Preparation, Isolation, and Characterization of Cutin Monomers and Oligomers from Tomato Peels

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Cutin in tomato peels was depolymerized in methanolic base to yield cutin monomers or a mixture of cutin oligomers. These products were isolated by typical solvent extraction methods or by precipitation, and the isolates were characterized by chromatographic and spectroscopic analyses. It was determined that the compositions of the isolates from both isolation procedures were similar, although solvent extraction gave higher yields. However, the precipitation method, which is easy to carry out and avoids the use of undesirable organic solvents, may be preferable in commercial processes for recovering these compounds.

Keywords: *Cutin; dihydroxyhexadecanoate; oligomers; methanolysis; tomato*

INTRODUCTION

Processing of fruits and vegetables for commodities such as juices or sauces results in the accumulation of large amounts of byproducts of little or no commercial value; orange pulp in the production of orange juice and tomato peels and seeds in the production of tomato juice are cases in point. Although orange pulp can be used to reconstitute orange juice to a more "natural" product, only a small fraction of the available pulp is used for such a purpose. Apple pomace and tomato peels, byproducts of apple and tomato juice production, are sold for minimal prices or, in some cases, given to farmers for use as animal feed. In effect, these materials have little commercial value to the processor and may even require that the processor pay for the disposal of these materials to be in compliance with environmental regulations. There are components of these waste products that are of potential value if they could be recovered economically. For example, tomato peels contain cuticular waxes, cutin, and other hydrophobic compounds such as lycopene (Baker et al., 1982). We have initially focused our attention on the isolation and modification of cutin monomers and oligomers from tomato peels.

Between 600 and 1200 million pounds of tomato waste were generated from tomato processing in 1994 (H. L. Durkin, personal communication, 1996). The waste is composed of seeds and skin, the latter containing primarily cuticular waxes and cutin, a polyester of primarily ω,9- and ω,10-dihydroxyhexadecanoic acids. These acids, which account for ~10 million pounds of the waste (H. L. Durkin, personal communication, 1996), if recovered economically, may be of commercial value in paints and coatings as a drying oil, plasticizer, wetting agent, or viscosity modifier (Derksen et al., 1995). They may also find use in the preparation of texturized oils as fat substitutes (Zaks et al., 1992). The isolation and characterization of n,16(n = 7−10)-dihydroxyhexadecanoic acid monomers and oligomers derived from tomato peels is the subject of this paper.

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MATERIALS AND METHODS

Isolation of Cuticle Lipids from Tomato Peels. Commercially available tomatoes (60.9 kg) were soaked in boiling water, and the peels that separated from the flesh of the tomato were isolated by hand. The peels were washed with water on a sintered funnel and then lyophilized (yield of dry peels = 53.7 g, 0.08%)

Exhaustive Extraction of Tomato Peels. Dried tomato peels (2.5 g) were stirred in 50 mL of MeOH overnight. After filtration, the filtrate was concentrated to dryness on a rotary evaporator. The extracted tomato peel was retreated with MeOH until no residue was obtained from the MeOH filtrates. The peels were then extracted with $CHCl_3$ and 50:50 $CHCl_3$/MeOH in a similar manner.

Preparation of Cutin Oligomeric Fractions from Tomato Peels. Lyophilized tomato peels (1 g) were exhaustively washed with MeOH and then treated with 20 mL of 1.5 M KOMe (prepared by dissolving KOH in MeOH) overnight at room temperature. Shorter reaction times (30−240 min) and weaker base (0.1 M KOMe) were used to prepare mixtures of different oligomeric composition. Two methods were used to isolate cutin methanolysates. In the extraction method the solutions were filtered, the retentate was washed with MeOH, and the combined filtrates were diluted with 2 volumes of H_2O. The solution was chilled and adjusted to pH 3.5 with 4 N HCl and then extracted twice with 50 mL of $CHCl_3$; the combined $CHCl_3$ extracts were back-extracted with H_2O (25 mL) and concentrated to dryness on a rotary evaporator. The residue was dissolved in EtOH for analysis. For the isolation of monomers by precipitation, the tomato peels were treated with 1.5 M KOMe and the solutions acidified and diluted with water as above; however, the solutions were then chilled in the ice bath for an additional 30 min after neutralization. The resultant precipitate was filtered, dried under vacuum, and dissolved in EtOH for analysis. The supernatant was extracted with 0.5 volume of $CHCl_3$ to recover any unprecipitated monomers. The recovery of ω,n-dihydroxyhexadecanoic acids as the diTMS methyl esters was determined by GC on a Varian 3700 gas chromatograph using 16-hydroxyhexadecanoic acid as an internal standard.

Oligomer Molecular Weight Distribution Analysis. Oligomer mixtures of tomato peel hydrolysates were fraction-

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Table 1. Extracts from Tomato Peel (2.4 g)

fraction	weight	composition
before methanolysis		
$CHCl_3$ solubles	27 mg	lipids[a,b]
MeOH solubles	37 mg	lipids, monosaccharides[b]
$CHCl_3$/MeOH solubles	8 mg	lipids, monosaccharides[b]
after methanolysis[c]		
$CHCl_3$ solubles	1.7 g	>90% cutin monomers[b]
residue	0.5 g	carbohydrate (>95%)[d]

[a] C_{15}−C_{32} saturated hydrocarbons, fatty acids, amyrins. [b] By GC/MS analysis of TMS, methyl ester derivatives and LC/APCIMS and LC/ESMS of acetylated methyl esters. [c] Overnight with 1.5 M aq KOMe. [d] By CP-MAS NMR.

ated on a 50.8 × 1 cm Sephadex LH-20 (Pharmacia) column eluted with MeOH at a flow rate of 1 mL min^{-1}. Fractions from the LH-20 column were collected and analyzed by HPLC size exclusion chromatography on a Shodex GPC K-803 20 cm polystyrene column eluted with $CHCl_3$ at a flow rate of 1 mL min^{-1} using a Hewlett-Packard 1050 chromatograph and a Varex ELSD MK lll evaporative light scattering detector. The HPLC column was calibrated with polystyrene standards (Sigma).

Compositional Analysis. Monomer composition of extracts was determined by gas chromatographic electron impact mass spectrometry analysis (GC/EIMS) on a Hewlett-Packard 6890 system. Chromatography was carried out on an SPB1 30 M capillary column temperature programmed as follows: 125 °C for 5 min, 4 °C/min to 205 °C, 10 °C/min to 280 °C, 280 °C for 15 min. Extracts were chromatographed underivatized, as trimethlysilyl derivatives [prepared with BSTFA (Supelco)] and as trimethylsilyl derivatives of methyl-esterified (with BF_3/methanol) mixtures. 16-Hydroxyhexadecanoic acid was used as an internal standard.

Cutin monomer and oligomer samples were also characterized by liquid chromatography/mass spectrometry (HPLC/MS). Analysis was carried out on a Hewlett-Packard 5989A MS Engine interfaced to a Hewlett-Packard 1050 liquid chromatograph with a Hewlett-Packard 59987A module for electrospray ionization (HPLC/ESMS) and for atmospheric pressure chemical ionization (HPLC/APCI) or a Hewlett-Packard 59980B particle beam for chemical (NH_3) ionization (HPLC/CIMS) and electron impact ionization (HPLC/EIMS). The samples were methyl-esterified as above and then acetylated with acetic anhydride in pyridine (1:1) at 70 °C for 30 min. Chromatographic conditions were as follows: column, Chrompack Hypersil 5 ODS column (10 cm); solvent programming, ACN/MeOH (90:10) (A) and MeOH (B) from 1:1, A/B (isocratic for 5 min), to 100% A in 15 min at a flow rate of 0.5 mL/min for HPLC/ESMS and HPLC/CIMS analyses. The ions used for selective ion monitoring were DP1, 409 $[M + 23]^+$; DP2, 721 $[M + 23]^+$; DP3, 1028 $[M + 18]^+$; DP4, 1340 $[M + 18]^+$, and DP5, 1658 $[M + 18]^+$.

NMR spectra for solid samples (CP-MAS) were obtained on a Bruker MSL 300 spectrometer.

RESULTS AND DISCUSSION

The results from mass spectral and NMR analysis of dry tomato peel that had been exhaustively extracted with organic solvents prior to methanolysis (treatment with 1.5 M KOMe in MeOH) indicate that it is a rich source of cutin (Table 1). Exhaustive extraction with a regime of organic solvents (Table 1) prior to methanolysis removed only minor amounts of material, which were shown by GC/EIMS and HPLC/CIMS to be composed of high molecular weight hydrocarbons and other compounds that have previously been identified in cuticle waxes (Baker, 1982). One of the tomato peel samples we analyzed contained significant amounts of 2-hydroxydocosanoic and 2-hydroxytetracosanoic acid in the $CHCl_3$/MeOH extracts of the unmethanolyzed peel. These compounds may result as artifacts from the degradation of cerebriosides or ceramides of cells attached to the cuticle (Kojima et al., 1991). We have also identified in the $CHCl_3$ extract of the same sample γ-amyrin [which to our knowledge has not been reported as a tomato cuticle component but has been found in Spanish broom (Musgrave et al., 1952)] as well as α- and β-amyrin.

The major components of the chloroform extract, after methanolysis, were methyl 9,16- and 10,16-dihydroxyhexadecanoate (∼75%) with minor amounts of the 8,16- and 7,16- isomers and methyl 16-hydroxyhexadecanoate. The yield and purity of the methyl ester fraction depended on the method of preparation and isolation. The most commonly used method of isolation usually involves $CHCl_3$ extraction (Walton, 1990), which would be undesirable for a commercial process. We therefore investigated other methods of monomer isolation that we believed to be both economically and environmentally preferable. Initially, we examined continuous extraction of an acidified aqueous methanolic monomer mixture with hexane; however, low monomer yields were obtained because of the limited solubility of the monomers in this solvent. Precipitation of the tomato peel methanolysate appeared to be an attractive alternative method in that no additional organic solvents would be necessary (only water would have to be added after neutralization). The precipitate can be isolated by centrifugation or filtration, and carbohydrate, protein, and other polar contaminants would remain in the aqueous layer. The results of four separate extractions using both methods indicated that, although the precipitate was of comparable purity to the $CHCl_3$-extracted material, the percent yield of monomer determined by GC analysis of the di-OTMS methyl esters was only 40%, or about half that obtained by extraction (40.3 ± 7.1 versus 72.0 ± 0.2). Monomers could be recovered by $CHCl_3$ extraction of the supernatant; therefore, further modifications in the precipitation procedure are necessary to obtain yields equivalent to $CHCl_3$ extraction.

The tomato peel residue, after methanolysis and $CHCl_3$ extraction, was predominantly carbohydrate by NMR analysis (Figure 1) (Fenwick et al., 1996; Pacchiano et al., 1993), which indicates that all of the cutin was converted to monomers with overnight methanolysis using 1.5 M KOMe. The residue did not contain pectin as there was no observable resonance for the C6-carboxyl of pectin (∼175 ppm). The overall yield of dihydroxyhexadecanoic acids from dry tomato peels by the precipitation procedure was ∼25%, and that for the extraction procedure was ∼48%.

Another consideration that we have addressed is the manipulation of tomato peel degradation to produce oligomeric mixtures that would have desirable physical properties. Treatment of tomato peel with 0.1 M KOMe overnight yielded an oligomeric mixture that ranged in degree of polymerization (DP) from monomer up to approximately DP8 as determined by size exclusion HPLC on LH-20 using light-scattering detection (Figure 2). We previously reported the production of cutin oligomers by partial methanolysis in KOMe (Osman et al., 1995). At the time of this paper we did not have light-scattering detection capabilities for HPLC. Our HPLC/MS capabilities were limited to particle beam interface for EIMS or CIMS, and we were able to observe and identify only the monomer and dimer of

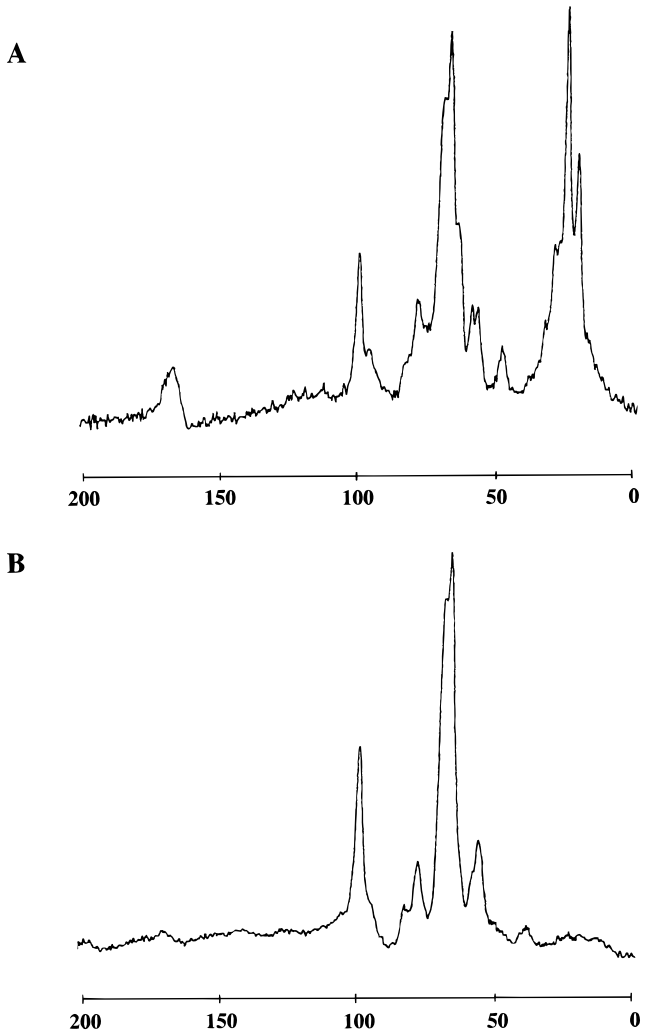

Figure 1. CP-MAS NMR of (A) dried tomato peel and (B) dried tomato peel residue after treatment with 1.5 M KOMe in CH_3OH, neutralization, and $CHCl_3$ extraction.

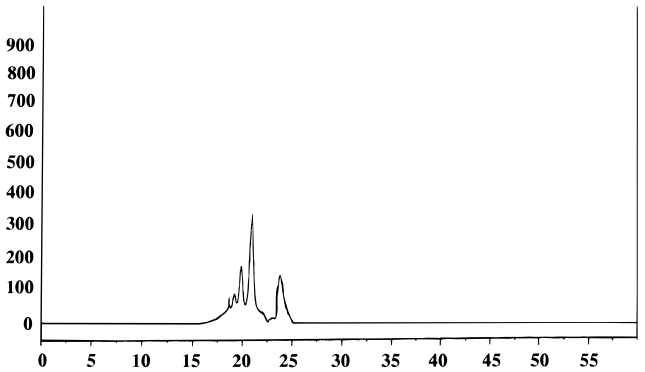

Figure 2. Size exclusion HPLC of partially hydrolyzed tomato peel.

dihydroxyhexadecanoic acid. We have now reexamined oligomer production using HPLC/ESMS and HPLC/APCIMS, which has enabled us to observe higher degrees of polymerization than was previously possible. The products were analyzed as the O-acetylated methyl esters. The oligomers were identified by molecular weight and the number of hydroxyls which, in turn, was determined by comparing mass spectra of acetylated to deacetylated products. For example, the methyl ester of the acetylated dimer has a molecular weight of 698;

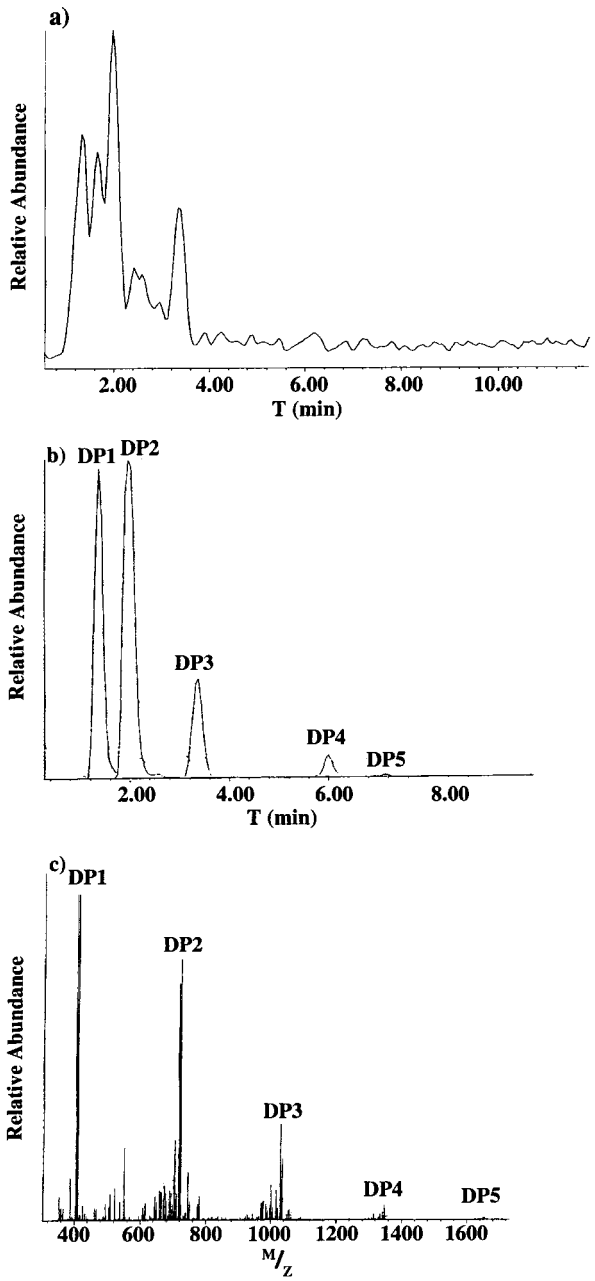

Figure 3. HPLC/ESMS of partially hydrolyzed tomato peel: (a) total ion chromatogram; (b) selective ion monitoring of oligomers; (c) ions observed from 0.7 to 11 min in LC/ESMS run. For HPLC conditions see Materials and Methods.

acetylation with deuterated (d_6) acetic anhydride would increase the molecular weight by 9 mass units to 707 because the dimer has three hydroxyls. Observation of a pseudomolecular ion corresponding to MW 698 in the unlabeled sample at the same retention volume as the pseudomolecular ion corresponding to MW 707 in the deuterium-labeled sample is considered to be compelling evidence that the compound is a dimer of dihydroxyhexadecanoic acid. In this manner we were able to identify oligomers up to DP5 (Figure 3) using ESMS detection, which we found to be more sensitive than LC/APCI. The ratio of monomer to oligomers released during treatment with 0.1 M KOMe was dependent on reaction time (Table 2). The same trend in molecular weight distribution was observed by open column size exclusion chromatography on LH-20 and HPLC size exclusion chromatography on a polystyrene column.

Table 2. Effect of Reaction Time on the Composition of Tomato Peel Methanolysates[a]

reaction time (min)	DP1	DP2	DP3	DP4	DP5
0	1	0	0	0	0
15	1	0.75	<0.1	<0.1	<0.1
30	1	0.9	0.1	0.1	<0.1
60	1	1.0	<0.1	<0.1	<0.1
120	1	0.6	<0.1	<0.1	<0.1

[a] Relative amounts (to DP1) are given because sufficient quantities of standards were not available to determine absolute values.

In conclusion, a lipid fraction containing predominantly cutin monomers or oligomers can be obtained by controlled depolymerization of the cutin in methanolic KOMe. The desired lipids can be isolated by solvent extraction or precipitation. Analysis of both the $CHCl_3$ extracts and the precipitates indicates that they are very similar in composition; however, higher yields were obtained with $CHCl_3$ extraction, and the yield of monomer extraction is about twice that obtained by precipitation. Further research is planned on improving precipitation yields as this would be the preferred method of isolation from a cost and environmental standpoint.

ABBREVIATIONS USED

MeOH, methanol; KOMe, potassium methoxide; EtOH, ethanol; $CHCl_3$, chloroform; ACN, acetonitrile; BSTFA, bis(silyltrifluoroacetamide); GC/EIMS, gas chromatography/electron impact mass spectrometry; HPLC/MS, liquid chromatography/mass spectrometry; HPLC/ESMS, electrospray ionization; HPLC/APCI, atmospheric pressure chemical ionization; HPLC/CIMS, chemical ionization; HPLC/EIMS, electron impact ionization.

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