Table II. Reproducibility of Absolute Peak Areas for Food Additives

food additive	1	2	3	4	5	6	$\bar{x}$	s	% SD
DHA	1329	1372	1132	1370	1434	1250	1314.3	108.0	8.22
BHA	3091	2685	2706	2755	2798	2507	2757.0	191.6	6.95
BHT	3589	2911	2960	2979	3078	2837	3059.0	271.5	8.88
TBHQ	1617	1617	1449	1435	1820	1506	1574.0	144.1	9.15
Ionox-100	2857	2656	2639	2799	2861	2661	2745.5	105.0	3.82

Table III. Change of Food Additive Concentration in Sesame Oil 3 through Various Reaction Times at 25 °C

time, min	DHA	BHA	BHT	TBHQ	Ionox-100
30	0.81	0.70	0.98	1.00	0.68
60	1.13	0.74	0.85	0.92	0.68
90	0.94	0.71	0.83	0.79	0.73
120	1.04	0.72	0.83	0.87	0.72
150	0.95	0.70	0.82	0.83	0.72
180	1.13	0.72	0.83	0.85	0.68

Table IV. Food Additives (ppm) Found in Commercial Vegetable Oils

vegetable oil	DHA	BHA
salad oil 1	ND[a]	ND
salad oil 2	ND	ND
salad oil 3	0.9	ND
salad oil 4	ND	ND
salad oil 5	ND	ND
corn salad oil	ND	ND
sesame oil 1	ND	ND
sesame oil 2	ND	ND
sesame oil 3	ND	ND
sesame oil 4	ND	ND
sesame oil 5	6.3	ND
spigadro oil 1	4.8	0.4
spigadro oil 2	4.5	0.4
olive oil	ND	ND
peanut oil	5.0	0.5

[a] ND = not detected.

the peaks of DHA, BHA, BHT, TBHQ, and Ionox-100. These results are also supported clearly by Figure 1C.

For a standard solution containing 5 ppm of each of the food additives in acetone, the results regarding to the reproducibilities of retention time and peak area in gas chromatograms are as shown in Table I; the standard deviation was very low, generally less than 0.06 %. As Table II shows, the absolute reproducibility in area counts for five food additives was just over 9% relative standard deviation, for six analyses. It might be said that the reproducibility of the absolute areas is also very good. This means that the external standard method can be used for these vegetable oils with confidence.

For five kinds of food additives, the relationship between reaction time and the percentage of residual food additive fraction against the amounts of added food additives to sesame oil 3 is as shown in Table III. It was found that the decrease of DHA concentration does not appear except at 30 min, but BHA, BHT, TBHQ, and Ionox-100 show a 70–80% concentration range, and these figures do not change throughout the reaction times. Therefore, BHA, BHT, TBHQ and Ionox-100 seemed to react with some components or denatured components in vegetable oils.

The concentration of DHA and BHA in 15 commercial vegetable oils is shown in Table IV. Some vegetable oils contained 4–5 ppm of DHA and less than 5 ppm of BHA. But, BHT, TBHQ, and Ionox-100 are not detected in all samples.

If the peak height, which is 3 times as high as the noise peak, is considered suitable for the quantitation, the lower limits of detection are estimated to be 0.5 ppm for DHA, TBHQ, and Ionox-100 and 0.3 ppm for BHA and BHT.

Registry No. DHA, 520-45-6; BHA, 25013-16-5; BHT, 128-37-0; TBHQ, 1948-33-0; Ionox-100, 88-26-6.

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Increased Specificity in Sorbic Acid Determination in Stored Dried Prunes

Two thermal degradation products produced from sugars in processed prunes during drying and protracted storage were isolated and identified by gas chromatography–mass spectroscopy (GC–MS). The compounds were 5-(hydroxymethyl)-2-furaldehyde and 2-furaldehyde. The gas chromatographic procedure was specific for sorbic acid and quantitation was not affected by these and other degradation compounds, but they appear to have affected to varying degrees the spectral methods used in this study.

Prior to 1950 dried prunes were stored and marketed at a water activity of about 0.65 (16–18% moisture), which obviated any need for preservatives. However, prunes are currently marketed at a water activity of 0.75 or greater (>28% moisture) to provide a product with a more tender texture. Food products stored at this water activity must be treated in some manner so that microbial proliferation will not occur. The main commercial procedure at present

Table I. Sorbic Acid Analysis on Stored Prunes Using Various Analytical Procedures and Butyl Levulinate Analysis (Expressed on a 30% Moisture Basis)

storage		ppm[a]					
		sorbic acid analytical procedure[b]					
temp, °C	time, months	1	2	3	4	5	butyl levulinate
	0	300	350	330	360	370	0
1	30	340	350	340	370	370	0
21	11	230	370	250	290	240	20
21	30	150	230	210	260	110	90
32	11	220	280	290	230	170	60
32	30	140	500	380	200	110	160

[a] Mean value of duplicate analysis. [b] 1, spectral (visible), chloroform extraction; 2, spectral (visible), filtered, aqueous extract; 3, spectral (UV), chloroform extraction; 4, spectral (UV), distillation; 5, gas chromatographic, methylene chloride extraction.

consists of dipping or spraying the fruit with the antimicrobial compound sorbic acid.

Sorbic acid and its potassium salt have long been used as effective fungistatic agents in foods. Sorbic acid is a generally recognized safe (GRAS) compound, and even in laboratory studies on dogs and rats it exhibited no physiological effect when it comprised up to 5% of their diet (Deuel et al., 1954). However, because of increased usage of food additives, many countries have placed rigid limits on the sorbic acid content of imported products. For this reason it is essential to be able to determine accurately the sorbic acid content of a product.

Two factors have to be considered in connection with sorbic acid analysis. First, sorbic acid when dry is stable but in aqueous solution becomes susceptible to oxidation (Arya, 1980). Sorbic acid in freshly processed prunes is concentrated mostly on the surface (Bolin et al., 1980) and can react with available atmospheric oxygen. The rate of oxidation is temperature dependent. Second, among the existing analytical procedures, most are nonspecific and can be affected by thermal degradation compounds formed in the product during dehydration and especially storage. These sugar decomposition compounds occur in products such as orange juice (Dinsmore and Nagy, 1971; Meydav and Berk, 1978) and glucose solution (Fukuchi et al., 1977).

This study was undertaken to determine if these compounds were formed in prunes during protracted storage and the extent of their effect on spectral and gas chromatographic analytical results.

EXPERIMENTAL SECTION

Sample Preparation. Commercially processed French prunes (size 11 per 100 g) that had been dipped in a 1.8% potassium sorbate solution were obtained for the storage study. The water activity of the processed prunes was 0.78 (30% moisture), and they contained approximately 400 ppm of sorbic acid. After the prunes were mixed thoroughly, they were put into 1-L glass containers, sealed and stored at 1, 21, and 32 °C.

Analyses. The samples were removed after 11 and 30 months of storage and analyzed for sorbic acid by four spectrophotometric and one gas chromatographic method (Table I). Two of the spectrophotometric methods are based on measurements in the visible region; one utilized a two-step extraction procedure (Stafford, 1976), and another used a simple water extraction (Nury and Bolin, 1962). Two methods employ measurement in the ultraviolet region at 253 nm; one includes a distillation step (Alderton and Lewis, 1958), and the other includes a two-step extraction (Stafford, 1976). The fifth was a gas chromatographic procedure (Stafford and Black, 1978). Moisture was determined by the Association of Official Agricultural Chemists (1978) procedure for dried fruits.

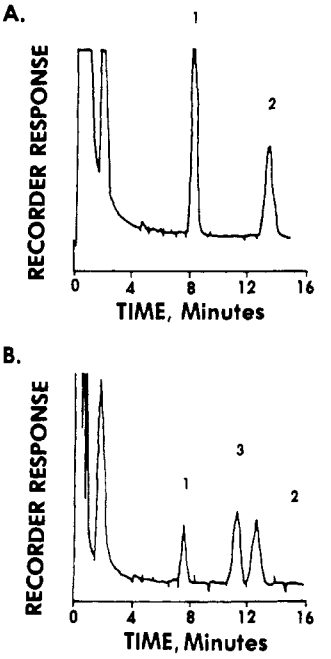

Figure 1. Chromatograms from GC sorbic acid analyses on a 1.8 m × 2.0 mm i.d. glass column packed with 80–100-mesh Chromasorb W, HP, coated with 10% AT-1000 (Alltech Associates). The column was operated isothermally at 135 °C. Chromatogram A: sample stored at 1 °C for 30 months. Chromatogram B: sample stored at 32 °C for 30 months. Identification: 1, *n*-butyl ester of sorbic acid; 2, *n*-butyl ester of decanoic acid; 3, *n*-butyl ester of levulinic acid.

A Finnigan 4500 GC/MS/Data System was used for gas chromatographic separation and mass spectral examination of the unknown sample components. A split injector, operated at 250 °C with a 30:1 split ratio, was used for sample introduction. A fused silica capillary column, coated with SP-2340 (50 m × 0.22 mm i.d.; Chrompack) and operated at 30 psi of head pressure (helium), was held at 100 °C for 0.1 min after sample injection, then was temperature programmed at 5 °C/min to 200 °C, and was held at that temperature for 0.1 min. The exit end of the chromatographic column extended directly to the ion source entrance through a heated zone held at 250 °C. The mass spectrometer was operated in the electron impact mode, at −70 eV. The ion source temperature was 180 °C, emission current was 0.30 mA, and the multiplier voltage was −1250 V. A 1-s scan rate was used, over a 33–450 amu mass range.

RESULTS AND DISCUSSION

Analysis of freshly processed prunes and of samples held near freezing for the duration of the storage study gave similiar sorbic acid results with all methods listed; however, when 21 and 32 °C storage samples were analyzed, results were variable (Table I).

In the gas chromatographic procedure a new peak occurred when the samples that had been stored at 21 and 32 °C for 11 or 30 months were analyzed (Figure 1B). This peak was identified by GC–MS as the *n*-butyl ester of levulinic acid, comprising up to 160 ppm as determined by peak area of the FID detector response. This indicated that 5-(hydroxymethyl)furfural (HMF) was present in the stored prunes, since it is the first thermal degradation product of hexose sugars (glucose → HMF → levulinic acid). Also, we found that in the preparation of butyl

esters using boron trifluoride butanol (14%/wt) HMF was converted to the butyl ester of levulinic acid. The stored prunes also contained furfural, which is the thermal degradation product of a pentose sugar (fructose → furfural → furoic acid). The presence of furfural was confirmed by GC–MS of an underivatized chloroform extract, concentrated by the method of Stafford et al. (1978). This is not seen in the gas chromatogram (Figure 1) because most of the furfural present in prunes would be removed from the prune extract in the two-step extraction procedure used for the GC method, and any remaining furfural would be lost when the extract was taken to dryness prior to the derivatization step.

The presence in stored dried prunes of furfural, HMF, and possibly levulinic and furoic acid, which are oxidation products of HMF and furfural, respectively, did not affect the quantitation of sorbic acid by the gas chromatographic procedure because of its specificity. The procedure involves the formation of the *n*-butyl derivative of sorbic acid, which has a definite, reproducible, gas chromatograph relative retention time (Figure 1), and uses decanoic acid as an internal standard, to aid quantitation. Other compounds could coelute with the butyl sorbate or butyl decanoate, but in the capillary GC–MS analyses of stored and unstored samples only spectra consistent with the presence of *n*-butyl sorbate and *n*-butyl decanoate were recorded. No mass spectral evidence was found for coelution of any interfering components in the GC peaks for these two compounds.

The visible absorption spectrophotometric methods (1 and 2) rely on the reaction of 2-thiobarbituric acid (TBA) and a product produced by the oxidation of sorbic acid, which gives a colored compound having an absorption maximum at 530 nm. However, as shown by Jennings et al. (1955), numerous aldehydes and other compounds (Sinnhuber et al., 1958) react with TBA to produce a highly pigmented product. Therefore, HMF and furfural could cause high results when these methods are used. Method 1 would tend to reduce the presence of these carbonyl compounds with the extraction steps. In method 2 any carbonyl compounds would be measured.

Quantitation based upon UV absorption measurements can also produce erroneous results, unless the sample is sufficiently pure or is purified to exclude other interfering UV-absorbing compounds, for example, compounds produced during storage. In method 3, levulinic and furoic acid and possibly small amounts of HMF and furfural would be extracted along with any sorbic acid present in the sample. In method 4 furfural would be steam distilled over to the distillate and be measured as sorbic acid. Any additional furfural produced during the distillation would also be distilled. The absorption maxima of these compounds, except sorbic acid, are in the 280-nm range, but the absorption band shoulder extends into the UV region of interest for the analysis of sorbic acid.

These results indicate that a more specific analytical procedure for determining sorbic acid, such as the previously discussed GC method, should be used to analyze prunes or other products containing sugars stored for prolonged periods of time. A high-performance liquid chromatography (HPLC) method similar to the one developed by McCalla et al. (1977) for use with wine would prove even more desirable, since the time-consuming derivatization step required for the GC method could be eliminated.

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Registry No. 5-(Hydroxymethyl)-2-furaldehyde, 67-47-0; 2-furaldehyde, 98-01-1; sorbic acid, 110-44-1; levulinic acid, 123-76-2.

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Cyclitols of Soybean Leaves

Some minor cyclitols of soybean [*Glycine max* (L) Merrill] leaves are identified as D-(+)-ononitol (D-4-*O*-methyl-*myo*-inositol), sequoyitol (5-*O*-methyl-*myo*-inositol), and bornesitol. They were characterized by GC–MS, ^{13}C NMR spectroscopy, and polarimetry.

Soybean cyclitols are interesting because they constitute the major soluble carbohydrates in soybean (Phillips and Smith, 1974) and because they may play a role in nitrogen fixation in the nodules (Streeter and Bosler, 1976; Streeter, 1980; Kawai and Kumazawa, 1982).

Pinitol (D-3-*O*-methyl-*chiro*-inositol) is the predominant soybean cyclitol. Plouvier (1950) discovered pinitol in soybean leaves wherein pinitol may be 0.1–0.7% of the fresh leaf weight (Phillips and Smith, 1974; Dougherty, 1976; Streeter, 1980; Binder and Kogan, 1981). In soybean