Identification of Erythritol by HPLC and GC–MS and Quantitative Measurement in Pulps of Various Fruits

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Erythritol in pulps of various fruits was first identified and quantitatively determined by high-performance liquid chromatography (HPLC) using a CK08SH packed column, 1% H_3PO_4 eluent, and a differential refractometer and by gas chromatography–mass spectrometry (GC–MS) using a 5% SP-2340 column and electron-impact ionization mode. Fruits were washed well to avoid contamination from peel. Pulps were carefully isolated from peel by knife and then homogenized. Determination by HPLC revealed 0.0022–0.0047% of erythritol in watermelon, melon, pears, and grapes, but not in apples, banana, cherry, and peach. The amount was very small compared to that contained in fermented foods.

Erythritol, which is 70–80% as sweet as sugar (Sasaki, 1988), is present in fermented foods such as Japanese sake (Onishi and Saito, 1960; Masuda et al., 1967), wine (Dubernet et al., 1974; Sponholz and Dittrich, 1985), soy sauce (Onishi and Saito, 1959), and miso bean paste (Shindou et al., 1988); in plants such as mushroom (Yoshida et al., 1984, 1986) and lichen (Huneck et al., 1967; Huneck and Follmann, 1967); and in animal blood (Britton, 1967; Roboz et al., 1984), urine (Haga et al., 1972; Imanari et al., 1975), and sperm (Ahluwalia and Graham, 1966; Clark et al., 1967; Storset et al., 1978).

It is believed that erythritol is derived mainly from microorganisms during the process of fermentation in food products and from the diet in animals. On the other hand, sugar and sugar alcohol in the pulp greatly affect the taste of fruits; however, there has been no report on the existence of erythritol in the pulp.

Accordingly, we conducted the present study and confirmed the existence of erythritol in pulp by high-performance liquid chromatography (HPLC) using CK08SH resin, which we established (Shindou et al., 1988). This paper reports the results of the study.

EXPERIMENTAL SECTION

Samples and Reagents. Fruits were purchased at a market in Omiya City, Japan. All reagents used were of Premier grade. If unavailable, those of the best grade available were used.

Conditions for HPLC and GC–MS. (*1*) *HPLC*: apparatus, Model 201 HPLC (Japan Millipore Ltd.); column, CK08SH (cation-exchange resin, 8 mm (i.d.) × 500 mm; Mitsubishi Chemical Industries Ltd.); eluent, 1% H_3PO_4; flow rate, 1 mL/min; detector, differential refractometer (Model R-401, Japan Millipore); integrator, Model D-2000 chromatointegrator (Hitachi Co.).

(*2*) *GC–MS*: apparatus, Model M-80A GC–MS (Hitachi Co.); ion source temperature, 180 °C; ionization voltage, 20 eV; ion accelerating voltage, 3 kV; column, 5% SP-2340, Chromosorb W AW-DMCS, 60–80 mesh, 3 mm (i.d.) × 200 mm, glass column; column temperature 180 °C; injection temperature 220 °C; interface temperature 220 °C; carrier, He, 50 mL/min.

Preparation of Erythritol Standard Solution. A 100-mg portion of erythritol was weighed precisely and added with distilled water to obtain 100 mL. To 10 mL of this solution was added 0.5 mL of 1,4:3,6-dianhydro-D-glucitol solution (50 mg/ML in water) as internal standard (IS).

Preparation of Sample for HPLC. Fruits were washed well under the running water to avoid contamination from the surface of the fruit. An apple, a banana, a cherry, a peach, a pear, and a grape were peeled off, pitted by knife or hand, and then homogenized. A melon and watermelon were cut into small pieces, and pulp's parts were isolated from peel and seed and then homogenized. Therefore we used only the edible part for analysis.

(*1*) *Watermelon.* A 100-g sample of the homogenized watermelon sample was combined with precisely 0.5 mL of IS and then centrifuged at 3000 rpm for 10 min. The supernatant was filtered, added with 0.5 g of activated carbon, agitated well, and then filtered again.

(*2*) *Melon and Other Fruits.* A 100-g sample of the homogenized fruit was combined with precisely 0.5 mL of IS and further combined with 50 mL of 80% ethanol. After thorough agitation, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was filtered through filter paper and then concentrated under reduced pressure. The residue was dissolved in 50 mL of water and then filtered.

Filtrates obtained in (1) and (2) were then added, respectively, to a mixed resin (MB-3; Japan Organo Co.), desalinated, and then filtered. The filtrates were concentrated under reduced pressure, filtered through a membrane filter (Millex-HV; 0.45 μm, 25-mm i.d.; Japan Millipore), and subjected to HPLC.

Preparation of Sample for GC–MS. A fraction with the same retention time as erythritol in HPLC was collected, concentrated with ethanol under reduced pressure, dried, and solidified. The fraction was combined with 0.5 mL of pyridine and 1 mL of acetic anhydride and then acetylated for 1 h on boiling water bath. The reaction mixture was chilled and extracted with 30 mL of ether, and the ether layer was washed 10 times with 20 mL of distilled water. The ether layer was dried under reduced pressure, and the residue was dissolved again in 1 mL of ether to obtain the sample for GC–MS.

RESULTS AND DISCUSSION

Identification of Erythritol in Watermelon. A sample obtained from watermelon was applied to the high-peformance liquid chromatograph. A small peak, with retention time 13 min (the same as that of erythritol), appeared as shown in Figure 1.

The fraction containing the peak was collected, concentrated, dried, reacted, and applied to the gas chromatograph. The result is shown in Figure 2. The reten-

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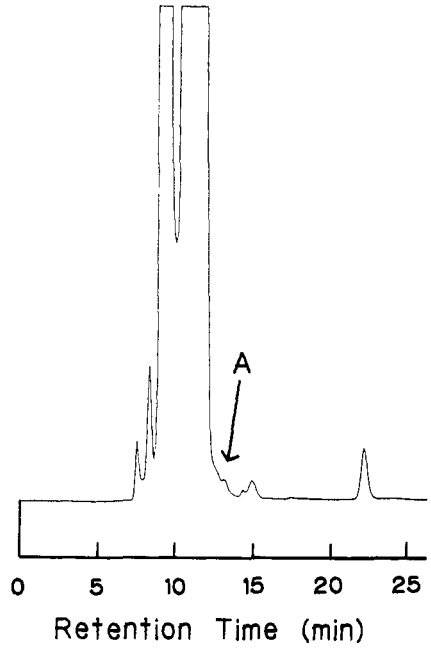

Figure 1. Liquid chromatogram of sugars and sugar alcohols in watermelon.

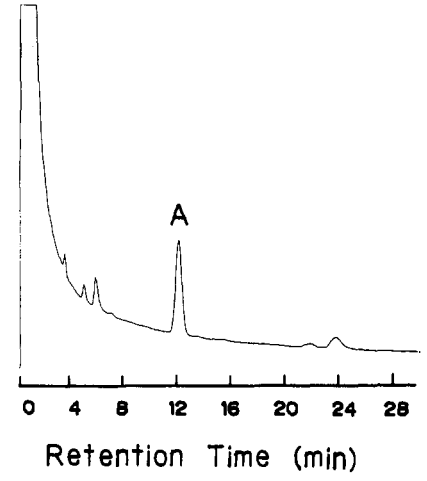

Figure 2. Gas chromatogram of acetyl derivatives isolated as peak A indicated in Figure 1.

tion time was identical with that of the gas chromatographed acetylated compound of erythritol.

Figure 3 shows the results of the mass spectrometry of erythritol and acetylated compound. As is clear from Figure 3, the acetylated derivative of the erythritol fraction obtained from watermelon showed a mass spectrum identical with that of the acetylated derivative of erythritol prepared separately. The above results confirm the presence of erythritol in watermelon.

Erythritol Amounts in Watermelon and Other Fruits. Since erythritol was demonstrated to be present in watermelon, we conducted further experiments to measure erythritol contents in banana, cherry, melon, peach, apples, pears, and grapes. Table I shows the result.

As we reported previously (Shindou et al., 1988), 100 mg of erythritol was present in 1 kg of fermented foods. However, we detected only 10 mg/kg of erythritol in fruits. Since fruits were carefully washed to prevent contamination from the fruit surface, the erythritol detected must be in the fruit.

It is well-known that the amounts of sugar and sugar alcohol in fruits vary according to the degree of maturity and storage condition (Matsushita, 1971; Komiyama et al., 1985). But the relationship of erythritol and maturity in fruits is not known well, and biosynthesis of erythritol in fruits is also not known. We have succeeded in quantitative determination of erythritol in fruits in this study, and we suppose that our analytical method will be a step toward elucidating the biosynthesis mechanism, maturity relationship, and taste quality.

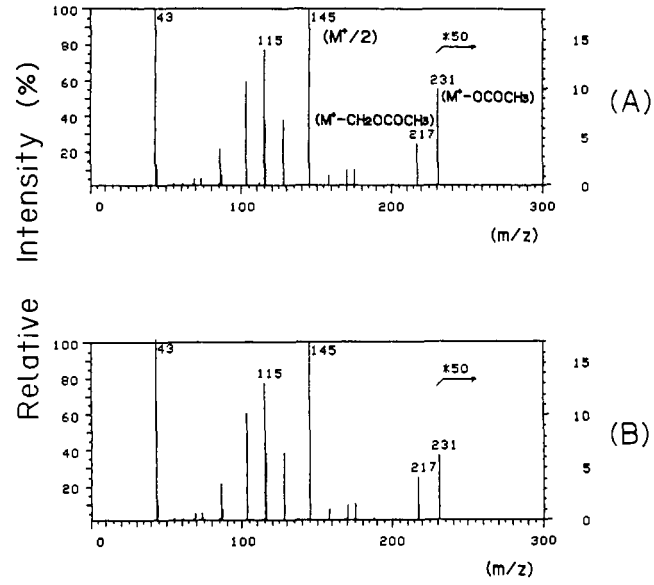

Figure 3. Electron-impact mass spectra of (A) erythritol tetraacetate and (B) acetyl compounds isolated as peak A indicated in Figure 2.

Table I. Erythritol Contents in Pulps of Various Fruits

species of fruit (local name)	erythritol, w/w %
watermelon	
Kouou maz	0.0022
melon	
Masuku melon	0.0047
peach	
Hakuto	ND[c]
pear	
Kousui	0.0040
Younashi	ND
apple	
Star king	ND
Sun fuji	ND
grape	
Muscat	0.0042
Kyohou	ND
banana[a]	ND
cherry[b]	ND

[a] Imported from The Philippines. [b] Imported from the United States. [c] Not detected.

Registry No. Erythritol, 149-32-6.

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Volatile Components from Bartlett and Bradford Pear Leaves

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Volatile components from whole leaves of Bartlett and Bradford pear were collected in a glass tube packed with Tenax and Carbotrap by passing a stream of air through a container of pear leaves and then into the trap. The trap was thermally desorbed onto a capillary GC column, and the compounds were identified by means of mass spectral data and Kovats retention indices. The compounds that were identified in all samples include (*E*)-β-ocimene, β-caryophyllene, and (*E*,*E*)-α-farnesene. Perillene and (*Z*,*E*)-α-farnesene were also found in all samples but were only tentatively identified. α-Copaene was a major component found in the Bartlett samples but not in the Bradford samples. Linalool (tentatively identified) was a medium to large peak and was found in the Bradford samples but not in the Bartlett samples. Compounds listed in this publication have not been reported previously as volatiles from pear leaves.

Psylla pyricola Forester (pear psylla) is an oligophagous insect that is quite specific in the selection of its host (Burts, 1970). It is known to infest only pear, quince, and chess grass (Madsen et al., 1962; Glass, 1969). Pear psylla exists in two distinct forms, and the summer form is one of the most destructive insect pests attacking pear orchards in the United States and Europe (Butt and Stuart, 1986). Losses in pear production are caused by direct defoliation, lower quality of the fruit, and "psylla shock", i.e., reduced quantity of fruit the year following an infestation. Psylla attacks most species of pear but with different levels of success (Chang and Philogene, 1976). Some experiments indicate that certain cultivars of pear are preferred targets for adult feeding (Chang and Philogene, 1978; Westigard et al., 1970), while other studies suggest an ovipositional preference for susceptible pear trees and nymphal antibiosis (mortality) on resistant pear trees (Harris, 1973). Although nonvolatile components from pear leaves have been studied (Challice and Williams, 1968a,b) and the volatiles from pear fruit have been investigated (Jennings et al., 1960), volatile organic compounds emanating from the foliage of pear trees have not been examined yet. These may, in fact, play a role

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