

## Separation and Identification of Furanic Compounds in Fruit Juices and Drinks by High-Performance Liquid Chromatography Photodiode Array Detection

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An HPLC method was developed for the simultaneous separation and identification of furanic compounds as the possible degradation products of sugars and ascorbic acid on heating. 5-(Hydroxymethyl)furfural (5-HMF), furfural, 2-furoic acid, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), 2-acetylfuran, and six unknown compounds were separated and detected in fruit juice concentrates with or without added vitamin C, wine, beer, and beverage samples. 5-HMF, furfural, and 2-furoic acid were detected in all samples analyzed. DMHF was detected in all samples except the cola. In contrast, 2-acetylfuran was detected in the cola sample only. In the fruit juice concentrates with added vitamin C, the contents of furfural, 2-furoic acid, and DMHF were higher than in all other samples.

**Keywords:** *Furanic compounds; fruit juices and drinks; identification; HPLC*

### INTRODUCTION

Heat treatment of foods containing sugars and ascorbic acid may result in nonenzymatic browning or Maillard reaction, which causes a change in the flavor, color, and nutritional value of the foods. 5-(Hydroxymethyl)furfural (5-HMF) and furfural are the principal degradation products of the hydrolysis of hexoses and pentoses, respectively (Espinosa-Mansilla et al., 1992). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) is one of the putative degradation products of sugars (Naim et al., 1993) in the presence of amines or amino acids (Blank and Fay, 1996). DMHF can also be generated directly from hexoses (Blank and Fay, 1996). Like furfural, DMHF is an important flavor compound that can be found in various fruits (Krammer et al., 1994; Sanz et al., 1994). Although formed by a side reaction, DMHF might significantly contribute to the overall flavor due to its low threshold values (Blank and Fay, 1996). Eiserich et al. (1992) identified volatile compounds from a Maillard model system that possessed antioxidative properties. Their results showed that DMHF displayed the strongest antioxidative activity. According to Lee and Nagy (1988a), 2-acetylfuran and furfuryl alcohol were also the degradation products of sugars. In the Maillard model system consisting of cysteine and glucose, 2-acetylfuran is one of the major thermal degradation products of glucose (Yeo and Shibamoto, 1991).

Ascorbic acid is readily decomposed to form furfural (Solomon et al., 1995) and other products, which are the same as the intermediates in the Maillard browning of pentoses (Davies and Wedzicha, 1994). The results of Rodriguez et al. (1991) showed that the accumulation of furfural was concurrent with the degradation of ascorbic acid observed during storage of an alcoholic

orange juice beverage. Sawamura et al. (1994) and Kimoto et al. (1993) have proved the occurrence of 2-furoic acid as one of the degradation products of an aqueous solution of dehydroascorbic acid, the oxidation form of ascorbic acid.

The rapid qualitative and quantitative analyses of these furanic compounds, one class of heterocyclic compounds that have been reported in a wide variety of food systems (Yeo and Shibamoto, 1991), have been a central problem in food and clinical chemistry and in biotechnology. The reversed-phase HPLC method has been used to determine the contents of 5-HMF and furfural in apple juices and concentrates (Blanco-Gomis et al., 1991), commercial brandies and caramels (Villalón-Mir et al., 1992), and coffee (Chambel et al., 1997). Albala-Hurtado et al. (1997) described a reversed-phase HPLC method, using an isocratic mobile phase and variable-wavelength absorbance detection means, to measure free and total furfural compounds in infant milk formulas. Lo-Coco et al. (1994) used the HPLC method, based on the formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds and subsequent reversed-phase separation of these derivatives, to determine the contents of furfural and 5-HMF in processed citrus juices. Bonn (1984) studied the elution behavior of oligosaccharides, monosaccharides, and sugar degradation products, furfural and 5-HMF, on series-connected ion-exchange resin columns. In addition, an anion-exclusion column was used to separate and determine the content of 5-HMF in honey and fruit juices (Kim and Richardson, 1992). Walsh et al. (1997) used the reversed-phase HPLC method to determine the content of DMHF in orange juice. However, no studies concerning the simultaneous determination of 5-HMF, furfural, DMHF, 2-furoic acid, and other furanic compounds have been reported. The aim of the present study is to develop an HPLC method for the simultaneous separation and analysis of these furanic compounds in fruit juice concentrates and drinks.

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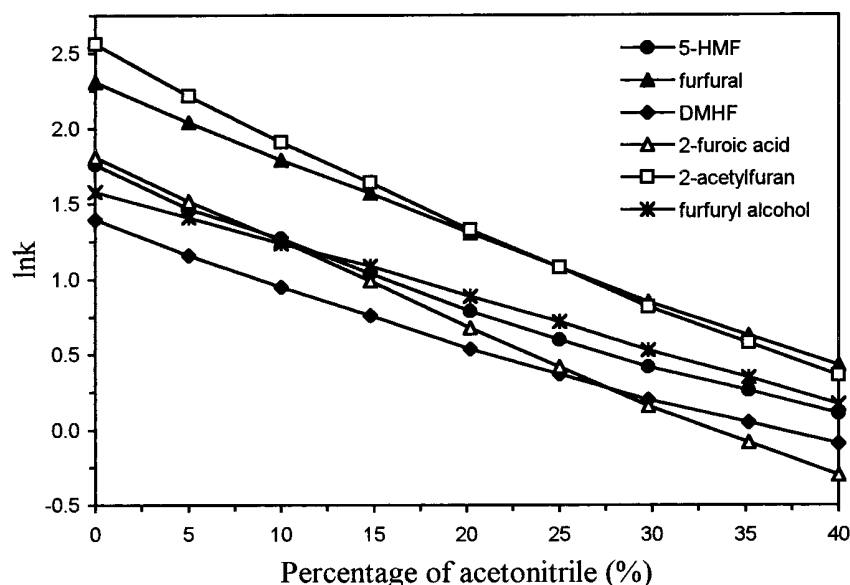


Figure 1. Effect of acetonitrile on the retention behaviors of furanic compounds.

#### EXPERIMENTAL PROCEDURES

**Chemicals and Reagents.** Acetonitrile (HPLC grade) was purchased from BDH Laboratory Supplies (Poole, England). 5-HMF, furfural, DMHF, 2-furoic acid, 2-acetylfuran, and furfuryl alcohol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Fruit juice concentrates and drinks were purchased from local stores.

**HPLC Method.** HPLC was conducted on a Waters liquid chromatograph equipped with two 510 pumps. The samples filtered through 0.45- $\mu$ m GHP Acrodisc GF filters were separated at room temperature by using a Bio-Rad Aminex HPX-87H hydrogen form cation-exchange resin-based column (300  $\times$  7.8 mm), packed with sulfonated divinylbenzene-styrene copolymer with a particle size of 9  $\mu$ m. The mobile phase consisted of acetonitrile and 0.005 M sulfuric acid aqueous solution. The flow rate was set at 0.6 mL/min. The samples were injected with a Rheodyne 7725i valve with a 20- $\mu$ L loop. A 996 photodiode array detector (PDA) (Waters) was used for the simultaneous detection of furanic compounds. The tridimensional chromatogram was recorded from 190 to 400 nm. Peaks were measured at wavelengths of 254 and 280 nm, respectively, to facilitate the detection of these compounds. All computations were performed using a Waters Millennium 2010 data system.

#### RESULTS

**Optimization of the Mobile Phase.** An Aminex HPX-87H column was chosen for the separation of 5-HMF, furfural, DMHF, 2-furoic acid, 2-acetylfuran, and furfuryl alcohol. As furanic compounds retained strongly on ion-exchange resin column, acetonitrile was chosen as an organic modifier that could lessen the retention times of furanic compounds (Yuan et al., 1995, 1996). The solvent mixture containing 0.005 M sulfuric acid aqueous solution and acetonitrile was tested for its ability to elute and separate these compounds.

The relative proportion of acetonitrile and sulfuric acid aqueous solution in the mobile phase was varied to effect separation. The effect of acetonitrile on the retention behavior of furanic compounds was investigated by adding acetonitrile to the mobile phase at concentrations up to 40% (v/v). The result is shown in Figure 1. While increasing the content of acetonitrile in mobile phase, the retention times of furanic compounds decreased. As the effects of acetonitrile on the

Table 1. Recoveries of Standard Addition in Fruit Juice

compd	content (mg/L $\pm$ SD)	amt added (mg/L)	found (mg/L $\pm$ SD)	recovery (%)
5-HMF	21.9 $\pm$ 0.3	8.0	30.1 $\pm$ 0.4	102.5
		15.0	36.4 $\pm$ 0.5	96.7
furfural	8.8 $\pm$ 0.2	8.0	17.2 $\pm$ 0.4	105.0
		15.0	23.2 $\pm$ 0.5	96.0
DMHF	2.0 $\pm$ 0.1	8.0	9.7 $\pm$ 0.5	96.3
		15.0	17.2 $\pm$ 0.9	101.3
2-furoic acid	10.7 $\pm$ 0.3	8.0	18.5 $\pm$ 0.5	97.5
		15.0	25.9 $\pm$ 0.7	101.3
2-acetylfuran	nd <sup>a</sup>	8.0	7.6 $\pm$ 0.2	95.0
		15.0	15.1 $\pm$ 0.4	100.7
furfuryl alcohol	nd	8.0	7.5 $\pm$ 0.2	93.8
		15.0	13.7 $\pm$ 0.4	91.3

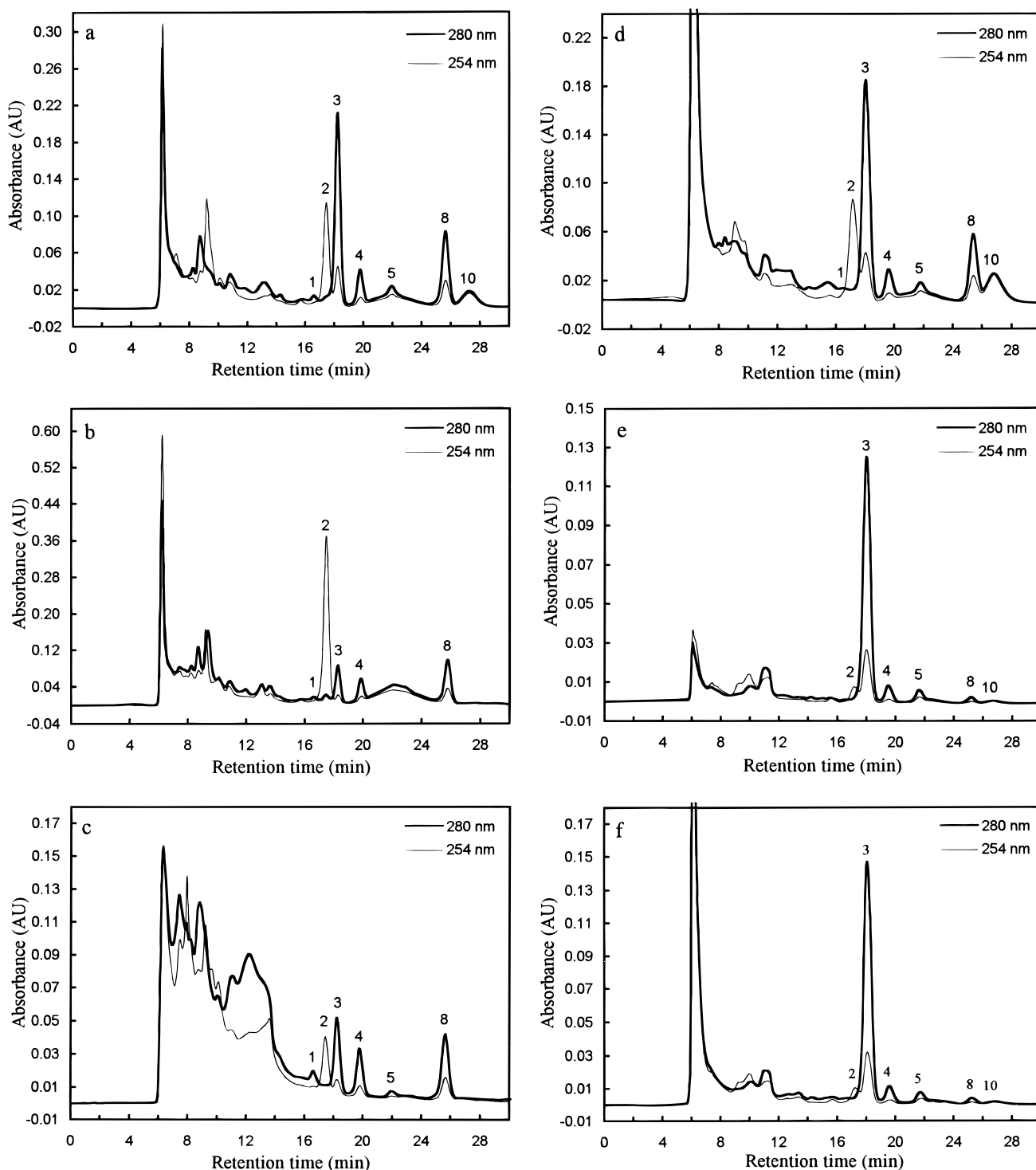
<sup>a</sup> nd, not detected.

retention of these furanic compounds on the separation column were different, the elution order and selectivity of these compounds would change with the addition of acetonitrile. The result indicates that the separation of these furanic compounds on this ion-exchange column was carried out according to the mechanism of reversed-phase partitioning.

While the acetonitrile concentration was between 15 and 23%, 5-HMF, DMHF, 2-furoic acid, and furfuryl alcohol were well separated. The lower concentration of acetonitrile was beneficial to the separation of furfural and 2-acetylfuran. Therefore, the mobile phase containing 19% (v/v) acetonitrile and 81% (v/v) 0.005 M sulfuric acid aqueous solution was used for the quick separation of furanic compounds in the fruit juices and drinks. For the determination of 2-acetylfuran, a lower concentration of acetonitrile (16%) in the mobile phase was chosen.

**Linearity and Recovery.** The calibration curves of the peak/area ratio against the concentration were obtained by using standard solutions from 0.1 to 100 mg/L. 5-HMF, furfural, DMHF, 2-furoic acid, 2-acetylfuran, and furfuryl alcohol presented linearity in this range, and the correlation coefficients ( $r^2$ ) were between 0.9992 and 0.9998.

Recovery was determined by using the standard addition method to demonstrate the accuracy of analysis. Known amounts of standards were added to the



**Figure 2.** HPLC chromatograms of (a) apple juice concentrate, (b) pear juice concentrate, (c) orange juice concentrate, (d) apple grape juice concentrate, (e) white grape juice concentrate, (f) red grape juice concentrate, (g) white wine, (h) red wine, (i) beer, and (j) cola samples.

samples. Two spiking levels were used, and five determinations were carried out. The contents of six furanic compounds in the samples and the recoveries of these compounds are shown in Table 1.

**Application.** As an application, the furanic compounds in some commercial drinks and fruit juice concentrates with or without added vitamin C were separated and analyzed. The sample was directly injected without any sample pretreatment, except filtration. In the analysis of the fruit juice concentrate and

drink samples, 5-HMF, furfural, DMHF, 2-furoic acid, and 2-acetylfuran were detected and identified by comparing the retention times and the absorbance spectra obtained from the samples (photodiode array) with those of authentic standards. The chromatograms of separation for these samples at the wavelengths of 254 and 280 nm by PDA are shown in Figure 2. In all samples analyzed, 11 compounds were detected. No furfuryl alcohol was detected (at 215 nm) in these samples. The retention times and absorption maxima

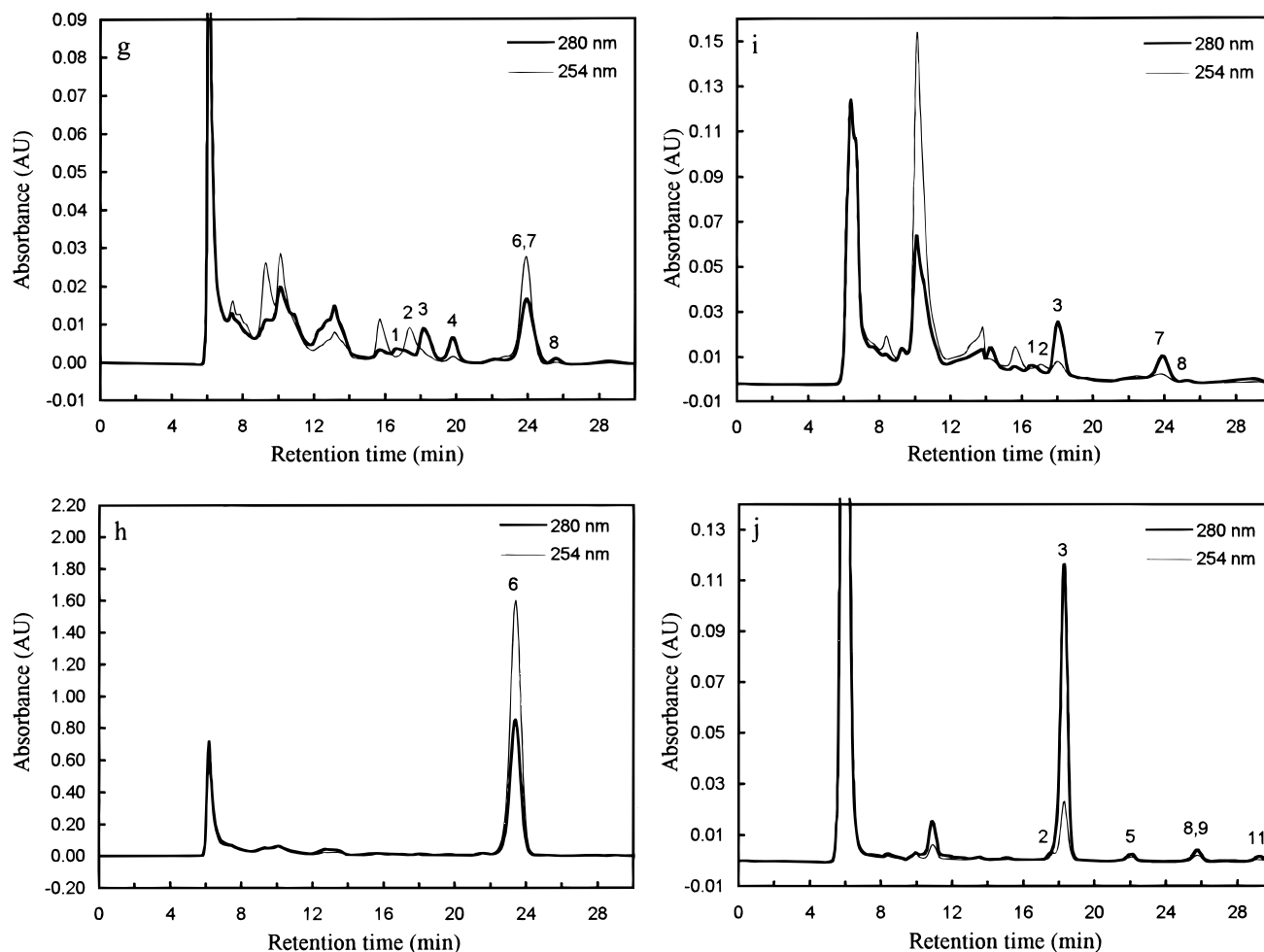


Figure 2 (continued)

**Table 2. Retention Times and Absorption Maxima of Furanic Compounds in Fruit Juice Concentrates and Drinks**

peak no.	retention time (min)	absorption max (nm)	compd identified	detection limit (mg/L)
1	16.6	287.7	DMHF	0.03 (at 280 nm)
2	17.4	(219.4), 252.3	2-furoic acid	0.005 (at 254 nm)
3	18.2	(228.8), 284.1	5-HMF	0.003 (at 280 nm)
	19.0	215.9	furfuryl alcohol	3 (at 215 nm)
4	19.8	(230.0), 296.0		
5	22.0	(227.7), 274.7		
6	23.4	261.7		
7	23.9	220.6, 274.7		
8	25.6	(227.6), 277.0	furfural	0.004 (at 280 nm)
9	25.8	(225.3), 273.5	2-acetylfuran	0.004 (at 280 nm)
10	26.8	215.9, 267.6		
11	28.9	(227.7), 291.2		

of these compounds and the identification of the five furanic compounds are summarized in Table 2. The contents of the five furanic compounds and the results of detection for the other six compounds are shown in Table 3.

## DISCUSSION

The reversed-phase HPLC system has been employed for the determination of 5-HMF and furfural, but extensive sample treatment is required to remove some compounds that eluted with the furanic compounds by the reversed-phase HPLC method and interfered with the determination for some complex food samples, for

example, tomato juice (Kim and Richardson, 1992) and citrus juice (Lee et al., 1986; Lo-Coco et al., 1994). The chemical complexity of the orange juice makes it difficult to completely resolve all of the juice components from DMHF (Walsh et al., 1997). The results of Kim and Richardson (1992) indicated that the quantitation of 5-HMF in tomato juice by the reversed-phase HPLC was subject to an uncertainty because of the interfering peaks, and accurate determination of 5-HMF without extensive sample treatment (for example, precipitation) was possible by the ion-exclusion chromatography (IEC) method. Furanic compounds retained strongly on the ion-exchange resin column (Bonn, 1985) and were eluted late from the ion-exclusion column (Kim and Richardson, 1992). Therefore, under the present chromatographic separation conditions, the furanic compounds could be well separated with some compounds possibly interfering with the determination, especially for orange juice (Figure 2c).

The selectivity and elution order of these furanic compounds would change with the variations of the percentage of acetonitrile in the mobile phase as shown in Figure 1. While the retention times increased when the percentage of acetonitrile was decreased, the separation of furanic compounds could not be improved significantly. A gradient elution procedure could not make the separation better. Therefore, an isocratic elution system was used.

As shown in Table 3, 5-HMF, furfural, and 2-furoic

**Table 3. Analysis Results of Furanic Compounds in Fruit Juice Concentrates and Drinks (Milligrams per Liter)**

sample	1, DMHF	2, 2-furoic acid	3, HMF	4 <sup>a</sup>	5 <sup>a</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8, furfural	9, 2-acetyl furan <sup>a</sup>	10 <sup>a</sup>	11 <sup>a</sup>
apple juice <sup>b</sup>	5.0	10.7	19.0	+	+	—	—	9.4	—	+	—
pear juice <sup>b</sup>	4.1	33.4	6.3	+	+	—	—	7.0	—	—	—
orange juice <sup>b</sup>	4.0	3.4	4.2	+	+	—	—	3.7	—	—	—
apple grape juice <sup>b</sup>	2.0	10.7	21.9	+	+	—	—	8.8	—	+	—
white grape juice	0.8	0.9	14.2	+	+	—	—	0.3	—	+	—
red grape juice	0.8	1.1	16.9	+	+	—	—	0.4	—	+	—
white wine	1.3	0.9	1.0	+	—	+	+	0.1	—	—	—
red wine	1.0	1.8	1.3	+	—	+	+	0.3	—	—	—
beer	2.6	0.4	3.0	—	—	—	+	0.1	—	—	—
cola	—	0.3	9.5	—	+	—	—	0.2	0.2	—	+

<sup>a</sup> +, detected; —, not detected. <sup>b</sup> With added vitamin C.

acid were detected in all samples analyzed, indicating the occurrence of these three furanic compounds as the degradation products of sugars and ascorbic acid in fruit juice concentrates and drinks on heating. The results of Lee and Nagy (1988b) suggested that ascorbic acid played a major role in the browning of canned grapefruit juice during storage. In four commercial fruit juice concentrate samples with added vitamin C (apple juice, pear juice, orange juice, and apple grape juice), the contents of furfural (3.7–9.4 mg/L) and 2-furoic acid (3.4–33.4 mg/L) were significantly higher than those of other samples (0.1–0.4 and 0.3–1.8 mg/L, respectively). Our results showed that the apple juice and grape juice samples contained higher 5-HMF (14.2–21.9 mg/L) than the other fruit juices. In the study of Fuleki et al. (1994), 5-HMF was not detected in the authentic apple juices; it was produced in the juice and its concentrate as a result of thermal stress during heat processing, concentration, and storage.

The formation of DMHF from hexoses such as glucose and fructose is more difficult than that from 6-deoxyhexoses due to the necessity of reduction which can occur in the presence of ascorbic acid (Haleva-Toledo et al., 1997). In heat-processed foods containing hexoses, DMHF is often detected (Buttery and Ling, 1997). The result of determination showed that DMHF was detected in all samples analyzed but cola. In four commercial fruit juice concentrate samples with added vitamin C, the contents of DMHF were higher (2.0–5.0 mg/L) than in the other samples except beer (2.6 mg/L). DMHF is generally unstable in air or in aqueous solution; the addition of ascorbic acid might prevent DMHF from further degradation (Haleva-Toledo et al., 1997). Guedes de Pinho and Bertrand (1995) studied the influence of the vinification method on DMHF levels in wine. In all cases, the vinification with skin contact led to a decrease in DMHF concentrations. The use of pectolytic enzymes with  $\beta$ -glucosidic secondary activities could increase the DMHF levels (Guedes de Pinho and Bertrand, 1995).

Of all the samples analyzed, 2-acetyl furan was detected only in the cola drink sample. Under the present separation conditions, 2-acetyl furan could not be separated with furfural. As furfural and 2-acetyl furan had similar spectra, it is difficult to identify these two compounds according to their spectra by PDA while the two compounds were not separated. For the separation and determination of samples containing simultaneously furfural and 2-acetyl furan, decreasing the concentration of acetonitrile in the mobile phase might improve the separation of 2-acetyl furan and furfural

(Figure 1). When the concentration of acetonitrile was <16%, 2-acetyl furan and furfural could be well separated.

Furfuryl alcohol was considered to be a degradation product of sugars (Lee and Nagy, 1988a), but in the present study, no furfuryl alcohol was detected in any of the samples analyzed. The absorption of furfuryl alcohol at its maximal absorption wavelength of 215.9 nm was much weaker than the absorption of other furanic compounds. Therefore, the low concentration (<3 mg/L) of furfuryl alcohol could not be detected.

In addition to 5-HMF, furfural, 2-furoic acid, DMHF, and 2-acetyl furan, six unknown compounds were detected in all samples analyzed. Compound 4 (peak 4), with a maximum absorption wavelength of 296.0 nm, was detected in all samples analyzed but cola and beer. The content of compound 4 was higher in fruit juice samples, especially in the fruit juice samples with added vitamin C. Perhaps this compound was also one of the degradation products of ascorbic acid. Compound 5 (peak 5), which gave a spectrum similar to that obtained from furfural or 2-acetyl furan, was detected in all samples analyzed but alcoholic drinks. Compound 6 (peak 6), having a maximum absorption wavelength of 261.7 nm, was detected only in wine, particularly red wine. Compound 7, which was detected in beer, occurred also in wine. Although coeluted with compound 6, compound 7 could still be identified from compound 6 in red and white wines by PDA. Compound 10 (peak 10), having maximum absorption wavelengths of 215.9 and 267.6 nm, was detected in apple and grape juice samples. Compound 11 (peak 11), with a maximum absorption wavelength of 291.2 nm, was detected in the cola sample only. Research is continuing in this department to further identify and characterize these unknown compounds.

## CONCLUSIONS

The developed method for the simultaneous separation and determination of furanic compounds shows a satisfactory result for fruit juice concentrate and drink samples. This method is rapid and simple since extensive sample pretreatment is not necessary. This analytical method could be useful in the analysis and identification of furanic compounds in commercial fruit juice concentrates and drinks during processing and storage and would also contribute to browning mechanism studies.

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