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Effect of raw and cooked onion dietary fibre on the antioxidant activity of ascorbic acid and quercetin

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ABSTRACT

The effect of cell walls (dietary fibre) from raw and cooked onions (*Allium cepa* L. red skinned variety) on two dietary antioxidants, L-ascorbic acid (AA) and quercetin, was investigated. Cell walls isolated from onion parenchyma tissues were incubated with AA or quercetin in HEPES buffer at pH 6.5 and 37 °C for 2 h. The resulting supernatants were analysed by the ferric reducing antioxidant power (FRAP) assay and cyclic voltammetry (CV). Results show that onion cell walls effectively reduced AA degradation, but provided no protective effect against quercetin degradation, and may increase the degradation. This suggests some type of favourable interaction of the cell wall components with AA but not with quercetin. Cooking facilitates the extraction of cell wall polysaccharides and hence influences the extent of interaction of cell wall components and antioxidants. The redox behaviour of the antioxidants, the polysaccharide components of cell walls, and their relative extractabilities all appear to have important influences on the interactions.

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1. Introduction

In a meal, a variety of foods is consumed together. Consequently, food components, such as dietary fibres (DFs), natural antioxidants, proteins and lipids, are likely to interact with each other during digestion (Harris & Ferguson, 1999; O'Connor, Sun, Smith, & Melton, 2003a, 2003b). These interactions may be beneficial, non-beneficial, or even detrimental. In previous work, cell walls from a range of fruits have been found to protect ascorbic acid from oxidation (Motomura & Yoshida, 2003). Subsequently, apple cell walls and their polysaccharides were shown to affect the antioxidant activity of quercetin and L-ascorbic acid (Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008). In particular, antioxidant activities could be enhanced or reduced by interaction with the plant cell wall components that make up dietary fibre.

Onions are one of the most widely consumed vegetables. Worldwide, ∼5.5 kg of onions is consumed annually per person. Onions contain relatively large amounts of quercetin (Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2008; Ewald, Fjelkner-Modig, Johansson, Sjöholm, & Akesson, 1999), which are absorbed by humans as the aglycone and possibly as their glycosides (Nemeth et al., 2003; Sesink, O'Leary, & Hollman, 2001). Ten different glycosides of quercetin have been detected in onions (Slimestad, Fossen, & Vagen, 2007) and five have been reported (Bonaccorsi et al., 2008) in red onions. The L-ascorbic acid (AA) content of onions varies from 50 to 100 mg/kg fresh weight (Food Standards Agency, 2002; Lawande, 2001). Thus, understanding the interactions between onion cell walls and natural antioxidants should benefit the development of new food products and nutraceuticals.

Parenchyma cells constitute most of the onion tissues consumed in the human diet. Parenchyma cell walls consist largely of cellulose, non-cellulosic polysaccharides (including pectin and xyloglucan) and water, as well as some glycoproteins and other smaller molecules, including phenolic acids (O'Neill & York, 2003). Pectin consists of a polygalacturonic acid backbone with regions of long side chains composed of galactose and arabinose (RG-I) and regions of short irregular side chains (RG-II). The carbohydrate composition of the cell walls of raw onions has been determined (Ng, Smith, & Waldron, 1998; Redgwell & Selvendran, 1986) and their physicochemical properties have been investigated (Ha, Apperley, & Jarvis, 1997; Ng et al., 2000; Smith, Harris, Melton, & Newman, 1998). While some are eaten raw in sandwiches and salads, onions are commonly cooked before eating. Hence, it is surprising how little work has been done on the cell walls of cooked onions. Common cooking methods such as steaming, boiling, microwaving and pressure-cooking increase the extractability and solubility of plant cell wall polysaccharides (Greve, McArdle, Gohlke, & Labavitch, 1994; Lecain, Ng, Parker, Smith, & Waldron,

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1999; Ratnayake, Hurst, & Melton, 2003). Boiling in water causes a greater loss of some cell wall polymers (e.g. pectic polysaccharides) than does steaming (Quach, Melton, Harris, Burdon, & Smith, 2001; Ratnayake et al., 2003). In this study, microwave- and steam-cooking were used.

We have investigated the effect of onion cell walls on the antioxidant activity of L-ascorbic acid and quercetin. The neutral monosaccharide (NM) and uronic acid (UA) compositions of the isolated cell walls from raw and cooked onions were determined. FRAP (Pulido, Bravo, & Saura-Calixto, 2000) and cyclic voltammetry (CV) (Kilmartin, 2001) assays were used to examine the redox behaviours of the antioxidants in the absence and presence of onion cell walls.

2. Materials and methods

2.1. Materials and solution preparations

Red onions (*Allium cepa* L.) were purchased from a local supermarket. Quercetin dihydrate, tripyridyltriazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemicals (St Louis, MO). Ascorbic acid was purchased from Scharlau Chemicals (Spain). *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), was purchased from BDH Chemicals, Poole, UK.

A solution of AA (0.50 mM, 50 mL) was prepared and stored in the dark. Quercetin solution (0.25 mM) was prepared by adding 0.0042 g 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 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.

2.2. Isolation of onion cell walls

The isolation method of Melton and Smith (2005a) without dithiothreitol was used to achieve a mild aqueous procedure, thereby avoiding the introduction of compounds such as phenols and other reducing agents that might interfere with FRAP and CV studies. Organic solvents were not used as they alter the physical structure of cell walls, and possibly their properties (Thimm, Burritt, Ducker, & Melton, 2000), so instead the cell walls were kept fully hydrated throughout their isolation. All cell walls were freshly isolated, and those used in antioxidant experiments were never dried. Portions of the isolated cells walls were freeze dried for determination of monosaccharide composition and dry weight. All materials and solutions were pre-cooled (⩽4 °C).

2.2.1. Raw onion cell walls

The terminal bud, the stem apex, the dry outer skin, the outer fleshy leaf and the innermost leaves were removed. The epidermal monolayer of cells on the concave surface of each remaining fleshy leaf was discarded. The selected tissue (120 g), containing predominantly parenchyma cells (as determined microscopically) was cut into small pieces, frozen in liquid nitrogen and then ground to a coarse powder with a mortar and pestle. The resulting coarse powder underwent further rupture in a ring grinder (1 min per portion). The fine powder was transferred to a beaker in an ice bath, 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 collected by filtration on 50 μm nylon mesh and washed with HEPES buffer. The cell wall isolation was continued as previously described (Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008).

2.2.2. Cooked onion cell walls

To examine the effect of cooking, cell walls were isolated from steam- and microwave-cooked onions. All raw and cooked samples originated from the same batch of homogeneous fresh onion tissues. Onions were chopped into slices $4 \times 4 \times 0.5\ cm^3$ prior to cooking. For steam-cooking, selected slices (120 g) were placed on a nylon sieve, covered and steamed (10 min) over boiling Milli-Q water (100 mL). The steam-cooked tissues were immediately frozen in liquid nitrogen for cell wall isolation. Alternatively, selected slices of raw onions (120 g) were cooked in a microwave oven for 1 min operating at 1000 W (Microwave RA-620, Mitsubishi, Japan), and then immediately frozen in liquid nitrogen for cell wall isolation.

Cooked onion cell walls were isolated in the same way as raw onion cell walls, except the first and second ring grinding steps were 1.5 and 3.5 min per portion, respectively.

2.3. Monosaccharide composition of the cell walls

The neutral monosaccharide (NM) compositions (as their alditol acetates) of the isolated cell walls from raw and cooked onions, following hydrolysis by trifluoroacetic acid (2 M, 121 °C, 1 h) and H_2SO_4 (72%, 30 °C, 2 h, then: 1 M, 100 °C, 3 h) for cellulose, together with their uronic acid contents (as galacturonic acid), were determined using reported procedures (Melton & Smith, 2005b, 2005c).

2.4. Incubation of cell walls and antioxidants

Incubations were carried out at 37 °C, pH 6.5 for 2 h in a shaking bath. Samples were prepared in duplicate (Table 1) and contained HEPES (15 mM), AA (0.125 mM) or quercetin (0.0625 mM), and freshly isolated, never-dried cell walls (0.1 g/mL) from raw or cooked onions. HEPES buffer was used here in the preparation of cell walls because, unlike phosphate and citrate, it does not complex metal ions. Supernatants were obtained by centrifugation (1000g, 5 min) for FRAP and cyclic voltammetry.

2.5. FRAP assay

FRAP assays were performed in triplicate according to the method of Pulido et al. (2000). The change in the antioxidant activity was expressed as "Percentage Change", calculated as in equation:

$$\text{Change in activity } (\%) = \frac{A_{\text{antioxidant-cell wall}} - (A_{\text{antioxidant}} + A_{\text{cell wall}})}{A_{\text{antioxidant}} + A_{\text{cell wall}}} \times 100 \qquad (1)$$

where *A* is the absorbance.

2.6. Cyclic voltammetry

Cyclic voltammetry was performed as previously described (Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008)

Table 1
Preparation of incubation samples (in duplicate) for FRAP and cyclic voltammetry

Sample	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 sample	HEPES buffer (15 mM, pH 6.5), AA (0.125 mM), isolated cell walls (1 g)
Quercetin control	HEPES buffer (15 mM, pH 6.5), quercetin (0.0625 mM)
Quercetin–cell wall sample	HEPES buffer (15 mM, pH 6.5), quercetin (0.0625 mM), isolated cell walls (1 g)
Cell wall control	HEPES buffer (15 mM, pH 6.5), isolated cell walls (1 g)

using a glassy carbon disk as the working electrode and a Ag/AgCl reference electrode. Samples were flushed with N_2 before scanning. The scan rate was 100 mV s^{-1} with a sensitivity of 1 μA/V. The variation of the peak potential between repeat runs was within 2–3 mV. Samples were prepared in duplicate and analysed in triplicate. The variation of peak potential between repeat runs was within 2–3 mV.

Before quantitative treatment, the solvent background curve for HEPES buffer was subtracted from that of the sample curve. "% 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.

2.7. Statistical analysis

Before calculation of mean values, Q tests were conducted at the 90% confidence level. At least two replicate determinations were retained for each datum point.

3. Results and discussion

3.1. Monosaccharide composition of cell walls

The monosaccharide compositions of the isolated cell walls from raw and cooked red onions are similar (Table 2) with slightly higher polygalacturonic acid content in the steamed CWs. Previously, it had been shown that the uronic acid in raw onion cell walls consisted of 97% galacturonic acid and 3% glucuronic acid (Smith et al., 1998). The composition of the red onion cell walls is comparable with those reported previously for other onion varieties, both raw (Ng et al., 1998; Redgwell & Selvendran, 1986; Smith et al., 1998) and processed (Lecain et al., 1999). The yields (dry weight) of cell walls from 120 g of onion were for raw 1.30 ± 0.06 g, steamed 0.97 ± 0.01 g, and microwaved 1.02 ± 0.01 g.

3.2. FRAP assay

Cell walls from raw and cooked onions inhibited the AA degradation effectively, but exerted little protective effect on quercetin degradation (Table 3). Cooked onion cell walls stabilized AA better than raw onion cell walls. Cell walls from the two types of cooked onions had similar effects on the antioxidants with those from steamed onions, exerting a slightly greater protective effect than those that had been microwaved.

The effect of cell walls on antioxidant activity of AA and quercetin appears to be associated with the monosaccharide composition (including the UA content) of the cell wall polysaccharides. Cell walls isolated from raw onion contained more non-cellulosic NMs (48.3 w/w% dried cell walls) and less UA (24.2 w/w% dried cell walls), than those isolated from microwaved onion (41.2 and 25.3 w/w% dried cell walls, respectively) and steamed onion (40.9 and 27.4 w/w% dried cell walls, respectively) (Table 2). The 2 h incubation also facilitated the release of cell wall components into the buffer. UA and NMs (principally galactose, glucose and xylose) were detected in the supernatant of the incubated onion cell walls. Cooking can be expected to increase the extractability of polysaccharides, especially pectins (Lecain et al., 1999; Ng & Waldron, 1997) and other compounds such as polyphenols (Ewald et al., 1999).

3.3. Trolox equivalent antioxidant capacity values

FRAP values were converted to Trolox equivalent values using:

$$\text{TEAC}_{\text{FRAP}}\text{value (mM)} = \frac{\text{Absorbance}}{1.46 \times \text{Concentration}_{\text{antioxidant}}\ (\text{mM})} \quad (2)$$

After incubation, the $\text{TEAC}_{\text{FRAP}}$ value of AA in the presence of onion cell walls (1.22 mM) retaining 92% of the AA antioxidant activity, was considerably higher than that of AA alone (0.34 mM). This result shows that the isolated onion cell walls (dietary fibre) protect AA antioxidant activity very effectively. However, little difference was seen for the $\text{TEAC}_{\text{FRAP}}$ value of quercetin in the absence (2.29 mM) or presence (2.31 mM) of isolated onion cell walls. Both values were lower than that of quercetin alone before incubation (3.53 mM), suggesting that onion cell walls did not provide either beneficial or detrimental effects on the antioxidant activity of quercetin. Onion cell walls were more effective than apple cell walls (Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008) in protecting AA but they had virtually the same effect on quercetin.

3.4. Cyclic voltammetry

Antioxidants can be oxidised at an electrode, and the more powerful the reducing agent, the lower is its positive oxidation potential. As well as the characteristic oxidation potential ($E_{p,a}$), the anodic current ($I_{p,a}$), reflects the concentration of the antioxidant. An antioxidant may exhibit more than one anodic peak if further oxidations occur, as was observed with quercetin (Fig. 1b,

Table 2
Neutral monosaccharide and uronic acid content of the raw or cooked onion cell walls expressed as mg/g dried cell walls

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
Monosaccharide mg/g dried cell walls								
Raw	7.7 ± 0.1	5.8 ± 0.3	18.7 ± 0.8	27.9 ± 1.4	3.8 ± 0.0	259 ± 4	160 ± 1.1	242 ± 2.0
Microwaved	5.8 ± 1.8	4.4 ± 0.2	14.5 ± 1.0	25.7 ± 5.1	3.0 ± 0.2	192 ± 12	167 ± 1.2	253 ± 1.0
Steamed	5.6 ± 0.6	3.9 ± 0.4	10.4 ± 0.8	22.8 ± 2.1	4.6 ± 1.9	185 ± 14	176 ± 4.3	274 ± 1.0

Data are expressed as a mean value ± standard deviation. NM results are means of duplicate determinations, UA (as GalA) results are means of 4 determinations (2 × duplicate).

Table 3
Changes in the activity of L-ascorbic acid and quercetin measured by FRAP assay after incubation with raw or cooked onion cell walls, calculated using Eq. (1)

Cell wall Samples	Change in ascorbic acid activity (% per mg dry basis)	Change in quercetin activity (% per mg dry basis)
Raw	+(2.2 to 2.9), mean = +2.6, n = 12	−(0.2 to 0.3), mean = −0.2, n = 12
Microwaved	+(2.7 to 3.2), mean = +3.0, n = 5	−(0.1 to 0.2), mean = −0.1, n = 5
Steamed	+(2.9 to 3.5), mean = +3.2, n = 5	−(0.1 to 0.2), mean = −0.1, n = 5

Data are based on the dry weight of cell walls and expressed as a range of the calculated results after statistical analysis by the Q tests (at 90% confidence level). "n" refers to the number of different cell wall samples isolated at different times. Each cell wall sample was used twice, for preparation of duplicate incubation mixtures, and each incubation mixture was measured three times by a FRAP assay.

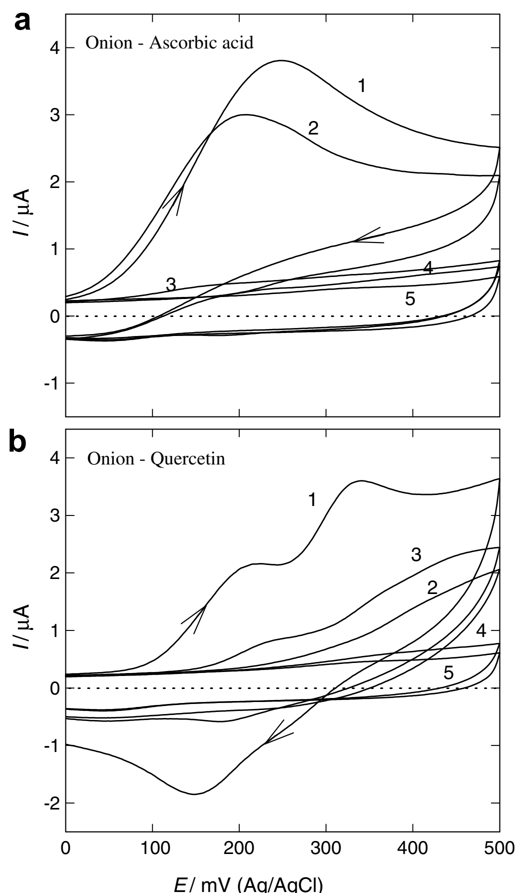

Fig. 1. Cyclic voltammograms of (a) Ascorbic acid aqueous samples; (b) Quercetin aqueous samples. Curve: 1, Antioxidant before incubation (pH 6.5, 37 °C); 2, Antioxidant + raw onion cell walls after incubation; 3, Antioxidant after incubation; 4, Raw onion cell walls after incubation; 5, Buffer solution background (HEPES 15 mM).

curve 1). The half-peak potential ($E_{p/2}$) is where the current reaches half of its maximum. $E_{p,a} - E_{p/2}$ characterises the sharpness of a peak, with the faster the oxidation the sharper the peak. The area under the anodic current (up to 500 mV), $Q_{500,}$ is a measure of the total antioxidant capacity (Chevion, Roberts, & Chevion, 2000).

On the reverse scan, the current at the cathodic peak ($I_{p,c}$) reflects the ease with which the oxidised form of the antioxidant is reduced to its original form. The oxidation of quercetin and its degradation products could be partially reversed (Fig. 1b, curve 1, both directions, as indicated by arrows), while ascorbic acid was irreversibly oxidised (Fig 1a, curve 1, both directions, as indicated by arrows).

Fig. 1a and b show the cyclic voltammograms for AA and quercetin controls before (curves A1 and B1) and after incubation (curves A3 and B3), for onion cell wall controls after incubation (curves A4 and B4), for AA–/quercetin–onion cell wall samples after incubation (curves A2 and B2), and for the buffer solution background (A5 and B5). Results from the CV experiments show that fresh raw onion cell walls noticeably stabilised AA antioxidant activity, but exerted little protection on quercetin activity.

Fig. 1a shows the anodic peak for AA before incubation (curve A1) is not symmetrical: at higher potential ($E_{p,a}$) there is an additional response that can be attributed to degradation products of AA, which are capable of being oxidized. After incubation with the cell walls (curve A2) the AA anodic peak is reduced and there is a greater proportion of the broad peak at higher potential. The AA alone lost most of its reducing power after incubation (curve A3). Q_{500} values before and after incubation were 10.3 and 0.78 µC, respectively (Table 4). However, 82% of the reducing power was retained if the incubation involved onion cell walls (curve A2). In a comparable experiment with apple cell walls, 33% of the reducing power was retained (Sun-Waterhouse et al., 2008).

Table 4
Cyclic voltammetry parameters for raw onion cell wall–antioxidant samples, antioxidant controls, and cell wall controls in HEPES buffer

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
QU + CW after incubation	*	*	*	*	1.89
AA before incubation	248.5	114.5	3.81	–	10.3
AA control after incubation	*	*	*	*	0.78
AA + CW after incubation	208.0	104.5	3.00	–	8.47
CW after incubation	*	*	*	*	0.36

Abbreviations used: QU represents aqueous quercetin. AA refers to ascorbic acid. $E_{p,a}$ (anodic peak potential); $E_{p/2}$ (potential at half the peak height); $I_{p,a}$ (anodic peak current); $I_{p,c}$ (cathodic peak current); Q_{500} (charge passed to 500 mV). The values in parentheses refer to the second anodic peak. * no accurate data were obtained due to broad peaks. – $I_{p,c}$ value was not available due to an irreversible wave.

AA, with a 2,3-endiol group in its structure, has strong reducing power in aqueous solution. When the endiol group of AA is oxidized, DHA is formed and it is easily hydrolysed to 2,3-diketo-L-gulonic acid (DKG) (Nakata, Morita, Sawada, & Takagi, 1992).While DKG does not have any reducing power, the broad anodic peak observed at high potential could arise from its subsequent degradation products (Sun-Waterhouse et al., 2008). Moreover, such compounds will have a range of antioxidant activities with the FRAP assay. The similarity of curves A1 and A2 in the cyclic voltammogram (Fig. 1a) suggests that in the presence of onion cell walls, the majority of AA has been retained or possibly regenerated (Kennedy, White, Warner, Lloyd, & Rivera, 1989; Takagi, Morita, & Nakata, 1991).

Fig. 1b shows two anodic peaks in the cyclic voltammogram of the quercetin solution (curve B1). These two peaks were attributed to the *ortho*-diphenol group on the B-ring and the hydroxyl group at C-3 on the C-ring of quercetin. Incubation seemed to facilitate the loss of these groups (curves B2 and B3), particularly when the incubation involved onion cell walls (curve B2), probably due to further oxidation and/or degradation. From the Q_{500} values it can be seen that the onion cell walls have a detrimental effect on the antioxidant activity of quercetin with 21% retained, compared with 38% in the absence of the cell walls. Apple cell walls show a negative effect on quercetin activity (Sun-Waterhouse et al., 2008) but the onion CWs are even less protective.

In spite of the relatively large amounts of ascorbic acid and quercetin present in onion, there is little indication that they are present in the isolated cell walls. We assume they had no opportunity to interact with the cell walls during the isolation procedure. In the case of ascorbic acid, with its high water-solubility, this premise is easily understood. It is pertinent to note that in chopped onions there was no change in the amount of quercetin after standing for 60 min at room temperature (Makris & Rossiter, 2001).

4. Concluding remarks and perspectives

Results from FRAP and CV experiments suggested there is an interaction between onion cell walls and ascorbic acid *in vitro*. Both

procedures showed onion cell walls have a greater protective effect on ascorbic acid antioxidant activity than apple cell walls (Sun-Waterhouse et al., 2008). There are a number of possible explanations. The cell walls have different compositions; onion walls have less arabinose and xylose but comparable uronic acid levels compared with apple walls. In previous work on apple cell walls (Motomura & Yoshida, 2003; Sun-Waterhouse et al., 2008) it was found that the soluble pectin from the walls and in particular polygalacturonic acid exerted the greatest protective effect on ascorbic acid. Indeed, solubility appears to be a key factor. Cooking the onions increased the uronic acid content of the soluble material in the incubation mixture and the related protective effect. Moreover, we found that after the two hour incubation of raw onion cell walls alone in the pH 6.5 HEPES buffer, 73% more polygalacturonic acid had been extracted from the cell walls compared with apple walls. The polygalacturonic acid portion of pectin may form a complex with the ascorbic acid via Ca^{2+} ions, which are the major cations in cell walls, or it could complex multivalent metal ions (copper, iron) that would otherwise catalyse the oxidation of ascorbic acid. It has been shown (Dumville & Fry, 2003) that L-ascorbic acid added to cell walls in the presence of Cu^{2+} ions undergoes oxidation, and as a consequence there is an increased solubilisation of pectin. Although this may be happening to some extent in our study, it is not the dominant influence, possibly because the effect observed by Dumville and Fry (2003) was greatest at pH 3.7 and minimal at pH 5.7, while we worked at pH 6.5.

Understanding the interaction of dietary fibre and quercetin is more problematical. The FRAP assay indicates onion cell walls have no effect on the antioxidant activity of quercetin. The same loss of activity occurred with or without onion walls. Likewise apple cell walls had little or no effect on the FRAP antioxidant activity of quercetin (Sun-Waterhouse et al., 2008). It should be noted that quercetin has much greater ability than ascorbic acid to reduce Fe^{3+} in the FRAP assay (Pulido et al., 2000). In contrast, cyclic voltammetry results suggest there was a greater loss of quercetin antioxidant activity when onion or apple cell walls were present, but for onion the loss was larger. If the CV results are considered as more reliable they suggest that the presence of onion or apple cell walls cause an additional decrease in quercetin antioxidant activity (Q_{500} of 3.37 μC alone compared with 1.89 μC with onion walls), which might be due to quercetin binding to the insoluble portion of the cell walls, which are removed by centrifugation before the antioxidant activity is measured. In an updated model of plant primary cell walls (Bootten, Harris, Melton, & Newman, 2004), the cellulose microfibrils are not completely coated with xyloglucan or other hemicelluloses, so they could be available to bind quercetin. Selective binding of polyphenols by isolated apple cell walls has been reported by Le Bourvellec, Le Quere, and Renard (2007) who found catechin and epicatechin (which have the same ortho-diphenol B-ring structure as quercetin) were bound. Another possibility is the cell wall isolate contains an oxidising agent or enzyme such as polyphenyloxidase or peroxidase. As no specific precautions were taken to inactivate enzymes in the cell wall isolation procedure, the presence of enzymes cannot be excluded.

Quercetin can form complexes with iron and copper ions (El Hajji, Nkhili, Tomao, & Dangles, 2006; Makris & Rossiter, 2000; Moridani, Pourahmad, Bui, Siraki, & O'Brien, 2003) and this has been suggested as the reason for their ease of metal-catalysed oxidation. The mildly alkaline conditions (pH 7.4; pH 8) typically used favour complex formation and hence oxidation of quercetin (such high pH values are not expected to be found in plant cell walls *in vivo*). A possible explanation of why the presence of cell walls leads to the additional loss of quercetin observed by CV is that quercetin successfully competes with the polygalacturonic acid portion of pectin for iron and copper ions originating in the cell walls. Indeed, a recent paper Jin et al. (2007) reported that a crude phenolic extract removed iron from root cell walls. Once bound to the quercetin, the metal ions could catalyse its oxidation.

Because the polysaccharide composition and cell wall architecture vary among fruits and vegetables, the interactions of different dietary fibres with antioxidants are likely to vary in both type and extent. A more challenging question is whether such interactions between dietary fibres and antioxidants occur in the human digestive tract.

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