JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Apple Cell Walls and Their Extracts on the Activity of Dietary Antioxidants

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The effect of dietary fiber in the form of apple cell walls and pectin extracts on natural antioxidants was examined. Cell walls (CW), isolated from apples (*Malus domestica* Borkh. cv. "Pacific Rose"), were incubated with ascorbic acid (AA) or quercetin in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 6.5) at 37 °C for 2 h. The resulting supernatants were characterized by a ferric reducing antioxidant power (FRAP) assay and cyclic voltammetry (CV). The experiments were repeated with pectin isolated from the apple cell walls and commercial pectins and showed that polysaccharide preparations stabilized AA effectively but offered little protection against quercetin oxidation. The water-soluble components from cell walls appeared to be responsible for the observed effects of cell-wall polysaccharide preparations on antioxidant activity.

KEYWORDS: Antioxidant activity; apple cell walls; pectin; dietary fiber; ascorbic acid; cyclic voltammetry; FRAP assay; quercetin

INTRODUCTION

The benefits of daily consumption of fruit and vegetables to human health are well-documented (*1, 2*). Dietary fibers (DFs), in the form of plant cell walls, and natural antioxidants in fruit and vegetables are among the putative causes of these health benefits. When DFs and natural antioxidants are consumed together, they are likely to interact with each other during their movement through the human gastrointestinal tract. However, little is known about the specific interactions between these food components. Renard et al. (*3*) reported apple cell walls bound procyanidins but not hydroxycinnamic acids or (−)-epicatechin. The interactions may or may not be beneficial to human nutrition and health. An improved understanding of these interactions may lead to strategic improvements in food product development and consumption practices.

There has been much debate on the definition of DFs, as well as the choice of methods used for characterizing them (*4, 5*). A general feature of all of these definitions is that plant cell walls are considered to be the major source of DF (*6*). Cell walls of fruit and vegetables consist of cellulose and noncellulosic polysaccharides (including pectic polysaccharides and xyloglucans), glycoproteins, water, and other smaller molecules, including phenolic acids (*7*). Pectic polysaccharides are a complex group comprising mainly homogalacturonans and rhamnogalacturonans (RG-I), neutral polysaccharides, arabinans, galactans, and arabinogalactans, which may be attached to the rhamnose residues of RG-I. Pectic polysaccharides have varying solubilities in aqueous solutions, depending upon their structures and interactions with other polysaccharides in the cell wall (*7*).

Apples contain large amounts of pectic polysaccharides in their cell walls (*3, 8–10*). Apples also contain significant amounts of AA and quercetin, both of which have antioxidant capability (*11, 12*). Antioxidants are reductants; hence, their antioxidant activity can be gauged by their reducing power against a particular oxidant (*13*) and cyclic voltammetry (CV) (*14*).

We examined the effect of cell walls isolated from apple parenchyma tissue, a water-soluble pectin fraction, and commercial pectins on the antioxidant activities of ascorbic acid (AA) and quercetin.

MATERIALS AND METHODS

Chemicals. Pacific Rose apples were purchased from a local supermarket. Quercetin dihydrate, pectins [from apple and citrus peel, containing 74 and 86% polygalacturonic acid (polyGalA), respectively], polyGalA from oranges, tripyridyltriazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemicals (St Louis, MO). AA was purchased from Scharlau Chemicals (Spain). *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), iodine, and potassium iodide were purchased from BDH Chemicals, Poole, U.K., and *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) was purchased from Acros Organics, Morris Plains, NJ.

Preparation of Antioxidant Solutions. A solution of AA (0.50 mM, 50 mL) was prepared and stored in the dark. The quercetin solution (0.25 mM) was prepared by adding 0.0042 g of quercetin dihydrate to 40 mL of Milli-Q water to form a suspension. The suspension was saturated with N_2 to remove dissolved O_2. Dissolution was facilitated

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10.1021/jf072670v CCC: $40.75
Published on Web 12/14/2007

Table 1. Preparation of Incubation Samples (in Duplicate) for FRAP and CV Analysis

sample name	10 mL incubation mixture
blank	HEPES buffer (15 mM, pH 6.5)
AA control	HEPES buffer (15 mM, pH 6.5), AA (0.125 mM)
AA–cell-wall material, commercial pectin, or polyGalA sample	HEPES buffer (15 mM, pH 6.5), AA (0.125 mM), freshly isolated cell walls (1 g), or commercial pectin or polyGalA
quercetin control	HEPES buffer (15 mM, pH 6.5), quercetin (0.0625 mM)
quercetin–cell-wall material, commercial pectin, or polyGalA sample	HEPES buffer (15 mM, pH 6.5), quercetin (0.0625 mM), freshly isolated cell walls (1 g), or commercial pectin or polyGalA
cell-wall material, commercial pectin, or polyGalA control	HEPES buffer (15 mM, pH 6.5), freshly isolated cell walls (1 g), or commercial pectin or polyGalA

by adjusting the pH of the solution to ~9.5 (using 0.01 M KOH), followed by gentle sonication. The final volume was adjusted to 50 mL with Milli-Q water, and the solution was stored in the dark.

Isolation of Apple Cell Walls. The isolation procedure of Melton and Smith (*15*) was used with some modifications. The modified method is a mild aqueous procedure, the use of which avoided the introduction of compounds, such as phenols and other reducing agents that could interfere with ferric reducing antioxidant power (FRAP) and CV studies. All solutions and materials used were precooled (≤4 °C).

Fresh apples were peeled and cored. Edible tissue (120 ± 1 g), containing predominantly parenchyma cells with unlignified primary walls (as confirmed microscopically after staining with toluidine blue O (*16*)), was cut into small pieces, immediately frozen in liquid nitrogen, and then ground with a mortar and pestle. The resulting fine powder was transferred to a beaker on ice and then homogenized in HEPES buffer (4 × 55 mL, 20 mM, pH 6.5) using an Ultra-Turrax (2 × 1 min, 24 000 rpm, IKA Werke, Janke and Kunkel, Staufen, Germany). The homogenate was filtered through a 50 μm nylon mesh to collect the cell walls, which were then washed with HEPES buffer and ground with further buffer in a ring grinder (2 × 2 min, Bench Top Ring Mill, Rocklabs Ltd., Auckland, New Zealand). The cell walls were collected by filtration on an 11 μm nylon mesh. The isolated walls were washed with HEPES buffer, and the filtrates were discarded. Batches of isolated cell walls were (i) washed with Milli Q water (2 × 100 mL) and used for incubation studies or (ii) treated with CDTA to extract pectic polysaccharides, or (iii) retained for monosaccharide analysis. The monosaccharide compositions of the isolated cell walls and CDTA extract were determined as their alditol acetates following trifluoroacetic acid hydrolysis (*17*). The measurement of uronic acid content was as reported previously (*18*).

Incubation of Cell-Wall Materials and Antioxidants. Incubation mixtures prepared in duplicate (**Table 1**) contained AA (0.125 mM) or quercetin (0.0625 mM), HEPES (15 mM), and never-dried cell walls (0.1 g/mL) or freeze-dried cell wall material (2 mg/mL). The mixtures were incubated at 37 °C and pH 6.5 for 2 h in a covered shaking bath (70 rpm). Supernatants were collected by centrifugation (1000*g*, 5 min) for FRAP and CV analysis.

Effect of Cell-Wall Materials from Sequential Extraction on AA and Quercetin Activity. Freshly isolated cell walls (1 g) in HEPES buffer (10 mL, 18.75 mM, pH 6.5) were incubated at 37 °C for 2 h. The first HEPES extract was collected by centrifugation (1000*g*, 5 min). A second extract was obtained by adding HEPES buffer (10 mL) to the pellet, incubating at 37 °C for 2 h, and collecting the supernatant by centrifugation. This procedure was repeated another 3 times to give 5 extracts. An aliquot (1 mL) of each extract was incubated (37 °C, 2 h) with 0.25 mL of AA or quercetin solution or water. The final concentrations were HEPES (15 mM) and AA (0.125 mM) or quercetin (0.0625 mM). Antioxidant controls were prepared, which contained no cell walls. The resulting samples were analyzed in duplicate by the FRAP assay.

A separate set of experiments was performed on freshly isolated cell walls (1 g) in HEPES (15 mM) in the presence of either AA (0.125 mM) or quercetin (0.0625 mM) and was incubated for the longer time of 2.5 h.

Incubation of Commercial Pectins and PolyGalA with Antioxidants. Solutions of pectin (1.25 mg/mL) in Milli-Q water and polyGalA [1.25 mg/mL in 1% (w/v) KOH] at pH 6.5 (adjusted using 0.01 M HCl) were prepared. Pectins or polyGalA were incubated with AA or quercetin at 37 °C for 2 h. Each sample contained HEPES (15 mM) and AA (0.125 mM) or quercetin (0.0625 mM) (**Table 1**). The resulting samples were examined by the FRAP assay.

FRAP Assay. Assays were performed on a double-beam UV-1240 spectrophotometer (Shimadzu, Japan). The FRAP reagent was prepared 2 h before each assay. Experiments were performed in triplicate. The absorbance (*A* value) measured at 593 nm after 12 min versus a blank is termed the antioxidant activity of a sample. Trolox was used to prepare a standard curve. The FRAP value was converted to the Trolox equivalent value, $TEAC_{FRAP}$ value (mM), which is the concentration of Trolox solution with an equivalent antioxidant potential to that of the antioxidant solution tested at 1 mM concentration.

The change in the antioxidant activity was expressed as "percentage change", calculated using eq 1, where *A* represents absorbance in the FRAP assay.

$$\text{percentage change} = \frac{A_{\text{antioxidant−cell-wall material}} - (A_{\text{antioxidant}} + A_{\text{cell-wall material}})}{A_{\text{antioxidant}} + A_{\text{cell-wall material}}} \times 100 \quad (1)$$

Cyclic Voltammetry. Cyclic voltammograms were recorded using a Bioanalytical Systems 100A electrochemical analyzer. The working electrode (a 3 mm glassy carbon disk electrode MF-2066) was freshly

Table 2. Changes in L-Ascorbic Acid or Quercetin Activity Measured by FRAP Assay after Incubation with Apple Cell-Wall Materials, Commercial Pectins, and Polygalacturonic Acid Calculated Using eq 1[a]

polysaccharide samples	change in ascorbic acid activity (% per mg dry basis)	change in quercetin activity (% per mg dry basis)
fresh cell walls (never-dried)	+(1.6−1.9), mean = +1.8, n = 13	−(0.3−0.6), mean = −0.4, n = 13
freeze-dried cell walls	+(1.5−2.3), mean = +1.9, n = 8	−(0.4−0.6), mean = −0.5, n = 8
CDTA fraction	+(2.8−3.0), mean = +2.9, n = 4	−(0.4−0.5), mean = −0.45, n = 4
commercial citrus pectin	+(8.2−9.6), mean = +8.9, n = 3	−(1.3−1.4), mean = −1.3, n = 3
commercial apple pectin	+(7.1−8.5), mean = +7.8, n = 3	−(1.2−1.4), mean = −1.3, n = 3
commercial polyGalA	+(12.5−14.7), mean = +13.6, n = 3	−(1.3−1.5), mean = −1.4, n = 3

[a] Data are based on the dry weight of cell-wall material or polysaccharide and expressed as a range of the calculated results after statistical analysis by the *Q* tests (at 90% confidence level). "*n*" is the number of different cell-wall samples prepared at different times. Each cell-wall polysaccharide sample was used twice for the preparation of duplicate incubation mixtures, and each incubation mixture was measured by the FRAP assay in triplicate.

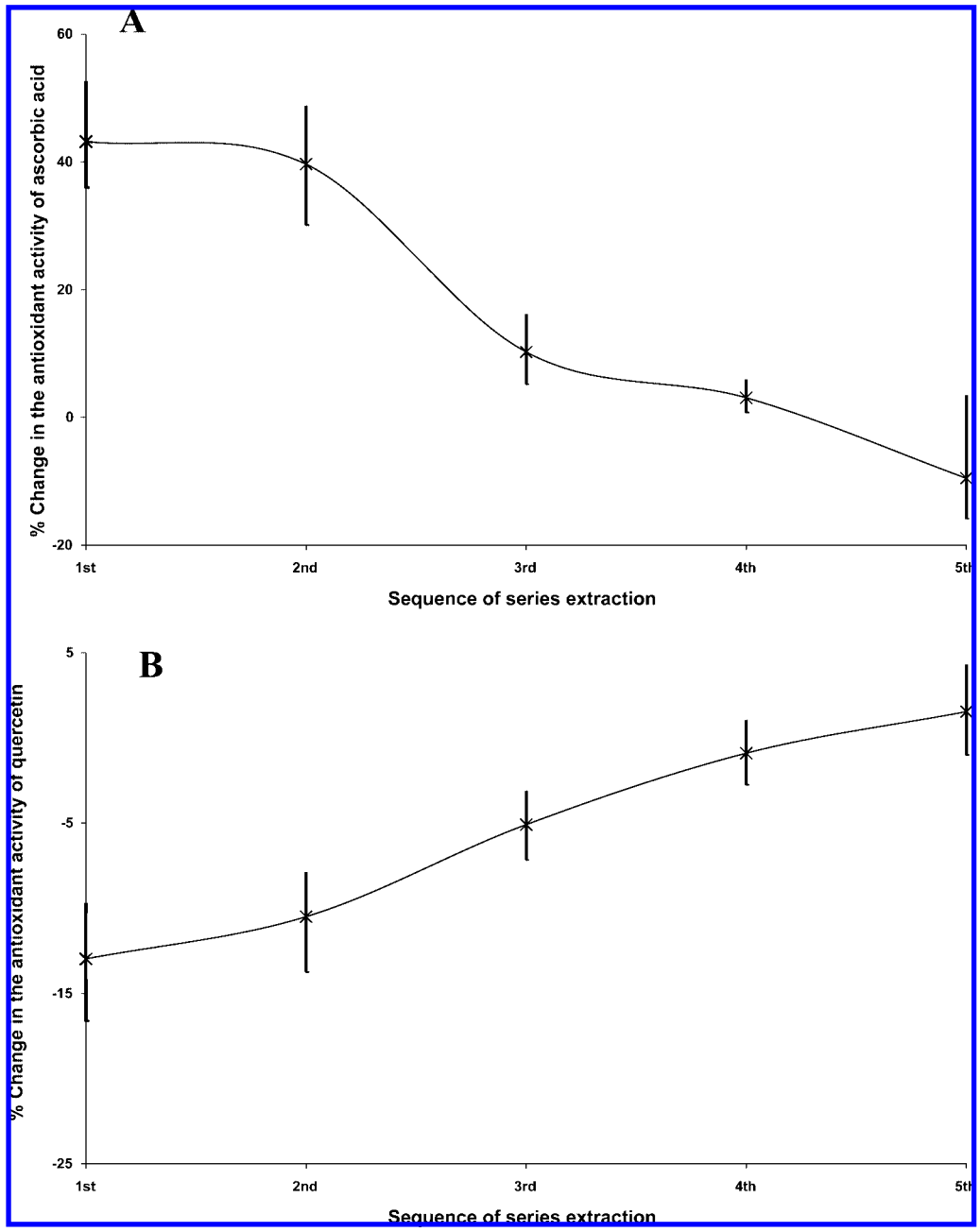

Figure 1. Effect of a series of HEPES extracts from freshly isolated never-dried apple cell walls on L-ascorbic acid (**A**) or quercetin activity (**B**). The mean values (×) and the range of values (bars) are shown. The experimental uncertainty of each datum point was approximately ±5%.

Table 3. Effects of the Freshly Isolated Never-Dried Apple Cell-Wall Suspension and Its Aqueous Supernatant on L-Ascorbic Acid or Quercetin Activity by the FRAP Assay[a]

sample	change in AA activity (%)	change in quercetin activity (%)
cell walls (2 h of incubation)	+(84 ± 4), $n = 3$	−(15.0 ± 0.7), $n = 3$
cell walls (2.5 h of incubation)	+(82 ± 4), $n = 3$	−(10.7 ± 0.5), $n = 3$
supernatant of cell-wall HEPES mixture (2.5 h of incubation)	+(73 ± 3), $n = 3$	−(7.7 ± 0.5), $n = 3$

[a] Data are expressed as (means ± standard deviations), and "*n*" is the number of cell-wall samples prepared at different times. Each incubation sample was assayed in duplicate.

abraded for 30 s using 3 μm alumina powder (PK-4 polishing kit) between runs to remove contaminants. The electrode potential was recorded against a Ag/AgCl reference electrode (+207 mV versus standard hydrogen electrode), and the current was taken against the response because of the buffer solution (blank). An aliquot of sample (20 mL) was added to the cell, and N_2 was flushed through the solution (2 min) prior to scanning to remove dissolved O_2. The scan was taken from −100 to +500 mV, with a scan rate of 100 mV s^{-1} and sensitivity of 1 μA/V. The variation of the peak potential between repeat runs was within 2–3 mV.

Before quantitative treatment, the solvent background curve (with only HEPES buffer present) was subtracted from that of the sample curve. The "percentage change" in the antioxidant activity was also expressed as Q_{500} values by replacing the absorbance (A) values in eq 1 with corresponding Q_{500} values.

Statistics. Q tests were conducted at the 90% confidence level.

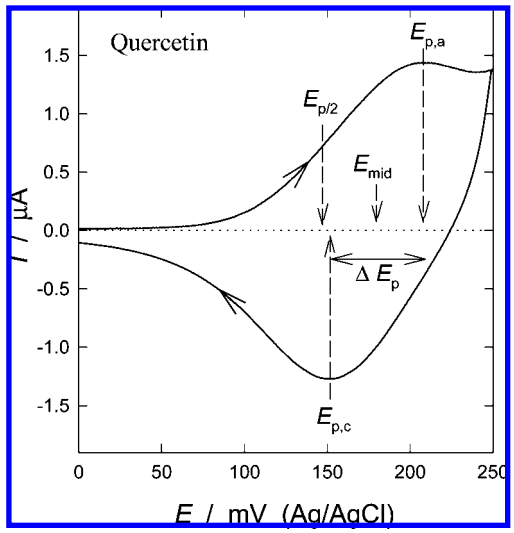

Figure 2. Cyclic voltammogram of quercetin over the potential range of 0−250 mV, showing the reversible nature of the first oxidation process.

RESULTS AND DISCUSSION

The monosaccharide composition of the isolated cell walls of Pacific Rose apples was Rha, 1.5; Fuc, 2.2; Ara, 18.0; Xyl, 22.0; Man, 0.6; Gal, 37.8; Glc, 17.9 mol %, and the uronic acid content was 239.0 mg/g. The recovery of total sugars was 773 mg/g of dry CW. The monosaccharide composition of the CDTA extract was Rha, 2.9; Fuc, 1.2; Ara, 19.0; Xyl, 33.3; Gal, 31.0; Glc, 12.6 mol %, with a uronic acid content of 282 mg/g. These results are comparable to those reported for other apple varieties (*3, 8–10*).

FRAP Assay and Antioxidant Activity. The FRAP assay was selected to detect the difference in the total antioxidant activity of AA and quercetin, in the absence and presence of apple cell walls or pectic polysaccharides. Freeze-dried cell walls gave virtually the same results as freshly isolated never-dried cell walls. Nonetheless, we chose to work with cell walls that were kept fully hydrated at all times, to lessen the possibility of their differing from cell walls in nature. In the presence of isolated cell walls and the CDTA fraction (**Table 2**), AA activity was retained but quercetin activity was lowered. Similar results were found for pectin and polyGalA. Apple and citrus pectins contain 74 and 86% polyGalA, respectively, and a higher polyGalA content seemed to be associated with a greater increase in AA activity. The commercial polyGalA exerted the strongest effect on AA or quercetin activity, indicating that the homogalacturonan backbone rather than the neutral side chains of the pectic polysaccharides is implicated in the effect on antioxidant activity.

The activity of quercetin was affected in a different manner from that of AA. We assume that the factors influencing AA activity were also playing roles in the observed quercetin activity, although the induced effects were of opposite sign. The relative extractabilities of polysaccharides and the functional groups on polysaccharides may contribute, as well as the presence of polyphenols (*7*) from the walls.

The effect of the relative extractabilities and solubilities of cell-wall components on the AA or quercetin activity is seen in parts **A** and **B** of **Figure 1**, respectively. The percent change in the antioxidant activity of AA and quercetin became smaller as the extraction sequence progressed, except for the second extraction. The results of the second extraction were comparable to those for the first extraction, implying that the interacting components continued to be solubilized over several hours and were then either exhausted or held in the walls, because they were insoluble under the current conditions (*15*). After the fourth extraction, little effect was seen, indicating that solubilization of components with protective capability had ceased.

Results shown in **Table 3** further support the hypothesis that water-soluble components leached from the cell walls affect the antioxidant activity of AA or quercetin. A difference was observed between the antioxidant activity profiles for cell walls suspended in the aqueous mixture or the supernatant of this mixture, when incubated with AA and quercetin for 2.5 h.

While trace amounts of polyphenolic compounds were present in the supernatants (*19*), the monosaccharide composition of the supernatants from the incubation mixture of cell walls and HEPES buffer (*19*) indicated the presence of predominantly pectic polysaccharides. Pectic polysaccharides vary in their structure, thus influencing intermolecular interactions and water solubility (*7*).

Apples contain both AA and quercetin. Although nearly all of the AA originally present in the apples would have been removed by the isolation and incubation steps carried out prior to the FRAP assay, quercetin and other polyphenols remained after cell-wall isolation and incubation, as evidenced by the low antioxidant activity detected in apple cell-wall controls and high-performance liquid chromatography (HPLC) data (*19*).

Trolox Equivalent Capacity of AA and Quercetin. The level of an antioxidant detected depends upon the technique used. Thus, antioxidant activity values are often converted to Trolox equivalent values to aid the comparison. In this study, some FRAP values have been converted to Trolox equivalent values using eq 2

$$\mathrm{TEAC_{FRAP}\ value\ (mM)} = \frac{\mathrm{absorbance}}{1.46 \times \mathrm{concentration_{antioxidant}\ (mM)}} \quad (2)$$

The $\mathrm{TEAC_{FRAP}}$ value of AA in the presence of the isolated apple cell walls (0.93 mM) after the 2 h of incubation was much higher than that of AA alone (0.34 mM). All of these values were much lower than that of AA alone before incubation (1.33 mM). These results support the idea that apple cell walls may help to maintain the antioxidant activity of AA. In contrast, little difference was seen between the $\mathrm{TEAC_{FRAP}}$ values of quercetin in the absence (2.29 mM) or presence (2.35 mM) of apple cell walls, with both values being much lower than that of quercetin alone before incubation (3.53 mM). This change could have resulted from the degradation of quercetin to products such as protocatechuic acid (3,4-dihydroxyphenylacetic acid) during the incubation (*19*). The degradation products are known (*20*) to have much lower TEAC values than quercetin.

Cyclic Voltammetry. CV of complex mixtures provides a measure of the total antioxidant status. The ability of antioxidants to be oxidized allows them to display unique electrochemical properties and be characterized by the current–potential relationships exhibited at an inert electrode. The potential is scanned at a controlled rate, and the current produced by oxidations or reductions is recorded continuously. Compounds with the same chemical characteristic give a similar response, although the CV response may vary considerably with a small change in structure; e.g., having two hydroxyl groups on a benzene ring in the *meta* rather than the *ortho* position can shift the oxidation potential by several hundred millivolts (*21*). The reversibility of the reaction at the carbon electrode will vary

Table 4. CV Parameters for Freshly Isolated Never-Dried Cell-Wall−Antioxidant Samples, Antioxidant Controls, and Cell-Wall Controls in HEPES Buffer[a]

sample	$E_{p,a}$ (mV)	$E_{p,a} - E_{p/2}$ (mV)	$I_{p,a}$ (μA)	$I_{p,c}/I_{p,a}$	Q_{500} (μC)
QU before incubation	208.0 (341.5)	57.9	2.16 (3.60)	0.86	8.96
QU control after incubation	232.5 (370.5)	63.7	0.80 (1.71)	0.73	3.37
AA before incubation	248.5	114.5	3.81	*b*	10.3
AA control after incubation	*c*	*c*	*c*	*c*	0.78
QU plus apple after incubation	*c*	*c*	*c*	*c*	2.90
AA plus apple after incubation	318	132.2	1.63	*b*	3.37
apple after incubation	*c*	*c*	*c*	*c*	0.51

[a] QU represents quercetin, and AA represents ascorbic acid. $E_{p,a}$ refers to the anodic peak potential; $E_{p/2}$ refers to potential at half the peak height; $I_{p,a}$ refers to the anodic peak current; $I_{p,c}$ refers to the cathodic peak current; and Q_{500} refers to the charge passed to 500 mV. The values in parentheses refer to the second anodic peak. The "apple" term refers to their cell walls. Three cell-wall samples were prepared at different times. Each incubation sample was analyzed in duplicate by CV. [b] The $I_{p,c}$ value was not available because of an irreversible wave. [c] No accurate data were obtained because of broad peaks.

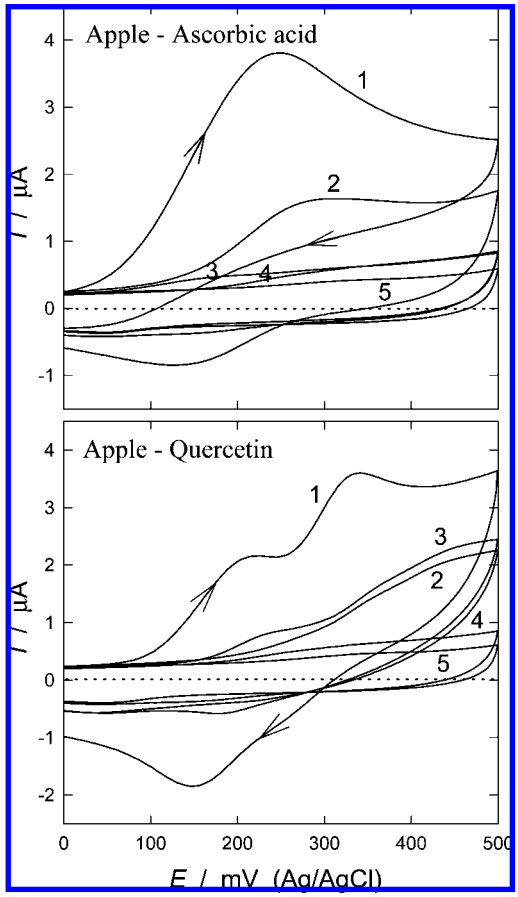

Figure 3. Cyclic voltammograms of aqueous samples (series from top to bottom): 1, antioxidant before incubation (pH 6.5, 37 °C); 2, antioxidant plus cell-wall materials after incubation; 3, antioxidant after incubation; 4, cell-wall materials after incubation; 5, solvent background (15 mM HEPES).

with different antioxidants. The oxidation of quercetin and its degradation products could be partially reversed at the carbon electrode, while AA was irreversibly oxidized, because the oxidation product in this case cannot be reduced at the carbon electrode.

Figure 2 shows a cyclic voltammogram for quercetin. The anodic peak potential ($E_{p,a}$) and the potential at which the current reaches half of its maximum ($E_{p/2}$), the half-peak potential, indicate the reducing strength of the particular structural group in a sample (*21*). Generally, the lower the oxidation potential under the same conditions, the more powerful is the antioxidant as a reducing agent (*22*). For a reversible wave, in which the oxidation and reduction occur easily and rapidly at the electrode, $|E_{p,a} - E_{p/2}|$ characterizes the sharpness of the peaks. On the reverse scan, the current at the cathodic peak ($I_{p,c}$) reflects the ease with which the oxidized form of an antioxidant can be reduced back to its original form. $I_{p,a}$, the intensity of the anodic current, reflects to some extent the concentration of the antioxidant. For a fully reversible system, a value of $I_{p,c}/I_{p,a}$ approaches a value of 1.0 (*21*), and in our case, the value is 0.86. The integrated area under the anodic current, Q_{500} (after subtraction of background spectra), provides a good evaluation of the total antioxidant capacity (*22*).

The parameters in **Table 4** were obtained from the current–potential curves (**Figure 3**). In a solution containing AA alone, AA had decomposed by the end of incubation to compounds with undetectable reducing power. Consequently, the AA solution lost most of its reducing power (Q_{500} values before and after incubation were 10.3 and 0.78 μC, respectively). However, 33% reducing power was retained if the incubation involved apple cell walls. In the presence of apple cell walls, the degradation product 2,3-diketo-L-gulonic acid was possibly converted, under decarboxylation pathways, into the reducing compounds L-erythrulose (*23*), L-threose (*24, 25*), L-threosone (*26*), L-xylose (*27*), and xylosone (*28*). Under the current incubation conditions, these intermediates had distinctive redox properties and consequently exhibited varied antioxidant activities in FRAP assays. They are probably responsible for the broad peak seen in the CV at high potential.

Two broad anodic peaks were present in the cyclic voltammogram of aqueous quercetin solution (208 and 342 mV) prior to incubation (**Figure 3**). After correction for variations in the concentration and pH, these two potentials lay in the range of 150−310 mV, consistent with the presence of both *ortho*-diphenol (catechol) and *meta*-diphenol groups. Incubation seemed to facilitate the loss of the above groups, particularly when the incubation involved apple cell walls. In the case of quercetin controls, the loss of the cyclic voltammogram response might result from further oxidative and/or degradative fragmentation, rapid chemical rearrangement of quercetin after oxidation, and stabilization of the quinone intermediate. These processes might also be involved in the case of cell-wall−quercetin incubation. Additionally, interactions between polysaccharides and phenolic compounds are possible and would be dependent upon the composition of the cell-wall preparation. These interactions might include complexation of phenolic compounds with polysaccharides (*29*) and oxidative coupling between carbohydrates and quinone methides of phenols (*30*). Quercetin and AA have very different chemical structures, and it is to be expected that they would interact differently with apple cell walls.

The effects of apple cell walls on the antioxidant activity of AA or quercetin [+(2.3−2.5) and −(0.36−0.40), respectively] in CV and [+(1.6−1.9) and −(0.36−0.40), respectively] in FRAP assays were similar, but larger changes in the value were observed by CV. The difference between CV and FRAP results may be due to the different reaction mechanisms involved. CV was conducted from −100 to 500 mV, while the FRAP assay occurs at a relatively low oxidation potential (~300 mV). Compounds with high oxidation potentials respond poorly in the FRAP assay (*21, 31*).

Apple cell-wall components largely stabilized AA antioxidant activity but offered little protection against quercetin degradation. Thus, the effect of the interactions between plant cell-wall polysaccharide preparations and natural antioxidants may be beneficial or nonbeneficial, depending upon the nature of antioxidants and plant cell-wall materials. Commercial pectins and polyGalA also stabilized the AA antioxidant activity, and neither had protective effect on quercetin. The water-soluble pectic polysaccharides derived from apple cell walls appear to be responsible for the observed effects of cell-wall materials on AA and quercetin and antioxidant activities.

We have demonstrated a potential for interactions between components of a meal, which may slow the degradation of some but not all nutrients. Such information is useful in furthering the understanding of the science behind current advice for consuming a diet high in fruit and vegetables and in the design of foods containing added phytochemicals.

ABBREVIATIONS USED

AA, ascorbic acid; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid; CV, cyclic voltammetry; CW, cell wall; DF, dietary fiber; FRAP, ferric reducing antioxidant power; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; polyGalA, polygalacturonic acid; QU, quercetin; RG-I, rhamnogalacturonan 1; TPTZ, tripyridyltriazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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Received for review September 10, 2007. Revised manuscript received November 9, 2007. Accepted November 13, 2007. The NZ Agricultural and Marketing Development Trust (AGMARDT) is thanked for the award of a doctoral scholarship to D.S.-W.

JF072670V