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Note

Relative effectiveness of ion-exchange and lead acetate precipitation methods in isolating pear sugars and acids*

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In investigating the changes of sugars and acids in pears during ripening and processing, a method was needed for their separation, identification, and quantitation. Several workers[1–4] have used a procedure based on the precipitation of acids as their lead salts from ethanolic fruit extracts followed by recovery of sugars from the residual supernatant; sugars and acids are subsequently analyzed as their trimethylsilyl (TMS) derivatives by gas chromatography (GC). Wagener *et al.*[5] found the method to be unsatisfactory for quantitation of grape acids and Weissberger *et al.*[6] reported similar results for cocoa beans. This note compares the effectiveness of lead acetate precipitation and ion-exchange procedures in the analysis of pear sugars and acids.

EXPERIMENTAL

GC–mass spectrometry (MS) equipment and conditions

A dual-column Varian Aerograph Model 200 gas chromatograph with hydrogen flame-ionization detectors was used for GC analyses. Two 3 m × 2 mm I.D. glass columns were packed: one with 5% SE-52 (for sugar analysis) and the other with 3% SE-30 (for acid analysis) on 80–100 mesh Chromosorb W HP. GC operating conditions common to both columns were: injector temperature 190°, detector temperature 250° and nitrogen carrier gas flow-rate of 25 ml/min. For the sugars, the SE-52 column was operated isothermally at 165° for 14 min, then programmed at 12°/min to 250° and held. For acids, the SE-30 column was programmed from 100 to 250° at 6°/min and held. The retention times and peak areas were determined with a Hewlett Packard Model 3380A recording integrator. TMS derivatives of sugars or acids were separated by GC prior to their entry into the ion source of the mass spectrometer. A differentially pumped magnetic mass spectrometer (Varian MAT CH-7), operated at 70 eV and a scanning speed of 2 sec in conjunction with a System Industries 150 data system, was used for MS analyses.

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Materials and methods

Wedge-shaped longitudinal sectors were cut peel to core from each of 5 Bartlett pears and 50 g representative samples were homogenized for 5 min in a Waring Blendor with 150 ml 95% ethanol. After standing for 60 min the extract was centrifuged (10 min, 2000 *g*), the supernatant decanted, the residue washed (2 × 25 ml) with 80% ethanol and the supernatants combined and made up to 250 ml. Sugars and acids of a 5-ml aliquot were separated by precipitation of the acids as their lead salts. This precipitate and an aliquot of the supernatant together with their respective internal standards [100 μl 0.2% (w/v) rhamnose for sugars and 100 μl 1% (w/v) tartaric acid for acids] were prepared for derivatization as described by Heatherbell[1].

Sugars and acids from a similar extract were isolated by the following ion-exchange procedure. A cation-exchange column (4 ml Dowex 50W-X4, 200–400 mesh, H^+) and anion-exchange column (9 ml Dowex 1-X8, 200–400 mesh, CH_3COO^-) were connected in series. The extract was applied and the columns washed with water til the sugars were eluted. The eluate was made to two 1- and a 100-ml aliquot removed and made up to 250 ml. The 1-ml aliquots plus the rhamnose internal standard were prepared for derivatization as previously described. Organic acids were recovered by treating the 1-X8 column with 250 ml 10 *N* formic acid and washing with water till neutrality. The eluate was made to one 1, a 100-ml aliquot taken to dryness on a rotary evaporator, and the residue taken up in 10 ml distilled water. Tartaric acid internal standard was added to a 2-ml aliquot, taken to dryness, and prepared for derivatization as previously described.

Preparation of TMS derivatives

Sugars were derivatized by adding 300 μl of "Tri Sil" (Pierce, Rockford, Ill., U.S.A.), mechanically shaking for 5 min and heating at 70° for 20 min followed by 15 min shaking to complete derivatization. Acids were derivatized by adding 300 μl of "Tri Sil", shaking for 5 min and heating at 50° for 30 min. The vials were centrifuged for 5 min and 2 μl of the sugar or acid supernatants were injected into the GC–MS system.

Calibration and calculations

Relative detector response factors (*K*) for the major pear sugars and acids were determined by using the procedure described by Heatherbell[1] for the following concentration ranges: fructose, 10–80 mg/100 ml; sorbitol, 10–50 mg/100 ml; glucose and sucrose, 50–300 mg/100 ml; malic acid, 50–150 mg/100 ml; phosphoric, citric and quinic acids, 10–50 mg/100 ml.

Percent recovery of sugars and acids from the ion-exchange column were determined from the following solution of standards which is representative of their concentration in pears: fructose, 4.0 g; sorbitol, 2.5 g; glucose, 1.5 g; sucrose, 1.5 g; malic acid, 0.08 g; phosphoric, citric and quinic acids, 0.02 g. The compounds were dissolved in 200 ml deionized water, placed on the ion-exchange columns, and eluted, dried, derivitized and chromatographed as described for pear extracts. Recoveries were calculated on the basis of the known weights of each component.

RESULTS AND DISCUSSION

GC–MS analyses of TMS ethers of Bartlett pear sugars isolated by either lead salt precipitation or ion exchange gave identical chromatograms (Fig. 1), the identities of fructose, glucose, sorbitol, sucrose, xylose and inositol being confirmed. In addition, galactose, reported as a trace sugar in pears (Ash and Reynolds[9]) was identified by GC, however, MS confirmation could not be obtained. Contrary to the report by Mohler and Schmolek[10] that trace amounts of arabinose are present in pear juice, no GC–MS evidence of its presence in Bartlett was found.

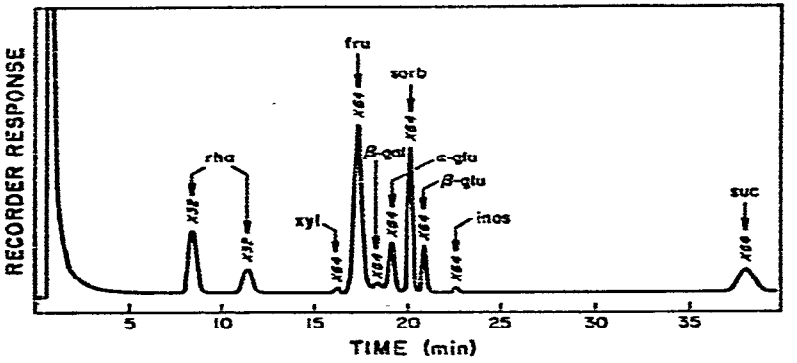

Fig. 1. GC separation on SE-52 column of TMS ethers of Bartlett pear sugars prepared either by lead salt precipitation or ion-exchange method; rha = rhamnose (int. std.); xyl = xylose; fru = fructose; β-gal = β-galactose; α-glu = α-glucose; sorb = sorbitol; β-glu = β-glucose; inos = inositol; suc = sucrose.

The chromatogram of pear acids isolated by precipitation of their lead salts is shown in Fig. 2. The mass spectrum for peak 8 contained extraneous ions to that of citric acid and it would appear that peak 8 is a mixture of citric acid and fructose as they co-chromatograph on the SE-30 column. Heatherbell[1] estimated that fructose could contribute to error in the order of 10% for citric quantitation in a model system

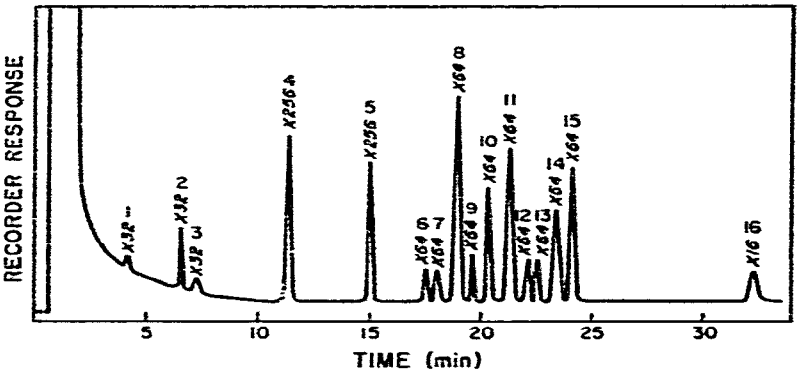

Fig. 2. GC separation on 3% SE-30 column of TMS ethers of Bartlett pear acids prepared by precipitation of their lead salts; 1 = glycolic; 2 = phosphoric; 3 = succinic; 4 = malic; 5 = tartaric (int. std.); 6 and 7 = unidentified; 8 = fructose plus citric; 9 = unidentified; 10 = quinic; 11–15 = unidentified; 16 = chlorogenic (identification by MS).

containing 10% fructose and 0.02% citric. One could expect an even higher contamination in pears with their high fructose (7.9–9.3 g/100 g) and low citric (0.10–0.16%) content[11]. The mass spectra for peaks 7, 10, 11, 12, 13 and 14 did not match that of the acids with corresponding retention times and they contained ions characteristic of TMS ethers of sugars. Pears have a relatively high pH (4.1–4.6) in comparison with other fruits[12] which may be a contributing factor to this method being less effective with pears than with other fruits.

GC–MS analysis of TMS derivatives of Bartlett pear acids separated by ion exchange (Fig. 3) indicate complete isolation of sugars from acids. The following acids were identified: glycolic, succinic, phosphoric, malic, citric, quinic, mucic and chlorogenic. Notably, phosphoric acid, which has not been reported in pears, was separated by both lead salt precipitation and ion exchange and identified by GC–MS. The recovery of sugars and acids from ion exchange columns was determined and is reported in Table I, along with their K values.

The lead salt precipitation method, while effective for sugar determinations, appears to be inappropriate for pear acids. The ion-exchange procedure for separation of sugars and acids in preparation for quantitative chromatographic analysis is recommended as the better method for pears with their high sugar, high pH, and low acidity.

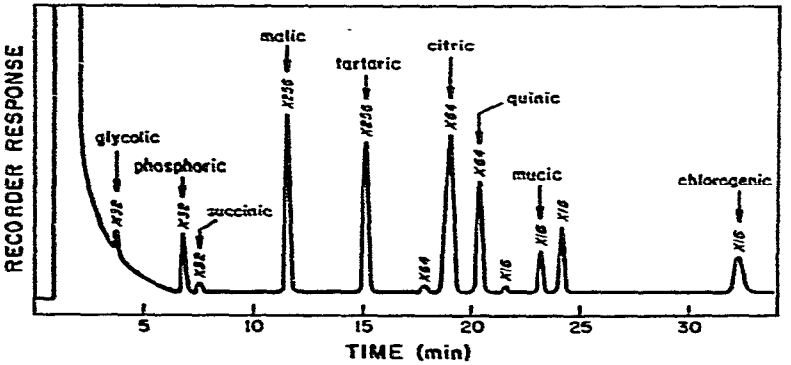

Fig. 3. GC separation on 3% SE-30 column of TMS ethers of Bartlett pear acids prepared by ion exchange: glycolic, phosphoric, succinic, malic, tartaric (int. std.), unidentified, citric, quinic, unidentified, mucic, unidentified, chlorogenic.

TABLE I

DETECTOR RESPONSE FACTORS (K) AND RECOVERY OF MAJOR SUGARS AND ACIDS OF BARTLETT PEARS FROM ION-EXCHANGE COLUMNS

Sugar or acid	*K*	*Recovery (%)*
Fructose	0.82	96
Sorbitol	1.54	102
Glucose	1.56	100
Sucrose	1.14	100
Malic	1.13	91
Citric	0.80	75
Phosphoric	1.11	98
Quinic	1.53	98

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