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Rapid Sample Preparation Method for HPLC Analysis of Capsaicinoids in Capsicum Fruits and Oleoresins

Vincent K. Attuquayefio and Ken A. Buckle*

A fast miniblender and commercially available Sep-pak filter cartridges have enabled sample preparation time of capsicum fruit for capsaicinoid analysis to be reduced from several hours to about 5 min. Sample purity is also greatly improved, with almost all the coloring matter and other lipid contaminants removed. The procedure gives a recovery of about 98%, and the results for capsaicinoids content are in agreement with more elaborate, time-consuming techniques. A typical extraction and HPLC analysis is about 25 min/sample.

For the objective analysis of the pungent principles (capsaicinoids) of capsicum fruits, sample preparation should provide complete extraction of the principles without interfering coextractives. In most cases this requires extensive and costly sample preparation and thus enhances the popularity of the less objective organoleptic methods for pungency assessment (Todd, 1958; Hartman, 1970; Todd et al., 1977).

As part of an extensive examination of the capsaicinoids in fresh and processed capsicums, a rapid method for capsaicinoid extraction, cleanup, and separation by high-performance liquid chromatography (HPLC) was sought. Capsicum samples extracted in chloroform or other solvents produce extracts containing a variety of interfering materials that affect resolution and sensitivity, necessitate column flushing after every run, and tend to reduce column life (Chiang, 1986). To assure faster analysis, longer column life, and more uniform resolution over several runs, a sample preparation technique was developed that reduced the level of interfering contaminants yet still enabled rapid and accurate analysis.

MATERIALS AND METHODS

Preliminary Experiments. Fresh samples of capsicum fruits (*Capsicum annuum* var. Yatsubusa) were obtained from capsicum plants grown in a glass house at The University of New South Wales, Kensington, Australia (UNSW). Samples were sun-dried and freeze-dried at the UNSW, and oleoresin capsicum was donated by Mauri Foods Australia Pty Ltd. Preliminary experiments indicated that direct high-speed blending of capsicum fruit tissue was effective in extracting the capsaicinoids. Subsequently, acetone, chloroform, methanol, methanol/0.1 N HCl (80:20, v/v), methanol/1% acetic acid (90:10, v/v), and acetonitrile were examined from the point of view of yield, ease, and effectiveness of extract cleanup. Parameters studied included capsaicinoid content and color of extract, the nature of the extracts with respect to the position of the coarse particulates, and the degree of chromatographic interference produced by the extract.

The HPLC procedure for capsaicinoids used a Waters Associates liquid chromatograph equipped with a 6000A pump, a U6K injector, an RCM module fitted with C_{18} Rad-Pak column (8-mm i.d., 5-μm pore size), Lambda-Max 480 detector set at 280 nm and 0.01 aufs, and mobile phase of methanol/water (63:37, v/v) at a flow rate of 3.5 mL/min.

Standard solutions of capsaicin (10 mg %, Merck) were prepared in the extracting solvent diluted with different volumes of water to give serial polarity gradings. The standards were introduced in 10-mL portions onto the Waters Associates Sep-pak filters previously conditioned in a solvent and the effluents assayed for the presence of capsaicinoids. The solvent containing no trace of capsaicinoids was of the desired polarity. A lower polarity solvent was chosen to remove selectively the capsaicinoids from the Sep-pak while avoiding the elution of relatively nonpolar constituents such as pigments and lipids. The Sep-pak containing capsaicinoids was washed with acetonitrile/water mixtures of lower polarity than the established wash solvent. The effectiveness of these solvents in eluting the capsaicinoids was determined by assaying a further 1.0 mL of acetonitrile wash from the Sep-pak for residual capsaicinoid.

Final Sample Preparation Method. *Extraction.* Dehydrated ground capsicum (1.0 g) or 10 g of fresh capsicum is blended with 10 mL of acetonitrile for 2 min. For oleoresins, 0.1 g of sample is dissolved in 2.0 mL of hexane and the capsaicinoids are transferred into 10 mL of acetonitrile by solvent partitioning. A 1.0-mL aliquot of the acetonitrile extract is taken for cleanup as with ground and fresh samples.

Cleanup. A C_{18} Sep-pak is conditioned with about 5 mL of acetonitrile followed by 5 mL of double-distilled water.

Department of Food Science and Technology, The University of New South Wales, Kensington, NSW 2033, Australia.

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Table I. Extraction of Capsaicinoids and Degree of Interference with Different Solvents

solvent	total capsaicinoid content, mg/100 g	color	particulates	interference on chromatogram[a]
acetone	313 ± 3	deep red	settled	++
chloroform	307 ± 1	deep red	floating	++
methanol	301 ± 3	light red	settled	++
methanol/HCl	314 ± 3	light red	suspended	++++
methanol/HOAc	307 ± 4	light red	suspended	++++
acetonitrile	310 ± 3	light red	settled	+

[a] Key: + = slight; ++++ = severe.

Table II. Capsaicinoid Retention and Elution on C_{18} Sep-pak Filters

acetonitrile/water, v/v	capsaicinoid in effluent from Sep-pak[a] first	final
1:9	–	+
3:7	+	+
5:5	+	+
7:3	+	+
9:1	+	+
10:1	+	–

[a] Key: + = capsaicinoid present; – = capsaicinoid not detectable.

The extract (1.0 mL) is diluted with 9 mL of water and injected into the conditioned Sep-pak. The capsaicinoids are then eluted with 4 mL of acetonitrile followed by 1.0 mL of acetonitrile containing 1% acetic acid.

RESULTS AND DISCUSSION

Extraction Technique. Table I shows the effectiveness of several solvents for yield of capsaicinoids, the extent to which coloring contaminants were extracted, the ease with which aliquots could be taken as related to the level and type of solids, and subsequent interference during HPLC analysis. Acetone is reported to give the highest yields (Sankarikutty et al., 1978); however, acetone also extracted significant levels of pigments as indicated by the deep red of the extract, and lipid materials. Chloroform, used by Jurenitsch et al. (1979), gave reasonable yields but also extracted red pigments, interfered in subsequent HPLC analysis, and provided difficulties when taking an aliquot as the particulates tended to float on top of the clear extract. Methanol extracted less capsaicinoids than either acetone or chloroform but also extracted less pigments and produced extracts in which particulates settled to the bottom of the clear extract. Methanol/0.1 N HCl (80:20, v/v) and methanol/1% acetic acid (90:10, v/v; Johnson et al., 1982) produced extracts that were difficult to handle during subsequent cleanup stages and also caused erratic shifts in the base line during HPLC analysis, although Johnson et al. (1982) reported high yields for acidified methanol.

Extraction of capsicum tissue in acetonitrile gave a clear, light-colored extract with particulates that settled well, a yield of capsaicinoids comparable to those of the other solvents (mean 98.2 ±0.3%), and an extract that was easily and effectively cleaned by Sep-pak filtration (Table II). Acetonitrile also enabled uniform extraction conditions for both capsicum fruit and capsicum oleoresin samples. With oleoresins, the capsaicinoids were partitioned completely into acetonitrile after dissolving in hexane (Johnson et al., 1982) and subsequently cleaned up by Sep-pak filtration in the same manner as for capsicum tissue extracts. Fukuba and Murota (1985) recently showed that Sep-pak silica and C_{18} cartridges could be used to cleanup extracts of spices (clove, star anise, cinnamon) for HPLC determination of tocopherols.

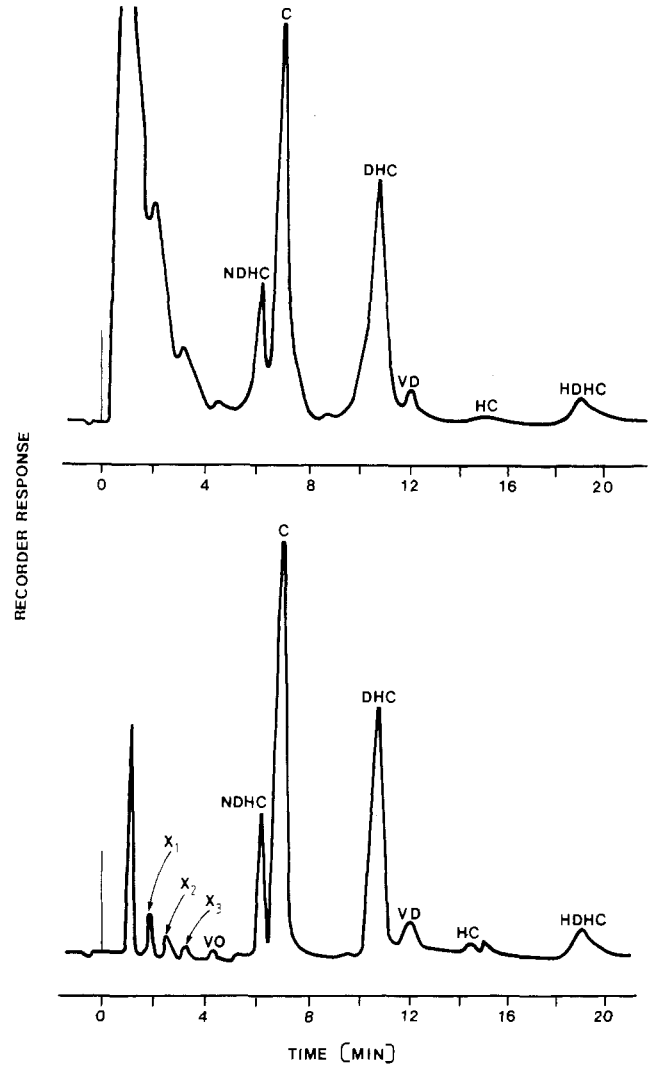

Figure 1. HPLC chromatogram of capsaicinoids in capsicum fruit: (top) before and (bottom) after Sep-pak cleanup. Key: VO, vanillyloctanamide; NDHC, nordihydrocapsaicin; C, capsaicin; DHC, dihydrocapsaicin; VD, vanillyldecanamide; HC, homocapsaicin; HDHC, homodihydrocapsaicin; X_1–X_3, unknown.

Sample Cleanup. When capsaicinoids dissolved in a 1:9 mixture of acetonitrile/water were injected into a Sep-pak cartridge, no trace of capsaicinoids could be detected in the effluent (Table II). Acetonitrile (4 mL) subsequently removed capsaicinoids from the Sep-pak, leaving no detectable residue. Unlike the other solvent mixtures tested, larger volumes did not improve significantly the recovery rate (mean 98.2 ± 0.6%). However, an additional 1.0 mL of acetonitrile containing 1% acetic acid overcame the slight turbidity that resulted with some fresh capsicum extracts.

HPLC Chromatogram of Cleaned Up Extracts. As shown in Figure 1, most interference was reduced and resolution of the capsaicinoid peaks was improved; there was almost complete elimination of interference associated with the void volume. The separated capsaicinoids include vanillyloctanamide (VO), nordihydrocapsaicin (NDHC), capsaicin (C), dihydrocapsaicin (DHC), vanillyldecanamide (VD), homocapsaicin (HC), and homodihydrocapsaicin (HDHC). The unknown peaks X_1–X_3 probably include norcapsaicin and vanillylheptanamide.

CONCLUSION

The method described for capsaicinoid extraction, cleanup, and HPLC separation is rapid, efficient, and reliable. The need for prolonged heat extraction is eliminated, and the time for sample cleanup is reduced from several hours to about 2 min. Total sample preparation takes about 5 min, with reduced requirements for sample and solvents and reduced chromatographic interference. The need for regular column flushing is eliminated as the load of contaminants is reduced significantly; because the Sep-paks are disposable, there is reduced chance of cross-contamination. Total time for extraction and quantitation of the capsaicinoids is about 25 min.

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Registry No. C, 404-86-4; VO, 58493-47-3; NDHC, 28789-35-7; DHC, 19408-84-5; VD, 31078-36-1; HC, 58493-48-4; HDHC, 20279-06-5; norcapsaicin, 61229-08-1; vanillylheptanamide, 89575-10-0.

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Rapid Extraction Method for Reproducible Analysis of Aroma Volatiles

M. F. Kok, F. M. Yong,* and G. Lim

A simple rapid method for extraction of volatile compounds from an aqueous sample using a J&W liquid–liquid extractor is described. *n*-Pentane was the solvent used to extract the volatiles, and the extract was subjected to high-resolution gas–liquid chromatographic analysis without further concentration. Linalool and 1-heptanol were used as model components. Average recoveries of 20 ± 2% and 5 ± 1% were obtained for linalool and 1-heptanol, respectively. Addition of 5% NaCl to the aqueous sample increased the recovery of both linalool and 1-heptanol to 30 ± 2% and 10 ± 2%, respectively.

The three basic procedures usually used in the analysis of aroma volatiles of commercial fruit juices or essences and those produced by microrganisms or plants are (i) sample preparation that includes isolation or extraction and concentration of volatiles; (ii) separation of volatiles, e.g., by gas–liquid chromatography (GLC); and (iii) identification and/or quantitation of volatiles.

The problem in qualitative and quantitative analysis of minute amounts of volatiles produced by microorganisms lies in the difficulties in isolating them before gas chromatography analysis (Nabeta and Sugisawa, 1983). The extraction procedures would require large quantities of sample and large volumes of solvents. Rapid monitoring of odorous volatile materials produced in a fermentation process is also not possible. Hence, sample preparation remains one of the critical areas in aroma volatiles analysis.

Alberola and Izquierdo (1978) reviewed the different methods for extracting volatile components from orange juice. The problems involved in sample preparation were also studied by Sugisawa (1981). Solvent extraction of volatiles followed by subsequent concentration by blowing nitrogen gas or air over the extract is commonly used (Kemp et al., 1972; Lanza et al., 1976; Yong et al., 1985). Distillation at atmospheric or reduced pressure to concentrate aroma extracts is also used (Collins and Halim, 1977; Sprecher and Hanssen, 1983). The Lickens–Nickerson method (Schultz et al., 1977; Au-Yeung and McLeod, 1981) is unsuitable for extraction of volatiles that are thermally unstable, and a cooked flavor has been observed after prolonged distillation (Gholap and Bandyopadhyay, 1984). Extraction of linalool and citronellal by the Likens–Nickerson method at atmospheric pressure causes these substances to be unstable even though the recovery and reproducibility of extraction are good (Alberola and Izquierdo, 1978). A microextraction method developed by Rhoades and Miller (1965) and adsorption on a porous polymer were used by Lund and Bryan (1977) and Lund and Dinsmore (1978).

Most of the above-mentioned methods could cause qualitative and quantitative changes to the sample during sample preparation. It is therefore often difficult to relate the chemical composition derived from gas chromatograms of the extracts to aroma quality.

In this paper we report a rapid extraction method for reproducible analysis of aroma volatiles using a J&W liquid–liquid extractor. Jennings (1981) first reported the availability of such a simple glass extractor for qualitative compositional analysis of aroma volatiles. Though Jennings (1981) regarded it as a semiquantitative extraction method that could be very useful in industrial quality control, no data were presented to show its reproducible semiquantitative nature. Since there is a need for a simple and rapid sample preparation method for use in monitoring aroma formation by microorganisms in a fermentation process where many samples of small volumes had to be taken from the culture broth for qualitative and quantitative evaluation, the suitability of using the J&W liquid–liquid extractor for such a purpose provided the

Department of Biochemistry, Faculty of Medicine (M.F.K., F.M.Y.), and Department of Botany, Faculty of Science (G.L.), National University of Singapore, Singapore 0511.

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