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Activity and Concentration of Polyphenolic Antioxidants in Apple Juice. 1. Effect of Existing Production Methods

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Apples are an important source of flavonoids in the human diet. The effect of processing apples into juice on polyphenolic antioxidant content and activity is described. Raw juice obtained from Jonagold apples by pulping and straight pressing or after pulp enzyming had an antioxidant activity that was only 10 and 3%, respectively, of the activity of the fresh apples. The levels of flavonoids and chlorogenic acid in the juice were reduced to between 50% (chlorogenic acid) and 3% (catechins). Most of the antioxidants were retained in the pomace rather than being transferred into the juice. Apparently, most of the antioxidant compounds are absorbed to the solid matter of the pomace. In apple juice, 45% of the total measured antioxidant activity could be ascribed to the analyzed antioxidants. For three apple cultivars tested (Elstar, Golden Delicious, and Jonagold), the processing methods had similar effects. The results indicate that processing can have a major impact on the bioactivity of products.

KEYWORDS: Antioxidant activity; quercetin glycosides; catechins; phloridzin; anthocyanins; chlorogenic acid; processing; apple juice; cultivar

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. Those compounds, if derived from plants, are described as phytochemicals. 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 relations with aging diseases such as coronary heart diseases and cancer have been described (*1*−*3*). This is ascribed to their function as antioxidants, or in modulating enzyme activity (*4*).

To satisfy a growing demand by consumers for products with a high content of bioactive components, it is necessary to know the influence of the various stages in the food production chain on the presence of these compounds in the product. Cultivation methods (*5*), choice of raw material (*6*), industrial processing, storage (*7*), distribution, and final processing by the consumer may all affect the final concentrations and the bioactivity of the product (*8*). Knowledge of these aspects will provide the food processor with information that can be used in product optimization with respect to health-protecting compounds. This should be performed in a way that will not affect traditional quality aspects, such as color and taste.

The three most important groups of flavonoids present in apple and apple products are flavanols or catechins, flavonols, and anthocyanins (*9*), with the main representatives (−)-epicatechin, quercetin glycosides, and cyanidin galactoside, respectively. These compounds all belong to the group "polyphenolics", together with procyanidins, which consist of oligomeric and polymeric catechins (*10*), phloridzin and phloretin xyloglucoside (dihydrochalcones), and chlorogenic acid and *p*-coumaroylquinic acid (phenolic acids), which are also present in apple (*11*). Besides their contribution to potential health benefits, flavonoids contribute to the color and taste of apples. Their (bio)chemical and physical properties are important in processing.

Catechins are colorless and oxidation sensitive. Normally they occur as aglycons, but sometimes they are esterified with gallic acid (*12*). However, galloylated catechins have not been detected in apple (*13*). Catechins are, together with chlorogenic acid, good substrates for the enzyme polyphenoloxidase (*11*, *14*). This enzyme plays a role in the browning of fruits caused by damage, such as cutting. In the presence of oxygen, these substrates form brown complexes.

Flavonols are usually present in the plant as glycosides and are colorless or light yellow in color (*12*). They are not substrates

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for polyphenoloxidase, because of the steric hindrance of the sugars bound to the C3 atom in the flavonol skeleton (*15*).

Anthocyanins are glycosylated or acylated anthocyanidins and are more water-soluble than flavonols and flavanols. They are important plant pigments and contribute to the purple and red colors of flowers and fruit. They accumulate in the vacuoles of epidermal and subepidermal cells (*16*). Anthocyanins are unstable during chemical and physical processing. Their color is strongly pH-dependent (*17*).

After processing of apples to apple juice, a low flavonoid concentration is found in the juice. In commercially available apple juice, only 2.5 mg/L quercetin was detected (*18*), while in apples the concentration was 36 ± 19 mg/kg (analyzed as aglycon) (*19*). To know whether this loss in flavonoid content is due to degradation of these compounds during processing or to other factors, it is necessary to look more precisely into the apple juice production process. The basic processing method for apple juice consists of the following steps (*20*): apple fruit selection (on the basis of cultivar, maturity, and quality), washing and inspection, crushing, milling, or slicing to pulp, pressing (or extraction) to raw juice, clarification/filtration, pasteurization, and packaging.

To obtain a higher juice yield, enzymatic treatment of the pulp before pressing is often applied (*21*). Pectolytic enzymes are used to increase the pressability by degrading pectins in the cell walls. Before addition of pectolytic enzymes, the pulp can be aerated to allow oxidation of polyphenols. This may prevent inhibition of pectolytic enzymes by these compounds.

It is likely that the industrial practice of enzyming and oxidizing polyphenols may affect the antioxidant activity of the juice, as the polyphenols are important contributors to antioxidant activity. It is known that the polyphenol concentration indeed is affected by these treatments. Enzymed versus straight pressed Golden Delicious apple juice prepared by Schols et al. (*22*) showed a reduction in the levels of epicatechin, catechin, phloridzin, and chlorogenic acid. In apple juices from three different cultivars prepared by pulp enzyming, Spanos et al. (*7*) found no quercetin glycosides, a low catechin concentration (0−4 mg/L), 1−9 mg/L epicatechin, 5−12 mg/L phloridzin, and 17−59 mg/L chlorogenic acid, depending upon the cultivar. Others reported the presence of traces of quercetin glycosides in apple juice following commercial scale pressing (*23*) and in apple juice prepared in a domestic juice processor (*24*). In commercially available apple juice, no catechin or epicatechin was detected (*25*).

The objective of the present study was to investigate the effects of the various steps of existing production methods on flavonoid content and antioxidant activity of apple juice. Straight pressing of apple pulp is compared with enzyme treatment of the pulp before pressing. Three different apple cultivars that can be used in apple juice production were tested: Jonagold, Golden Delicious, and Elstar.

MATERIALS AND METHODS

Chemicals. Kaempferol, myricetin, quercetin dihydrate, and rutin trihydrate were purchased from Fluka Chemicals (Zwijndrecht, The Netherlands); chlorogenic acid, phloridzin, (±)-catechin, and (−)-epicatechin were from Sigma Chemicals (Zwijndrecht, The Netherlands); and quercetin 3-arabinoside, hyperoside, isoquercitrin, quercitrin, and ideainchloride were from Carl Roth GmbH and Co. (Karlsruhe, Germany). Quercetin 3-arabinofuranoside was obtained from Apin Chemicals (Oxfordshire, United Kingdom) and reynoutrin from Plantech (Reading, United Kingdom). L-(+)Ascorbic acid and iron(II) sulfate heptahydrate were obtained from Merck (Darmstadt, Germany). The enzyme Rapidase BE Super was supplied by Gist-Brocades International B.V. (Delft, The Netherlands). All other chemicals were of analytical or HPLC grade purity.

Apple Cultivars and Harvest and Storage Conditions. Three main apple cultivars grown in The Netherlands were used: Jonagold, Elstar, and Golden Delicious. Apples were harvested from commercial orchards in 1998, which were the same as described in Van der Sluis et al. (*6*).

The day of harvest was predicted using variety-specific models (*26*) that predict the optimal harvest date for Jonagold apples for long-term storage in controlled atmosphere (CA). This means that at the time of harvest, apples have not reached the stage of complete maturity suitable for immediate consumption.

Fruits from all apple cultivars were picked at one time, from the outer layer of the trees, avoiding the tops and bottoms. Apples from the inner layer of the tree were not used because their total flavonoid concentration is much lower due to lower light levels (*5*). Trees at the border of the orchard were avoided as well.

Applied CA storage conditions for Jonagold, Elstar, and Golden Delicious were 1.5 °C, 1.2% O_2, and 2.5% CO_2. After 3.5 months of storage, the apples were transported to MATFORSK, Norway, for processing of juice.

Sample Preparation. Flavonoid standards were dissolved in methanol. Fresh apple samples were taken as follows. Four apples were chosen at random. After cleaning with water and removal of stalks, the whole apples were cut to pieces with a knife and subsequently ground under liquid nitrogen in order to prevent oxidation. Duplicate samples were taken from each four-apple sample. At various stages during the apple juice production, duplicate samples were taken and also ground under liquid nitrogen. All frozen samples were stored immediately at −20 °C until lyophilization. Dry weight was determined from the sample weight before and after lyophilization. Lyophilized samples were stored at −20 °C in the dark until analyzed.

All samples were extracted before HPLC analysis and antioxidant activity determination. Lyophilized sample (0.5 g) was extracted with 10 mL of methanol, while juice samples were diluted to 50% with methanol. The samples were sonicated for 30 min, followed by 10 min of centrifugation at 2500 rpm. The supernatant was filtered through a 0.45-μm filter.

Quantification of Flavonoids by HPLC. Quercetin glycosides, catechins, chlorogenic acid, phloridzin, and cyanidin galactoside were determined in the apple samples by HPLC as described by Van der Sluis et al. (*6*).

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.

Quercetin glycosides were analyzed separately and presented as group "total Q-glycosides", which consists of the compounds Q-3-Ga, Q-3-Ru, Q-3-Gl, Q-3-Xy, Q-3-Ar, and Q-3-Rh (see Abbreviations Used). The catechins were also analyzed separately and presented as group "total catechins", which consists of the compounds catechin and epicatechin. Quercetin in aglycon form was not detected in any of the apple samples.

Antioxidant Activity Determination. Antioxidant activity was determined by an in vitro method in which lipid peroxidation (LPO) is induced in rat liver microsomes (*27*). The reaction is initiated by Fe^{2+} (10 μM) and ascorbic acid (200 μM). Reaction products are measured spectrophotometrically by the thiobarbituric assay ($A_{540-620}$). Inhibition of LPO is an indication of the antioxidant activity. The mean absorbance reading ($A_{540-620}$) ± standard deviation of the blanks was 1.026 ± 0.145 (in 70 repetitions collected on 6 experimental days).

The antioxidant concentration at which 50% inhibition of LPO occurs (IC_{50}) was calculated from triplicate determination of six different antioxidant concentrations ranging from no (0%) to full (100%) inhibition of LPO.

Apple Juice Production. Different types of apple juice were prepared. Starting weights of about 25 kg were used. Apples were cleaned by washing, stalks were removed, and the fruit was cut in two, all during a 30-min period. Apple pulp was prepared by quick slicing in a dicing machine (BL-1000, Eillert B.V., The Netherlands); this took

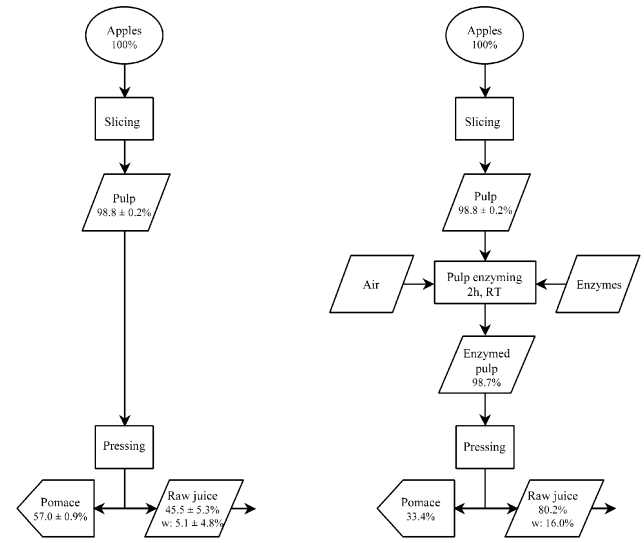

Figure 1. Processing schemes and standardized weights of apple fractions during Jonagold apple juice production. Comparison of straight pressing (a) and pulp enzyming (b). All percentages are related to the fresh apple fraction. Apple pulp particle size, 3 × 3 × 10 mm; w, amount of water in the raw juice that was added to standardize the obtained juice to 12 °Brix.

about 15 min. Two apple pulp particle sizes were prepared (3 × 3 × 10 mm versus 3 × 3 × 3 mm). Straight pressed apple juice was prepared by immediate pressing of apple pulp. Pulp-enzymed juice was prepared after addition of pectolytic enzymes (200 ppm Rapidase BE Super) to apple pulp (particle size 3 × 3 × 10 mm) and leaving the mixture for 2 h at room temperature with continuous stirring before pressing. Apple pulp was pressed in a Bucher-Guyer juice press (three times pressing to 160 bar). The pressing process lasted for about 80 min for the straight pressing juice and 50 min for the pulp-enzymed juice.

During apple juice production, samples of the apple fractions (fresh apple, pulp, pomace, and raw juice) were taken. All fractions were weighed, and mass balances were composed. From the mass balances, standardized mass balances were calculated in which the weight of the apple fractions was corrected for sample taking. No correction was made for losses that remained in the equipment during the production. The degree Brix of the obtained raw apple juices was determined, and in the standardized mass balances the raw juices were adjusted with water to 12 °Brix. This degree Brix level is higher than the 10.2 °Brix minimum value set for single strength apple juice not from concentrate (*28*).

Calculations. *Mass Balances.* Mass balances and compound mass balances were calculated for all processing steps described in **Figure 1**. An overall mass balance (eq 1a) describes the effect of washing and grinding of fresh apples to pulp. Compound mass balances were used for the 10 different flavonoids and chlorogenic acid; they were composed of the standardized weights of the apple fractions and the concentrations of those compounds present in the fractions using eq 1b.

The effect of washing and grinding:

$$\text{standardized mass balance:} \quad m_f = m_p + m_l \quad \text{(kg)} \quad \text{(1a)}$$

$$\text{compound mass balance:} \quad m_{f,i} \times c_{f,i} = m_{p,i} \times c_{p,i} + m_{l,i} \times c_{l,i} \quad \text{(mg)} \quad \text{(1b)}$$

with m the weight of the apple fraction (kg) and c the concentration of compound i ($i = 1-11$) in the apple fraction (mg/kg of fw). Apple fractions are represented as f, fresh apple; p, pulp or ground apple; and l, loss.

The effect of pressing can be described by eqs 2a and 2b:

$$\text{standardized mass balance:} \quad m_p = m_{pc} + m_{srj} + m_l \quad \text{(kg)} \quad \text{(2a)}$$

$$\text{compound mass balance:} \quad m_{p,i} \times c_{p,i} = m_{pc,i} \times c_{pc,i} + m_{srj,i} \times c_{srj,i} + m_{l,i} \times c_{l,i} \quad \text{(mg)} \quad \text{(2b)}$$

Apple fractions are represented as p, pulp or ground apple; pc, pomace; srj, standardized raw juice (12 °Brix); and l, loss.

Equations 3a and 3b describe the standardization of raw juice to a chosen degree Brix:

$$\text{standardized mass balance:} \quad m_{rj} + m_w = m_{srj} \quad \text{(kg)} \quad \text{(3a)}$$

$$\text{compound mass balance:} \quad m_{rj,i} \times c_{rj,i} + m_{w,i} \times c_{w,i} = m_{srj,i} \times c_{srj,i} \quad \text{(mg)} \quad \text{(3b)}$$

Apple fractions are represented as rj, raw juice; w, water; and srj, standardized raw juice.

Antioxidant Activity. The antioxidant activity of the apple samples is derived from the IC_{50} value (in grams of fw per liter), which gives the concentration of the antioxidant sample (an individual compound or food extract) at which 50% inhibition of lipid peroxidation occurs. The measured (or actual) antioxidant activity of an apple sample is expressed as the dilution factor that gives 50% inhibition ($DF_{50,sample}$) according to eq 4:

$$DF_{50,sample} = \frac{1000}{IC_{50,sample}} \quad (4)$$

where $IC_{50,sample}$ is the IC_{50} value of the apple sample (g of fw/L).

From the concentrations of compounds in a food product and the IC_{50} values of these individual compounds, it is theoretically possible to calculate the antioxidant activity of that product. Assumptions are 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 (*29*). To predict the antioxidant activity of the apple fractions from its composition, eq 5 and the IC_{50} values of 11 standard components as given by Van der Sluis et al. (*27*), recalculated to milligrams per liter, were used.

The calculated (or predicted) antioxidant activity of a mixture of known antioxidants is described by eq 5. The ratio in eq 5 equals the calculated dilution factor of the mixture of components to give 50% inhibition of lipid peroxidation ($DF_{50,mixture}$). In the ideal situation, where the mentioned assumptions are valid, the measured $DF_{50,sample}$ (eq 4) equals the calculated value (eq 5).

$$\frac{\sum_{i=1}^{n} C_i}{IC_{50,mixture}} = \sum_{i=1}^{n} \frac{C_i}{IC_{50,i}} = DF_{50,mixture} \quad (5)$$

where C_i is the concentration of component i (mg/kg of fw) and $IC_{50,i}$ is the IC_{50} value of component i (mg/L).

Antioxidant activity values of raw juices with values of $DF_{50,sample}$ below 30 are within the detection limit of the method. The values are obtained by extrapolation in the assay in the case where it was not possible to compose a concentration range that provided a range from 0% to 100% inhibition in the LPO assay. Therefore, these values might be less accurate. For lyophilized samples, this detection limit was 70.

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

RESULTS AND DISCUSSION

Existing Juice Production Methods: Straight Pressing and Pulp Enzyming. Straight pressed apple juice was prepared following the processing scheme described in **Figure 1a**. This figure shows the standardized weights (in % related to the fresh

Table 1. Mass Balances, Concentrations of Flavonoids and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight), and Antioxidant Activities of Apple Fractions during Processing of Jonagold Apple Juice: Straight Pressing versus Pulp Enzyming

							straight pressing						pulp enzyming		
	apples ($n = 5$)			pulp ($3 \times 3 \times 10$ mm) ($n = 4$)			pomace ($n = 3$)			standardized raw juice ($n = 3$)			enzymed pulp ($n = 1$)	pomace ($n = 1$)	standardized raw juice ($n = 1$)
	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD			
standardized mass balance (kg)	25.0	±	0	24.7	±	0.0	14.2	±	0.2	11.4	±	1.3	24.7	8.4	20.1
included added water (kg)										1.3	±	1.1			4.0
concentrations (mg/kg of fw)															
Cy-Ga	10	±	3	8	±	2	14	±	2	3	±	0	6	11	2
phloridzin	46	±	17	36	±	7	63	±	11	4	±	1	25	46	2
chlorogenic acid	202	±	33	178	±	16	170	±	20	133	±	15	100	87	63
total Q-glycosides	109	±	25	103	±	17	179	±	30	13	±	1	98	186	11
total catechins	186	±	26	176	±	13	173	±	19	16	±	1	74	105	6
measured activity: $1000/IC_{50}$ (L/mg of fw)	202.3	±	67.6[a]	195.0[b]			203.9[b]			21.2	±	18.4[a]			6.2[b]
calculated activity: $\sum(C/IC_{50})$	72.7	±	11.3	68.4	±	5.8	78.0	±	9.7	9.5	±	1.2	36.9	57.8	4.7
explained activity: calculated/measured	36%			35%			38%			45%					76%

[a] $n = 2$. [b] $n = 1$.

apple) of the apple fractions during processing. The initial degree Brix values of the juices were between 14.2 and 15.0, and the raw juices were adjusted to 12 °Brix for standardization purposes by adding water. The addition of standardization water causes the sum of the weights of the pomace and the standardized raw juice to be more than 100%.

Due to the low apple juice yield obtained by straight pressing of apple pulp (46% for Jonagold apples in a Bucher-Guyer press), pectolytic enzymes are often applied in industrial apple juice production to facilitate pressing and to increase yield. The processing scheme and standardized weights for the production of pulp-enzymed apple juice are given in **Figure 1b**. The juice yield increased to 80% for Jonagold apples when the pectolytic enzymes were applied. An alternative way to increase juice yield is to reduce the particle size of the pulp before pressing. In the present study, the juice yield increased from 45.5% to 59.2% when the particle size of the pulp was reduced from $3 \times 3 \times 10$ mm to $3 \times 3 \times 3$ mm. It is possible to increase the juice yield further by adding water to the pomace and applying a second pressing cycle (*22*).

The use of eqs 1a−3a enabled the calculation of the losses that occurred in apple juice production. The losses in the various apple fractions caused by material remaining in the equipment can be calculated from **Figure 1**. Slicing caused a loss of 1% of the apple starting weight, pressing a loss of about 3% (when no correction for the degree Brix values of the juices is made). In the present processing trial, these losses were small compared to the biological variation of flavonoid and chlorogenic acid concentration in apples (10−30%, **Table 1**).

To compare the two apple juice production methods with respect to their ability to give juice with high bioactivity, levels, and activity of antioxidants of pulps, data for pomaces and juices are presented in **Table 1**.

Apple Pulp. Slicing of the apples did not affect chlorogenic acid and flavonoid (cyanidin galactoside, phloridzin, total quercetin glycosides, total catechins) concentration or antioxidant activity (**Table 1**). Pulp enzyming caused decreases in phloridzin (31%), chlorogenic acid (44%), and total catechin (58%) concentrations. These compounds are oxidation sensitive and are known substrates for polyphenoloxidase. In the 2 h of pulp enzyming with continuous stirring, oxidation occurs. Cyanidin galactoside and total quercetin glycoside concentration were not affected by the extended oxidation period during enzyming, most likely because these compounds are not substrates for polyphenoloxidase (*15*, *17*).

The calculated antioxidant activity of enzymed pulp, based upon the analyzed antioxidants and using eq 5, was lower than that of the untreated pulp, due to the loss in total catechins and chlorogenic acid. Phloridzin does not contribute to the antioxidant activity with the method applied (*6*).

Pomace. The concentrations of all analyzed compounds were higher in the pomaces than in the corresponding juices (**Table 1**). Similarly, Price et al. (*23*) reported higher levels of quercetin glycosides in pomace than in juice. Lu and Foo (*30*) reported the presence of phloretin-2′-xyloglucoside and 3-hydroxyphloridzin in apple pomace from undescribed origin, in concentrations of 12% and 19% of that of phloridzin, respectively. They concluded that apple pomace contained a high level of polyphenols which could be commercially exploited.

Comparisons of both pomaces in the present study showed the same pattern as found in the apple pulp from which they were produced. The pomace from the pulp enzyming process was lower in phloridzin, chlorogenic acid, and total catechin concentration, while cyanidin galactoside and total quercetin glycoside concentration were unchanged in both pomaces. Due to the lower levels of chlorogenic acid and total catechins, the calculated antioxidant activity was approximately 25% lower for the pulp enzyming process than for the straight pressing process. The compound concentrations in the pomaces cannot be directly compared to their concentrations in the corresponding pulps, because pressing causes a split of the pulp into two fractions: the pomace and the raw juice. The use of compound mass balances prevents this problem.

Raw Apple Juice. Chlorogenic acid was found to have the highest concentration in the juice compared with the other compounds analyzed (**Table 1**). This indicates that during pressing only a small fraction of the analyzed compounds other than chlorogenic acid is extracted into the juice. The differences in water solubility between the different flavonoid groups and the chlorogenic acid might contribute to this observation. Chlorogenic acid is the most water-soluble compound, which explains the highest yield for this compound in the juice.

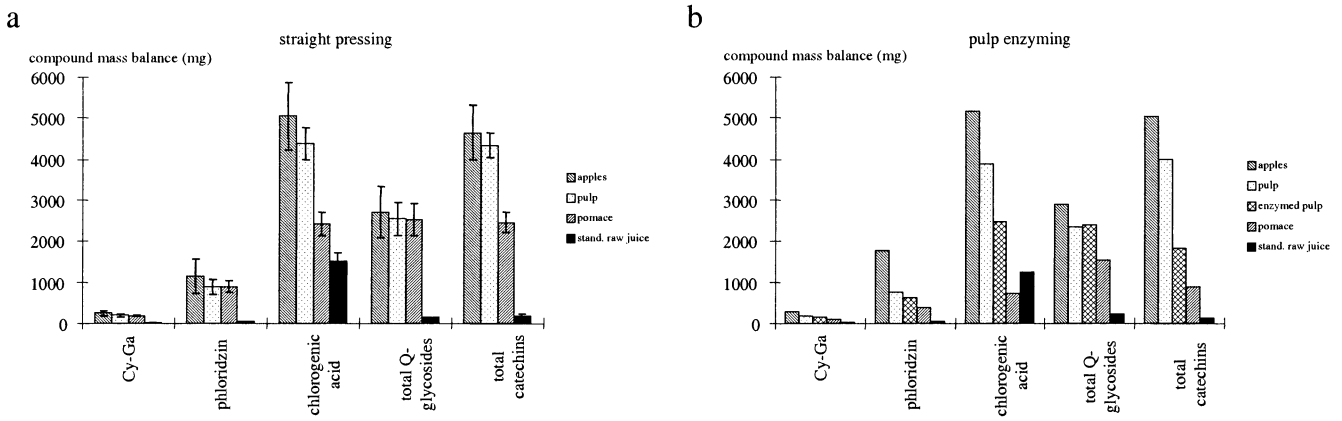

Figure 2. Compound mass balances of apple fractions in Jonagold apple juice production. Comparison of straight pressing (a, $n = 3$) versus pulp enzyming (b, $n = 1$). Starting weight was 25 kg. In panel a, each set of four bars shows (left to right) apples, pulp, pomace, and standardized raw juice. In panel b, each set of five bars shows (left to right) apples, pulp, enzymed pulp, pomace, and standardized raw juice.

Furthermore, flavonols and dehydrochalcones are mainly located in the "solid" parts of the fruits (such as the skin and seeds), which complicates their extraction.

In both raw juices, the levels of flavonoids and chlorogenic acid were reduced 2−30-fold when compared to those in the fresh apple fraction (depending on the different groups studied: quercetin glycosides 10-fold lower, phloridzin 10−20-fold lower, anthocyanins 5 times lower, and chlorogenic acid 2 times lower). The most striking reduction (30-fold) was that of the catechins in the juice obtained with enzyme treatment and aeration. Simultaneously, an extensive loss of antioxidant activity was observed. For both raw juices, the measured and calculated antioxidant activities were only a small fraction of that of the fresh apples they were produced from. The juice obtained by straight pressing or by incorporating an enzymatic treatment and oxidation step had a measured antioxidant activity that was only 10% and 3% of the original activity of fresh apples, respectively. The measured antioxidant activity of the raw juices was within the lower detection limits of the analytical method.

Flavonoid concentrations and antioxidant activity of apple juice prepared from 3 × 3 × 3 mm apple pulp did not significantly differ from those of juice prepared from the larger sized apple pulp (for which data are given in **Table 1**). Therefore, lowering the apple particle size did not improve the apple juice production method with respect to retaining polyphenolic antioxidant content and activity.

Mass Balances during Juice Production. The compound mass balances of components present in the various apple fractions of both treatments are presented in **Figure 2**. Equations 1b−3b were used for calculations. Chlorogenic acid was equally distributed between the pomace and the juice, while quercetin glycosides, total catechins, phloridzin, and cyanidin galactoside remained preferentially in the pomace. This indicates that, for most of the analyzed antioxidant components in apples, adsorption to the solid matter of the pomace is favored. Renard and co-workers (*31*) reported that procyanidins are able to bind to the cell wall matrix, whereas hydroxycinnamic acids and epicatechin are not.

Catechins are the most vulnerable compounds during apple juice production. The compound mass balance shows that the total amount of catechins in raw juice and pomace was 43% lower than the amount present in fresh apple or pulp (80% lower in the case of pulp enzyming). This loss may be explained by the oxidation sensitivity of the catechins. On the other hand, all quercetin glycosides could be traced back in the mass balance. The amount of quercetin glycosides present in the raw juice and pomace together was equal to the amount present in the fresh apple or in the pulp. In both apple juice production methods, slicing and pressing did not affect the quercetin glycosides. The pressing only caused a "partitioning" of these compounds between the pomace and the juice, in such a way that 93% of the quercetin glycosides was present in the pomace (from straight pressing). Phloridzin behaved similarly to the quercetin glycosides, and more than 77% of the initial level remained in the pomace.

The level of cyanidin galactoside was low in the apples and thus also in the various fractions from the juice processing, compared with the other polyphenolics analyzed. No significant losses occurred throughout the juice processing. In the straight pressing process, about 10% of the cyanidin galactoside occurred in the raw juice, while 80% remained in the pomace.

The use of compound mass balances was extremely useful in locating processing steps that affect the concentrations of compounds contributing to the antioxidant activity of the product. Its use revealed that, besides the partitioning phenomenon that caused losses during the production, another process, most probably oxidation (especially of the catechins), resulted in considerable losses of the antioxidant compounds of the apples.

Contribution of Compounds to the Measured Antioxidant Activity. Comparison of the measured antioxidant activity with the calculated antioxidant activity using eqs 4 and 5 (**Table 1**) showed that only about 35% of the antioxidant activity in fresh apples, pulp, and pomace and 45% in the raw juice can be ascribed to the analyzed components. In **Figure 3**, the relative contribution of each analyzed flavonoid and of chlorogenic acid to the measured antioxidant activity of the various fractions in the apple juice production is given. For all fractions, the group of "total catechins" was the most important contributor to the antioxidant activity. In straight pressed apple juice, the contribution to the measured antioxidant activity was 25% in the fresh apples, the pulp, and the pomace, and in the raw juice the contribution was 30%. The group "total quercetin glycosides" was the second most important contributor to the measured antioxidant activity, with 6%, 8%, and 12% in the fresh apples, the pulp, and the pomace, respectively. In the raw juice, however, chlorogenic acid was the second most important contributor, with 21% of the measured antioxidant activity, followed by total quercetin glycosides, with 12%. The contribution of cyanidin galactoside in all apple fractions was below 2%. Phloridzin did not contribute at all to the measured

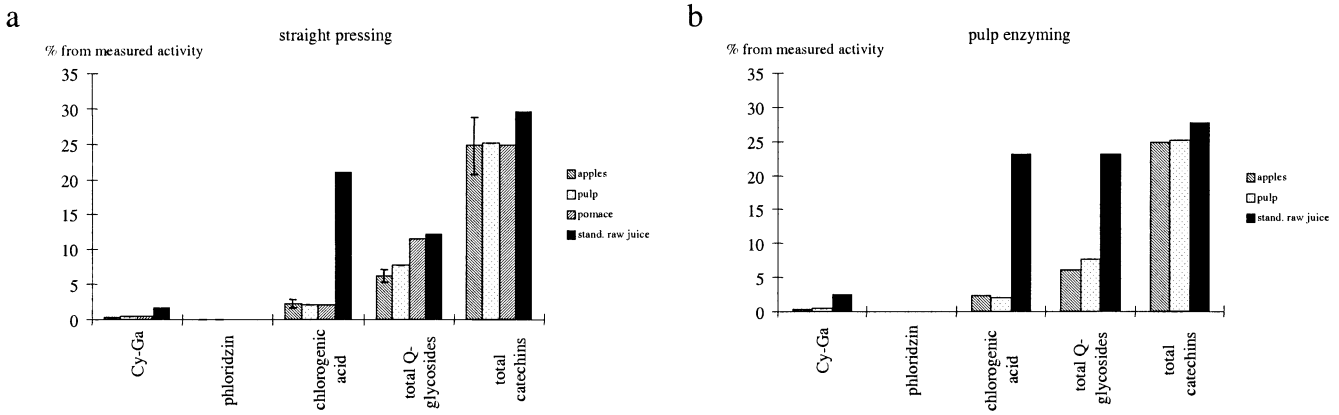

Figure 3. Calculated contribution of flavonoids and chlorogenic acid to the measured antioxidant activity of apple fractions in Jonagold apple juice production. Comparison of straight pressing (a, $n = 2$) versus pulp enzyming (b, $n = 1$). In panel a, each set of four bars shows (left to right) apples, pulp, pomace, and standardized raw juice. In panel b, each set of three bars shows (left to right) apples, pulp, and standardized raw juice.

antioxidant activity in any of the apple fractions, due to the low antioxidant activity of phloridzin in the assay (*27*).

The pulp after enzyming and the pomace were not analyzed for their antioxidant activity, but the results of the other fractions from the processing of pulp-enzymed apple juice were similar to those seen for straight pressed apple juice. The only difference occurred in the raw juice, where there was a higher (76%) total explained antioxidant activity (**Table 1**). The contribution of the group "total quercetin glycosides" was higher than that in straight pressed apple juice. This is explained by the higher decrease in the catechins by this method, as was discussed before.

To summarize these findings: the contribution of analyzed compounds to the measured antioxidant activity of fresh Jonagold apple, apple pulp, and pomace was total catechins > quercetin glycosides > chlorogenic acid > cyanidin galactoside ≫ phloridzin. In both raw juices, the contribution was total catechins > chlorogenic acid > quercetin glycosides > cyanidin galactoside ≫ phloridzin.

Comparison of antioxidant activity data of apple juice is, to a certain extent, possible with the findings of Miller et al. (*32*). They analyzed total antioxidant activity of apple juice from a not further specified production process, but with no added vitamin C. In their method, 51.4% of the total antioxidant activity was explained by analyzed compounds, with chlorogenic acid (32%) and phloridzin and phloretin glycosides (11%) as the most important contributors to the total antioxidant activity. In contrast to our results, quercetin glycosides were not detected in that study, and epicatechin was detected only in a negligible amount. Also, their use of a test system based on radical scavenging ability ($ABTS^{\bullet+}$) instead of the capacity to inhibit LPO may explain the discrepancies seen between the two studies. It has been suggested that antioxidant activity may differ between these assays, since they are based on different chemical and physical principles for monitoring oxidation (*33*). Phloridzin clearly shows differences in reactivity in both assays.

Antioxidant Activity Unaccounted For. A large proportion of the measured activity could not be ascribed to the analyzed antioxidants in the present study. The explanation for this difference could be the occurrence of synergism between the various antioxidant components. Further, the presence of compounds other than those analyzed might contribute to the antioxidant activity in apples and their products. These compounds could be procyanidins, which possess good antioxidant properties (*34*) and whose presence has been reported in apples (*35*−*37*) and in pomace (*38*). Other possibilities are carotenoids (*39*) and vitamins (*40*).

Table 2. Concentrations of Flavonoids and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight) and Juice Yields of Raw Apple Juices Produced by Straight Pressing, from Three Apple Cultivars

compound	Elstar ($n = 1$)	Golden Delicious ($n = 2$)	Jonagold ($n = 3$)
Cy-Ga	4	2 ± 1	3 ± 0
phloridzin	6	3 ± 1	4 ± 1
chlorogenic acid	48	17 ± 14	133 ± 15
total Q-glycosides	8	7 ± 2	13 ± 1
total catechins	33	5 ± 0	16 ± 3
juice yield (%) (12 °Brix)	34	42 ± 2	46 ± 5
included water (%)	3	4 ± 5	5 ± 4

In orange juice and black currant drink, 5% and 24%, respectively, of the antioxidant activity remained unaccounted for (*41*). In these juices, added vitamin C was the main contributor to the measured antioxidant activity. In the apple juices of the present study, no vitamin C was added. Miller et al. (*32*) reported that the contribution of ascorbic acid to the measured antioxidant activity of apple juice was only 1%. Similarly, Gardner et al. (*42*) reported that the contribution of total carotenoid content to the antioxidant activity of various fruit juices (apple juice included) was negligible.

To resolve the characteristics of the compounds that are providing extra antioxidant activity, the correlation of the unaccounted antioxidant activity with the calculated activity of all analyzed antioxidants in the fractions in Jonagold apple juice produced by straight pressing in the present study was determined. As can be seen in **Figure 4a**, a high correlation was obtained for total catechins ($R^2 = 0.994$). The correlation between explained and unaccounted antioxidant activity from total quercetin glycosides (**Figure 4b**, $R^2 = 0.753$) and cyanidin galactoside (**Figure 4c**, $R^2 = 0.716$) was less profound. Low correlation was found with chlorogenic acid activity (**Figure 4d**, $R^2 = 0.797$).

The high correlation for the explained and unaccounted antioxidant activity for the catechins indicates that the unknown antioxidant compound(s) behaved quite similarly to the catechins in the juice production process. Rice-Evans and Miller (*43*) suggested that unaccounted antioxidant activity presumably is derived from unidentified polyphenols and phenolic acids as

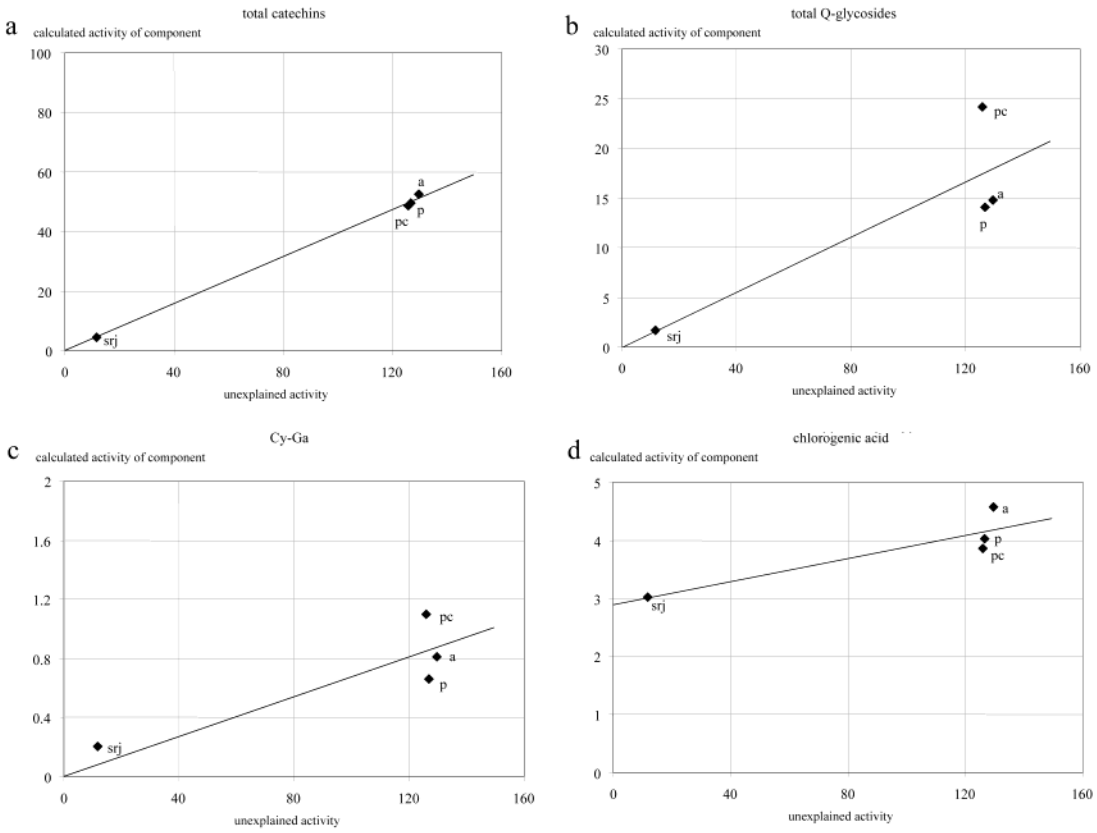

Figure 4. Correlation of the unaccounted antioxidant activity with the calculated activity of selected components of apple fractions in Jonagold apple juice produced by straight pressing. Symbols with letters are defined as follows: a, fresh apple; p, apple pulp; pc, pomace; and srj, standardized raw juice.

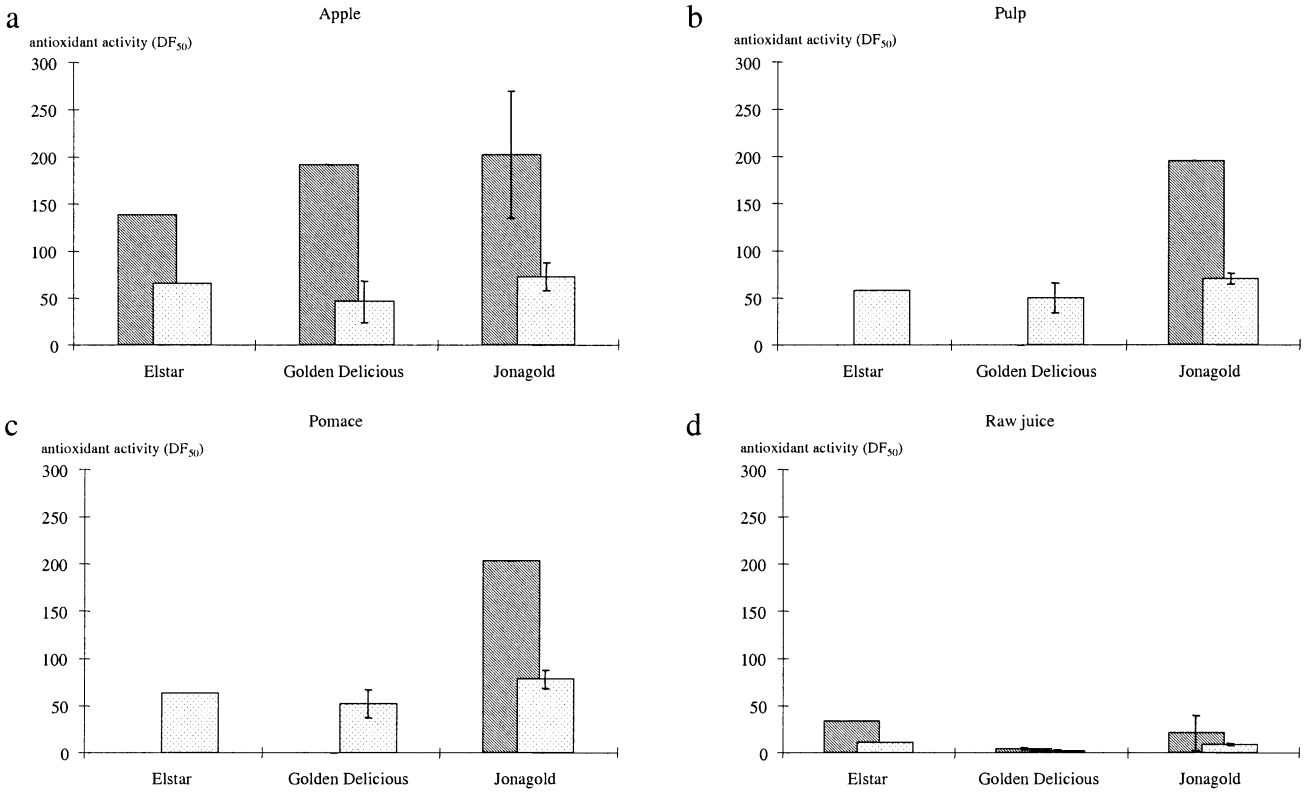

Figure 5. Antioxidant activity of various apple fractions during juice production (straight pressing). Comparison of three apple cultivars used as starting material. Striped bars represent measured antioxidant activity. Dotted bars represent antioxidant activity calculated from composition.

well as polymers formed from them. The results of the present study may thus add more specificity to this explanation by suggesting that the unidentified compounds could be polymeric catechins, such as procyanidins (*38*).

These findings demonstrate that it is necessary to measure the antioxidant activity of potentially important intermediate fractions during processing with the purpose of locating processing steps that affect unanalyzed compounds that are important for the antioxidant activity of a product. This information can be used for product and process optimization.

Choice of Apple Cultivar in Apple Juice Production. Three apple cultivars (Elstar, Golden Delicious, and Jonagold), which all can be used as starting material for apple juice, were compared. In **Table 2**, the flavonoid and chlorogenic acid concentrations of the raw apple juices as well as the juice yield from the three apple cultivars are given. Elstar apples seemed to give a lower juice yield than Golden Delicious and Jonagold apples, but the difference was not significant.

The juices prepared from the three cultivars had low concentrations of all analyzed compounds compared to the concentrations in the fresh apples. This confirms the finding that flavonoids preferably remain in the pomace and are hardly extracted into the juice. The concentrations of polyphenolic compounds in Elstar and Golden Delicious apples have been described previously (*6*). The low flavonoid and chlorogenic acid contents of the raw apple juices indicate that the apple juice production method itself appears to have a greater influence on the concentration of these compounds than the variation between cultivars.

Figure 5 shows the antioxidant activity of the various apple fractions occurring in juice manufacture from these cultivars. The measured antioxidant activity of the raw apples and their juices, as well as the antioxidant activity calculated from the apples' composition, are given. There were no significant differences in antioxidant activity between the three cultivars for any of the apple fractions tested. This is also valid for the calculated antioxidant activity of the four apple fractions, with the exception of the calculated antioxidant activity of raw juice produced from Golden Delicious apples, which was lower. The measured antioxidant activity of the raw juices was only 17%, 6%, and 13%, respectively, of the initial activity of fresh Elstar, Golden Delicious, and Jonagold apples.

Possible Enhancement of Antioxidant Activity of Apple Juice. The results of the present study clearly show that processing can have a major impact on the potential health benefits of a product. Therefore, studying the effects of processing methods on both levels of active compounds and biological activity of the different fractions occurring during processing will provide tools for finding the most efficient steps to improve a product with respect to its healthiness.

The fact that flavonoids and other compounds that contribute to antioxidant activity preferentially remain in the pomace offers a possibility for apple juice optimization with respect to flavonoid content and antioxidant activity. These compounds are not deteriorated or lost during juice manufacture, but remain in the pomace, which normally is a waste from the apple juice production. Thus, it is a challenging option to search for methods in which the flavonoids may be extracted from the pomace and later added to the final apple juice.

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.

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