Polyphenolic Antioxidants in Apples. Effect of Storage Conditions on Four Cultivars

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Abstract

Consumers' interest in the relationship between diet and health is increasing. From a production chain perspective, 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 consumed as fresh apples or used in processed apple products, were compared with regard to flavonol, catechin, phloridzin, and chlorogenic acid compositions and antioxidant activities. Jonagold apples possessed the highest flavonoid concentration and the highest antioxidant activity. In the four apple cultivars, 35% to 50% of the measured antioxidant activity could be ascribed to the compounds mentioned above. Total catechin concentration was the main contributor to the measured antioxidant activity in the analysed cultivars. Long-term storage, both at refrigerated temperatures and under controlled atmosphere conditions, was found to minimally influence flavonoid concentration. Antioxidant activity was not affected by the applied storage conditions.

INTRODUCTION

There is a growing interest in compounds in food which possess a possible health protecting capacity and which were previously regarded as non-nutrients. They hardly contribute to the nutritional value of the product, but might play an important role in maintaining human health. Flavonoids are an example of these compounds and in epidemiological studies inverse relationships between the levels of the compounds and ageing diseases, such as coronary diseases and cancer, have been described (Middleton and Kandaswami, 1994; Hertog et al., 1993). The benefits of the compounds are ascribed to their function as antioxidants, or modulators of enzyme activity (Parr and Bolwell, 2000).

The most important flavonoids present in apple and apple products are catechins, flavonols, and anthocyanins (Lister et al., 1994; Figure 1). Catechins are present in the apple skin and flesh (Arts et al., 2000). Flavonols are detected in the apple skin, with minor concentrations in the flesh (Price et al., 1999; Van der Sluis et al., 2001). Anthocyanins are present only in the skin in most apple varieties. Other compounds in apple which possess antioxidant activity are chlorogenic acid (a phenolic acid) and phloridzin (a dehydrochalcone).

The aim of this research was to provide a concept for an integrated approach in the development of health foods by setting up and using measurement systems to investigate health claims associated with these foods. The effect of storage and processing on the health benefits of apples and apple products is used as a case study. In this paper, the effect of storage (controlled atmosphere (CA) and cold air storage), on polyphenolic antioxidants and their activity will be described.

MATERIAL AND METHODS

Apple Cultivars and Storage Conditions

Four apple cultivars grown in the Netherlands were used: Jonagold, Elstar, Golden Delicious and Cox's Orange Pippin. The day of harvest was determined using variety-specific models (de Jager and Roelofs, 1996) that predict the optimal harvest date for Jonagold apples for long-term CA storage. Fruits were picked from the outer canopy, avoiding the top and bottom of the tree. Apples from the inner canopy of the tree were not used, since their total flavonoid concentration is much lower due to lack of light (Awad et al., 2001). Trees at the border of the orchard were avoided as well.

CA storage conditions of Jonagold, Golden Delicious and Elstar were 1.2% O_2 + 2.5% CO_2 at 1.5 °C. Cox's Orange was stored in 1.2% O_2 + 0.7% CO_2 at 4.0 °C. In cold air storage all apple cultivars were kept at 4°C. Apples were stored in 12 kg plastic boxes. Samples were taken regularly throughout the storage period.

Sample Preparation and HPLC Analysis

Four apples were chosen at random and in duplicate. The apples were cut into pieces with a stainless steel knife and subsequently ground to a fine powder (grinder: ATO-MSE mix) in liquid nitrogen. The frozen samples were stored immediately at -20°C until lyophilisation. Lyophilised apple samples were stored at -20°C as well.

Lyophilised apple samples (0.5 g) were extracted with 10 mL methanol and sonicated for 30 min followed by 10 min centrifugation at 2500 rpm. The supernatants were filtered through a 0.45-µm filter. Quercetin glycosides, catechins, chlorogenic acid, phloridzin and cyanidin galactoside in the supernatants were determined by HPLC (Van der Sluis et al., 2001)

Antioxidant Activity

Antioxidant activities in the supernatants were determined by an in vitro measurement in which lipid peroxidation (LPO) was induced in rat liver microsomes. The reaction was initiated by the addition of 10 µM Fe^{2+} and 200 µM ascorbic acid. Reaction products were measured spectrophotometrically by the thiobarbituric assay (A_{540}–A_{620}). Inhibition of LPO was an indication of the antioxidant activity (Van der Sluis et al., 2000).

The IC_{50} value gives the concentration of the antioxidant sample (an individual compound or food extract) at which 50% inhibition of lipid peroxidation occurs. The lower an IC_{50} value, the higher a compound's antioxidant activity. Each IC_{50} value was based on triplicate determination of six different antioxidant concentrations ranging from no to full inhibition of lipid peroxidation.

The antioxidant activity of apple samples is described as follows: $\left(\frac{1000}{IC_{50,sample}}\right)$, in which $IC_{50,sample}$ is the IC_{50} value of the apple sample (in g fresh wt/L).

RESULTS AND DISCUSSION

Varietal Differences in Apples

Four apple cultivars were compared with respect to their polyphenolic antioxidant composition and their antioxidant properties, before subjecting them to storage treatments. Figure 2a shows that Jonagold had the highest total catechin, total quercetin glycoside, and chlorogenic acid concentrations. The sum of all analysed compounds in the four cultivars showed the following order: Jonagold > Golden Delicious > Elstar > Cox's Orange.

The group "total q-glycosides" is composed of q-3-galactoside, q-3-rutinoside, q-3-glucoside, q-3-xyloside, q-3-arabinoside and q-3-rhamnoside. The group "total

catechins" consists of catechin and epicatechin. Cy-ga: cyanidin galactoside. Chlor.acid: chlorogenic acid. Mean ± SD are presented.

The IC_{50} values of standard compounds representing components detected in apple are presented in Figure 2b. Quercetin (aglycon) possessed the highest antioxidant activity.

Jonagold apples possessed the highest antioxidant activity (Figure 2c), followed by Elstar and Cox's Orange. Golden Delicious apples had the lowest antioxidant activity.

From the concentrations of compounds in an apple sample and the IC_{50} values of these individual compounds it is theoretically possible to calculate the antioxidant activity of that apple sample. Assumptions are made that no synergistic, antagonistic or other matrix effects play a role and that all compounds with antioxidant activity in the food product are known and detectable (Dekker et al., 1999). Calculated antioxidant activity values are also given in Figure 2c. In the four apple cultivars, 35% to 50% of the measured antioxidant activity can be ascribed to their total catechin, total quercetin glycoside, cyanidin galactoside, chlorogenic acid, and phloridzin contents. However, 50% to 65% of the antioxidant activity of the apples remains unexplained. Other compounds, such as procyanidins or vitamins, that contribute to the antioxidant activity have been reported to be present in apples (Lee and Mattick, 1989; Mayr et al., 1995; Guyot et al., 1997). The existence of synergism between compounds might also explain the discrepancy between measured and calculated antioxidant activity.

The contribution of flavonoids and chlorogenic acid to the measured antioxidant activity differed between cultivars (Figure 2d). In all cultivars, the group of 'total catechins' was the most important contributor (29% to 39%). The group 'total quercetin glycosides' was the second greatest contributor to the measured antioxidant activity, explaining 5% to 8% of the activity. Chlorogenic acid comprised only 1% to 3% of the measured antioxidant activity. The contribution of cyanidin galactoside in all apple cultivars was even lower. Phloridzin did not contribute to the measured antioxidant activity, due to its high IC_{50} value (thus low activity), in the assay.

Storage at Controlled Atmosphere

CA storage did not have a significant influence (at the 5% level) on the total quercetin glycoside, phloridzin and cyanidin galactoside concentrations for any of the four cultivars (Figure 3). However, CA storage caused a significant decrease in chlorogenic acid in Jonagold apples. Total catechin concentration remained fairly stable in Jonagold, Golden Delicious, and Cox's Orange, but in Elstar apples a small but significant increase was observed on the last date. In all apple cultivars, substantial amounts of polyphenolic antioxidants were present after the storage period. The storage time of Cox's Orange apples (24 weeks), however, was much shorter than that of the other three cultivars (48-52 weeks), since they began to develop brown spots and softened earlier.

Flavonoids appeared to be quite stable during CA storage. Awad and de Jager (2000) reported no significant changes during 30 weeks of storage under regular and CA conditions.

Cold Air Storage

Cold air storage did not have a significant effect on the total quercetin glycoside, phloridzin and cyanidin galactoside concentrations in the four cultivars (Figure 4), just as was observed during CA storage. Total catechin concentrations however, underwent a decline in Jonagold and a small increase in Cox's Orange. Chlorogenic acid concentration did not significantly change during 25 weeks cold air storage, except in Cox's Orange.

Effect of Storage on Antioxidant Activity

Cold air storage or storage under CA conditions did not affect the antioxidant activity of apple fruit (Figure 5).

CONCLUSION

Jonagold apples contained the highest level of flavonols and catechins and possessed the highest antioxidant activity, followed by Elstar and Cox's Orange. Golden Delicious apples had the lowest antioxidant activity. Antioxidant activity of the apple cultivars was not significantly affected by storage at near-optimal CA conditions, nor in air at refrigerated temperature. This indicates that CA and cold air storage do not apparently compromise antioxidant activity, so the potential health benefits are maintained under these storage conditions.

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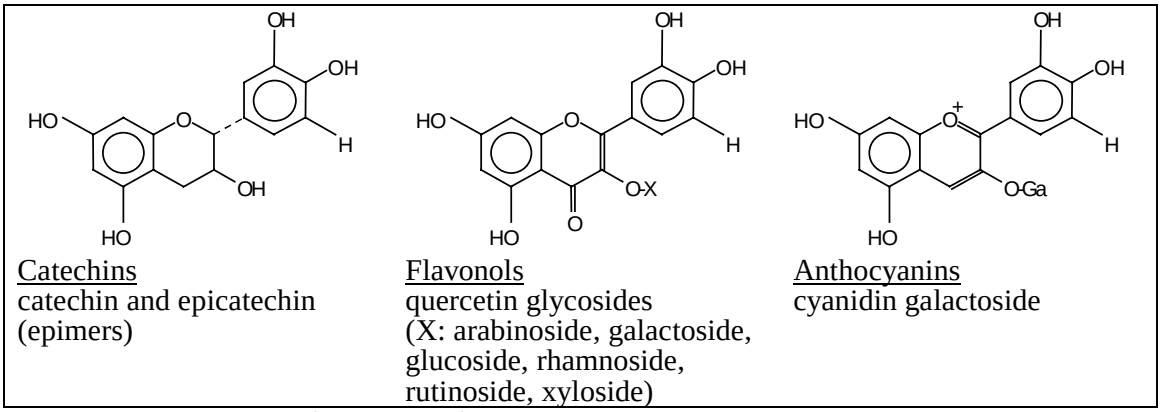
Figures



Catechins
catechin and epicatechin (epimers)

Flavonols
quercetin glycosides (X: arabinoside, galactoside, glucoside, rhamnoside, rutinoside, xyloside)

Anthocyanins
cyanidin galactoside

Fig. 1. Basic structure of catechins, flavonols, and anthocyanins.

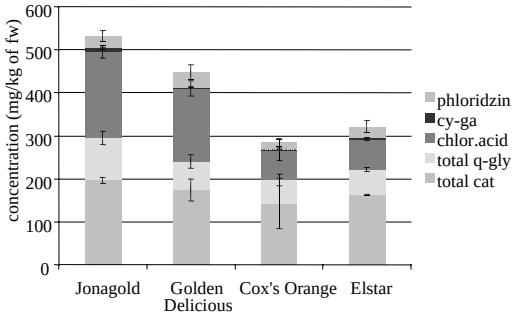


Fig. 2a. Variation in flavonoid and chlorogenic acid concentration (mg/kg on a fresh weight basis) of four apple cultivars at harvest time on a fresh weight basis (harvest years 1996, 1997, 1998).

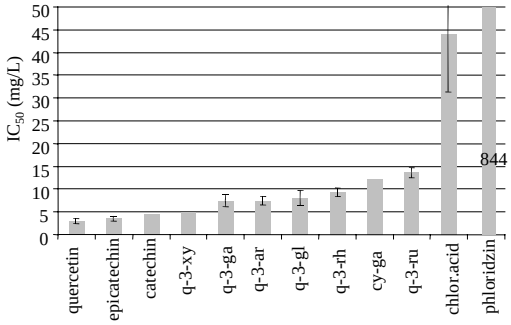


Fig. 2b. IC_{50} values (mg/L) of standards representing compounds detected in apple. Based on Van der Sluis et al., (2000).

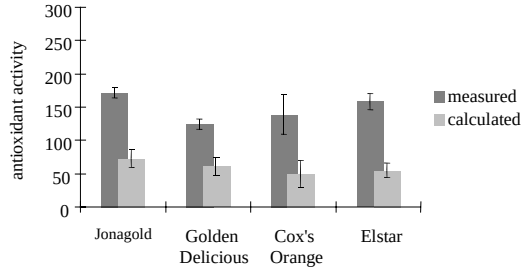


Fig. 2c. Comparison of the calculated antioxidant activity with the measured antioxidant activity of apple (extracts).

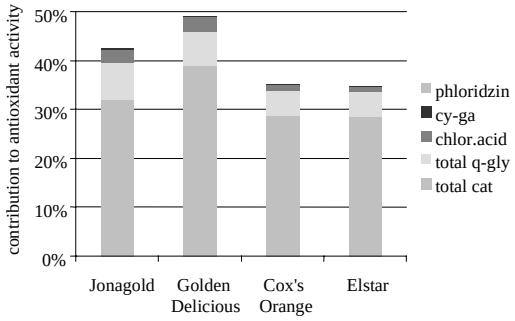


Fig. 2d. Contribution of selected polyphenolics to the measured antioxidant activity of apple.

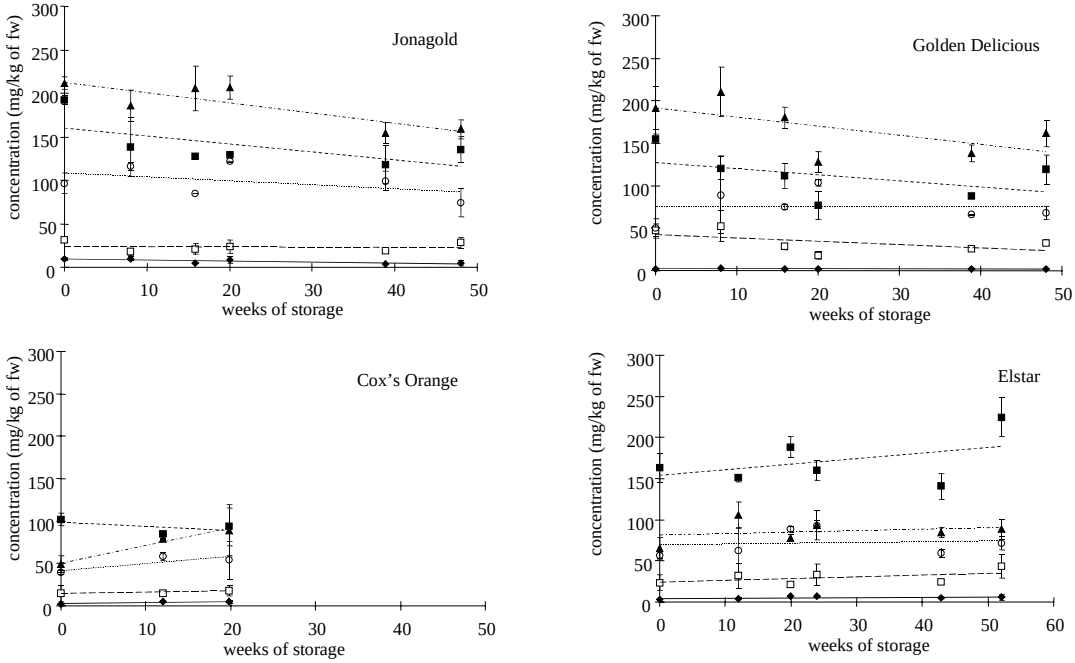


Fig. 3. Flavonoid and chlorogenic acid concentration (mg/kg on a fresh weight basis) of four apple cultivars (harvest year 1997) during CA storage. Apple samples were taken in duplicate (mean ± SD).
Cyanidin-galactoside (—◆—), phloridzin (- - - -), chlorogenic acid (-˙ -˙ ▲-˙ -˙), total quercetin glycosides (˙˙˙❍˙˙˙), total catechins (---■---).

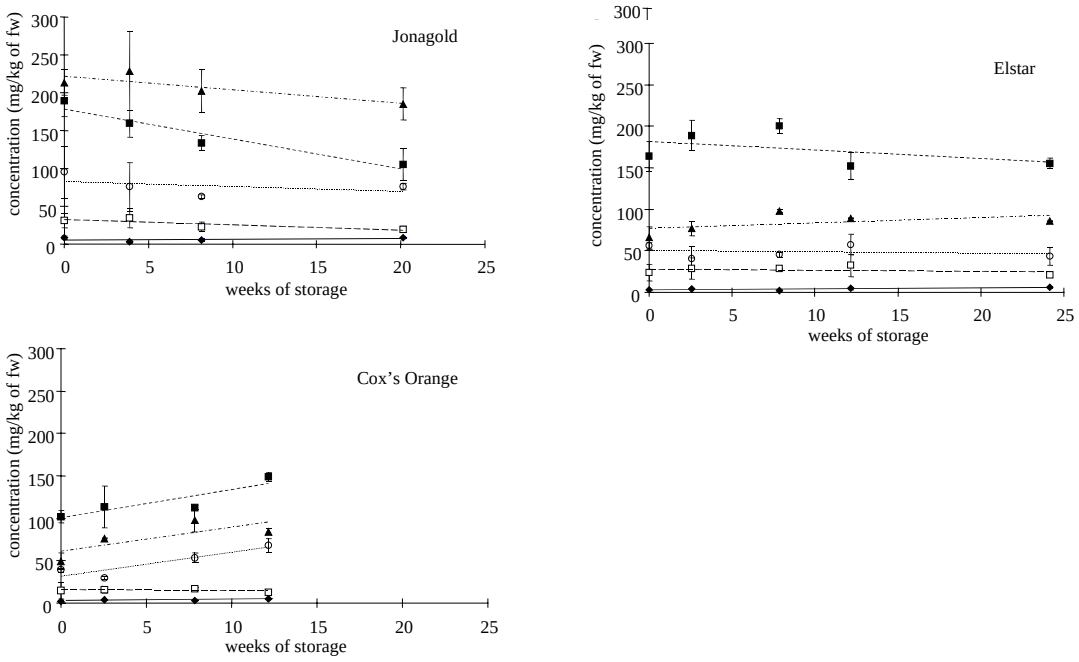


Fig. 4. Flavonoid and chlorogenic acid concentration (mg/kg on a fresh weight basis) of four apple cultivars (harvest year 1997) during cold air storage. Apple samples were taken in duplicate (mean ± SD).
Cyanidin-galactoside (—◆—), phloridzin (- - - -), chlorogenic acid (-· -· ▲-· -·), total quercetin glycosides (···○···), total catechins (---■---).

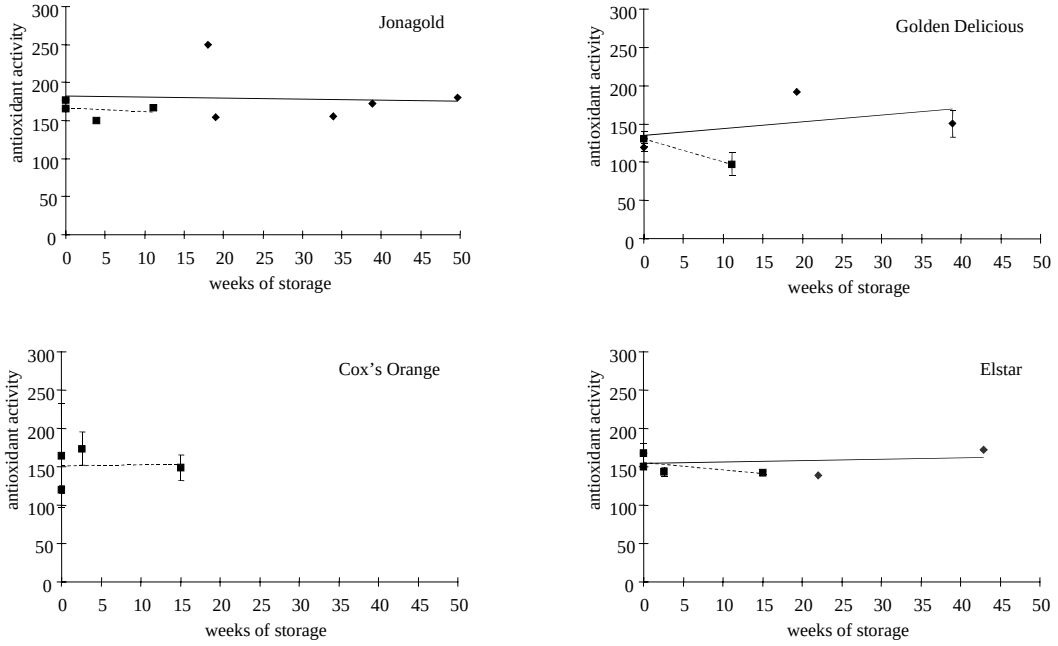


Fig. 5. Antioxidant activity of four apple cultivars during CA storage (—◆—) and cold air storage (---■---). Samples were taken at least in duplicate (mean ± SD).