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Activity and Concentration of Polyphenolic Antioxidants in Apple Juice. 2. Effect of Novel Production Methods

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There is a great interest in food components that possess possible health-protecting properties, as is the case with flavonoids. Previous research showed that conventional apple juice processing resulted in juices poor in flavonoids and with a low antioxidant activity. This paper shows that it is possible to improve flavonoid content in juice and its antioxidant activity by applying an alcoholic extraction either on the pulp or on the pomace. The levels of flavonoids and chlorogenic acid in enriched juice were between 1.4 (chlorogenic acid) and 9 (quercetin glycosides) times higher than in conventional apple juice. In enriched juice the antioxidant activity was 5 times higher than in conventional apple juice, with 52% of the antioxidant activity of the originating fruits present. The novel processing method had similar effects for three apple cultivars tested (Elstar, Golden Delicious, and Jonagold). The taste and color of enriched juice were different from those of conventional juice.

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

INTRODUCTION

There is growing interest in food components that possess possible health-protecting properties, such as flavonoids, which are polyphenolic antioxidants. Epidemiological studies showed inverse relationships between some of these compounds and aging diseases such as coronary heart diseases and cancer (*1*, *2*). This is ascribed to their function as antioxidants or by modulation of enzyme activity (*3*). Consumption of 700 mL of apple juice increased plasma antioxidant activity in human volunteers (*4*).

Being secondary plant metabolites, flavonoids are present in fruits and vegetables. In apple and apple products the most important groups of polyphenolic antioxidants present are flavonols (with quercetin glycosides as the main representative), flavanols (or catechins) and their oligo- and polymers, and anthocyanins (*5*). Furthermore, dihydrochalcones (e.g., phloridzin) and phenolic acids (such as hydroxycinnamic acids and, in particular, chlorogenic acid) are present in apple (*6*).

Before consumption, fruits and vegetables may undergo different forms of processing. Cultivation methods, industrial processing, storage, distribution, and final processing by the consumer all will affect the final concentration of flavonoids in the product and their bioactivity (*7*). Therefore, it is important for a food processor to have insight in these factors, because it provides information that can be used in product and process design and optimization.

Previous work showed that processing apples into juice results in big losses of flavonoids. Conventional apple juice production (straight pressing of apple pulp or pressing after pulp enzyming) resulted in a juice poor in flavonoids and with only 3−10% of the antioxidant activity of the fruit they were produced from (*8*). Several possibilities exist within the juice production chain to enhance the flavonoid content of apple juice: by choice of the raw material or production methods and processing conditions or by adding additional flavonoids from other sources to the juice.

Using conventional cross-breeding or even by genetic modification, it seems to be possible to enhance the flavonoid content of apples. Examples of genetic manipulation of antioxidants in plant foods are described, but in the case of flavonoids the interrelation of the flavonoid classes makes it difficult to predict the outcome of overexpressing enzymes in the biosynthetic pathway, which is an option for the manipulation (*9*). However, Muir et al. (*10*) succeeded in producing transgenic tomato lines that contained up to 78-fold more flavonols in the peel, mainly quercetin-3-rutinoside. The tomato flesh did not accumulate significant amounts. Furthermore, the concentration of flavonoids in apple fruit skin can be increased by optimizing fertilization in the orchard, especially that of nitrogen (*11*).

The possibility of improving the flavonoid content of apple juice by adjustments in production and processing methods

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seems to be easier and faster to implement than changes in the raw material itself. Effects of various production methods on the flavonoid content of apple juice are to some extent already described in the literature. Spanos and co-workers (*12*) compared the effect of straight pressing and diffusion extraction at various temperatures on the content of quercetin glycosides, catechin, epicatechin, and phloridzin in apple juice produced from Red Delicious apples. They reported a 3−5-fold increase in catechin, epicatechin, and phloridzin content, an almost 2-fold increase in chlorogenic acid content, and an increase from 0 mg/L quercetin glycosides in apple juice obtained by straight pressing to 38 mg/L in apple juice obtained by diffusion extraction at 73 °C (*12*). In diffusion extraction the elevated temperatures inactivate the enzyme polyphenol oxidase. Oxidation products are hardly formed, and a larger part of the polyphenols originally present in the apple is found in the juice (*13*). Disadvantages of this method are that deviation in aroma and taste may occur and that the juice is more susceptible to browning (*13*).

Schols and co-workers (*14*) showed that it was possible to increase the catechin, epicatechin, phloridzin, and chlorogenic acid concentration of apple juice produced from Golden Delicious apples by liquefaction (300 ppm of pectolytic enzyme, 4 h, 45 °C) of the apple pulp. The effect on quercetin glycosides was not determined.

The fact that in conventional apple juice production methods >80% of the quercetin remains in the pomace suggests that the production process may be optimized (*15*). At any time the effect of changes in production and processing methods on other quality factors (such as taste, color, and keepability), production efficiency, economic feasibility, and consumer acceptance should be taken into account. In addition, restrictions imposed by food laws regarding the final product should be obeyed.

We explore the possibility of performing a specific extraction on the apple pulp or on the pomace. In flavonoid analysis alcoholic extraction is often used when plant samples are prepared for analysis (*16*). Flavonoids have to be removed from the matrix in which they are present, which is exactly the same objective in flavonoid-rich apple juice production.

In this paper the design of a process to obtain an apple juice with an enhanced content of polyphenolic antioxidants is presented. Enriched apple juice was produced by performing an extra extraction on pomaces obtained in conventional juice production methods. Pomaces obtained after straight pressing of apple pulp and after enzyme treatment of the pulp before pressing were compared as starting materials. The effects of pomace extraction on polyphenolic antioxidant content as well as antioxidant activity were assessed. Three different apple cultivars were tested: Jonagold, Golden Delicious, and Elstar. Antioxidant activity determined in a microsomal oxidation assay was used as a measure of bioactivity for apple and apple products. The activity of the analyzed compounds in the juice was calculated and compared to the measured one.

MATERIALS AND METHODS

Materials. Chemicals, apple cultivars (Jonagold, Golden Delicious, and Elstar), harvest year (1998, if not stated otherwise), harvest conditions, and storage conditions were the same as described in Van der Sluis et al. (*8*).

Methods. Sampling and sample preparation, extraction before HPLC analysis, and antioxidant activity determination are described in Van der Sluis et al. (*8*). Extracted pomace samples were lyophilized, juice samples were not. The same extract was used for both HPLC analysis and antioxidant activity determination.

Quantification of flavonoids by HPLC and HPLC equipment are described earlier (*17*). Quercetin glycosides were analyzed separately and presented as the 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. The catechins were also analyzed separately and presented as the group "total catechins", which consists of the compounds catechin and epicatechin. Quercetin in aglycon form was not detected in any of the apple samples.

The antioxidant concentration at which 50% inhibition of lipid peroxidation occurs (IC_{50}) was calculated from triplicate determination of six different antioxidant concentrations ranging from no to full inhibition of lipid peroxidation, which was assessed by measuring thiobarbituric acid reactive species (TBARS) after heating. Absorption was read at 540 nm (color) versus 620 nm (turbidity correction) by an ELISA reader (*18*).

Apple Juice Enrichment. *Pulp Extraction.* Alcoholic pulp extraction was performed at small scale (starting weights of fresh apples of ∼1.5 kg). Jonagold apples (harvest year 1997) were cleaned by washing, stalks were removed, and the fruits were cut in four pieces. Apple pulp was prepared by quick slicing in a domestic food processor (Braun). Alcohol (methanol, ethanol, or propanol) was added in a 1:1 proportion (on weight basis) to the pulp, which was then extracted for 90 min in a 30 °C water bath and stirred now and then. Extracted apple pulp was pressed in a hydraulic manual press using a cheesecloth saturated with water. The obtained juice was concentrated to 75 °Brix using a rotary evaporator and diluted to 12 °Brix with water.

Pomace Extraction. Pomace extraction was performed at pilot plant scale (starting weights of fresh apples of ∼25 kg) and at small scale (starting weights of fresh apples of ∼1.5 kg).

At pilot plant scale pomaces and raw juices as produced in Van der Sluis et al. (*8*) were used as basic material, with the same variables: straight pressing (A) versus pulp enzyming (B); and apple pulp particle size of 3 × 3 × 10 mm (A1) versus 3 × 3 × 3 mm (A2). Unless stated otherwise, A1 was the standard procedure.

Pomaces were extracted in a stirring tank with ethanol (4 h at room temperature) in 1:1 proportion and under continuous stirring. Extracted pomaces were carefully pressed in a Bucher−Guyer juice press. From each batch a juice containing ethanol was obtained, and the ethanol was removed using a rotary evaporator by concentration to 50−60 °Brix. In this concentrate no ethanol could be detected by smelling. The obtained concentrate was diluted with water to 12 °Brix, and this diluted extract was added to the earlier produced raw juice.

During enriched apple juice production, samples were taken of the most important fractions that occurred in the process chain (extracted pomace, concentrated extract, diluted extract, and enriched juice). All fractions were weighed, and mass balances were composed (see below under Calculations). Mass balances were standardized by correcting for sample taking (weight). No correction was made for losses that remained in the equipment during the production. The °Brix values of the obtained concentrated extract, diluted extract, and enriched juice were determined and in the standardized mass balances adjusted with water to 50, 12, and 12 °Brix, respectively.

Sensory Evaluations. Potential differences in taste between the obtained conventional and enriched juice were evaluated using the triangle difference test. The members of an untrained 12-member panel were each presented with two triangles, each consisting of three samples. Panelists were asked to indicate if any sample differed in taste from the other two. The color of the juices was masked by a cover of aluminum foil and by using red light. For a panel of 12 members, each examining two triangles, differences between the taste of conventional and enriched juice would be significant at the 5, 1, and 0.1% levels if the odd sample was identified 13, 15, and 17 times, respectively (*19*).

The colors of raw juice and enriched apple juice were compared using a Tricolor LMF3 spectrophotometer in order to determine the L^*, a^*, and b^* values.

Calculations. *Mass Balances.* Mass balances and compound mass balances were calculated for all of the processing steps described in **Figure 1**. The balances of the starting material (apples, pulps, pomaces, and raw juices) were described earlier (*8*). An overall mass balance (eq 1a) describes the effect of pomace extraction. Compound mass balances were used for the 10 different flavonoids and chlorogenic acid; they were composed of the standardized weights of the apple fractions

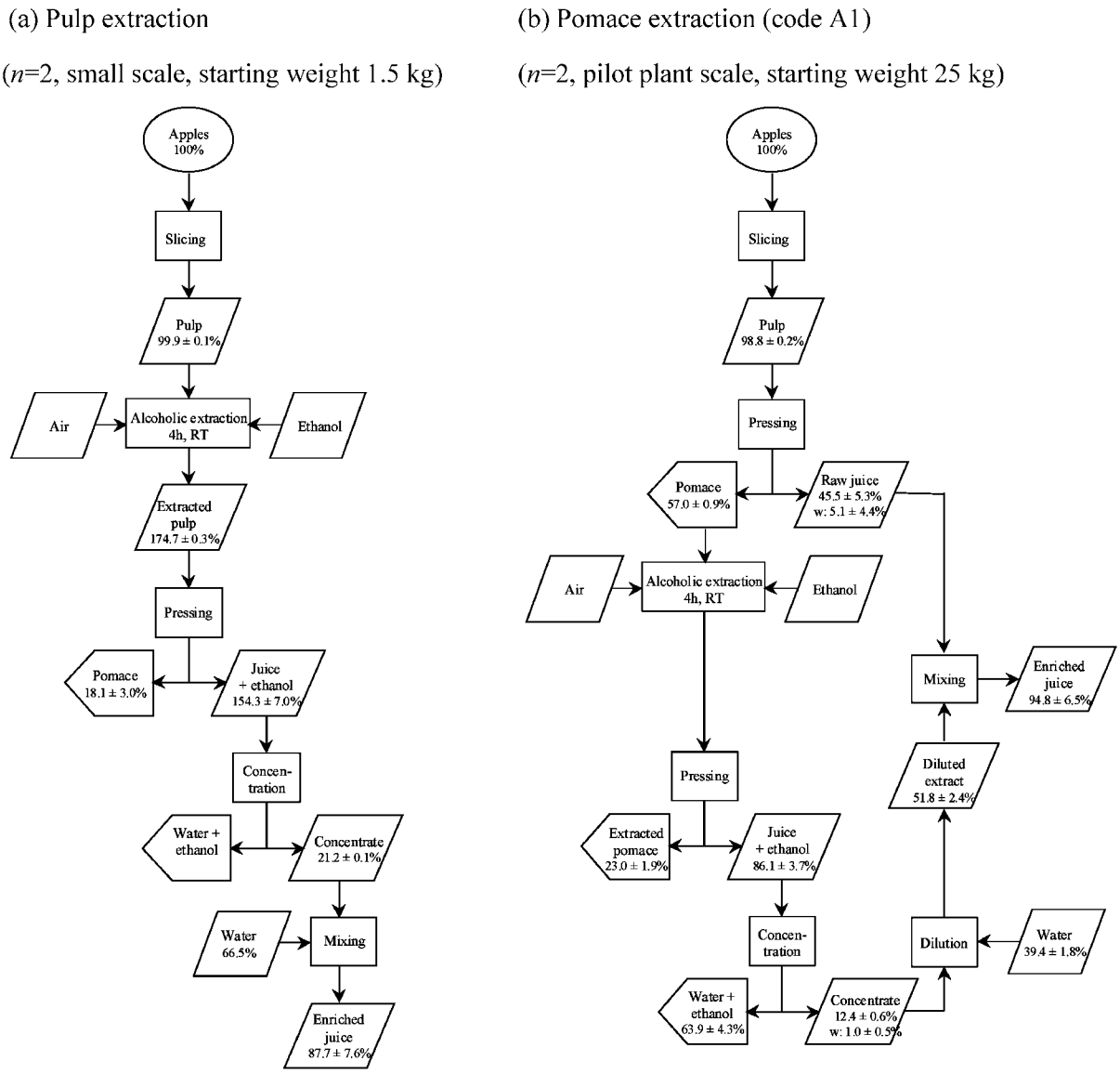

Figure 1. Process schemes and standardized weights of apple fractions during flavonoid-rich apple juice production; comparison of ethanolic pulp extraction (**a**) and pomace extraction (**b**). All percentages are related to the fresh apple fraction. w, amount of water that was added for standardization purposes; A1, pomace and raw juice obtained by straight pressing (particle size of pulp was 3 × 3 × 10 mm). Values are mean ± SD.

and the concentrations of those compounds present in the fractions using eq 1b.

effect of pomace extraction

standardized mass balance: $m_{pc} + m_e = m_{epc} + m_{ej} + m_l$ (kg) (1a)

compound mass balance:

$$m_{pc,i}c_{pc,i} + m_{e,i}c_{e,i} = m_{epc,i}c_{epc,i} + m_{ej,i}c_{ej,i} + m_{l,i}c_{l,i}\ (\text{mg}) \quad (1b)$$

m is the weight of apple fraction (kg) and c the concentration of compound i ($i = 1-11$) in the apple fraction (mg/kg of fresh weight). Fractions are represented as subscripts pc, pomace; epc, extracted pomace; ej, ethanolic juice; e, ethanol; and l, loss. $C_{(e),i} = 0$.

The production of a flavonoid-rich concentrate from the ethanolic juice by rotary evaporation is described by

standardized mass balance: $m_{ej} = m_{co} + m_{(w+e)} + m_l$ (kg) (2a)

compound mass balance:

$$m_{ej,i}c_{ej,i} = m_{co,i}c_{co,i} + m_{(w+e),i}c_{(w+e),i} + m_{l,i}c_{l,i}\ (\text{mg}) \quad (2b)$$

Fractions are represented as subscripts co, concentrate (50 °Brix), and w+e, water containing ethanol.

The obtained concentrate is further processed to a diluted extract according to

standardized mass balance: $m_{co} + m_w = m_{de} + m_l$ (kg) (3a)

compound mass balance:

$$m_{co,i}c_{co,i} + m_{w,i}c_{w,i} = m_{de,i}c_{de,i} + m_{l,i}c_{l,i}\ (\text{mg}) \quad (3b)$$

Fractions are represented as subscripts de, diluted extract (12 °Brix), and w, water. $C_{w,i} = 0$.

The production of an enriched juice is described by

standardized mass balance: $m_{de} + m_{srj} = m_{serj} + m_l$ (kg) (4a)

compound mass balance:

$$m_{de,i}c_{de,i} + m_{srj,i}c_{srj,i} = m_{serj,i}c_{serj,i} + m_{l,i}c_{l,i}\ (\text{mg}) \quad (4b)$$

Apple fractions are represented as subscripts serj, standardized enriched raw juice (12 °Brix), and srj, standardized raw juice (12 °Brix).

Antioxidant Activity. The calculated (or predicted) antioxidant activity of a mixture of known antioxidants and the measured antioxidant activity of an apple sample were derived as described earlier (*8*). To predict the antioxidant activity of the apple fractions from its composi-

Table 1. Mass Balances (Kilograms), Concentrations of Flavonoids and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight), and Antioxidant Activity of Apple Fractions during Jonagold Apple Juice Production (Extraction of Pomaces A1 and B with Ethanol)

		straight pressing (A1)[a]			pulp enzyming (B)[a]		
Jonagold	apples ($n = 5$), mean ± SD	extracted pomace ($n = 2$), mean ± SD	standardized concentrated extract ($n = 2$), mean ± SD	standardized enriched juice ($n = 2$), mean ± SD	extracted pomace ($n = 1$)	standardized concentrated extract ($n = 1$)	standardized enriched juice ($n = 1$)
standardized mass balance (kg)	25.0 ± 0	5.8 ± 0.5	3.1 ± 0.1	23.7 ± 1.6	4.8	1.7	26.9
included added water (kg)			0.2 ± 0.1			–1.2	
concen (mg/kg of fw)							
Cy-Ga	10 ± 3	6 ± 1	44 ± 0	7 ± 1	8	36	6
phloridzin	46 ± 17	18 ± 0	207 ± 14	35 ± 3	30	261	25
chlorogenic acid	202 ± 33	55 ± 6	869 ± 64	180 ± 1	52	589	99
total Q-glycosides	109 ± 25	89 ± 0	768 ± 34	117 ± 2	128	1068	96
total catechins	186 ± 26	59 ± 0	432 ± 21	67 ± 7	58	317	40
measd activity [1000/IC_{50} (L/mg of fw)]	202.3 ± 67.6[b]	87.2[c]		104.3 ± 3.0[b]			49.1[c]
calcd activity [Σ (C/IC_{50})]	72.7 ± 11.3	30.4 ± 0.2	248.2 ± 11.7	39.5 ± 1.7	35.6	249.8	27.0
explained activity (calcd/measd, %)	36	35		38			55

[a] A1, pomace and raw juice obtained by straight pressing (particle size of pulp was 3 × 3 × 10 mm); B, pomace and raw juice obtained by pulp enzyming. [b] $n = 2$. [c] $n = 1$.

tion, the IC_{50} values of 11 standard components as given by Van der Sluis et al. (*18*) were recalculated to milligrams per liter.

Statistical Analysis. Statistical analysis was performed on the original data by one-way analysis of variance for juice yields and by two-way analysis of variance with replications for concentrations, with significance level $\alpha = 0.05$ using the statistical package from Microsoft Excel. Data are represented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Novel Apple Juice Production Methods. To optimize flavonoid content and antioxidant activity of apple juice, adjustments in production methods were tested. Various solvents such as methanol, ethanol, and propanol were compared for their capacity to remove flavonoids from the matrix in which they are present. In flavonoid analysis these solvents are often used, perhaps partly diluted with water (*20*, *21*).

Pulp Extraction. At first the solvents were applied directly on the pulp, as shown in the process scheme described in **Figure 1a**. Using ethanol, the juice yield was 88 ± 8%, and the concentration of quercetin glycosides in the juice was 65 ± 32 mg/kg of fresh weight ($n = 2$). With methanol ($n = 5$) and propanol ($n = 2$) these values were not significantly different (data not shown). Comparison with conventionally produced apple juice (juice yield 46 ± 5% and quercetin glycoside concentration 13 ± 1 mg/kg of fresh weight) (*8*) shows that it was possible to improve flavonoid content in apple juice and the juice yield considerably by applying an alcoholic extraction on the apple pulp. In food production methanol and propanol can be used as extractants if the residual level is below 10 mg/kg (*22*), and ethanol can be used if its use leads to the presence of residues in only technically unavoidable amounts that present no danger to human health (*22*, *23*). Therefore, ethanol was chosen to be used in further apple juice experiments.

Pomace Extraction. It is more effective to perform an alcoholic extraction on the pomace, due to the high concentration of flavonoids that remains there. Because of the reduced weight, less alcohol is needed in pomace extraction. A flavonoid-rich solution can be created, from which the alcohol can be removed. This extract can be concentrated and added to the conventionally produced apple juice, at any desired stage and level, and other potential processes in which a loss of flavonoids might occur can be avoided.

Pomace extraction was performed following the process scheme described in **Figure 1b**. This figure shows the standardized weights (in percentage related to the fresh apple) of the apple fractions during processing. The first stages in this process scheme have been described before (*8*), and whenever comparisons are made with conventionally produced pomaces and raw juices, the reader is referred to that paper.

After pomace extraction, the enriched juice yield was 95 ± 6% (**Figure 1b**), which is again considerably higher than in conventional production. After extraction of pomaces A2 (from smaller sized pulp) and B (from pulp enzyming), the juice yields were 105 and 108%, respectively, which indicates that for the juice yield it does not matter what type of preparation the pomace and raw juice are subjected to. It also indicates that pulp enzyming will not be necessary for obtaining a higher juice yield. Due to standardization to 12 °Brix, the juice yields can be >100%. For comparison, the pomace was extracted with water, which gave a final juice yield of 83 ± 0% ($n = 2$).

In **Table 1** the mass balances, concentrations of flavonoids and chlorogenic acid, and antioxidant activity of apple fractions during enriched apple juice production from Jonagold apples are presented. Pomaces were obtained after straight pressing or pulp enzyming and were then used for extraction with ethanol. The levels of flavonoids and chlorogenic acid in the enriched juices obtained from pomace A1 were all higher than in the corresponding raw juices described earlier (*8*). Chlorogenic acid concentration was 1.4 times higher; cyanidin galactoside, 2.8; total catechins, 4.1; phloridzin, 8.9; and quercetin glycosides, 9.0 times higher than in conventional apple juice. Comparable results were obtained for enriched juices from pomaces A2 (data not shown) and B. However, the levels of all analyzed compounds were lower in enriched juice from pomace B compared to those in juice from pomace A1, caused by the lower levels that were present in pomace B itself.

This indicates that it is possible to enhance the polyphenolic antioxidant content of apple juice by performing an alcoholic extraction on the pomace and to use this extract to enrich the earlier obtained raw juice. Lu and Foo (*24*) also suggested that apple pomace be exploited commercially as a source for polyphenols, because it contains high levels of quercetin glycosides, phloridzin, and epicatechin (in total 6.5 g/kg of dry matter). Wolfe and Liu (*25*) succeeded in producing a value-

added food ingredient out of apple peel, because of its high antioxidant content.

After alcoholic pomace extraction, not all of the analyzed compounds were extracted completely from the pomace. The resulting extracted pomaces still contained 50% of the concentration of quercetin glycosides present in the original pomace; for cyanidin galactoside, total catechins, chlorogenic acid, and phloridzin, these values were 44, 34, 33, and 29%, respectively.

(a) Mass Balances in Pomace Extraction. The use of eqs 1a−4a enabled the calculation of the losses that occurred in the enriched juice production. The losses in the various processing steps caused by material remaining in the equipment can be calculated from **Figure 1**. Pressing of the extracted pomace caused a loss of 5% of the apples starting weight, which was small compared to the biological variation of flavonoid and chlorogenic acid concentration in apples (10−30%, from **Table 1**). Concentrating in order to remove the alcohol caused a weight loss of ∼30%, which is quite large but not unexpected using rotary evaporation. Dilution of the concentrate to 12 °Brix and mixture of the diluted extract with the raw juice to obtain the enriched juice caused a loss of 3%. When the apple juice productions were performed on a small scale, the losses were relatively higher; however, it is expected that on an industrial scale, the losses will be much smaller and that the concentration procedure can be optimized.

The compound mass balances (in milligrams) of components present in the various apple fractions in enriched apple juice production (from straight pressing or pulp enzyming) are presented in **Figure 2**. Enriched apple juices were prepared by ethanolic pomace extraction. Standardized mass balance and the concentrations as mentioned in **Table 1** were used as input. The total amount of all the analyzed compounds present in the extracted pomaces (A1 and B) was very low compared to the amount present in the original apples or in the pomaces prior to the extraction. In the pomace extracted after straight pressing (**Figure 2a**) only 19% of the amount of quercetin glycosides, 14% of the cyanidin galactoside, 7% of the total catechins, 6% of the chlorogenic acid, and 9% of the phloridzin that were present in the originating apples were found. In the enriched juice 102% of the amount of total Q-glycosides, 84% of the chlorogenic acid, 71% of the phloridzin, 67% of the cyanidin galactoside, and 34% of the total catechins that were present in the originating apples were detected. This indicates that the analyzed antioxidant components present in the pomace were extracted by ethanol from the pomace into the enriched juice. Furthermore, due to the low weight of the extracted pomace (which was ∼20% of the weight of the originating apples) and the use of compound mass balances, it can be seen that only low amounts of the components of interest remain there. This makes the need for optimization of the pomace extraction less than what might have been concluded from looking at the concentrations of these components only.

(b) Antioxidant Activity and Contribution of Compounds. **Table 1** shows that the antioxidant activity of the extracted pomace was 43% of the activity of the pomace before extraction as well as of the activity of the fresh apples, which showed the same activity. The standardized enriched apple juice had an antioxidant activity that was 4.9 times higher than that of the corresponding raw juice. This indicates that compounds that possess antioxidant activity indeed could be extracted from the pomace and transferred into the juice, but apparently the extraction still was not complete.

Enriched juice obtained from Jonagold apples from pomaces A1 and B had antioxidant activities that were 52 and 24%,

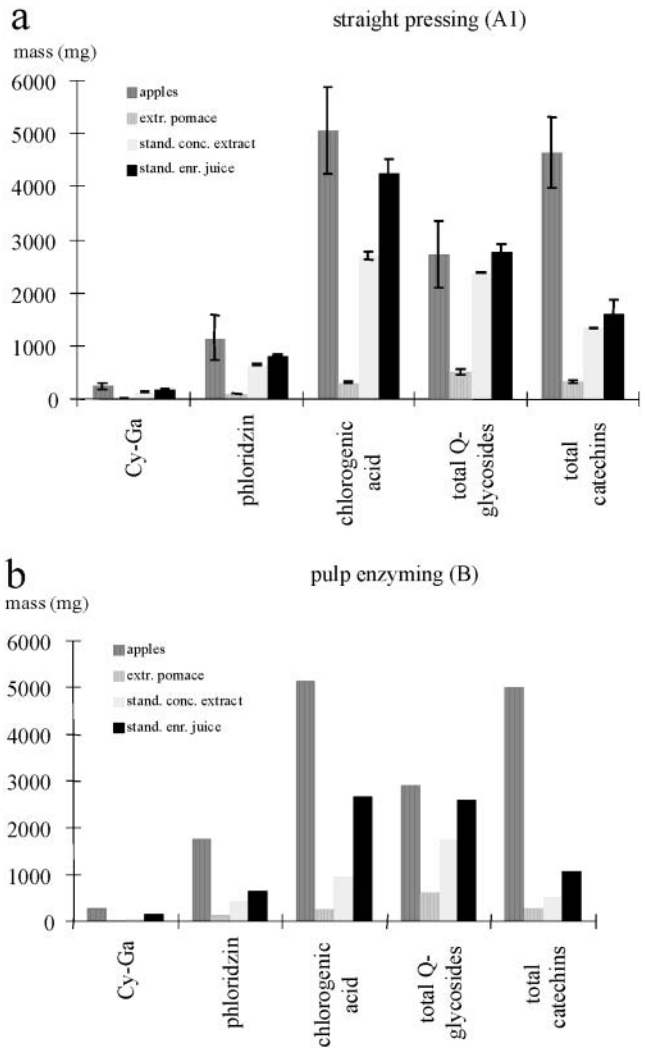

Figure 2. Compound mass balances of apple fractions in Jonagold apple juice production, prepared by ethanolic pomace extraction after straight pressing (**a**, mean ± SD, $n = 2$) or pulp enzyming (**b**, $n = 1$). Each set of four bars shows (left to right) apples, extracted pomace, standardized concentrated extract, and standardized enriched juice.

respectively, of the activity of the fresh apples. Therefore, it can be concluded that the pulp-enzyming procedure significantly lowered the antioxidant activity of the juice.

Figure 3 shows the calculated contribution of flavonoids and chlorogenic acid to the measured antioxidant activity of the various fractions occurring in enriched apple juice production. Ethanolic pomace extractions performed on pomaces A1 (from $3 \times 3 \times 10$ mm sized pulp) and B (from pulp enzyming) were compared. The contributions of the analyzed compounds to the antioxidant activity of the extracted pomace A1 and its corresponding enriched juice were 35 and 38% respectively. Of these compounds the group of "total catechins" was the most important with contributions of 19 and 18%, respectively. The group "total quercetin glycosides" was the second most important contributor to the measured antioxidant activity, at 14 and 15%, respectively. Chlorogenic acid, cyanidin galactoside, and phloridzin hardly contributed to the measured antioxidant activity. After pulp enzyming and ethanolic pomace extraction (B), the contribution of the analyzed compounds to the antioxidant activity of the enriched juice was 55%, with a contribution of total quercetin glycosides of 26% and of total catechins of 23%. The findings considering the contribution of the analyzed compounds to the measured antioxidant activity

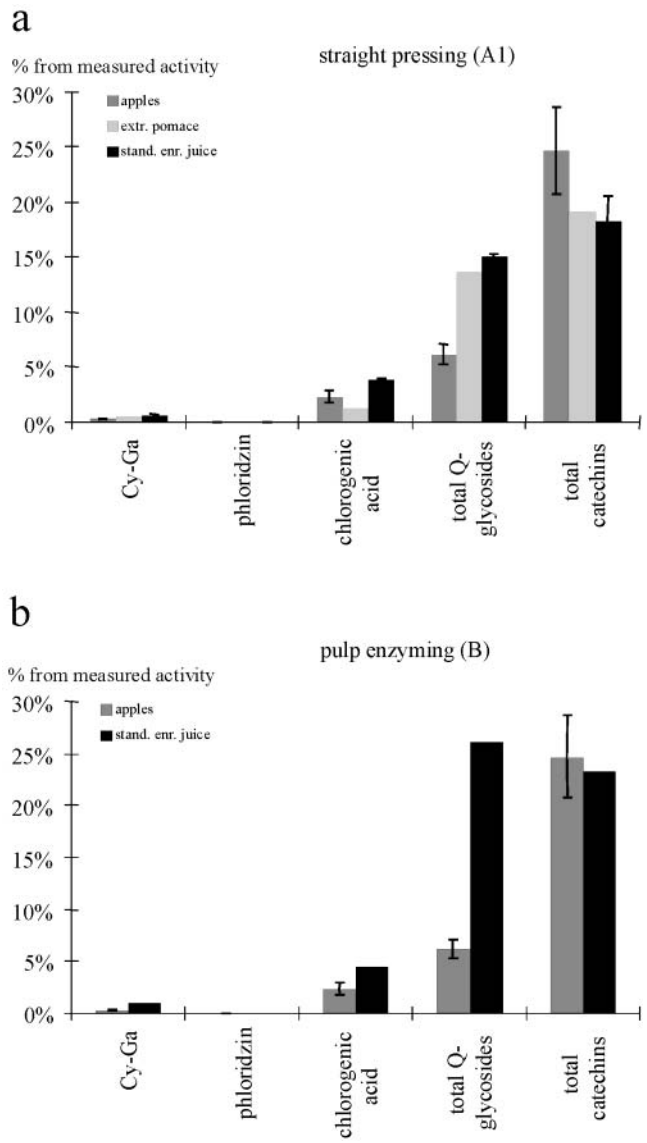

Figure 3. Calculated contribution of flavonoids and chlorogenic acid to the measured antioxidant activity of apple fractions in Jonagold apple juice production, prepared by ethanolic pomace extraction after straight pressing (**a**, mean ± SD, $n = 2$) or pulp enzyming (**b**, $n = 1$). In panel **a**, each set of three bars shows (left to right) apples, extracted pomace, and standardized enriched juice. In panel **b**, each set of two bars shows (left to right) apples and standardized enriched juice.

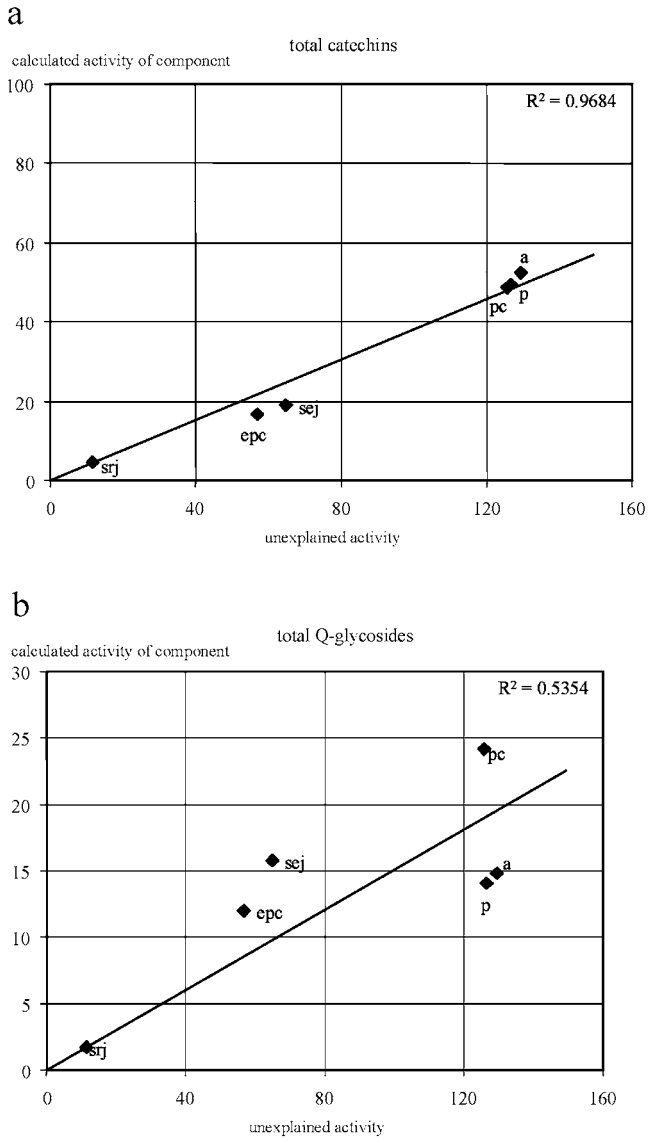

Figure 4. Correlation of the unexplained antioxidant activity with the calculated antioxidant activity of selected components of apple fractions in standardized enriched juice from pomace A1. Jonagold apples were used. Lines are trend lines. Unexplained activity is measured antioxidant activity minus calculated antioxidant activity. Symbols with letters are defined as follows: a, fresh apple; p, apple pulp; pc, pomace; srj, standardized raw juice; epc, extracted pomace; sej, standardized enriched juice.

of the apple fractions occurring in enriched apple juice production correspond to the findings described earlier (*8*).

(c) Unaccounted for Antioxidant Activity. As was the case in the previous study (*8*), a large proportion of the measured activity could not be ascribed to the analyzed antioxidants. One possible explanation for this difference could be the occurrence of synergism between the various antioxidant components. To see if interaction between the analyzed antioxidants existed, mixtures composed of standard compounds in the same concentrations as found in enriched apple juice samples were tested for their antioxidant activity. The enriched apple juices used for these experiments showed antioxidant activity values (expressed as DF_{50}) of 105.2 ± 12.8 ($n = 3$), and had calculated antioxidant activity values of 20.1 ± 2.4 ($n = 3$). The mixtures composed of standard compounds in the same concentrations as found in the enriched apple juice showed antioxidant activity values of 15.2 ± 1.9 ($n = 3$), which was not significantly different from the calculated antioxidant activity ($p = 0.052$). Thus, no interaction was observed between the analyzed antioxidants.

A second hypothesis for the observed discrepancy is the presence of compounds other than those analyzed that contribute to the antioxidant activity of extracted pomaces and enriched juices. These compounds could be procyanidins, carotenoids, and/or vitamins (*8*). In the apple fractions occurring in conventional apple juice production a high correlation between the unexplained and calculated antioxidant activities of catechins was shown (*8*), indicating that the unknown antioxidant compound(s) behaved quite similarly to catechins. Therefore, the correlation of the unexplained antioxidant activity with the calculated activity of catechins and of the other analyzed antioxidants present in the extracted pomace and enriched juice were also determined. Values for the fractions occurring earlier in the production process were added as well (*8*). **Figure 4a** shows that a high correlation was again obtained for total

Table 2. Concentration of Flavonoids and Chlorogenic Acid (Milligrams per Kilogram of Fresh Weight) and Yields of Concentrates and Flavonoid-Rich Apple Juices (Pomaces A1 Were Extracted with Ethanol); Comparison of Three Apple Cultivars[a]

	Elstar ($n = 1$)	Golden Delicious ($n = 2$)	Jonagold ($n = 2$)
concentrate			
Cy-Ga	31	21 ± 9	44 ± 0
phloridzin	154	183 ± 0	207 ± 14
chlorogenic acid	330	426 ± 69	869 ± 64
total Q-glycosides	487	491 ± 173	768 ± 34
total catechins	443	286 ± 67	432 ± 21
concentrate yield (%) (50 °Brix)	14	15 ± 1	12 ± 1
enriched juice			
Cy-Ga	7	3 ± 0	7 ± 1
phloridzin	30	22 ± 6	35 ± 3
chlorogenic acid	69	58 ± 21	180 ± 1
total Q-glycosides	89	69 ± 35	117 ± 2
total catechins	81	27 ± 16	67 ± 7
juice yield (%) (12 °Brix)	92	104 ± 8	95 ± 6

[a] Harvest 1998. Pomace and raw juice (A1), obtained by straight pressing (particle size of pulp was 3 × 3 × 10 mm).

catechins ($R^2 = 0.968$). The correlation between unexplained antioxidant activity and calculated antioxidant activity of total quercetin glycosides (**Figure 4b**, $R^2 = 0.535$) and of cyanidin galactoside ($R^2 = 0.686$) was less profound. Low correlation was found for chlorogenic acid activity ($R^2 = 0.344$). This indicates that in the enriched apple juice production process the unknown antioxidant compound(s) behaved quite similarly to the group of "total catechins". Foo and Lu (*26*) reported the presence of procyanidins (epicatechin polymers) in apple pomace, and most probably these compounds are extracted into the juice during alcoholic pomace extraction.

Choice of Apple Cultivar in Enriched Apple Juice Production. Pomaces from three different apple cultivars (Elstar, Golden Delicious, and Jonagold) were used as starting material for the production of enriched apple juice. **Table 2** shows the concentration of flavonoids and chlorogenic acid together with the yields of the concentrates and enriched juices produced from pomaces A1, which were extracted with ethanol. From all three cultivars enriched juice yield was at least 2 times higher than conventionally produced juice yield (*8*). The concentration of the analyzed compounds was considerably enhanced as well. The differences in compound concentrations resulted from differences in levels already existing in the fresh apples. Jonagold was the apple cultivar with highest concentration of analyzed compounds, with a total of 532 mg/kg of fresh weight, followed by Golden Delicious and Elstar with total concentrations of 448 and 321 mg/kg of fresh weight, respectively (*17*). In the produced concentrates and in the enriched juices from Jonagold apples total concentrations were highest as well. Therefore, the proposed novel process of pomace extraction can be used for different apple cultivars with similar effects.

In **Figure 5** the antioxidant activities of apple and enriched juice prepared by ethanolic pomace extraction (A1) are shown. The measured antioxidant activity and the antioxidant activity calculated from the sample composition are given. The measured antioxidant activity of enriched Jonagold juice was significantly higher ($p = 0.007$) than that produced from Elstar and Golden Delicious apples. The measured antioxidant activities of the enriched juices were 51, 16, and 52%, respectively, of the initial activity of fresh Elstar, Golden Delicious, and Jonagold apples. The measured antioxidant activities of the enriched juices from

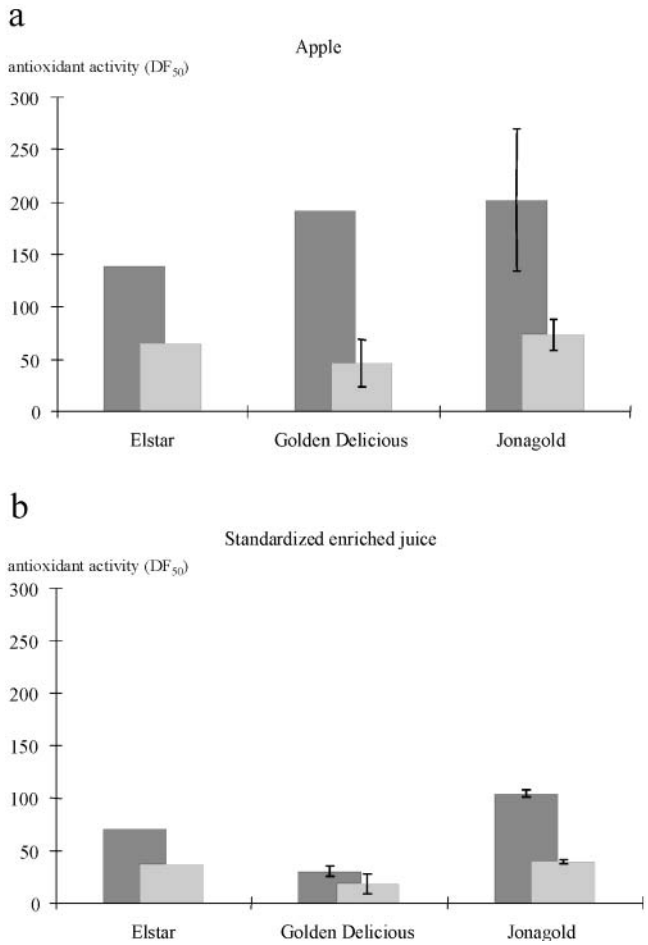

Figure 5. Antioxidant activity of apple and enriched juice prepared by ethanolic pomace extraction (A1); comparison of three cultivars used as starting material. Dark gray bars represent measured antioxidant activity. Light gray bars represent antioxidant activity calculated from composition. Values are mean ± SD. DF, dilution factor.

Elstar, Golden Delicious, and Jonagold apples were 2, 7, and 5 times higher than that of the conventionally produced juices described earlier (*8*). The contributions of the analyzed compounds to the measured antioxidant activity of the enriched juices were 52, 59, and 38% in enriched juices from Elstar, Golden Delicious, and Jonagold apples, respectively. The proposed novel process of pomace extraction increased the antioxidant activity of enriched apple juices produced from three apple cultivars by a factor of between 2 and 7; therefore, the method can be used for different apple cultivars.

Sensory Properties of Raw and Enriched Juice. To determine whether differences in taste existed between conventional and enriched Jonagold apple juices, they were evaluated using the triangle difference test. The untrained panelists identified the odd sample 18 times in a preliminary session, and after one training session 22 times; therefore, the tastes of conventional and enriched juices were significantly different at the 0.1% level. Panelists were not asked questions about preference, degree of difference, acceptance, or type of difference after identification of the odd sample.

The color of raw and enriched Jonagold apple juice can be described by the following L^*, a^*, and b^* values. For raw apple juice they are 46.3, 20.4, and 53.5, respectively, and for enriched juice, 54.2, 14.2, and 50.2. This indicates that both juices have an orange-brownish color. However, visually the enriched juice would be described as more reddish than the raw juice.

The concentrated extracts obtained from Jonagold and Elstar apples looked very red, but the concentrated extract obtained from Golden Delicious apples was green. This difference in color most probably cannot be explained only by the lower cyanidin galactoside concentration present in Golden Delicious concentrated extract.

Technological Implications. We showed that it is possible to enhance the activity and content of polyphenolic antioxidants in apple juice by performing an alcoholic extraction on the pomace or pulp, followed by removal of the solvent from the obtained extract. This might be performed by multistage evaporation. Juice yield was improved considerably as well.

The antioxidant activity of the final juice was 5 times increased and the concentration of polyphenolic antioxidants up to 9 times increased compared to conventional processing. Sensory evaluation showed that the enriched juice tasted different, but preferences were not determined. It is expected to be more adstringent than conventional juice, due to the presence of the extracted polyphenols (*27*). The color of the enriched juice was slightly different (more reddish) from that of conventional juice, which may be beneficial in marketing an enriched juice versus a conventional one.

Advantages of pomace extraction over pulp extraction are that less alcohol is needed and that apart from facilities for the pomace extraction only minor adjustments in the usual production facilities are needed. In both cases the production process will take more time than in conventional apple juice production, because of the extra time needed for extraction.

A hot water extraction of the pomace may be investigated as an alternative to the use of alcoholic pomace extraction. Extraction of the pomace with water at an elevated temperature might also produce a flavonoid-rich solution that can be added to the raw juice immediately or after concentration. As mentioned before, Spanos and co-workers (*12*) showed that diffusion extraction performed on apple pulp at elevated temperatures indeed increased flavonoid content of the juice 3−5-fold.

When using alcohols as extractant in food production, legislation and good manufacturing practices should be considered (*23*). Furthermore, the solvent should be recovered from the residue (*28*) to meet environmental legislation.

Pomace extraction most probably has less influence on the taste of the obtained juice than pulp extraction, because the largest amount of the juice will be produced in the conventional and therefore known manner. During the evaporation process necessary to remove the alcohol from the extract, some of the aroma compounds may be lost, which will affect the taste of the final enriched juice. The use of aroma trapping may prevent the loss of aroma compounds and provide a tasty enriched apple juice.

The proposed novel production process can be a valuable approach to the design of new types of apple juice with an enhanced health-protecting capacity.

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.

NOTE ADDED AFTER ASAP

Equation 2b was modified April 28, 2004, from the original ASAP posting of April 14, 2004.

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