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Relationship between Apple Peel and the Whole Fruit Antioxidant Content: Year and Cultivar Variation

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Antioxidants are usually considered with regard to plant defense mechanisms due to the oxidative stress or their importance for human health or both. In the present research, a comprehensive study was made to test the relationship between the antioxidant content in apple peel and the whole fruit in two growing seasons. Antioxidants were mainly localized in the apple peel, but cultivars exhibited very high biodiversity in the distribution pattern. High or very high correlation coefficients between apple peel glutathione reductase and catalase activity as well as ascorbate, glutathione, and anthocyanins concentrations and the whole fruit were discovered on the basis of both fresh and dry weight basis in two growing seasons: 2004 and 2005. In the case of ascorbate peroxidase activity and phenolics or flavonol contents, it was proven only in 2005. It seems that apple peel could be a good marker of health values as well as antioxidant potential of apple fruit. Additional arguments, as compared to the previous study, were incorporated to support the suggestion of glutathione reductase as worth considering as an environmental stress marker and/or signalling molecule.

KEYWORDS: ***Malus domestica*** **Borkh; antioxidant distribution; ascorbate; thiol compounds; phenolics; glutathione reductase**

INTRODUCTION

Every species and cultivar possesses its own genetically dependent chemical characteristics, which may be modified by different external factors. At the tissue level, there are significant qualitative and quantitative differences between the antioxidant contents of seeds, epidermal, and subepidermal layers (peel) and the internal tissue (cortex) (*1*, *2*). According to many authors, apple phytocompounds are mainly localized in the skin. As compared to flesh, the peel was richer in phenols (*3*−*7*), ascorbic acid, and glutathione (*1*, *2*) or antioxidative enzyme activity (*2*). The highest contents of almost all of the quantified phenols such as flavonols, flavanols, procyanidins, dichydrochalcones, and hydroxycinnamates are displayed in peel (epicatechin, procyanidin B2, and phloridzin occurred as the most abundant) in comparison with the pulp (*6*). In peels, flavonols, flavanols, and procyanidins constituted the top contributors (∼90%) of the total antioxidant activity, whereas in pulps antioxidant activity was mainly derived from flavanols (monomers and polymers) and hydroxycinnmates (*5*, *8*, *9*). Another, although indirect (synergistic or antagonistic action may occur), proof of higher peel antioxidant content is the comparison of the total antioxidant activity of peel and pulp. In spite of different methods used, the peel had a significantly higher total antioxidant capacity than the flesh (*8*−*10*) or peel followed by flesh with skin and flesh (*11*). Phenolics are the most frequently examined group containing more powerful antioxidants, highly correlated with the total antioxidant activity (*8*, *9*).

Antioxidants are under investigation mainly because of two reasons, an active part in plant expression of defense mechanisms due to the oxidative stress and increasing evidence suggesting that they can be nutritionally important for human health. The peel is commonly selected to explore antioxidant metabolism and defense mechanisms, because this part of the fruit is a primary target on unfavorable environmental factors (biotic and abiotic stress). At that time, accumulation of antioxidant in apple peel frequently occurs. Additionally, more distinct differences between tested source of variation (also among cultivars) could be observed after separating epidermal and subepidermal layers from the internal tissue. The whole fruit antioxidant capacity is more interesting with respect to the nutritional value of apple because apple peel represents, at the maximum, up to 10% of the whole fruit (*12*). The health benefits and consumption of apple are attributed to its bioactive constituents and their availability throughout the year. The contribution of apple in the daily intake of total flavonoids, total phenolics, and antioxidants has been estimated lately from the most common fruit in the American diet, and apple has been classified as the second highest (*13*). Hence, the examination of apple antioxidants is involved in apple breeding programs as well as in searching of quantitative trait loci in the *Malus* genome.

Although a number of studies have been made in this issue, they are often restricted to a few cultivars and/or tested

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Table 1. Harvest Date of Analyzed Cultivars in the Years 2004–2005

cultivar	year 2004	year 2005	cultivar	year 2004	year 2005
Clivia	6 X	NF[a]	McIntosh	23 IX	3 X
Cox'Orange	1 X	NF[a]	Monroe	6 X	3 X
Elan	28 IX	3 X	Mutsu	21 X	NF[a]
Elstar	23 IX	3 X	Pilot	14 X	10 X
Fiesta	13 X	14 X	Pinova	14 X	10 X
Fuji	28 X	3 XI	Prima	1 X	3 X
Gala	28 IX	3 X	Priscilla	14 X	28 X
Gloster	21 X	28 X	Red Rome	13 X	28 X
Golden Delicious	21 X	14 X	Rubin	23 IX	3 X
Granny Smith	28 X	3 XI	Spartan	28 IX	NF[a]
Idared	21 X	28 X	Starking Delicious	25 X	3 XI
Jonamac	1 X	3 X			

[a] NF, trees did not bear fruit.

compounds, predominantly phenolics, and most of them were limited only to one growing season. According to our knowledge, the question still not answered is the following: How high is the correlation between the apple peel and the whole fruit antioxidant properties? If the peel antioxidant content is a good, statistically justified marker of the whole fruit antioxidant capacity, it can be used for many types of investigations in the future and/or to compare different data from the current literature. Previous research made on a wide range on genetical resources of *Malus* showed very high biodiversity in antioxidant contents that were both cultivar- and seasonal-dependent (*14*). Thus, more extensive studies were conducted to answer the questions: What the is distribution pattern, to what extent do cultivars differ in the antioxidant content between the apple peel and the whole fruit, and is there any correlation between antioxidants themselves? To give more credible answers to these questions as well as to assess the influence of the year, investigations on apple peel and the whole fruit were conducted in two vegetation seasons, 2004 and 2005, on 23 and 19 apple cultivars, respectively, also known as common progenitors in Europe.

MATERIALS AND METHODS

Plant Material and Meteorological Information. The examined cultivars of apple were obtained from the Experimental Orchard of the Department of Pomology and Basic Natural Sciences in Horticulture of Warsaw Agricultural University—SGGW in Warsaw—Wilanow. The localization and the way of tree cultivation have already been described (*14*). Twenty-three apples cultivars in 2004 and 19 apple cultivars in 2005 were harvested successively as they ripened from September 23 to October 28, 2004, and from October 3 to November 3, 2005 (**Table 1**). Fruits were picked when their starch indexes reached values of 6−8.

The mean temperature and rainfall from the meteorological station of Warsaw−Wilanow Orchard in 2004 and 2005 seasons are shown in **Figure 1**. As compared to the multiannual average (data not shown), both vegetation periods were rather dry and the year 2004 was hotter as compared to 2005.

Sample Preparation. Apple peels and whole fruits were tested. Five replicates for chemical analyses as well as dry weight contents for each cultivar and type of tissue were prepared. Each of them included tissue samples from two fruits. The apple was cut into four sections, along a longitudinal axis, to remove the core and seeds, and next, "sectors" from opposite sides were cut into small pieces. Simultaneously, the other two parts of the fruit were peeled with a peeler and both types of tissues were immediately put into liquid nitrogen. The first sample described was referred to as whole apple fruit (ca. 5% of this tissue constituted the apple peel), and the second was referred to as the apple peel. A thin layer of apple flesh remained adhered to the peel (contamination by apple flesh on FW basis was ∼30−35%), and the peel sample was therefore considered the epidermic zone of the apple fruit. The taken samples were stored at −80 °C until analysis. Directly before analysis, apple tissues were ground to a fine powder in liquid nitrogen.

The proportion of apple peel taken using the way described above was calculated after weighting the whole fruit and its apple peel. On the basis of the fresh matter, the peel represented 6.33 ± 0.87% of the whole apple fruit, on average. A weak negative correlation was found between the weight of the fruit and the peel content. The dry weight (DW) was obtained by oven drying the samples at 105 °C for a period of 3 days.

Determination of Bioactive Constituents. *Apparatus and Chemicals.* The high-performance liquid chromatography (HPLC) from Waters Co., System Breeze with binary solvent delivery system (1525), degasser, an autosampler with thermostat with the scale 4−40 °C (M 717 PLUS), scanning fluorescence detector (M 474) and 2-channel UV/vis detector (M 2487), and the thermostat for the column 5−85 °C (Peltier) were used. Spectrophotometer measures were conducted using a Lambda Bio 10 spectrophotometer from Perkin-Elmer Co. Glutathione (GSH), γ-glutamyl-cysteine (γ-GC), and L-ascorbate (AA) were from Sigma. L-Cysteine (CYS) was purchased from Fluca. Monobromobimane (thiolyte) was from Calbiochem, and cyanidin-3,5-di-glukoside chloride was from Chromadex. All other chemicals were of analytical or HPLC grade purity.

Enzyme Activities. The tissues (100 mg) ground in liquid nitrogen were suspended in 5 mL of 100 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.8) containing Triton X-100 (0.5%), insoluble polyvinylpolypyrrolidone (PVPP), and ascorbate (5 mM). The mixture was centrifuged at 48000*g* for 20 min at 4 °C. Ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT) activities were measured in a total volume of 1 mL by monitoring the decrease in absorbance at 290, 340, and 240 nm, respectively, and calculating the appropriate absorption coefficients (*15*−*17*).

Extraction of Thiol Compounds and Ascorbate. Frozen apple powder was homogenized in 0.1 M HCl containing PVPP and centrifuged at 21900 *g* for 20 min at 4 °C.

GSH. The total GSH (GSH + GSSG, reduced and oxidized glutathione, respectively, referred to as GSH in the text) concentration was determined in the supernatant after reduction with DTT (DL-dithiothreitol) and derivatization with monobromobimane. Monobromobimane derivatives were detected fluorometrically at 480 nm by excitation at 380 nm. During the same analysis, CYS and γ-GC were also determined (*18*).

Ascorbate. The total ascorbate [the sum of AA and dehydroascorbate (DHAA), referred to as ASC] was measured after complete oxidation of AA to DHAA with ascorbate oxidase. DHAA was derivatized with *o*-phenylenediamine, and the reaction product was detected as a fluorescent compound (350/450 nm) (*19*).

Phenolics. Extraction was made in the mixture of methanol, formic acid, and distilled water (50:1.5:48.5). After centrifugation (24000*g*, 4 °C, 10 min), the supernatant was filtered through a 0.45 μm membrane and diluted with 10% ethanol. For the estimation of some subgroups of phenolics, the absorbance of the extracted solution was read at 280, 360, and 520 nm to measure total phenolics, flavonols, and anthocyanins, respectively. Gallic acid, quercetin, and cyanidin-3,5-di-glucoside chloride were used as standards for total phenolics, flavonols, and anthocyanins, respectively (*20*). A more detailed description of all chemical analysis is also presented in a previous paper (*14*).

Statistical Analysis and the Way of Presenting Data. To test the distribution of antioxidant in the apple as well as the influence of the growing season and genotype, obtained results were elaborated by three-way factorial analysis of variance (ANOVA) of Startgraphics Plus 4.1. For this target, the results received from 19 cultivars that fruited in both years were used. The significance of differences between means of main effect (cultivar, type of tissue or year, and interactions) was evaluated using Tukey's honestly significant difference (HSD) procedure, at the 5% probability level. Because of a wide description of "year effect" on apple peel antioxidant content in the former manuscript (*14*), currently I focused mainly on the distribution pattern and the extent of differences between cultivars and some global effects, including

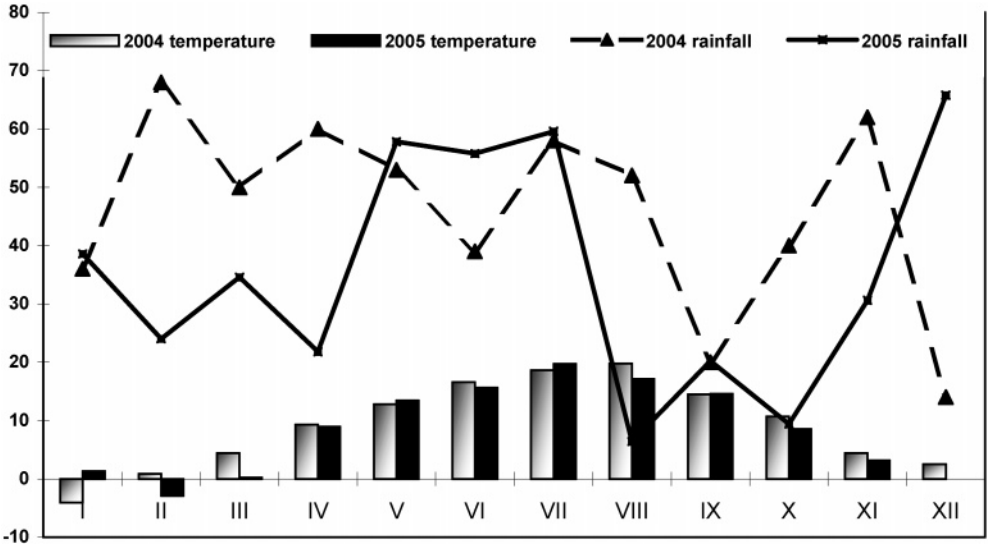

Figure 1. Mean month temperature and rainfall for the field of Warsaw–Wilanow Station, where apple trees were grown in 2004 and 2005 seasons.

Table 2. Summary of Analysis of Variance (ANOVA) for Components Tested; Values of F for Particular Sources of Variation and Their Significance

	source of variation			interactions			
component	cultivar (A)	tissue type (B)	year (C)	AB	AC	BC	ABC
df	18	1	1	18	18	1	18
GR	20.4[a]	318.5[a]	1001.7[a]	8.53[a]	24.88[a]	306.31[a]	6.23[a]
APX	25.4[a]	200.9[a]	26.3[a]	6.51[a]	8.36[a]	0.83[d]	5.24[a]
CAT	43.8[a]	45.8[a]	364.7[a]	8.63[a]	42.18[a]	11.61[a]	2.46[a]
ASC	55.6[a]	2274.7[a]	36.4[a]	27.45[a]	9.32[a]	26.09[a]	4.67[a]
CYS	14.0[a]	8.31[b]	302.8[a]	4.33[a]	7.32[a]	0.02[d]	4.89[a]
GSH	28.0[a]	214.9[a]	104.8[a]	2.68[a]	6.55[a]	17.85[a]	1.08[d]
phenolics	24.1[a]	630.3[a]	161.4[a]	9.29[a]	9.50[a]	133.54[a]	7.36[a]
flavonol	14.9[a]	432.1[a]	9.32[b]	11.23[a]	6.24[a]	31.84[a]	5.28[a]
anthocyanins	57.8[a]	715.1[a]	4.06[c]	27.30[a]	21.28[a]	20.16[a]	6.65[a]
DW	77.4[a]	5631.2[a]	2.01[d]	22.71[a]	29.14[a]	95.70[a]	10.09[a]

[a] Significant at $\alpha = 0.001$. [b] Significant at $\alpha = 0.01$. [c] Significant at $\alpha = 0.05$. [d] NS, not significant.

growing season. Another important reason is that the relationship between the apple peel and the whole fruit (P/WF) did not change considerably throughout the tested years. Differences between calculated P/WF relationship for particular years not exceeding 30% were obtained for most of the tested cultivars in relation to ASC, GSH, CYS, CAT, and APX. The year effect was predominantly linked to the fact that in a given year, the content of compound in general significantly increased or decreased in both tested tissues, so P/WF was similar in both tested years. Special attention was put on the correlation between the antioxidant apple peel and the whole fruit as well as between tested compounds. The last might help in explanation of the influence of the growing season on antioxidant contents. Correlation coefficients were calculated separately for each tested year to take advantage of more cultivars analyzed in 2004 and/or to notice the expected differences between years since growing seasons had a significant impact on the constituent tested. Linear regression analyses were computed using the regression procedure in Microsoft Excel for Windows.

RESULTS

Statistical Analysis—Global ANOVA Results. The influence of almost all principal factors (cultivar, tissue type, and growing season), as well as their interactions at very high probability level, was statistically proven (**Table 2**). The most significant effect exhibited was tissue type and growing season, especially with regard to DW and ASC or GR and CAT, respectively.

Table 3. Content of Dry Weight in Apple Peel and the Whole Fruit Depending on Cultivar (%, Average Value for 2004 and 2005)

cultivar	P[a]	WF[b]	P/WF[c]
Elan	22.0	16.3	1.35
Elstar	21.7	17.1	1.27
Fiesta	22.7	15.2	1.49
Fuji	23.7	15.3	1.55
Gala	21.6	14.7	1.47
Gloster	24.1	15.5	1.55
Golden delicious	24.4	17.1	1.43
Granny Smith	23.5	15.4	1.53
Idared	21.0	14.2	1.48
Jonamac	23.4	16.1	1.45
McIntosh	21.2	13.8	1.54
Monroe	23.1	16.7	1.38
Pilot	26.7	18.9	1.41
Pinova	27.0	**19.3**	1.40
Prima	**18.8**	**13.6**	1.38
Priscilla	21.0	17.0	**1.24**
Red Rome	21.7	14.4	1.51
Rubin	19.9	15.1	1.32
Starking Delicious	**28.5**	15.5	**1.84**
average	22.9	15.9	1.44
difference at $\alpha = 0.05$	SD[d]		

[a] Peel. [b] Whole fruit. [c] P/WF, the content in apple peel divided by whole fruit content. [d] SD, significant differences between means of tested types of tissue. Bolds refer to the highest and lowest values.

Simultaneously, the *F* value for these factors is in a very wide range, from an insignificant year effect on DW content up to 1002 for GR activity and from 8.31 (CYS) up to 5631 (DW) in the case of influence of tissue type. Contrary to this, the genotype displayed comparable influence with respect to antioxidant concentration. Among interactions between main factors, the tissue type GR activity strongly depended on growing season followed by phenolics and DW content.

Dry Matter Content. The content of dry matter differed significantly between the apple peel and the whole fruit (**Table 3**). On the average, fresh matter of the peel and whole apple fruit consisted of 22.9 and 15.9% of dry matter, respectively. Cultivars considerably differed in the content of dry matter. On the basis of average value for both tested years, this parameter amounted from 18.8 (Prima) to 28.5% (Starking Delicious) and from 13.6 (Prima) to 19.3% (Pinova) for apple peel and the whole fruit, respectively. The increase of dry matter from 24 (Priscilla) to 84% (Starking Delicious) in the peel, as compared

Table 4. Distribution of Ascorbate and Thiol Compounds in Apple Peel and the Whole Fruit Depending on Cultivar (μmol g^{-1} DW; Average Value for 2004 and 2005)

	AA + DHAA			GSH + GSSG			CYS		
cultivar	P[a]	WF[b]	P/WF[c]	P	WF	P/WF	P	WF	P/WF
Elan	10.2	3.21	3.18	0.285	0.207	1.38	0.042	0.035	1.20
Elstar	9.1	4.46	2.04	**0.171**	0.116	1.47	0.044	0.048	0.92
Fiesta	**19.5**	**8.19**	2.38	0.312	0.274	1.14	0.041	0.041	1.00
Fuji	9.8	3.96	2.47	0.291	0.220	1.32	0.036	0.031	1.16
Gala	8.8	3.56	2.47	0.219	0.205	**1.07**	0.036	0.033	1.09
Gloster	8.5	2.67	3.18	0.375	0.264	1.42	0.050	**0.069**	0.72
Golden Delicious	15.4	6.88	2.24	0.195	0.130	1.50	0.034	0.027	1.26
Granny Smith	16.6	4.14	4.01	**0.395**	0.294	1.34	0.053	0.043	1.23
Idared	16.9	4.82	3.51	0.298	0.183	1.63	0.039	0.037	1.05
Jonamac	7.6	**2.48**	3.06	0.271	0.157	1.73	0.032	0.024	1.33
McIntosh	**3.9**	5.33	**0.73**	0.206	0.135	1.53	0.026	**0.021**	1.24
Monroe	10.0	3.73	2.68	0.214	0.147	1.46	**0.025**	0.032	0.78
Pilot	14.7	6.82	2.16	0.331	0.198	1.67	0.050	0.042	1.19
Pinova	11.1	4.36	2.55	0.178	0.109	1.63	0.035	0.026	1.35
Prima	11.9	3.76	3.16	0.272	0.157	1.73	0.051	0.035	1.46
Priscilla	11.5	3.74	3.07	0.323	0.188	1.72	**0.057**	0.038	1.50
Red Rome	12.8	4.20	3.05	0.360	0.290	1.24	0.041	0.051	0.80
Rubin	18.0	3.47	**5.19**	0.232	**0.104**	**2.23**	0.038	0.024	**1.58**
Starking Delicious	7.6	3.08	2.47	0.309	**0.315**	0.98	0.027	0.040	**0.68**
average	11.8	4.36	2.71	0.276	0.194	1.42	0.040	0.037	1.08
difference at $\alpha = 0.05$	SD[d]			SD			SD		

[a] Peel. [b] Whole fruit. [c] P/WF, the content in apple peel divided by whole fruit concentration. [d] SD, significant differences between means of tested types of tissue. Bolds refer to the highest and lowest values.

with the whole fruit, was noted. The biggest differences in the content of dry matter between apple peel and the whole fruit were discovered for Starking Delicious, followed by Fuji, Gloster, McIntosh, Granny Smith, and Red Rome, namely, between 84 and 51%. Priscilla, Elstar, Rubin, Elan, Monroe, and Prima showed the lowest differences in DW content between the apple peel and the whole fruit (variation from 24 to 38%).

Distribution of Antioxidant Concentration and Enzyme Activities between Apple Peel and the Whole Fruit. *Ascorbate.* Tissue type was the most prominent factor that affected total ASC content followed by cultivar and growing season (see **Table 2**). The concentration of ASC in apple peel amounted to 11.8 μmol g^{-1} DW on average (**Table 4**). As compared with the whole fruit, where the content of this antioxidant reached 4.36 μmol g^{-1} DW, the peel concentration was 171% higher. Depending on genotypes, the content of ASC in apple peel was from 2.0 (Elstar) to 5.2 (Rubin)—times higher on dry matter basis, as compared with the whole fruit (an exception was McIntosh, where the whole fruit ASC content was higher as compared with the peel). Furthermore, the increase of ASC in apple peel, as compared with the whole fruit, exceeded 100% concerning all of the tested cultivars. The concentration of ASC in apple peel varied between 3.9 and 19.5 for McIntosh and Fiesta cultivars, respectively, and for the whole fruit from 2.48 (Jonamac) to 8.19 (Fiesta) μmol g^{-1} DW. It means 5.0- and 3.3-fold variations between cultivars for apple peel and the whole fruit, respectively. Similar to the present study in the case of whole fruit, a 3.6-fold variation in mean ascorbate content, based on 31 cultivars, was noted by Davey and Coulemans (*21*). Planchon et al. (*22*) presented as high as an 8.8-fold variation testing Belgian apple genetic resources; however, authors included in the survey old apple cultivars that contained several times more ascorbic acid than commercial ones. Besides, the cited results concerned the fresh weight of the whole apple fruit.

The lowest differences between the epidermic zone and the whole fruit were characteristic for Pilot, followed by Golden Delicious and Fiesta. Hence, apples of these cultivars are a rich source of ascorbic acid throughout the whole fruit. Opposite to this, Rubin, Granny Smith, and Idared exhibited the most significant differences between the apple peel and the whole fruit. Interestingly, both groups of cultivars mentioned above were characterized as the richest sources of ASC.

Thiol Compounds. As compared with the whole fruit, the total GSH content in the apple peel was 42% higher on average (**Table 4**). The increase, exceeding the medium value, concerned over half of the tested cultivars, and the highest difference between the tested tissues was exhibited by Rubin. The GSH content amounted from 0.171 (Elstar) to 0.395 (Granny Smith) and from 0.104 (Rubin) to 0.315 μmol g^{-1} DW (Starking Delicious) for peel and the whole fruit, respectively. The biggest variation between cultivars was exhibited by the whole fruit as compared with the peel (3.0- and 2.3-fold variation, correspondingly). Only the whole fruit and apple peel of Starking Delicious, followed by Gala, had nearly the same content of GSH. The bigger biodiversity in GSH content was displayed by Davey and Coulemans (*21*) cultivars, 4.4-fold variation in fresh weight of the whole apple fruit.

The concentration of CYS, one of the amino acid-building GSH compounds, was only 8% higher in apple peel in comparison to the whole fruit on average. These results were affected by a few cultivars (Pinova, Prima, Priscilla, and Rubin). In general, most of the tested cultivars exhibited differences between the apple peel and the whole fruit CYS content below 30%. That parameter was influenced by tissue type in the lowest degree level (see **Table 2**).

Phenolic Compounds. The total phenolics concentration was 117% higher in apple peel in comparison to the whole fruit and ranged from 3.05 to 9.54 mg g^{-1} DW and from 1.57 to 4.57 for peel and the whole fruit, respectively (**Table 5**). Rubin displayed the lowest whereas Idared and Starking Delicious displayed the highest concentration of global phenolics. Apart from tissue type and growing season, a high F value for interaction between them also appeared (**Table 2**). Taking into account examined parts of fruit, the extent of differences between cultivars was nearly the same, regardless of the tissues

Table 5. Distribution of Some Subgroups of Phenolics in Apple Peel and the Whole Fruit Depending on Cultivar (mg g^{-1} DW; Average Value for 2004 and 2005)[a]

	phenolics[a]			flavonols[b]			anthocyanins[c]		
cultivar	P[d]	WF[e]	P/WF[f]	P	WF	P/WF	P	WF	P/WF
Elan	4.23	1.77	2.39	1.32	0.72	1.83	0.44	0.15	2.93
Elstar	5.85	2.76	2.12	2.02	0.50	4.04	0.84	0.26	3.23
Fiesta	3.40	2.04	1.67	1.46	0.92	1.59	0.45	0.26	1.73
Fuji	4.24	2.48	1.71	2.14	0.70	3.06	ND	ND	ND
Gala	5.64	2.98	1.89	1.94	0.73	2.66	0.52	0.28	1.86
Gloster	6.73	2.29	2.94	3.11	0.64	4.86	1.76	0.44	4.00
Golden Delicious	3.42	2.19	1.56	1.24	0.44	2.82	ND	ND	ND
Granny Smith	9.31	2.95	**3.16**	**5.95**	0.63	**9.44**	**0.14**	**0.07**	2.00
Idared	**9.54**	3.97	2.40	3.40	**1.42**	2.39	**2.17**	**0.62**	3.50
Jonamac	3.86	2.77	**1.39**	**1.12**	0.71	1.58	1.21	0.26	4.65
McIntosh	7.71	3.62	2.13	4.28	1.28	3.34	0.80	0.18	4.44
Monroe	7.62	2.53	3.01	4.22	0.87	4.85	1.33	0.40	3.33
Pilot	4.04	2.46	1.64	1.52	**0.37**	4.11	0.35	0.13	2.69
Pinova	4.28	2.90	1.48	1.41	0.99	**1.42**	0.23	0.32	**0.72**
Prima	7.49	2.41	3.11	2.46	0.48	5.13	1.47	0.12	**12.3**
Priscilla	6.98	2.71	2.58	3.34	0.57	5.86	0.68	0.16	4.25
Red Rome	6.90	3.26	2.12	2.46	0.91	2.70	0.82	0.26	3.15
Rubin	**3.05**	**1.57**	1.94	1.19	**0.37**	3.22	0.48	0.31	1.55
Starking Delicious	9.42	**4.57**	2.06	2.97	0.74	4.01	1.48	0.36	4.11
average	5.98	2.75	2.17	2.50	0.74	3.38	0.82	0.24	3.42
difference at $\alpha = 0.05$	SD[g]			SD			SD		

[a] Expressed as equivalent of gallic acid. [b] Expressed as equivalent of quercetin. [c] Expressed as equivalent of cyanidin-3,5-di-glucoside. [d] Peel. [e] Whole fruit. [f] P/WF, the content in apple peel divided by whole fruit concentration. [g] SD, significant differences between means of tested types of tissue. Bolds refer to the highest and lowest values.

tested (3.1- and 2.9-fold variation for peel and the whole fruit, respectively). Similar results were obtained by Tsao et al. (*5*); 2.6- and 3.4-fold variation between the apple peel and the flesh was recorded, respectively; eight cultivars were tested. Likewise, 11 organically and 11 integrated production growing cultivars showed great differences between the peel and the apple pulp different phenolic compounds content (*12*).

However, the range of the differences between the tested parts of fruit was very high and amounted from 39 (Jonamac) to 216% (Granny Smith). Eleven of 19 cultivars exhibited differences between the tested tissues higher than 100%. The concentration of flavonols and anthocyanins was considerably higher in apple peel as compared with the total phenolics. The content in both groups reached more then 300% of the whole fruit concentration, but again, it was highly dependent on the tested cultivar. The lowest whole fruit and peel flavonol differences, below 100%, were noted for Pinova, next Jonamac, Fiesta, and Elan. For the remaining examined cultivars, the increase of apple peel flavonols ranged from 139 (Idared) up to 844% (Granny Smith). The flavonol content ranged from 1.12 (Jonamac) to 5.95 (Granny Smith) for peel and from 0.37 (Pilot and Rubin) to 1.42 mg g^{-1} DW (Idared) in the whole apple fruit. Anthocyanins were predominantly localized in the red and dark-red apple peel. The richest sources of anthocyanins were Idared, next Gloster, Starking Delicious, and Monroe, irrespective of the tested part of fruit. Anthocyanins were not detected in Golden Delicious and Fuji apples, and only low levels were detected in Granny Smith. In the case of Fuji, the growing season in our climatic conditions is too short for it to develop red color of the skin. Overall, the color of its apple was fair pink with the ground color green to yellow. Surprisingly, Priscilla (red color of skin) showed trace amounts, below the medium concentration, and Elstar (red to orange color) displayed the anthocyanins content above the average value.

Enzyme Activities. The major differences between the enzyme activity of apple peel and the whole fruit were demonstrated for glutathione reductase (GR). As compared with the whole fruit, the 50% increase of GR activity was recorded in the apple peel (**Table 6**). This mean value was exceeded by most of the examined cultivars. However, it should be added, such big differences became apparent only in 2004. The major differences were obtained for Fiesta, where GR peel activity reached 235% of the whole one. The GR activity amounted from 13.6 (McIntosh and Prima) to 31.5 (Priscilla) and from 8.88 (Pinova) to 19.5 nkat g^{-1} DW (Fuji) for peel and the whole fruit, respectively. Contrary to all other tested parameters, the APX activity, except for Elan and Elstar, was considerably higher in the whole fruit as compared with the peel. The whole fruit APX activity reached ca. 139% of apple peel activity. Starking Delicious, followed by Fuji, Gloster, and Gala, was characterized by the highest differences between two tested types of tissues. The APX activity ranged from 221 to 909 and from 426 to 1234 nkat g^{-1} DW for apple peel and the whole fruit, respectively. Gala showed the lowest and Prima the highest APX activity, irrespective of tissue tested. As in the case of APX, the superior part of the examined cultivars was characterized by higher CAT activities in the whole fruit as compared to the peel; however, the increase was not so high (7%). The increase in the whole fruit as compared with the peel ranged from 0 (without any differences; Prima, Priscilla) to 40% (Starking Delicious). Gloster, followed by Rubin, Jonamac, and Pinova, demonstrated slightly higher CAT activity in the apple peel. The CAT activity ranged from 24.7 to 44.6 in the apple peel and from 25.2 to 46.5 nkat g^{-1} DW in the whole fruit. Regardless of the tested part of fruit, the lowest CAT activity was displayed by Pilot and the highest by Idared. Cultivar variations of the apple peel GR and CAT activities were close to the whole fruit, namely, 2.3- and 2.2-fold variation for peel and the whole fruit GR activity, respectively, and 1.8-fold variation for CAT, irrespective of tissue tested. For APX, the biggest differences appeared for peel (4.1-fold) and then the whole fruit (2.9-fold variation).

Effect of Growing Season. Significantly higher concentrations of thiol compounds, total phenolics, flavonols, as well as APX and CAT activities were recorded in the year 2005 (**Table**

Table 6. Antioxidative Enzyme Activities in Apple Peel and the Whole Fruit Depending on Cultivar and Type of Tissue (nkat g^{-1} DW; Average Value for 2004 and 2005)

	GR			APX			CAT		
cultivar	P[a]	WF[b]	P/WF[c]	P	WF	P/WF	P	WF	P/WF
Elan	18.1	12.8	1.41	590	475	1.24	32.1	33.2	1.03
Elstar	15.6	15.5	1.01	593	471	**1.26**	36.6	40.4	0.91
Fiesta	25.6	10.9	**2.35**	633	860	0.74	37.1	39.9	0.93
Fuji	26.4	**19.5**	1.35	285	652	0.44	33.6	40.7	0.83
Gala	14.6	15.6	**0.94**	**221**	**426**	0.52	29.7	34.1	0.87
Gloster	26.8	14.9	1.80	376	823	0.46	33.6	29.1	**1.15**
Golden Delicious	24.6	13.5	1.82	584	612	0.95	26.1	32.5	0.80
Granny Smith	28.3	15.2	1.86	601	809	0.74	33.7	34.6	0.97
Idared	28.1	17.5	1.61	714	1014	0.70	**44.6**	**46.5**	0.96
Jonamac	20.9	12.8	1.63	742	891	0.83	40.8	36.8	1.11
McIntosh	**13.6**	13.9	0.98	665	864	0.77	36.3	45.0	0.81
Monroe	14.7	8.98	1.63	635	838	0.76	35.3	41.7	0.85
Pilot	17.1	11.3	1.51	459	654	0.70	**24.7**	**25.2**	0.98
Pinova	16.5	**8.88**	1.85	526	795	0.66	29.2	27.6	1.06
Prima	**13.6**	12.5	1.09	**909**	**1234**	0.74	41.6	41.5	1.00
Priscilla	**31.5**	17.0	1.85	716	867	0.83	30.0	30.3	0.99
Red Rome	20.1	15.8	1.27	625	913	0.68	26.1	34.3	0.76
Rubin	18.3	10.6	1.73	774	1100	0.70	40.9	36.4	1.12
Starking Delicious	19.4	15.9	1.22	433	1078	**0.40**	29.2	40.9	**0.71**
average	20.7	13.8	1.50	583	809	0.72	33.80	36.3	0.93
difference at $\alpha = 0.05$	SD[d]			SD			SD		

[a] Peel. [b] Whole fruit. [c] P/WF, the content in apple peel divided by whole fruit concentration. [d] SD, significant differences between means of tested types of tissue. Bolds refer to the highest and lowest values.

Table 7. Growing Season Effect on Concentration of Tested Phytocompounds

	year		relationship
compound	2004	2005	(2005/2004)[a]
ASC (μmol g^{-1} of DW)	1.51 a[e]	1.34 b	0.89
CYS (μmol g^{-1} of DW)	0.029 b	0.048a	1.66
GSH (μmol g^{-1} of DW)	0.207 b	0.263 a	1.27
phenolics[b] (mg g^{-1} of DW)	3548 b	5185 a	1.46
flavonols[c] (mg g^{-1} of W)	1486 b	1754 a	1.18
anthocyanins[d] (mg g^{-1} of W)	552 a	508 b	0.92
GR (nkat g^{-1} of DW)	23.4 a	11.2 b	0.48
APX (nkat g^{-1} of DW)	655 b	737 a	1.13
CAT (nkat g^{-1} of DW)	31.5 b	38.6 a	1.23
% DW	19.3 a	19.4 a	1.01

[a] Years 2004/2005, the average content obtained in 2004 divided by 2005, which corresponds to an average value for 19 cultivars irrespective of tissue tested. [b] Expressed as equivalent of gallic acid. [c] Expressed as equivalent of quercetin. [d] Expressed as equivalent of cyanidin-3,5-di-glucoside. [e] Values marked with the same letters within lines did not differ significantly.

Table 8. Correlation Coefficient between Antioxidant Content in Apple Peel and the Whole Fruit on Fresh and Dry Weight Basis Depending on Vegetation Season

	2004[a]		2005[b]	
compound	FM	DW	FM	DW
CYS	0.669[c]	0.489[d]	0.56[e]	0.421[f]
GSH + GSSG	0.872[c]	0.694[c]	0.883[c]	0.893[c]
AA + DHAA	0.687[c]	0.651[d]	0.707[c]	0.736[c]
phenolics	0.295[f]	0.343[f]	0.717[c]	0.662[d]
flavonols	0.119[f]	0.154[f]	0.582[d]	0.614[d]
anthocyanins	0.678[c]	0.741[c]	0.618[d]	0.641[d]
GR	0.660[c]	0.646[d]	0.709[c]	0.747[c]
APX	0.222[f]	0.163[f]	0.582[d]	0.700[c]
CAT	0.600[d]	0.524[d]	0.755[c]	0.893[c]

[a] 23, number of cultivars taken into account for correlation analysis. [b] 19, number of cultivars taken into account for correlation analysis. [c] Significant at $\alpha = 0.001$. [d] Significant at $\alpha = 0.01$. [e] Significant at $\alpha = 0.05$. [f] NS, not significant.

7). Contrary to this GR, ASC and anthocyanins contents significantly increased in 2004, as compared with 2005. To the most degree, growing season influenced GR activity followed by CYS and global phenolics. Vegetation period did not affect dry weight content. In spite of the fact that the intensity of reaction to the environmental conditions highly depended on the examined cultivar, almost all cultivars characterized the same direction of changes (data not shown). Only the effect of the growing season on flavonol concentration was highly inconsistent.

Correlation between Apple Peel Antioxidant Concentration and the Whole Fruit. High (at $p = 0.01$) or very high (at $p = 0.001$) correlation coefficients existed between GSH, ASC, and anthocyanins concentrations as well as GR and CAT activities in apple peel and the whole fruit expressed either on fresh or dry matter basis, regardless of vegetation season (**Table 8**). In the case of global phenolics, flavonols and APX activity correlation depended on growing season. For these compounds, the correlation coefficients were statistically proven only in 2005.

Correlation between GR and Other Bioactive Constituents. There was a positive correlation between GR activity and ASC and GSH concentration as well as CAT activity, but only in the first of the tested years was observed (**Table 9**). Obviously, a high correlation coefficient existed between flavonols, anthocyanins, and total phenolics and between cysteine and GSH content irrespective of tissue type or growing season. The concentration of ASC was negatively correlated with particular subgroups as well as with global phenolic concentrations, but not at a significant level (an exception, ASC-anthocyanins in 2005). Similar to Davey and Coulemans (*21*), a weak correlation across the group of tested cultivars was found between GSH and ASC. Fruits richest in mean total glutathione tended to have higher ASC concentrations.

DISCUSSION

The antioxidant contents in apple vary considerably depending on the tissue being analyzed. Results obtained from the analysis

Table 9. Correlation Coefficient between Antioxidants, Depending on Part of Fruit and Growing Season on Dry Weight Basis

	2004[a]		2005[b]	
	P[c]	WF[d]	P	WF
GR–GSH	0.653[e]	0.490[f]	0.374[h]	0.258[h]
GR–ASC	0.583[f]	0.295[h]	–0.140[h]	–0.427[h]
GR–CAT	0.577[f]	0.460[f]	–0.444[g]	0.101[h]
GR–APX	0.232[h]	0.170[h]	–0.075[h]	0.080[h]
GSH–ASC	0.354[h]	0.355[h]	0.285[h]	0.009[h]
GSH–CYS	0.621[f]	0.831[e]	0.515[g]	0.514[g]
ASC–phenolics	0.050[h]	–0.072[h]	–0.257[h]	–0.181[h]
ASC–flavonols	0.168[h]	–0.096[h]	–0.179[h]	–0.188[h]
ASC–anthocyanins	–0.166[h]	–0.204[h]	–0.444[g]	–0.211[h]
phenolics–flavonols	0.852[e]	0.651[f]	0.852[e]	0.586[f]
phenolics–anthocyanins	0.491[f]	0.469[f]	0.733[e]	0.541[g]
APX–CAT	0.558[f]	0.226[h]	–0.075[h]	0.106[h]

[a] 23, number of cultivars taken into account for correlation analysis. [b] 19, number of cultivars taken into account for correlation analysis. [c] Peel. [d] Whole fruit. [e] Significant at $\alpha = 0.001$. [f] Significant at $\alpha = 0.01$. [g] Significant at $\alpha = 0.05$. [h] NS, not significant.

of 12 apple cultivars (*1*) showed that, on average, the concentrations of hydrophilic antioxidant ascorbate and glutathione levels in the epidermis were ca. 7- and 3-fold higher, respectively, than in the underlying mesocarp. Similar results were presented in the other research work, where peel contained approximately five and three times higher ascorbate and glutathione contents, while GR, APX, and CAT activities were 3-, 2-, and 2-fold higher, correspondingly (*2*). Moreover, authors found that, in general, ascorbate and glutathione levels displayed an exponential type decrease in concentration with distance in from fruit surface (*1*). Total polyphenolics determined either by HPLC or measured by the Folin−Ciocalteu method were approximately three times higher in the peel as compared to the flesh (*5*). Among the wide range of phenolics, only the content of hydrocinnamics (chlorogenic and *p*-coumarylquinic acids) was higher or comparable to the peel (*4*−*6*, *8*), but the distribution pattern may be markedly determined by genotype (*12*) or growing region (*7*). According to Wolfe et al. (*11*), the total phenolics contents of the peels were significantly higher than the flesh and flesh + peel within all varieties, while the two last types of tissue mentioned did not differ significantly. All of these results were expressed on fresh matter basis.

In the present work, as compared with the whole fruit, apple peel contained ca. three, two, and 1.4 times higher concentrations of ASC, phenolics (higher differences existed for particular subgroups of phenolics: flavonols and anthocyanins, for both constituents 3.4-fold variation), and GSH, respectively. The differences were lower, but results were expressed on dry matter of the tested apple tissue. Out of the examined antioxidative enzymes, only GR exhibited the enhanced by 50% (on the average) activity in apple peel as compared with the whole fruit, but it was strongly dependent on growing season. Such a distribution pattern exhibited all or almost all examined cultivars, and the genetic biodiversity in the extent of differences in the antioxidant area that was present is both cultivar- and tissue-dependent, which is what, I hope, makes a great opportunity for nutritional or resistance enhancement during breeding procedures. The range of differences between the examined cultivar apple peel and the whole fruit antioxidant contents was as follows: 0−1130, 42−844, 39−216, 37−420, 2−123, and 1−135% for anthocyanins, flavonols and total phenolics, ASC, GSH, and GR, respectively.

Concentrations of thiol compounds, ascorbate, anthocyanins, as well as GR and CAT activities in apple peel highly correlated with the whole fruit contents based on both fresh and dry matter regardless of growing season. In the case of global phenolics and flavonols as well as APX activity, the correlation coefficient depended on growing season. An explanation could be that the distribution pattern may be a result of a large number of variously localized compounds and probably individually influenced by environment (*23*). Both flavonols and anthocyanins are mainly localized in the skin, and it is difficult to explain insignificant correlation coefficients obtained for flavonols in 2004. Supplementary analysis of these compounds will be done using an HPLC technique to explain it more exhaustively. The APX activity was the most changeable constituent throughout cultivars and tissues tested as well as from year to year. Anyway, high correlation coefficients that were proven for almost all tested constituents in two vegetation seasons between the apple peel and the whole fruit are interesting from methodological and practical points of view. Looking through the literature, I have not encountered such clear information on this issue. First of all, it gives information that apple peel is a good indicator of the whole apple bioactive constituents and may be used in a wide range of experiments in relation to physiological roles of antioxidant and/or health value of apple fruit. Moreover, this information supports justification of the possibility to compare results made on different parts of fruit that is frequently present in literature.

On the basis of the concentrations results, it maybe concluded that ASC, GSH, phenolics compounds (mainly flavonols and anthocyanins), as well as GR activity are predominantly present and operate in the epidermic zone of apple fruits. Enzymes such as APX and CAT are present and act throughout the whole apple fruit, or even, their activity is higher in the apple flesh. It is not surprising, as the skin is an important barrier to protect fruit against unfavorable biotic and abiotic environmental factors (*24*−*27*), but according to the present survey, it is not an uniform statement in relation to all apple genotypes as well as compounds. Concluding, each cultivar should be perceived by its own internal chemical composition pattern individually influenced by external factors and examined also with regard to its agronomic characters (e.g., disease or unfavorable environmental factors resistance).

Taking into account the GSH−ASC system, it seems that high or sometimes low content, but relatively stable throughout the years (Granny Smith, Idared, Gloster, Pilot, Fiesta, Rubin, Golden Delicious, or Starking Delicious cultivars) in ASC and/or GSH concentrations, is linked with the increasing possibility of GSH precursor (CYS) synthesis or increasing GR activity. Changes in the content of CYS or especially in GR activity in the cultivars mentioned above were very high. It also explained the higher year effect on these compounds as compared to GSH. GSH synthesis is controlled mainly by γ-glutamyl-cysteine synthetase activity and CYS availability (*28*). Besides, accumulation of CYS through the whole apple fruit suggests that there is higher intercellur transport of this compound than GSH and ascorbate. The latter are highly localized in the skin. A high GSH concentration in cells is maintained by GR, whereas dehydroascorbic acid is back to its reduced form predominantly by the cytosolic ASC−GSH pathway, including APX and GR as well as other antioxidative enzymes (*29*). However, it should be emphasized that the high correlation coefficient between these compounds strongly depended on growing season. Accepting my previous hypothesis about GR as an environmental stress factor (*14*), an additional argument can be incorporated to this, namely, that a highly significant positive correlation between GR activity and GSH, ASC concentrations as well as CAT

activity, existed only in 2004, both tested in apple peel or in the whole fruit. That year, GR, ASC, and anthocyanins were significantly higher, as compared to 2005. Therefore, it seemed that the enhanced hydrogen peroxide removal as well as other free radicals was needed. What is more, in 2005, most of these positive correlations become negative. Hence, the hypothesis is that GR may be considered as a stress signaling and transducing molecule in the apple peel for further study. Additionally, on average, the total pool of GSH was significantly lower in the 2004 as compared to the 2005 growing season. Weak correlations between GSH and ASC peel content appeared across both growing seasons. Also, according to Davey and Coulemans (*21*), cultivars with a higher ASC content tend to have a higher GSH concentration. The GSH system as a stress marker was reviewed by Tausz et al. (*25*) but with the more pronounced role of GSH, its redox state as well as multiple roles in plant metabolism (*30*). It seems that GR plays a key role in the overcompensation by increased regeneration of GSH together with its enhanced synthesis (increasing CYS content). Within the space of 2003−2005, when my genetic resources with regard to antioxidant properties of *Malus* were investigated, it seemed that 2003 and 2005 were similar using the GSH system as a stress marker. Nearly the same GR activity and ASC concentration in apple peel, on a fresh matter basis (data not shown), in these years were noted. Taking into account that together with GR activity only ASC and anthocyanins concentrations significantly increased in 2004, it may be temperature and/or light intensity or other aspects of the plant and soil environment (*31*, *32*). Unfortunately, in spite of the increase of ascorbate and anthocyanins that year, there was no linear relationship between ascorbate and anthocyanins, and what is more, the correlation coefficient was negative for both the peel and the whole fruit concentrations. Such a protective effect of phenolics (in particular anthocyanins) was previously suggested (*1*). It is presently unclear which weather or environmental factor or their period time of the vegetation season is critical. For the scientific statement, more detailed analysis of the weather and other environmental conditions should be done.

It seems interesting from the antioxidant potential point of view that Idared and Granny Smith were the richest in almost all tested constituents. Pilot and Fiesta were the richest and to the least degree influenced by growing season in their ASC and GSH contents, and it agrees with my previous results, especially in the case of Pilot (*14*). Besides, Granny Smith and Idared as well as McIntosh and Starking Delicious comprised the best source of phenolics.

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