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Note

Occurrence of L-sorbose in apple-cider vinegar

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Since 1852, when L-sorbose was discovered by Pelouze[1] in sorb juice that had been stored for over a year, only sparse references to its natural occurrence are to be found. Bertrand[2] isolated it from fermented juice of mountain ash (*Sorbus aucuparia* L.) berries, where it occurs as a secondary product, formed by the oxidation of D-glucitol (sorbitol) by such bacteria as *Acetobacter xylinum*. Nearly a century after its discovery, Martin and Reuter[3] reported "L-sorbose" (identified only as the phenylosazone) in an enzymic hydrolyzate of pectin from the skin of passion fruit (*Passiflora edulis*); however, neither Sherman *et al.*[4] nor Pruthi[5] was able to confirm this. Recently, Josefosson[6] has detected small proportions of a sorbose in sea water.

The presence in apples of appreciable proportions of D-glucitol has been known[7] since 1889, and the conversion of D-glucitol into L-sorbose by *Acetobacter* is the subject of an extensive literature. It was, therefore, reasoned that L-sorbose might be expected to be a product when apple cider containing D-glucitol is fermented by *Acetobacter* in the production of apple-cider vinegar; L-sorbose has now been identified as a constituent of apple-cider vinegar.

Because others had been unable to repeat Pelouze's work[1], Bertrand[2] carefully studied the transformations that occur in the juice of mountain-ash berries during the conversion of D-glucitol into L-sorbose and, as a result of his investigation he developed a method for the biochemical preparation of L-sorbose. Neither fermentation (with *Saccharomyces vini*) nor the molds which grew in the fermented juice produced sorbose. Bertrand[2] surmised that a vinegar fly (*Drosophila cellularis*) carrying *Acetobacter xylinum* had inoculated the medium, causing conversion of D-glucitol into L-sorbose. Other investigators[8–10], whose work had prompted Bertrand's study[2], had reported glucitol in the fresh berry juice, but could not find sorbose therein. In the present work, a glucitol, but no L-sorbose, was found in apple cider.

Terada *et al.*[11] reported a nonepimeric isomerization at C-5 of sorbose and fructose by *Acetobacter* species. As fructose was found in the apple cider, the possibility of its conversion into sorbose cannot be ignored. However, it seems logical to

conclude that most of the L-sorbose is formed from D-glucitol during the vinegar-making process, and that it might be expected as a product when *Acetobacter* and a substrate containing D-glucitol are involved.

EXPERIMENTAL

L-Sorbose from apple-cider vinegar. — Apple-cider vinegar (~500 ml) was passed through a column of Amberlite IR-120 (H^+) and then through one of Duolite A-4 (OH^-) ion-exchange resin, and the eluate was concentrated to ~15% of solids. This solution was examined for the presence of sugars by paper chromatography. By use of 5:1 phenol–water as the irrigant, and an orcinol reagent[12] for development, two zones were detected. One was faint-yellow and corresponded to sucrose, and the other was a large, yellow spot, R_{Fru} 0.77, a value identical with that of authentic sorbose. The major component was separated by chromatography on heavy paper, and eluted from the paper with water. Upon evaporation of the eluate, a dry crystalline mass (~300 mg) resulted; after recrystallization from ethanol, it had m.p. and mixed m.p. 165° with L-sorbose (m.p. 165°) [lit.[13] m.p. 166.5–167°], $[\alpha]_D^{25}$ −42.0° (*c* 2, water) [lit.[14] $[\alpha]_D^{25}$ −43.4° (equil., water)], and its i.r. spectrum (KBr) was identical to that of authentic sorbose.

Glucitol from apple cider. — Apple cider was concentrated to ~15% of solids. A portion of this solution was fermented with bakers' yeast; the fermented solution was treated with ion-exchange resins, and again concentrated to ~15% of solids. Aliquots of the nonfermented and fermented solutions were chromatographed with 8:2:1 ethyl acetate–pyridine–water and with 5:1 phenol–water, and developed with the orcinol reagent[12] and with a silver nitrate reagent[15]. Fructose was found in the unfermented solution, but sorbose was not detected, either before or after fermentation of the cider. Before fermentation of the cider, glucitol was not detected with any certainty (owing to the interference of a large pool of fermentable sugars); however, it was found in the fermented solution. A zone having an R_F value identical with that of authentic D-glucitol was detected with silver nitrate. Co-chromatography with known D-glucitol in the two solvent systems further verified the presence of a glucitol.

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