Activity and Concentration of Polyphenolic Antioxidants in Apple: Effect of Cultivar, Harvest Year, and Storage Conditions

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Consumers' increasing interest in the relationship between diet and health is a sign for food producers to pay more attention to potential health-protecting compounds in new product development and food processing. From a production chain perspective the choice of the raw material that is used is important for the health-protecting potential of the end product. Four apple cultivars (Jonagold, Golden Delicious, Cox's Orange, and Elstar), which can be used as fresh apples or in processed apple products, were compared with regard to flavonol, catechins, phloridzin, and chlorogenic acid concentrations and antioxidant activity. Jonagold apples possessed the highest flavonoid concentration and the highest antioxidant activity. To study seasonal differences, apples from three different harvest years were analyzed, but in three cultivars no effect on flavonoid concentration and antioxidant activity was observed. Long-term storage, both at refrigerator temperature and under controlled atmosphere conditions, was found not to influence flavonoid concentration or antioxidant activity.

Keywords: *Antioxidant activity; flavonoids; storage; apples; cultivar; harvest*

INTRODUCTION

Flavonoids are secondary plant metabolites present in fruits and vegetables. They belong to the group known as polyphenolics, and generally they occur in plants as glycosides. Over 6400 different structures have been identified, and this number continues to increase (*1*).

In epidemiological research some flavonoids are associated with protection against aging diseases (*2*). This may be ascribed to their action as antioxidants. Formation of oxygen radicals is supposed to play a key role in the development of cancer and coronary heart disease. Free radicals may attack biomolecules, such as lipids, proteins, or DNA, which can be prevented by antioxidants.

In the Dutch diet important sources of flavonoids are tea, onions, and apples (*3*). The most important flavonoids present in apple and apple products are flavanols or catechins, flavonols, and anthocyanidins (*4*). Flavonols are mainly present as quercetin glycosides; cyanidin galactoside is the most common anthocyanin, and (−)-epicatechin is the predominant form of the catechins. In contrast with flavonols and anthocyanidins, catechins are not glycosylated. They appear in monomeric form as well as in oligomeric form (procyanidins) (*5*). Furthermore, dihydrochalcones (e.g., phloridzin) and phenolic acids (in particular chlorogenic acid) are present in apple (*5*).

The dietary intake of flavonoids has been estimated as 1 g/day (*6*). More recently, new analysis techniques became available and Dutch flavonol and flavone intake was estimated on 23 mg/day (*7*). In the latter research catechins and anthocyanidins were excluded. This might cause an underestimation of the total flavonoid intake. Finnish flavonoid intake (catechins, flavonols, flavones, and flavanones) was estimated to be 55.2 mg/day, with anthocyanidins excluded (*8*).

To be a bioactive compound of interest, not only should the intake be sufficiently high but also the bioavailability (the absorption into the body) must be at a sufficient level to obtain active levels within the body. Polyphenolic antioxidants are absorbed in the human body to some extent; for example, 52% of the quercetin glycosides present in onions (*9*) and 33% of chlorogenic acid present in a supplement (*10*) are absorbed. Upon absorption of quercetin glycosides the compounds are hydrolyzed to quercetin and subsequently converted to quercetin glucuronides and sulfates in the human body (*11*).

Food producers are increasingly interested in developing new products with an increased level of certain health-protecting compounds to address the increasing interest of consumers in the relationship between diet and health. For this development not only it is relevant to know which health-protecting compounds are present in raw materials and in what concentrations, but also their bioactivity is of importance. Before consumption, fruits and vegetables in which bioactive compounds are present may undergo different forms of processing, which might affect the concentration and bioactivity of the health-protecting compounds in the product. From a production chain perspective all of the steps will have an effect on the final level and activity of bioactive compounds in the final product (*12*). Important aspects within the production chain of apple are the choice of the raw material [cultivar, growing conditions (climate,

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10.1021/jf001493u CCC: $20.00
Published on Web 07/12/2001

soil type, use of fertilizers), seasonal differences, and harvest and storage conditions], industrial processing, packaging, storage of the final product, and consumer processing. Until now studies have mainly focused on the influence of these factors on the polyphenolic composition of apples (*13*−*16*) and not on their bioactivity.

This paper is part of a project that studies the effects of storage and processing on flavonoids and chlorogenic acid in apple and apple products (*17*). Polyphenolic compounds as present in apple are strong antioxidants (*18*) and may function as such in preventing aging diseases; therefore, the antioxidant activity of apples was used as a measure of their bioactivity (*19*).

The biological variability within a certain cultivar of apple is investigated, and on the basis of this information a sampling protocol has been developed. Four apple cultivars (Jonagold, Golden Delicious, Cox's Orange, and Elstar) were compared with regard to polyphenolic composition and antioxidant activity.

To study potential seasonal differences in polyphenolic composition and antioxidant activity, apples from three different harvest years were analyzed.

To investigate the effect of apple storage, controlled atmosphere (CA) storage and cold storage were tested.

MATERIALS AND METHODS

Chemicals. Kaempferol, myricetin, quercetin dihydrate, and rutin trihydrate were purchased from Fluka; chlorogenic acid, phloridzin, (±)-catechin, and (−)-epicatechin were from Sigma; and quercetin 3-arabinoside, hyperoside, isoquercitrin, quercitrin, and ideainchloride were from Roth. Quercetin 3-arabinofuranoside was obtained from Apin chemicals and reynoutrin from Plantech. L-(+)Ascorbic acid and iron(II) sulfate heptahydrate were obtained from Merck. All other chemicals were of analytical or HPLC grade purity.

Apple Cultivars and Harvest and Storage Conditions. In this research the two main apple cultivars grown in The Netherlands were used (Jonagold and Elstar) as were two less important apple cultivars (Golden Delicious and Cox's Orange). Apples were harvested from commercial orchards in three growing seasons, 1996−1998, to investigate seasonal variability. Each year the apples were collected from the same beds in the same orchards. Fruit trees were planted in a four-row system, called a "Zeeuws bed", with a density of ∼3500 trees/ha, except for Jonagold harvest 1998, when apples were obtained from trees from a different orchard, planted in single rows (3300 trees/ha).

The day of harvest was predicted using variety-specific models (*20*) that predict the optimal harvest date for Jonagold apples for long-term storage in controlled atmosphere. This means that at the time of harvest apples have not reached the stage of complete maturity suitable for immediate consumption.

Fruits were picked at one time, from the outer layer, avoiding the tops and bottoms of the trees. Apples from the inner layer of the tree were not used because their total flavonoid concentration is much lower due to lack of light (*21*). Trees at the border of the orchard were avoided as well.

Applied CA storage conditions for Jonagold, Golden Delicious, and Elstar were 1.5 °C, 1.2% O_2, and 2.5% CO_2. Cox's Orange apples were stored at 4.0 °C, 1.2% O_2, and 0.7% CO_2.

In cold storage all apple cultivars were kept at 4 °C. The effect of combined storage (cold storage after a storage period of approximately 13 or 46 weeks in CA conditions) was determined as well.

Apples were stored in 12 kg boxes. Samples were taken regularly (four to seven times) throughout the storage period, until the apples lost their quality as assessed by loss of firmness or development of brown spots.

Sample Preparation. Flavonoid and chlorogenic acid standards were dissolved in methanol. Apple samples were taken as follows. Four apples (unless stated otherwise) were chosen at random and in duplicate. After cleaning with water, stalks were removed and the complete apples were cut into pieces by knife and subsequently ground to a fine powder (with an ATO-MSE mix) under liquid nitrogen to prevent oxidation. The frozen samples were stored immediately at −20 °C until lyophilization. Dry weight was determined from the sample weight before and after lyophilization. Lyophilized apple samples were stored at −20 °C until analyzed.

To determine the distribution of flavonoids and chlorogenic acid within an apple, apples were peeled with a potato knife; a thin layer of apple flesh remained adhered to the peel. Therefore, the peel can be considered as the epidermic zone of the apples.

To establish the effect of oxidation time on the flavonoids and chlorogenic acid in the apple samples before extraction, apples were sliced in a food processor and left at room temperature for 0−30 min before they were ground under liquid nitrogen.

Apple samples were extracted before HPLC analysis and antioxidant activity determination. Extraction conditions were optimized in order to be able to use the same extract in both determinations. Lyophilized sample (0.5 g) was extracted with 10 mL of methanol (unless stated otherwise) and sonicated for 30 min followed by 10 min of centrifugation at 2500 rpm. The supernatant was filtered through a 0.45-μm CA filter (Schleiger and Schuell).

Quantification of Flavonoids by HPLC. Quercetin glycosides, catechins, chlorogenic acid, phloridzin, and cyanidin galactoside were determined in the apple samples by an adaptation of the method described by Lister et al. (*4*). This method was adjusted for the use of a Merck Lichrosorb RP18 (4 × 250 mm, 5 μm) analytical column with guard column. A Spectra Focus scanning UV−vis detector, a Spectra System P2000 solvent programmer, and a Spectra System AS3000 autosampler from Spectra Physics were used. Integrator software was TSP version 3.0. Eluents, solvent gradient, flow rate, and column conditions were described before (*19*).

The individual compounds were identified and quantified by comparison with standard solutions of known concentrations and, if necessary, by comparison of spectra. Quercetin, its glycosides, and chlorogenic acid were monitored at 350 nm, phloridzin and the catechins were monitored at 280 nm, and cyanidin galactoside was monitored at 525 nm. The coefficients of variation for the slope of the calibration curves of the various compounds (16 replications) were as follows: chlorogenic acid, 2%; Q-3-galactoside, 5%; Q-3-rutinoside, 15%; Q-3-glucoside, 3%; Q-3-arabinoside, 5%; Q-3-rhamnoside, 16%; quercetin, 6%; catechin, 10%; epicatechin, 13%; phloridzin, 14%.

Antioxidant Activity Determination. Preparation of rat liver microsomes for antioxidant activity determination was performed as described earlier (*19*). After isolation, microsomal protein concentration, determined by Biuret assay with bovine serum albumin used as standard, was 7.2 mg/mL. Microsomes were diluted with phosphate buffer to 5 mg/mL protein before storage in 1 mL aliquots in liquid nitrogen.

Antioxidant activity was determined by measuring the inhibition of lipid peroxidation in rat liver microsomes by the apple samples (*19*). In the assay microsomal protein concentration was 0.5 mg/mL. Lipid peroxidation (LPO) was induced by adding ascorbic acid (final concentration = 0.2 mM in the assay) and $FeSO_4$ (final concentration = 0.01 mM in the assay). Antioxidant samples were 10 times diluted by administration to the assay. LPO was assessed by measuring thiobarbituric acid reactive species (TBARS) after heating, and absorption was read at 540 nm (color) versus 620 nm (turbidity correction) by an ELISA reader. The mean absorbance reading ($A_{540} - A_{620}$) ± standard deviation of the methanol blanks was 1.172 ± 0.129 (in 59 repetitions collected on 5 experimental days).

The concentration of the antioxidant sample at which 50% inhibition of LPO occurs (IC_{50}) was calculated from triplicate determination of six different antioxidant concentrations ranging from no to full inhibition of LPO.

Table 1. Flavonoid and Chlorogenic Acid Concentration (Milligrams per Kilogram of Fresh Weight) of a Jonagold Apple Sample Extracted by Four Different Extraction Solutions[a]

compound	water	50% MeOH	100% MeOH	15% HAc/MeOH
Q-3-Ga	11 ± 2[a]	30 ± 2[b]	27 ± 3[b]	28 ± 4[b]
Q-3-Ru	0 ± 0[a]	0 ± 0[b]	0 ± 0[b]	0 ± 0[b]
Q-3-Gl	2 ± 1[a]	6 ± 1[b]	6 ± 0[b]	6 ± 1[b]
Q-3-Xy	3 ± 1[a]	12 ± 3[b]	12 ± 1[b]	12 ± 3[b]
Q-3-Ar	5 ± 1[a]	23 ± 4[b]	26 ± 3[b]	24 ± 6[b]
Q-3-Rh	11 ± 1[a]	28 ± 8[b]	29 ± 8[b]	30 ± 10[b]
catechin	5 ± 3[a]	16 ± 1[b]	17 ± 2[b]	14 ± 3[b]
epicatechin	16 ± 5[a]	93 ± 14[b]	121 ± 7[c]	129 ± 14[c]
Cy-Ga	1 ± 1[a]	9 ± 1[b]	11 ± 1[b]	11 ± 1[b]
phloridzin	9 ± 2[a]	25 ± 6[b]	31 ± 2[b]	34 ± 5[b]
chlorogenic acid	61 ± 17[a]	173 ± 7[b]	195 ± 11[c]	219 ± 26[c]

[a] Extractions were performed in quadruplicate (mean ± SD). Values within a row having the same letter are not different at the 5% level.

Statistical Analysis. Statistical analysis of the data was performed on the original data by one-way analysis of variance (ANOVA) or regression analysis, with significance level $\alpha = 0.05$ using the statistical package from Microsoft Excel.

RESULTS AND DISCUSSION

Effect of Sample Taking. *Effect of Extraction Solution*. Extraction conditions were optimized in order to be able to use the same extract for HPLC analysis and antioxidant activity determination. The following extraction solutions were compared: water, 50% aqueous methanol, and 100% methanol (with or without 15% acetic acid). Hertog and co-workers (*3*) reported that 50% aqueous methanol was most efficient for flavonoid extraction. Lister et al. (*4*) used 100% methanol as extraction solution for proanthocyanidins and 15% acetic acid in methanol for the extraction of flavonols and anthocyanins.

Table 1 shows that water is not suitable for flavonoid extraction; this is the case for all of the compounds analyzed. Epicatechin and chlorogenic acid are better extracted by 100% methanol (with or without 15% acetic acid) than by 50% aqueous methanol. The extraction solution of 100% methanol (with or without 15% acetic acid) gives reproducible good yields, and no difference in extraction efficiency was observed in all compounds. For antioxidant activity determination it is better not to add acetic acid to the test system. Therefore, 100% methanol was chosen as extraction solution for HPLC analysis and antioxidant activity determination.

Effect of Oxidation Time before Extraction. During processing, apple samples brown very quickly by enzymatic oxidation of polyphenols. We have studied the effects of exposure to air during the sample treatment on the level of the components of interest. As expected, Figure 1 shows significant decreases in the levels of total catechins (−28%) and chlorogenic acid (−25%), which are known substrates for polyphenol oxidase (*22*). Phloridzin and cyanidin galactoside concentrations were not affected by oxidation. Figure 1 also shows that quercetin glycosides are not substrates for polyphenol oxidase. There seems to be a slight but significant increase (17%) in the total quercetin glycoside concentration.

In Figure 2 the effect of oxidation time on dry weight and antioxidant activity of Jonagold apple is depicted. The IC_{50} value shows a slight but significant increase (13%), which means that the antioxidant activity slightly

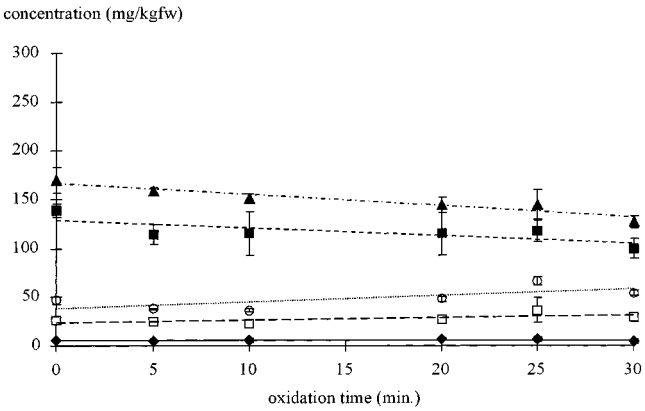

Figure 1. Effect of oxidation time (min) on flavonoid and chlorogenic acid concentrations (mg/kg of fw) of Jonagold apple, harvest 1997: Cy-Ga (◆), phloridzin (□); chlorogenic acid (▲); total Q-gly (○); total catechins (■). Triplicate extractions were performed.

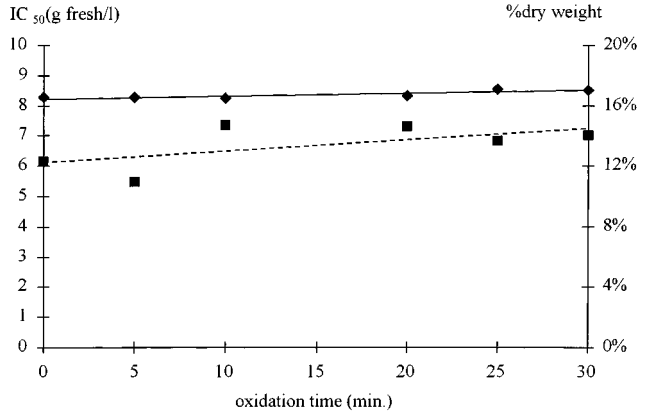

Figure 2. Effect of oxidation time (min) on antioxidant activity (g of fw/L) and percent dry weight of Jonagold apple, harvest 1997: antioxidant activity (■); % dry weight (◆).

Table 2. Distribution of Quercetin Glycosides, Phloridzin, and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight) in Jonagold Apple Peel and Flesh, Harvest 1996

compound	peel	flesh
Q-3-Ga	126 ± 22	0 ± 0
Q-3-Gl	20 ± 2	0 ± 0
Q-3-Xy	57 ± 7	1 ± 1
Q-3-Ar	159 ± 21	2 ± 2
Q-3-Rh	145 ± 7	7 ± 3
phloridzin	66 ± 3	14 ± 5
chlorogenic acid	148 ± 16	170 ± 43

lowers with increasing oxidation time. Therefore, it is important to ensure that no oxidation occurs during sample taking. The sample should not thaw during this process but remain frozen until lyophilization.

Variation in Flavonoid and Chlorogenic Acid Concentrations within an Apple. Flavonoids are not equally distributed throughout the apple. Table 2 shows that quercetin glycosides were almost exclusively found in the peel, but low concentrations were detected in the flesh, which is consistent with the findings of others (*14*, *15*). Phloridzin was present in both flesh and peel, although with a higher concentration in the peel. Chlorogenic acid was equally distributed over peel and flesh ($p = 0.583$). Phloridzin and chlorogenic acid distributions within an apple confirm the findings of Burda et al. (*23*). Catechins were not analyzed in this part of our study, but it has been reported that the

Table 3. Flavonoid and Chlorogenic Acid Concentration (Milligrams per Kilogram of Fresh Weight), Percent Dry Weight, and Antioxidant Activity of Four Individual Jonagold Apples and Jonagold Apple Samples Composed of Three or More Apples (Mean ± SD)

compound	1	2	3	4	mean	three apples	four apples	five apples	mean
Q-3-Ga	20	10	29	22	22 ± 7	33 ± 2	31 ± 10	21 ± 3	29 ± 9
Q-3-Ru	3	1	2	2	2 ± 1	in Q-3-Glu	in Q-3-Glu		in Q-3-Glu
Q-3-Gl	4	2	4	3	3 ± 1	7 ± 1	5 ± 1		6 ± 1
Q-3-Xy	9	7	14	11	11 ± 3	14 ± 3	11 ± 2	13 ± 2	12 ± 2
Q-3-Ar	29	20	41	30	32 ± 8	36 ± 3	35 ± 5	33 ± 5	35 ± 5
Q-3-Rh	38	30	53	37	41 ± 10	32 ± 0	30 ± 5	29 ± 3	31 ± 4
epicatechin	76	99	128	81	99 ± 26	nd[a]	nd	nd	nd
Cy-Ga	4	4	7	5	5 ± 3	nd	nd	nd	nd
phloridzin	13	28	24	15	20 ± 6	47 ± 4	56 ± 33	74 ± 26	57 ± 27
chlorogenic acid	126	176	200	167	173 ± 27	241 ± 25	240 ± 24	322 ± 3	256 ± 40
% dry wt	17.6	15.9	18.0	19.4	18.1 ± 1.4	15.8 ± 0.4	16.1 ± 1.1	19.9 ± 1.8	17.0 ± 2.1
IC_{50} (g of fw/L)	6.4	7.5	4.9	6.9	6.4 ± 1.1	nd	nd	nd	nd

[a] nd, not determined.

epicatechin concentration was higher in the skin than in the flesh (*23*). In all apple samples quercetin aglycon was absent.

Variation in Flavonoid and Chlorogenic Acid Concentration and Antioxidant Activity between Individual Apples. To determine the variation in flavonoid and chlorogenic acid concentrations and antioxidant activity between individual apples, four Jonagold apples were analyzed separately. Flavonoid concentrations are presented in Table 3. The variation in compound concentration between individual apples was 10−30%, depending on the compound. The largest variations were found in epicatechin and phloridzin concentrations. The same variation was observed in Elstar and Golden Delicious apples (data not shown). In Table 3 the dry weight and the antioxidant activity expressed as IC_{50} (the concentration of the antioxidant sample at which 50% inhibition of lipid peroxidation occurs) of the individual Jonagold apples are also presented. The variation in dry weight among the four Jonagold apples was 8%, and the variation in IC_{50} value was 17%.

Number of Apples in a Sample. Because of the observed variation in flavonoid and chlorogenic acid concentrations between individual apples, it is better to compose samples of more than one apple. Jonagold apple samples composed of three, four, or five apples were analyzed. Flavonoid and chlorogenic acid concentrations and dry weights are given in Table 2. Not much difference in the concentration of these compounds was found. Therefore, we chose duplicate sample taking with samples composed of four apples. In duplicate sample taking of four apples a variation of 10−30% existed, which is considered to be the biological variation.

We used whole apples in a sample, not just the peel where higher flavonoid concentrations are found, which has been done by many authors (*4*, *13*, *24*). Later in the project complete apples were processed, and for comparison purposes it was therefore necessary to analyze the concentration in the complete apple.

Seasonal Variability. Fruits of four apple cultivars were collected during three years to study the effect of seasonal variation on flavonoid concentration and antioxidant activity. Table 4 describes the means of the flavonoid concentration of the three harvest seasons.

In Jonagold apples the total quercetin glycoside concentration did not significantly differ over the three harvest years, although the total quercetin glycoside concentrations between the years 1996 and 1998 were significantly different ($p = 0.022$) and was highest in harvest year 1996. Total catechin, cyanidin galactoside, and chlorogenic acid concentrations were the same in the analyzed harvest years. Phloridzin concentration was highest in the 1998 harvest ($p = 0.004$).

Table 4. Concentration of Flavonoids and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight), Percent Dry Weight, and Antioxidant Activity of Four Apple Cultivars[a]

compound	Jonagold	Golden Delicious	Cox's Orange	Elstar
total Q-glycoside[b]	98 ± 16	67 ± 16	54 ± 15	60 ± 4
total catechins[c]	197 ± 17	173 ± 26	143 ± 59	162 ± 2
Cy-Ga[c]	8 ± 2	2 ± 0	4 ± 2	3 ± 0
phloridzin	28 ± 13	35 ± 16	14 ± 9	26 ± 15
chlorogenic acid	201 ± 15	171 ± 18	69 ± 25	70 ± 4
% dry wt	17.4 ± 0.4	17.1 ± 0.3	16.7 ± 0.4	16.8 ± 0.5
IC_{50}[c] (g of fw/L)	5.8 ± 0.3	8.0 ± 0.5	7.2 ± 1.6	6.3 ± 0.5

[a] Mean (± SD) of three harvest years (1996, 1997 and 1998). [b] The group total Q-glycosides is composed of Q-3-Ga, Q-3-Ru, Q-3-Gl, Q-3-Xy, Q-3-Ar, and Q-3-Rh. [c] Mean (± SD) of two harvest years (1997 and 1998). The group total catechins consists of catechin and epicatechin.

In Golden Delicious apples, concentrations of chlorogenic acid, total catechins, and cyanidin galactoside were the same at harvest time over the three years. Some variation in total quercetin glycoside concentration ($p = 0.041$) and phloridzin concentration ($p = 0.050$) was measured.

Cox's Orange apples seemed to be most sensitive to seasonal variation. Seasonal variation in chlorogenic acid and total catechin concentrations was significant ($p = 0.018$ and 0.043). For total quercetin glycoside, cyanidin galactoside, and phloridzin the significance was between the 5 and 10% levels ($p = 0.062$, 0.056, and 0.068, respectively).

Seasonal variation in total quercetin glycoside, cyanidin galactoside, chlorogenic acid, and total catechin concentrations of Elstar apples was not significant and therefore lower than the observed biological variation. However, seasonal variation was observed for phloridzin concentration in Elstar ($p = 0.006$).

The conclusions found for Jonagold and Elstar apples are similar to the findings for apple peels reported by Awad and de Jager (*25*), except that we observed seasonal variation in phloridzin concentration instead of cyanidin galactoside concentration.

Table 4 also gives the antioxidant activity of the four apple cultivars from the 1997 and 1998 harvests. In the four analyzed apple cultivars no seasonal effect on antioxidant activity was observed. Jonagold apples possessed the highest antioxidant activity, followed by

Table 5. Quercetin Glycoside and Catechin Profiles in Four Apple Cultivars[a]

	mean % of total quercetin glycosides			
	Jonagold	Golden Delicious	Cox's Orange	Elstar
Q-3-Ga	25 ± 3	23 ± 8	32 ± 4	33 ± 3
Q-3-Ru	1 ± 1	1 ± 1	2 ± 0	2 ± 0
Q-3-Gl	5 ± 2	7 ± 1	7 ± 2	10 ± 2
Q-3-Xy	11 ± 2	11 ± 3	10 ± 3	11 ± 2
Q-3-Ar	28 ± 1	27 ± 2	34 ± 2	31 ± 1
Q-3-Rh	30 ± 3	31 ± 3	15 ± 1	13 ± 2
	mean % of total catechins			
	Jonagold	Golden Delicious	Cox's Orange	Elstar
catechin	7 ± 1	6 ± 3	7 ± 3	7 ± 4
epicatechin	93 ± 1	94 ± 3	93 ± 3	93 ± 4

[a] Mean (± SD) of three harvest years (1996, 1997, and 1998).

Elstar and Cox's Orange. Golden Delicious apples showed the lowest antioxidant activity.

Varietal Differences and Quercetin Glycoside or Catechin Profiles. Table 4 shows that Jonagold had the highest quercetin glycoside concentration, followed by Golden Delicious, Elstar, and Cox's Orange at a 30−40% lower level. The same pattern was found in total catechin concentration, with a 10−20% lower total catechin concentration.

Quercetin glycoside concentrations are within the range described for eight different apple cultivars by Price and co-workers (*15*) [40−110 mg/kg of fresh weight (fw) after recalculation to quercetin glycosides], but Cox's Orange quercetin total glycoside content was lower than reported by them. In all cultivars, total catechin was higher than reported by Arts et al. (*26*). Varietal differences in chlorogenic acid are also high [30−430 mg/kg of fw (*27*)], and our findings are within this range.

In Table 5 the percentage of individual quercetin glycosides and catechins of their corresponding totals in the four cultivars is given. In Jonagold and Golden Delicious apples these quercetin glycoside profiles were similar; therefore, it is not possible to distinguish between these cultivars on the basis of their quercetin glycoside profile. In both cultivars Q-rhamnoside was the most abundant quercetin glycoside present, followed by Q-arabinoside and Q-galactoside. Q-xyloside, Q-glucoside, and Q-rutinoside had the lowest contributions (in decreasing order).

The quercetin glycoside profiles of Cox's Orange and Elstar apples were also similar to each other but differed substantially from those of Jonagold and Golden Delicious. In these cultivars Q-galactoside was the most abundant quercetin glycoside, followed by Q-arabinoside and Q-rhamnoside. Q-xyloside, Q-glucoside, and Q-rutinoside again had the lowest contributions (in decreasing order). All four apple cultivars showed very similar catechin profiles, with epicatechin as the predominant compound.

Storage. *CA Storage.* Storage at CA conditions did not have a significant influence on the total quercetin glycoside, phloridzin, and cyanidin galactoside concentrations in all apple cultivars (Figure 3). Chlorogenic acid and total catechin concentrations decreases were

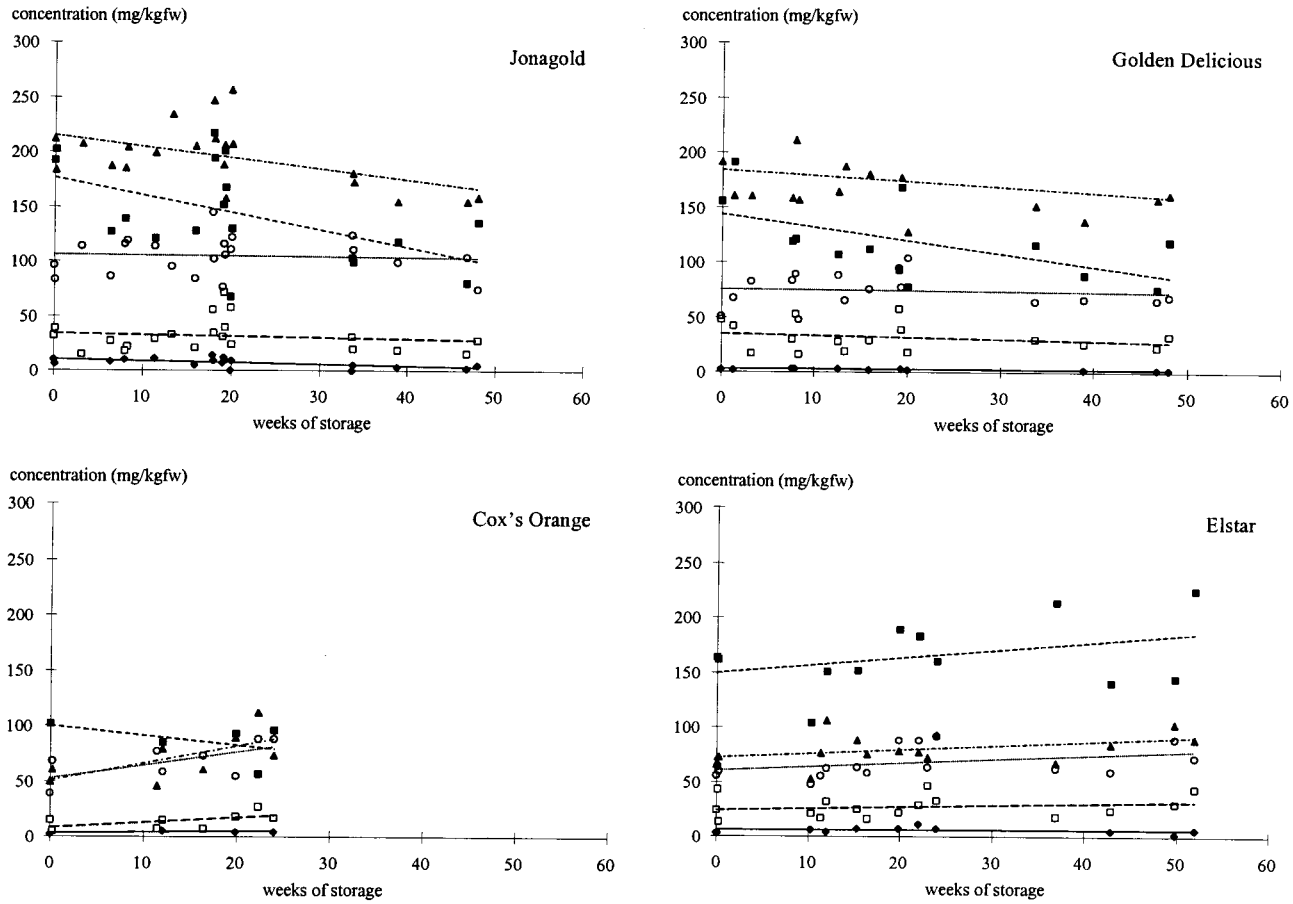

Figure 3. Flavonoid and chlorogenic acid concentrations (mg/kg of fw) of four apple cultivars (harvests 1996, 1997, and 1998) during storage in CA: Cy-Ga (◆); phloridzin (□); chlorogenic acid (▲); total Q-gly (○); total catechins (■). Apple samples were taken in duplicate (SD = 15−20%).

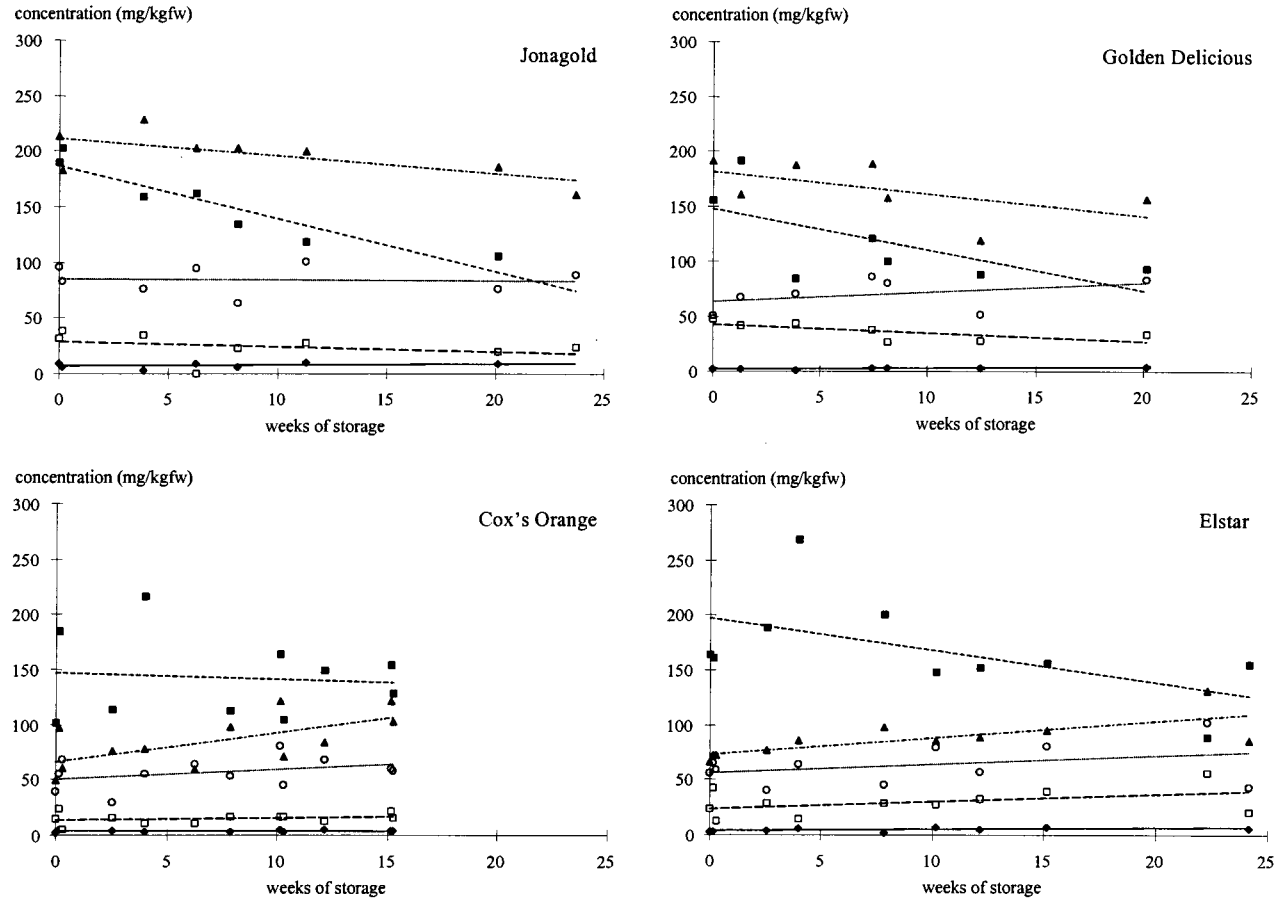

Figure 4. Flavonoid and chlorogenic acid concentrations (mg/kg of fw) of four apple cultivars (harvests 1996, 1997, and 1998) during cold storage: Cy-Ga (◆); phloridzin (□); chlorogenic acid (▲); total Q-gly (○); total catechins (■). Apple samples were taken in duplicate (SD = 15−20%).

significant in Jonagold apples. The observed decreases were not very high (18 and 40%, respectively), and after 52 weeks of storage, still substantial amounts were present. In Golden Delicious apples a small decrease in total catechin concentration was observed as well, but changes in chlorogenic acid concentration were not significant. In Cox's Orange and Elstar apples significant differences were observed in chlorogenic acid concentrations ($p = 0.03$ and 0.039, respectively, for the 3-year data), but in both cultivars this was observed in only one of the three years (1996 and 1998, respectively). Total catechin concentration remained fairly stable in Cox's Orange apples, and in Elstar apples small but significant changes were observed only in storage year 1997. The storage time of Cox's Orange apples (24 weeks) was much shorter than of the other three cultivars (48−52 weeks) as they began to develop brown spots and softened earlier.

Flavonoids appeared to be quite stable compounds during CA storage, as was observed by Awad and de Jager (*25*). They reported finding no significant changes upon 30 weeks of storage under regular and CA conditions.

Cold Storage. Cold storage was only possible for a shorter time compared to the storage at CA conditions. Figure 4 shows that cold storage did not have a significant effect on the total quercetin glycoside, phloridzin, and cyanidin galactoside concentrations in the four cultivars, corresponding to what was observed during CA storage. The chlorogenic acid concentration also did not significantly change during the 25 week period of cold storage. In total catechin concentrations, however, small but significant differences were observed in Golden Delicious (for only the year 1998), Cox's Orange (1997), and Elstar apples.

Antioxidant Activity during Apple Storage. IC_{50} values during storage of Jonagold, Golden Delicious, Cox's Orange, and Elstar apples for 1997 and 1998 are given in Figure 5. Cold storage or storage at CA conditions did not affect the antioxidant activity of the apple samples. The same differences in antioxidant potency among the four different cultivars were observed, as was described in Table 4.

In Table 6 mean concentrations of all apple samples collected during the three year (1996, 1997, and 1998) storage experiments (CA and cold storage) are given per cultivar. The sum of quercetin glycosides concentration shows that this value does not correlate with the order found in antioxidant activity differences. Jonagold possessed the highest quercetin glycoside concentration, followed by Golden Delicious. The lowest quercetin glycoside concentration was found in Cox's Orange and Elstar apples.

The total catechin concentration also did not show a correlation with the antioxidant activity of the various cultivars. Elstar had the highest total catechin concentration, followed by Jonagold. Golden Delicious and Cox's Orange had the lowest.

The sum of all analyzed compounds showed the following order: Jonagold > Golden Delicious > Elstar > Cox's Orange. Because Jonagold apples possessed the highest antioxidant activity, followed by Elstar, Cox's Orange, and then Golden Delicious apples, other compounds must be present in apples that contribute to the

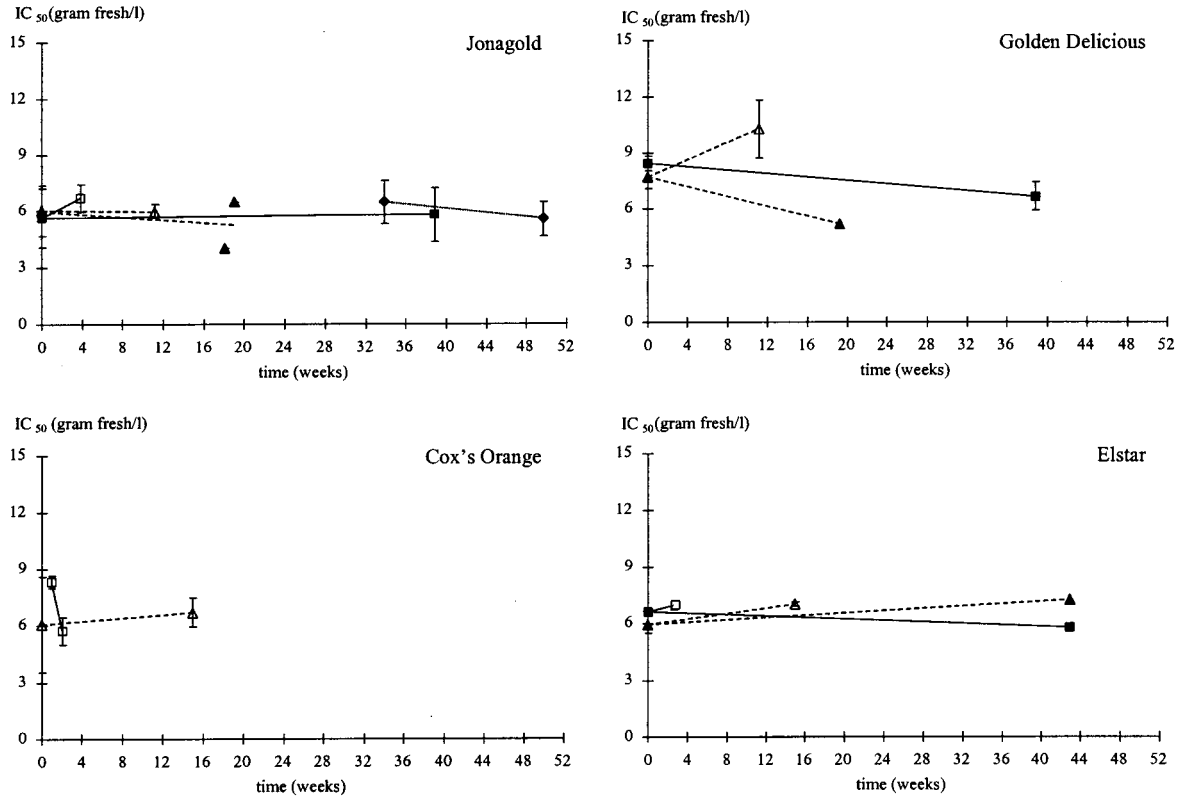

Figure 5. Antioxidant activity (g of fw/L) of four apple cultivars during storage in CA (solid symbols) and cold storage (open symbols): apples from harvest 1996 (◆), harvest 1997 (■), and harvest 1998 (▲). Samples were taken at least in duplicate (mean ± SD). Each IC_{50} value was based on triplicate determination of six different antioxidant concentrations ranging from no to full inhibition of lipid peroxidation.

Table 6. Comparison of Compositions and Antioxidant Activities of Four Apple Cultivars[a]

cultivar	total Q-glycosides (mg/kg of fw)	total catechins (mg/kg of fw)	sum of all compounds analyzed (mg/kg of fw)	IC_{50} (g of fresh/L)
Jonagold	95 ± 11	145 ± 37	467 ± 86	5.8 ± 0.8
Golden Delicious	67 ± 11	121 ± 29	385 ± 108	7.6 ± 1.9
Cox's Orange	64 ± 12	106 ± 47	265 ± 98	6.7 ± 1.1
Elstar	63 ± 12	152 ± 42	326 ± 100	6.6 ± 0.6

[a] Mean (± SD) of all apple samples during the 3 year (1996, 1997, and 1998) storage experiments (CA and cold storage).

antioxidant activity, such as procyanidins or vitamins, the presence of which has been reported (*28*−*30*). The existence of synergistic effects between compounds might be another explanation.

Implications for Product Development. If food producers want to pay more attention to possible health-protecting compounds in food processing and new product development, the choice of the raw material that is used is important. Of the four tested cultivars Jonagold apples possessed the highest antioxidant activity; therefore, this cultivar might be the most interesting choice of raw material to use in a product. Elstar apple antioxidant activity was lower, followed by Cox's Orange and Golden Delicious. No correlation between flavonoid concentration and antioxidant activity was found.

After choosing the apple cultivar with the highest antioxidant activity in the raw material, the producer has to take care that as much as possible of the active compounds remains in the product. The effects of storage have shown to be minor. For further processing to, for instance, apple juice, the producer should find the optimal way of processing to preserve as much as possible of these compounds and activity in the juice. This will be a subject of further research.

ABBREVIATIONS USED

Q-3-Ga, quercetin galactoside or hyperin; Q-3-Ru, quercetin rutinoside or rutin; Q-3-Gl, quercetin glucoside or isoquercitrin; Q-3-Xy, quercetin xyloside or reynoutrin; Q-3-Ar, quercetin arabinoside or avicularin; Q-3-Rh, quercetin rhamnoside or quercitrin; Cy-Ga, cyanidin galactoside or ideain.

ACKNOWLEDGMENT

We thank The Fruit Research Station in Randwijk, The Netherlands, for helping us to obtain the various apple cultivars during the research period. We are grateful to Simon Schouten and Jan Verschoor from The Agrotechnological Research Institute in Wageningen, The Netherlands, for kindly providing facilities for CA storage of the apple cultivars.

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Received for review December 19, 2000. Revised manuscript received May 23, 2001. Accepted May 30, 2001.

JF001493U