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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motivation for the present investigation. The model system presented here is the extraction of linalool and 1-heptanol dissolved in distilled water with *n*-pentane as an organic solvent. The extract is then subjected to gas–liquid chromatographic analysis without further treatment except for drying with anhydrous sodium sulfate. The method proved to be simple and convenient to use. Furthermore, it is a nondestructive method of extraction with good reproducibility for routine qualitative GLC analysis of aroma volatiles. As the results presented in this paper show, a component(s) in an aroma extract could be quantified by the use of a standard curve(s) obtained with an authentic sample(s) of the substance(s) to be quantified.

EXPERIMENTAL SECTION

Chemicals. *n*-Pentane, linalool, and 1-heptanol were of Fluka Guarantee grade. *n*-Pentane was redistilled before use. Anhydrous sodium sulfate and sodium chloride were of Merck AnalaR grade.

Extraction Method. A 5% NaCl solution (40 mL) with various concentrations of linalool or 1-heptanol was extracted with 200 μL of *n*-pentane in a J&W liquid–liquid extractor (obtainable from J&W Scientific, Rancho Cordova, CA). The extract was dried over anhydrous sodium sulfate in a 1-mL vial with a conical insert (available from Chrompak) for use with the Hewlett-Packard autosampler.

Analytical Method. The linalool and 1-heptanol extracted were analyzed with a HP Model 5880 gas–liquid chromatograph equipped with a flame ionization detector and an inlet splitter. The split ratio used was 20:1. The carrier gas nitrogen was set at 10 psi (μ 40 cm/s). Hydrogen and air flow rates were set at 20–25 and 400–450 mL/min, respectively. Of the *n*-pentane extract 1 μL was manually injected into a OV 101 fused silica capillary column of i.d. 0.2 mm and length 12.5 m. The temperature program used was as follows: initial oven temperature at 70 °C raised to 170 °C at 2 °C/min, isothermal for 10 min, raised to 190 °C at 10 °C/min, held isothermal for a further 10 min. Injector and detector temperatures were set at 170 and 250 °C, respectively.

RESULTS AND DISCUSSION

The calculations of recovery were made with reference to the standard curve of the amount of linalool or 1-heptanol added to 40 mL of aqueous standard solutions before extraction. A consistent recovery at various linalool and 1-heptanol concentrations (when these substances were present as single volatile components in an aqueous solution) is depicted by the linearity of the graphs as shown in Figure 1, parts a and b, respectively. The standard deviation (SD) obtained from six replicates was ±0.05 ppm. The minimum level of detectibility for linalool is approximately 0.01 ppm. Good reproducibility of the above-described extraction procedure was also obtained when an aqueous mixture of 1-heptanol and linalool at various concentrations was extracted (see Figure 2). This implied that 1-heptanol could be used as an internal standard in a system where the amount of linalool has to be determined.

Addition of NaCl into the aqueous medium is known to affect extraction recoveries (Sugisawa, 1981). In the present investigation, results showed that the recovery of linalool was improved by 15 ± 5% (SD) (Table I). The recovery of 1-heptanol in the presence of NaCl was double that obtained in the absence of NaCl. Although the recovery appears to be low, it is nevertheless higher than those reported by Sugisawa and Hirose (1981). In the simultaneous distillation adsorption (SDA) method as reported by Sugisawa and Hirose (1981), the recovery of linalool was less than 20% when *n*-pentane was used as

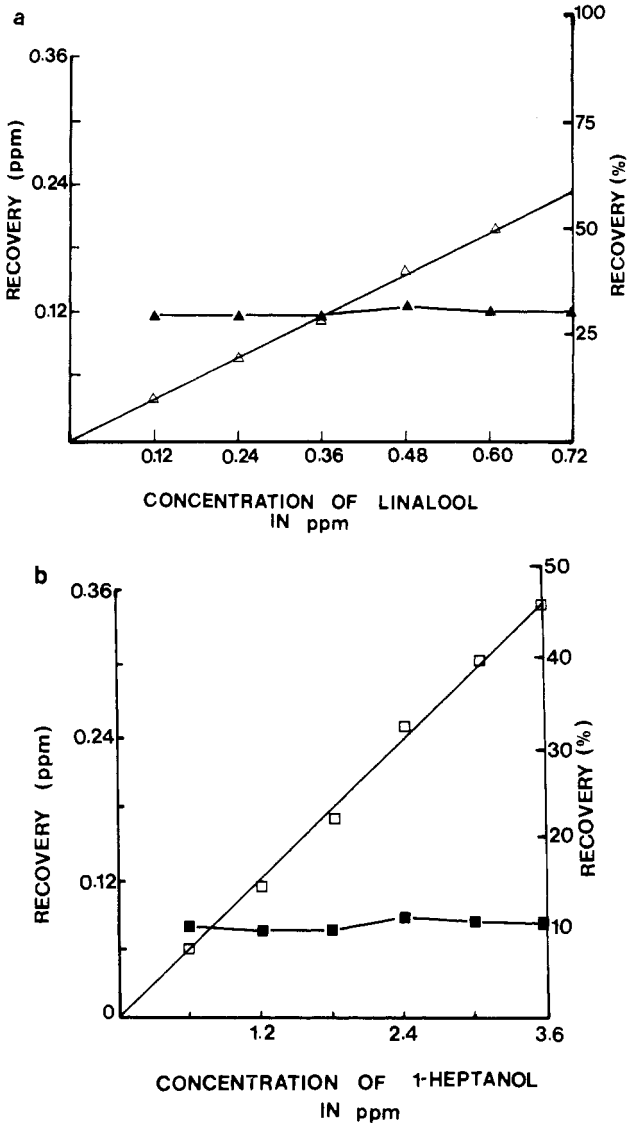

Figure 1. (a) Recovery of linalool in ppm (△) and in percent (▲) from 5% NaCl solution using pentane extraction in a J&W liquid–liquid extractor. (b) Recovery of 1-heptanol in ppm (□) and in percent (■) from 5% NaCl solution using pentane extraction in a J&W liquid–liquid extractor.

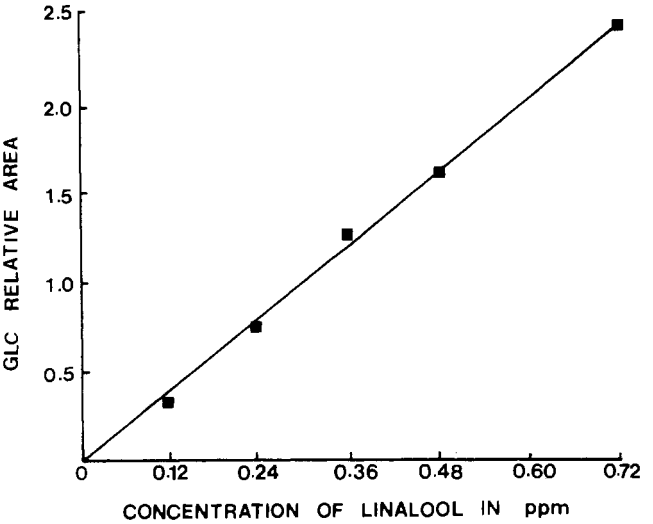

Figure 2. Relative area (linalool/1-heptanol) vs. concentration of linalool in ppm. (A concentration of 1.25 ppm of 1-heptanol as an internal standard was added to various concentrations of linalool.)

Table I. Effect of 5% NaCl on the Extraction of Linalool and Heptanol by Pentane

	concn in 40 mL aq medium, ppm	recovery ± SD,[a] % without NaCl	recovery ± SD,[a] % with NaCl
linalool	0.25	17 ± 1	31 ± 2
	0.50	18 ± 2	30 ± 2
	0.75	22 ± 3	32 ± 2
heptanol	1.00	6 ± 1	11 ± 2
	2.00	4 ± 1	10 ± 2
	2.50	4 ± 1	8 ± 1

[a] Standard deviation from six replicates.

the solvent. These workers also showed that the recovery could be improved if a more polar solvent such as diethyl ether was used to extract linalool instead of *n*-pentane; they obtained a recovery of about 70% when diethyl ether was used in their SDA method. Schultz et al. (1977) reported a recovery of about 60% for linalool with both solvents when they used the simultaneous distillation extraction (SDE) method. Bull et al. (1985) were able to extract volatiles from soya sauce with diethyl ether in a J&W liquid–liquid extractor. Soy sauce normally contains a high NaCl level (around 18%). With only 5% NaCl added to a salt-free aqueous sample, diethyl ether was found to be unsuitable because of its greater solubility in water. Hence, in our experiments *n*-pentane with its lower solubility and density was chosen as the solvent. A 3% NaCl solution was used by Rhoades and Miller (1965) in the microextraction of orange essence using diisopropyl ether as solvent. We found that at the level of 5% NaCl the separation of *n*-pentane extract from the aqueous sample was enhanced, the recovery was increased, no extraneous peak from NaCl was introduced, and the recovery was reproducible.

It should be noted that solvents with densities equal to or higher than that of water could not be used with the J&W liquid–liquid extractor. With this simple extraction method concentration of the extract was unnecessary. This has the further advantage of reducing the formation of artifacts possibly through oxidation, loss of important volatiles by vaporisation, and degradation of volatiles through thermal distillation. Therefore, this extraction method has great potential for extracting volatiles that are oxidizable, thermally degradable, and highly volatile.

The whole extraction procedure required about 5 min, which is therefore a much more rapid procedure than most other methods. Extraction with a J&W liquid–liquid extractor is definitely easier to perform compared to the SDA or SDE methods. This is especially advantageous when there are many samples to be analyzed as in the monitoring of aroma chemical production in submerged plant or microbial cell culture. Small sample volumes, only 40 mL, of the culture need to be withdrawn at timed intervals from the bioreactor for analysis.

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