

## Spectrophotometric Determination of Sorbic Acid in Apple Cider<sup>a</sup>

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### SUMMARY

**An improved method for measuring sorbic acid in cider is based on dilution of the sample and direct ultraviolet readings at 262 m $\mu$ . This method was compared with the distillation method for sorbic acid usually applied to cider. The latter method produced ultraviolet-absorbant materials that interfered with sorbic acid determinations and were shown to be affected by the sugar content of the cider and the distillation techniques. The ultraviolet-interfering materials in cider distillates have absorption peaks and chemical properties similar to those of 5-hydroxymethylfurfural.**

In this paper, "cider" refers to un-pasteurized unfermented juice from apples, not the heat-processed canned (or bottled) product. Freshly pressed cider has distinctive consumer appeal. Freezing, chemical preservation, and pasteurization have been studied (Marshall, 1947; Robinson *et al.*, 1958; Smock and Neubert, 1958; Tressler and Joslyn, 1954) as means of maintaining cider in good condition.

Smaller cider producers are particularly interested in the addition of chemical preservatives to extend the shelf life of cider during warm fall weather. Weaver *et al.* (1957), Robinson and Hills (1959), and Dryden and Hills (1959) found sorbic acid and its salts to be good preservatives, because their fungistatic properties increased storage life with the least flavor change. Therefore, it appeared desirable to be able to follow concentration changes in sorbic acid during the storage life of cider.

Distillation and spectrophotometric procedures have been developed and used by Melnick and co-workers (Gooding *et al.*, 1955; Luckmann and Melnick, 1955; Melnick and Luckmann, 1954) for sorbic acid determination in dairy products and other

foodstuffs. Other workers (Böhme and Bertling, 1959; Spanyol and Sänder, 1958; Hardon and Visser, 1958; Alterton and Lewis, 1958) have used alterations of these procedures for their studies on sorbic acid. This paper presents a simple and accurate method of determining sorbic acid in cider based on the ultraviolet absorption of diluted cider rather than cider distillates.

### MATERIALS AND METHODS

**Sorbic acid.** Commercial samples of sorbic acid and potassium sorbate from Union Carbide Chemicals Company were used (mention of companies or products does not imply endorsement by the United States Department of Agriculture over others not named). Ultraviolet absorption curves for carefully prepared solutions of known concentrations agreed with the published spectra of Alterton and Lewis (1958), Luckmann and Melnick (1955), and Melnick and Luckmann (1954).

**Cider.** Freshly pressed cider was obtained from a local producer. Fresh cider was compared with pectinol clarified, heated and unheated, fermenting and fermented samples, with and without added sorbic acid. Commercially bottled cider was also studied.

**Instrument.** Adsorption spectra in the 210–320-m $\mu$  region were recorded on a Cary Model 14 spectrophotometer in a 1-cm cell versus acidified distilled water.

**Sorbic acid distillation procedure.** Cider samples of 5–10 ml, with and without added sorbic acid, were placed in a 500-ml distilling flask containing 50 g of MgSO<sub>4</sub> · 7H<sub>2</sub>O, 50 ml of distilled water, and 1 g of citric acid. The electrically

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heated flask was connected to a Friedrichs condenser. When 45 ml of distillate had been collected in a graduated cylinder cooled in an ice bath, 50 ml of distilled water was added through the side opening of the flask. The distillation was stopped after an additional 45 ml was collected, and the condenser rinsed with 90 ml of hot water. The distillate and rinse water were diluted to 1000 ml with acidified distilled water (pH 3.2-3.5) to prepare the sample for ultraviolet absorption spectrum measurements.

Malic acid may be substituted for citric acid, or 50 ml of 0.1N H<sub>2</sub>SO<sub>4</sub> for the distilled water and citric acid, without affecting the results. An excess of acid (10 ml of conc. H<sub>2</sub>SO<sub>4</sub>) added to the distilling flask greatly increased the amount of interfering materials in the distillate. Variations in distilling techniques will affect the distillate of the test samples and their blanks by incomplete distillation or excessive pyrolysis.

**Dilution method.** Recovery errors are eliminated by this method, in which 1 ml of cider, with or without sorbic acid, was diluted to 100 or 200 ml by acidified distilled water (1 ml of 1N HCl per 100 ml of distilled water). The cider solution may be filtered through No. 50 Whatman paper, before or after dilution, to ensure clarity. The optimum sorbic acid concentration for measurement in a 1-cm cell is 1-3 ppm, which can be determined with an error no greater than  $\pm 1\%$ .

**Calculation of sorbic acid concentration.** For both the distillation and dilution procedures:

$$\text{ppm sorbic acid} = \frac{A_s - A_b}{.23} \times \text{dilution factor}$$

Where  $A_s$  = absorbance of cider + sorbic acid at 262 m $\mu$

$A_b$  = absorbance of cider blank at 262 m $\mu$

.23 = constant (absorbance of 1 ppm sorbic acid at 262 m $\mu$  in 1-cm cell)

$$\text{Dilution factor} = \frac{\text{diluted volume}}{\text{original volume}} \text{ of sample}$$

## RESULTS AND DISCUSSION

Spectrophotometric determination of sorbic acid in apple cider is based on the degree of ultraviolet absorbance of this substance at the wave length of maximum absorption. This maximum occurs at 262 m $\mu$ , and the absorbance increases proportionally with concentration, in agreement with Beer's Law.

Fig. 1 illustrates application of the dilution method for sorbic acid determination at various levels in cider. Curve A is for the

1% cider blank. Similarly curves B, C, D, and E respectively represent cider samples containing 23, 46, 91, and 182 ppm of sorbic acid. Because no heat is used, this method avoids the generation of irrelevant ultraviolet absorbant materials influenced by the cider's quality (clarified, pasteurized, fresh and/or fermenting) and no recovery losses are encountered.

However, many sorbic acid determination procedures are based on its recovery by distillation. These methods were applied to cider; and under some conditions, irrelevant materials interfere with the absorbance readings.

Fig. 2 and Table 1 are included in this paper to demonstrate problems in the application of the distillation procedure for sorbic acid recovery and determination from cider.

Fig. 2, curve A, indicates that fresh cider distillates contain materials that have a maximum absorption peak at 282 m $\mu$ . When sorbic acid is distilled from cider (B and C curves), peak absorption (between 262 and 282 m $\mu$ ) is shifted relative to the sorbic

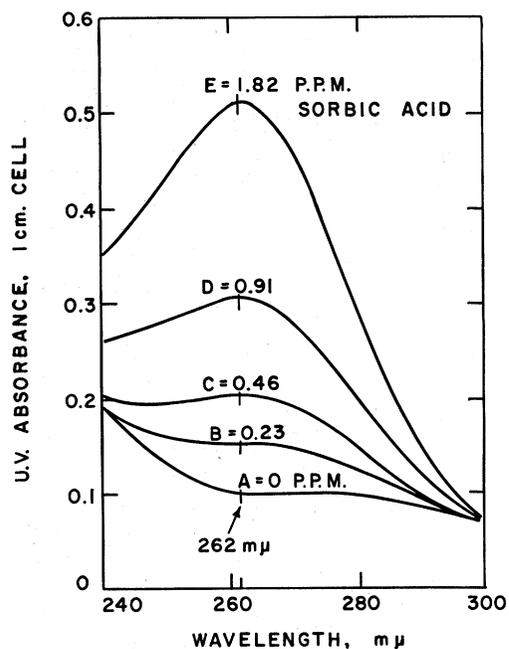


Fig. 1. Sorbic acid determinations in cider by dilution; dilution factor = 100. A) cider blank; B) cider + 23 ppm sorbic acid; C) cider + 46 ppm sorbic acid; D) cider + 91 ppm sorbic acid; E) cider + 182 ppm sorbic acid.

Table 1. Effect of microbiological activity on sorbic acid determinations in cider.

Storage time at room temperature (days)	By dilution			By distillation		
	Ultraviolet absorbance at 262 m $\mu$		Calculated sorbic acid content <sup>b</sup> (ppm)	Ultraviolet absorbance at 262 m $\mu$		Calculated sorbic acid content <sup>b</sup> (ppm)
	Sorbated-cider <sup>a</sup>	Blank-cider <sup>a</sup>		Sorbated-cider <sup>a</sup>	Blank-cider <sup>a</sup>	
0	0.428	0.068	156	0.440	0.100	148
4°	0.422	0.065	155 (154) <sup>d</sup>	0.414	0.050	158 (137) <sup>d</sup>
8°	0.420	0.058	157 (153) <sup>d</sup>	0.410	0.021	169 (135) <sup>d</sup>

<sup>a</sup> The sorbated-cider and its blank (same lot) were inoculated with spoilage organism from fermenting cider, then similarly sampled and analyzed by above methods. The cider was of late season and had a pH 3.95.

$$^b \frac{(\text{sorbated-cider}) - (\text{blank-cider})}{0.23} \times 100 = \text{ppm sorbic acid.}$$

<sup>c</sup> There was considerable microbiological activity indicated by gas pressure and rising bubbles in cider.

<sup>d</sup> Recalculated using the zero or initial blank values, the differences show that the distillation method is considerably affected by microbial quality changes in the cider (sorbated or blank), altering the value of its calculated sorbic acid content. The dilution method is not similarly affected.

acid-cider ratios. The effect is especially large at low sorbic acid levels because of superimposition of the spectra of the interfering cider distillate materials on that of

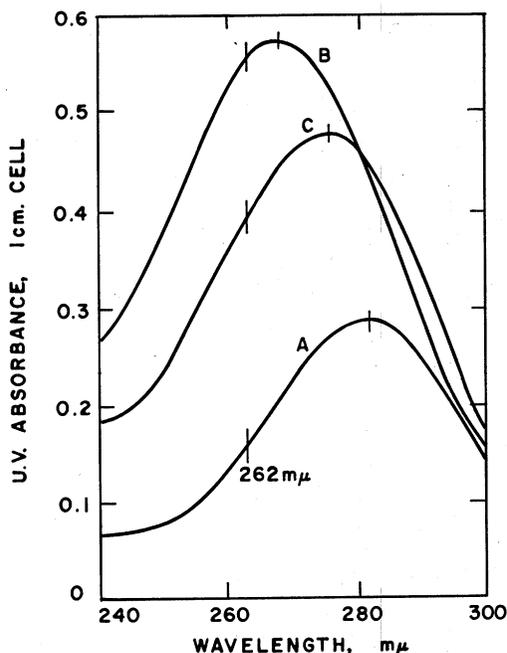


Fig. 2. Ultraviolet absorption spectra of sorbate-cider distillate; dilution factor = 100. A) cider blank; B) cider + 180 ppm sorbic acid; C) cider + 96 ppm sorbic acid.

sorbic acid. The quantity of interfering materials in the distillate is affected by the amount of cider distilled, the distillation procedure, and the quality of cider, but the amount of interfering materials in the distillation procedure can be kept constant by using a standardized procedure and cider of constant quality (prevention of microbial growth). Then the sorbic acid content can be calculated by the absorbance difference of a sorbated-cider minus its blank measured at 262 m $\mu$ . Thus, the sorbic acid contents of cider used for B and C curves of Fig. 2 respectively calculate to 174 and 103 ppm for cider containing 180 and 96 ppm.

However, when microbial growth alters cider quality, then varying amounts of interfering absorbing materials are distilled and we have no accurate basis for calculating the amount of recovered sorbic acid. This is demonstrated by Table 1.

From Table 1 it may be seen that microbial spoilage had less quantitative effect on the sorbic acid determined by dilution than by the distillation procedure, whether calculated using the original blank or one that had equal storage and microbial growth. Since the effect of microbial spoilage on fresh or sorbate-cider is evidently complex, accurate calculations for determination of sorbic acid levels cannot be made on dis-

tillates of cider in which microbial growth has occurred. This uncertainty is due to the variation in amounts of the interfering materials distilled from either blank or sorbated-cider which are changing in quality.

Sugars are known to produce ultraviolet-absorbing materials upon heating (Wolfrom *et al.*, 1948; Haas *et al.*, 1948); spectra of heated sugar solutions were compared with the interfering substances observed in cider distillates. A 1% solution of D-fructose, the predominant and least heat-stable sugar in apples (Smock and Neubert, 1958), was acidified as outlined in the dilution process. Spectra were obtained before and after 2 min of boiling. The unheated sample showed little absorption; the heated sample had at 282 m $\mu$  a strong maximum similar to that observed for cider distillates and boiled cider. Distillates of acidified fructose also exhibited maxima at 282 m $\mu$ , as did heated sucrose solutions. Increased absorption at 282 m $\mu$  in cider distillates can therefore result partly from sugar decomposition, which is accentuated by longer heating periods, higher temperatures, and addition of acid, but diminish if fermentation occurs.

Singh *et al.* (1948) and Wahhab (1948) have established that the principal decomposition product of sugars heated under acid

conditions is 5-hydroxymethyl-2-furfural, especially when hexoses are present. The similarity in the spectra of a commercial sample of hydroxymethylfurfural and a fresh cider distillate is shown in Fig. 3. Cider distillates gave a positive Molisch test and had similar paper chromatographic R<sub>f</sub> values compared to commercial 5-hydroxymethylfurfural, thus suggesting that the principal interfering material is 5-hydroxymethylfurfural.

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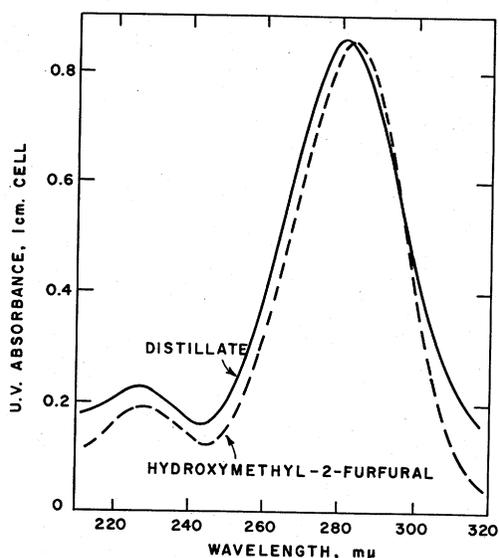


Fig. 3. Comparison of ultraviolet absorption curves for cider distillate and hydroxymethyl-furfural.

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