fraction (see Figure 2). The slope to the tangent line of the V_s curve at some x_2 describes the change in V_s resulting from a change in x_2. This slope, the ratio of these changes, can be related to partial specific volumes and the apparent specific volume parameters by the expressions

$$dV_s/dx_2 = (v_{s2} - V_s)/x_1 = v_{s2} - v_{s1} = \epsilon_1 - v^\circ_{s1} + (2\epsilon_2 + 3\epsilon_3 x_2 + 4\epsilon_4 x_2^2)x_2 \quad (24)$$

$$dV_s/dx_1 = (v_{s1} - V_s)/x_2 = -dV_s/dx_2 \quad (25)$$

In the dilute limit ($x_2 = 0$), dV_s/dx_2 is equal to $\epsilon_1 - v^\circ_{s1}$. In the high concentration limit ($x_2 = 1$), dV_s/dx_2 is equal to $\epsilon_1 - v^\circ_{s1} + 2\epsilon_2 + 3\epsilon_3 + 4\epsilon_4$.

Abbreviations Used: A, weight fraction of dry substance that is sulfated ash; DS, dry substance, weight fraction of nonaqueous components; DPi, saccharide consisting of i monomers (dextrose units); DP4+, component consisting of tetrasaccharides and saccharides of higher DP (degree of polymerization); dV_s/dx_2, derivative of specific volume with respect to the saccharide weight fraction; F, volume fraction of water in solution; q, number of saccharide components; R, ratio of water molecules to saccharide residues in solution; s, weight fraction of dry substance (saccharides plus ash) in solution; t, temperature in degree Celcius; t_0, base temperature, 20 °C; V, solution volume, mL; V_s, solution specific volume, mL/g; ${}^\phi V_{si}$, apparent specific volume of the ith saccharide in water, mL/g; v_{s1}, partial specific volume of water, mL/g; v°_{s1}, specific volume of pure water, i.e., 1.00177 mL/g at 20 °C; v_{si+1}, partial specific volume of only the ith saccharide in water, mL/g; w_1, weight water, g; w_2, weight saccharide, g; x_1, weight fraction of water; x_2, weight fraction of solute in binary mixture; x_{i+1}, weight fraction of the ith saccharide in solution; x_{ash}, weight fraction of ash in solution; α_1, α_2, and α_3, parameters associated with higher order thermal effects of dry substance; α_v, coefficient of thermal expansion of liquid, $(°C)^{-1}$; β_{ij}, temperature parameter of $(i-1)$ order in s for apparent specific volume of the jth saccharide; ϵ_{ij}, compositional parameter of $(i-1)$ order in s for apparent specific volume of the jth saccharide; Δ, difference in specific volume of water at temperature t and t_0, mL/g; ∂, symbol for partial differentiation; ash, ash component (on inorganic sulfate basis); s, specific; 1, water component; 2, saccharide solute in binary mixture; v, volume; ϕ, apparent; °, pure component.

ACKNOWLEDGMENT

We thank A. M. Wartman, T. D. Spawn, and M. A. Eliason for the assistance and cooperation that made this analysis possible.

Registry No. Dextrose, 50-99-7; fructose, 57-48-7; sucrose, 57-50-1.

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Received for review September 23, 1983. Accepted March 3, 1984.

High-Performance Liquid Chromatographic Determination of Furfural in Orange Juice

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A high-performance liquid chromatographic (HPLC) method to quantify the amount of furfural in orange juice has been developed. It was found necessary to separate furfural from interfering compounds by distilling the juice. The distillate was injected on a Du Pont Zorbax ODS column. The furfural was eluted with water–methanol (70:30 v/v). Detection was by absorbance at 280 nm. A linear response was obtained from 12 to 20 000 ppb. The minimum detectable level of furfural in orange juice is 2 ppb. Compared to traditional colorimetric methods of furfural analysis, the HPLC procedure was more precise, had a wider range, and could be run in less time with safer reagents.

Processed orange juice and orange concentrate are susceptible to color, flavor, odor, and nutritional changes if stored for extended periods above refrigerated (4 °C) temperatures. Many chemical tests have been suggested to indicate storage abuse (Dinsomore and Nagy, 1971; Nagy and Nordby, 1970). A test that is widely accepted as an indication of general flavor changes in citrus juices subjected to storage temperature abuse is the test for furfural (Dinsmore and Nagy, 1972, 1974). This test was chosen because furfural is virtually nonexistent in fresh citrus juice, whereas large amounts of furfural have been reported in citrus juices stored at improper temperatures (Kirchner and Miller, 1957; Rymal et al., 1968; Dinsmore and Nagy, 1971).

Furfural content is useful as an off-flavor indicator, although it does not cause the off-flavor developed during temperature abuse of citrus juices (Tatum et al., 1975). A strong relationship between flavor change in orange and grapefruit juice and furfural content has been shown (Nagy

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0021-8561/84/1432-0979$01.50/0 © 1984 American Chemical Society

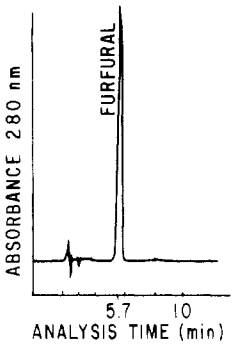

Figure 1. Typical chromatogram of furfural recovered from orange juice.

et al., 1972; Nagy and Randall, 1973; Dinsmore and Nagy, 1974). The use of furfural content as a flavor indicator becomes more important to food processors as aseptic processing and packaging of citrus juices becomes accepted. Aseptic packaging allows for higher temperatures during distribution and storage without microbial spoilage, but off-flavors and loss of nutritional quality develop as citrus products are exposed to these conditions. This makes the detection of furfural of increasing importance to the citrus processing industry.

The current method of furfural analysis in citrus juices (Dinsmore and Nagy, 1972, 1974) uses a colorimetric reaction between furfural and aniline in the presence of glacial acetic acid and stannous chloride. This method is time consuming, taking approximately 1 h for color development, and requires the hazardous chemical aniline (Christensen et al., 1974). The purpose of this study was to develop a high-performance liquid chromatographic (HPLC) method for furfural detection in citrus juices that is more sensitive, is less time consuming, and utilizes less hazardous materials.

MATERIALS AND METHODS

Sample Preparation. Orange juice samples, 200 mL, were distilled according to the method of Dinsmore and Nagy (1974). The first 10 mL of distillate was collected (5% of juice volume), mixed, and used for analysis.

Reagents and Chemicals. "Baker Analyzed" HPLC reagent-grade solvents (J. T. Baker Chemical Co., Phillipsburg, NJ) were used for all chromatography. Reagent-grade furfural was doubly distilled before use.

Chromatographic Conditions. Methanol–water (35:65 v/v) and a Du Pont (Wilmington, DE) Zorbax ODS column 4.6 mm i.d. × 25 cm at 1.0 mL/min separated furfural in aqueous juice distillates in less than 10 min. Chromatographic hardware consisted of a Waters Associates (Milford, MA) m-6000A pump, U-6K injector, Model 440 fixed-wavelength detector with the 280 nm aperature kit, and a Spectra-Physcis SP4270 recording integrator. Concentration factors were obtained from furfural standards and used to calculate concentration. Run time was 8.5 min.

RESULTS AND DISCUSSION

Chromatographic Separation. With a 35:65 (v/v) methanol–water mobile phase and the HPLC equipment used in this study, furfural elutes between 6 and 7 min at a flow rate of 1.0 mL/min (Figure 1). As seen in Figure 1, there are no or very few compounds in the distillate that elute near the solvent front. Therefore, the highest reasonable concentration of methanol was chosen to provide a complete separation of furfural from other compounds that elute near the solvent front and to provide a minimum analysis time. Acetonitrile may be used in place of methanol but is not recommended because of its greater toxicity and cost.

We have chosen to monitor column effluent using a fixed-wavelength detector with a 280-nm phosphorus filter because maximum absorbance of furfural (277 nm) is very close to 280 nm. This allowed a greater signal/noise (S/N) ratio by using this type detector compared to variable-wavelength detectors.

Furfural has been determined in a variety of food products by using TLC (Anet, 1962), GLC (Shimizu and Watanabe, 1979), and most recently HPLC (Hong et al., 1981; Jeuring and Kuppers, 1980; Alfonso et al., 1980). Most HPLC methods employ a C_{18} column and a water–methanol solvent system of varying composition. Hong et al. (1981) separated furfural produced by bacterial cultures using a 70:30 mixture of methanol–water and detected furfural at 254 nm. Alfonso et al. (1980) separated furfural from caramel solutions using a water–methanol gradient starting at 10% methanol and finishing at 60% methanol. Detection was at 277 nm. Jeuring and Kuppers (1980) separated furfural and hydroxymethyl furfural in spirits and honey using a 10:90 methanol–water mixture with detection at 285 nm. Honey, caramel, spirits, and bacterial cultures are relatively clean samples compared to citrus juices. Direct injection as used by Alfonso et al. (1980) and Jeuring and Kuppers (1980) was impractical for orange juice because of the many interfering compounds that coelute with furfural. These interfering compounds are numerous enough to make sample cleanup prior to injection necessary.

Even though furfural recoveries are not complete, one of the easiest sample cleanup methods is by distillation. As shown in Figure 1, distillation greatly simplifies the separation problem.

Distillation. Recovery of furfural by distillation from aqueous standards and spiked orange juice samples ranging from 100 to 10 000 ppb was measured. Average recovery of furfural ($N = 30$) from spiked orange juice samples was 37.7 ± 0.7% with a relative standard deviation of 1.9%. The recovery of furfural can be greatly improved by collecting a larger amount of the distillate. However, if more distillate is collected, the furfural concentration effect during distillation is lost. It should be noted that the furfural distillation procedure will affect the amount of furfural in the distillate. If a distillation procedure other than the one described is used, the furfural recovery should be established with standard solutions.

Recovery of 37.7 ± 0.7% furfural corresponds to the results from aqueous standards reported by Dinsmore and Nagy (1972, 1974) but is better than the recovery they reported for citrus juice samples. Dinsmore and Nagy (1974) demonstrated furfural recovery losses and increased variability when citrus juices were distilled due to interferences with the colorimetric reaction. Addition of stannous chloride to the colorimetric procedure decreased the interference from codistilled compounds and improved the stability of furfural from citrus juice samples. Average recovery of furfural from standards was 38%. Recovery of furfural from citrus juice was calculated to be 34% (Dinsmore and Nagy, 1974). The degree of variability was not reported, but Dinsmore and Nagy (1974) did report an increased distillation variability when furfural was distilled from citrus juices. Neither furfural recovery nor method precision were affected by codistilled compounds.

Comparison of Results. Since sample preparation was identical for HPLC and colorimetric procedures, a direct comparison of furfural values from the sample distillate was possible. To test the precision of the colorimetric and

Table I. Comparison of Colorimetric and HPLC Methods for Furfural Measurement in Aqueous Solutions[a]

standard solutions, ppb of furfural	colorimetric method, ppb	% error	HPLC method, ppb	% error
140	148	+5.7%	139	-0.7
234	224	-4.2	238	+1.7
374	365	-2.4	369	-1.3
515	507	-1.6	507	-1.6

[a] Values for both methods are the means of five analyses.

Table II. Comparison of Colorimetric and HPLC Methods for Measuring Furfural in Orange Juice[a]

orange juice sample	colorimetric method, ppb	HPLC method, ppb	% relative difference
1	1490[b]	1432	3.9
2	1392[b]	1361	2.2
3	715[b]	712	0.8
4	717[b]	718	0.1
5	690[b]	655	5.1
6	586[b]	623	6.3
7	386[b]	425	10.1
8	290	311	7.2
9	193	174	9.8
10	70	47	32.8

[a] Values are the mean of duplicate samples. [b] Denotes samples that required dilution prior to colorimetric analysis.

the HPLC procedure, multiple (N = 5) analyses were performed on the same distillate. The colorimetric method had a relative standard deviation of 5.5%, whereas the HPLC method had a relative standard deviation of 1.6%. This indicates the HPLC method is more precise than the colorimetric method in quantifying furfural from juice samples regardless of the sample preparation method.

A comparison was made between results obtained with HPLC and colorimetric procedures to quantify furfural (Table I). Aqueous furfural solutions were carefully made and used for both detection methods. The percent error for the colorimetric procedure ranged from -1.6 to +5.7%. The HPLC method had a range of +1.7 to -1.6%.

Calibration standards of 0.5, 1.0, and 2.0 ppm of furfural in the distilled sample are used in the colorimetric procedure. Assuming the suggested average furfural recovery of 34%, the lowest working detection limit of furfural in citrus juice samples would be about 75 ppb and an upper detection limit of 300 ppb. Juice samples that have more than 300 ppb would require dilution prior to analysis. Sample dilution is both time consuming and another possible source of error. The HPLC method was tested from 0 to 20 000 ppb of furfural and found to be linear through this range (r^2 = 0.9997). Because of the concentrating effect of the distillation, the lowest detection limit for HPLC furfural analysis is approximately 2 ppb in juice samples. The analysis of aqueous and orange juice samples spiked with furfural showed the HPLC method had the same percent error for juice samples as aqueous samples.

The relative difference between furfural content in 10 orange juice samples as determined by colorimetric and HPLC methods showed good correlation in most cases (Table II). Orange juice samples were not spiked with a standard furfural solution but rather were allowed to develop furfural by ambient temperature storage of aseptically packaged orange juice concentrate. There was good linear correlation between the two analytical procedures (r^2 = 0.998, Y intercept = -14.8, slope = 1.033). The largest difference was at the lowest furfural concentration. This is expected since the colorimetric test, unlike the HPLC procedure, is limited to approximately 75 ppb as its lowest working limit. Juice samples to be analyzed by the colorimetric method above 300 ppb of furfural required dilution. In many cases, several dilutions were needed to establish the correct furfural level before the colorimetric analysis could be performed. The relative difference between the colorimetric and HPLC methods increased if juice samples were not diluted before colorimetric analysis. This HPLC method has the potential of becoming a routine quality assurance test for packaged citrus products because it is more precise, is less time consuming, and uses safer reagents than the presently used method.

Registry No. Furfural, 98-01-1.

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Received for review February 15, 1984. Accepted June 25, 1984.