Application of the Porapak Q Column Extraction Method for Tomato Flavor Volatile Analysis

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The Porapak Q column method (PQM) was compared to the method of simultaneous distillation extraction (SDE) under reduced pressure for extraction of the volatile compounds produced by tomato cv. Momotaro. The PQM was found to be effective at trapping and isolating many low and high boiling point volatile compounds and at producing the very desirable natural ripe tomato flavor of extracts. The SDE method was less effective in isolating the higher boiling point volatile compounds and caused deterioration of volatile compounds due to the heating process that takes place during extraction, resulting in an unpleasant boiled green tomato flavor of extracts. The advantages of using the PQM are its simplicity and its high efficiency in isolating many volatile compounds from nonvolatile materials at room temperature. A total of 367 volatile compounds were isolated by the PQM. Of these, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 2- and 3-methylbutanol, and 2-phenylethanol were relatively more abundant than other compounds and (*Z*)-3-hexenal showed the highest relative amount.

KEYWORDS: Tomato flavor; Porapak Q column; volatile analysis; *Lycopersicon esculentum* Mill.

INTRODUCTION

Porapak Q (ethylvinylbenzene−divinylbenzene polymer) is a substance used to pack the column in gas chromatography (GC). The Porapak Q column method (PQM) was recently developed to concentrate volatile components in foods (*1*). It is an easy and rapid method compared to the conventional headspace sampling method and the steam simultaneous distillation extract (SDE) method. It is capable of (1) trapping micropolar and nonpolar compounds regardless of low or high boiling point, (2) concentrating volatile compounds in solvent, (3) extracting volatile compounds at room temperature, and (4) giving natural flavor to extract samples. It has been used for analyzing volatile compounds in green tea (*2*), *sake* (*3*), *Perilla frutescens* (*4*), and muskmelon (*5*, *6*).

The aroma of tomatoes plays an important role in consumer acceptability. The volatiles of tomatoes have been studied very thoroughly by the headspace sampling method. In these studies, the headspace of tomato puree was concentrated by flushing the puree with nitrogen and capturing the released volatiles on a trapping material (purge-and-trap method) (*7*, *8*), by concentrating the headspace volatiles at the head of the GC column using a combination of pressure and low temperature (*9*, *10*), by detecting volatile compounds present in the headspace over a sample of tomato with a sensor (electric nose technology) (*11*), or by the solid-phase microextraction method [adsorption of volatile compounds on SPME fiber coating (*12*)]. Compared to the Porapak Q column extraction method, these methods are relatively expensive and difficult. Moreover, it is difficult to isolate high boiling point volatile compounds from nonvolatile material by most of the conventional dynamic headspace sampling or SDE methods. Buttery et al. (*13*) developed a method involving high-flow dynamic headspace sampling with excess anhydrous sodium sulfate in purge-and-trap for analysis of Furaneol in tomato.

The aim of this study was to determine the usefulness of the Porapak Q method for the extraction of tomato volatiles. The volatile compounds of ripe tomato fruits obtained by the PQM were analyzed and compared to those obtained by the SDE method.

MATERIALS AND METHODS

Materials. Ripe tomato fruit (cv. Momotaro) was harvested from a greenhouse at Hiroshima Prefectural University, Hiroshima, Japan. Each tomato sample (100 g) consisted of pieces cut from three different tomatoes and was blended for 30 s. The blended mixture was then held for 3 min to produce the volatiles (*7*). After holding, the blended mixture was centrifuged for 20 min at 10000*g* and 0° C. The resulting supernatant was filtered by a glass filter vacuum (17G4, Pyrex Inc., Corning, NY) and then subjected to each extraction method.

Porapak Q Column Extraction Method. For the purposes of this study, we followed the procedure described by Shimoda et al. (*1*). The filtrate was immediately passed through a column (11 cm length × 2

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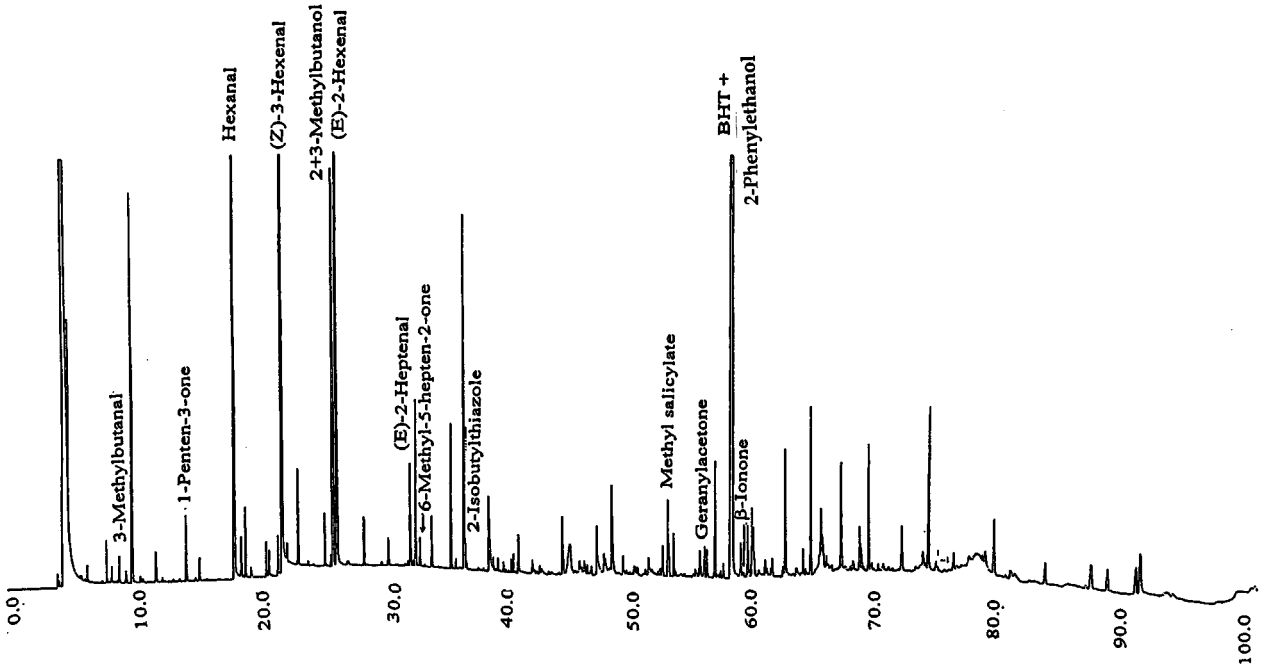

Figure 1. Capillary GC analysis (DB-Wax) of volatiles isolated from fresh tomatoes using the PQM.

cm i.d.) packed with 10 mL of Porapak Q (50−80 mesh, Waters Co., Milford, MA). After the column was washed with 100 mL of deionized water, adsorbed compounds were eluted with 80 mL of diethyl ether. The eluate was dried over excess anhydrous sodium sulfate for another 3 h after the addition of an internal standard (50 μL of 0.1% cyclohexanol) in order to remove the residual moisture in the extracts. The volume of the extracts was reduced to ~20 μL by evaporating the solvent under a gentle nitrogen stream. One microliter was drawn for volatile analysis by capillary GC/GC-MS. For quantitative analysis, the relative amount of volatile compounds was quantified relative to the peak area of the internal standard. The Porapak Q column was regenerated by washing with diethyl ether (60 mL), methanol (80 mL), and deionized water (100 mL).

Simultaneous Distillation Extraction Method. For the SDE method, we followed the procedure described by Shimoda et al. (*1*). A 2-L round-bottom flask was used as the sample flask to contain 100 mL of the filtrate and deionized water of an equivalent volume. A 200-mL V-shaped bottom flask containing 100 mL of diethyl ether was attached to the solvent arm of the SDE head. The vacuum line was closed when the sample started boiling. The separation of volatile compounds was carried out under reduced pressure (75 mmHg, 65° C) for 60 min. The condenser of the SDE head was cooled with a mixture of water and ethylene glycol at −5° C. After the addition of internal standard, the extract was dried over anhydrous sodium sulfate and concentrated to ~20 μL under a nitrogen stream before 1 μL was drawn for GC/GC-MS analysis.

Gas Chromatography Analysis. The composition of volatile samples was analyzed using a Shimadzu GC-17A (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a 60 m × 0.25 mm fused silica DB-Wax capillary column (J&W Scientific Inc., Folsom, CA) and FID detector. The oven temperature was held at 40 °C for 10 min, increased from 40 to 220 °C at 3 °C/min, and then held at 220 °C for 30 min. The carrier gas (He) flow velocity was 30 cm s^{-1}. Sample size was 1 μL split 1/20. The injector and detector temperatures were 230 and 250 °C, respectively.

Gas Chromatography—Mass Spectrometry Analysis. The composition of volatile samples was identified using a QP5050 GC-MS system (Shimadzu Co., Ltd.) with selected ion monitoring. The column and oven conditions were the same as those described for GC analysis. Compounds were identified primarily by comparing their mass spectra with the mass spectral data of standard compounds in the NIST library together with a Kovats index comparison of the obtained compounds with standard compounds.

RESULTS AND DISCUSSION

Our experimental results show that the Porapak Q column method successfully isolated tomato flavor. Fresh concentrated Porapak Q extracts possess a very desirable natural ripe tomato flavor, which differs greatly from the aroma of SDE extracts, which give an unpleasant boiled green tomato flavor. Similar results have also been reported in studies of green tea (*2*) and muskmelon (*5*). The PQM, therefore, has been shown to be more favorable for the extraction of odor concentrates in a manner that preserves their natural aromas.

Chromatograms of the volatile extracts of tomato fruits produced by Porapak Q are shown in **Figure 1**, and those produced by SDE are shown in **Figure 2**. Both qualitative and quantitative differences in the two extracts were observed. A total of 367 volatile compounds were isolated by the PQM, and 271 were isolated by SDE. Hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 2- and 3-methylbutanol, and 2-phenylethanol were relatively more abundant than other compounds in the Porapak Q extracts, whereas (*E*)-2-hexenal was relatively more abundant than other compounds in the SDE extracts. The PQM allowed high boiling point volatile compounds to be isolated from nonvolatile material at room temperature, whereas these high boiling point compounds were too difficult to isolate by using the SDE method. These results may be attributed to the fact that the PQM involves the use of porous polymer beads that trap many volatile compounds regardless of low or high boiling point, resulting in a natural tomato-like flavor of extracts. The SDE method, on the other hand, caused serious decomposition of volatile compounds during extraction and was not able to isolate the high boiling point compounds, leading to a change in the flavor of extracts. In SDE extracts, green aroma compounds such as hexanal, (*Z*)-3-hexenal, and (*E*)-2-hexenal were reduced by 2−4 times compared to the compounds found in Porapak Q extracts (data not shown). Kazeniac and Hall (*14*) explained that the reduction of "green" aroma compounds in tomato flavor might cause the appearance of the "cooked" aroma in tomato products via heating process.

The PQM for tomato volatile analysis after blending of the tomatoes and holding the mixture for 3 min involved the

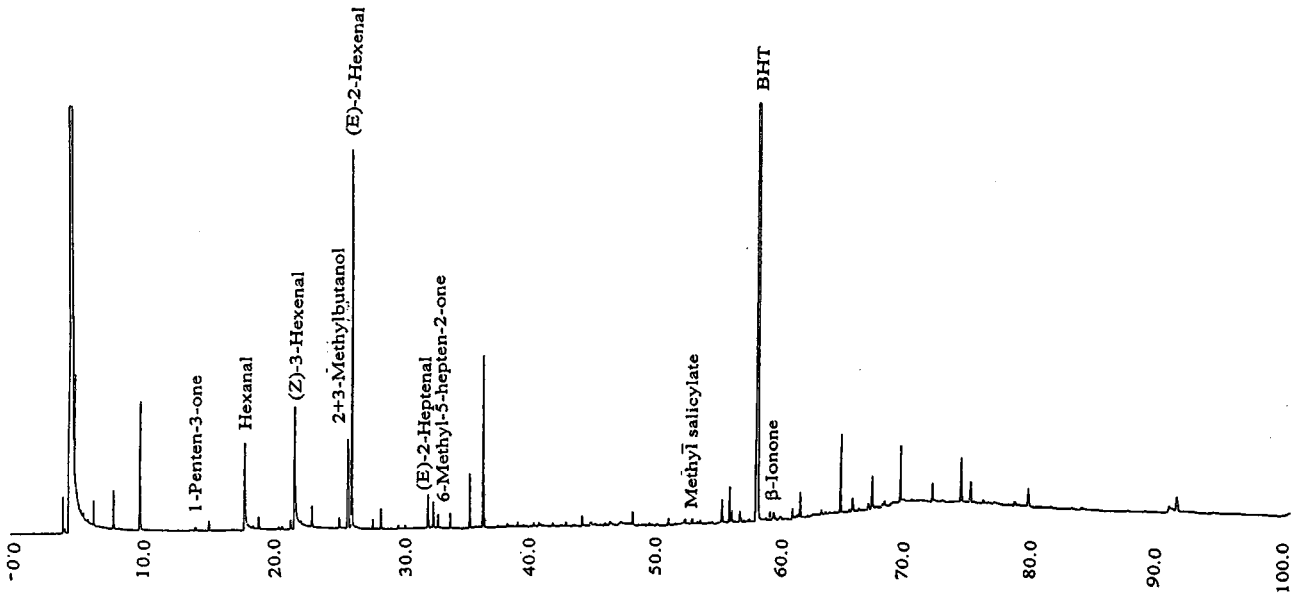

Figure 2. Capillary GC analysis (DB-Wax) of volatiles isolated from fresh tomatoes using the SDE method.

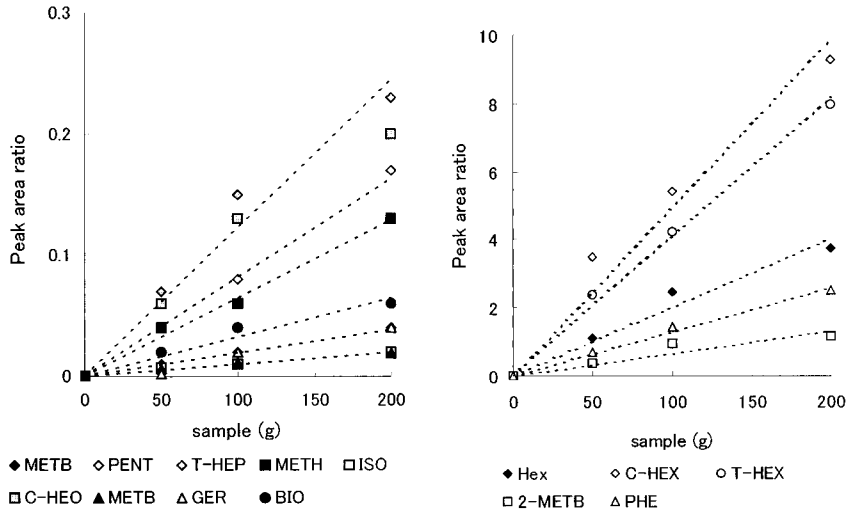

Figure 3. Relationship between sample volume and relative peak area of important volatile compounds in tomato sample by the PQM. Volatile compounds: *cis*-3-hexenal (C-HEX), *trans*-2-hexenal (T-HEX), hexanal (HEX), *cis*-3-hexenol (C-HEO), *trans*-2-heptenal (T-HEP), 1-penten-3-one (PENT), 2-isobutylthiazole (ISO), 6-methyl-5-hepten-2-one (METH), 3-methylbutanal (METB), β-ionone (BIO), 2-methylbutanol (2-METB), 3-methylbutanol (3-METB), methyl salicylate (METS), geranylacetone (GER), and 2-phenylethanol (PHE).

following steps: (1) centrifugation of the blended mixture at 0° C; (2) passing the filtrate through the Porapak Q column immediately, followed by washing with 100 mL of deionized water; (3) eluting the adsorbed compounds with 80 mL of diethyl ether; (4) addition of internal standard; (5) drying over excess anhydrous sodium sulfate to remove residual moisture; and (6) GC analysis of the Porapak Q extracts. (*Z*)-3-Hexenal showed the highest relative levels of volatile compounds isolated from the Porapak Q extracts. (*Z*)-3-Hexenal was previously reported both to have the highest concentration and greatest importance in the tomato flavor isolated by a headspace sampling technique and to be an unstable compound (*7*, *8*, *15*); it was apparently largely isomerized to *trans*-2-hexenal during isolation and analysis (*16*, *17*). Due to cis−trans isomerization, the level of *cis*-3-hexenal was lower and that of *trans*-2-hexenal was higher in the study carried out by Baldwin et al. (*9*). To avoid deterioration of *cis*-3-hexenal during headspace sampling (~60 min), it was therefore necessary to deactivate the enzyme system by adding $CaCl_2$ to the tomato homogenate (*7*). In the present study, the relative amount of (*Z*)-3-hexenal was 5.62 peak area ratio, whereas that of (*E*)-2-hexenal was 3.86 peak area ratio. With the Porapak Q column method, the tomato homogenate is centrifuged at 0 °C and is then immediately isolated by the Porapak Q column within 5−10 min, so both low temperature of sample and very short time during extraction may have diminished the conversion of *Z*-3-hexenal to *E*-2-hexenal. It has been reported that the important volatile compounds for fresh ripe tomato flavor are (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanal, 1-penten-3-one, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, 3-methylbutanal, β-ionone, 2- and 3- methylbutanol, methyl salicylate, geranylacetone, (*E*)-2-heptenal, and 2-phenylethanol (*7*), and these compounds were identified in the Porapak Q extracts in the present study. Our results also indicate that the PQM is able to extract alcohol, aldehyde, ketones, and nitrogen-containing compounds from fresh tomatoes.

The PQM was repeated five times, and the reproducibility of important tomato volatile compounds is shown in **Table 1**. With regard to the coefficient of variation of these compounds, that of (*Z*)-3-hexenal was relatively high (29.6%) because of

Table 1. Percent Reproducibility of Some Tomato Volatile Compounds by the Porapak Q Column Method

compound	% CV[a]	compound	% CV[a]
3-methylbutanal	13.5	6-methyl-5-hepten-2-one	15.7
1-penten-3-one	9.4	*Z*-3-hexenol	18.0
hexanal	13.4	2-isobutylthiazole	3.3
Z-3-hexenal	29.6	methyl salicylate	16.2
2-methylbutanol	16.0	geranylacetone	21.8
3-methylbutanol	17.9	2-phenylethanol	15.9
E-2-hexenal	13.9	β-ionone	16.9
E-2-heptenal	14.7		

[a] Coefficient of variation obtained from five experiments.

Table 2. Percent Recoveries of Some Tomato Volatile Compounds by the Porapak Q Column Method

	% recovery		
compound	I	II	III
3-methylbutanal	100	0	0
1-penten-3-one	100	0	0
hexanal	99.1	0.9	0
Z-3-hexenal	98.05	1.85	0
2- +3-methylbutanol	99.4	0.6	0
E-2-hexenal	98.4	1.6	0
E-2-heptenal	73.8	21.1	5.05
6-methyl-5-hepten-2-one	97.1	2.9	0
Z-3-hexenol	86.8	13.2	0
2-isobutylthiazole	95.9	4.1	0
methyl salicylate	100	0	0
geranylacetone	83.5	10.8	5.8
2-phenylethanol	99.99	0	0
β-ionone	79.9	14.0	6.1

its instability (*7*), whereas others ranged from 3.3 to 21.8%. The relationship between sample volumes (50, 100, and 200 g) and the relative peak area of volatile compounds in tomato fruit is shown in **Figure 3**. Peak area ratios of compounds increased relative to sample volume, and 10 mL of Porapak Q did not reach the limits of the relative peak area even after 200 g of tomato had been treated. Furthermore, because the composition ratio of the compounds changed only negligibly, it was clear that 10 mL of Porapak Q was sufficient to extract volatile compounds from 100 g of tomato fruit. **Table 2** shows the percent recoveries of some tomato volatiles, determined as their elution rates by using 40 mL of diethyl ether (repeated three times). The present study shows that >90% of the extractable tomato volatile compounds, except (*E*)-2-heptenal, (*Z*)-3-hexenol, geranylacetone, and β-ionone, were recovered in the first washing with 40 mL of diethyl ether, and all of these compounds were almost completely recovered during the second washing process. As most compounds were recovered during the second washing process, the volume of diethyl ether needed to elute the absorbed volatile compounds in Porapak Q was determined to be ~80 mL.

The data obtained in this study support the use of the Porapak Q column extraction method as a simple, reproducible, and efficient technique for the isolation of volatile compounds in horticultural flavor analysis (*4*−*6*). Its efficiency of trapping many volatile compounds regardless of low or high boiling point at room temperature (**Figure 1**) suggests that the PQM can preserve natural flavor and give more clearly information of horticultural flavor because its headspace flavor compound (low boiling point compound) and the flavor compound released while it is eaten (medium−high boiling point compound) can be isolated. Also, because only standard laboratory equipment and commercially available reagents are used, the Porapak Q column extraction method may become a satisfactory alternative technique for tomato flavor analysis.

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