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DETERMINATION OF ANTIOXIDANT ACTIVITIES OF STRAWBERRY TREE (*Arbutus unedo* L.) FLOWERS AND FRUITS AT DIFFERENT RIPENING STAGES

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Abstract. In this study, the antioxidant activities of Strawberry tree (*Arbutus unedo* L.) flowers at flowering period and fruits during ripening period were investigated, along with some ripening characteristics such as color, reducing sugar and acidity. Antioxidant activity characteristics were investigated using by the methods of DPPH (1,1-Diphenyl-2--picryl-hydrazyl) scavenging activity, β-carotene bleaching, reducing power, metal chelating capacity, superoxide anion scavenging and hydrogen peroxide scavenging activity in water, ethanol and methanol extracts. The total phenolic content of flowers in water extracts was found to be 232.38 ± 7.19 mg GAE · g^{-1} extract, and the DPPH activity was 81.3 ± 0.49% at 50 $\mu g \cdot ml^{-1}$ concentration. In the ripening stages, the fully red fruits were determined higher antioxidant capacity than green and yellow fruits, except H_2O_2 scavenging activity which was highest in green fruit. In correlation study, the highest relationship was found between total phenolic content with reducing power (r^2 = 0.987**, $P < 0.01$), while the lowest with H_2O_2 scavenging activity (r^2 = 0.519*, $P < 0.05$).

Key words: DPPH scavenging activity, β-carotene bleaching activity, reducing power, correlations

INTRODUCTION

The interest in berry fruits has increased worldwide because of their multiple health-promoting photochemicals. In addition to the usual nutrients, berry fruits are also rich in flavanols, anthocyanidins, proanthocyanidins, catechins, flavones, and glycosides. These components are capable of performing a number of antioxidant activity [Wang 2010].

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Arbutus unedo L., the Strawberry tree (Ericaceae family), is an evergreen shrub or small tree, and it is widely distributed in the Mediterranean region and in the North Africa. It grows wildly on dry rocky slopes and hillsides or in pine forests, particularly in the Taurus Mountains, 600 m above sea level in Turkey [Ayaz et al. 2000]. This fruits are suitable for the production of alcoholic beverages, jams, jellies and marmalades [Pallauf et al. 2008]. Also, *A. unedo* is frequently used in traditional medicine in some countries, such as Spain and Morocco [Ziyyat et al. 1997, Tahraoui et al. 2007]. In several Portuguese regions the fruits are eaten raw or made into liqueurs, as well as bark or roots decoctions, which are used as anti-inflammatory, laxative, carminative, digestive, odontalgic and cardiotonic [Barros et al. 2010].

In Turkey, important studies have been carried out to select the *A. unedo* genotypes of superior fruit quality from the Northwestern part of Turkey, as well as to prevent extinction and to allow extensive cultivation of the strawberry trees [Celikel 2008]. Some researchers investigated the nutritional properties of fresh strawberry fruits [Ozcan and Haciesrefogulları 2007]. Sugars, non-volatile compounds and phenolic acid composition were determined in the fruit samples harvested in Northern Anatolia [Ayaz et al. 2000]. The morphological and pomological characteristics of *A. unedo* fruits from Western Turkey have been determined by Seker et al [2004]. Seker and Toplu [2009] revealed that *A. unedo* fruits are good sources of minerals and ascorbic acid and they are high in phenolics, antioxidant capacity and low in soluble sugars.

The ripening process in fruits is considered important factor that affect the biosynthesis of phytochemicals. Therefore, a number of researches carried out concerning the effectiveness and relationships of ripening period on antioxidant capacity and polyphenolic content of berries. In a previous study, Ozgen et al. [2009] investigated the changes in chemical composition, total phenolic content and antioxidant activity of *Arbutus andrachne* fruit at different maturation stages. Recently, Oliveira et al. [2011] have investigated the influence of strawberry tree fruit ripening stage on antioxidant activity in Portugal. Concerning the antioxidant properties of *A. unedo* flower, however, we have not found a previous study. The objective of this study was to evaluate the antioxidant capacity of Strawberry tree flowers and to determine the antioxidant activity of fruits at three different ripening stages (green, yellow and red). Accordingly, in this work, the extracts of Strawberry tree flower and fruits were prepared by using water, methanol and ethanol for extraction solvents. The antioxidant properties of these extracts were evaluated through several biochemical assays; DPPH scavenging activity, β-carotene bleaching activity, reducing power, metal chelating capacity, superoxide anion scavenging activity and hydrogen peroxide scavenging activity methods.

MATERIAL AND METHODS

Strawberry tree (*Arbutus unedo* L.) flowers and fruits, which is native in Çanakkale province, Lapseki subprovince and Şevketiye village, were collected from at about 100–200 m above sea level at 2009. The area has a subtropical climate. The average annual rainfall is around 600 mm. August is the driest month. The mean temperature ranges from a minimum of -11.2°C in February to a maximum of 39°C in July accord-

ing to long years period (1975–2010). Fruits have been harvested at three different ripening stages (green, yellow and fully red). Strawberry flowers and fruits were kept in cooled bags for transportation to the laboratory. They were sorted visually according to their color and named as green fruit (GF), yellow fruit (YF) and red fruit (RF). All solvents and reagents used for this study were of analytical grade and obtained from various suppliers including Merck, Sigma-Aldrich, Riedel-de Haen.

Preparation of the extracts: For drying samples, freeze drying method used by a vacuum freeze dryer (Armfield; FT 33). Before freeze drying, the samples were frozen by blast and fluid bed freezer (Armfield; FT 36) at -40°C. Freeze dried samples were ground to fine powder by using Waring blender. To obtain methanol (ME) and ethanol extracts (EE), 5 g of ground samples were mixed with 250 ml of solvent and the mixture was shaken at 180 rpm during overnight at room temperature. Extracts were filtered through Whatman No. 4 filter paper and the residue was reextracted twice and filtered. All extracts were pooled and solvents were removed using a rotary evaporator (IKA-Werke GmbH & Co.KG) under vacuum at 40°C. To obtain the water extracts (WE), 10 g of freeze dried ground samples were heated with 200 ml of boiling water with a magnetic stirrer for 30 min. Then, the extract was filtered over Whatman No. 4 filter paper. The filtrates were frozen and lyophilized in a vacuum freeze dryer. All extracts were stored in a freezer at –20 ± 2°C for antioxidant activity determination.

Total phenolic content (TP) of extracts were determined using Folin-Ciocalteu reagent and gallic acid as a standard phenolic compound according to Slinkard and Singleton [1977]

DPPH radical scavenging activities were evaluated with 1,1-diphenyl-2-picryl--hydrazil ($DPPH^{\bullet}$) by using the Blois method [Blois 1958] and the absorbance was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601). The antiradical activity (%) was calculated using the equation: $(A_{control} - A_{sample} / A_{control}) \times 100$, where $A_{control}$ is the absorption of the DPPH solution and A_{sample} is the absorption of the DPPH solution after the addition of the sample. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against different extract concentration, ranging between 50–1000 $\mu g \cdot ml^{-1}$.

Total antioxidant assay by using β-carotene bleaching method test was carried out using the spectrophotometric method of Miller and Luiz-Larrea [2002] based on the ability to decrease the oxidative bleaching on β-carotene in a β-carotene/linoleic acid emulsion and the absorbance of the mixtures was read at 490 nm. Total antioxidant activity was expressed as percent of inhibition relative to the control after a 120 min incubation period [Al-Saikhan et al. 1995], as shown below:

$$TAA = 100(DR_C - DR_S)/DR_C$$

where: DR_C = degradation rate of control = $\ln(a/b)/120$;
DR_S = degradation rate of sample = $\ln(a/b)/120$;
a and b = absorbance of samples and controls at 0 and 120 min.

Reducing power was determined according to the Oyaizu [1986] method at 700 nm. The control was prepared without the sample. Increasing absorbance of the reaction mixture indicate increasing the reducing power.

Metal chelating capacities of ferrous ions by the extracts were measured according to Dinis et al [1994] with a slight modification. The samples were added to 50 µl of 2 mM $FeCl_2$ and the solution was incubated at room temperature for 30 minutes. After the incubation process, 100 µl of 5 mM ferrozine was added. The mixture was shaken vigorously and left at room temperature for 10 minutes. The absorbance of the solution was measured at 562 nm against the same mixture, without the sample, as a blank. The percentage of inhibition of ferrozine – Fe^{2+} complex formation was calculated using the following equation:

$$\text{Metal chelating activity } \%= [1 - (A_1/A_0)] \times 100,$$

where: A_0 – absorbance of the blank,
A_1 – absorbance of the samples.

The determination of superoxide radical scavenging activity was done according to Nishikimi et al [1972]. The method is based on generating a superoxide radical by $PMS/NADH/O_2$ system in the presence of NBT, which is gets reduced to nitrite. Decreasing of absorbance at 560 nm by the reactive mixture indicate an increasing in superoxide radical scavenging activity. The inhibition (%) of superoxide radical generation was calculated according to the following equation:

$$\text{Inhibition } \% = [(A_0 - A_1) / A_0] \times 100$$

where: A_0 – the absorbance of control,
A_1 – the absorbance of the samples.

Hydrogen peroxide scavenging activity of extracts were determined according to the method of Ruch et al [1984] and the absorbance value of the reaction mixture was measured at 230 nm. The percentage of H_2O_2 scavenging of extracts was calculated as:

$$[1 - (\text{Absorbance of the sample at 230 nm / Absorbance of the blank at 230 nm})] \times 100$$

Other analysis methods**:** Dry matter content of samples was determined by using a vacuum dryer at 70°C [Cemeroglu 2007]. Reducing sugar content was assayed according to Ross [1959] by using 2,4-dinitrophenol reactive and glucose as a standard. The measurements were made at 600 nm by a spectrophotometer (Hitachi UV/Vis - 2000). Color measurements of the fruits were performed by using Hunter-Lab tristimulus colorimeter (D25LT model). CIE L^*, a^* and b^* color parameters of fruit samples were measured from 10 points of every sample. In the CIE L^*, a^*, b^* color system, L^* value represent the lightness of color (0 = black, 100 = white), a^* represent the redness (positive values) or greenness (negative values) color and b^* represent the yellowness (positive values) and blueness (negative values) color [Anonymous 2006]. Titrable acidity was determined by titration with a 0.1 N sodium hydroxide solution up to titration-endpoint of pH 8.1 and was expressed as $g \cdot 100\ g^{-1}$ citric acids. The pH value was determined according to Cemeroglu [2007] by using the Fisher accument model 10 pH-meter.

Statistical analysis. Assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. All experiments were conducted as a completely randomised block design and performed in triplicate. Data were analyzed

by using MSTAT statistical program (Version 3.00/EM, Package Program) [Anonymous, 1982].

RESULTS AND DISCUSSION

The color parameters, namely L^*, a^* and b^* values of *A. unedo* flowers and fruits are given in table 1. As seen in table 1, the highest L^* value was determined in YF. Negative a^* values, ranging from -8.65 ± 0.17 in FL to -3.50 ± 0.31 in YF, indicated the changes of greenness and chlorophyll content of flowers and fruits. It is shown that, greenness degree of fruits decreased along with the ripening period. b^* values of fruits increased from FL to YF, then, decreased again in RF, along with the increasing redness (table 1). The highest L^* and b^* values was determined YF, and the decrease of these values in mature fruits at red stages correspond to those of Ozgen et al. [2009].

Table 1. Some characteristic properties of *A. unedo* L. flowers and fruits
Tabela 1. Niektóre cechy charakterystyczne kwiatów i owoców *A. unedo* L.

Ripening stages Stadia dojrzewania	Color parameters Parametry koloru			DM %	Reducing sugar Cukry redukujące $g \cdot 100\ g^{-1}$ DM	Acidity Kwasowość $g \cdot 100\ g^{-1}$	pH
	L^*	a^*	b^*				
FL	50.45±0.12^{b*}	-8.65±0.17^{d}	31.02±0.22^{c}	53.44±2.12^{a}	16.28±0.16^{b}	0.88±0.01^{c}	5.27±0.02^{a}
GF	44.55±0.20^{c}	-7.18±0.13^{c}	34.95±0.09^{b}	30.02±0.69^{d}	9.89±0.33^{d}	1.06±0.02^{b}	4.79±0.01^{b}
YF	79.15±0.06^{a}	-3.50±0.31^{b}	45.13±0.12^{a}	49.18±0.80^{b}	12.62±0.28^{c}	1.28±0.02^{a}	4.66±0.02^{b}
RF	41.18±0.06^{d}	31.99±0.25^{a}	30.13±0.22^{c}	43.84±1.07^{c}	25.88±0.28^{a}	1.07±0.03^{b}	4.71±0.01^{b}

*Different letters within each column is indicating significant differences at $P < 0.05$ level.
*Różne litery w każdej kolumnie wskazują na istotne różnice przy poziomie $P < 0,05$.

It is known that during the fruit development, from early ripening stage to the fully matured stage, a lot of chemical characteristics change by including conversion of polysaccharides to reducing sugar. In RF, reducing sugar content was found to be highest as seen in table 1. The sugar content of red fruit was determined to be lower than the results obtained by Ayaz et al. [2000] and Barros et al. [2010]. This difference may be due to the differences in climatic and regional growing conditions of the plants. On the other hand, Serce et al. [2010] determined that the total sugar content of strawberry tree fruit was 9.83 ± 0.09 $g \cdot 100\ g^{-1}$ fw and similar to our findings that if reducing sugar of RF is calculated in fresh weight (11.35 $g \cdot 100\ g^{-1}$ fw).

Throughout the fruit development, the lowest acidity was found in FL and the highest acidity in YF. Our results in RF confirm those of Celikel et al. [2008] who reported that acidities of the five selected strawberry tree genotypes changed in the range of 0.80 to 1.59%. Karadeniz et al. [1996] reported that acidities varied from 1.51 to 3.45% in strawberry tree genotypes, which is higher than our findings.

Table 2. The extraction yields and total phenolic contents of *Arbutus unedo* L. flowers and fruits
Tabela 2. Plony ekstrakcji oraz całkowita zawartość fenoli w kwiatach i owocach *Arbutus unedo* L.

Ripening stages Stadia dojrze-wania	Extraction yield (%) Plon ekstrakcji (%)			Phenolic content (mg GAE · g^{-1} extract) Zawartość fenoli (mg GAE · g^{-1} ekstraktu)			
	WE	ME	EE	WE	ME	EE	Average Średnio
FL	37.96±1.22^{b*}	53.80±1.17^{c}	55.03±2.67^{b}	232.38±7.19^{a}	207.59±0.78^{b}	166.67±2.19^{c}	202.21^{a}
GF	53.01±0.09^{a}	64.51±1.13^{b}	53.42±2.13^{b}	12.14±0.71^{de}	15.71±1.71^{d}	9.28±0.00^{e}	12.38^{b}
YF	53.67±1.06^{a}	75.42±2.31^{a}	56.08±1.85^{b}	5.24±0.41^{f}	9.04±0.41^{ef}	12.62±0.41^{de}	8.97^{c}
RF	50.34±2.06^{a}	76.42±2.25^{a}	70.34±1.05^{a}	12.14±1.43^{de}	14.28±0.71^{d}	14.29±0.01^{d}	13.57^{b}

*Different letters is indicating significant differences between interaction (ripening periods × extracts) at $P < 0.05$ level
* Różne litery w każdej kolumnie wskazują na istotne różnice przy poziomie $P < 0,05$

As seen in table 2, the extraction yield of ME was found to be highest in fruit extractions. The TP content of FL extracts was found to be significantly different from fruit extracts. As shown in table 2, after blooming period, the TP content of fruits decreased significantly, and, the lowest TP content of fruits was found in YF, according to average values. There was no significant differences between TP content of GF and RF in WE and ME, statistically ($P < 0.05$) (tab. 2). These results are in agreement with Ozgen et al. [2009], who determined that the total phenolic values were the highest at the red stage, followed by the green stage. On the other hand, Oliveira et al. [2011] reported that the highest phenolic content was at yellow stage. TP content of samples were significantly affected by the extraction solvent. While the ME of fruits had more TP, the WE of flowers had more TP content. This could be due to the differences in extractability of various phenolic compounds in different solvents.

The DPPH radical scavenging activity of extracts from flowers and fruits displayed significant differences ($P < 0.05$). As seen in Fig 1 (A), the highest value of 91.39% was determined in the FL extracts. The inhibition effects of FL extracts was found to be significantly high even at very low concentrations and these data for FL at 50 μg·ml^{-1} were 81.3 ± 0.49% for WE, 73.53 ± 0.76% for ME, and 64.54 ± 1.25% for EE. As seen in Fig 1 (B) the DPPH scavenging activities of extracts were concentration-dependent. The DPPH scavenging activities of RF at 1000 μg·ml^{-1} were determined to be 83.15 ± 0.75% in EE. The average DPPH activity of fruits was determined as the following sequence: RF > GF > YF. These differences occurring in ripening stages may explain due to the change in the phytochemical component amounts at different ripening stages. Our results correspond to Ozgen et al. [2009], who determined that the antioxidant activities were the highest at the red stages for both the TEAC and FRAP methods. DPPH scavenging activity exhibited significant differences by extraction solvents and extract efficiencies were determined in the order of ME > EE > WE.

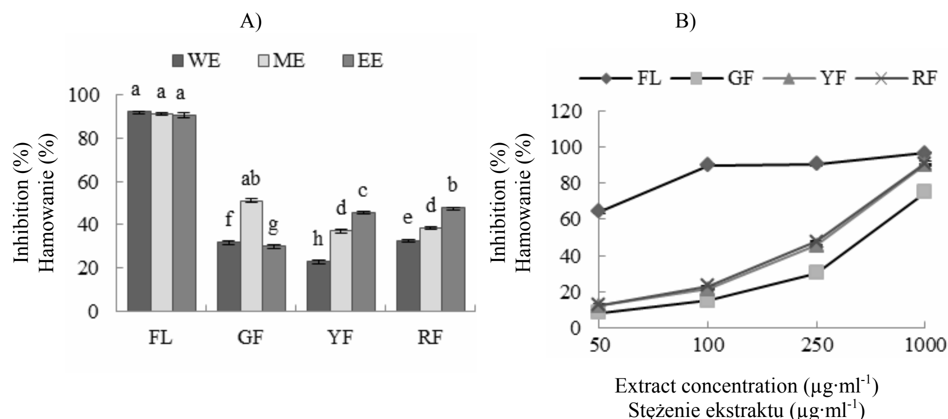

Fig. 1. A) DPPH scavenging activity of different extracts at 250 μg·ml^{-1}. *Different letters upper index within the column indicate significant differences at $P < 0.05$ level. Average value of extract activity; WE: 45.035b, ME: 54.59a, EE: 53.62a. Average value of ripening period; FL: 91.39a, GF: 37.77c, YF: 35.36d, RF: 39.80b; B) DPPH scavenging activity of the *A. unedo* ethanol extracts at different concentrations

Ryc. 1. A) Działanie oczyszczające DPPH w różnych ekstraktach przy 250 μg·ml^{-1}. *Różne litery w indeksie górnym w kolumnie wskazują na istotne różnice przy poziomie P < 0,05. Średnia wartość działania ekstraktu: WE: 45,035 b, ME: 54,59 a, EE: 53,62a. Średnia wartość okresu dojrzewania: FL: 91,39a, GF: 37,77c, YF: 35,36d, RF: 39,80 b; B) Działanie oczyszczające DPPH w ekstraktach etanolowych *A. unedo* przy różnych stężeniach

The EC_{50} value is the effective concentration which is required to decrease the initial DPPH concentration by 50% and the lower EC_{50} value reflects the better protective action. The FL extracts had the lowest EC_{50} value (tab. 3). According to statistical evaluation, in fruit ripening period, the average EC_{50} value of DPPH scavenging activities is the following order: RF < GF < YF. Our results for red fruits are agreement with Oliveira et al. [2011], who explained the lowest EC_{50} value of DPPH in ripened fruits.

The total antioxidant activities of extracts according to β-carotene bleaching method are demonstrated in Fig. 2 (A) and (B). The bleaching activity of FL was found to be significantly higher even at very low concentrations. These results obtained at 50 μg·ml^{-1} were 64.27 ± 1.14% for WE; 78.50 ± 2.48% for ME, 67.79 ± 0.62% for EE. During the ripening stages of fruits, the highest β-carotene bleaching activity was found in RF according to average value. The β-carotene bleaching activities of RF were determined to be 72.05 ± 1.61% for WE, 68.54 ± 2.05% for EE, 76.20 ± 3.20% for ME at 1000 μg·ml^{-1}, respectively. There was no significant difference between GF and YF, statistically. According to average values, solvent efficiencies were determined to be in the following order: ME > EE = WE (Fig. 2A).

EC_{50} values obtained from β-carotene bleaching method are given in table 3. According to statistical evaluation, the average EC_{50} values obtained β-carotene bleaching methods are in the following order: FL < RF < GF < YF. This result is similar to those of DPPH scavenging activity method.

Table 3. EC_{50} ($mg \cdot ml^{-1}$) values of DPPH and β-carotene bleaching activities of extracts obtained from *Arbutus unedo* L. flowers and fruits

Tabela 3. Wartości EC_{50} ($mg \cdot ml^{-1}$) DPPH i wybielającego działania β-karotenu w ekstraktach otrzymanych z kwiatów i owoców *Arbutus unedo* L.

Ripening stages Stadia dojrzewania	DPPH assay Próba DPPH				β-carotene bleaching assay Próba wybielania β-karotenem			
	WE	ME	EE	average value** wartość średnia**	WE	ME	EE	average value wartość średnia**
FL	0.030^{j*}	0.033^{j}	0.035^{j}	0.033^{d}	0.037^{g}	0.032^{g}	0.034^{g}	0.034^{d}
GF	0.514^{c}	0.243^{i}	0.590^{b}	0.449^{b}	0.452^{b}	0.102^{fg}	0.239^{d}	0.264^{b}
YF	0.701^{a}	0.473^{e}	0.325^{g}	0.499^{a}	0.510^{a}	0.157^{e}	0.317^{c}	0.328^{a}
RF	0.492^{d}	0.443^{f}	0.297^{h}	0.409^{c}	0.431^{b}	0.123^{f}	0.185^{e}	0.246^{c}

*Different letters indicate significant differences between interaction (ripening periods × extracts) at $P < 0.05$ level.
* Różne litery wskazują na istotne różnice w interakcji (okresy dojrzewania × ekstrakty) na poziomie $P < 0,05$.
**The average value of different extraction solvents.
** Średnia wartość różnych rozpuszczalników ekstrakcyjnych.

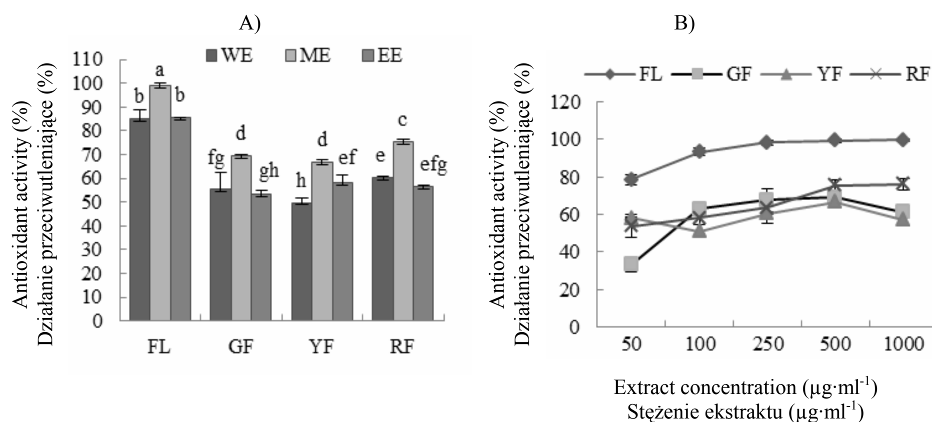

Fig. 2. A) β-carotene bleaching activities of the different extracts at 500 $\mu g \cdot ml^{-1}$. *Different letters upper index within the column indicate significant differences at $P < 0.05$ level. Average value of extract activity; WE: 62.62b, ME: 77.60a, EE: 63.3b. Average value of ripening period; FL: 89.82a, GF: 59.25c, YF: 58.25c, RF: 64.03b; B) β-carotene bleaching activities of the *A. unedo* L. methanol extracts at different concentrations

Ryc. 2. A) Działanie wybielające ß-karotenu w różnych ekstraktach przy 500 $\mu g \cdot ml^{-1}$. *Różne litery w indeksie górnym w kolumnie wskazują na istotne różnice przy poziomie $P < 0,05$. Średnia wartość działania ekstraktu: WE: 62,62b, ME: 77,60a, EE:63,3b Średnia wartość okresu dojrzewania: FL: 89,82a, GF: 59,25c, YF: 58,25c, RF: 64,03b; B) Działanie wybielające ß-karotenu w ekstraktach etanolowych *A. unedo* przy różnych stężeniach

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity. As seen in Fig. 3, the reducing powers of FL extracts were significantly different. According to average values, the reducing power was determined in the following order: FL > GF = RF > YF in samples. Regarding the extract efficiencies, higher reducing power was obtained from ME ($P < 0.05$). Our results are different from the research Oliveira et al. [2011], who explained the lowest reducing power in intermediate fruits.

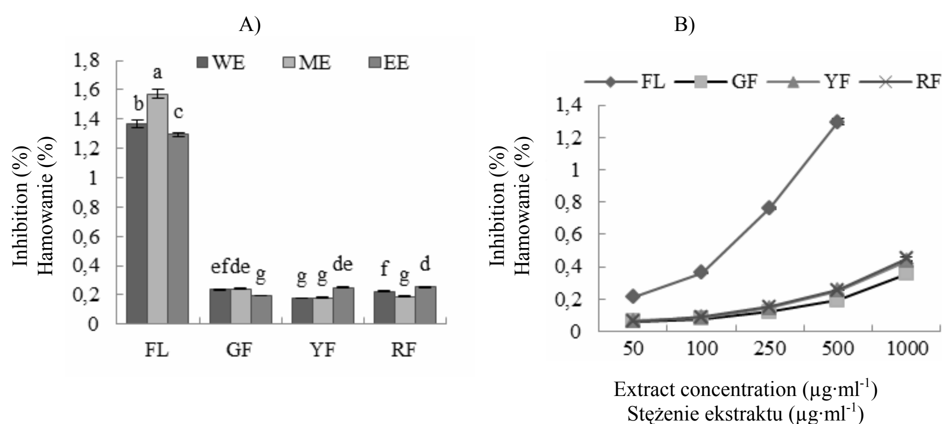

Fig. 3. A) Reducing power of the different extracts at 500 µg·ml^{-1}. *Different letters upper index within each column indicate significant differences at $P < 0.05$ level. Average value of extract activity; WE: 0.502b, ME: 0.545a, EE: 0.499b. Average value of ripening period; FL: 1.412a, GF: 0.223b, YF: 0.204c, RF: 0.222b; B) Reducing power of the *A. unedo* ethanol extracts at different concentrations

Ryc. 3. A) Siła redukująca różnych ekstraktów przy 500 µg·ml^{-1}. *Różne litery w indeksie górnym w kolumnie wskazują na istotne różnice przy poziomie P < 0,05. Średnia wartość działania ekstraktu: WE: 0,502b, ME: 0,545a, EE: 0,499b. Średnia wartość okresu dojrzewania: FL: 1,412a, GF: 0,223c, YF: 0,204c, RF: 0,222b; B) Siła redukująca ekstraktów etanolowych *A. unedo* przy różnych stężeniach

Superoxide is a reactive oxygen species, which can cause damage to cells and DNA, leading to various diseases. Therefore it has been proposed to measure the interceptive ability of the antioxidant extracts to scavenge the superoxide radicals [Vani et al. 1997]. The superoxide radical scavenging activities of FL extracts was found to be highest (80.43%) and significantly affected by ripeness level ($P < 0.05$) (table 4). At full maturity stage, superoxide radical scavenging activity of *A. unedo* significantly increased. According to average values, the highest activity was determined in RF (62.31%) and it was found significantly higher than in GF (39.36%) and YF (33.30%). Regarding the extract activities, WE exhibited significantly more activity than EE and ME ($P < 0.05$).

Metals chelation may provide important antioxidative effects by retarding metal-catalyzed oxidation reactive oxygen species [Gulcin et al. 2010]. As seen in table 4, this study revealed that the FL extracts possess low metal chelating capacity and the fruit extracts had no activity. The highest metal chelating capacity was found to be 8.78 ± 0.98% in ethanolic extracts at 250 µg·ml^{-1} concentration. To evaluate the potency

Table 4. Superoxide radical scavenging activity, metal chelating capacity and H_2O_2 scavenging activity of *A. unedo* L. flower and fruit extracts at different ripening stages

Tabela 4. Działanie oczyszczające rodnika nadtlenku, zdolność chelatowania metali oraz działanie czyszczące H_2O_2 ekstraktów kwiatów i owoców *A. unedo* L. na różnych etapach dojrzewania

Ripening stages Etapy dojrzewania	Superoxide radical scavenging activity (%)[a] Działanie oczyszczające rodnika nadtlenku (%)[a]				Metal chelating capacity (%)[b] Zdolność chelatowania metali (%)[b]			H_2O_2 scavenging activity (%)[c] Działanie oczyszczające H_2O_2 (%)[c]			
	WE	ME	EE	average value wartość średnia	WE	ME	EE	WE	ME	EE	average value wartość średnia
FL	82.00±0.20[a*]	79.57±0.40[a]	79.73±1.36[a]	80.43[a]	3.33±0.34[c]	6.55±0.39[b]	8.78±0.98[a]	26.40±0.65[de]	41.12±2.67[a]	22.59±2.50[b]	32.454[a]
GF	62.13±1.60[c]	32.63±1.27[f]	23.33±1.32[h]	39.36[c]	nd	nd	nd	28.03±0.69[cd]	28.25±1.50[bc]	25.03±0.20[ef]	27.108[b]
YF	44.83±1.62[e]	25.4±1.10[h]	29.67±0.72[g]	33.30[d]	nd	nd	nd	28.13±3.74[cd]	29.23±0.19[bc]	21.53±1.78[g]	26.33[bc]
RF	68.5±2.80[b]	58.53±1.10[d]	58.89±0.18[d]	62.31[b]	nd	nd	nd	25.03±0.39[ef]	29.13±0.09[bc]	23.41±0.05[f]	25.86[c]
[d]Avarage value Wartość średnia	64.12[a]	49.28[b]	47.91[c]					27.18[b]	31.94[a]	24.95[c]	

[a] Superoxide radical scavenging activities of all extracts were determined at 1000 $\mu g \cdot ml^{-1}$.
[b] Metal chelating activities of all extracts were determined at 250 $\mu g \cdot ml^{-1}$.
[c] H_2O_2 scavenging activities of all extracts were determined at 500 $\mu g \cdot ml^{-1}$.
[d] The average of different extraction solvents.
*Different letters is indicate the significant differences between interaction (ripening stages × extracts) at $P < 0.05$ level.
[a] Działanie oczyszczające rodnika nadtlenku we wszystkich ekstraktach wyznaczano przy 1000 $\mu g \cdot ml^{-1}$.
[b] Działanie chelatujące metali we wszystkich ekstraktach wyznaczano przy 250 $\mu g \cdot ml^{-1}$.
[c] Działanie oczyszczające H_2O_2 we wszystkich ekstraktach wyznaczano przy 500 $\mu g \cdot ml^{-1}$.
[d] Średnio dla różnych rozpuszczalników ekstrakcyjnych.
*Różne litery wskazują na znaczące różnice w interakcji (okresy dojrzewania × ekstrakty) na poziomie $P < 0,05$.

of the extract, the activity was compared to EDTA at same concentration. Metal chelating activity of EDTA was 67.90 ± 1.07% at this concentration. On the other hand, metal chelating capacity disappeared in ripening period and red fruits had no metal-chelating capability. In their research related to antioxidant activity of polyphenol and anthocyanin extracts, Sun et al. [2009] reported that anthocyanin extracts had no metal-chelating capability, indicating that no ortho-dihydroxy aromatic moiety (the key structure for chelating transition metals) existed in the B-ring of the anthocyanin molecules. However, they explained that polyphenol extracts exhibit some metal chelating capability and they refferred to the catechol group in B-ring of these polyphenol molecules [Brouillard and Dangles 1993]. In another research, Wang et al. [2010] determined a poor ferrous ion-chelating effect from the carotenoid extract (1.8%) while showing high DPPH activity (70.6%). Therefore, we can conclude that having metal chelating activity of FL extracts may be due to the strikingly higher phenolic contents than in fruits.

Table 5. Linear correlations of the different antioxidant characteristics of *A. unedo* flowers and fruits
Tabela 5. Korelacje liniowe różnych cech antyoksydacyjnych kwiatów i owoców *A. unedo*

	β-carotene bleaching activity Działanie wybielają-ce β-karotenu	DPPH scavenging activity Działanie oczyszcza-jące DPPH	Metal chelating capacity Zdolność do chela-towania metali	Reducing power Siła redukująca	Total phenolic content Całkowita zawartość fenoli	H_2O_2 scavenging activity Działanie oczyszcza-jące H_2O_2	Superoxide radical scavenging activity Działanie oczyszcza-jące rodnika nadtlenku
β-carotene bleaching activity Działanie wybielające β-karotenu	1.000	0.782**	0.793**	0.864**	0.849**	0.688**	0.552**
DPPH scavenging activity Działanie oczyszczające DPPH		1.000	0.818**	0.855**	0.906**	0.444**	0.633**
Metal chelating capacity Zdolność do chelatowania metali			1.000	0.904**	0.895**	0.671**	0.705**
Reducing power Siła redukująca				1.000	0.987**	0.581**	0.855**
Total phenolic content Całkowita zawartość fenoli					1.000	0.519**	0.764**
H_2O_2 scavenging activity Działanie oczyszczające H_2O_2						1.000	0.369*
Superoxide radical scavenging activity Działanie oczyszczające rodnika nadtlenku							1.000

* Indicates significant differences between the different characteristics. * r ($P < 0.05$) = 0.404 – * Wskazuje na znaczące różnice pomiędzy różnymi cechami *r ($P < 0,05$) = 0,404
** Indicates significant differences between the different characteristics. ** r ($P < 0.01$) = 0.515 (n = 34) – Wskazuje na znaczące różnice pomiędzy różnymi cechami** r ($P < 0,01$) = 0,515 (n = 34)

The scavenging of hydrogen peroxide which may result in great damage to cells is very important for antioxidant defence [Wang et al. 2007]. As seen in table 4, the extracts obtained from flowers and fruits were capable of hydrogen peroxide scavenging activity and the highest activity was determined at the ME of FL (41.12 ± 2.67%). Although WE and ME of yellow and green fruits did not show significant differences in statistically ($P < 0.05$), GF showed higher activity according to average values. Solvent efficiency was determined in the order of ME > WE > EE (tab. 4).

The results of the TP content and different antioxidant assays were compared with each other and evaluated statistically (tab. 5). According to correlation analyses, TP content is highly positively correlated with all characteristics. The highest relationship between TP content and antioxidant activity assays was found in reducing power ($r^2 = 0.987$**, $P < 0.01$) while the lowest relationship was found in hydrogen peroxide scavenging assay ($r^2 = 0.519$**, $P < 0.01$). The relationship of TP content with β-carotene bleaching activity ($r^2 = 0.849$**) and DPPH activity ($r^2 = 0.906$**) were found to be significantly important. Additionally, there was a significant correlation between the TP content and the superoxide radical scavenging activity ($r^2 = 0.764$**). The results of strong correlations between antiradical activity assays and TP content are in agreement with the results from obtained by Su and Chien [2007], Liu et al. [2008], Anastasiadi et al. [2010]. These investigations exhibit that the TP content plays an important role in antioxidant activity. According to antioxidant activity assays, DPPH scavenging activity and β-carotene bleaching activity are correlated positively and significantly with each other ($r^2 = 0.782$**). The highest correlations were determined between DPPH scavenging activity and reducing power ($r^2 = 0.855$**), and between reducing power and metal chelating capacity ($r^2 = 0.904$**). These results correspond to Zhang and Wang [2010].

CONCLUSIONS

In conclusion, this study has demonstrated that flower extracts of strawberry tree are phenolic rich and demonstrated significant antioxidant activity. After flowering period, antioxidant activity of strawberry tree fruits was affected by ripening stages. At the end of ripening period, an increase in antioxidant activity was determined again. According to average values, the methanol extracts of strawberry tree revealed a slightly higher activity than those of water extracts according to β-carotene bleaching method, DPPH scavenging activity and reducing power in contrast to superoxide anion scavenging capacity which showed highest activity in water extracts. Finally, the fully red fruits had higher antioxidant capacity than green and yellow fruits except H_2O_2 scavenging activity which was highest in green fruit. This study revealed that the FL extracts possess low metal chelating capacity and the fruit extracts had no activity. However, further studies are necessary to clarify the possible effects of phytochemical compounds and their bioavailability at different antioxidant activity characteristics in order to learn the real health benefits which can be offered by these compounds.

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OKREŚLENIE DZIAŁANIA PRZECIWUTLENIAJĄCEGO KWIATÓW I OWOCÓW DRZEWA TRUSKAWKOWEGO (*Arbutus unedo* L.) NA RÓŻNYCH ETAPACH DOJRZEWANIA

Streszczenie. W niniejszej pracy prześledzono działanie przeciwutleniające kwiatów drzewa truskawkowego (*Arbutus unedo* L.) w okresie kwitnienia oraz owoców w czasie dojrzewania wraz z niektórymi charakterystycznymi cechami dojrzewania, takimi jak kolor, cukier redukujący oraz kwaśność. Zbadano cechy charakterystyczne działania antyok-

sydacyjnego, posługując się następującymi metodami: oczyszczającą DPPH (1,1-Difenyl--2-pikryl-hydrazyl), wybielaniem β-karotenem, siłą redukującą, zdolnością metali do chelatowania, oczyszczaniem anionami nadtlenku i nadtlenkiem wodoru w wodzie oraz ekstraktach alkoholu etylowego i metylowego. Stwierdzono, że całkowita zawartość fenoli w kwiatach w ekstraktach wodnych wynosi 232,38±7,19 mg GAE/g ekstrakt, zaś działanie DPPH wynosiło 81,3±0,49% przy stężeniu 50 $\mu g \cdot ml^{-1}$. W stadiach dojrzewania u całkiem czerwonych owoców stwierdzono wyższą zdolność antyoksydacyjną niż w owocach zielonych i żółtych, oprócz oczyszczającego działania H_2O_2, które było najsilniejsze w owocach zielonych. W badaniu korelacji najsilniejszą zależność stwierdzono pomiędzy całkowitą zawartością fenoli przy mocy redukującej (r^2 = 0.987**, $P < 0.01$), najsłabszą zaś przy działaniu oczyszczającym H_2O_2 (r^2 = 0.519*, $P < 0.05$).

Słowa kluczowe: działanie oczyszczające DPPH, działanie wybielające β-karotenu, siła redukująca, korelacje

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