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Great Differences in Antioxidant Properties Exist between 56 Apple Cultivars and Vegetation Seasons

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The contents of ascorbate, thiols, and phenolic compounds and antioxidative enzyme activity were measured in the apple peel of 56 genotypes after harvest in two vegetation seasons, 2003 and 2004. The main reason of great interest in these bioactive compounds is their well-established physiological role in all living systems. The biggest differences between tested genotypes were noted for ascorbate peroxidase and glutathione reductase (GR) activity, followed by total ascorbate, phenolics, and glutathione concentration; the least difference was observed in the case of catalase. A large cultivar variation was noted in the anthocyanins and flavonols contents. Distinguishing the cultivars with the lowest, highest, relatively stable or those in which antioxidant content greatly differed depending on growing seasons was attempted. The GR activity is proposed as an environmental stress marker of apple fruit.

KEYWORDS: ***Malus domestica*; Rosaceae; apple; phenolics; glutathione; ascorbate; antioxidative enzyme activity**

INTRODUCTION

The presence of active oxygen species (AOS) is an inseparable feature of life in an oxygen atmosphere, and as consequence plants have evolved antioxidant defense system to keep AOS under control. The level of AOS in plant tissues is regulated, among others, by the capacity of scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR), and the lipid-soluble (e.g., α-tocopherol, carotenoids) and water-soluble (ascorbate, glutathione, flavonoids) antioxidants (*1*). Those compounds are involved in plant response to biotic and abiotic stress factors such as temperature extremes, ozone exposure or UV radiation, water status, or salinity, which has been reported in many scientific papers. Similar to growing plants, tolerance to oxidative stress of pre- and postharvest fruits and vegetables was associated with such factors as water- and lipid-soluble antioxidant capacities. As fresh fruits experience many types of stress during growth and development, processing, storage, and transport, increasing research is being generated on how bioactive compounds are associated with pre- and postharvest fruit quality and marketability. External as well as internal factors (such as genotype) or both can affect the antioxidant content and enzyme activity (*2*). It is expected that crop cultivars with higher antioxidant potential will have better stress resistance, nutritional quality, yield, and storage characteristics. In general, stress-tolerant plants or plant parts may initially contain or can generate more antioxidants during unfavorable environmental conditions and/or produce fewer AOS than more sensitive species or cultivars. Oxidative stress is also involved in fruit ripening and senescence. In apple, the early stages of fruit ripening are crucial to the storage behavior of the fruit and affect its postharvest life (*3*). Hence, we decided to test the majority of our *Malus* genetic resources with regard to the content of the main antioxidant compounds in apple fruit, and an attempt was made to find the connection between antioxidant concentration and other apple quality traits.

A major class of phytochemicals found in apples comprises a variety of phenolic compounds, especially flavonoids and ascorbate as well as glutathione (*4*−*9*). The concentration and composition of phytochemicals in apples vary greatly between the apple peel and the apple flesh (*6*, *10*−*13*). Depending on variety, the apple peel contained from 2 to 6 times more phenolic compounds and from 2 to 3 times more flavonoids (*14*). The predominantly found phenolic compounds in the apple peel included phenolic acids (hydroxycinnamic acid derivatives such as chlorogenic acid or caffeic acid) and the flavonoids. The flavonoids are a large class of compounds, generally occurring as glycosides. Glycosylation renders them less reactive toward AOS and more water soluble (*15*). The flavonoids included several families: monomeric and oligomeric flavanols (catechin, epicatechin, and procyanidins), flavonols (quercetin conjugates), and flavones (rutin), in addition to phloridzin and phloretin glycosides known as chalcones (*4*, *6*). The red coloration of the skin is mainly due to the anthocyanidin cyanidin-3-galactoside. Various phenolics showed different antioxidant activities. The highest contribution to the total antioxidant activity among the major apple phenolics exhibited quercetin, followed by epicatechin and procyanidin B2 (*7*).

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Table 1. Harvest Date of Analyzed Cultivars in the Years 2003–2004

cultivar	year 2003	year 2004	cultivar	year 2003	year 2004	cultivar	year 2003	year 2004
Alwa	Oct 23	Oct 21	Jester	Sept 9	Sept 13	Redgold	Oct 3	Oct14
Arlet	Sept 19	Sept 28	Jonamac	Sept 26	Oct 1	Rosana	Sept 24	Sept 23
Auralia	Oct 8	Oct 21	King of the Pippins	Sept 26	Oct 1	Rubin	Sept 26	Sept 23
Britemac	Sept 9	Sept 13	Liberty	Sept 26	Oct 13	Rubinette	Oct 8	Oct 13
Cortland	Oct 4	Oct 1	Ligol	Sept 26	Oct 14	Ruby	Oct 23	Oct 28
Cox's Orange Pippin	Oct 7	Oct 1	Ligolina	Oct 7	Oct 14	Šampion	Sept 19	Sept 24
Egeria	Sept 16	Sept 17	McIntosh	Sept 16	Sept 23	Sawa	Sept 12	Sept 17
Elstar	Sept 19	Sept 23	Melodie	Oct 4	Oct 4	Selena	Sept 16	Sept 23
Fiesta	Sept 19	Oct 13	Melrose	Oct 23	Oct 21	Sława Pobieditieliam	Aug 28	Sept 6
Freedom	Sept 22	Oct 6	Monroe	Sept 19	Oct 6	Spartan	Sept 26	Sept28
Fuji	Oct 23	Oct 28	Mutsu	Oct 23	Oct 21	Spencer	Oct 23	Oct 21
Gala	Sept 16	Sept 28	Nova Easygro	Sept 26	Oct 1	Topaz	Oct 4	Oct 13
Gloster	Oct 8	Oct 21	Odra	Oct 7	Oct 13	U 1065	Oct 1	Oct 6
Golden Delicious	Oct 8	Oct 21	Pilot	Oct 4	Oct 14	U 1165	Oct 1	Oct 13
Gorjaczkowski Seedling	Oct 23	Oct 21	Pinova	Oct 4	Oct 14	U 633	Sept 16	Sept 28
			Priam	Sept 16	Sept 23	Undine	Oct 7	Oct 6
Granny Smith	Oct 23	Oct 28	Prima	Sept 9	Oct 1	Vanda	Sept 24	Oct 4
Haralson	Oct 4	Oct 14	Priscilla	Oct 4	Oct 14	Wealthy	Sept 1	Oct 1
Honeygold	Sept 16	Oct 21	Rajka	Oct 4	Oct 4	Witos	Sept 9	Sept 13

Phenolics play a crucial role in determining the sensory and nutritional quality of fresh apple, act as a substrate for browning enzymes, possess almost ideal chemical structures as antioxidants, free radical scavengers, and metal chelators, and are more powerful in comparison to vitamin C. They are implicated in pathogen resistance or some physiological disorders. Anthocyanins are also involved in the protection of fruit against UV and excessive sun irradiation (*15*−*18*).

Ascorbate and glutathione are among major hydrophilic, low molecular weight antioxidants present in plant tissues. The particular relationship between L-ascorbic acid (AA) and reduced glutathione (GSH) is present during hydrogen peroxide removal known as the Halliwel−Asada cycle, where GSH is used as reducing power for the enzymatic regeneration of dehydroascorbic acid (DHAA) (*1*, *19*). The capacity of this cycle is dependent on the concentration of the antioxidant and the related enzyme activities, and in an acclimation reaction the increase of these elements was frequently observed. Progressive oxidation and degradation of the ascorbate and glutathione concentrations are inseparable elements of the inability of AOS removal and, eventually, senescence and cell death (*20*).

According to Davey et al. (*13*), the AA and GSH levels in the exocarp (epidermal) tissue were 6.7- and 2.8-fold higher, respectively, than those in the underlying mesocarp (on the basis of a mean value for 12 cultivars). According to our unpublished results made on 25 cultivars, on average, the glutathione reductase (GR), catalase (CAT), ascorbate, cysteine, γ-glutamylcysteine, glutathione, and phenolics were approximately 2.9, 1.5, 4.4, 1.7, 2.1, 2.1, and 2.5 times higher in the apple peel as compared to the whole apple fruit. Thus, we decided to test only apple peel to obtain clearer differences among tested cultivars.

The aim of this research was to evaluate the concentration of the total pool of ascorbate and glutathione (the sum of reduced and oxidized forms), some subgroups of phenolic compounds (flavonols, anthocyanins, and total), and some antioxidative enzyme activities [GR, ascorbate peroxidase (APX), CAT] in the peel of 56 genotypes of apple throughout the 2003−2004 vegetation seasons. Monitoring of such a great number of cultivars during two years allowed, on the one hand, the extent to which the measured parameters are influenced by the environment to be noted and, on the other hand, the cultivars with more "stable" and more "variable" elements of antioxidant apparatus to be marked.

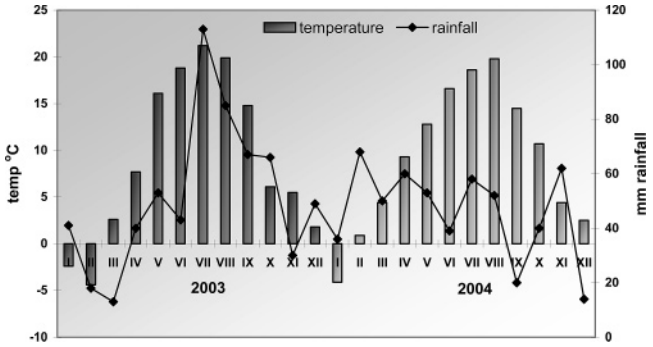

Figure 1. Mean month temperature and rainfall for the field of the Warsaw-Wilanow Station, where apples were grown in the 2003 and 2004 seasons.

MATERIALS AND METHODS

Reagents and Apparatus. GSH, γ-glutamylcysteine (γ-GC), and AA, were from Sigma. l-Cysteine (CYS) was purchased from Fluka. Monobromobimane (thiolyte) was from Calbiochem and cyanidin-3,5-diglucoside chloride from Chromadex. All other chemicals were of analytical or HPLC grade purity. The HPLC was from Waters Co., System Breeze with a binary solvent delivery system (1525), degasser, autosampler with thermostat with the scale 4−40 °C (M 717 PLUS), scanning fluorescence detector (M 474), two-channel UV−vis detector (M 2487), and the thermostat for the column 5−85 °C (Peltier).

Plant Material and Meteorological Information. The research was performed on apples 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 orchard is located in the postglacial valley of the Vistula River, 52° N and 21° E. The soil was maintained as herbicide strips with rows of trees and sward between them. The orchard received standard horticultural practices. Apples were harvested successively as they ripened from August 28 to October 23 and from September 6 to October 28 in 2003 and 2004, respectively (**Table 1**). The research included apples of 53 cultivars and 3 clones, U 633, U 1165, and U 1065 (for simplicity in this paper also called cultivars).

The Experimental Orchard in Warsaw-Wilanow, where research was conducted, possesses a meteorological station. Mean temperatures and rainfall in 2003 and 2004 seasons are shown in **Figure 1**. The meteorological data indicate the great differences between the tested seasons. The vegetation period in 2004, as compared to 2003, was rather

cold and dry. Hence, great differences in harvest dates for particular cultivars in examined years were noted (**Table 1**). Compared to multiannual average (data not shown), the vegetation period in 2003 was considered to be hot and that in 2004 to be dry.

Sample Preparation. Apples were peeled with a potato knife, as a thin layer of apple flesh remained adhered to the peel [contamination by apple flesh on a fresh matter (fm) basis was ~30%]; the peel sample should be considered to be the epidermic zone of the apple fruit. Samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Directly before analysis apple tissues were ground to a fine powder in liquid nitrogen. Chemical analyses were made in three replicates for each cultivar; each of them included apple peel from two fruits.

Extraction and Determination of Enzymatic Activities. Ground tissues were suspended in 100 mM potassium phosphate 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. Activity analyses of APX, GR, and CAT were carried out in a total volume of 1 mL.

The activity of APX was measured by monitoring the decrease in absorbance at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}). The assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 8 mM ascorbate, 20 mM H_2O_2, and enzyme extract (*21*). GR activity was monitored at 340 nm (extinction coefficient of 6.2 mM^{-1} cm^{-1}) in the mixture containing 500 mM HEPES (pH 8.0), 5 mM EDTA, 1.25 mM NADPH, 5 mM oxidized glutathione (GSSG), and enzyme extract (*19*). CAT activity was calculated from the fall in absorbance at 240 nm (extinction coefficient of 39.4 mM^{-1} cm^{-1}) in the supernatant containing 50 mM potassium phosphate buffer (pH 7), 10 mM H_2O_2, and the enzyme extract (*22*). Blank rates in the absence of extract were determined for each test system and subtracted during calculation. For each analysis the reaction was initiated by adding the enzyme extract.

Measurements of Low Molecular Weight Thiols 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. Total glutathione (GSH + GSSG) concentration was determined in the supernatant after reduction with dl-dithiothreitol (DTT) 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. Thiol derivatives were separated on a Symmetry C_{18} column (250 mm × 4,6 mm, 5 μm, Waters) by applying a solution of 10% methanol containing 0.25% (v/v) glacial acetic acid (solvent A, pH 4.3) and 90% methanol with the same acetic acid concentration (solvent B, pH 3.9); the flow rate was 1 mL min^{-1} (*23*).

Total ascorbate (sum of the ascorbate and dehydroascorbate) was measured after complete oxidation of ascorbate to dehydroascorbate with ascorbate oxidase (*24*). Dehydroascorbate is derivatized with *o*-phenyldiamine, and the reaction product was detected as a fluorescent compound (350/450 nm). The flow rate was 1 mL min^{-1}, the eluent 20% MeOH + 800 mM K_2HPO_4 (pH 7.8), and the column the same as for glutathione. The results were calculated with a standard curve.

Quantification of Phenolics. For the estimation of some subgroups of 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. The method consisted of placing 250 μL of sample or standard in a test tube and adding the same volume of 0.1% HCl in 95% ethanol and 4500 μL of 2% HCl. The absorbance of the solution was then read at 280, 360, and 520 nm to measure total phenolics, flavonols, and anthocyanins, respectively. Gallic acid, quercetin, and cyanidin-3,5-diglucoside chloride were standards used for total phenolics, flavonols, and anthocyanins, respectively. Standards were prepared in 96% ethanol except for gallic acid, which was made in 10% ethanol (*25*).

Statistical Analysis. Because the performed two-way analysis of variance exhibited significant differences between tested years for almost all tested parameters, the presented results were elaborated by a one-way factorial ANOVA of Statgraphics Plus 4.1. separately for each tested year. The significance of differences between cultivar means was evaluated using the Newman−Keuls test; a 5% probability level was used to indicate the significance of differences.

RESULTS

Ascorbate. The average concentration of apple peel ascorbate (AA + DHAA) ranged from 926.6 (cv. McIntosh) to 5202.2 (cv. Rubin) nmol g^{-1} of fm and from 1299.8 (cv. Britemac) to 5537.0 (cv. Fiesta) nmol g^{-1} of fm in 2003 and 2004, respectively (**Table 2**). Taking into consideration the 10 cultivars with the lowest and the 10 cultivars with the highest concentrations of ascorbate (denoted hereafter as groups of high and low given compound contents) in both years, four and five occurred in 2003 and 2004, respectively. Namely, cvs. McIntosh, Britemac, Alwa, and Fuji were in the group with the smallest amounts of ascorbate, as opposed to cvs. Rajka, Topaz, Pilot, Šampion, and Melodie, which exhibited a high concentration in both seasons. The amount of ascorbate in 2004 was considerably higher than that in 2003. In both vegetation seasons cultivars in which the content of ascorbate exceeded the mean value were nearly the same and amounted to approximately 41% (2003) and 43% (2004). The increase of ascorbate content above 50% in 2004 in comparison to the former one appeared in 13 cultivars, whereas there were only 2 cultivars with such increasing concentration of ascorbate in 2003. The highest rises of ascorbate content in 2004 were exhibited by cvs. Melrose, Granny Smith, Fiesta, Jester, McIntosh, and Gorjaczkowski Seedling, namely, 223, 121, 91, 82, 80, and 72%, respectively. Of those cultivars McIntosh and Melrose had the lowest concentration of ascorbate in 2003. The differences, not exceeding 10%, between cultivars in both tested years showed ~29% of analyzed genotypes. The lowest differences were noted for cv. Rubinette (0.5%) followed by cvs. King of the Pippins, Redgold, and Witos (1%), Freedom (2%), Ligol, Pilot, Prima, Ruby, and Selena (4%), Britemac and Šampion (5%), Auralia and Priscilla (8%), and Sawa and Undine (9%). In the above-mentioned group the majority contained between low and medium concentrations of ascorbate, but three cultivars were characterized by high amounts of ascorbate, namely, Šampion, Pilot, and Ligol, on average 4646.0, 4162.9, and 3580.6 nmol g^{-1} of fm, respectively.

Low Molecular Weight Thiols. The concentrations of cysteine and glutathione (GSH + GSSG) in both tested years were nearly the same, on average, 6.40 and 56.1 nmol g^{-1} of fm, respectively (**Table 2**). The concentration of cysteine ranged from 2.67 (U 1065) to 10.40 (Melodie) and from 1.27 (U 633) to 10.07 nmol g^{-1} of fm (Pilot) in 2003 and 2004, respectively. The content of γ-GC after harvest was rather low; the same mean value for both years was recorded, namely, 0.3 nmol g^{-1} of fm (data not shown). Glutathione content amounted to from 32.1 (Pinova, 2003) and 29.3 (U 633, 2004) to 104.8 (2003) and 109.9 (2004) nmol g^{-1} of fm for Gorjaczkowski Seedling. The lowest concentration of glutathione was recorded for the U 633, McIntosh, and Sława Pobieditelіam, and the highest in both tested years was found for Gorjaczkowski Seedling followed by Granny Smith, Cox's Orange Pippin, Melodie, and Pilot, on average. The last four specified cultivars were simultaneously characterized by the highest cysteine content. The lowest concentration of cysteine in both tested years (below 5 nmol g^{-1} of fm) was found for U 633, followed by U 1065, McIntosh, Spencer, and Spartan; in general, genotypes with a high content of glutathione (>60 nmol g^{-1} of fm) were also rich in cysteine (over the average value).

Compared to ascorbate there were no obvious differences exceeding 70% between tested cultivars in consecutive years

Table 2. Concentrations of Ascorbate, L-Cysteine, and Glutathione Depending on Cultivar and Harvest Year[a]

	ascorbate (AA + DHAA) (nmol g^{-1} of fm)		L-cysteine (nmol g^{-1} of fm)		glutathione (GSH + GSSG) (nmol g^{-1} of fm)	
cultivar	2003	2004	2003	2004	2003	2004
Alwa	1421.8a–c	1832.8a–c	7.60b–h	6.17b–h	85.9h–k	59.8d–l
Arlet	2673.3a–i	4255.5i–o	7.47b–h	6.00b–h	53.5a–g	41.8a–f
Auralia	2823.6b–i	2622.1a–h	7.53b–h	8.43d–j	50.6a–g	78.8k–m
Britemac	1366.1a–c	**1299.8**a	6.23a–g	5.77b–h	55.2a–h	47.0a–h
Cortland	1823.9a–g	2519.1a–g	9.07f–h	9.03g–j	73.4e–j	63.4e–m
Cox's Orange Pippin	4492.5j–l	2921.7a–j	8.47c–h	8.53e–j	87.2i–k	76.8i–m
Egeria	1693.8a–f	2114.0a–e	6.90b–h	5.53b–h	46.0a–g	46.1a–h
Elstar	4255.0i–l	2564.1a–g	7.57b–h	5.57b–h	48.3a–g	32.5a–c
Fiesta	2905.4b–i	**5537.0**o	7.17b–h	9.10g–j	69.5c–j	65.4f–m
Freedom	2795.8b–i	2734.3a–i	5.63a–g	5.67b–h	49.2a–g	41.5a–f
Fuji	1401.4a–c	2045.6a–e	4.70a–d	5.13b–f	56.2a–i	58.4c–l
Gala	1292.2a–c	2158.1a–e	6.53b–g	7.00b–j	53.2a–g	41.6a–f
Gloster	1638.6a–e	2015.0a–e	5.13a–f	8.57e–j	43.7a–f	64.4f–m
Golden Delicious	3302.1d–j	4377.7j–o	5.03a–f	8.30c–j	35.5a–c	55.3b–k
Gorjaczkowski Seedling	1582.6a–e	2724.7a–i	4.90a–e	8.37d–j	**104.8**k	**109.9**n
Granny Smith	1850.9a–g	4092.7g–n	7.50b–h	9.23h–j	79.4g–j	84.7m
Haralson	2250.6a–g	3545.1c–m	5.57a–f	4.93b–e	68.3c–j	63.4e–m
Honeygold	3268.6d–j	2265.8a–f	4.87a–e	6.53b–j	47.7a–g	50.3a–h
Jester	1839.2a–g	3344.1b–m	5.07a–f	5.83b–h	46.5a–g	49.5a–h
Jonamac	2036.6a–g	1808.9ab	7.07b–h	6.57b–j	70.3d–j	59.8d–l
King of the Pippins	3003.1c–i	2972.0a–k	6.70b–h	8.97f–j	37.2a–d	61.9e–m
Liberty	1486.1a–c	2137.5a–e	8.00c–h	8.10b–j	55.2a–h	46.3a–h
Ligol	3513.6g–k	3647.5e–m	7.83c–h	6.77b–j	65.8a–j	45.8a–h
Ligolina	1984.5a–g	3187.5b–m	7.73c–h	6.30b–i	46.9a–g	42.3a–f
McIntosh	**926.6**a	1666.3ab	4.43a–c	4.87b–e	42.6a–f	37.1a–d
Melodie	3955.0h–l	5398.8no	**10.40**h	8.53e–j	76.3f–j	82.6lm
Melrose	1132.4ab	3652.6e–m	5.13a–f	5.40b–h	54.3a–h	70.0g–m
Monroe	2428.3a–h	2807.4a–j	7.30b–h	5.80b–h	68.7c–j	41.8a–f
Mutsu	2438.0a–h	3879.6f–m	6.23a–g	6.80b–j	66.3b–j	54.5a–k
Nova Easygro	1417.3a–c	2091.6a–e	3.60ab	7.20b–j	52.9a–g	77.0j–m
Odra	2829.5b–i	3219.1b–m	5.20a–f	4.93b–e	42.0a–f	51.8a–i
Pilot	4242.7i–l	4083.0g–n	8.70d–h	**10.07**j	72.3e–j	82.0lm
Pinova	2427.8a–h	3192.8b–m	5.37a–f	6.63b–j	**32.1**a	42.5a–f
Priam	1890.1a–g	2415.8a–f	8.90e–h	5.47b–h	61.5a–j	43.7a–f
Prima	1861.2a–g	1927.6a–d	6.83b–h	5.10b–f	42.6a–f	35.1a–d
Priscilla	2140.1a–g	2310.5a–f	9.53gh	8.43d–j	60.9a–j	58.7d–l
Rajka	3455.8g–k	4184.3h–n	8.20c–h	5.93b–h	73.6e–j	61.8e–m
Redgold	2009.8a–g	1990.1a–e	6.57b–g	4.90b–e	62.3a–j	54.2a–k
Rosana	1909.4a–g	2634.7a–h	6.20a–g	4.60b–d	46.9a–g	44.2a–g
Rubin	**5202.2**l	3852.0f–m	6.27a–g	5.50b–h	46.9a–g	42.6a–f
Rubinette	2921.2b–i	2907.0a–j	5.97a–g	8.83f–j	52.7a–g	81.9lm
Ruby	1992.7a–g	2063.2a–e	5.07a–f	5.73b–h	89.5jk	58.1c–l
Šampion	4769.4kl	4522.6k–o	7.10b–h	4.60b–d	63.0a–j	66.1f–m
Sawa	2011.0a–g	2200.7a–e	7.10b–h	4.53b–d	46.3a–g	31.2ab
Selena	2194.3a–g	2105.0a–e	6.10a–g	9.90ij	44.9a–f	70.1h–m
Sława Pobieditieliam	1526.9a–d	2174.0a–e	5.33a–f	6.20b–h	41.3a–e	41.3a–f
Spartan	1613.7a–e	1343.4a	4.97a–e	4.46bc	68.8c–j	44.3a–g
Spencer	1567.6a–e	2541.6a–g	4.47a–c	4.93b–e	56.1a–i	41.9a–f
Topaz	3402.9f–k	4596.9l–o	5.67a–g	5.33b–g	50.8a–g	64.9f–m
U 1065	1595.9a–e	2130.2a–e	**2.67**a	4.27b	36.1a–d	52.9a–j
U 1165	2917.7b–i	3598.0d–m	6.70b–h	4.37b	71.8e–j	63.7f–m
U 633	1856.5a–g	2461.5a–g	4.47a–c	**1.27**a	33.9ab	**29.3**a
Undine	3326.7e–j	3043.3b–l	5.07a–f	5.17b–f	37.5a–d	34.6a–d
Vanda	2521.7a–h	3159.0b–m	7.50b–h	6.70b–j	55.0a–h	63.1e–m
Wealthy	2920.0b–i	4683.2m–o	5.07a–f	5.67b–h	40.1a–e	42.5a–f
Witos	1862.8a–g	1879.4a–c	5.03a–f	6.63b–j	50.8a–g	57.9c–l
av	2428.0	2918.5	6.40	6.40	56.6	55.5

[a] Means followed by the same letters do not differ significantly. Bold type indicates the highest and lowest values

in the case of glutathione and only a few examples for cysteine (Gorjaczkowski Seedling, Nova Easygro, and U 633). However, the small tendencies of the increase of this thiol compound could be observed in 2004. As a consequence, ~46% of the cultivars tested that year had above average contents of glutathione as compared to only 41% in the former year.

There were 16 cultivars in which differences between seasons in the content of glutathione did not exceed 10%: Egeria and Sława Pobieditieliam (0%); Fuji and Priscilla (4%); Gorjaczkowski Seedling and Honeygold (5%); Fiesta, Jester, Rosana, and Wealthy (6%); Granny Smith (7%); Haralson, Melodie, and Undine (8%); and Rubin (10%). Of this group Melodie, Granny Smith, and Gorjaczkowski Seedling were among the richest in glutathione in both 2003 and 2004.

Phenolic Compounds. The average concentration of apple peel phenolics ranged from 777.8 (Vanda) to 1842.9 (Haralson) μg g^{-1} of fm and from 476.2 (Rubin) to 2349.5 (Haralson) μg g^{-1} of fm in 2003 and 2004, respectively (**Table 3**). On average, a considerably higher phenolics content was noted in 2003. Among 10 cultivars with the lowest and 10 cultivars with the

Table 3. Concentrations of Some Subgroups of Phenolics Depending on Cultivar and Harvest Year[a]

	phenolics[b] (μg g^{-1} of fm)		flavonols[c] (μg g^{-1} of fm)		anthocyanins[d] (μg g^{-1} of fm)	
cultivar	2003	2004	2003	2004	2003	2004
Alwa	1581.5f–m	1049.4a–d	222.3ab	314.0a–c	256.3l–u	183.8b–i
Arlet	1458.4c–m	1270.7b–g	667.4g–k	694.9b–g	195.0e–p	155.4a–h
Auralia	1019.7a–i	672.1ab	348.0a–g	340.5a–d	92.9a–g	nd[e] a
Britemac	1549.7e–m	964.6a–d	350.1a–g	325.1a–c	100.4a–h	100.4a–h
Cortland	1408.8b–m	878.7a–d	404.1a–h	296.5a–c	183.0e–p	229.8g–j
Cox's Orange Pippin	1512.8e–m	1120.0a–d	699.0h–k	599.1a–g	71.6a–e	144.7a–h
Egeria	1265.1a–m	1317.8b–g	371.1a–g	698.4b–g	263.5m–u	144.4a–h
Elstar	927.8a–e	1055.0a–d	262.3a–c	492.8a–f	114.5a–j	165.7a–i
Fiesta	880.4a–d	826.0a–d	256.6a–c	398.1a–e	149.3b–m	150.0a–h
Freedom	1207.1a–l	1949.1hi	462.4a–k	976.7g	129.0a–k	235.4g–j
Fuji	1630.2h–m	1029.5ad	765.1k	398.7a–e	94.9a–g	148.8a–h
Gala	875.0a–d	944.1a–d	177.2a	362.7a–d	37.5a–c	138.1a–h
Gloster	1503.6e–m	1243.9a–f	513.1b–k	789.4d–g	340.9t–w	458.3k
Golden Delicious	1130.9a–k	962.7a–d	293.0a–d	349.2a–d	41.5a–d	nd a
Gorjaczkowski Seedling	1465.3c–m	1194.0a–f	628.9e–k	674.3a–g	248.6k–u	141.2a–h
Granny Smith	1697.1k–m	1843.5f–i	530.1b–k	**1525.1**h	36.4a–c	58.1a–e
Haralson	**1842.9**m	**2349.5**i	614.5d–k	976.2g	168.3e–o	234.8g–j
Honeygold	961.7a–f	1028.2a–d	289.0a–d	484.3a–f	**9.9**a	nd a
Jester	983.9a–g	1242.9a–f	283.3a–d	629.5a–g	27.9ab	58.4a–e
Jonamac	1132.8a–k	799.3a–d	487.2a–k	358.6a–d	379.9w	224.1e–j
King of the Pippins	1666.6k–m	1910.0g–i	597.3d–k	727.5c–g	176.1e–p	56.6a–e
Liberty	1662.9j–m	2286.6i	744.7jk	655.5a–g	**516.7**y	501.5k
Ligol	812.9ab	997.5a–d	302.0a–d	524.6a–f	112.3a–j	168.3a–i
Ligolina	1034.7a–i	707.2ac	287.9a–d	289.8a–c	106.0a–i	51.9a–d
McIntosh	1489.9d–m	1073.9a–d	566.6c–k	630.5a–g	183.8e–p	136.4a–h
Melodie	1622.2h–m	939.0a–d	575.6c–k	431.8a–f	345.2t–w	137.4a–h
Melrose	1510.4e–m	1486.2c–h	510.8b–k	503.4a–f	281.2n–w	344.5j
Monroe	1543.9e–m	962.8a–d	**773.5**k	553.0a–f	335.8s–w	272.8h–j
Mutsu	1438.9c–m	774.0a–d	466.5a–k	**215.6**a	30.6ab	nd a
Nova Easygro	1524.4e–m	1050.3a–d	429.7a–i	359.3a–d	165.2d–n	63.0a–g
Odra	1384.7a–m	1798.6e–i	508.4b–k	668.2a–g	357.1uw	**560.2**k
Pilot	1024.2a–i	743.7a–d	255.8a–c	301.9a–c	77.0a–f	37.4a–c
Pinova	1024.5a–i	761.1a–d	319.8a–e	379.8a–d	118.8a–j	37.6a–c
Priam	1305.4a–m	957.6a–d	334.4a–f	343.9a–d	194.7e–p	186.0b–i
Prima	1164.1a–l	1204.3a–f	444.6a–j	340.7a–d	232.4i–t	267.5h–j
Priscilla	1755.1lm	798.4a–d	656.8f–k	325.8a–c	295.1p–w	113.9a–h
Rajka	999.4a–h	710.9a–c	554.5c–k	391.9a–e	167.5e–o	169.0a–i
Redgold	1508.6e–m	1094.6a–d	603.2d–k	516.5a–f	317.8r–w	310.8ij
Rosana	1151.8a–l	1314.7b–g	475.8a–k	606.9a–g	193.0e–p	197.5c–j
Rubin	1026.7a–i	**476.2**a	355.7a–g	257.3ab	226.2h–s	111.1a–h
Rubinette	1091.2a–k	984.9a–d	350.5a–g	562.6a–f	122.0a–j	56.1a–e
Ruby	1688.9k–m	929.7a–d	379.6a–g	225.3a	160.7c–n	**22.5**ab
Šampion	1001.6a–h	1149.6a–e	438.1a–j	404.9a–e	117.2a–j	114.8a–h
Sawa	1446.1c–m	1354.2b–g	552.0b–k	529.6a–f	286.9o–w	272.0h–j
Selena	1375.8a–m	1280.9b–g	594.3d–k	632.8a–g	212.2g–r	118.6a–h
Sława Pobieditieliam	1611.3g–m	950.9a–d	399.2a–h	362.2a–d	139.5b–l	90.5a–g
Spartan	1266.8a–m	1113.6a–d	506.4b–k	446.2a–f	236.0j–t	317.5ij
Spencer	1381.9a–m	731.9a–c	522.8b–k	444.7a–f	149.0b–m	nd a
Topaz	919.7a–e	1040.5a–d	301.0a–e	395.4a–e	108.9a–j	174.0b–i
U 1065	1030.0a–i	832.6a–d	291.5a–d	354.6a–d	205.6f–r	228.1f–j
U 1165	1020.7a–i	1384.5b–h	356.9a–g	396.7a–e	138.5b–l	272.6h–j
U 633	1087.9a–k	1076.8a–d	311.8a–e	471.9a–f	316.7r–w	216.4d–j
Undine	1645.7i–m	835.1a–d	724.8i–k	419.5a–f	133.4a–l	86.8a–g
Vanda	**777.8**a	1287.2b–g	387.8a–h	826.7e–g	126.6a–k	147.6a–h
Wealthy	1046.9a–j	1521.7d–h	374.6a–g	846.0fg	40.8a–d	141.0a–h
Witos	851.5a–c	782.5a–d	285.1a–d	305.8a–c	86.1a–g	86.6a–g
av	1284.1	1137.6	447.4	509.9	177.4	172.2

[a] Means followed by the same letters do not differ significantly. Bold type indicates the highest and lowest values [b] Expressed as equivalent of gallic acid. [c] Expressed as equivalent of quercetin. [d] Expressed as equivalent of cyanidin-3,5-di-glucoside. [e] Not detected.

highest concentrations of phenolic compounds, only two and four occurred in both the 2003 and 2004 seasons, respectively. The highest amount of phenolics was measured for Haralson followed by Liberty, King of the Pippins, and Granny Smith and the lowest for Rajka and Witos, on average. Of all analyzed cultivars, seven showed resistance to apple scab (V_f resistance gene): Freedom, Liberty, Priam, Priscilla, Sawa, Topaz, and Witos. These genotypes contained totally different phenolics as well as flavonol concentration from low (Witos and Topaz) to medium (Priam and Priscilla) to high (Sawa, Freedom, and Liberty), on average, for the two examined years. However, irrespective of the season Witos was in the group with the lowest phenolics concentration, whereas Liberty was in the group with the highest. Among scab-resistant cultivars, the biggest difference in the total phenol content between the years (>100%) was obtained for Priscilla. Nearly the same content of phenolics was noted for Sawa and Witos—the differences were below 10% between 2003 and 2004 for all subgroups of phenolics. Other cultivars, in which total phenol content did not depend on the vegetation period, were Egeria, Gala, Granny Smith, Honeygold,

Melrose, Prima, Selena, and U 633. A great influence of the year was noted when differences between mean values of phenolics of above >70% were exhibited for Melodie, Mutsu, Rubin, Ruby, Spencer, and Undine.

In general, genotypes with the highest phenolics concentration had simultaneously a high flavonol content. As opposed to the phenolics, the average value of flavonol concentration was considerably higher in 2004, especially in the cases of Egeria, Elstar, Gala, Granny Smith, Jester, Ligol, Vanda, and Wealthy, which contained >70% higher flavonol content as compared to 2003. Contrary to the above-mentioned cultivars, Fuji, Mutsu, Undine, and Priscilla had considerably higher flavonol contents in the first tested year. The dark-red and red apple fruits had the highest anthocyanins concentrations. Hence, Sawa, Monroe, Redgold, Gloster, Odra, and Liberty were the richest in anthocyanins, on average, for the two examined years. The lowest concentration, <100 μg g^{-1} of fm, was found mainly in the fruit of Honeygold, Mutsu, Golden Delicious, and Auralia, with an orange blush of the skin, followed by Pilot, Pinova, Gala, and Rubinette (orange to red color of the skin), but also in this group were cultivars with red skins such as Ligolina, Witos, Wealthy, and Ruby.

On average, 177.4 and 172.2 μg g^{-1} of fm anthocyanins were obtained for 2003 and 2004, respectively. The increase of anthocyanins concentration in 2003 was especially noted for Egeria, Gorjaczkowski Seedling, Nova Easygro, Priscilla, Selena, Ligolina, and Melodie. In that year also small amounts of anthocyanins were detected for cultivars with orange skin such as Auralia, Golden Delicious, Honeygold, and Mutsu, whereas in 2004 they were below the detection limit of the method used. Steady levels of these phytochemical contents throughout both tested years were exhibited by Britemac, Fiesta, Liberty, Priam, Rajka, Redgold, Rosana, Šampion, Sawa, and Witos—most of them with dark-red and red skin color.

Enzyme Activitiy. The average concentration of apple peel GR activity ranged from 1.07 (Nova Easygro) to 7.77 (Rubinette) nkat g^{-1} of fm and from 2.23 (Sawa) to 14.54 (Rubinette) nkat g^{-1} of fm in 2003 and 2004, respectively (**Table 4**). Hence, compared to 2003, >2 times higher activity of GR in 2004 was noted, on average. Differences above 70% between the years were presented by ~73% of the cultivars among all tested, and from this group more that half exhibited >100% higher GR activity in 2004 than in the former year. In only two cases, Selena and Sawa, was a meaningful increase obtained in 2003, whereas Alwa, Elstar, Gala, Odra, Rajka, and Rubin showed very small differences in GR activity between the examined years. Not so high as in the case of GR, but for almost all cultivars the increase of CAT activity was also measured; on average, a 28% increase of CAT activity in 2004 in comparison with 2003 was recorded. The activity of CAT amounted from 3.97 (Prima) to 8.87 (Šampion) nkat g^{-1} of fm and from 4.64 (Sława Pobiediteliam) to 11.13 (Melodie) nkat g^{-1} of fm in 2003 and 2004, respectively (**Table 3**). Ten of 56 examined genotypes had very similar CAT activities in both years: Britemac, Egeria, Fuji, Gloster, Golden Delicious, Honeygold, Mutsu, Nova Easygro, Odra, and Spencer—the differences between the years for these cultivars did not exceed 10%. The year effect was the lowest in the case of APX activity. Compared to 2003, the increase of APX activity in 2004 amounted to ~19%. Considerably higher, in comparison with GR, and comparable to CAT activity, a number of cultivars (~20%) exhibited similar APX activities in both 2003 and 2004, namely, Cox's Orange Pippin, Egeria, Freedom, Golden Delicious, Honeygold, Jonamac, Liberty, Ligol, Melodie, Pinova, Priam, Vanda, and Witos. The lowest APX activity was obtained for Topaz (25.6 nkat g^{-1} of fm) and Gala (26.9 nkat g^{-1} of fm) and the highest for Šampion (200.7 nkat g^{-1} of fm) and U 633 (192.6 nkat g^{-1} of fm) in both 2003 and 2004, respectively. It is interesting that 40 cultivars in 2004 had a higher APX activity than the average value, whereas only 20 cultivars did so in 2003.

DISCUSSION AND CONCLUSIONS

Both natural and synthetic antioxidants were shown to enhance product stability, quality, and shelf life. Besides, the literature is now demonstrating that some antioxidants present in food provide important health benefits including the delay of cardiovascular diseases, some cancers, arthritis, and Alzheimer's disease (*26*, *27*). Hence, fruit quality research includes not only studies of sensory elements followed by the development of reduced browning genotypes and the investigation of postharvest attributes as well as disease resistance but also the examination of antioxidant content and composition. However, the latter is discussed as an important factor in relation to many formerly mentioned traits (*2*, *8*, *9*, *17*, *18*, *28*).

The distinct differences between apple cultivars in phytochemical content were found in relation to smaller (*4*, *6*, *14*) and greater numbers of cultivars (*7*−*9*). Also, this study shows the significant differences between examined genotypes of apple in antioxidant content. Mean contents of total ascorbate, glutathione, and phenolics represented 5.6−4.3-, 3.3−3.7-, and 2.4−4.9-fold variations, respectively, in their concentration depending on the year, whereas GR, APX, and CAT activities exhibited 7.3−6.5-, 7.8−7.2-, and 2.2−2.4-fold variations, respectively. Davey and Keulemans (*8*) testing 31 cultivars of apple noted 3.6- and 4.6-fold variations for total ascorbate and total glutathione, respectively. However, they analyzed a whole apple fruit. In the case of phenolics, 5.4-fold variation in total phenolics concentration was recorded (*7*). The years 2003 and 2004 considerably differed with regard to mean temperature and rainfall; therefore, it could also be noted which compounds and cultivars exhibited the highest intensity of responses to changing weather conditions. We tried to distinguish the cultivars with the lowest, highest, and relatively stable concentrations or those in which antioxidant concentrations greatly differed depending on the growing seasons. Irrespective of the year, high contents of ascorbate were obtained for Rajka, Topaz, Pilot, Šampion, and Melodie; for glutathione, Gorjaczkowski Seedling, Granny Smith, Cox's Orange Pippin, Melodie, and Pilot; and in the case of phenolics, Haralson, Liberty, King of the Pippins, and Granny Smith. Simultaneously, among these cultivars the differences in the given compound content in a successive year were rather small, especially for glutathione and, in general, they did not exceed 30%. It was noted that it is difficult to find cultivars "rich" or "poor" in all of the tested phytochemicals and additionally in both years. Pilot and Melodie had the highest ascorbate and glutathione contents and McIntosh the lowest, irrespective of growing season. Granny Smith was in the group of the highest contents of glutathione and phenolics. Compared to phenolics, there were more cultivars described as "stable" in relation to the concentration of ascorbate and glutathione despite tested year. It is interesting that, in the case of ascorbate, in this group there were rather cultivars with low or medium levels of this compound. As opposed to this, the "stable" cultivars with respect to glutathione concentration were predominantly those with the content of over the mean value or rather high.

The glutathione system as a stress marker in plant ecophysiology is discussed by Tausz et al. (*20*). They suggested an initial stress response in relation to changes in the glutathione redox

Table 4. Antioxidative Enzyme Activity Depending on Cultivar and Harvest Year

	GR (nkat g^{-1} of fm)		APX (nkat g^{-1} of fm)		CAT (nkat g^{-1} of fm)	
cultivar	2003	2004	2003	2004	2003	2004
Alwa	2.49a–k	2.67ab	67.1a–g	152.1f–k	5.46a–g	7.97b–m
Arlet	3.05a–n	8.42g–m	94.6a–g	148.4f–k	5.91a–h	8.81f–m
Auralia	4.04i–o	9.62j–n	133.3c–h	113.9b–k	5.22a–g	6.37a–i
Britemac	1.57a–e	4.48a–f	53.4a–e	124.8c–k	5.58a–g	5.76a–g
Cortland	2.79a–l	6.91c–m	105.9a–g	74.6a–g	4.63a–e	8.50e–m
Cox's Orange Pippin	3.26b–n	6.51c–k	82.5a–g	87.8a–i	6.75a–j	7.62a–l
Egeria	1.51a–e	5.96b–j	147.0f–h	137.5d–k	8.62ij	8.22d–m
Elstar	3.57e–n	3.76a–d	86.4a–g	122.6c–k	4.10ab	6.50a–j
Fiesta	2.19a–j	10.35k–n	44.5a–c	171.4i–k	4.96a–g	9.93k–m
Freedom	3.96i–o	7.30d–m	81.1a–g	87.6a–i	5.41a–g	7.60a–l
Fuji	2.35a–k	9.82k–n	128.0c–h	75.0a–g	7.65g–j	7.82a–l
Gala	4.22j–p	4.05a–e	84.3a–g	**26.9**a	4.30a–c	5.13a–d
Gloster	5.78p–t	9.01i–m	126.9c–h	75.9a–g	7.38e–j	7.64a–l
Golden Delicious	5.91p–t	10.28k–n	156.5gh	143.7e–k	5.91a–h	5.45a–e
Gorjaczkowski Seedling	2.80a–l	12.83no	100.9a–g	156.0f–k	5.79a–g	8.98f–m
Granny Smith	1.71a–g	10.73mn	53.1a–e	150.0f–k	5.30a–g	8.34d–m
Haralson	2.88a–m	6.84c–l	90.0a–g	41.9ab	6.38a–j	9.06g–m
Honeygold	2.00a–i	7.18d–m	137.5d–h	125.8c–k	5.80a–g	5.66a–f
Jester	1.71a–g	4.45a–f	74.8a–g	116.3b–k	5.18a–g	7.79a–l
Jonamac	6.01r–t	7.88f–m	149.9f–h	158.1g–k	6.59a–j	9.12h–m
King of the Pippins	6.86s–u	8.08f–m	81.9a–g	65.2a–e	6.82b–j	8.96f–m
Liberty	3.84g–o	9.02i–m	102.5a–g	99.8a–j	4.82a–f	7.74a–l
Ligol	4.42k–r	8.77h–m	142.3e–h	149.9f–k	7.57f–j	11.05m
Ligolina	1.30a–d	7.93f–m	93.9a–g	108.4b–j	6.79a–j	8.04b–m
McIntosh	1.80a–h	3.42a–c	42.3a–c	104.8b–j	4.76a–e	6.16a–h
Melodie	3.37d–n	12.56no	107.6a–g	107.4b–j	5.43a–g	**11.13**m
Melrose	1.91a–i	10.33k–n	122.1b–h	158.3g–k	5.92a–h	8.75f–m
Monroe	1.64a–f	4.75a–g	31.4ab	169.1i–k	4.38a–d	6.16a–h
Mutsu	1.89a–i	10.53l–n	47.8a–d	58.3a–d	5.02a–g	5.20a–d
Nova Easygro	**1.07**a	5.23a–h	145.9e–h	79.5a–h	6.42a–j	6.86a–l
Odra	5.53o–s	5.11a–h	134.5c–h	54.7a–c	7.37e–j	7.86a–l
Pilot	2.94a–n	6.88c–m	144.8e–h	109.4b–j	5.33a–g	6.99a–l
Pinova	2.75a–l	7.00c–m	154.2f–h	162.2h–k	5.56a–g	7.80a–l
Priam	1.45a–e	5.33a–i	138.7d–h	141.0e–k	6.56a–j	8.35d–m
Prima	2.54a–k	3.61a–d	74.5a–g	113.6b–k	**3.97**a	4.9a–c
Priscilla	3.48d–n	8.30g–m	74.7a–g	136.9d–k	4.50a–d	6.62a–k
Rajka	6.19r–u	6.75c–l	32.2ab	116.5b–k	8.51h–j	6.75a–l
Redgold	4.94n–r	10.15k–n	88.9a–g	139.2d–k	6.39a–j	9.77j–m
Rosana	3.76f–o	7.65e–m	119.4b–h	178.3jk	5.19a–g	9.08g–m
Rubin	6.14r–u	5.63a–i	120.1b–h	136.7d–k	5.96a–h	7.51a–l
Rubinette	**7.77**u	**14.54**o	85.3a–g	156.8g–k	7.04c–j	9.34h–m
Ruby	1.60a–f	7.04c–m	106.6a–g	158.3g–k	3.99a	6.33a–i
Šampion	7.34tu	9.88k–n	**200.7**h	143.3e–k	**8.87**j	10.01lm
Sawa	3.09a–n	**2.23**a	124.7c–h	71.9a–f	5.73a–g	4.85a–c
Selena	7.19s–u	6.04b–j	87.2a–g	154.2f–k	8.76ij	7.08a–l
Sława Pobieditieliam	1.15a–c	2.76ab	155.5gh	98.7a–j	5.34a–g	**4.64**a
Spartan	2.95a–n	3.73a–d	91.8a–g	103.9c–j	4.26a–c	5.34a–e
Spencer	2.85a–l	9.02i–m	53.7a–e	145.7e–k	6.76a–j	7.04a–l
Topaz	2.70a–k	10.16k–n	**25.6**a	105.2b–j	4.95a–g	8.79f–m
U1065	1.66a–f	7.06c–m	93.8a–g	109.5b–j	6.26a–i	9.68i–m
U1165	2.70a–k	9.97k–n	148.8f–h	77.4a–g	7.59f–j	4.81ab
U633	3.32c–n	9.63j–n	63.3a–g	**192.6**k	4.71a–e	10.00lm
Undine	4.81l–r	7.45e–m	61.2a–f	137.9d–k	7.18d–j	8.11c–m
Vanda	4.90m–r	9.65j–n	85.2a–g	80.2a–h	6.51a–j	9.39h–m
Wealthy	1.10ab	8.64h–m	128.6c–h	112.6b–k	6.27a–i	8.24d–m
Witos	3.92h–o	5.97b–j	130.9c–h	129.0c–k	7.61f–j	8.76f–m
av	3.40	7.50	100.0	118.9	6.00	7.69

[a] Means followed by the same letters do not differ significantly. Bold type indicates the highest and lowest values

state; an acclimation is marked by an increased GSH content, increased related enzyme activity, and/or a greater redox status of GSH. The latter is interpreted as overcompensation leading to enhanced regeneration of GSH. If we accept the weather conditions of 2004 as unfavorable, there was no distinct decrease or increase of glutathione and its precursors content in this season as a stress response factor, in comparison to the former year. Contents of these compounds were nearly the same, regardless of the year and, in general, irrespective of cultivar, whereas the activity of antioxidative enzymes considerably increased, especially with regard to GR—the enzyme evolved in the reduction of GSSG, followed by CAT and APX. GR activity in 2004 was 2 times higher than in 2003, on average, and a bigger or smaller increase than the average value was presented by 51 genotypes of 56 tested. Although we did not determine the GSH/GSSG redox state, it seems that GR activity may be a better and more convenient stress marker of apple fruit.

The relationship between ascorbate−glutathione content and extent of photooxidative damage is also suggested (*29*). Taking into account different parts of a single fruit (*13*), AA concentrations were highest in tissues that had been exposed to higher

light intensities (red exocarp) and, opposed to this, GSH levels were generally lower in this area and vice versa. Moreover, total phenolics content of red exocarp was 1.7-fold higher in comparison with green, but without qualitative differences between the two types of tissues. Other authors (*13*) concluded that enhanced stress resistance of red, highlighted acclimated exocarp is due to the antioxidant activities of phenolics and, in particular, anthocyanins. Also, Planchon et al. (*9*) reported that ascorbic acid content depended not only on maturity but also on fruit position in the tree, fruit size, and fruit skin color. In the present results there was no clear relationship between the overcolor of the skin (blush of apple) and ascorbate or glutathione level. In the group of cultivars with high ascorbate concentration (>3400 nmol g^{-1} of fm—the mean value for two years), there were mainly fruits with orange-red overcolor of the skin, for example, Elstar, Cox's Orange Pippin, Topaz, Pilot, Fiesta, and Rubin. Red and dark-red apple peels were predominantly noted among cultivars with high glutathione concentration (>60 nmol g^{-1} of fm, on average)—Melrose, Šampion, Nova Easygro, Jonamac, Haralson, Rajka, Cortland, Alwa, Ruby, and Gorjaczkowski Seedling. The same remark as for glutathione concerns phenolics and obviously anthocyanins. A positive correlation between phenolics and flavonols content ($R = 0.5705$) and weaker correlations between ascorbate and GR ($R = 0.1952$), between phenolics and anthocyanins ($R = 0.1923$), between glutathione and GR ($R = 0.1125$), between ascorbate and glutathione ($R = 0.0339$), or between ascorbate and APX ($R = 0.0105$) were recorded. The weak negative correlation between ascorbate and different subgroups of phenolics, especially anthocyanins ($R = 0.0711$), was noted.

This work supported the same conclusion as in the Davey and Keulemans paper (*8*) in relation to harvest date and vitamin C content. Namely, cultivars with the highest ascorbate concentration were harvested later in the season. It is suggested that the combination of high AA and GSH contents and late ripening is possibly partially responsible for storage behavior in apple cultivars. Contrary to this, Podsêdek et al. (*28*) reported that summer cultivars showed relatively high ascorbic acid content.

To adapt some cultivars to production systems that are more environmentally friendly or to use them in breeding programs for the relationship between bioactive compound and their redox state, characteristics that are responsible for or may act as signal-transducing molecules in disease resistance are also looked for (*9*, *17*, *30*). However, in our study a comparison of seven cultivars resistant to apple scab showed that the total phenolics content is a rather weak tool to mark scab resistant genotypes. Mayr et al. (*17*) reported that a high concentration of flavan-3-ols was found in those orchards which were not infected with *Venturia inaequalis*.

It is difficult to discuss or perceive all connections in such a great number of cultivars, but we hope that the knowledge of the concentration of enzymatic and nonenzymatic antioxidants, which are associated with the regulation of a wide range of metabolic functions, could be used across many disciplines, especially in the genetic improvement of apples, for example, in an integrated analysis of progenitors and breeding families to identify QTL alleles improving antioxidant content in fruit, or by other researchers, for example, in physiological areas. A high content of secondary metabolites in new cultivars described by Schmitz-Eiberger (*7*) indicates that the content of secondary plant ingredients can be enhanced by breeding. Looking through the presented antioxidant characteristics, it could sometimes be difficult to find the connection between their content and other quality parameters. This may suggest that they are not universally involved in the quality trait of each cultivar. The difficulties originate from a large variety of antioxidant compounds, probably many not identified so far, and still fragmentary knowledge about their synergistic or antagonistic effects.

ABBREVIATIONS USED

AA, L-ascorbic acid; DHAA, dehydroascorbic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; CYS, L-cysteine; γ-GC, γ-glutamylcysteine; GR, glutathione reductase; APX, ascorbate peroxidase; CAT, catalase; SOD, superoxide dismutase; AOS, active oxygen species.

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