Journal of Chromatography, 624 (1992) 503–509
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 506

Separation and determination of 5-hydroxymethyl-2-furaldehyde and 2-furaldehyde in fruit juices by micellar electrokinetic capillary chromatography with direct sample injection

Danilo Corradini* and Claudio Corradini

Istituto di Cromatografia del CNR, Area della Ricerca di Roma, P.O. Box 10, I-00016 Monterotondo Stazione (Rome) (Italy)

ABSTRACT

The separation of 5-hydroxymethyl-2-furaldehyde (5-HMF) and 2-furaldehyde (2-FA), which are recognized indices of deteriorative changes in some commercially processed foods, was investigated by micellar electrokinetic capillary chromatography (MECC), employing sodium dodecyl sulphate as the anionic surfactant. The effects of micellar concentration, temperature and the addition of methanol or acetonitrile on the migration times and selectivity were investigated. MECC was successfully applied to the determination of 5-HMF and 2-FA in grapefruit juice by an internal standard method without any sample pretreatment.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) is a rapidly developing, highly efficient separation technique based on micellar solubilization and electrokinetic migration in open-tubular capillaries, which is related to capillary electrophoresis [1–4]. A wide variety of applications of MECC in many areas of analytical chemistry have been reported, including the determination of drugs [5–7], antibiotics in plasma [8,9] and chiral compounds of pharmaceutical interest [10–12].

We are interested in the development of small-scale analytical methods for substances that are involved in the deterioration reaction occurring during food processing and storage. Two main chemicals that are generated in the non-enzymic browning process of fruit juices, 2-furaldehyde (2-FA) and 5-hydroxymethyl-2-furaldehyde (5-HMF), have been proposed as general indices of the deterioration of food quality during heating processes, *i.e.*, concentration, pasteurization or storage [13–16], although it was shown that they do not directly contribute to the perceptible off-flavour. Traditional approaches for the control and determination of 5-HMF and 2-FA include spectrophotometric and chromatographic procedures.

Spectrophotometric methods have been used for the determination of both 5-HMF and 2-FA in fruit juices [13,14], honey [15] and caramel [16]. These methods have the disadvantage of the instability of the coloured complex formed, the time required and the use of hazardous chemicals.

Chromatographic techniques used include thin-layer chromatography [17], gas chromatography [18] and, more recently, high-performance liquid chromatography (HPLC) [19–23] using UV detection at 280–285 nm. However, the presence of interfering peaks complicates the HPLC separation of 5-HMF and 2-FA and sample pretreatment, such as distillation [19], extraction [22] or clarification [20], is needed before HPLC.

This paper presents the results of a study of the electrophoretic conditions for the separation and determination of 5-HMF and 2-FA by MECC. The effects of micellar concentration, temperature and the addition to the running buffer of an organic modifier on migration times and selectivity were

0021-9673/92/$05.00

studied. The application of MECC to the identification and determination of 2-FA and 5-HMF in commercially processed grapefruit juice is also described.

EXPERIMENTAL

Equipment and procedure of MECC

Separations were carried out on a P/ACE 2100 HPCE instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a fused-silica capillary cartridge (75 μm I.D. $\times$ 375 μm O.D.) with a total length of the capillary of 37 cm (30 cm to the detector). Prior to use the capillary was pretreated successively with 0.1 *M* HCl and 0.1 *M* NaOH for 30 min each, then rinsed with 0.2 *M* phosphate buffer (pH 7.5) and the running buffer. To maintain good peak shapes and reproducible migration times, the capillary tube was rinsed with 0.2 *M* phosphate buffer (pH 7.5) for 1 min and then with the running buffer for 2 min each time before a sample solution was injected. The capillary tube temperature was maintained at the experimental value to within $\pm$0.1°C by means of a fluorocarbon liquid continuously circulated through the cartridge.

A deuterium light source with a 280-μm bandpass filter was used and absorbance was monitored at a range of either 0.006 or 0.010 a.u.f.s. Injection was made by nitrogen pressure for 2.5 s. All experiments were carried out applying a constant voltage of 10 kV with the anode at the inlet and the cathode at the outlet side. Data analysis and collection were accomplished using the Beckman Gold System software, version 6.01.

Materials

Sodium dodecyl sulphate (SDS) of electrophoresis purity reagent grade was purchased from Bio-Rad Labs. (Richmond, CA, USA); analytical-reagent grade phosphoric acid, hydrochloric acid and sodium hydroxide and HPLC-grade water, methanol and acetonitrile were all obtained from Carlo Erba (Milan, Italy).

5-Hydroxymethyl-2-furaldehyde, 2-furaldehyde and 2-furyl methyl ketone (2-acetylfuran) were purchased from Aldrich (Milwaukee, WI, USA). Micellar solutions were prepared by dissolving SDS in phosphate buffer, which was prepared by titrating a solution of 50 m*M* phosphoric acid with 0.1 *M* sodium hydroxide to pH 7.5. The solutions were filtered through a Type HA 0.22-μm membrane filter (Millipore, Bedford, MA, USA) and degassed by sonication before use.

All fruit juices, honey and spirits were purchased from a local store.

Procedure for quantitative analysis

A 2.0 mg/ml stock solution of 5-HMF and 2-FA in methanol–water (10:90, v/v) and 10 mg/ml of 2-furyl methyl ketone (2-FMK) in water as the internal standard solution were prepared daily. The stock solution was diluted to produce working standard solutions at five different concentrations within the range 0.1–50.0 μg/ml. An appropriate volume of internal standard solution was added to each solution to give a concentration of 10.0 μg/ml of 2-FMK. Calibration graphs were plotted based on the linear regression analysis of the peak-area ratios.

Grapefruit juice (exactly 25 ml) was diluted to 50.0 ml with water after the addition of the internal standard solution to give a concentration of 10 μg/ml of 2-FMK.

RESULTS AND DISCUSSION

Optimization of separation

We first investigated the effect of SDS concentration on the migration behaviour of the two furanic compounds. Both 5-HMF and 2-FA are non-ionic solutes and consequently in the capillary zone electrophoresis mode (*i.e.*, when the SDS concentration in the running buffer is zero) they migrated with almost the same velocity as methanol, which was the tracer of the electroosmotic flow.

On addition of SDS to the running buffer, the migration times of 5-HMF and 2-FA became longer than that of methanol, allowing the separation of the two samples. Above the critical micellar concentration (CMC), addition of SDS increases the micelle concentration with the monomer concentration remaining constant [24]. The plots in Fig. 1 show that the migration times of the solutes increased with increase in SDS concentration, although the electroosmotic flow was not changed significantly over the whole SDS concentration range. This means that the migration time of the two neutral solutes is proportional to the phase ratio of the micellar to the

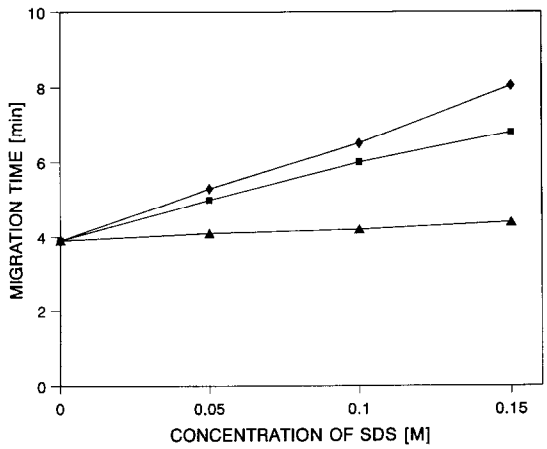

Fig. 1. Effect of SDS concentration on migration time. Buffer, 0.05 *M* phosphate (pH 7.5); capillary, fused silica, 370 mm × 0.075 mm I.D.; length of the capillary used for the separation, 300 mm; applied voltage, 10 kV; temperature, 25°C; detection wavelength, 280 nm. ■ = 5-HMF; ◆ = 2-FA; ▲ = methanol.

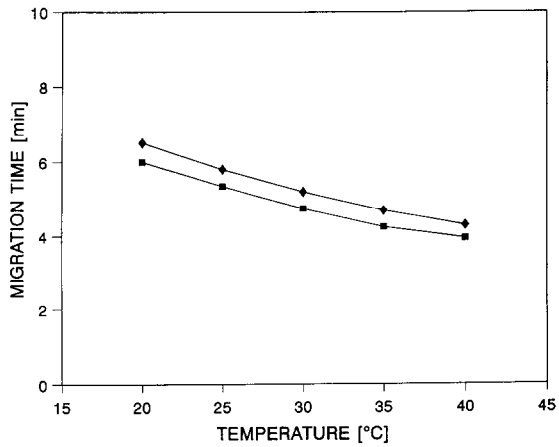

Fig. 2. Dependence of migration times on temperature. Running buffer, 0.050 *M* phosphate (pH 7.5) containing 0.10 *M* SDS. Other conditions and symbols as in Fig. 1.

aqueous phase, which is proportional to the micelle concentration.

The migration time of 2-FA was delayed more than that of 5-HMF with increasing SDS concentration, because 2-FA was more easily incorporated into SDS micelles owing to its higher hydrophobicity. At an SDS concentration of 0.10 *M* the two examined samples were well resolved within 7 min.

Increasing the capillary temperature from 20 to 40°C gave a large reduction in the migration times (see Fig. 2), whereas the resolution slightly decreased above 30°C. A decrease in the buffer viscosity, an increase in the CMC of SDS and changes in the distribution coefficient of the solutes between the micellar and aqueous phases were probably responsable for the observed results [2,25].

In addition to the parameters mentioned above, the effect of an organic modifier on the migration behaviour of 5-HMF and 2-FA was investigated by adding methanol or acetonitrile to the running buffer at concentrations up to 60%. These organic solvents were added to the running buffer on a volume/volume basis in a volumetric flask with appropriate amounts of SDS and phosphate buffer from stock solutions so that dilution to the mark gave the same concentration of SDS and phosphate ions in each solution.

As illustrated in Fig. 3, the migration times of 5-HMF and 2-FA increased with increase in either the methanol or acetonitrile concentration. However, the increase was much lower for the acetonitrile system. This effect is attributed to the increase in both the electroosmotic flow and the electrophoretic velocity of the micelles [26]. The electroosmotic flow is affected by variations in the viscosity and dielectric constants of the running buffer and by the change in the net charge of the capillary wall, which appear to be reduced with the addition of an organic modifier [27].

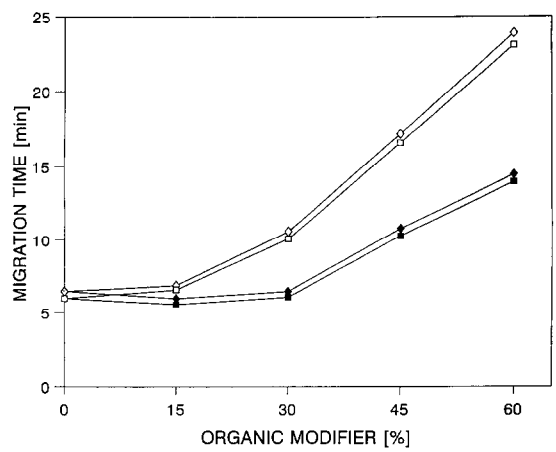

Fig. 3. Migration time as a function of the percentage of organic modifier. Open symbols, methanol; closed symbols, acetonitrile. □, ■ = 5-HMF; ◇, ◆ = 2-FA. Other conditions as in Fig. 2.

For 5-HMF and 2-FA, the migration order and selectivity were not influenced by the addition of methanol or acetonitrile, probably because these electrically neutral solutes have nearly the same structure. However, the peak broadening effect that occurred as the migration time of the two solutes increased with increase in the organic modifier concentration was larger in the acetonitrile than in the methanol system.

Qualitative and quantitative analysis

On the basis of the results reported above, a 0.050 *M* phosphate buffer solution of pH 7.5 containing 0.10 *M* SDS was selected for qualitative and quantitative analysis of 5-HMF and 2-FA in fruit juices. The temperature of the capillary cartridge was maintained at 20°C.

In order to examine the reproducibility of the migration times, the mean value, the standard deviation (S.D.) and the relative standard deviation (R.S.D.) of the migration time were calculated from the electropherograms obtained by five repeated injections of a sample solution containing equimolar amounts of 5-HMF and 2-FA and the internal standard. The results are reported in Table I and show that the R.S.D.s were better than 0.95% for the three compounds.

Quantification was performed by an internal standard method. A number of possible internal standards were explored for the simultaneous determination of 5-HMF and 2-FA. 2-FMK was selected as the internal standard as it was well resolved from both 5-HMF and 2-FA and met the following criteria which were evaluated in assessing a suitable

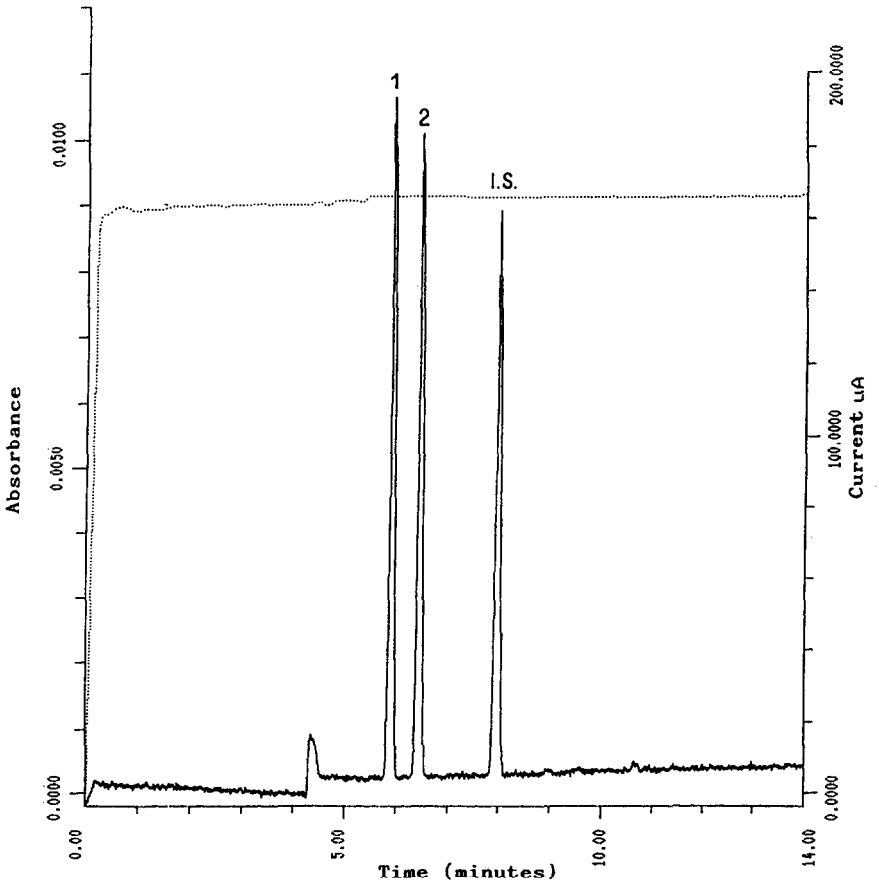

Fig. 4. Separation of (1) 5-HMF, (2) 2-FA and (I.S., internal standard) 2-FMK by MECC with 0.10 *M* SDS in 0.050 *M* phosphate buffer (pH 7.5). Capillary, fused silica (370 × 0.075 mm I.D.); length of capillary used for the separation, 300 mm; applied voltage, 10 kV; temperature, 20°C; detection wavelength, 280 nm.

TABLE I

REPRODUCIBILITY OF MIGRATION TIMES

Five repeated injections. Conditions as in Fig. 4.

Sample	Migration time (min)			R.S.D. (%)
	Individual values	Mean	S.D.	
5-HMF	5.95, 5.98, 6.04, 6.02, 5.93	5.98	0.046	0.77
2-FA	6.46, 6.50, 6.58, 6.55, 6.43	6.50	0.062	0.95
2-FMK	7.88, 7.91, 8.04, 8.02, 7.94	7.96	0.069	0.87

TABLE II

REPRODUCIBILITY OF PEAK-AREA AND PEAK-HEIGHT RATIO WITH RESPECT TO THE INTERNAL STANDARD

Five repeated injections. Conditions as in Fig. 4.

Sample	R.S.D. (%)	
	Peak-area ratio	Peak-height ratio
5-HMF	1.25	3.53
2-FA	0.58	2.40

candidate: absorption in the region of 280 nm, solubility in aqueous solution and chemical similarity to 5-HMF and 2-FA.

A typical electropherogram of these compounds together with the internal standard is shown in Fig. 4. The reproducibility of the determination of 5-HMF and 2-FA was investigated by repeated injection ($n = 5$) of an equimolar sample solution containing the internal standard. As shown in Table II, the peak-area ratio mode gave a higher reproducibility than the peak-height ratio mode. The calibration graphs for 5-HMF and 2-FA obtained by the

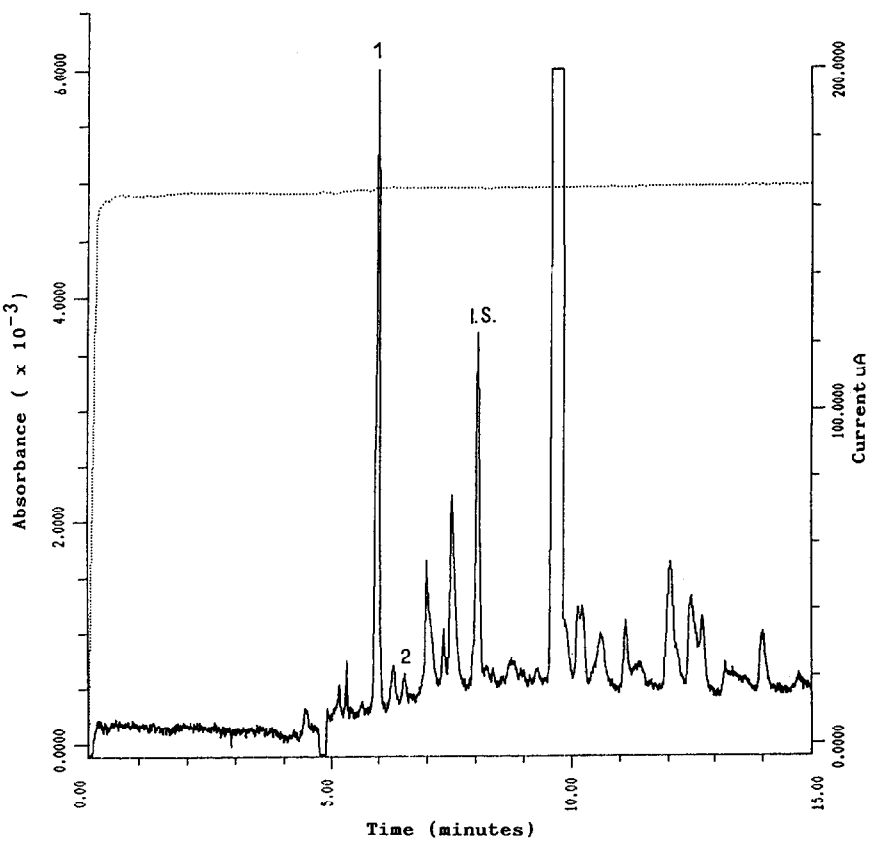

Fig. 5. Electropherogram of an industrial processed grapefruit juice. Conditions as in Fig. 4. (1) 5-HMF; (2) 2-FA; (I.S.) 2-FMK.

TABLE III

REPRODUCIBILITY OF THE DETERMINATION OF 5-HMF AND 2-FA IN AN INDUSTRIAL PROCESSED GRAPEFRUIT JUICE

Conditions as in Fig. 4.

Analyte	Amount found (μg/ml)			R.S.D. (%)
	Individual values	Mean	S.D.	
5-HMF	15.406, 15.127, 14.960, 15.542, 14.881	15.123	0.212	1.400
2-FA	0.602, 0.628, 0.596, 0.622, 0.615	0.612	0.013	2.189

peak-area ratio method showed excellent linearity over the concentration range 0.1–50 μg/ml with correlation coefficients $r = 0.99982$ and 0.99985, respectively, and nearly passed through the origin.

Determination of 5-HMF and 2-FA in fruit juices

Both 5-HMF and 2-FA are recognized indicators of the quality deterioration of fruit juices during the heating process, *i.e.*, concentration, pasteurization or storage [28]. As an application, we determined the amount of 5-HMF and 2-FA in a commercial grapefruit juice. The grapefruit juice was directly injected onto the capillary tube without any sample pretreatment, except that it was filtered through a 0.22-μm membrane filter after addition of the internal standard solution. The assay results are summarized in Table III and a typical electropherogram is shown in Fig. 5.

CONCLUSIONS

This study has shown that MECC, employing SDS as the anionic surfactant, is an effective method for the qualitative and quantitative analysis of 5-HMF and 2-FA in grapefruit juice from the viewpoint of successful separation, high accuracy, high reproducibility and short analysis time. It is also notable that no sample pretreatment is necessary.

Preliminary attempts to apply this technique to other processed foods have indicated that it can be employed for the determination of 5-HMF and 2-FA in honey, spirits and other fruit juices and concentrates.

ACKNOWLEDGEMENT

This work was supported by the CNR (Consiglio Nazionale delle Ricerche), Rome, Progetto Strategico "Characterization, Control and Analysis of Food".

REFERENCES

1 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
2 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
3 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
4 J. H. Knox and I. H. Grant, *Chromatographia*, 24 (1987) 135.
5 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 498 (1990) 313.
6 M. T. Ackermans, F. M. Everaerts and J. L. Beckers, *J. Chromatogr.*, 585 (1991) 123.
7 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 2773.
8 T. Nakagawa, Y. Oda, A. Shibukawa and H. Tanaka, *Chem. Pharm. Bull.*, 36 (1988) 1622.
9 T. Nakagawa, Y. Oda, A. Shibukawa and H. Tanaka, *Chem. Pharm. Bull.*, 37 (1989) 707.
10 S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403.
11 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 234.
12 A. S. Cohen, A. Paulus and B. L. Karger, *Chromatographia*, 24 (1987) 15.
13 S. Meydav and Z. Berk, *J. Agric. Food Chem.*, 26 (1978) 282.
14 H. L. Dinsmore and S. Nagy, *J. Food Sci.*, 37 (1972) 768.
15 J. W. White, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 509.
16 F. C. Alfonso, G. E. Martin and R. H. Dyer, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 1037.
17 R. E. Berry and J. H. Tatum, *J. Agric. Food Chem.*, 13 (1965) 588.
18 J. Shimizu and M. Watanabe, *Agric. Biol. Chem.*, 43 (1979) 1365.
19 J. E. Marey and R. L. Rouseff, *J. Agric. Food Chem.*, 32 (1984) 979.

20 H. S. Lee, R. L. Rouseff and S. Nagy, *J. Food Sci.*, 51 (1986) 1075.
21 Z. F. Li, M. Sawamura and H. Kusunose, *Agric. Biol. Chem.*, 52 (1988) 2231.
22 D. Blanco Gomis, M. D. Gutierrez Alvarez, L. Sopena Naredo and J. J. Mangas Alonso, *Chromatographia*, 32 (1991) 45.
23 H. J. Kim and M. Richardson, *J. Chromatogr.*, 593 (1992) 153.
24 K. L. Mittal (Editor), *Micellization, Solubilization and Microemulsion*, Vol. 1, Plenum Press, New York, 1977.
25 A. S. Cohen, S. Terabe, J. A. Smith and B. L. Karger, *Anal. Chem.*, 59 (1987) 1021.
26 J. Gorse, A. T. Balchunas, D. F. Swaile and M. T. Sepaniak, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1988) 554.
27 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.
28 R. L. Handwcrk and R. L. Colcman, *J. Agric. Food Chem.*, 36 (1988) 231.