ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol., Technol. Aliment. 10(4) 2011, 443-454
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

ANTIOXIDANT ACTIVITY OF APPLES – AN IMPACT OF MATURITY STAGE AND FRUIT PART*

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Background. Recently, many studies have been oriented towards improving methods and efficiency of antioxidants recovery from different fruit and their wastes. The aim of the study was to evaluate antioxidant potential of apple seeds and peel, which constitute the fruit industry wastes, and compare it to apple flesh. Antioxidant activity of apples at different maturity and storage stage were analysed too.
Material and methods. The Idared and the Šampion cultivars of apples were used in the study. Antioxidant activity was estimated using ABTS and DPPH assays, and polyphenols profile was determined by HPLC method.
Results. Seeds of analysed apple cultivars were characterised by a significantly higher antioxidant capacity and by higher concentrations of polyphenols analysed when compared to their peel and flesh. There were present two predominant compounds: phloridzin in seeds (84% and 72%) and quercetin glycosides in peels (54% and 38%, Idared and Šampion cultivars, respectively). No quercetin glycosides in seeds were found. The capacity to scavenge an ABTS radical, but not DPPH, decreased during ripening of apples, while cold storage resulted in enhanced antioxidant potential.
Conclusion. It can be concluded that unripe apples together with apple seeds and peel (fruit industry wastes) constitute a valuable source of polyphenols.

Key words: antioxidant capacity, HPLC, maturity stage of fruit, peel, polyphenols, seeds

INTRODUCTION

Free radicals and reactive oxygen species (ROS) can react with lipids, proteins, sugars, and nucleic acids, causing inactivation of enzymes, changes in genetic material and tissue damage [Bergamini et al. 2004, Valko et al. 2007]. Human organism has devel-

*This work was supported by the PBZ KBN 094/P06/2003/28 of State Committee for Scientific Research.

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oped different antioxidant mechanisms but their efficiency can be insufficient. Especially low level of antioxidant protection together with enhanced free radical and ROS production was observed in samples collected from smokers, elders, and patients with some disorders, as well as among people subjected to health-threatening agents [Michota-Katulska 2000]. Quite efficient scavengers of free radicals are polyphenols and some vitamins, such as ascorbic acid, tocopherols, carotenoids and retinol [Sroka et al. 2005]. Antioxidants are present in fruits, vegetables, herbaceous plants, cereals, leguminous plants, juices, wine, and tea [Rice-Evans et al. 1997, Aherne and O'Brien 2002, Manach et al. 2004, Pourmorad et al. 2006].

The antioxidant supplementation is a generally accepted method of prolonging the stability and storage life of food products, in particular the ones including fat. It is also the way of increasing antioxidants intake with daily diet. However, the artificial compounds with antioxidant properties, like butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT), have a limited allowance for food due to their negative impact on human health [Yamaki et al. 2007]. The growing demand for natural antioxidants observed in food industry forces the search for new sources of these compounds.

Fruits and vegetables wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and/or utilized. On the other hand, they are very rich in bioactive compounds, which are considered to have a beneficial effect on health [Duda-Chodak and Tarko 2007, Tarko et al. 2009 b]. For the last decade, efforts have been put to improve methods and efficiency of antioxidants recovery from different fruits and their wastes [Tarko et al. 2009 a].

The authors put forward the hypothesis that the level of antioxidant compounds in apples changes during ripening and storage of fruit, influencing the efficiency of antioxidants recovery. Moreover, the concentration of individual polyphenols is highly diversified between different parts of fruit suggesting that isolation of particular component should be preceded by the detailed analysis of polyphenols profile. In the present investigation the differences between individual parts of apple fruit (seeds, peel, and flesh), as well as at different stages of maturity and storage were analysed.

MATERIAL AND METHODS

Chemicals. Diammonium salt of the 2,2'-azino-bis (3-ethylbenzothiazoline-6--sulfonic) acid (ABTS diammonium salt); 2,2'-diphenyl-1-picrylhydrazyl (DPPH); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); a phosphate buffer (PBS): 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride; pH 7.4 at a temperature of 25°C; and the enzymes: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidase. All the chemicals listed, as well as HPLC standards, were purchased from the SIGMA-Aldrich Company (Germany). The chemicals: potassium iodide, potassium persulfate ($K_2S_2O_8$) and methanol (analytically pure) were obtained from the POCh Company (Poland), and a 96% ethanol from the ChemPur Company (Poland).

Investigated materials. Two apple cultivars, Idared and Šampion, originating from a pomologic orchard run by the University of Agriculture in Krakow situated in Garlica Murowana, near Krakow, were used in the investigation. A representative sample con-

sisted of minimum 8 ripe fruits of each cultivar picked from the central axes of four different trees. The Starch Index method was used to determine the harvest maturity of the apples.

Antioxidant activity and polyphenols profile were analysed at different stages of apples maturity and storage. At the ensuing stages of maturity (1st stage: on 17th July, and it was 80 days after beginning of blooming; 2nd stage: day 120th; 3rd stage: day 143rd; 4th stage: day 160th, which was harvest maturity stage), fruits were picked directly from trees. Fruits at the storing stages (5th and 6th stages) were obtained from the cold store after 64 and 112 days of storage. The apples deprived of seed cores were cut into fine pieces and lyophilised. They were stored at a temperature of –20°C until the analysis.

Antioxidant activity and polyphenols profile were assessed also in individual parts of apple fruits (peel, seeds, flesh) picked, from the trees, at their harvest maturity stage.

Starch index. Each fruit was cut in halves, and the cut surface was immersed in a solution of 10 g of potassium iodide and 2.5 g of iodine crystals. Using a starch index chart [Cowgill et al. 2007], a starch index value (1-9) was assigned to each fruit, where 1 = total surface stained and 9 = no stain. Apples with scores of 4, 5, and 6 were considered mature.

Methanol extracts. A portion of the lyophilized sample was placed in a container of the laboratory mill and grounded (2 × 12 seconds). An amount of 25 cm^3 methanol was poured over a 0.500 g ground lyophilisate and mixed for 2 h with a magnetic stirrer (500 rpm). The whole mixture was seeped and centrifuged for 10 minutes (1467 × g, 20°C), and the supernatants obtained were collected into twisted test-probes. Those methanol extracts were then stored in a freezer (–20°C) until the analysis.

ASSESSMENT OF THE ANTIOXIDANT ACTIVITY

ABTS assay. The antioxidant activity was assayed on the basis of a protocol described earlier [Duda-Chodak et al. 2010] with some modifications incorporated. The ABTS radical was generated during a chemical reaction between the 7 mM aqueous solution of diammonium salt of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid and the 2.45 mM potassium persulfate. The solution was kept at a room temperature in darkness throughout the night, in order to complete the reaction and to stabilize the ABTS cation-radical. To investigate extracts, a concentrated solution of the radical was diluted by a phosphate buffer (PBS), its pH being 7.4, so as to obtain a final absorbance of the solution, measured at a wave length of 734 nm, of A = 0.70 ±0.02 ($ABTS_{0.7}$).

An amount of 0.1 cm^3 of the properly diluted extract investigated or of Trolox solutions (their concentration ranging from 0 to 10 mg × 100 cm^{-3}) was added to a 1 cm^3 $ABTS_{0.7}$; next, the absorbance was measured in the 6^{th} minute upon the completed mixing. The antioxidant capacity of extracts under study was calculated using a standard curve drawn up for solutions of the synthetic vitamin E (Trolox) and expressed as mg Trolox × 100 g^{-1} of fruit fresh weight. All determinations were performed in triplicate.

DPPH assay. The scavenging capacity of DPPH radical was assessed by the method described earlier [Duda-Chodak et al. 2010]. An amount of 0.2 cm^3 of the extract analysed (adequately diluted with a re-distilled water) or Trolox solutions (their concentrations ranging from 0 to 2.5 mg × 100 cm^{-3}) was added to 0.8 cm^3 of a 225 μM solution

of DPPH (in ethanol) and, then, the rate of absorbance disappearance was measured at a wave length of 515 nm in the 10th minute upon the mixing of reagents in a cuvette. The antioxidant capacity of methanol extracts was calculated using a standard curve developed for Trolox, and expressed as mg of Trolox × 100 g^{-1} of fresh weight. All determinations were performed in triplicate.

Qualitative and quantitative analysis of polyphenols. The polyphenols in the apples investigated were determined by the HPLC method described earlier [Duda-Chodak et al. 2010]. 2 g of apple lyophilisate was extracted three times with 80% aqueous solution of methanol in order to obtain 50 cm^3 of extract (ultrasonic bath, 40 kHz, BAS-10, BAS, Warsaw, Poland, 15 minutes). The extracts were then filtered through a Schott G4 funnel and centrifuged (10 min, 14 000 rpm). The extracts were analysed by high performance liquid chromatography (HPLC), on a Merck-Hitachi L-7455 apparatus with a diode array detector (DAD). Separation was performed on a Synergi Fusion RP-80A 150×4.6 mm (4 μm) Phenomenex column (Torrance, CA, USA) thermostated at 30°C. The mobile phase consisted of 2.5% acetic acid (solution A) and acetonitrile (solution B), applied in a gradient changing linearly from 0% B to 25% B during 36 minutes. The column was then washed with the pure solution A. The flow of the liquid phase was 1 $cm^3 \times min^{-1}$, and the detection was conducted at four wavelengths: 280 nm (flavanols), 320 nm (phenolic acids), 360 nm (flavonols) and 520 nm (anthocyanins). In order to identify the compounds, retention times of the compounds under analysis and standard compounds were compared. In addition, enzymatic hydrolysis of flavonol glycosides and cyanidin glycosides in a citrate buffer solution (citric acid and sodium citrate, pH 5), was performed for identification. The disappearance of single peaks in the chromatogram and formation of the corresponding aglycones was observed using HPLC after 1-hour incubation at 38°C with a specific enzyme: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidase. The calibration curves were made from (–)epicatechin, (+)catechin, chlorogenic acid, phloridzin, isoquercitrin, and cyanidin-3-glucoside as standards. Procyanidin B2, C1, and B1 used as standards were obtained by the method of Oszmiański and Bourzeix [1995]. For those analytes where no standard was available, standards of the same family were used; thus quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-arabinoside, quercetin-3-xyloside and quercetin-3-rhamnoside were quantified as quercetin-3-glucoside (isoquercitrin); phloretin-2-glucoside as phloridzin; *p*-coumaric derivative as *p*-coumaric acid; and caffeic acid derivatives as chlorogenic acid. Results were expressed as mg × 100 g^{-1} of fresh weight. The assays were performed in duplicate. The standard error of the method was determined in preliminary determinations and was below 10%.

Statistical analysis. The results were shown as an arithmetic mean (± standard deviation) of three independent determinations. A single-factor Analysis of Variance test (ANOVA) with a *post hoc* Tukey test was applied to perform a statistical analysis. A Kołmogorov-Smirnov test was applied to examine the normality of distribution. Differences were considered to be significant at $p < 0.05$. The HPLC analysis was performed in duplicate. The maximum error of the method was determined in preliminary determinations and was below 10%. All statistical calculations were performed using GraphPad InStat version 3.01 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Changes in the antioxidant activity and in the polyphenols profile during the ripening and storage of the fruit

The results revealed that the ability to scavenge an ABTS radical decreased during the ripening period of the apples (from 693 to 306 mg of Trolox × 100 g^{-1} of fresh fruit weight) (Fig. 1). On the contrary, during fruit storage in a cold store, antioxidant activity

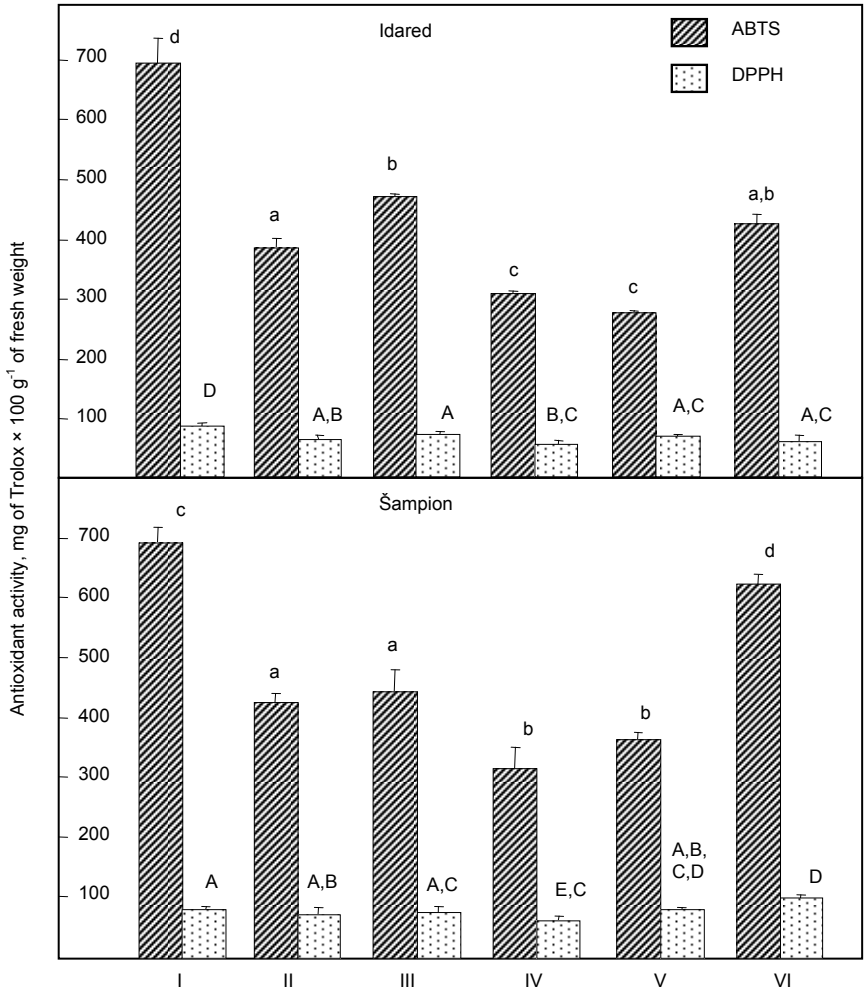

Fig. 1. Antioxidant activity of the Idared and Šampion apple cultivars at different stages of maturity (I-IV) and storing (V-VI) (mean value ±SD, n = 3). The same letters by the columns denote the lack of statistically significant differences at $p < 0.05$; a, b, c, and d as for ABTS; A, B, C, and D as for DPPH (the statistical analysis was performed within the range of a given cultivar)

increased and reached a level of 424 or 621 mg of Trolox × 100 g^{-1} of fresh weight of apple (Idared and Šampion cultivar, respectively). When carrying out experiments with a DPPH radical applied, the differences were minor or statistically insignificant. At individual stages of the apple ripeness, the changes in the amounts of polyphenol compounds, confirmed by the HPLC analysis, were correlated with the changes in the antioxidant activity assessed using a method with ABTS (r = 0.94 for the Idared cultivar, and r = 0.82 for the Šampion cultivar).

Table 1. Polyphenols concentration at subsequent stages of ripening and storing the apples of the Idared and Šampion cultivars, mg × 100 g^{-1} of fresh weight

Cultivar	Idared						Šampion					
Stage	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Chlorogenic acid	28.63	16.27	14.61	12.38	13.60	12.85	9.28	5.82	3.71	2.74	5.31	4.63
p-Coumaryl-quinic acid	1.98	0.89	0.71	0.56	0.69	0.66	2.60	1.44	1.03	0.74	1.35	0.95
(+)Catechin	1.98	0.56	0.27	1.33	0.41	2.31	1.22	0.72	0.17	1.72	1.35	2.51
(–)Epicatechin	16.22	6.75	5.99	2.92	3.93	4.90	22.17	11.52	8.60	10.93	10.87	11.54
Procyanidin B2	6.85	5.40	5.47	3.88	4.78	4.52	10.44	8.50	8.38	5.32	10.10	10.58
Procyanidin B1	2.11	1.50	1.31	0.64	0.83	1.21	1.70	1.32	2.17	1.35	1.21	1.53
Procyanidin C1	4.41	1.86	2.42	1.26	1.90	3.85	4.58	2.85	3.95	2.47	6.12	6.50
Phloretin xyloglucoside	0.99	0.84	0.80	0.11	0.47	0.58	2.94	1.57	1.36	0.65	0.69	1.13
Phloridzin	7.45	3.43	2.78	2.05	2.24	NA	3.82	0.23	1.27	1.01	1.03	NA
Quercetin glycosides												
rutinoside	0.10	0.06	0.16	0.05	0.20	0.09	0	0	0	0	0	0
galactoside	4.31	2.11	3.15	1.51	2.79	1.68	3.11	1.27	2.05	1.88	3.34	2.07
glucoside	0.39	0.23	0.38	0.16	0.34	0.19	0.52	0.28	0.40	0.39	0.56	0.38
xyloside	1.96	0.92	1.05	0.62	0.75	1.36	1.34	0.58	0.65	0.54	0.87	1.66
fructorhamnoside	5.40	2.37	2.74	NA	NA	NA	3.45	1.43	1.59	1.31	NA	NA
rhamnoside	1.88	0.81	0.87	0.57	0.79	0.51	1.85	0.83	0.89	0.74	0.93	0,88
arabinoside	NA	NA	NA	1.59	1.92	0.67	NA	NA	NA	NA	2.21	0.73
Cyanidin-3-galactoside	NA	NA	NA	0.38	NA	1.20	NA	NA	NA	NA	NA	0.67
Total	84.68	44.00	42.71	29.98	35.63	38.41	69.03	39.73	36.23	31.78	45.96	46.78

NA – a non-assayed compound.
The maximum error of the measurements was < 10%.

Whilst the fruits were ripening, the contents of chlorogenic acid, (–)epicatechin, procyanidin B2, and phloridzin considerably decreased (Table 1). On the other hand, when the apples were stored in a cold store, it was proved that the content of procyanidin C1 increased in the apples, and, additionally, in the case of the Šampion cultivar, the contents of procyanidin B2 and chlorogenic acid rose, and as for the Idared cultivar: (–)epicatechin and catechin amounts rose during its storage.

Antioxidant properties and content of polyphenol compounds in individual parts of the fruit

The seeds of the apple cultivars examined (Idared and Šampion) were characterised by a significantly higher antioxidant capacity (Fig. 2), as well as by higher concentration of the polyphenols analysed (Table 2) compared to the peel and flesh of the apples.

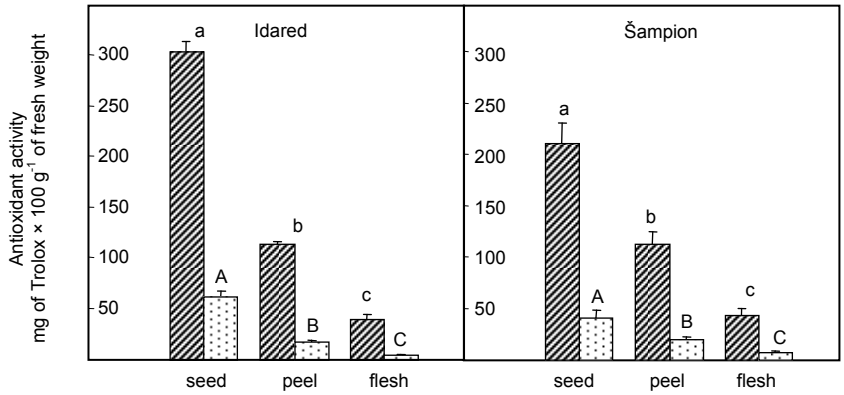

Fig. 2. Antioxidant activity of particular parts of the fruit of the Idared and Šampion apple cultivars (mean value ±SD, n = 3). The same letters by the columns denote the lack of statistically significant differences at $p < 0.05$; a, b, c, and d as for ABTS; A, B, C, and D as for DPPH (the statistical analysis was performed within the range of a given cultivar)

The analysis of composition of the polyphenols using a HPLC method showed that, as for the seeds, the phloridzin predominated and its amount ranged from 72% (Šampion cultivar) to 84% (Idared cultivar) of all assayed antioxidants, and the chlorogenic acid amounted to 15% and 10%, respectively. No quercetin glycosides were found in seeds (Table 2), although they were a significant fraction of the polyphenols in apple peels. As for the Idared cultivar, quercetin galactoside (20%), quercetin fructoramnoside (18%), and (–)epicatechin (13%) predominated, whereas in the Šampion apple peels, the most abundant were: procyanidin B2 (33% of all the polyphenols assayed), quercetin galactoside and quercetin fructoramnoside (15 and 11%, respectively). In the flesh of the apple cultivars examined (Šampion and Idared), the following compounds prevailed: chlorogenic acid (14% and 53%, respectively), (–)epicatechin (29% and 10%), and procyanidin B2 (28% and 14%). In that part of the apples, no, or only trace amounts of quercetin glycosides were detected.

Table 2. Polyphenols concentration in individual parts of the fruits of Idared and Šampion cultivars, mg × 100 g^{-1} of fresh weight

	Seeds		Peel		Flesh	
	Idared	Šampion	Idared	Šampion	Idared	Šampion
Chlorogenic acid	50.29	51.80	7.15	1.12	11.69	5.34
p-Coumaryl-quinic acid	0.16	0.36	1.26	0.70	0.59	1.26
(+)Catechin	1.25	1.19	1.31	1.56	0.76	1.37
(–)Epicatechin	3.87	2.08	18.15	9.91	2.26	10.70
Procyanidin B2	11.39	9.71	9.74	38.39	3.12	10.37
Procyanidin B1	4.47	3.81	4.97	3.78	0.75	1.55
Procyanidin C1	4.14	2.32	7.67	8.65	1.60	4.59
Phloretin xyloglucoside	5.83	26.97	4.20	2.23	0.09	0.71
Phloridzin	438.89	256.97	10.01	6.06	1.24	0.91
Quercetin glycosides						
rutinoside	0 (0)	0	0.68	0	0	0
galactoside	0 (0)	0	27.74	18.10	0	0.02
glucoside	0 (0)	0	3.06	3.37	0	0.05
xyloside	0 (0)	0	9.46	4.88	0	0.07
fructorhamnoside	0 (0)	0	25.50	12.65	NA	NA
rhamnoside	0 (0)	0	9.78	5.55	0	0.37
arabinoside	NA	NA	NA	NA	0	0.14
Cyanidin-3-galactoside	NA	NA	NA	NA	0	0
Total	520.28	355.22	140.69	116.94	22.09	37.42

NA – a non-assayed compound.
The maximum error of the measurements was < 10%.

DISCUSSION

Fruits, including apples, contain many flavonoids and phenolic acids. Owing to their widespread availability and relatively low prices, apples appear to be a basic source of antioxidants in the Polish diet. The main compounds with antioxidant properties present in apples are polyphenols. The general opinion is that their concentration depends on the species and cultivar of a fruit, as well as on the fruit maturity degree, cultivation methods, soil and climatic conditions, and insolation [Podsędek et al. 2000, Sluis et al. 2001, Kondo et al. 2002, McGhie et al. 2005, Duda-Chodak et al. 2010]. The apples used in the experiments originated from the same orchard so the impact of mentioned factors can be excluded.

It was demonstrated that the antioxidant properties of apples, both Idared and Šampion cultivar, diminished along with the ripening of the fruit (Fig. 1). In particular, the drop in the contents of chlorogenic acid and epicatechin was very clear-cut. It means that unripe apples are more valuable raw material for polyphenols extraction, especially chlorogenic acid and (–)epicatechin, than apples at harvest maturity stage. Interestingly, that when the apples investigated were stored in a cold store, the share of procyanidins, (+)catechin and (–)epicatechin grew. Similar phenomena were observed by Robards et al. [1999] and Kondo et al. [2002] in their research. They confirmed that, generally, concentration of flavonoids and chlorogenic acid droped whilst the fruits ripen. The increase in the polyphenol content in the apples studied, during their storage, could be explained by the fact that polyphenols evolve from a bounded form (for example bound with the cell walls) into a free form, and, as an effect, a higher extraction efficiency is obtained. Furthermore, the enzyme that plays a key role in the biosynthesis of ethylene, the ACC-oxidase is stimulated by cold in the peel. Ethylene induces its own synthesis and the synthesis of phenylalanine ammonia lyase, which is the basic enzyme in the biosynthesis of flavonoids [Perez-Ilzarbe et al. 1997]. The elevated level of polyphenols in the apples after storage indicates that fruit remaining in cold stores at the end of the season can be still valuable material. Although they are not suitable for direct consumption they can serve for antioxidants recovery.

The issue of changes in the chemical composition during storage of the fruit is also very important from the point of view of the fruit processing industry. According to Awad and Jager [2003], a profile of phenol compounds undergoes changes during the storage of fruits; the amount of catechins, epicatechins, and phenolic acids in the fruits decreases during the storing of fruits. Among other authors, Robards et al. [1999] and Sluis et al. [2003], found the decrease in the chlorogenic acid content. Koleśnik et al. [1977] received different results and confirmed that the concentration of anthocyanins and flavanols increased during the storage. Other investigations prove that the storage, both in a cold store and under the conditions of controlled atmosphere, does not impact the antioxidant activity and the content of polyphenols in apples [Sluis et al. 2001, Manach et al. 2004]. On the other hand, it is difficult to compare individual research results since the observations were carried out under changing conditions of experiments and referred to different apple cultivars.

It should be highlighted that the antioxidant activity rates as achieved in the experiments with an ABTS radical involved were averagely five-fold higher than the respective rates obtained with a DPPH radical applied. This is attributed to the dissimilar nature of the two radicals, since they enable the determination of hydrophobic antioxidant substances only (as in the case of DPPH) or of hydrophilic and hydrophobic (as in the case of ABTS).

Antioxidant potential of apple seeds is very high, reaching almost 3000 mg Trolox × 100 g^{-1} of fresh weight of Idared seeds. The values obtained for peels were more than twice lower, and flesh antioxidant potential did not exceed 500 mg Trolox × 100 g^{-1}. In the hitherto literature references [Boyer and Liu 2004, Chinnici et al. 2004 a], there is unanimity among the researchers that there are significant differences with regard to antioxidant activity and composition of polyphenols among seeds, peel and flesh of the fruit. Though, the results achieved (Table 2) do not completely and fully comply with the former reports. As a matter of fact, Lu and Foo [1998] proved that phloridzin is the basic antioxidant in the seeds of Royal Gala apples followed by chlorogenic acid, phloretin-2-xyloglucoside, and quercetin glycosides. It is rather difficult to unambigu-

ously affirm whether or not the deficiency of quercetin glycosides, as confirmed in the seeds examined, is caused by cultivar differences or by other reasons. According to the research outcomes published in other papers, quercetin glycosides occur only, and almost exclusively, in apple peels [Sluis et al. 2001, Boyer and Liu 2004], and, together with phloretin and procyanidins constitute a quantitatively outweighing component of the peels [Oszmiański and Lee 1994, Chinnici et al. 2004 b]. On the other hand, Kondo et al. [2002] proved that in the peels of Fuji, Oorin, and Redfield apples, phloridzin and chlorogenic acid predominated, and, that their concentrations were much higher in apple peel than in apple flesh. In the experiments accomplished, polyphenols contained in peels of the two examined apple cultivars: Šampion and Idared, showed essentially higher concentrations compared to the fruit flesh of those two cultivars; this fact conforms to the expectations and earlier observations by Guyot et al. [2002] and Chinnici et al. [2004 a]. But it should be stressed that in the research cited, both in the peels and the flesh, procyanidins represented the main fraction.

CONCLUSIONS

The research accomplished confirmed a significant diversity in the polyphenol contents and in the antioxidant activity related to them. The polyphenol profile is characteristic for both the cultivar, maturity stage and the particular part of fruit. In the seeds of apples, phloridzin is a predominating polyphenol compound, whereas chlorogenic acid, (–)epicatechin, and procyanidin B2 in the apple flesh. Quercetin glycosides are a significant fraction of polyphenols in the apple peels, but, as for seeds and flesh, they are either found in trace amounts only or they are not found at all. The seeds of apples are characterised by a higher concentration of polyphenols and a higher antioxidant activity than peels and flesh of the apples. Moreover, it was confirmed that as the apples ripened, the content of polyphenols and the antioxidant features of fruit decreased. On the other hand, the storage of ripe fruit can contribute to improving their pro-health value. We conclude that unripe apples together with apple seeds and peel (fruit industry wastes) constitute a valuable source of polyphenols.

REFERENCES

Aherne S.A., O'Brien M., 2002. Dietary flavanols: chemistry, food content, and metabolism. Nutrition 18, 75-81.

Awad M.A., Jager A., 2003. Influences of air and controlled atmosphere storage on the concentration of potentially healthful phenolics in apples and other fruits. Postharvest Biol. Technol. 27, 53-58.

Bergamini C.M., Gambetti S., Dondi A., Cervellati C., 2004. Oxygen, reactive species and tissue damage. Curr. Pharm. Des. 10 (14), 1611-1626.

Boyer J., Liu R.H., 2004. Apple phytochemicals and their health benefits. Nutr. J. 3, 5-20.

Chinnici F., Bendini A., Ganiani A., Riponi C., 2004 a. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. 52, 4684-4689.

Chinnici F., Ganiani A., Natali N., Riponi C., Galassi S., 2004 b. Improved HPLC determination of phenolic compounds in cv. Golden Delicious apples using a monolithic column. J. Agric. Food Chem. 52, 3-7.

Cowgill W., Clements J., Compton J., 2007. Painless and efficient maturity testing. Available on the Internet: http://www.umass.edu/fruitadvisor/clements/articles/sitest.htm (Retrieved 2009--07-13).

Duda-Chodak A., Tarko T., 2007. Antioxidant properties of different fruit seeds and peels. Acta Sci. Pol., Technol. Aliment. 6 (3), 29-36.

Duda-Chodak A., Tarko T., Satora P., Sroka P., Tuszyński T., 2010. The profile of polyphenols and antioxidant properties of selected apple cultivars grown in Poland. J. Fruit Ornam. Plant Res. 18 (2), 39-50.

Guyot S., Bourvellec C., Marnet N., Drilleau J.F., 2002. Procyanidins are the most abundant polyphenols in dessert apples at maturity. Lebensm. Wiss. Technol. 35, 289-291.

Kolesnik A., Elizarowa L.G., Starodubsteva T.V., Afanasyeva V.S., Erakhina T.S., 1977. Changes in polyphenols during storage of fruits and vegetables. Prikl. Biokhim. Mikrobiol. 13, 333--338.

Kondo S., Truda K., Muto N., Ueda J., 2002. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. Sci. Hortic. 96, 177-185.

Lu Y., Foo L.Y., 1998. Constitution of some chemical components of apples seed. Food Chem. 61 (1/2), 29-33.

Manach C., Scalbert A., Morand C., Rémésy C., Jimenez L., 2004. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 79, 729-747.

McGhie T.K., Hunt M., Barnett L.E., 2005. Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. J. Agric. Food Chem. 53 (8), 3065-3070.

Michota-Katulska E., 2000. Antyoksydanty – wybrane aspekty zdrowotne [Antioxidants – selected health aspects]. Żywn. Żyw. Prawo Zdrow. 3, 331-337 [in Polish].

Oszmiański J., Bourzeix M., 1995. Preparation of catechin and procyanidin standards from hawthorn (*Crategus azorus* L.) and pine (*Pinus mesogeensis* Fieschi) barks. Pol. J. Food Nutr. Sci. 4/45 (2), 91-98.

Oszmiański J., Lee C.Y., 1994. Frakcjonowanie i hydroliza niektórych glikozydów flawonoidowych ze skórek jabłkowych [Fractionation and hydrolysis of some flavonoid glycosides from apple peels]. Zesz. Nauk. AR Wroc. 244, Techn. Żywn. 7, 105-112 [in Polish].

Perez-Ilzarbe J., Hernandez T., Estrella I., Vendrell M., 1997. Cold storage of apples (cv. Granny Smith) and changes in phenolic compounds. Z. Lebensm. Unters. F. A, 204, 52-55.

Podsędek A., Wilska-Jeszka J., Anders B., Markowski J., 2000. Compositional characterization of some apple varieties. Eur. Food Res. Technol. 210, 368-372.

Pourmorad F., Hosseinimehr S.J., Shahabimajd N., 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African J. Biotech. 5 (11), 1142--1145.

Rice-Evans C., Miller N.J., Paganga G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 4 (2), 152-159.

Robards K., Prenzler P.D., Tucker G., Swatsitang P., Glover W., 1999. Phenolic compounds and their role in oxidative process in fruits. Food Chem. 66, 401-436.

Sluis van der A., Dekker M., de Jager A., Jongen W.M.F., 2001. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J. Agric. Food Chem. 49, 3606-3613.

Sluis van der A., Dekker M., Jongen W.M., 2003. Polyphenolic antioxidants in apples. Effect of storage conditions on four cultivars. Acta Hortic. 600, 533-540.

Sroka Z., Gamian A., Cisowski W., 2005. Niskocząsteczkowe związki przeciwutleniające pochodzenia naturalnego [Low molecular weight antioxidants of natural origin]. Post. Hig. Med. Dośw. 59, 34-41 [in Polish].

Tarko T., Duda-Chodak A., Ignacok M., 2009 a. Odzysk związków przeciwutleniających z roślinnych surowców odpadowych [Recovery of antioxidant compounds from plant waste]. Przem. Ferm. Owoc.-Warz. 10, 18-22 [in Polish].

Tarko T., Sobusiak J., Duda-Chodak A., 2009 b. Sposoby wykorzystania odpadów przemysłu owocowo-warzywnego [Various possibilities of utilization of fruit and vegetables industry wastes]. Przem. Ferm. Owoc.-Warz. 3, 32-34 [in Polish].

Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., Telser J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39 (1), 44-84.

Yamaki K., Taneda S., Yanagisawa R., Inoue K., Takano H., Yoshino S., 2007. Enhancement of allergic responses *in vivo* and *in vitro* by butylated hydroxytoluene. Toxicol. Appl. Pharmacol. 223, 164-172.

WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE JABŁEK – WPŁYW STADIUM DOJRZAŁOŚCI ORAZ CZĘŚCI OWOCU

Wstęp. Ostatnio wiele badań jest ukierunkowane na ulepszenie metod oraz zwiększenie wydajności odzyskiwania przeciwutleniaczy z różnych owoców i ich odpadów. Celem pracy było określenie potencjału antyoksydacyjnego nasion i skórek jabłek, będących częstym odpadem przemysłu owocowego, oraz porównanie go z właściwościami miąższu. Analizowano także właściwości antyoksydacyjne jabłek w różnych stadiach dojrzałości i etapach przechowywania.

Materiał i metody. W badaniach użyto jabłek odmian Idared i Šampion. Aktywność antyoksydacyjną oceniano metodami z rodnikiem ABTS oraz DPPH, a profil związków polifenolowych określano metodą HPLC.

Wyniki. Nasiona analizowanych odmian charakteryzowały się znacznie większą aktywnością antyoksydacyjną i wyższym stężeniem związków polifenolowych w porównaniu ze skórkami i miąższem tych owoców. Dominującym składnikiem w nasionach była florydzyna (84% i 72% wszystkich badanych polifenoli u odmiany Idared i Šampion), a w skórkach – glikozydy kwercetyny (54% i 38%). Nie wykazano obecności glikozydów kwercetyny w nasionach. Zdolność do wygaszania rodnika ABTS, ale nie DPPH, malała w trakcie dojrzewania owoców, natomiast przechowywanie w chłodni skutkowało zwiększeniem potencjału antyoksydacyjnego jabłek.

Wnioski. Na podstawie uzyskanych wyników można wnioskować, że niedojrzałe jabłka, a także nasiona i skórki jabłek (odpady z przemysłu owocowego) są cennym źródłem związków polifenolowych.

Słowa kluczowe: właściwości antyoksydacyjne, HPLC, stadium dojrzałości owocu, skórki, nasiona, polifenole

Received – Przyjęto: 3.03.2011

Accepted for print – Zaakceptowano do druku: 17.05.2011

For citation – Do cytowania: Duda-Chodak A., Tarko T., Tuszyński T., 2011. Antioxidant activity of apples – an impact of maturity stage and fruit part. Acta Sci. Pol., Technol. Aliment. 10(4), 443-454.