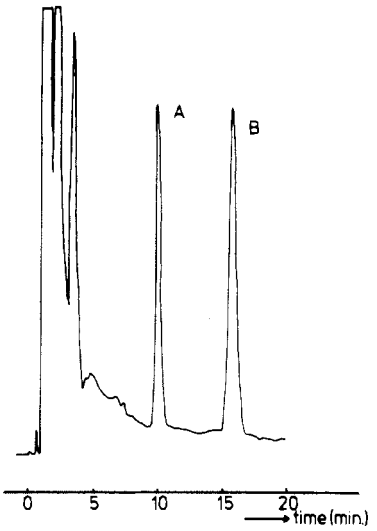

Figure 1. Gas chromatogram of a Brazilian soybean meal, after treatment with cyanogen bromide and titanium trichloride. A = MeSCN. B = EtSCN (=standard).

of Table IV. Some of the values obtained by ion-exchange chromatography are higher than those obtained by the cyanogen bromide/titanium trichloride method, and the reason for this may be the limitations that are inherent with the cyanogen bromide reaction. Some values obtained by ion-exchange chromatography, however, may be rather high, as a result of interfering substances. The procedure developed is very suitable for routine analysis, and up to 60 analyses may be performed in 3 working days. A constraining factor is the time of 20 min for one gas chromatographic analysis. The data presented in Table IV show a substantial amount of methionine sulfoxide in proteins that have been treated with peroxide, e.g., the oxidized casein or the Promine D. Little or not sulfoxide was found in other industrially processed proteins, like soybean meal, meat meal tankage, gelatin, or one of the soy isolates.

Registry No. Methionine, 63-68-3; methionine sulfoxide, 454-41-1; cyanogen bromide, 506-68-3; titanium trichloride, 7705-07-9.

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High-Performance Liquid Chromatographic Determination of Sodium Benzoate When Used as a Tracer To Detect Pulpwash Adulteration of Orange Juice

James F. Fisher

Sodium benzoate was added as a tracer to orange pulpwash. Orange juice diluted with the marked pulpwash was examined by high-performance liquid chromatography for evidence of sodium benzoate. The sample preparation was accomplished with a Sep-PAK C-18 cartridge. Resolution of the sodium benzoate was carried out with a radial compression separation system and an 8-mm C-18 cartridge. Detection was at 230 nm.

Orange pulpwash, a byproduct of the citrus industry, is suspected of being added as a diluent (adulterant) to orange juice. So that this practice can be identified, sodium benzoate, a commonly used food additive and not native to citrus, has been added as a tracer to orange pulpwash.

A practical high-performance liquid chromatographic (HPLC) procedure has been developed to quantitatively identify this marker in both pulpwash and adulterated orange juice. The samples undergo a short preliminary cleanup procedure with final separation of the sodium benzoate by HPLC.

The FDA has approved the use of sodium benzoate at a concentration not to exceed 100 ppm (0.01%) as a tracer in citrus pulpwash when reconstituted to 11.8 °Brix. The Brix value represents the total soluble solids in citrus products [*Natl. Bur. Stand. (U.S.), Circ.*, 1946]. The Florida Citrus Commission has set the lower limit at 50 ppm.

Methods in the literature for the determination of sodium benzoate (Association of Official Analytical Chemists, 1980; Mandrou and Bressolle, 1980; Bennett and Petrus, 1977; Archer, 1980) all entail certain unsatisfactory con-

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ditions such as extensive sample preparation, lengthy analysis times, and ion-exchange chromatography and, in general, are not convenient for routine analysis.

The object of this work was to design a simple, fast assay for sodium benzoate in citrus pulpwash and juice. The following procedure was developed and used in this laboratory. In the method described below Florida frozen concentrated orange juice (FCOJ) samples adulterated with pulpwash, which contained sodium benzoate, were detected when examined by HPLC.

MATERIALS AND METHODS

Apparatus. A Model ALC 202 high-performance liquid chromatograph (HPLC) with a Model 6000A pump and U6K injector (Waters Associates, Milford, MA) was used. The recorder was a two-pen Soltec Model B-281 (Soltec Corp., Encino, CA). A Laboratory Data Control UV–visible spectrophotometer, Model 1202 Spectro Monitor II (Laboratory Data Control, Riviera Beach, FL), an ultrasonic cleaner (Cole-Parmer, Model 8845-6), an International Clinical Centrifuge (Model CL), and a C-18 Sep-PAK cartridge (Waters Associates, Milford, MA) with a 5.0-mL Luer Lok syringe were used. A digital mini pH meter, Model 88 (Markson Scientific, Inc., Del Mar, CA), and pH strip, colorpHast (American Scientific Products), were used.

Standard Sample Employed as Criteria of Identity. The authentic sodium benzoate (Sigma, St. Louis, MO) was examined by HPLC and gave one peak, thus establishing its chemical purity and retention time in this chromatographic system.

Cartridge and Eluting System. A Waters Associates radial compression separation system, Model RCM-100 (RCSS), with a Radial-PAK 10-μm particle C-18 cartridge (8 mm × 10 cm) equipped with a C-18 guard column was the analytical system. A Sep-PAK C-18 cartridge was used for the sample cleanup. The eluting system employed with the RCM-100 was (A) acetonitrile–water (25:75 v/v) adjusted to pH 2.5–3.0 with 85% phosphoric acid. The acetonitrile was Baker analyzed reagent for HPLC. The water was distilled and deionized. The solvent system was degassed with an ultrasonic cleaner.

Sep-PAK C-18 Cartridge Preparation. The long end of the cartridge was attached to the Luer tip of a 5.0-mL syringe barrel and washed with 5.0 mL of acetonitrile, followed by 5.0 mL of 1% phosphoric acid. The flow rate was 9–11 mL/min. This rate was also applied to the elution sequence.

Sample Preparation. *Concentrated Pulpwash.* The sample of pulpwash concentrate to be analyzed for sodium benzoate was reconstituted with water to 11.8 °Brix. The amount of water used depended upon the pulpwash Brix value. The diluted pulpwash was well mixed and then centrifuged for 5 min at a relative centrifugal force (RCF) (*g*) of 3170 (top speed). This removed most of the material that would plug the column. One milliliter of the supernatant liquid was diluted to 5 mL with water. This 1:5 diluted supernatant liquid was adjusted to a pH of 1–2 with 1 drop of 85% phosphoric acid. This assured that the benzoate present was in the benzoic acid form. The sample was then ready to be put through a Sep-PAK.

Orange Juice. A sample of adulterated single-strength orange juice to be analyzed for sodium benzoate was centrifuged as above and 5.0 mL of the supernatant liquid adjusted to a pH of 1–2 with 1 drop of 85% phosphoric acid. This sample was then ready for the Sep-PAK.

Sample Cleanup on Sep-PAK. One milliliter of either the pulpwash or adulterated orange juice from above was pipetted into the syringe and passed through a prepared Sep-PAK cartridge by means of the syringe. The benzoic acid concentrates near the head of the cartridge. Five milliliters of 1% phosphoric acid was pumped through the Sep-PAK by means of a syringe and the effluent was discarded. It did not contain benzoate. Four milliliters of a sodium hydroxide solution, 0.025 g/100 mL of water (pH 11.3), was placed in the barrel of the 5.0-mL syringe and 1.0 mL pumped through the Sep-PAK and discarded. Then 2.0 mL of the sodium hydroxide solution was pumped through the Sep-PAK. This 2.0 mL of eluate was adjusted to a pH of 1–2 with 1 drop of 85% phosphoric acid and used for the HPLC analysis. An examination of the Sep-PAK after the 2.0 mL of sodium hydroxide elution showed that the cartridge did not retain sodium benzoate.

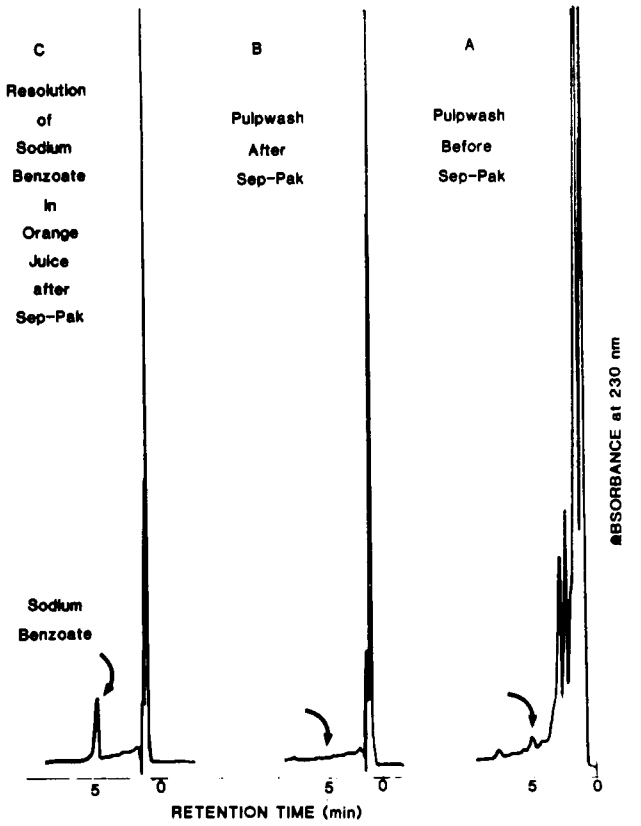

Figure 1. Pulpwash without sodium benzoate before and after cleanup on the Sep-PAK and the resolution of sodium benzoate in adulterated orange juice. For experimental details, see the text.

High-Performance Liquid Chromatographic (HPLC) Resolution of Sodium Benzoate. A 50-μL portion of the above sample was examined by HPLC using the RCSS with a radial-PAK C-18 cartridge, 8 mm × 10 cm, and solvent system A. Detection was accomplished at an attenuation of 0.08 aufs at 230 nm and a time constant of 5 s. The recorder chart speed was 30 cm/h. The temperature was between 22 and 24 °C. The flow rate was 3.0 mL/min. The injection of acetonitrile and phosphoric acid, as a blank, into the chromatographic system did not produce any of the chromatographic peaks.

Figure 1 shows pulpwash without sodium benzoate before and after cleanup on the Sep-PAK (chromatograms A and B, respectively). The resolution of sodium benzoate in adulterated orange juice after the Sep-PAK is shown in chromatogram C. As seen in chromatogram B (after the Sep-Pak) the area in the region of 5 min is devoid of peaks that are present in chromatogram A. The peak at a retention time of 5 min seen in chromatogram C is due to sodium benzoate at a concentration of 3.0 ppm.

Preparation of Sodium Benzoate Standard Curve. By use of standard sodium benzoate solutions from 1.0 to

10.0 ppm in 1.0-ppm increments and a constant injection volume of 50 μL, a linear detector response was obtained from 0.05–0.5 μg (r = 0.997).

Addition of Sodium Benzoate to Pulpwash. Sodium benzoate (18.6 mg) in 1.0 mL of water was added slowly with stirring to 38.0 mL (48.5 g) of 60 °Brix pulpwash that had been cooled to 10 °C. This mixture was then diluted with 199.0 mL of water which resulted in a 75-ppm concentration of sodium benzoate in 11.8 °Brix pulpwash and was maintained at 10 °C for 16 h before use.

Percent Recovery, Precision, and Distribution. The reliability of the procedure was determined by fortifying 10 identical samples of pulpwash with sufficient sodium benzoate to provide concentrations of from 10 to 100 ppm in 10-ppm increments. The addition of the appropriate amount of sodium benzoate to the pulpwash and the analysis were performed as described above.

The precision of the method was established with five replicate sodium benzoate determinations from a pulpwash sample containing 75 ppm of sodium benzoate prepared as above.

Distribution studies were carried out on 60 °Brix pulpwash containing sufficient sodium benzoate so that when reconstituted to 11.8 °Brix the sodium benzoate concentration would be 75 ppm. This labeled pulpwash was placed in a narrow cylinder with a large-bore stopcock, and samples withdrawn from five different levels. A similar experiment was conducted under industrial conditions by removing samples of 60 °Brix pulpwash labeled with sodium benzoate as above from a 3000-gal tank. The samples were taken at seven levels: bottom, 500 gal, 1000 gal, 1500 gal, 2000 gal, 2500 gal, and surface. The analysis for sodium benzoate was as described above.

RESULTS AND DISCUSSION

A quantitative HPLC procedure was developed for sodium benzoate in Florida orange juice which works equally well for orange pulpwash, Brazilian and Mexican orange juice, or tangerine juice.

The sample preparation is to simplify the analysis by removing interferences from a complex sample matrix such as citrus juice and also, in the case of the pulpwash sample, to reduce the amount of sodium benzoate to a manageable concentration. A single-strength orange juice sample does not require dilution since the sodium benzoate concentration in the pulpwash has been reduced by the orange juice. However, if the suspect sample is an orange juice concentrate, then it must be reconstituted to 11.8 °Brix but further dilution is unnecessary. The finding of sodium benzoate at any concentration in orange juice is evidence of pulpwash adulteration.

The 1:5 dilution of pulpwash samples prior to the Sep-PAK cleanup and the subsequent recovery of the sodium benzoate present in 1.0 mL of this diluted sample which has been further diluted to 2.0 mL by elution from the Sep-PAK result in a 1:10 dilution. Therefore, values in ppm obtained for these samples from the Sep-PAK by using the standard curve must be expressed as a multiple of 10 to represent the sodium benzoate in the pulpwash.

When using a Sep-PAK C-18 cartridge with aqueous samples, it is necessary to prewet the cartridge with a water-miscible solvent such as acetonitrile used in this procedure. The aqueous 1% phosphoric acid acidifies the cartridge to help retain the benzoate as benzoic acid.

The sample cleanup on the Sep-PAK depends upon pH adjustment of the sample to effect selective adsorption on and elution from the Sep-PAK. The sample is placed on the Sep-PAK as benzoic acid (pH 1–2). The benzoic acid is adsorbed near the head of the C-18 Sep-PAK which retains nonpolar material. The 1% phosphoric acid removed acid-soluble interferences from the cartridge. The elutant is changed to sodium hydroxide (pH 11.3) which converts the benzoic acid to sodium benzoate. This polar material is readily removed from the C-18 Sep-PAK. The Sep-PAK retains neutral substances which could hinder the analysis of the benzoate.

The eluting system of pH 2.5–3.0, used with the C-18 RCSS, is a compromise between the pK of 4.2 for benzoic acid and a reasonable pH above 2.0 for the safe operation of the cartridge.

Under conditions of maximum sensitivity 0.05 μg (1.0 ppm) of sodium benzoate will give a signal to noise ratio of 18:1. If pulpwash contains the minimum amount of sodium benzoate (50 ppm) and the adulterated orange juice contains only 10% of this pulpwash, then we have 5.0 ppm of sodium benzoate in the diluted orange juice. The 1:10 dilution that takes place during the sample cleanup results in a 0.5-ppm sodium benzoate concentration in the test sample. This amount of sodium benzoate can be reliably detected by this procedure. Thus, 10% adulteration of orange juice with sodium benzoate labeled pulpwash can be detected. Under the conditions of this assay, the capacity factor, k', was 6.6 for the sodium benzoate and the number of theoretical plates achieved was 2100. The range of sodium benzoate found in the five repeatability experiments was 70–78 ppm with a mean of 74 and a standard deviation of ±2.7.

The 10 pulpwash samples that were fortified with 10–100 ppm of sodium benzoate in 10-ppm increments gave recoveries of 10, 20, 32, 42, 52, 64, 69, 83, 88, and 98 ppm of sodium benzoate, respectively. These recoveries and particularly those at the lower end indicate that sodium benzoate is not irreversibly adsorbed or lost in the matrix of the pectin present in the pulpwash. This conclusion is further supported by the repeatability experiment.

Sodium benzoate was found to be uniformly distributed throughout the pulpwash in both laboratory- and industrial-scale operations. In the industrial-scale experiment, the sodium benzoate distribution was in the range of 68–82 ppm (less than ±10% variation from the added sodium benzoate) with a mean of 73 ppm and a standard deviation of ±5.6. The distribution of sodium benzoate on the laboratory scale was slightly better.

At the pH of the pulpwash (about 4) the sodium benzoate is in part converted to benzoic acid; therefore, an equilibrium between sodium benzoate and benzoic acid is established in the pulpwash. The benzoate that precipitates does so as a very fine crystal. Good agitation evenly disperses this precipitate throughout the pulpwash. The presence of benzoate in the pulpwash is not obviously visible.

The procedure is simple and fast (30 min/sample) and even faster on a multiple sample basis in routine analyses.

Registry No. Sodium benzoate, 532-32-1.

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