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Activity and Concentration of Polyphenolic Antioxidants in Apple Juice. 3. Stability during Storage

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Kinetic data are reported describing the stability of various classes of polyphenolic antioxidants in an apple juice enriched in these compounds as a function of storage temperature and oxygen concentration. The most thermally sensitive compounds were the various quercetin glycosides and epicatechin, whereas phloridzin and chlorogenic acid were more stable. The quercetin glycosides showed differences in their stability: quercetin galactoside ≈ quercetin rhamnoside > quercetin glucoside/rutinoside > quercetin arabinoside. The effect of the presence of oxygen on the degradation rates was clear for only quercetin and to a lesser extent for epicatechin. Accelerated shelf-life testing of enriched apple juice during 4 days at 80 °C showed decreases in the antioxidant activity of 20−40%. The parameters obtained were used to predict the stability at different storage conditions. Calculations showed that polyphenolic antioxidants and antioxidant activity of enriched apple juice will be quite stable at ambient or refrigerated storage conditions up to half a year.

KEYWORDS: Antioxidant activity; quercetin glycosides; catechins; phloridzin; anthocyanins; chlorogenic acid; storage; apple juice; kinetics; shelf-life

INTRODUCTION

Six classes of polyphenols are present in apples: flavonol glycosides; catechins; anthocyanins; dihydrochalcones (phloretin glucoside and xyloglucoside); phenolic acids (chlorogenic acid and *p*-coumaroylquinic acid); and procyanidins (*1*).

From epidemiological research, the intake of fruits and vegetables has been widely acknowledged to be inversely related to cancer incidence and cardiovascular diseases (*2*, *3*). Antioxidative, anti-inflammatory, antimutagenic, and anticarcinogenic properties and capacity to modulate some key cellular enzyme functions are ascribed to many phenolic compounds (*4*). Therefore, these compounds might play a role in relation to human health (*5*).

Polyphenolic intake (phenolic acids, flavonols, catechin monomers, proanthocyanidins, flavanones, anthocyanins) is estimated at 1 g/day, but the structural diversity of polyphenols makes the estimation of their content in food difficult and, therefore, the estimation of daily intake as well (*6*). From this 1 g/day, phenolic acids provided ∼30% and flavonoids accounted for the remainder. In a Western diet catechins from apples contribute 5−12% to their daily intake (*7*) and flavonols, 10% (*8*).

Apples can be further processed to, for example, apple juice, apple cider, or applesauce. In conventional apple juice production, juice poor in flavonoids and with only 3−10% of the antioxidant activity of the originating fruit is obtained (*9*). Applying an alcoholic extraction either on the pulp or on the pomace makes it possible to improve flavonoid content in apple juice as well as its antioxidant activity (*10*).

During the storage of apple juice or apple juice concentrates various changes may occur, for example, as a result of browning and degradation reactions. Comparison of apple juices prepared from concentrates that were not stored with concentrates that were stored at 25 °C for 9 months showed that quercetin glycoside and phloridzin concentrations decreased 54 and 32%, respectively. In the juice from the not-stored concentrate 2.9 mg/L of catechin and 6.1 mg/L of epicatechin was present, but in the juice from the stored concentrate these compounds were not detected at all (*11*). A decrease of flavonol glycosides in juice concentrates upon 90 days of storage at 30 °C was reported by van Buren et al. (*12*). Furthermore, carton-laminated packed commercial apple juices showed declines in phenolic acids and flavonoid content as well as in TEAC value after 11 months of storage at room temperature (*13*). This indicates that storage of apple juice or juice concentrates affects flavonoid concentration and antioxidant activity in apple juice.

Using apple juice with increased flavonoid content gives the opportunity to study the behavior of polyphenolic antioxidants in apple juice during storage at normal and elevated temperatures. Therefore, the aim of the present study was to evaluate the effects of storage at 4 °C, at ambient temperature, and under

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conditions for accelerated shelf-life testing on the concentration and antioxidant activity of various polyphenolic compounds found in enriched apple juice. To distinguish between oxidative and nonoxidative breakdown reactions, storage was done with varying oxygen concentrations. A kinetic modeling approach was used to study the reaction pathways of breakdown and conversions of polyphenolic antioxidants and to be able to translate the results of the accelerated shelf-life testing into the stability of compounds under normal conditions.

MATERIALS AND METHODS

Materials. Chemicals were the same as described earlier (*9*). Jonagold apples were used to produce apple juice and were harvested from commercial orchards in the years 1996 and 1998. Apples were harvested as described before (*9*) and stored until use (1−6 months) at controlled atmosphere (CA) conditions (1.5 °C, 1.2% O_2, and 2.5% CO_2), which is optimal for long-term storage.

Methods. *Apple Juice.* Apple juice used in cold storage and storage at room temperature was prepared by pomace extraction as described earlier (*10*). About 1.5 kg of Jonagold apples (harvest year 1998) was cleaned, and pulp was prepared using a domestic food processor. Apple pulp was pressed in a hydraulic manual press using a cheesecloth (two times pressing to 100 bar). Raw juice was obtained, and pomaces were extracted with ethanol (4 h at room temperature) in 1:1 proportion (on weight basis), under continuous stirring. Extracted pomaces were pressed in a hydraulic manual press. Juice containing ethanol was obtained, and the ethanol was removed in a rotary evaporator by concentration to 50−60 °Brix. The obtained concentrate was diluted with water to 11 °Brix, and this diluted extract was added to the earlier produced raw juice. The storage experiments started immediately after apple juice production.

Apple juice used in the accelerated storage experiments was prepared according to the pulp extraction method described earlier (*10*). About 1.5 kg of Jonagold apples (harvest year 1996) was cleaned by washing, stems were removed, and the fruit was cut in four pieces. Apple pulp was prepared by quick slicing in a domestic food processor (Braun). Apple pulp was aerated for 60 min. Methanol was added in a 1:1 proportion (on weight basis) to the pulp, which was then extracted for 45 or 90 min in a 30 °C shaking water bath. No difference was observed between the extraction times of 45 and 90 min (data not shown). Extracted apple pulp was pressed in a hydraulic manual press. The obtained juice was concentrated to 65−75 °Brix using a rotary evaporator and diluted to 11 °Brix with water. Apple juice was used immediately or stored at −20 °C for a maximum of 2 weeks until use in the storage experiments.

Storage Experiments. (a) Cold Storage and Storage at Room Temperature. Sterilized glass bottles were filled with 100 mL of apple juice (raw or enriched juice, noncentrifuged, pasteurized for 30 s at 90 °C) and sealed. Bottles were stored in a cold room (4 °C) or at room temperature (20 °C). For sampling at different times, different bottles were used.

(b) Accelerated Storage. Three flasks filled with 170−350 mL of apple juice each were placed in a water bath of 70, 80, or 90 °C or in a glycerol/water (1:1) bath of 100 °C. Each of the flasks was continuously flushed with nitrogen, air, or oxygen, which was bubbled straight into the juice, with a flow rate of ∼100 mL/min. The flow rate was measured by an electrical flow meter. The flasks were connected to condensers in order to minimize possible losses of water and volatiles from the apple juice by evaporation.

Sampling and Analysis. In cold storage and storage at room temperature an aliquot of apple juice was taken from a bottle and stored immediately at −20 °C until analysis. Apple juice (5 mL) was extracted with 5 mL of methanol and sonicated for 30 min followed by 10 min of centrifugation at 2500 rpm. The supernatant was filtered through a 0.45-μm CA filter (Schleicher and Schuell). Extractions were performed in duplicate. The same extract was used for both HPLC analysis and antioxidant activity determination.

During the accelerated storage experiments apple juice samples (20−25 mL) were taken regularly and stored immediately at −20 °C until lyophilization. Dry weight was determined from the sample weight before and after lyophilization. Lyophilized apple samples were stored at −20 °C until analyzed. Apple juice samples were extracted in duplicate before HPLC analysis. Lyophilized sample (0.5 g) was extracted with 10 mL of 15% acetic acid in methanol and sonicated for 30 min followed by filtration through a 0.45-μm CA filter (Schleicher and Schuell).

Quantification of flavonoids by HPLC and HPLC equipment was as described earlier (*14*). Flavonoid and chlorogenic acid standards were dissolved in methanol.

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 (*15*). Antioxidant activity was expressed as DF_{50}, which corresponds to $1000/IC_{50}$ (*9*). The higher the DF_{50} value, the higher the antioxidant activity of a sample is.

Modeling and Statistical Analysis. Analyses were performed at least in duplicate, and results were expressed on a fresh weight basis. The analyzed polyphenols (chlorogenic acid, phloridzin, epicatechin, quercetin, Q-3-Ga, Q-3-Gl/Ru, Q-3-Ar, Q-3-Rh) were all expressed as millimolar in the juice. Oxygen concentrations were calculated from the temperature-dependent Henry coefficients [derived from Rooney and Daniels (*16*)], based on the gas composition that was led through the juice (0, 21, and 100% oxygen) and were also expressed as millimolar.

Reaction kinetics was studied by multiresponse modeling using the determinant criterion (*17*). Multiresponse modeling implies that more than one reactant or product is taken into account. The determinant criterion is then more suitable than the familiar least-squares criterion. The software package Athena Visual Workbench (www.athenavisual-.com) was used for numerical integration of differential equations as well as parameter estimation of the rate constants in the differential equations following minimization of the determinant in order to obtain the reaction kinetic parameters (rate constant k and activation energy E_a).

RESULTS AND DISCUSSION

Cold Storage and Storage at Room Temperature. Figure 1 shows the effect of storage of raw apple juice and enriched apple juice at 4 °C or at 20 °C for 1 month. During storage the °Brix values of the apple juices remained unchanged (11.3 ± 0.1 for raw apple juice and 11.2 ± 0.1 for enriched apple juice) at both storage conditions. Also, the pH did not change (values between 3.6 and 3.7). During storage at 4 °C and at 20 °C no significant changes were observed at a 5% significance level in concentrations of total quercetin glycosides, total catechins, chlorogenic acid, phloridzin, and cyanidin galactoside. During the storage time no quercetin aglycon was formed. The above indicates that a 1-month storage of apple juice in a refrigerator or even at room temperature will not lower the concentration of the present polyphenolic antioxidants.

Stability of the Polyphenolic Antioxidants at Elevated Temperatures. In accelerated storage experiments the stability of eight polyphenolic antioxidants was studied in enriched apple juice, and the results are shown for three oxygen pressures (0, 21, and 100%) at 80 °C in **Figure 2**. Breakdown of quercetin glycosides showed no dependency on oxygen pressure. A clear difference in degradation rate between the various glycosides was observed (**Figure 2a−d**). The stability decreased in the order Q-Ga ≈ Q-Rh > Q-Gl/Ru > Q-Ar. During the incubations an increase in the amount of quercetin aglycon (Q) was observed (**Figure 2e**). The aglycon was present at only very low amounts initially (<3 μM) but was formed during the incubation, presumably by the hydrolysis of the various quercetin glyco-

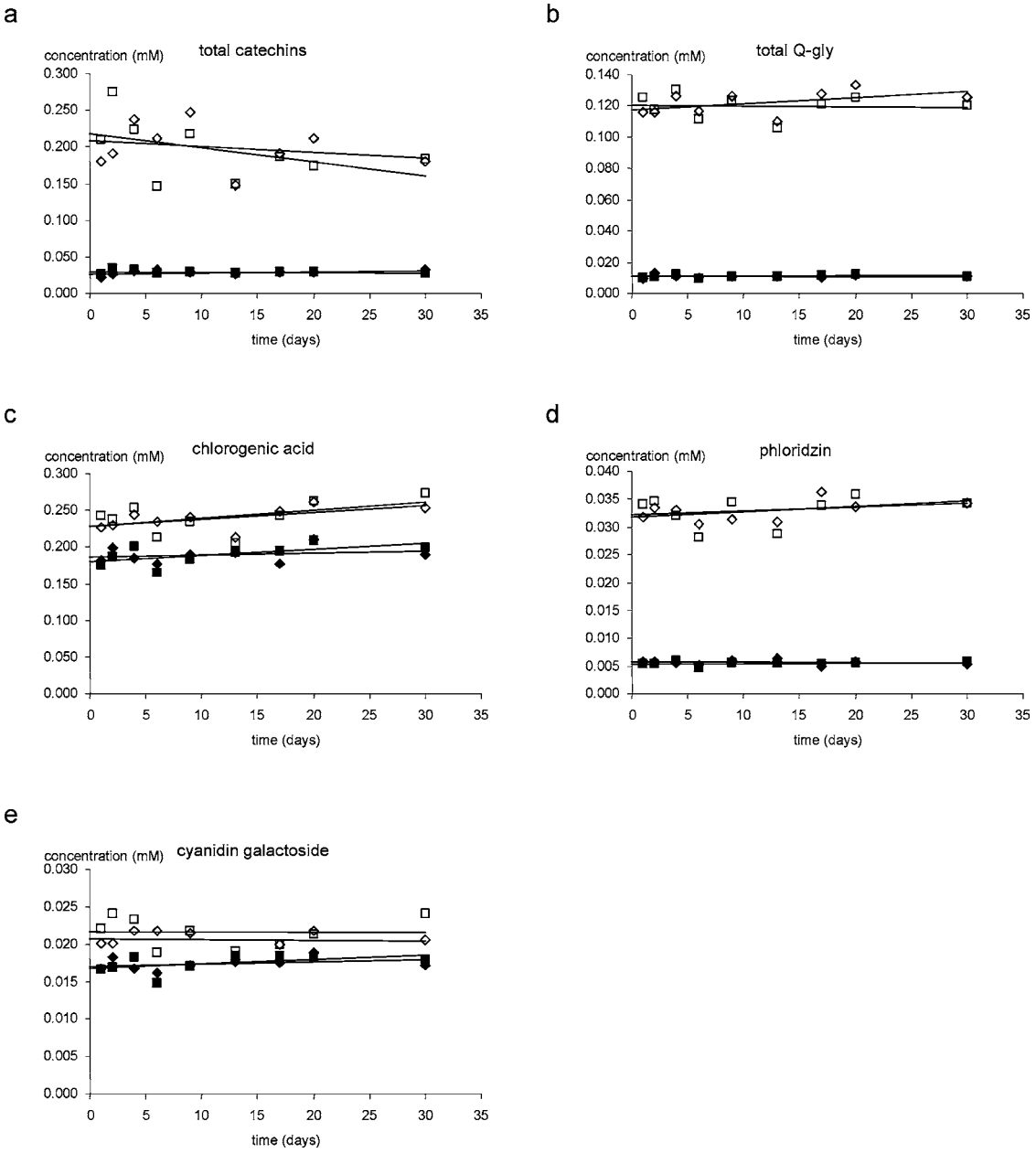

Figure 1. Effect of storage conditions on raw apple juice (solid symbols) and enriched apple juice (open symbols). Juices were stored at 4 °C (◇) or at 20 °C (□) for 1 month. Enriched apple juice was prepared by ethanolic pomace extraction. SD = 1–20%. Lines are linear trend lines. The group "total Q glycosides" consists of the compounds Q-3-Ga, Q-3-Ru/Gl, Q-3-Ar, and Q-3-Rh. The group "total catechins" consists of the compounds catechin and epicatechin.

sides. Acid hydrolysis of quercetin glycosides is a well-known phenomenon, but, to our knowledge, has not been described in apple juice before. In **Figure 2e** it is seen that the formed quercetin aglycon was not stable but was further degraded. This degradation of quercetin showed a clear dependency on the amount of oxygen, indicating an oxidative pathway. Phloridzin and chlorogenic acid (**Figure 2f**,**g**) showed minor degradation rates. Both compounds showed some 20−30% decrease in levels over 100 h of incubation at 80 °C. The presence of oxygen did not have a big effect, if any, on the stability of these compounds. Epicatechin degradation profiles showed a decrease of 80% of the initial values after 50−100 h (depending on the oxygen pressure). The concentration remained stable at this 20% level. For epicatechin in chocolate it has been observed that epicatechin can be formed from the polymerized forms (procyanidins), which are also present in the product, at elevated temperatures (*18*). Because procyanidins are present in considerable concentrations in apple [0.5−1 g/kg (*19*)], they are expected to be present in high levels in enriched apple juice as well; therefore, it can be assumed that the seemingly stable remaining levels are in fact a quasi steady state as a consequence of a formation and a degradation reaction.

Incubation of the apple juices at various temperatures (70, 80, 90, and 100 °C) for different oxygen pressures (0, 21, and 100%) showed a clear effect of temperature on the stability of all polyphenolic antioxidants studied. In **Figure 3** results of Q-Rh at 0% oxygen are shown as an example. At 70 °C ∼50% breakdown was observed after 100 h of incubation, whereas at 100 °C >95% breakdown was observed after 50 h.

Antioxidant Activity. The effect of incubation at 80 °C at various oxygen pressures on the antioxidant activity of the apple juice is shown in **Figure 4**. The activity is expressed as the dilution factor (DF_{50}) to reach 50% inhibition of the lipid oxidation in the microsomal assay. A higher DF_{50} value

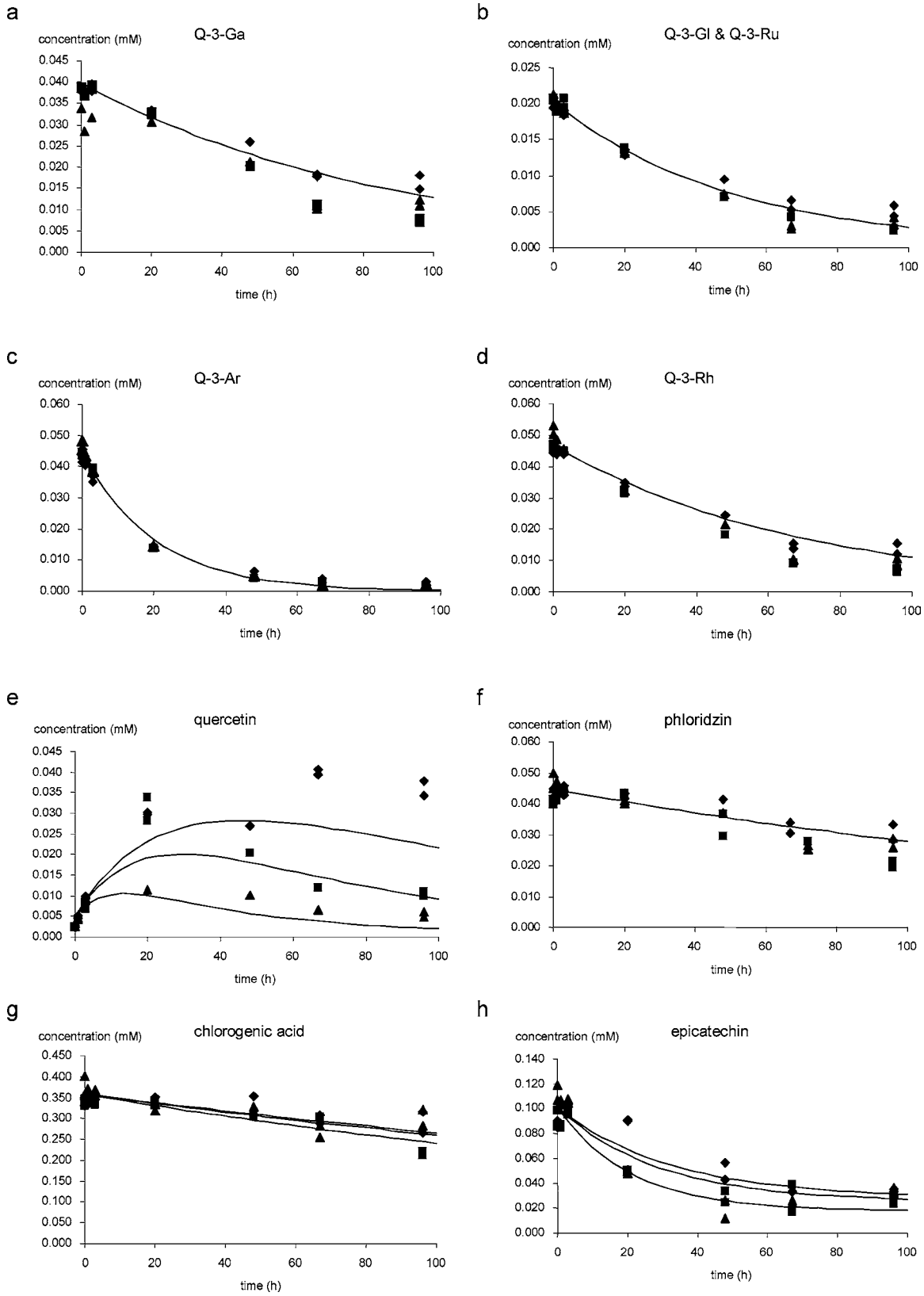

Figure 2. Effect of storage conditions on enriched apple juice. Juices were stored at 80 °C while bubbled with 0% O_2 (◆), 21% O_2 (■), or 100% O_2 (▲), for 4 days. Enriched apple juice was prepared by methanolic pulp extraction. Values are from duplicate extractions. Lines are fitted lines using eqs 5–8. Estimated parameters are given in **Tables 1**–**3**.

therefore indicates a higher antioxidant activity of the juice. It can be seen that the antioxidant activity was reduced after incubation of 100 h at 80 °C with 20% (0 and 21% oxygen) until 40% (100% oxygen). Except for the 0% oxygen incubation an initial increase in the antioxidant activity was observed in the first hours of incubation. The reason for this is not clear. It is known that the formation of quercetin aglycon by hydrolysis of quercetin glycosides results in an increase in activity, because the aglycon has around twice the antioxidant activity compared to the average quercetin glycoside activity (*15*). The time frame

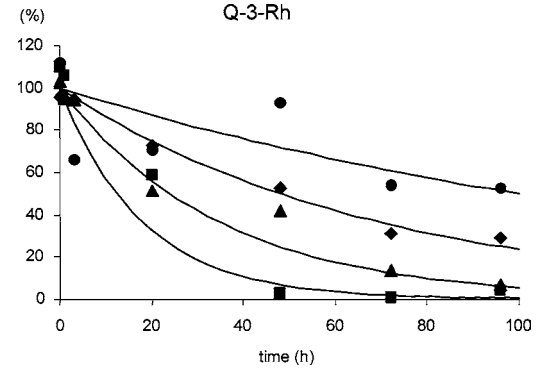

Figure 3. Effect of temperature on the stability of Q-Rh in enriched apple juice stored at 0% oxygen. Juices were incubated at 70 °C (●), 80 °C (◆), 90 °C (▲), or at 100 °C (■). Enriched apple juice was prepared by methanolic pulp extraction. Lines are fitted lines.

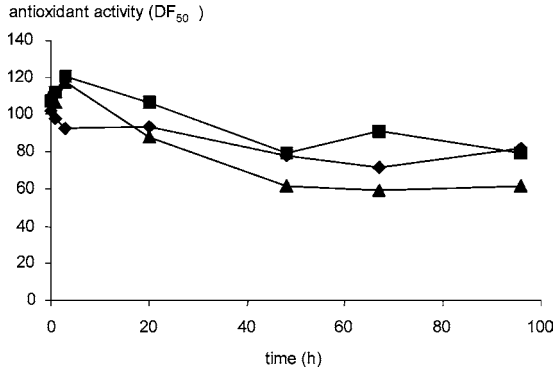

Figure 4. Effect of storage on the antioxidant activity of enriched apple juice. Juices were stored at 80 °C while bubbled with 0% O_2 (◆), 21% O_2 (■), or 100% O_2 (▲), for 4 days. Enriched apple juice was prepared by methanolic pulp extraction.

Table 1. Estimated Reference Rate Constants for the Hydrolysis of Quercetin Glycosides and Their Activation Energies

compound	$k_{h,70}$ (h^{-1})	$E_{a,h}$ (kJ/mol)
quercetin galactoside	2.2×10^{-3} (0.2×10^{-3})[a]	78 (5)
quercetin glucoside/rutinoside	4.3×10^{-3} (0.3×10^{-3})	66 (3)
quercetin arabinoside	11.8×10^{-3} (0.7×10^{-3})	57 (3)
quercetin rhamnoside	2.9×10^{-3} (0.2×10^{-2})	74 (4)

[a] Values in parentheses indicate the 95% confidence interval.

of this reaction, however, is much longer than that of the observed increase. Because also in some of the analyses of the individual quercetin glycosides a slight increase was observed in the first few hours of incubation, an explanation of the increase could be an increase in extractability of the freeze-dried samples after heating. This phenomenon could be due to protein denaturation leading to reduced complex formation between protein and flavonols.

Modeling the Degradation Reactions of the Polyphenolic Antioxidants. The complete data set of all the analyzed concentrations after incubation of the apple juice for the different conditions (time, temperature, and oxygen pressure) was used to estimate the Arrhenius parameters for all of the reactions as described in eqs 1−10 below. This was a total of 206 time/temperature/oxygen combinations with each seven (or eight at 80 °C) different polyphenolics analyzed. The results of this analysis are shown in **Tables 1** and **2**, which show the estimates of the reference rate constants and their activation energies together with their 95% confidence intervals as determined by the multiresponse fitting procedure described in the modeling section under Materials and Methods. The modeling results will be discussed for each group of components separately.

The nonenzymatic degradation of polyphenols can be divided in oxidative degradation and nonoxidative degradation. The proposed pathways of the two types of degradation are shown in the general reaction scheme

$$\mathrm{PP} \xrightarrow{k_d} \mathrm{X}_1 \tag{1}$$

$$\mathrm{PP} + \mathrm{O}_2 \xrightarrow{k_o} \mathrm{X}_2 \tag{2}$$

where PP indicates polyphenol, X_n indicates breakdown products, k_d is the nonoxidative degradation rate constant, and k_o is the oxidative degradation rate constant.

In the case of quercetin glycosides also a hydrolysis reaction may be involved in the degradation pathway:

$$\mathrm{QG} \xrightarrow{k_h} \mathrm{Q} + \mathrm{G} \tag{3}$$

QG is quercetin glycoside, Q is quercetin, G is sugar residue, and k_h is the hydrolysis rate constant.

Both the quercetin glycoside and the aglycon can subsequently be degraded as described by reactions 1 and 2.

In the case of epicatechin a steady state was observed after an initial breakdown. This can be modeled by taking into account the possibility of the formation of epicatechin from polymeric forms (procyanidins):

$$\mathrm{PC} \xrightarrow{k_f} \mathrm{EC} \tag{4}$$

PC indicates procyanidins, EC indicates epicatechin, and k_f is the formation rate constant.

The reaction schemes were translated into the following differential equations:

$$\frac{\mathrm{d}[\mathrm{PP}]}{\mathrm{d}t} = -k_{d,\mathrm{PP}}[\mathrm{PP}] - k_{o,\mathrm{PP}}[\mathrm{PP}][\mathrm{O}_2] \tag{5}$$

$$\frac{\mathrm{d}[\mathrm{QG}_n]}{\mathrm{d}t} = -k_{h,\mathrm{QGn}}[\mathrm{QG}_n] - k_{d,\mathrm{QGn}}[\mathrm{QG}_n] - k_{o,\mathrm{QGn}}[\mathrm{QG}_n][\mathrm{O}_2] \tag{6}$$

$$\frac{\mathrm{d}[\mathrm{Q}]}{\mathrm{d}t} = +\sum_1^4 k_{h,\mathrm{QGn}}[\mathrm{QG}_n] - k_{d,\mathrm{Q}}[\mathrm{Q}] - k_{o,\mathrm{Q}}[\mathrm{Q}][\mathrm{O}_2] \tag{7}$$

$$\frac{\mathrm{d}[\mathrm{EC}]}{\mathrm{d}t} = +k_f[\mathrm{PC}] - k_{d,\mathrm{EC}}[\mathrm{EC}] - k_{o,\mathrm{EC}}[\mathrm{EC}][\mathrm{O}_2] \tag{8}$$

The PP are chlorogenic acid, phloridzin, and epicatechin. k_d is the nonoxidative degradation rate constant, and k_o is the oxidative degradation rate constant.

The temperature dependence of the reaction rate constants was described by the Arrhenius equation

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \tag{9}$$

which was rearranged to

$$k = k_{ref} \exp\left[\left(\frac{E_a}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{10}$$

The temperature of 70 °C was used as the reference temperature (T_{ref} = 343 K). Estimation of the k_{ref} and E_a of all reactions

Table 2. Estimated Reference Rate Constants for the Nonoxidative and Oxidative Degradation of Chlorogenic Acid, Phloridzin, and Quercetin Aglycon and Their Activation Energies

compound	$k_{d,70}$ (h^{-1})	$E_{a,d}$ (kJ/mol)	$k_{o,70}$ (h^{-1})	$E_{a,0}$ (kJ/mol)
chlorogenic acid	1.8×10^{-3} (0.3×10^{-3})[a]	52 (8)	1.0×10^{-4} (0.6×10^{-4})	187 (25)
phloridzin	2.3×10^{-3} ($0.5 \cdot 10^{-3}$)	73 (10)	0	
quercetin aglycon	1.1×10^{-2} (0.1×10^{-2})	31 (5)	3.8×10^{-2} (0.6×10^{-2})	30 (8)

[a] Values in parentheses indicate the 95% confidence interval.

Table 3. Estimated Rate Constants for the Nonoxidative and Oxidative Degradation of Epicatechin and the Formation Rate Constant at 80 °C

compound	$k_{d,80}$ (h^{-1})	$k_{o,80}$ (h^{-1})	$k_{f,80}$[PC] (mM/h)
epicatechin	31.2×10^{-3} (5.2×10^{-3})[a]	11.3×10^{-3} (8.6×10^{-3})	8.7×10^{-4} (2.0×10^{-4})

[a] Values in parentheses indicate the 95% confidence interval.

was done by fitting the equations simultaneously for the experimental data of all four temperatures investigated. The initial concentrations were estimated by the fitting procedure as well, to allow for uncertainty in the experimental observation at $t = 0$.

Quercetin Glycosides and Quercetin Aglycon. The stability of the four different quercetin glycosides and quercetin aglycon was modeled simultaneously because the formation of quercetin aglycon in the juice was a direct result from the hydrolysis rate of the quercetin glycosides. It was not possible to estimate the individual nonoxidative and oxidative reaction rate constants and hydrolysis rate constants for all different glycosides separately, because of the high correlation between them. Therefore, it was assumed that the individual quercetin glycosides had a degradation rate which is the summation of the nonoxidative degradation reaction rate and the hydrolysis rate of quercetin. The oxidative degradation reaction was neglected because no effect of oxygen pressure on the stability of the quercetin glycosides was observed at all temperatures studied (**Figure 2**). The ratio between breakdown and hydrolysis of the glycosides was assumed to be constant for all quercetin glycosides and was estimated by the modeling procedure. This resulted in the estimation that 43.5 ± 2.0% of the observed breakdown of quercetin glycosides occurred through hydrolysis, resulting in the formation of quercetin aglycon. This percentage showed no temperature dependence in the range of 70−100 °C. The hydrolysis rate constants (**Table 1**) of the individual glycosides varied with a factor of 5. The stability decreased in the order quercetin galactoside ≈ quercetin rhamnoside > quercetin glucoside/rutinoside > quercetin arabinoside. The quercetin aglycon was formed during the incubations and was subsequently degraded both oxidatively and nonoxidatively, with the oxidative reaction being dominant at high oxygen pressures (see also **Figure 2**). The fit of the data of quercetin, as shown in **Figure 2e**, showed an underestimation for the data of the anaerobic incubations at 80 °C. The deviations at the other temperatures were less marked and showed a small underestimation at 70 and 80 °C and a small overestimation at 100 °C. These deviations could indicate that an additional reaction pathway is involved for the nonoxidative degradation of quercetin.

Phloridzin. The main degradation route for phloridzin was the nonoxidative pathway. No significant effect of different oxygen pressures was observed at any temperature studied (**Table 2**).

Chlorogenic Acid. The main degradation route at temperatures lower than 90 °C for chlorogenic acid was the nonoxidative pathway. The oxidative degradation was observed at only 90 and 100 °C. This resulted in a quite large confidence interval for the oxidative reaction rate constant and its activation energy. A high correlation coefficient was also observed between these two parameters (−0.978). At normal storage conditions the nonoxidative reaction will be dominant.

The above does not include enzymatic degradation. At ambient conditions oxidative degradation of chlorogenic acid and catechin by the enzyme polyphenol oxidase is also possible, as chlorogenic acid and epicatechin are good substrates for this enzyme (*12*); however, at elevated temperatures, the enzyme will be inactivated.

Table 2 shows that the degradation rate constants for phloridzin and chlorogenic acid are a factor 10 lower than that of quercetin aglycon, which indicates that phloridzin and chlorogenic acid are more stable than quercetin aglycon upon heating.

Epicatechin. Epicatechin degradation was investigated at only 80 °C; therefore, no effect of temperature on the reaction rate could be analyzed. As mentioned in the discussion of the general observations of the trends, a stable level of ∼20% of the compound remained in the juice after the initial breakdown. This behavior could be modeled by taking into account the formation of epicatechin from the hydrolysis of its polymeric form (procyanidins). Procyanidins were not analyzed in the present study, but it is expected that enriched apple juice contains high levels of these compounds. Because this is most probably higher than the concentration of epicatechin, it was assumed that the hydrolysis rate remained constant during the incubations. From the parameter estimation it can be concluded that the nonoxidative degradation is slightly higher than the oxidative degradation at 100% oxygen (**Table 3**).

Prediction of the Effect of Ambient Storage on the Levels of Polyphenolic Antioxidants in Apple Juice. With the estimated rate constants and their activation energies it was possible to predict the effect of practical storage conditions on the level of polyphenolic antioxidants in apple juice. This is shown in **Figure 5**, where the effect of 200 days of storage at 20 °C in the absence of oxygen is shown. According to this simulation both chlorogenic acid and quercetin glycosides declined roughly by 40%, a small amount of quercetin aglycon was formed, and phloridzin remained quite stable. In carton-laminated packed commercial apple juices a decline of phenolic acids (5−21%) and in flavonoid content (14−18%; quercetin glycosides and phloridzin together) is reported after 11 months of storage at room temperature (*13*), which is roughly half of our prediction for enriched apple juice.

To check if it was possible to use the estimated rate constants and their activation energies for temperatures that lie outside

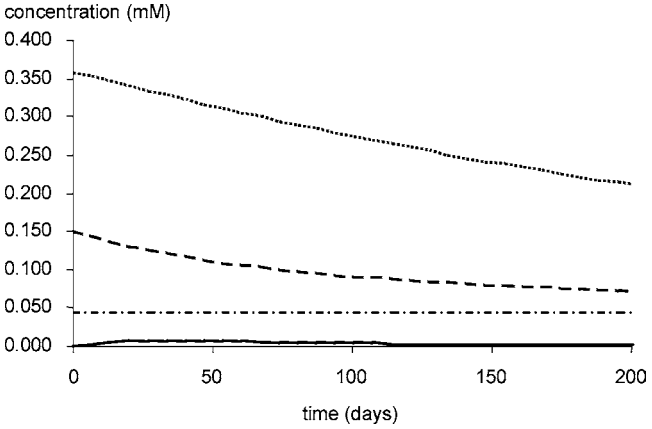

Figure 5. Prediction of the effect of practical storage conditions (200 days at 20 °C in the absence of oxygen) on the level of chlorogenic acid (•••), total quercetin glycosides (- - -), phloridzin (-•••-), or quercetin aglycon (—) in enriched apple juice. Estimated rate constants and their activation energies were used as input.

the range of the measured temperatures (70−100 °C), predicted changes in polyphenolic antioxidant concentrations were compared with measured ones (as shown in **Figure 1**). The predicted decreases in quercetin glycoside and chlorogenic acid concentration after 30 days of storage at room temperature were 17 and 7%, respectively, where in the experiment a stability of these compounds at 20 °C was observed. Phloridzin remained stable in both the model predictions and the measurements. The storage experiment showed no formation of quercetin aglycon at 20 °C, where the model predicted the formation of a few micromoles. This indicates that for quercetin glycosides the prediction of breakdown rates slightly overestimates reality during extrapolation to lower temperatures.

Prediction of the Effect of Heat Treatments on the Level of Quercetin in Apple Juice. With the estimated rate constants and their activation energies it was also possible to predict the effect of various heat treatments such as pasteurization and sterilization on the level of antioxidants in apple juice. Data were extrapolated to heat treatments of maximal 1 h at 140 °C, and as an example **Figure 6a** shows the effect of heating time and temperature on the decrease in quercetin content. Whereas quercetin glycoside concentration decreased, quercetin aglycon concentration increased (as mentioned earlier), those two effects were added in **Figure 6a**. This figure shows that pasteurization [bottles, 10−20 min in water of 80−90 °C (*20*)] will not affect quercetin concentration in apple juice, nor will sterilization (90 s at 120 °C). After 1 h at 120 °C a decrease in quercetin glycoside concentration of 12% will be observed, which is a treatment one will not find in practice.

In **Figure 6b** the effect of heat treatments on the calculated antioxidant activity of quercetin glycosides and quercetin aglycon is shown, using the concentrations given in **Figure 6a** and the antioxidant activity of the individual compounds reported earlier (*15*).

The calculated antioxidant activity is derived as follows

$$\sum_{i=1}^{n} \frac{C_i}{\mathrm{IC}_{50,i}} \tag{11}$$

where C_i is the concentration of component i (mM) and $\mathrm{IC}_{50,i}$ is the IC_{50} value of component i (mM).

Figure 6b shows that the decrease in calculated antioxidant activity is lower than the decrease in concentrations predicted as a result of heat treatments, due to the formation of quercetin aglycon, which has an almost 2-fold higher antioxidant activity than its corresponding glycosides (*15*). After 1 h at 120 °C, a decrease of only 5% in calculated antioxidant activity will be observed. In the mentioned calculated antioxidant activity only the contribution of quercetin glycosides and quercetin aglycon is taken into account; in this case the contribution of other antioxidants and their possible interaction are not included.

Practical Implications. It has been possible to obtain information about the stability of polyphenolic antioxidants from accelerated storage experiments. Using kinetic modeling, predictions about the stability of quercetin glycosides, chlorogenic acid, and phloridzin in apple juice during storage and various heat treatments could be made. It showed that the results of the predictions are in accordance with our own results, and the compounds are stable during normal storage temperatures (4 and 20 °C). Furthermore, it was predicted that quercetin glycosides can withstand 1.5 min at 140 °C or 1 h at 70 °C without major degradation.

Upon heating, the various quercetin glycosides and epicatechin were the most sensitive compounds, whereas phloridzin and chlorogenic acid were more stable. Quercetin glycosides showed differences in their stability. The decrease in concentration as a consequence of heat treatments is higher than the decrease in calculated antioxidant activity.

The prediction does not include enzymatic degradation of chlorogenic acid at lower temperatures; however, at elevated temperatures, the enzyme polyphenol oxidase will be inactivated. Quercetin glycosides are not substrates for polyphenol oxidase.

Substantial differences have been observed in terms of activation energies for the various polyphenolic antioxidants

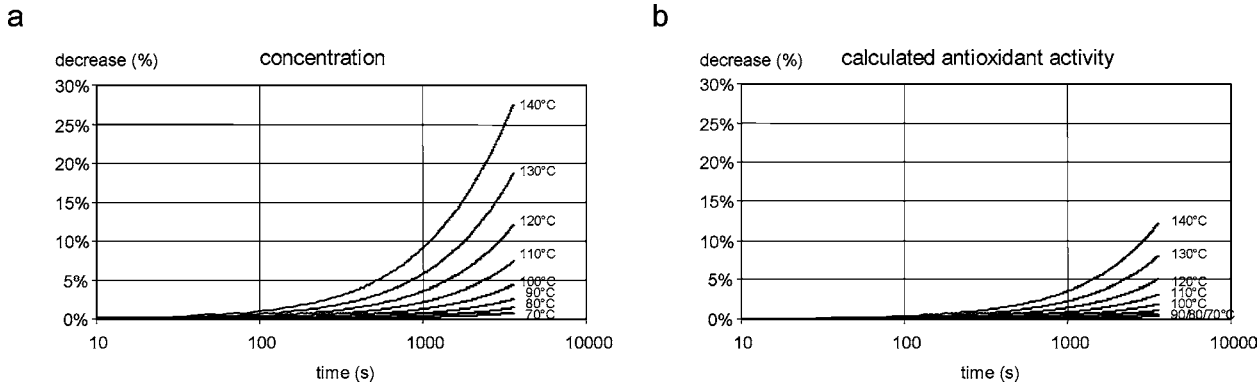

Figure 6. Prediction of the effect of heat treatments (such as pasteurization) in the presence of 21% oxygen on the decrease in concentration and calculated antioxidant activity of total quercetin glycosides including quercetin aglycon in apple juice. Estimated rate constants and their activation energies were used as input.

studied. This can be used for optimizing heat treatments, for example, regarding a specific compound of interest.

The effect of the presence of oxygen on the degradation rates was clear for only quercetin aglycon and to a lesser extent for epicatechin, which indicates that there was not much of an effect of oxygen concentration observed and that with respect to packaging and heat treatments no special precautions regarding oxygen have to be taken.

ABBREVIATIONS USED

Cy-Ga, cyanidin galactoside or ideain; EC, epicatechin; k_d, nonoxidative degradation rate constant; k_o, oxidative degradation rate constant; k_f, formation rate constant; PC, procyanidins; PP, polyphenol; Q, quercetin; QG, quercetin glycoside; Q-3-Ga, quercetin galactoside or hyperin; Q-3-Ru, quercetin rutinoside or rutin; Q-3-Gl, quercetin glucoside or isoquercitrin; Q-3-Ar, quercetin arabinoside or avicularin; Q-3-Rh, quercetin rhamnoside or quercitrin; X_n, breakdown products.

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