ciocaltau reagent were mixed and incubated for 3 min in dark. Afterwards, 2 mL of distilled water and one mL of 20% Na_2CO_3 were added, and then the mixture was mixed and incubated in the dark for 60 min. The control sample was also prepared by replacing the content of the extract with its solvent. All samples were centrifuged for 10 min at 3000 rpm at room temperature. Based on the spectrum (spectrophotometer Cary 300, Varian) of the tested extract the maximum value of the wavelength was estimated, at which the absorbance readings were performed. The results were expressed as equivalent mg of gallic acid per 100 g of fresh mass (mg GAE/100 g FM). A standard curve for gallic acid was plotted under the same conditions as the studied samples. All determinations were performed five times (n=5).

Determination of total flavonoid content

Each extract was analyzed for total flavonoid content according to the previously reported colorimetric method with modifications [Jia *et al*., 1999]. One mL of a methanolic solution of extracts at a suitable concentration was mixed with one mL of a methanolic solution of 2% $AlCl_3 \times 6H_2O$. After 10 min incubation the absorbance of the solution was measured at 430 nm with a spectrophotometer (Cary 300, Varian). The results were expressed as equivalent mg of rutin per 100 g of fresh mass (mg RE/100 g FM) and compared with the rutin standard curve, which was made under the same conditions. All determinations were performed five times (n=5).

Determination of anthocyanins content

Total anthocyanin amount, calculated as cyanidin 3-O-glucoside, was determined by means of the pH differential method [Giusti & Wrolstad, 2001], using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) and sodium acetate buffer, pH 4.5 (0.4 mol/L). At pH 1.0 anthocyanins occur in the colored oxonium or flavylium form, whereas at pH 4.5 they are predominantly in the colorless carbinol form. The first 1.0 mL aliquot of the extract was transferred to a 100 mL volumetric flask, diluted to a volume of pH 1.0 buffer and mixed. The second 1.0 mL aliquot of the extract was placed in a 100 mL volumetric flask, diluted to the volume with pH 4.5 buffer and mixed. The solutions were allowed to stand at a room temperature for 20 min. Absorption was measured with a spectrophotometer (Cary 300, Varian) at 510 and 700 nm, using the formula: $A = (A_{510}\text{-}A_{700})_{pH\ 1.0} - (A_{510}\text{-}A_{700})_{pH\ 4.5}$. Results were expressed as equivalent mg of cyanidin 3-O-glucoside per 100 g of fresh mass (mg C3GE/100 g FM). All determinations were performed in six replicates (n=6).

Analytical HPLC of phenolics and iridoids

Phenolics and iridoids were determined by the method described by Kucharska [2012], using the Dionex HPLC system (USA) equipped with the diode array detector model Ultimate 3000. Compounds were monitored at wavelengths: 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (phenolic acids), and 245 nm (iridoids). The results were calculated as % of cyanidin 3-O-glucoside, quercetin 3-O-glucoside, chlorogenic acid, ellagic acid, gallic acid, *p*-coumaric acid, and loganic acid. All determinations were performed in duplicate.

Estimation of partition coefficient

1-octanol:water (P) partition coefficient

Estimation of the 1-octanol:water (P) partition coefficient was done according to Nenadis *et al.* [2003]. In brief, 3 mL of 1-octanol and a proper amount of each extract were kept at 37°C for 30 min and an UV spectrum was then run. The absorbance value was recorded at the maximum wavelength (A_0). Equal volumes of organic and aqueous phases were vortexed (2500 rpm) for 1 min and centrifuged for five min, 1500×g, at a room temperature. After 30 min, the UV spectrum of the organic layer (A_x) was recorded. The partition coefficient P was calculated according to the formula: $P = [A_x / (A_0 – A_x)]$. A solution of 1-octanol saturated with water was used as the blank. All determinations were performed five times (n=5).

Liposome PC: buffer (K_d) partition coefficient

The procedure for determining the liposome PC:buffer (K_d) partition coefficient was described by Tammela *et al.* [2004]. This method is based on fluorescence quenching of the DPH probe and determines the partition coefficient (K_d – dissociation constant) of the test compounds to model bilayer membrane systems comprised of phosphatidylcholine (PC) as the bilayer-forming phospholipid. For this experiment liposomes were prepared in the same manner as described earlier. The fluorescent DPH probe was used at 0.1 μmol/L concentration. The DPH stock solution was prepared in DMF. Partition coefficients were determined, assuming the use of the Stern-Volmer equation, from the fluorescence intensity of the DPH probe as a function of the tested compound concentration:

$$\frac{1}{(F_0 / F) - 1} = \frac{K_d}{[PC]} \cdot \frac{1}{[Sub]} + \frac{1}{[PC]}$$

where: F_0 and F are the fluorescence intensities observed in the absence and in the presence of extract (Sub), respectively, [PC] – concentration of phosphatidylcholine in the probe, [Sub] – extract concentration in mg/mL. A straight line was obtained from the plot $1 / [(F_0 / F) – 1]$ *versus* 1 / [Sub], of which K_d values were obtained by the gradual addition of the test extract into vesicles with a fluorescent probe. The fluorescence experiment was conducted using a spectrofluorimeter with a built-in polarization attachment (SFM-25, Kontron Instruments, Zürich, Switzerland). The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were as follows: λ_{ex}=360 nm and λ_{em}=425 nm. All determinations were performed in six replicates (n=6).

Statistical analysis

Means and standard deviation were calculated from the data obtained from five to six experiments. These data were compared using Duncan's multiple range test. Data were analyzed by one-way analysis of variance (ANOVA) using Statistica (Version 8.0). Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The inhibitory effect of the extracts on peroxidation of the phosphatidylcholine liposome membrane is illustrated in Figure 1. Results are expressed in terms of the IC_{50} parameter, which indicates the concentration of an extract that causes 50% reduction of liposome oxidation. Membrane lipids were oxidized using three different inductions: AAPH compound, Fe(II)/ascorbic acid mixture and UVC irradiation. In the case of AAPH-induced oxidation, significantly ($p<0.05$) greater protection of liposome membranes occurred with the extract *B. vulgaris* L. (0.14±0.01 mg/mL) compared to *M. aquifolium* Nutt. (0.34±0.03 mg/mL) and *C. mas* L. (1.13±0.01 mg/mL). In this case free radicals were developed as a result of successive thermal decomposition of AAPH molecules that consisted in the breaking of a double bond between nitrogen atoms, with resultant formation of two free radicals. The rate at which this reaction occurred was most probably low because of the high efficiency of their recombination by extract components. Significantly lower membrane protection was observed in the case of liposome oxidation induced by UVC radiation. There were no statistically significant differences ($p<0.05$) between extracts of *M. aquifolium* Nutt. (2.35±0.10 mg/mL) and *B. vulgaris* L. (2.69±0.04 mg/mL). The antioxidant activity of the *C. mas* L. extract was however significantly lower (6.17±0.06 g/mL). This might be due to the relatively low ability of the extracts to scavenge the hydroxyl radicals that arise as a result of UV irradiation. Hydroxyl radicals are highly reactive radicals, capable of attacking most biological substrates; for example carbohydrates, DNA, polyunsaturated fatty acids and proteins [Shih & Hu, 1999]. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from phospholipid membranes and thus inducing peroxidic reaction of lipids [Karawita *et al*., 2005]. During UVC irradiation, aside from the mentioned •OH, the singleton oxygen arises. UVC may directly transfer photons from a triplet oxygen to a singlet state. This possibility is energetically feasible since the energy content of UVC photon-mole is ~ 226 kJ/mol, whereas that of singlet oxygen is ~93 kJ/mol above the ground state, but achievable only when oxygen concentration is high [Shih & Hu, 1999].

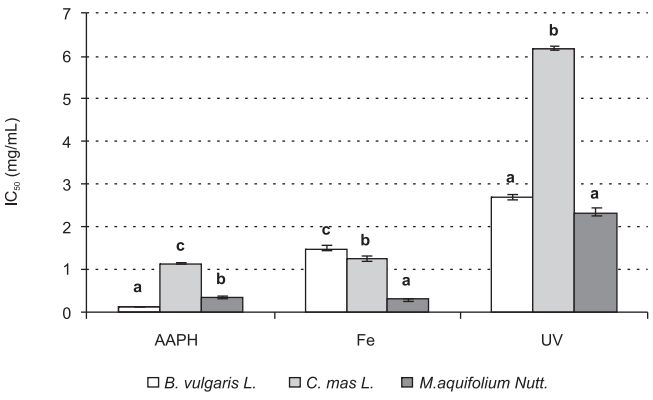

FIGURE 1. Comparison of IC_{50} (mg/mL) of PC oxidation induced by AAPH, Fe (II)/ascorbic acid ions (Fe) and UV light for the compounds studied: *B. vulgaris* L., *C. mas* L. and *M. aquifolium* Nutt. Data are the mean±SD from three independent experiments. The mean significant difference of a–c is $p<0.05$ by Duncan's test.

In the case of Fe(II)/ascorbic acid as an oxidation inducer, values of the IC_{50} parameter were the lowest for *M. aquifolium* Nutt. (0.29±0.03 mg/mL). Statistically significant differences ($p<0.05$) were found between antioxidant activities of *C. mas* L (1.24±0.07 mg/mL) and *B. vulgaris* L. (1.50±0.05 mg/mL). In the presence of iron ions the lipid peroxidation process may be accompanied with a reinitiating phenomenon, meaning that lipid peroxides – *i.e.* nonradical products of peroxidation – would decompose into free-radical products. Such a decomposition is initiated by ions of transition metals (especially iron and copper):

$$LOOH + Fe(II) \rightarrow Fe(III) + HO^- + LO^\bullet \text{ and } LOOH + + Fe(III) \rightarrow Fe(II) + H^+ + LOO^\bullet$$

Fe(III) ions react with lipid peroxides slower than Fe(II) ions. However, lipid peroxidation can be initiated only when both Fe(II) and Fe(III) are present, neither Fe(II) nor Fe(III) alone being able to promote the peroxidation of polyunsaturated fatty acids. The absolute Fe(III): Fe(II) ratio determines the rate of lipid peroxidation, the highest activity being associated with a ratio approaching 1:1 [Minotti & Aust, 1987].

The antioxidant potential of methanolic extracts to protect liposome membrane against oxidation was compared with the antioxidant activity of Trolox, a water-soluble derivative of vitamin E (Table 1). Analyses demonstrated the highest TEAC values of the trimers in the case of liposomal membranes oxidation induced by AAPH. The highest value of TEAC was obtained for *M. aquifolium* Nutt. and *B. vulgaris* L., which for *M. aqufolium* Nutt. accounted for 40% of the *B. vulgaris* L value. TEAC value for *Cornus mas* L. in this case was about 3 times lower in comparison to *M. aquifolium* Nutt. In other cases, *i.e.* the oxidation of Fe(II)/ascorbic acid or UV radiation, the TEAC value was significantly lower reaching even less than unity.

The DPPH$^\bullet$, a stable nitrogen-centered free radical, is frequently used for the estimation of free radical-scavenging ability [Brand-Williams *et al*., 1995]. Analyses showed a significantly higher ($p<0.05$) DPPH$^\bullet$ scavenging activity for *B. vulgaris* L. extract (4.18±0.21 mmol TE/100 g FM) compared to that of *M. aquifolium* Nutt. (2.96±0.12 mmol TE/100 g FM) and *C. mas* L. (1.96±0.02 mmol TE/100 g FM) (Figure 2). The activity of *C. mas* L. in reducing DPPH$^\bullet$, according to West *et al.* [2012], was expressed as IC_{50} parameter equal to 3.8 μL/mL. However, the DPPH$^\bullet$ method is only capable of indicating the potential antioxidant activity, it does not utilize a food/biologically-relevant oxidizable substrate and, consequently, no direct information on protective performance can be determined [Dorman *et al*., 2003b]. Therefore, the ability of the samples to inhibit peroxidation of the liposome membrane was determined, as phospholipids are believed to play a principal role in oxidative deterioration and the off-flavor development in food products.

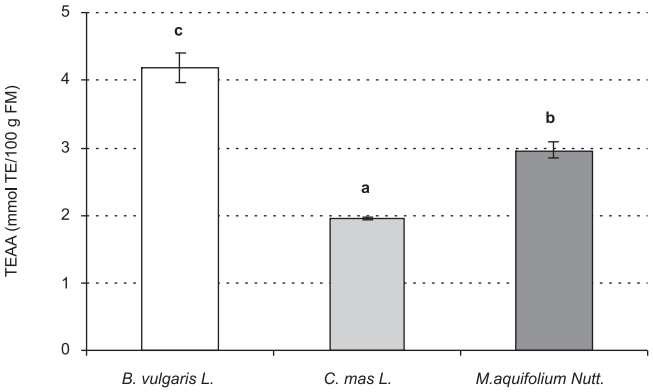

FIGURE 2. DPPH$^\bullet$ free radical scavenging properties of extracts from *B. vulgaris* L., *C. mas* L. and *M. aquifolium* Nutt., expressed as a value of TEAA in mmol TE/100 g FM. Data are the mean±SD from five independent experiments. Differences are statistically significant. Capacities to scavenge free radicals are denoted with the letters *a*, *b, c* ($p<0.05$).

The FRAP assay is based on measurement of the ability of a substance to reduce Fe(III) to Fe(II). The reducing power is another antioxidant defense parameter commonly used to establish the oxidation potential of plant extracts. Different studies have indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with their antioxidant activity [McCune & Johns, 2002; Dorman *et al*., 2003a]. The data presented in this study suggest that components in *C. mas* L., *M. aquifolium* Nutt. and *B. vulgaris* L. which are soluble in the acidic aqueous methanol extracts are capable of electron donation and therefore should be able to donate electrons to unstable free radicals, converting them into more stable non-reactive species, with the *B. vulgaris* L. extract components being the most effective electron donators. The methanolic extract of *B. vulgaris* L. possessed the highest FRAP, which was significantly higher than the value observed for the *M. aquifolium* Nutt. and *C. mas* L. extracts. The reduction potential of *C. mas* L. extract was also evaluated by Pantelidis *et al*. [2007] and Tural & Koca [2008], but differences in the assay protocol made the comparison of the data impossible.

Summarized data of the concentrations of total polyphenols, flavonoids and anthocyanins in three extracts are presented in Table 2. A significantly higher ($p<0.05$) content of polyphenols was found in the *B. vulgaris* L. extract (1024.31±15.20 mg GAE/100 g FM) compared to *M. aquifolium* Nutt. extract (806.19±6.44 mg GAE/100 g FM). The *C. mas* L. extract had the lowest content of total polyphenols. The total polyphenol content in cornelian cherries grown in Turkey ranges between 280 and 580 mg/100 g FM [Tural & Koca, 2008]. The *Cornus mas* grown in the Czech

TABLE 1. Comparison of TEAC (mmol TE/100 g FM) and FRAP values (μmol TE/1 g FM) for the plant extracts studied obtained for phosphatidylcholine membranes (PC). Phosphatidylcholine liposome oxidation was induced by AAPH or Fe(II)/ascorbate (Fe) acid mixture incubation or UV radiation. Data are the mean±SD of triplicate samples. Different letters (a–c) within the same column indicate significant differences at $p<0.05$ by Duncan's test.

Extract	FRAP (μmol TE/1 g FM)	TEAC (mmol TE/100 g FM)		
		AAPH	Fe	UV
B. vulgaris L.	20.09 ± 0.88^b	24.17 ± 1.54^c	0.71 ± 0.02^a	0.45 ± 0.01^b
C. mas L.	13.81 ± 0.60^a	3.00 ± 0.03^a	0.87 ± 0.05^a	$0.20\pm0.2\cdot10^{-2a}$
M. aquifolium Nutt.	12.37 ± 0.42^a	9.94 ± 0.74^b	3.72 ± 0.14^b	0.51 ± 0.02^c

TABLE 2. Comparison of total phenolics content (mg GAE/100 g FM), flavonoids content (mg RE/100 g FM) and anthocyanins content (mg C3GE/100 g FM) in crude methanolic extracts of *B. vulgaris* L., *C. mas* L. and *M. aquifolium* Nutt. Data are the mean±SD from five independent experiments. Different letters (a–c) within the same column indicate significant differences at $p<0.05$ by Duncan's test.

Extract	Total phenolic concentration (mg GAE/100 g FM)	Flavonoid concentration (mg RE/100 g FM)	Anthocyanin concentration (mg C3GE/100 g FM)
B. vulgaris L.	1024.31 ± 15.20^{c}	86.03 ± 1.84^{c}	45.89 ± 2.17^{a}
C. mas L.	339.36 ± 4.59^{a}	63.86 ± 3.04^{a}	49.94 ± 2.24^{a}
M. aquifolium Nutt.	806.19 ± 6.44^{b}	78.17 ± 1.81^{b}	226.63 ± 4.46^{b}

GAE – gallic acid equivalent; RE – rutin equivalent; C3GE – cyanidin 3-O-glucoside equivalent.

Republic contains higher amounts of total polyphenols, up to 210 mg/100 g FW [Rop *et al*., 2010]. The Bulgarian cultivars of cornelian cherries contain comparable to other reports amounts of total polyphenols in the range 280–460 mg/100 g FM [Marinova *et al*., 2005]. Total anthocyanin content in *Cornus mas* was reported as being on average 223.0 mg per 100 g [Pantelidis *et al*., 2007] and between 95 and 107 mg/100 g FM [Tural & Koca, 2008]. The broad range of anthocyanin concentrations reported for Polish cultivars of cornelian cherries in this study (27–160 mg/100 g FM) and in previous papers is due to great differences in pigmentation between the cultivars of cornelian cherries [Kucharska, 2012].

Flavonoids, including anthocyanins, as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging properties. A significantly higher ($p<0.05$) content of flavonoids in the methanolic extract of *B. vulgaris* L. (86.03±1.84 mg RE/100 g FM) was found than in *M. aquifolium* Nutt.(78.17±1.81 mg RE/100 g FM) and *C. mas* L. (63.86±3.04 mg RE/100 g FM). At the same time the extract of *M. aquifolium* Nutt. contained the highest level of anthocyanins. No significant differences ($p<0.05$) were found between anthocyanins contents of the *B. vulgaris* L. and *C. mas* L. extracts. In the present work the crude extract of *B. vulgaris* L. (with the highest content of total polyphenols and flavonoids) showed weak capability to protect liposome membranes against peroxidation initiated by iron ions. According to Chen & Ahn [1998], the free-radical scavenging ability of antioxidants, UV absorption and/or Fe(II)-chelation properties of natural phenolics contributed significantly to the control of lipid oxidation induced by UV or Fe(II).

The antioxidant capacity of extracts towards liposomes oxidized by AAPH ($TEAC^{AAPH}$), UV ($TEAC^{UV}$) and free radical scavenging (DPPH•) was highly correlated with the total content of polyphenols (r=0.920, r=0.869, r=0.966) and flavonoids in the extracts (r=0.935, r=0.848, r=0.975). There was also a correlation between total polyphenols and flavonoids content and the reducing power (r=0.617, r=0.648, respectively). This fact may suggest a mechanism of the antioxidant ability of the extracts when liposomes are oxidized with AAPH and UV radiation. The free radicals AAPH• and •OH are most probably reduced by electrons of the extract molecules or the hydrogen atom.

$TEAC^{UV}$ was also correlated with anthocyanins content (r=0.648). The antioxidant potential of plant extracts is associated with the concentration and composition of polyphenolic compounds [Rop *et al*., 2010]. Such a high correlation proves that in both cases of membrane oxidation, by AAPH• and •OH radicals, the effectiveness of their reduction is directly proportional to the number/concentration of biologically-active molecules in the extracts responsible for that process. Protection of PC liposomes against the AAPH free radical, which was more effective in the presence of extracts, can also be explained by the extracts' membrane-surface localization, distinct from the deeper penetration, for example, by flavones [Gabrielska *et al*., 2007]. This facilitates a better acceptability of the extract, when dissolved in water, of particles during AAPH free radical scavenging. In the case of the $TEAC^{Fe}$ parameter no correlation was obtained with the total contents of polyphenols and flavonoids except the anthocyanins, where the correlation was high (r=0.999). It shows that the anthocyanins present in the methanolic extracts of *B. vulgaris, C. mas* and *M. aquifolium* exhibit a differentiated ability to chelate Fe(II) ions, which affects the protection of the liposome membrane in the case of peroxidation induced by Fe(II)/ascorbic acid. The highest protection of liposome membranes was observed in the case of *M. aquifolium* extract, the one which exhibited the highest content of anthocyanins. The percentage of anthocyanins identified by HPLC in the sample extracts is summarized in Table 3. The predominant an-

TABLE 3. Main substances content (%) in extracts of *B. vulgaris* L., *C. mas* L., and *M. aquifolium* Nutt.

Compounds	Content (%) of main substances in extract		
	B. vulgaris L.	*C. mas* L.	*M. aquifolium* Nutt.
Anthocyanins	**4.2** in this: pg-3-glu (77%)	**17.3** in this: pg-3-gal (63 %) cy-3-gal (36 %) dp-3-gal (< 1 %)	**18.4** in this: cy-3-rut (43 %) dp-3-rut (30 %)
Flavonols	**3.1** in this: qc-3-glu (57 %)	**1.7** in this: qc-3-glucur (73 %) kempf-3-gal (27 %)	**4.2** in this: qc-3-glu (57 %)
Phenolic acids	**93.7** in this: chlo. ac. (78 %)	**6.2** in this: ellagic ac. (44 %) gallic ac. (37 %) p-coumaroylhex. (15 %)	**77.4** in this: chlo. ac. (88 %)
Iridoids	no	**74.8** in this: loganic ac. (69 %) cornuside (8 %)	no

pg-3-glu – pelargonidin 3-*O*-glucoside; pg-3-gal – pelargonidin 3-*O*-galactoside; cy-3-rut – cyanidin 3-*O*-rutinoside; dp-3-rut – delphinidin 3-*O*-rutinoside; dp-3-gal – delphinidin 3-*O*-galactoside; cy-3-gal – cyanidin 3-*O*-galactoside; qc-3-glu – quercetin 3-*O*-glucoside; qc-3-glucur – quercetin 3-*O*-glucuronide; kempf-3-gal – kempferol 3-*O*-galactoside; chlo. ac. – chlorogenic acid; ellagic ac. – ellagic acid; gallic ac. – gallic acid; loganic ac. – loganic acid; *p*-coumaroylhex. – *p*-coumaroylhexoside.

thocyanin in *B. vulgaris* L. was pelargonidin 3-*O*-glucoside, whereas the dominant phenolic acid was chlorogenic acid. In the methanolic extract of *Cornus mas* the following anthocyanins were predominating: pelargonidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside and delphinidin 3-*O*-galactoside. A similarly high content of pelargonidin 3-*O*-galactoside and cyanidin-3-*O*-galactoside in the extract of *C. mas* L. was reported by Vareed *et al.* [2006]. Previous studies showed that the extracts from fruits of *C. mas* L. contained five anthocyanins, identified by paper chromatography, spectrophotometer and peroxide oxidation analysis, as listed above, and cyanidin-3-*O*-rhamnosylgalactosie and pelargonidin-3-*O*-rhamnosylgalactoside [Du & Francis, 1973]. The latter work showed that the extract from *C. mas* L. contained a mixture of three compounds: delphinidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside and pelargonidin 3-*O*-galactoside [Seeram *et al*., 2002]. The work of Tural & Koca [2008] showed that *C. mas* L. contained three anthocyanins: cyanidin 3-*O*-glucoside, cyanidin-3-rutinoside and pelargonidin 3-*O*-glucoside. The most abundant phenolic acids present are: ellagic acid, gallic acid and *p*-coumaroylhexoside. Iridoids are represented by loganic acid and cornuside. Among the flavonols present in the sample extracts quercetin 3-*O*-glucuronide was the predominant one. In turn, kaempferol 3-*O*-galactoside was found in *C. mas* L. extract. In the methanolic extract of *M. aquifolium* the predominating anthocyanins included: cyanidin 3-*O*-rutinoside and delphinidin 3-*O*-rutinoside. Finally, among flavonols present in the sample extracts the predominating one was quercetin 3-*O*-glucoside.

To characterize the type of interaction between components in methanolic extracts and liposome membranes, the partitioning between water and 1-octanol was determined (Table 4). The affinity of the compounds to 1-octanol shows their relative hydrophobicity. The P values presented in Table 4 indicate that components in the prepared extracts exhibited lower affinity to 1-octanol than to the aqueous phase, suggesting that their hydrophobic properties are limited and thus they have a low possibility to incorporate into the hydrophobic part of PC liposomes. The fact that the extracts exhibit lower affinity to the organic phase, penetrating that phase according to the sequence: *C. mas* L. < *B. vulgaris* L. < *M. aquifolium* Nutt., confirms literature data that anthocyanins, one of the main extract components, exhibit greater affinity to water and dissolve better in polar solvents [Kong *et al*., 2003]. No statistically significant differences were found between affinity to the organic phase for *B. vulgaris* L. and *M. aquifolium* Nutt.

The ability of the extracts to associate with the liposome membrane was determined using lipid vesicles with the DPH probe. It was expected that higher values of dissociation constants (K_d) would correlate with shallow membrane penetration by extract components. The calculated dissociation constants K_d (Table 4) indicate that extracts affinity to PC liposome membranes follows the sequence: *B. vulgaris* L. > *M. aquifolium* Nutt. > *C. mas* L. This relation is close to that for the 1-octanol:water partition coefficient. The lower K_d (in mg/mL) values for *B. vulgaris* and *M. aquifolium* relative to K_d for *Cornus mas* (statistically significant differences, $p<0.05$) suggest greater affinity of the two extracts to PC liposome membranes. The values of K_d for quercetin and luteolin are, for instance: 7.5±0.1 and 7.2±0.1 μmol/L, respectively [Tammela *et al.*, 2004].

TABLE 4. The 1-octanol: water (P) and the liposome PC: buffer (K_d) partition coefficients of *B. vulgaris* L., *C. mas* L. and *M. aquifolium* Nutt. methanolic extracts.

Extract	P	K_d (mg/mL)
B. vulgaris L.	0.57 ± 0.06^b	1.16 ± 0.07^a
C. mas L.	0.44 ± 0.06^a	2.55 ± 0.08^b
M. aquifolium Nutt.	0.59 ± 0.13^b	1.57 ± 0.10^a

In this study we additionally noted a high correlation between K_d values and the content of phenols ($r=-0.999$) and flavonoids ($r=-0.998$) in the extracts. It seems that the extracts' capacity to form associates with the PC liposome membrane is related to the chemical structure of antioxidants rather than to their concentration in the extract. These correlations indicate also the essential role of anchorage in the membrane (most probably in the hydrophilic region near the membrane surface) of antioxidant molecules, as then they can effectively scavenge free radicals that attack the membrane from the aqueous phase. We observed a strong correlation between the antioxidant activity against lipid peroxidation, induced by AAPH ($TEAC^{AAPH}$) and UV radiation ($TEAC^{UV}$), and the K_d parameter ($r=-0.908$, $r=-0.883$, respectively). The negative correlation means that the higher the affinity to the membrane of liposomes (low K_d value) the stronger the antioxidant properties (greater TEAC value) shown by the extracts. Flavonoids, in particular their subclass anthocyanins, that impart the yellow, red orange and blue color to fruits could become important in replacing synthetic pigments by natural ones as ingredients of functional foods [Pawlowska *et al*., 2010; Popović *et al*. 2012]. The possible application of *C. mas* L. extract instead of synthetic compounds was suggested by West *et al.* [2012]. The consumption of *Cornus* spp. fruits has a potential to contribute to the overall health condition [Vareed *et al.*, 2007].

CONCLUSIONS

The studies revealed a differentiated activity of methanolic extracts of *B. vulgaris* L., *M. aquifolium* Nutt. and *C. mas* L., towards phosphatidylcholine liposomes that depended on the extract concentration and the oxidizing factor.

It has been shown that the antioxidant activity of the extracts was positively correlated with total polyphenol and flavonoid contents and the compound's ability to incorporate into the liposome membrane. This means that the protection of the lipid membrane by an extract's constituent against attack by free radicals is the better the greater its concentration and depth of incorporation into the membrane.

The *B. vulgaris* L. and *M. aquifolium* Nutt. (barberry and mahonia) components of crude extracts showed particularly strong antioxidant activity (against AAPH and UV induced liposome oxidation) and antiradical properties (in DPPH test). These *in vitro* results may suggest the potential application of barberry and mahonia extract as preservatives (though obtained by ex-

traction with ethanol, 50–80%) to retard the free radical-mediated degradation of susceptible components, in particular those containing unsaturated fatty acids.

Barberry, mahonia and cornelian cherry growing in southern Poland are attractive fruits for consumption. As the source of biologically-active compounds (anthocyanins – mahonia>cornelian cherry≥barberry; flavonols – barberry>mahonia>cornelian cherry; phenolic acids – barberry>mahonia>>cornelian cherry; and in the case of cornelian cherry – iridoids) these fruits can potentially be used in the production of new processed fruit.

ACKNOWLEDGEMENTS

The work was supported from the Ministry of Science and Higher Education funds on science in 2007–2010 as a research-development project No R05 049 02, and partly funded as statutory research by the Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences.

REFERENCES

1. Benzie I.F.F., Strain J.J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., 1996, 239, 70–76.
2. Brand-Williams B., Cuvelier M.E., Berset C., Use of a free radical method to evaluate antioxidant activity. Lebens.-Wiss. Technol., 1995, 28, 25–30.
3. Buege J.A., Aust S.D., Microsomal lipid peroxidation. Methods Enzymol., 1978, 52c, 302–310.
4. Chen X., Ahn D.U., Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe2+ or ultraviolet light. JAOCS, 1998, 75, 1717–1721.
5. Dorman H.J.D., Peltoketo A., Hiltunen R., Tikkanen M.J., Characterization of the antioxidant properties of de-odourised aqueous extracts from selected *Lamiaceae* herbs. Food Chem., 2003a, 83, 255–262.
6. Dorman H.J.D., Kosar M., Kahlos K., Holm Y., Hiltunen R., Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. J. Agric. Food Chem., 2003b, 51, 4563–4569.
7. Du C. –T., Francis F.J., Anthocyanins from *Cornus mas*. Phytochemistry, 1973, 12, 2487–2489.
8. Fatehi M., Saleh T.M., Fatehi-Hassanabad Z., Farrokhfal K., Jafarzadeh M., Davodi S., A pharmacological study on *Berberys vulgaris* fruit extract. J. Ethnopharmacol., 2005, 102, 46–52.
9. Gabrielska J., Korzeniowska M., Wojdyło A., Antioxidative effect of plant extracts and flavones on liposome and erythrocyte membranes. Pol. J. Food Nutr. Sci., 2007, 57, 145–150.
10. Giusti M.M., Wrolstad R.E., Characterization and measurement of anthocyanins by UV-visible spectroscopy. 2001, *in*: Current Protocols in Food Analytical Chemistry (eds. R.E. Wrolstad, T.E. Acree, H. An, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz, C.F. Shoemaker, P. Sporns), 1st ed., John Wiley & Sons, Inc., New York, pp. F1.2.1–F.2.13.
11. Gładkowski W., Chojnacka G., Kiełbowicz G., Psarski B., Trziszka T., Wawrzeńczyk C., Characteristics of phospholipid fractions isolated from the yolks of eggs from Lohman Brown hens and traditional local hen breed "zielonozka kuropatwiana". Przemysł chem., 2009, 88, 432–435 (in Polish).
12. Gutteridge J.M.C., Free radical in disease processes. Free Rad. Res. Comm., 1993, 19, 141–158.
13. Halliwell B., Gutteridge J.M.C., Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. Lancet, 1984, 1, 1396–1398.
14. Jacob R.A., Burri B.J., Oxidative damage and defense. Am. J. Clin. Nutr., 1996, 63, 985S-990S.
15. Ji X., Li Y., Liu H., Yan Y., Li J., Determination of the alkaloid content in different parts of some *Mahonia* plants by HPLC. Pharm. Acta Helv., 2000, 74, 387–391.
16. Jia Z., Tang M., Wu J., The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 1999, 64, 555–559.
17. Karawita R., Siriwardhana N., Lee K., Heo M., Yeo I., Lee Y., Reactive oxygen species scavenging, metal chelation, reducing power and lipid peroxidation inhibition properties of different solvent fractions from *Hizikia fusiformis*. Eur. Food Res. Technol., 2005, 220, 363–371.
18. Koncić M.Z., Kremer D., Karlović K., Kosalec I., Evaluation of antioxidant activities and phenolic content of *Berberis vulgaris* L. and *Berberis croatica* Horvat. Food Chem. Toxicol., 2010, 48, 2176–2180.
19. Kong J.M., Chia L.S., Goh N.K., Chia T.F., Brouillard R., Analysis and biological activities of anthocyanins. Phytochemistry, 2003, 64, 923–933.
20. Kucharska A.Z., Active compounds of cornelian cherry fruit (*Cornus mas* L.). Monografia CXL 5111, Wyd. UP we Wrocławiu, 2012, p. 18 (in Polish).
21. Liu R.H., Health benefits of fruit and vegetables are from additive and synergistic combination of phytochemicals. Am. J. Clin. Nutr., 2003, 78S, S517-S520.
22. Liu R.H., Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr., 2004, 134, 3479S-3485S.
23. Marinova D., Ribarova F., Atanassova M., Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metallurgy, 2005, 40, 255–260.
24. McCune L., Johns T., Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. J. Ethnopharmacol., 2002, 82, 197–205.
25. Minaiyan M., Ghannadi A., Mahzouni P., Jaffari-Shirazi E., Comparative study of *Berberis vulgaris* fruit extract and berberine chloride effects on acetic acid-induced colitis in rats. Iran. J. Pharmaceut. Res., 2011, 10, 97–104.
26. Minotti G., Aust S.D., The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. J. Biol. Chem., 1987, 262, 1098–1104.
27. Nenadis N., Zafiropoulou I., Tsimidou M., Commonly used food antioxidant: a comparative study in dispersed systems. Food Chem., 2003, 82, 403–407.
28. Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis Gr., Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem., 2007, 102, 777–783.
29. Pawlowska A., Camangi F., Braca A., Quali-quantitative analysis of flavonoids of *Cornus mas* L. (Cornaceae) fruits. Food Chem., 2010, 119, 1257–1261.

30. Popović B.M., Štajner D., Slavko K., Sandra B., Antioxidant capacity of cornelian cherry (*Cornus mas* L.) – Comparison between permanganate reducing antioxidant capacity and other antioxidant methods. Food Chem., 2012, 134, 734–741.
31. Račková L., Oblozinsky M., Kostalova D., Kettmann V., Bezakova L., Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. J. Inflamm., 2007, 4, 1–7.
32. Rop O., Mleck J., Kramarova D., Jurikova T., Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition. Afr. J. Biotechnol., 2010, 9, 1205–1210.
33. Seeram N.P., Schutzki R., Chandra A., Nair M.G., Characterization, quantification, and bioactivities of anthocyanins in Cornus species. J. Agric. Food Chem., 2002, 50, 2519–2523.
34. Shih M., Hu M., Relative roles of metal ions and singlet oxygen in UVA-induced liposomal lipid peroxidation. J. Inorg. Biochem., 1999, 77, 225–230.
35. Tammela P., Laitinen L., Galkin A., Wennberg T., Heczko R., Vuorelam H., Slotte J.P., Vuorela P., Permeability characteristics and membrane affinity of flavonoids and alkyl gallates in Caco-2 cells and in phospholipid vesicles. Arch. Biochem. Biophys., 2004, 425, 193–199.
36. Tural S., Koca I., Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. Sci. Hortic., 2008, 116, 362–366.
37. Turkmen N., Sari F., Velioglu S., Effects of extraction solvents on concentration activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem., 2006, 99, 835–841.
38. Wang J., Sun B., Cao Y., Tian Y., Li X., Optimalization of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chem., 2008, 106, 806–810.
39. West B. J., Deng S., Jensen C.J., Palu A.K., Berrio L.F., Antioxidant, toxicity, and iridoid tests of processed Cornelian cherry fruits. Int. J. Food Sci. Technol., 2012, 47, 1392–1397.
40. Vareed S.K., Reddy M.K., Schutzki R.E., Nair M.G., Anthocyanins in *Cornus alterifolia, Cornus controversa, Cornus kousa* and *Cornus florida* fruits with health benefits. Life Sci., 2006, 78, 777–784.
41. Vareed S.K., Schutzki R.E., Nair M.G., Lipid peroxidation, cyclooxygenase enzyme and tumor cell proliferation inhibitory compounds in *Cornus kousa* fruits. Phytomedicine, 2007, 14, 706–709.
42. Yilmaz K.U., Ercisil S., Zengin Y., Sengul M., Kafkas E.Y., Preliminary characterization of cornelian cherry (*Cornus mas* L.) genotypes for their physic-chemical properties. Food Chem., 2009, 114, 408–412.

Submitted: 26 October 2012. Revised: 26 March 2013. Accepted: 23 May 2013. Published on-line: 10 December 2013.