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Institute of Crop Science and Resource Conservation, Horticultural Science, University of Bonn

Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions

Anne Matthes, Michaela Schmitz-Eiberger

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Summary

The benefits of fruits and vegetables are often attributed to their high antioxidant content. Research supports a role of secondary plant metabolites particulary polyphenols in the prevention of degenerative diseases e.g. cardiovascular diseases and cancer. Apple fruit are an important source of secondary plant metabolites and one of the major phenol sources being consumed during the whole year. The present investigation was undertaken to determine antioxidant capacity in selected apple cultivars depending on cultivar and different modes of postharvest storage. Additional storage at 20 °C was tested to simulate the conditions at the consumers' home (shelf life). Antioxidant capacity differed between the cultivars. Cold storage (1 °C) for 4.5 months increased the antioxidative capacity and polyphenol content in most of the cultivars. Shelf life led to a decrease in polyphenol content and in antioxidant capacity. Storage under controlled atmosphere led to low increases of both antioxidant capacity and polyphenol content. In some cultivars polyphenol content remained stable. After the shelf life period lower values for antioxidant capacity were determined, in combination with no changes in phenol content. Correlation analysis showed a positive correlation between total phenols and antioxidant capacity (TEAC-Value). Lipophilic antioxidants decreased during storage.

Storage experiments indicated that a high content of polyphenols and antioxidants can be sustained by optimal storage conditions, these fruit may contribute to an antioxidant rich diet and may impart health benefits.

Introduction

In the mid-1990s the first epidemiological studies were published in which the intake of fruits and vegetables was inversely correlated to the mortality of cardiovascular diseases and other degenerative diseases (HERTOG et al., 1993a; SCALBERT et al., 2005b). Current evidence strongly supports the role of polyphenols in prevention of cardiovascular diseases, cancers and osteoporosis and assumes a contribution of polyphenols in preventing neurodegenarative diseases and diabetes mellitus (SCALBERT et al., 2005b). Increased formation of oxygen radicals, which may result in attack of different biomolecules such as lipids of biomembranes, DNA and other proteins, may be related to this age-related diseases. The protective effects of fruit and vegetable consumption may be attributed to the antioxidant properties of secondary plant metabolites such as carotenes, ascorbic acid and polyphenols, which are due to their ability to act as metal chelators, reductants, hydrogen donators and singulett oxygen quenchers (RICE-EVANS, 1995). Some animal and in-vitro studies support this relationship (EBERHARD et al., 2000; HALLIWELL et al., 2005).

Especially polyphenols belong to the most important bioactive components; a direct positive correlation between phenol content and antioxidative capacity was reported several times (KALT et al., 1999; SCHMITZ-EIBERGER et al., 2003). Recent studies reported that cells respond to polyphenols by a direct interaction with receptors and enzymes that modify the redox status of the cell (SCALBERT et al., 2005a). HALLIWELL et al. (2005) reported that phenols might exert positive effects directly in the gastrointestinal tract, including binding of prooxidant iron, scavening of reactive nitrogen, chlorine and oxygen species. Therefore, phenolic compounds commonly found in fruits and vegetables improve the quality and nutrional value for the consumer, and moreover the significance of the content of secondary plant metabolites will rise for the food producers in the future.

Total polyphenol intake is estimated to about one gram per day for people who eat several servings of fruit and vegetables during the day. That means the intake is ten times higher than that of ascorbic acid (SCALBERT and WILLIAMSON, 2000; MANACH et al., 2004). Yet not only the total intake should be high but also the bioavailability and metabolism of various substances should be taken into consideration in order to evaluate their biological activity in the body.

In Central Europe apples are an important product in the plant food market as they are available at markets the whole year, and for that reason they are one of the most important sources for secondary metabolites besides coffee, tea and onion in the Dutch diet (HERTOG et al., 1993b). Polyphenols are not homogenously distributed in apple fruit, higher phenol content was found in the peel than in the flesh (LATTANZIO et al., 2001; SOLOVCHENKO and SCHMITZ-EIBERGER, 2003; WOLFE et al., 2003). The main phenols in the flesh consisted of catechins, procyanidines, phloretin glycosides and chlorogenic acid, the peel contains higher amounts of these components and quercetin glycosides (BURDA et al., 1990; VAN DER SLUIS et al., 2001). During storage of apples changes of the content of bioactive components may occur as a result of metabolic degradation, respiration and synthesis processes. Especially during storage at ambient temperature in supermarkets or at consumers' homes there might be changes affecting the quality of the fruits. Changes might be attributed to diseases and physiological disorders. Fruit phenolics have also been implicated in pathogen resistance (MAYR et al., 1997) and some physiological disorders occuring during storage (LATTANZIO et al., 2001), so that a high phenol content can enhance postharvest disease resistance. Several studies on the impact of cold storage or controlled atmosphere on polyphenol composition of apple fruits have been carried out (AWAD and DE JAGER, 2000; VAN DER SLUIS et al., 2001; NAPOLITANO et al., 2004). It can be concluded from these studies, that polyphenols are stable during storage, however other studies observed an increase. Up to now it has not often been investigated in how far shelf life influences the content of secondary plant metabolites and antioxidant capacity.

The aim of this study was to determine polyphenol content and the antioxidative capacity of selected apple cultivars and to evaluate whether the phenol content correlates with antioxidant capacity. Furthermore we wanted to evaluate the effects of different storage conditions and the influence of shelf life on antioxidant content.

Materials and methods

Plant material and storage conditions

Fruit of 19 apple cultivars (*Malus domestica,* Borkh.) were cultivated at the centre of competence Klein-Altendorf, Germany. The 'AW 106'

cultivar is a new selection developed by the centre of competence. Streif-Index was used to determine maturity and as a consequence the optimal harvest date for every cultivar. The index was calculated as fruit firmness/ [soluble solid content x starch degradation value] (HÖHN et al., 1990). The harvest lasted from the beginning of September ('Elstar', 'Gala', 'Honeycrisp') to the end of October ('Fuji Kiku'). Subsequent to harvest the fruit were washed and the antioxidants extracted. To minimize variation ten fruits were randomly selected for analysis.

For storage experiment fruit were stored in controlled atmosphere conditions (CA) (1.5 °C, 1.2% O_2, 2.5% CO_2) or in a cold chamber (1 °C) for 4.5 months. Subsequent to this, some fruits were stored at shelf life conditions (20 °C) for two weeks to simulate the conditions at the consumers' home or during the handling in the production chain. Storage experiments were conducted with six cultivars: 'Wellant', 'Golden Delicious', 'Honeycrisp', 'Mairac', 'Pinova' and 'Topaz'.

Preparation of extracts

Extraction of hydrophilic antioxidants was carried out with a potassium phosphate buffer (10 mM di-potassium hydrogen phosphate (K_2HPO_4), 10mM potassium dihydrogen phosphate (KH_2PO_4), pH 7.1) containing 10 mM sodium dietyldithiocarbamate trihydrate (DIECA) and 2mM ethylenediaminetetraacetic acid (EDTA). Peel and pulp of a composite sampling of ten fruit was homogenized with buffer in a relation 1:1.5 w/v with a Retsch (Retsch, Haan, Germany) grinder. The extracts were prepared from the same ratio of peel and pulp for each cultivar. The core was removed before extraction. After incubation for four hours at room temperature, the homogenates were centrifuged at 4 °C for 15 min at 3500 rpm. The supernatant was collected and stored at -80 °C until analysis.

The preparation of the lipophilic extract was carried out as described by SCHMITZ and NOGA (2000). Two gram of chilled pulp were homogenized with 3 mL hexane using a Retsch homogenizer. Samples were centrifuged at 4 °C for 10 min at 4000 rpm, the supernatants were collected, blown up with nitrogen and filled up to 2 mL with hexane. Until analysis the extracts were stored at -80 °C.

Analysis of antioxidant capacity and total phenol content

The determination of the antioxidant capacity of the water-extract was performed as reported by MILLER et al. (1993) with some modifications. The method is based on the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) to form a radical cation $ABTS^{\cdot+}$ in the presence of hydrogen peroxide and metmyoglobin. The radical has a maximum of absorbance at 734 nm and a direct scavening of the formed radical by hydrogen-donating antioxidants resulted in a decrease of absorbance at 734 nm. The absorbance was measured after incubating for 6 min from start of the reaction in a Lambda 5/15 spectrophotometer (Perkin Elmer). 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), a water-soluble vitamin E analogon, was used as a standard and results were expressed as mg Trolox g^{-1} FW.

Total phenols were measured using Folin-Ciocalteau reagent (SINGLETON and ROSSI, 1965). An aliquot of 0.5 mL of the sample was mixed with 0.5 mL of destilled water and 0.5 mL of the Folin-Ciocalteau reagent was added. After 30 sec 5 mL of aqueous sodium carbonate were added, the mixture was incubated for 30 min at room temperature in the dark. Extinction was measured at 720 nm. Total phenol content was calculated using gallic acid as standard. Results were expressed as mg gallic acid units (GAU) 10^{-2} g^{-1} FW.

The determination of the antioxidant capacity of lipophilic extracts was determined according to CHEVOLLEAU et al. (1992). This method is based on the decoloration of β-carotene by radicals that are generated during the oxidation of linoleic acid at 50 °C. One milligram β-carotene was solved in 10 mL chloroform. To 5 mL of the carotine-chloroform-solution 20 mg linolic acid and 200 mg Tween 40 were added. After vaporization of chloroform with nitrogen the substances were filled up with distilled water to 100 mL.

An aliquot of 200 µL of every sample were vaporized with nitrogen, then mixed with 5 mL of the carotene-chloroform solution. The solutions were incubated in a 50 °C water bath and the extinction was measured every 30 min at 470 nm for a period of two hours. Antioxidant capacity was calculated as mE g^{-1} FW.

Statistical analysis

Analyses were performed at least in duplicate. The experimental data were analysed using the statistic program SPSS 12.0 for Windows (Munich, Germany). The statistical analysis was performed by one way Analysis of Variance (ANOVA), with a significance level of $\alpha = 0.05$. Comparisons of mean values were performed by the Tukey-Test. A normal distribution and variance homogeneity was implied. Any correlation was calculated as Pearson's coefficient of correlation.

Results

Total phenol content of apple cultivars ranged between 25 - 44.3 mg GAU 10^{-2} g^{-1} FW and a cultivar variation was noticed (Tab. 1). At harvest total phenol content was highest in the cultivars 'Cox Orange', 'Fuji Kiku', 'Rubinette' followed by 'Delia', 'Pinova' and 'Jonagold'. Lowest contents were measured in the cultivars 'AW 106', 'Braeburn' and 'Rubens' (Tab. 1).

'Pinova' and 'Topaz' fruit stored under CA conditions for 4.5 months showed a slight increase in total phenol content (Fig. 1). This was not the case for the cultivars 'Wellant', 'Honeycrisp' and 'Golden

Tab. 1: Polyphenol content (mg GAU 10^{-2} g^{-1} FW), antioxidant capacity of hydrophilic extracts (mg Trolox g^{-1} FW; TEAC-values) and lipophilic extracts (mE g^{-1} FW) in selected apple cultivars at harvest (Mean ± SE).

Cultivar	Total phenols	TEAC-value	Lipophilic antioxidant capacity
Cox Orange	44.3 ± 2.1	11.1 ± 0.0	4.1
Fuji Kiku	37.1 ± 0.0	13.8 ± 0.4	29.1
Rubinette	35.8 ± 1.5	11.1 ± 0.1	31.2
Delia	35.3 ± 0.6	8.3 ± 0.6	60.7
Pinova	35.1 ± 1.3	11.7 ± 0.5	27.4
Jonagold	34.3 ± 0.8	10.2 ± 0.1	17.2
Diwa	34.1 ± 0.4	11.3 ± 0.1	31.1
Topaz	33.1 ± 0.6	9.5 ± 0.3	15.9
Wellant	32.7 ± 2.0	9.7 ± 0.2	200.2
Cameo	31.4 ± 0.4	10.0 ± 0.1	18.2
Mairac	31.3 ± 0.8	7.5 ± 0.1	16.3
Grenstar	29.3 ± 0.0	6.9 ± 0.4	19.8
Elstar	29.2 ± 1.1	8.3 ± 0.2	23.1
Honeycrisp	28.6 ± 0.2	9.6 ± 0.2	2.7
Golden Delicious	27.3 ± 0.2	9.0 ± 0.5	50.5
Kanzi	26.0 ± 0.6	8.3 ± 0.2	33.8
AW 106	25.7 ± 0.1	6.7 ± 0.1	56.8
Braeburn	25.1 ± 0.5	2.5 ± 0.2	34.9
Rubens	25.0 ± 0.3	7.2 ± 0.2	37.5

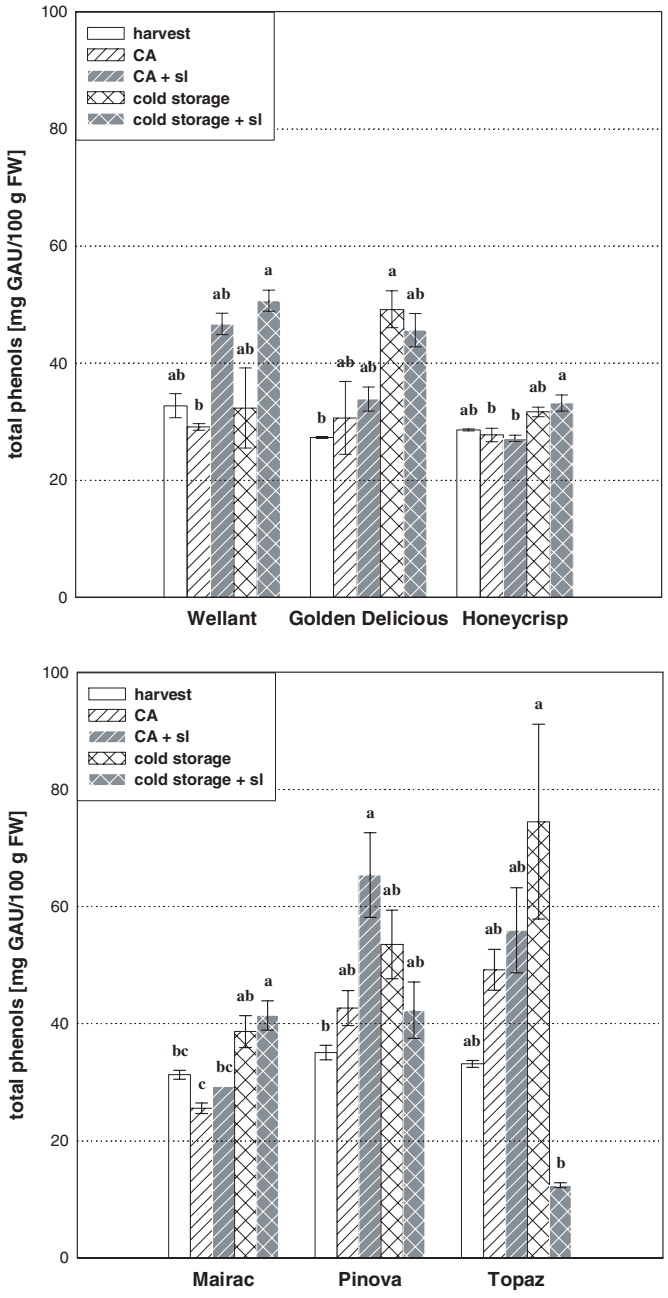

Fig. 1: Phenolic compounds in different apple cultivars at harvest, after cold and CA storage and after an additional shelf life (sl) period (Mean ± SE). Values followed by different letters within a cultivar are significantly different at 5% significance level.

Delicious', in which phenol content remained stable and for the cultivar 'Mairac' in which phenol content declined. During an additionally shelf life period in most cultivars no differences in polyphenol content were observed. Just in 'Wellant' and 'Pinova' fruit higher concentrations were found after the shelf life period in comparison to that determined after CA-storage (Fig. 1). Phenol concentration was affected by cold storage, which resulted in an increase in 'Pinova' and 'Topaz' fruit and a significant increase in the cultivar 'Golden Delicious'. In the other cultivars phenol concentration was constant during storage. Subsequent shelf life at ambient temperature led to different reactions in the fruit. In four cultivars ('Golden Delicious', 'Pinova', 'Mairac' and 'Honeycrisp') phenol content did not change, an increase was observed for 'Wellant' and a significant decrease was determined in 'Topaz', phenol content was only 50% in comparison to that measured at harvest. Total phenol content was more dependent on the cultivar but on storage conditions (Fig. 1).

Antioxidant capacity of the hydrophilic extract was measured using the TEAC-method. Highest TEAC-values at harvest were measured in 'Fuji Kiku', 'Pinova' and 'Cox Orange' followed by 'Rubinette' and 'Diwa'. The lowest antioxidant capacity was measured in 'Braeburn' (Tab. 1).

Stored cultivars showed an increase in TEAC-values if stored for 4.5 months irrespective of the storage condition (Fig. 2). This could not be observed in cold stored 'Pinova' fruit and in CA-stored 'Golden

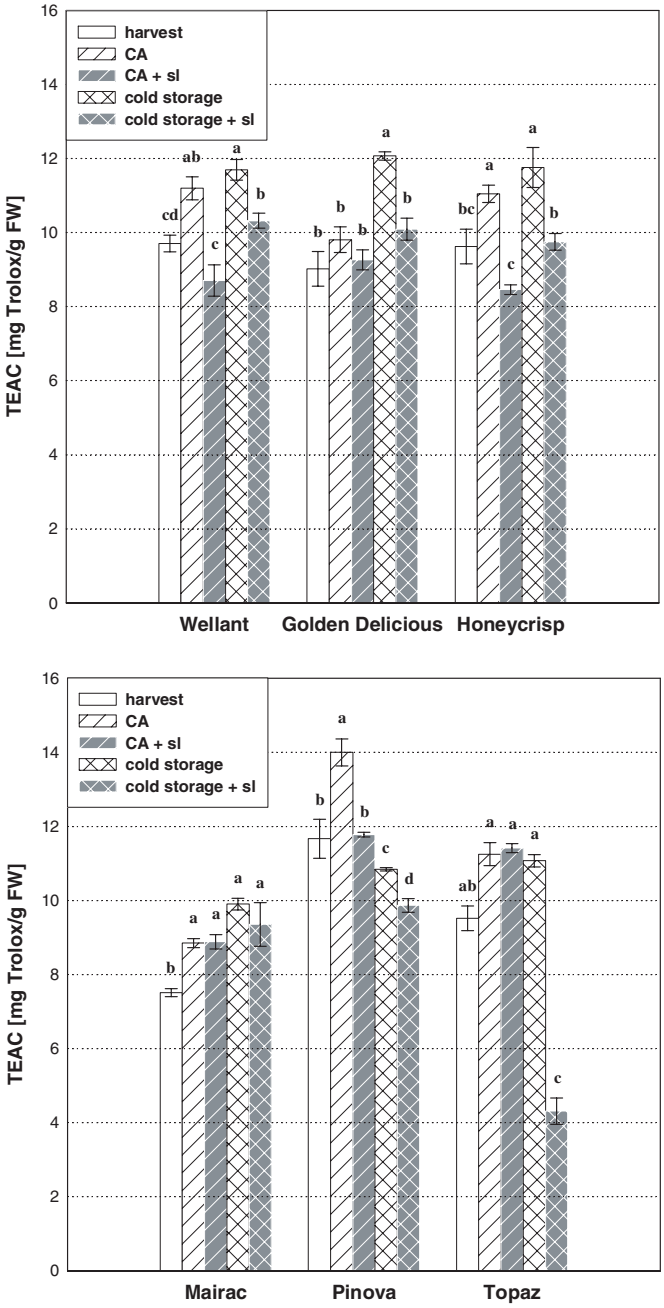

Fig. 2: TEAC-values in different apple cultivars at harvest, after cold and CA storage and after an additional shelf life (sl) period (Mean ± SE). Values followed by different letters within a cultivar are significantly different at 5% significance level.

Delicious' where antioxidant capacity remained unchanged. Storage at 20 °C for two weeks led to a significant decrease of antioxidant activity in most cultivars. For the cultivars 'Mairac', 'Golden Delicious' (CA-stored) and 'Topaz' (CA-stored) no changes in TEAC-values could be observed (Fig. 2).

Correlation analysis showed that total phenol content was positively correlated to the antioxidant activity (Tab. 2). A strong correlation between TEAC-values and the total phenol content was found after harvest, cold and CA-storage with additional shelf life. A moderate relationship was calculated for the fruit after CA-storage and no correlation was found after cold storage (Tab. 2).

Antioxidant capacity of the lipophilic extract was highest in the cultivar 'Wellant', followed by 'Delia', 'AW 106' and 'Golden Delicious'. Lowest antioxidant capacity was found in the cultivars 'Cameo', 'Jonagold', 'Mairac' and 'Topaz' (Tab. 1).

Lipophilic antioxidants decreased during storage in the cultivars 'Wellant', 'Pinova' and 'Topaz'. In fruit of 'Mairac', 'Honeycrisp' and 'Golden Delicious' highest antioxidant capacity was determined after cold storage in comparison to the values at harvest and after CA-storage. An additional shelf life period led to a further decrease in antioxidant capacity. Tendentiously higher values were found in cold stored fruit in comparison to fruit stored under CA conditions (Tab. 3).

Discussion

Phenolic compounds are important secondary plant metabolites and contribute to any health promoting effects of fruits and vegetables. Food producers and consumers spend increasing interest in the amount of certain health promoting substances. Therefore a close investigation is necessary to find out whether this metabolites can be enhanced by breeding and the selection of special cultivars. As apples are distributed the whole year it is important to investigate if this metabolites remain stable during storage and in how far any degradation processes can be decelerated by improving the storage conditions and in how far degradation processes are influenced by shelf life.

Several studies reported on the content of different bioactive components in apple fruit. These studies were mainly focused on the peel of the fruit, because higher concentrations of polyphenols were found there (LATTANZIO et al., 2001; SOLOVCHENKO and SCHMITZ-EIBERGER, 2003; WOLFE et al., 2003). We used whole apples, because usually apple fruit are consumed as whole fruits and water-soluble antioxidants are distributed in the pulp respectively. The variation of single components of polyphenols between individual apples was 10-30% in a study conducted by VAN DER SLUIS et al. (2001), so we used composite samples.

Polyphenol content was shown to be cultivar dependent. SCHMITZ-EIBERGER et al. (2003) investigated bioactive components in 31 apple cultivars. They found a large cultivar variation with highest contents in cultivars used for must and juice production. Some of the results of the current study are consistent with that of 2003 (e.g. low TEAC- and Folin-values in the cultivar Braeburn), contradictionary results were e.g. obtained for the cultivar 'Pinova' and 'AW 106'. This showed that the antioxidant content of apple fruit is affected by environmental conditions which support the biosynthesis of polyphenols, so that content may vary at different harvest years. In conflict to this VAN DER SLUIS et al. (2001) observed no seasonal effect on antioxidant capacity and flavonoid concentration in four apple cultivars when they compared the results of three different harvest years.

Polyphenols seem to be stable during storage as shown here and in several other studies. AWAD and DE JAGER (2000) observed no significant changes in flavonoid content upon storage for 30 weeks under CA and cold conditions in the skin of the apple cultivars 'Jonagold' and 'Elstar'. There were no significant differences in flavonoid concentration between fruit stored under CA and regular conditions (0 °C). Except for catechin the data of AWAD and DE JAGER (2000) showed that flavonoid and chlorogenic acid concentration remained constant during two weeks shelf life. LATTANZIO et al. (2001) determined a decrease in phenolics, particulary phloridzin after ten days of shelf life in 'Golden Delicious' fruit. VAN DER SLUIS et al. (2001) reported that CA and storage in a cold chamber did not influence flavonoid concentration and antioxidant activity of whole apple fruit of various cultivars. This is consistent with the findings of GOLDING et al. (2001) who found no great changes in the concentration of the major phenolics during long-term storage. NAPOLITANO et al. (2004) observed an increase in the flesh of 'Annurca' apple fruit in antioxidant activity during cold storage, which was correlated with the concentrations of catechin and phloridzin. In disagreement to this BURDA et al. (1990) found a decrease of catechin, but total phenol concentration stayed at a constant level. Another study detected a decrease in anthocyanins with simultaneously increasing phenol content (LEJA et al., 2001). LEJA et al. (2001) reported an increase in total phenol content and a doubling of antioxidant activity after four months of storage in a cold chamber and under CA conditions. An additional storage at 16 °C led to a further increase in total phenol content and to maintainance of antioxidant activity. The present results are different from

Tab. 2: Pearson's correlation coefficients (r) between phenolic compounds and antioxidant capacity (TEAC-values) of selected apple cultivars.

	Phenolic compounds				
	harvest	CA	CA + sl	Cold storage	Cold storage + sl
Antioxidant capacity	0.761 **	0.5	0.82 **	0.072	0.876 **

** significant at $p < 0.01$

Tab. 3: Lipophilic antioxidant capacity (mE g^{-1} FW) of selected apple cultivars at harvest, after cold and CA-storage and after additional shelf life (sl) period (Mean).

Cultivar	Harvest		CA		CA + sl		Cold storage		Cold storage + sl	
Wellant	200.2	a*	10.2	bc	13.4	b	12.7	b	6.6	c
GD	50.5	b	6.2	b	14.5	b	340.2	a	4.8	b
Honeycrisp	2.7	d	5.7	cd	15.3	b	21.7	a	6.8	c
Mairac	16.3	a	6.5	a	0	a	117.2	a	3.3	a
Pinova	27.4	a	12.7	bc	2.5	c	22.1	ab	2.2	c
Topaz	15.8	a	5.7	bc	0.3	c	12.7	ab	3.9	c

* Means with same letters within a cultivar are not significantly different, Tukey-test ($p \leq 0.05$).

those reported by TARROZI et al. (2004) who found lower phenol values in the peel of apple fruit after cold storage for three months, no further effect was seen after six month. No effect of cold storage was shown for gallic acid concentration in the pulp of the fruit. NAPOLITANO et al. (2004) reported a decrease of antioxidant capacity in a water extract of apples during storage of the fruit, which was related to the ascorbic acid degradation occuring during storage or any polymerization of free phenols into less water-soluble polymers. This effect was also shown here, even though the polyphenol content was constant during shelf life the TEAC-values decreased. This could be explained by lower amounts of synergistic antioxidants e.g. ascorbic acid (data not shown) and other lipid-soluble antioxidants, which could also be seen by lower antioxidant activity of lipophilic extracts after storage.

An increase of polyphenol content could be due to ethylene action. This phytohormone stimulates the activity of the key enzyme in polyphenol biosynthesis, the phenylalanine ammonium lyase (PAL), which leads to formation of polyphenols (BLANKENSHIP and UNRATH, 1988; LEJA et al., 2001; NAPOLITANO et al., 2004). PAL activity has been shown to have a direct influence on total flavonoids in apples (LISTER et al., 1996). LEJA et al. (2001) found a marked ethylene evolution in stored apple fruit, especially when stored in air, and reported on increased polyphenol contents during storage. TOMAS-BARBERAN and ESPIN (2001) reported that PAL activity is higher at lower temperatures. At the same time activity of enzymes responsible for polyphenol degradation, polyphenoloxidase, are inhibited by lower temperatures. LEJA et al. (2001) stated that an increase in phenol content could be due to lower activity of polyphenoloxidase at low temperatures, so that oxidation processes were minimalized. Increases in polyphenol content during shelf life can be explained by a higher ethylene production which results in a stimulation of PAL (NAPOLITANO et al., 2004). However, REYES (2003) indicated that ethylene and temperature did not significantly affect accumulation of polyphenols. But in his study phenol amount in purple flesh potatoes increased through mechanical damage, that led to activation of polyphenol biosynthesis probably via signalling pathways such as salicylic and jasmonic acids.

A high number of correlation studies can be found in literature. In consistence with our results a good correlation between antioxidant capacity and total phenol content was found in several studies (KALT et al., 1999; ARNOUS et al., 2001; SCHMITZ-EIBERGER et al., 2003). In contrast to this no correlation between the antioxidant capacity and any single phenolic compounds was found (ARNOUS et al., 2001; VAN DER SLUIS et al., 2001; SCHMITZ-EIBERGER et al., 2003). The lacking correlation after cold storage might be explained by synthesis of any other antioxidants during cold storage, so that antioxidant capacity was maintained.

Antioxidant activity is a result of several phytochemicals present in the fruit and their synergistic effects. The benefits of fruit and vegetable consumption can not be attributed to a single compound but to synergistic and additive effects between different phytochemicals (LIU, 2003). The contribution of ascorbic acid to the total antioxidant activity of fruits was determined to be generally lower than 15% (WANG et al., 1996), other authors calculated a contribution of ascorbic acid to antioxidant capacity of only 0.4% (KALT et al., 1999; EBERHARD et al., 2000; SCHMITZ-EIBERGER et al., 2003; WOLFE et al., 2003). However, synergistic effects between polyphenols and other antioxidants were described in earlier studies (FRYER, 1992; SAUCIER and WATERHOUSE, 1999; EBERHARD et al., 2000). Previous studies showed that the antioxidant and antiproliferative activities of apples are the consequence of synergistic activities of phenolics rather than ascorbic acid (EBERHARD et al., 2000; ARNOUS et al., 2001). Much attention has recently been paid to the possible health benefits of dietary phenolics which have stronger antioxidant activities than ascorbic acid. There is increasing evidence that phenolics can be absorbed into the human body in amounts that are sufficient to exert antioxidant properties in vivo. First studies showed a bioavailability of 52% of quercetin glycosides present in onions (HOLLMANN et al., 1997) and of 33% of chlorogenic acid present in a supplement (OLTHOF et al., 2001). The relative bioavalibility of quercetin from apples was only one-third of that from onions (HOLLMANN et al., 1997). That shows that bioavailability depends on the amount and kind of metabolite present in the food source and the food itself. Nevertheless further investigations have to be executed to investigate the absorption of different secondary metabolites, their tissue uptake, plasma concentrations and transport as well as metabolism and elimination, so that the metabolites can be identified that exert potential health benefits.

Conclusions

We conclude that polyphenolics vary among cultivars, they remain relatively stable during cold storage and storage at controlled atmosphere. Shelf life led to lower concentrations of antioxidants, which indicates that metabolism and turnover is higher at room temperature. The results obtained in the present study showed that bioactive components can be preserved by storage and can therefore obtain the high nutrional quality of apple fruits for the consumer. Consuming cultivars with high content of antioxidants even after storage may contribute to an antioxidant rich diet and may impart health benefits.

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Address of the authors:
Dipl. oec. troph. Anne Matthes and Priv.-Doz. Dr. Michaela Schmitz-Eiberger, University of Bonn, Institute of Crop Science and Resource Conservation, Horticultural Science, Auf dem Huegel 6, D-53121 Bonn, Germany
E-mail: amatthes@uni-bonn.de