Esterified Phenolics of the Cell Walls of Chufa (*Cyperus esculentus* L.) Tubers and Their Role in Texture

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Chufas (*Cyperus esculentus*) are edible tubers that, like Chinese waterchestnut (CWC), are very crisp when raw and do not soften when cooked. The present study compares the mechanical properties of chufas with those of potato and CWC in relation to the carbohydrate and phenolic compositions of the cell walls. The cutting toughness of raw chufa was higher than that of raw CWC and potato; its value decreased on boiling, as also observed with CWC, but remained over twice that of raw potato. Chufa cell walls were rich in xylose, arabinose, glucose, uronic acid, and galactose, with minor quantities of mannose. The cell walls of the parenchyma exhibited a uniform pH-dependent autofluorescence indicating the presence of cinnamic acid derivatives. Analysis of these revealed that peeled tuber cell walls are rich in ferulic acid, whereas *p*-coumaric acid dominates the monomeric phenol fraction of the skin. Cell wall material from both skin and peeled tubers contains a significant amount of different diferulic acids (~20% of the wall ferulic acid), consisting mainly of the 8−*O*−4′-, 8−5′-, and 5−5′-dimers. These are potentially available to form thermally stable cross-links between polysaccharides within the wall and between cells. This may confer thermal stability of texture.

Keywords: *Chufa; cell wall; mechanical properties; phenolics*

INTRODUCTION

Previous work on Chinese waterchestnut (CWC) (Parker and Waldron, 1995; Parr et al., 1996; Waldron et al., 1997a) and beet root (Waldron et al., 1997b) has indicated that resistance to softening during cooking (thermal stability of texture) may be a consequence of cross-links between diferulates attached to the cell wall polymers involved in cell adhesion. In CWC, noted for its crisp texture even when canned, the dimers are attached to arabinoxylans. Thus, despite loss of pectic material from the walls during cooking, the cells do not separate. In beet root, the dimers cross-link pectic polysaccharides, so treatments that degrade the pectin eventually allow the tissue to soften and the cells to separate (Waldron et al., 1997b; Ng et al., 1998).

Like CWC, chufa (*Cyperus esculentus*), or tiger nut, is a member of the Cyperaceae, a family that has been identified as having phenolic acids bound to cell walls (Harris and Hartley, 1980). It is a sedge, sometimes regarded as a weed, which produces small edible tubers on its fibrous roots, and is grown mainly in southern Europe, Africa, and the United States. These tubers are rich in oil (Winton and Winton, 1935; Linssen et al., 1988), and their most popular usage is in the preparation of "Horchata de Chufa", a milky-looking, oil-rich, aqueous extract with an almond-like flavor (Primo and Lafuente, 1965). Because of their crisp texture and pleasant taste, chufas are commonly eaten raw as a snack, but we have observed that crispness is maintained after thermal treatments.

Although chufas have recently been identified as a new source of dietary fiber (Linssen et al., 1989), the phenolic composition of the cell walls in relation to their mechanical properties has not been investigated. In the present study, the mechanical properties (toughness) of raw and cooked chufa tubers, as measured with a cutting test, have been compared with those of raw and cooked potato (with no wall-bound phenolics) and CWC (with phenolic-rich walls). The morphology of chufa tubers, and the carbohydrate and phenolic compositions of purified cell wall material (CWM) have also been examined in order to identify possible associations with thermal stability of texture.

MATERIALS AND METHODS

Source of Materials. Dried tubers of chufa (*C. esculentus* L.) were a gift from Dr. A. Femenia. They were soaked in distilled water at 4 °C for 16 h and then peeled thinly with a scalpel to produce separate samples of skin and peeled tubers. Soaked chufas were also boiled for 2 h in distilled water.

Potato (*Solanum tuberosum* cv. Bintje) and fresh and canned CWC (*Eleocharis dulcis*) were obtained from a local supplier. Potatoes were steamed at 95 °C for 10, 20, and 30 min. Fresh CWC were also steamed at 95 °C for 20 min.

All reagents, unless otherwise stated, were of AnalaR grade.

Microscopy. *Tissue Morphology.* Transverse sections of chufa tubers were cut using a razor blade and then sonicated in distilled water for 2 min to remove the contents of the surface cells. Some sections were stained overnight in very dilute toluidine blue (C.I. 52040, BDH Chemicals Ltd., Poole, U.K.; 1 drop of 0.05% stain in 15 mL of distilled water). Other sections were stained with phloroglucinol HCl (Sigma-Aldrich Co. Ltd., Poole, U.K.; 2−3 drops of 1% phloroglucinol in 95% ethanol, followed by 1 drop of 25% HCl) to locate lignin.

Resin Embedding. Cortex tissue of chufa was chopped into 1 mm^3 pieces and fixed for 4 h in 3% glutaraldehyde (25% EM grade, Agar Scientific Ltd., Stansted, U.K.) in 0.05 M cacodylate buffer (pH 7.2; Agar Scientific Ltd.). The pieces were washed three times in buffer and then post-fixed in 1% aqueous osmium tetroxide (BDH Chemicals Ltd.) for 4 h. The blocks were dehydrated through an ethanol series, transferred

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Table 1. Carbohydrate Composition of CWM from Skin and Peeled Tubers of Chufa

	yield (% wet wt)	carbohydrate composition (μg/mg)								total (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	
skin	20.2	2.2	0.7	59.6	238.6	1.0	12.3	185.9	77.5	577.7
peeled tubers	7.9	6.3	3.7	165.9	169.6	1.1	54.0	305.6	146.0	852.1

to acetone, and then infiltrated and embedded in Spurr resin (Agar Scientific Ltd.). Sections, 1−2 μm thick, were cut with glass knives, stained with toluidine blue (1% in 1% borax, pH 11), and examined and photographed using a Leitz Ortholux II microscope (Ernst Leitz GmbH, Wetzlar, Germany).

Autofluorescence of Cell Walls. Transverse sections of the skin and cortex of chufa were cut by hand with a razor blade and mounted in distilled water or 20 mM NH_4OH (pH >10). The autofluorescence of these sections, and also of cells separated by incubation in 0.5 M TFA (Sigma-Aldrich Co. Ltd.) at 100 °C, was examined and photographed using a Leitz Ortholux II fluorescence microscope with an HBO 50 W mercury arc lamp and an exciter and barrier filter combination with transmissions of 340−380 nm and >430 nm, respectively.

Scanning Electron Microscopy (SEM). Pieces of raw and cooked chufas were fractured, fixed in 3% glutaraldehyde (see above), dehydrated in an ethanol series, and then transferred to 100% acetone and critical point dried (CPD) (Polaron, Watford, U.K.). The tissue pieces, fracture surfaces uppermost, were mounted on aluminum stubs with silver conducting paint (Agar Scientific Ltd.). These samples, together with transverse sections of sonicated cortex tissue dried down onto glass coverslips, were sputter-coated with a layer of gold ~25 nm thick (Emitech Ltd., Ashford, U.K.) and examined and photographed using a Leica Stereoscan 360 scanning electron microscope (LEO, Cambridge, U.K.).

Mechanical Testing. Raw and heat-treated chufas, CWC, and potatoes were peeled, and the storage parenchyma was cut into rectangular pieces. The width of each piece was measured before it was positioned on the lower platen of the testing machine.

A single-edged razor blade, length = 39 mm, depth = 12.5 mm, thickness = 0.25 mm, was gripped such that the cutting edge was horizontal in the upper tensile testing grip of a Stable Microsystems (Godalming, U.K.) TAXT2 testing machine with a 5 kg load cell. The blade was driven downward at a speed of 0.05 mm s^{-1} to within 1−2 mm of the lower surface of the sample. The force−distance data were recorded by the testing machine for six replicates. The cutting toughness was defined as the energy to cut a unit area of the sample, which reduces to the equilibrium force divided by the sample width.

Preparation of CWM. Samples (100 g) of skin or peeled tubers of chufa were blended in 1.5% sodium dodecyl sulfate (SDS; Sigma-Aldrich Co. Ltd.) containing 5 mM $Na_2S_2O_5$ (Sigma-Aldrich Co. Ltd.) with an Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol (Sigma-Aldrich Co. Ltd.) were added to reduce foaming. The homogenate was filtered through a 100 μm nylon mesh (John Stannier and Co., Manchester, U.K.), and the residue was ball-milled (Pascall ball mill, Fisher Scientific, Loughborough, U.K., 0.5 L pot) at 0 °C in 0.5% SDS containing 3 mM $Na_2S_2O_5$ for 2 h at 60 rpm to facilitate removal of the bulk of remaining intracellular contents. After the homogenate had been filtered through 75 μm nylon mesh, the residue was suspended in cold water containing 3 mM $Na_2S_2O_5$, homogenized for 5 min, and refiltered. The procedure was repeated three times until each cell wall residue was free of starch granules as assessed by light microscopy after staining with iodine/potassium iodide. The CWM was stored as a frozen suspension at −20 °C. Prior to the extraction of phenolics, each cell wall sample of skin and peeled tubers was further extracted with hot ethanol (100 °C) for 1 min to remove any alcohol-soluble phenolics, washed three times with acetone, and then air-dried.

Sugar Analysis of CWM. Neutral sugars of CWM of skin and peeled tubers of chufa were analyzed as described previously by Parr et al. (1997). All analyses were carried out in duplicate, and the standard deviations of the data were <2%. Sugars were released from CWM by dispersion in 72% H_2SO_4 (Fisher Scientific U.K.) for 3 h followed by dilution to 1 M and hydrolysis for 2.5 h at 100 °C (Selvendran and O'Neill, 1987). Neutral sugars were reduced with $NaBH_4$ and acetylated according to the method of Blakeney et al. (1983) using 2-deoxyglucose (Sigma-Aldrich Co. Ltd.) as an internal standard. Alditol acetates were quantified by gas chromatography as described in Parr et al. (1997).

Uronic acids were determined colorimetrically according to a modification of the method of Blumenkrantz and Asboe-Hansen (1973) in which each sample was dispersed in 72% H_2SO_4 for 3 h at room temperature, diluted to 1 M H_2SO_4, and hydrolyzed for 1 h at 100 °C prior to analysis.

Analysis of Phenolic Acids of CWM. Phenolic acids were extracted from the CWM of skin and peeled tubers of chufa and determined as described by Waldron et al. (1996). CWM (1 g) was sequentially extracted with 0.1 M NaOH (1 h; HPLC grade; BDH Chemicals Ltd.), 0.1 M NaOH (Sigma-Aldrich Co. Ltd.; 24 h), 1 M NaOH (24 h), and 2 M NaOH (24 h) under N_2 (O_2-free) at room temperature in darkness. The supernatant was filtered (GF-A, Whatman, Maidstone, U.K.) and neutralized with HCl (Fisher Scientific U.K.). *trans*-Cinnamic acid (Sigma-Aldrich Co. Ltd.) was added as an internal standard, and the filtrate was extracted three times with ethyl acetate (Fisher Scientific U.K.; HPLC grade). The ethyl acetate extracts were combined and dried under a stream of N_2. The sample was dissolved in 50% methanol (200 μL; HPLC grade; BDH Chemicals Ltd.) and analyzed by HPLC as described in Waldron et al. (1996).

Klason Lignin of CWM. Samples of CWM of skin and peeled chufa tubers were dispersed in 72% (v/v) H_2SO_4 (BDH Chemicals Ltd.) for 3 h at 20 °C followed by dilution to 1 M and hydrolysis for 2.5 h at 100 °C. The residues were recovered by filtration through 35 μm nylon mesh, washed (three times with water and once with acetone), and dried overnight. The Klason lignin was then quantified gravimetrically.

Vortex-Induced Cell Separation (VICS). Transverse sections (approximately 10 × 10 × 1 mm) of raw and cooked chufa tubers were sequentially extracted with 0.05 M *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA; Sigma-Aldrich Co. Ltd.), then 0.05 M Na_2CO_3 (Sigma-Aldrich Co. Ltd.), and increasing concentrations of KOH (Sigma-Aldrich Co. Ltd.) as described by Parker and Waldron (1995). After each extraction, the tendency for cell separation was determined by placing two tissue sections into each of two screw-capped test tubes with 3 mL of water, vortexing the tissue sections for 1 min, and shaking the tubes vigorously 10 times. The following scores (number of "+") were assigned according to the degree of disruption. (0) each tissue section intact; (1) each tissue section broken into 3−5 clumps; (2) each tissue section broken into 6−7 clumps; (3) tissue sections broken into many clumps, some separate cells; (4) tissue sections disrupted into clumps of ~20−30 cells or less, many separated cells; (5) tissue completely disrupted, many clumps <5−10 cells, mostly single separated cells (total VICS). Intermediate values were apportioned if necessary.

Treatment of Tissues in Hot, Dilute Acid. Fresh and cooked chufa cortex were extracted in acid at 100 °C (0.5 M H_2SO_4, 0.5 M HCl, or 0.05 M TFA) as described by Parker and Waldron (1995).

RESULTS AND DISCUSSION

Morphology of Chufa Tubers. Fresh or rehydrated chufa tubers are up to 30 mm long, brown on the outside with three encircling leaf scars, and white inside with dark root initials (Figure 1a). Chufas can be stored dry (Figure 1b) and rehydrated by soaking without losing

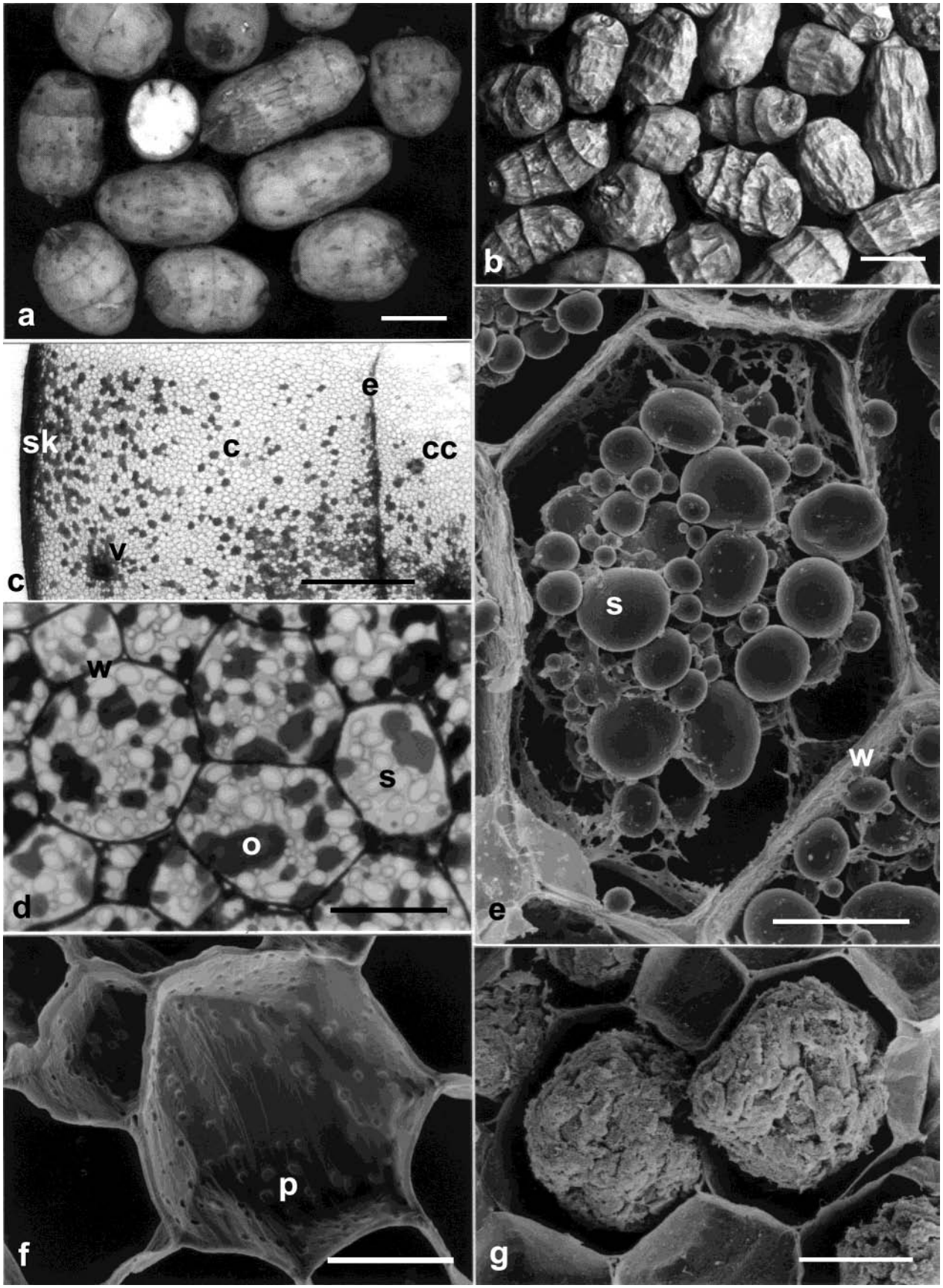

Figure 1. Morphology of chufa tubers: (a) soaked whole and cut raw tubers, bar = 10 mm; (b) dry tubers, bar = 10 mm; (c) transverse section showing skin (sk), cortex (c), endodermis (e), central cylinder (cc), and vascular strand (v), bar = 1 mm; (d) section through parenchyma cells showing walls (w), starch (s), and oil (o) reserves, bar = 50 μm; (e) scanning electron micrograph of parenchyma showing starch (s) and thick cell walls (w), bar = 20 μm; (f) sonicated section of parenchyma showing thick cell walls and pit areas (p), bar = 20 μm; (g) fracture surface of tuber cooked for 2 h showing cell contents, bar = 20 μm.

Table 2. Total Esterified Phenolic Acids (Micrograms per Gram) of CWM from Skin and Peeled Tubers of Chufa

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	skin	tuber	skin	tuber	skin	tuber	skin	tuber
monomeric components								
p-hydroxybenzoic acid	8.3	5.0	6.0	0.0	6.3	0.0	6.7	7.0
vanillic acid	14.9	6.7	25.3	8.0	17.7	5.8	18.6	3.0
p-hydroxybenzaldehyde	337.0	50.9	134.0	15.9	39.3	8.2	26.9	4.0
vanillin	62.0	46.0	68.7	34.0	62.5	24.2	48.5	15.5
p-trans-coumaric acid	617.0	183.0	3239.0	635.0	6801.0	1416.0	4228.0	479.0
trans-ferulic acid	291.0	1193.0	2025.0	2284.0	1095.0	536.0	404.0	106.0
p-cis-coumaric acid	28.9	0.0	85.8	0.0	244.0	37.8	121.0	0.0
cis-ferulic acid	37.1	124.0	142.7	218.0	53.8	27.0	18.0	3.5
dimeric components								
8−8′ AT diFA	0.0	0.0	0.0	127.0	0.0	240.0	0.0	0.0
8−5′ diFA	0.0	0.0	22.3	99.0	0.0	0.0	33.3	0.0
5−5′ diFA	0.0	115.8	180.4	262.0	49.6	32.1	0.0	5.0
8−*O*−4′ diFA	20.9	829.0	384.1	507.0	94.5	41.1	27.8	6.8
8−5′−(B) diFA	3.6	600.0	178.2	199.0	0.0	0.0	0.0	0.0
unidentified components								
unidentified A	38.6	611.0	167.0	101.0	48.2	8.1	0.0	0.0

Table 3. VICS of Chufa Parenchyma

chemical extractant	temp (°C)	time	VICS
0.05 M Na_2CO_3	1	16 h	
0.05 M Na_2CO_3	20	16 h	
0.05 M Na_2CO_3	100	30 min	+++++
0.1 M KOH	20	16 h	+
0.1 M KOH	100	30 min	+++++
0.25 M KOH	20	16 h	++
0.25 M KOH	100	30 min	+++++
0.5 M KOH	20	16 h	++
0.5 M KOH	100	30 min	+++++
1 M KOH	20	16 h	+++
1 M KOH	100	30 min	+++++
8 M KOH	20	5 h	+++++
8 M KOH	100	5 min	+++++

their crisp texture. Most of the cells contain starch and oil, which can be removed from sections by sonication to reveal the morphology of the tuber (Figure 1c). The tough outer skin (sk; Figures 1c and 2a) consists of a thin epidermis and longitudinally arranged, thick-walled stone cells and sclerenchyma fibers (sc; Figure 2a). The walls of the stone cells stain a cherry-red color with phloroglucinol HCl, indicating the presence of lignin. The endodermis (e; Figure 1c) forms a distinct brown circle separating the outer cortex (c; Figure 1c) from the inner central cylinder (cc; Figure 1c). The parenchymatous cells of the outer cortex and central cylinder are traversed by small vascular strands (v; Figure 1c). The parenchyma contains starch granules (s; Figure 1d,e) and in excess of 20% oil (Primo and Lafuente, 1965) located in 1−2 μm droplets that tend to coalesce into larger pools (o; Figure 1d). The cell walls (w) are robust and stain strongly with toluidine blue (Figure 1d) but not with phloroglucinol HCl. When fractured raw chufa tissue is critical point dried, the oil is removed (Figure 1e) so that the starch (s) and walls (w) can be clearly seen in the broken-open cells. The tough walls with numerous pits (p; Figure 1f) do not distort when cell contents are removed by sonication. After boiling for 2 h, fracture surfaces of chufa reveal that the cells have not separated but show the cell walls broken, revealing the gelatinized contents within the cells (Figure 1g). Cell wall breakage also occurs in cooked CWC (Parker and Waldron, 1985).

Autofluorescence of Cell Walls. The cell walls of raw and cooked chufas autofluoresce strongly when irradiated with UV light. In the skin (sk; Figure 2a), the lignin-rich stone cells gave an unusual faint mauve

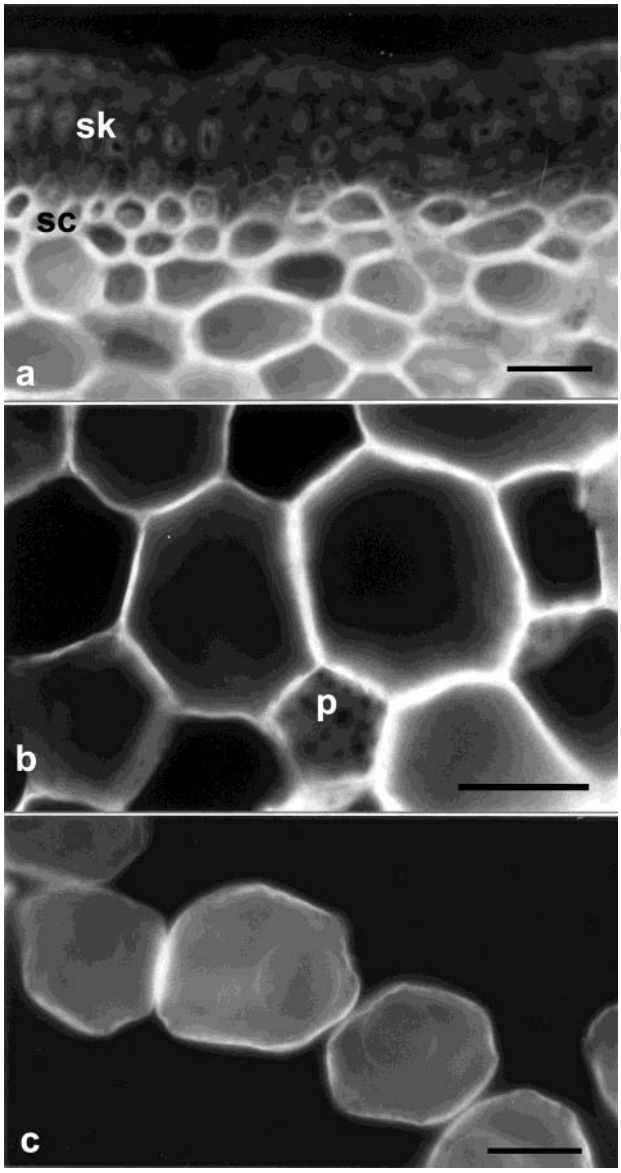

Figure 2. Autofluorescence of cell walls in sections of chufa under UV light at pH >10: (a) cells of the skin (sk), sclerenchyma (sc), and outer cortex, bar = 50 μm; (b) cells of the central cylinder, bar = 50 μm; (c) parenchyma cells after separation in 0.05 M TFA at 100 °C, bar = 50 μm.

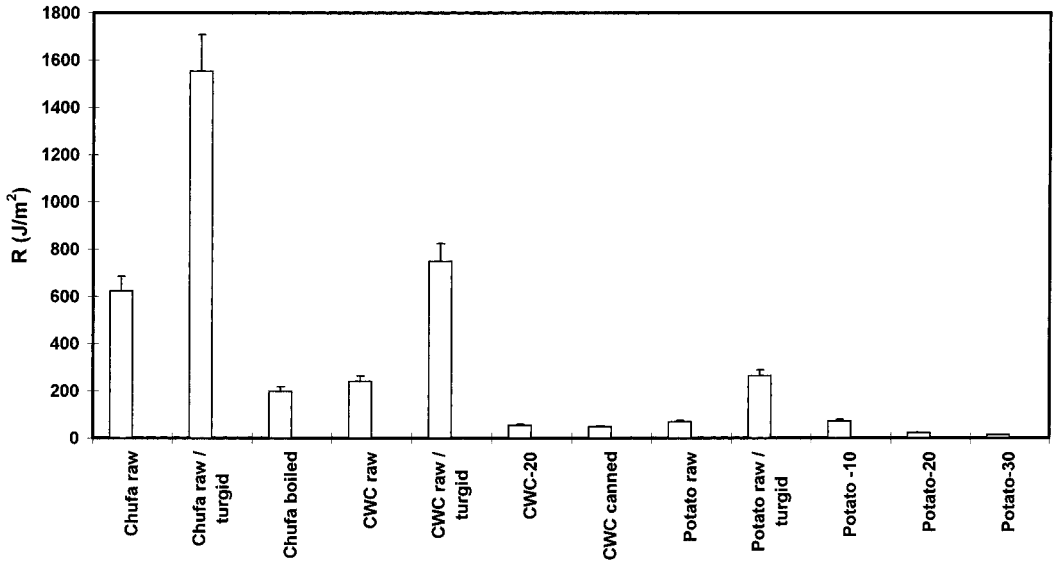

Figure 3. Cutting energy, R, of raw and heat-treated chufas, CWC, and potatoes as measured with a razor test.

autofluorescence in distilled water, and this intensified at pH >10. The underlying sclerenchyma (sc; Figure 2a) showed some blue autofluorescence in water and mauve and green autofluorescence at pH >10, indicating the presence of ferulic compounds. The autofluorescence of the cell walls of the parenchyma of the cortex and central cylinder (Figure 2b) was also strongly pH-dependent (PDA), changing from blue to green as the pH was raised. The autofluorescence of the parenchyma is similar to that shown by CWC and is indicative of the presence of simple phenolic acids such as ferulic acid (Harris and Hartley, 1976; Parker and Waldron, 1995). The mauve autofluorescence was not seen in CWC but may reflect the high *p*-coumaric acid content of the skin. Treatment with 0.05 M TFA at 100 °C did not reduce PDA but caused the cells to separate (Figure 2c).

Mechanical Properties. The toughness of raw and cooked chufas was compared with that of raw and cooked CWC and potato. Toughness may be measured using the wedge-indentation test provided various criteria are met, which equate the work done solely to the energy opening the crack (Atkins and Mai, 1985). However, several reservations regarding this approach were raised recently by Hiller and Jeronimidis (1996), who refined the approach using successive (cyclic) loading and unloading to remove hysteresis losses. They demonstrated that a razor-cutting test gave toughness values comparable with those obtained from their cyclically-loaded wedge tests (Hiller and Jeronimidis, 1996). Therefore, in this study we have adopted the razor test to measure toughness.

Parenchyma tissue from raw chufas was very tough, with a cutting energy of 623 $J \cdot m^{-2}$ (Figure 3). Raw CWC and potato tissue were much less tough, with cutting energies of 240 and 68 $J \cdot m^{-2}$, respectively. These results are in keeping with those of Hiller and Jeronimidis (1996), who reported a mean cutting energy of 82 $J \cdot m^{-2}$ for raw, turgid, potato tissue, which fell to 33 $J \cdot m^{-2}$ in flaccid samples. In raw tissues of chufa, CWC, and potato, cutting resulted in the breakage of cell walls as illustrated for chufa in Figure 1e,f. The high toughness value for chufa parenchyma is probably due to the robust cell walls, which are ~3 μm thick except in the pit fields, where the walls are thinner (p; Figures 1f and 2b). In contrast, the cell walls of raw CWC and potato parenchyma are ~1 μm thick.

Because flaccidity affected toughness, the effect of maximizing turgor pressure by soaking tissue overnight in distilled water was investigated. In all three vegetable tissues, the toughness increased >2-fold and in the case of chufa rose from 623 to >1500 $J \cdot m^{-2}$ (Figure 3).

Steaming potatoes at 95 °C for 10, 20 and 30 min resulted in a considerable reduction in toughness (Figure 3), and the softening of the tissues involved cell separation as previously noted (Ng and Waldron, 1997). Heat-treated (canned) CWC was considerably less tough than fresh CWC (Figure 3). However, it was almost as tough as fresh potato and retained its characteristic crisp texture. This was consistent with the observation that, in canned CWC, the adhesion between cells is maintained and the cell walls rupture when the tissue is fractured (Waldron et al., 1997a). Cooking chufa tubers for 2 h resulted in the toughness decreasing to 198 $J \cdot m^{-2}$; this is still very tough compared with raw potato. As in canned CWC, the cells of cooked chufa do not readily separate, and cutting results in the cells being broken open to reveal the gelatinized cell contents (Figure 1g).

Chemical Composition of CWM of Skin and Peeled Tubers of Chufa. *Carbohydrate Composition.* CWM was prepared from both the skin and peeled tubers of chufa, and the yields, on a wet weight basis, were approximately 20 and 8%, respectively (Table 1). The absence of intracellular starch from CWM was confirmed by the lack of staining with iodine/potassium iodide. The bulk (~85%) of the CWM of peeled tubers consists of carbohydrates: mainly glucose, xylose, arabinose, galactose, and uronic acid with minor quantities of mannose (Table 1). The high yield of arabinose and xylose indicates that these cell walls contain large quantities of arabinoxylan hemicelluloses. The non-carbohydrate component of the CWM of peeled tubers probably consists of coprecipitated intracellular and cell-wall protein. Klason lignin comprised <2% of the CWM and probably originates from the xylem vessels in the vascular strands of the peeled tubers and the sheaths of roots arising from the endodermis.

In contrast, the CWM of the skin contains much higher levels of xylose and less arabinose and galactose (Table 1) and has a lignin content of >20%. These results are consistent with acid phloroglucinol-indicated

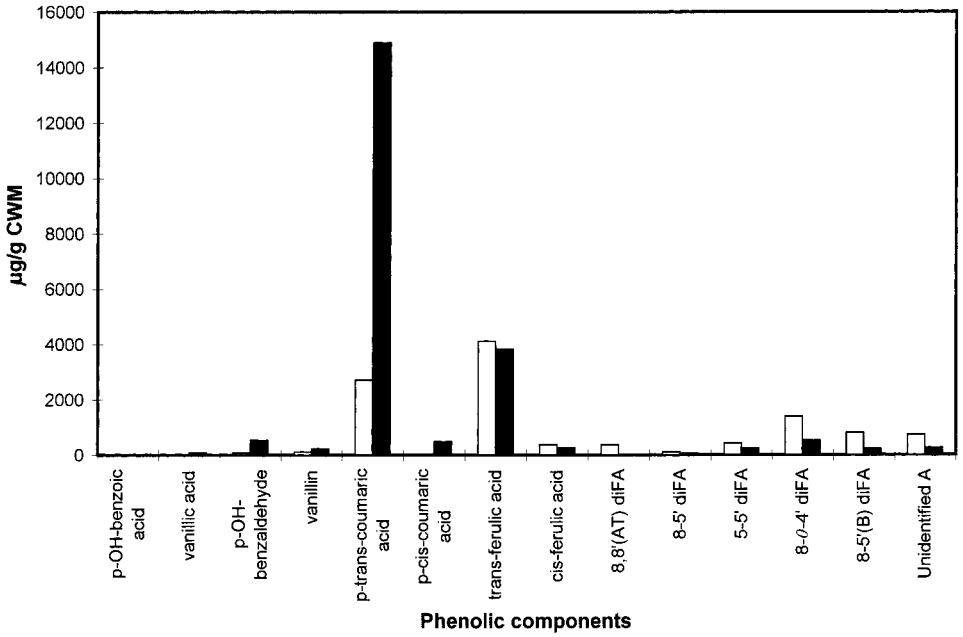

Figure 4. Phenolic components of CWM from skin (■) and peeled tubers (□) of chufa.

lignin of the stone cells of the skin (not shown) and with the findings of Linssen et al. (1989), who suggested that the overall lignin content of chufa tubers originates from the tough outer skin.

Analysis of Alkali-Labile Phenolics. The alkali-labile phenolic components of CWM from skin and peeled tubers were extracted with increasing strengths of alkali and analyzed by HPLC-DAD. Total levels of phenolics are shown in Figure 4. Ferulic acid (FA) is the most abundant esterified phenolic component in the CWM of peeled tubers; *p*-coumaric acid (pCA) is present at approximately two-thirds the FA level. FA is present in similar amounts in the skin but at much lower levels than pCA, which predominates. The results of sequential extraction of the phenolics in alkali are shown in Table 2. The bulk of the alkali-labile FA from CWM of skin and peeled tubers is released by 0.1 M NaOH, whereas the largest release of pCA occurs in 1 M NaOH (Table 2). The precise chemical significance of these differences is not clear; previous studies of grasses have demonstrated that pCA is less-readily released by dilute alkali than is FA (Hartley and Morrison, 1991). The release of phenolic esters is probably influenced by their accessibility, and coextraction of other cell-wall components may be a prerequisite. For example, the stone cells of the skin are highly lignified with very thick walls (Figure 2a), and it is possible that the lignin matrix needs to be opened up with a strong alkali before the pCA phenolic esters can be released.

In addition to FA, five forms of diferulic acid were detected and quantified (Table 2; Figure 4). Their presence is consistent with their proposed involvement in thermal stability of cell adhesion in these tissues (Waldron et al., 1997a). The most abundant dimer is 8−*O*−4′ diferulic acid, and the levels are highest in the peeled tubers. As in cell walls of CWC and beet root, the bulk of the ferulate dimers are extractable in 0.1 M NaOH except for the 8−8′ diferulic acid (aryltetralin form), which is solubilized mainly by 1 M NaOH (Table 2).

Small quantities of several other phenolic momomers were identified including *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzaldehyde, and vanillin. In addition,

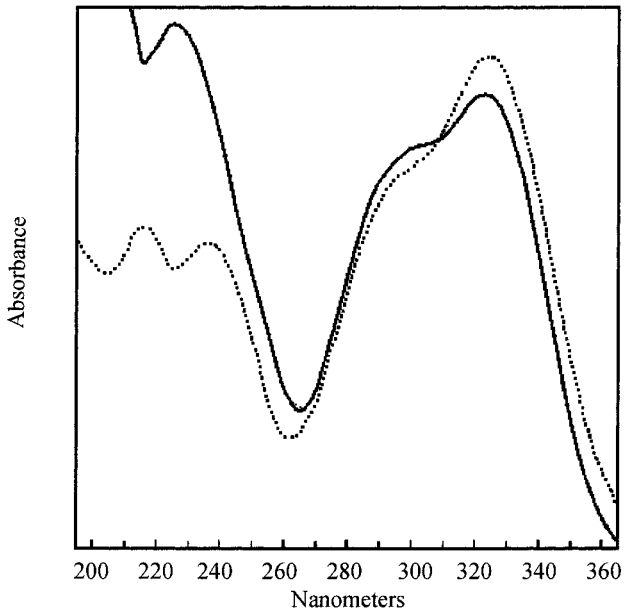

Figure 5. Absorbance spectrum of phenolic component "unidentified A" (—) and FA (· · ·).

there were several unidentified peaks. Although the level of most of these is negligible, "unidentified A" in Table 2 and Figure 4 is of interest. The levels of this component have been estimated using the response factor of the nearest identified component, 5−5′ diferulic acid. Its absorbance spectrum is not dissimilar to that of FA (Figure 5) and, because it elutes among the diferulates, it may prove to be a novel dimeric moiety.

VICS. Sections of raw chufa tissue were subjected to a variety of extraction procedures designed to cleave, selectively, chemical bonds in the cell walls. The tendency of the cells to separate in controlled vortexing conditions (VICS) provides some indication of the chemistry of cell−cell adhesion (Parker and Waldron, 1995).

Effect of Hot Water and Chelating Agents. Extraction of raw or cooked sections of chufa with hot water (pH 5.5, 100 °C, 2 h) had no effect on VICS. Furthermore, extraction of these tissues with CDTA at 20 °C for 16 h

or at 100 °C for 2 h had no effect either. This indicates that, as in CWC (Parker and Waldron, 1995), cell adhesion in raw and cooked chufa is not due to the cross-linking of pectic polysaccharides by calcium ions.

Effect of Alkali. The CDTA-extracted tissues were subsequently subjected to a series of less-severe extractions, developed initially to extract cell wall polysaccharides with minimal degradation (Redgwell and Selvendran, 1986). Extraction in Na_2CO_3 (0.05 M, 1 and 20 °C) for up to 16 h had no effect on VICS. However, heating Na_2CO_3-extracted sections in the dilute base (100 °C, 30 min) resulted in complete VICS (Table 3) with an accompanying reduction in the level of PDA. In this respect, chufa is similar to CWC (Parker and Waldron, 1995), but the separated cells of chufa lack the specific pattern indicating the number and packing of adjacent cells that is characteristic of CWC (Parker and Waldron, 1995). In addition, VICS was partially induced after extraction in 0.1 M KOH for 16 h. In 0.25 M KOH over the same period, total VICS was achieved. VICS was accompanied by a visible reduction in PDA, and the separated cells retained their angular shape and overall integrity. Again, these results are similar to those for CWC (Parker and Waldron, 1995) and suggest that polymers containing autofluorescent phenolic compounds may be involved in cell adhesion.

Effect of Hot Acid. Extraction of fresh or cooked chufa tuber cortex with 0.5 M H_2SO_4, 0.5 M HCl, or 0.05 M TFA at 100 °C resulted in total VICS within 25, 25, and 90 min, respectively (Figure 2c). These results are similar to findings for CWC (Parker and Waldron, 1995). Separation of chufa parenchyma in hot, dilute (0.05 M) TFA (Figure 2c) is consistent with the involvement of arabinose-containing polysaccharides in cell adhesion. Such conditions will hydrolyze arabinofuranose glycosidic linkages (Fry, 1988) but not glycopyranosidic linkages, which exhibit much lower hydrolysis rates (Aspinall, 1973; Lindberg et al., 1975; Fry, 1988).

Hence, the thermal stability of chufa tubers, particularly the parenchyma cells which comprise the bulk of the tubers, may be similar to that of CWC. The parenchyma cell walls contain significant quantities of FA and diferulic acid, and cell separation in alkali in which these components are extracted is accompanied by a general decrease in PDA. Very dilute, hot acid may also induce cell separation, indicating that arabinoxylans may be involved, but hot chelating agents do not, which suggests that calcium cross-linking in pectin is not limiting. Chufa tubers, therefore, provide a further example of a tissue in which cross-linking of cell wall polymers by diferulic acid may be associated with thermal stability of cell adhesion.

CONCLUSIONS

(1) Parenchyma tissue of chufa tubers is very tough compared with that of CWC and potato; the toughness of all three is more than doubled by increasing the turgidity of the tissue.

(2) Chufa parenchyma tissue remains very tough even after extensive heat treatment. This is due to the maintenance of cell adhesion and is similar to that found in CWC.

(3) The cell walls of peeled tubers are rich in arabinose and xylose, indicating the presence of arabinoxylan hemicelluloses. The significant quantities of ferulic and diferulic acid in the walls gives rise to an intense autofluorescence, which is pH-dependent. The parenchyma cells are not lignified.

(4) Stone cells in the skin of chufa are highly lignified, rich in *p*-coumaric acid, and exhibit a mauve autofluorescence.

(5) Parenchyma cells of chufa do not separate in hot solutions of chelating agents. However, as in CWC, separation may be induced using chemical treatments that will de-esterify a large proportion of ferulic and diferulic acid esters or treatments that will hydrolyze arabinofuranose glycosidic linkages.

(6) These observations are consistent with the involvement of diferulic acid cross-linked arabinoxylans in the thermal stability of texture in chufa parenchyma.

LITERATURE CITED

Aspinall, G. O. Degradation of polysaccharides. In *Elucidation of Organic Structures by Physical and Chemical Methods II*; Bentley, K. W., Kirkby, G. W., Eds.; Wiley-Interscience: New York, 1973; pp 379−450.

Atkins, A. G.; Mai, Y. W. *Elastic and Plastic Fracture: Metals, Polymers, Ceramics, Composites, Biological Materials*; Ellis Horwood: Chichester, U.K., 1985.

Blakeney, A. B.; Harris, P. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharides analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

Fry, S. C. *The Growing Plant Cell Wall: Chemical and Metabolic Analysis*; Longman Scientific and Technical: Harlow, U.K., 1988; pp 119−124.

Harris, P. J.; Hartley, R. D. Detection of bound ferulic acid in cell walls of the gramineae by ultraviolet fluorescence microscopy. *Nature* **1976**, *259*, 508−510.

Harris, P. J.; Hartley, R. D. Phenolic constituents of the cell walls of Monocotyledons. *Biochem. Syst. Ecol.* **1980**, *8*, 153−160.

Hartley, R. D.; Morrison, W. H. Monomeric and dimeric phenolic acids released from cell walls of grasses by sequential treatment with sodium hydroxide. *J. Sci. Food Agric.* **1991**, *55*, 365−375.

Hiller, S.; Jeronimidis, G. Fracture in potato tuber parenchyma. *J. Mater. Sci.* **1996**, *31*, 2779−2796.

Lindberg, B.; Lonngren, J.; Svensson, S. Specific degradation of polysaccharides. *Adv. Carbohydr. Chem. Biochem.* **1975**, *31*, 185−240.

Linssen, J. P. H.; Kielman, G. M.; Cozijnsen, J. L.; Pilnik, W. Comparison of chufa and olive oils. *Food Chem.* **1988**, *28*, 279−285.

Linssen, J. P. H.; Cozijnsen, J. L.; Pilnik, W. Chufa (*Cyperus esculentus*): A new source of dietary fibre. *J. Sci. Food Agric.* **1989**, *49*, 291−296.

Ng, A.; Waldron, K. W. Effect of steaming on cell wall chemistry of potatoes (*Solanum tuberosum* cv. Bintje) in relation to firmness. *J. Agric. Food Chem.* **1997**, *45*, 3411−3418.

Ng, A.; Harvey, A. J.; Parker, M. L.; Smith, A. C.; Waldron, K. W. The effect of oxidative coupling on the thermal stability of texture and cell-wall chemistry of beetroot (*Beta vulgaris*). *J. Agric. Food Chem.* **1998**, *46*, 3365−3370.

Parker, M. L.; Waldron, K. W. Texture of Chinese Water Chestnut: Involvement of cell wall phenolics. *J. Sci. Food Agric.* **1995**, *68*, 337−346.

Parr, A. J.; Waldron, K. W.; Ng, A.; Parker, M. L. The wall-bound phenolics of Chinese Water Chestnut (*Eleocharis dulcis*). *J. Sci. Food Agric.* **1996**, *71*, 501−507.

Parr, A. J.; Ng, A.; Waldron, K. W. Ester-linked phenolic components of carrot cell walls. *J. Agric. Food Chem.* **1997**, *45*, 2468−2471.

Primo, E.; Lafuente, B. This is Horchata. *Proc. 1st Int. Congr. Food Sci. Technol.* **1965**, *4*, 687−691.

Redgewell, R.; Selvendran, R. R. Structural features of cell wall polysaccharides of onion *Allium cepa*. *Carbohydr. Res*. **1986**, *157*, 183−199.

Selvendran, R. R.; O'Neill, M. A. Isolation and analysis of cell walls from plant material. *Methods Biochem. Anal*. **1987**, *32*, 25−153.

Waldron, K. W.; Parr, A. J.; Ng, A.; Ralph, J. Cell wall esterified phenolic dimers: identification and quantification by reverse phase high performance liquid chromatography and diode array detection. *Phytochem. Anal*. **1996**, *7*, 305−312.

Waldron, K. W.; Smith, A. C.; Parr, A. J.; Ng, A.; Parker, M. L. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends Food Sci. Technol*. **1997a**, *8*, 213−221.

Waldron, K. W.; Ng, A.; Parker, M. L.; Parr, A. J. Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. *J. Sci. Food Agric*. **1997b**, *74*, 221−228.

Winton, A. L.; Winton, K. B. *The Structure and Composition of Foods. Vol. II, Vegetables, Legumes, Fruits*; Wiley: New York, 1935.

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