Application of Porapak Q Column for a Sample Preparation of Volatile Chemicals from Brewed Coffee

Kazuyoshi Aino, Matt Hengel and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California, Davis, CA, USA

*Corresponding Author: Takayuki Shibamoto, Department of Environmental Toxicology, University of California, Davis, CA 95616, USA.

Abstract

Volatile chemicals were isolated from brewed coffees using solid-phase, Porapak Q. The chemicals isolated were identified by gas chromatography and gas chromatography/mass spectrometry. Total 51 chemicals were identified; including 8 alcohols, aldehydes and ketones; 7 pyrroles; 2 pyridines; 11 pyrazines; 9 furans; 2 acids; 3 phenols; and 2 miscellaneous compounds. Two major flavor components of a brewed coffee, maltol and 3-pridinol were isolated by Porapak Q but not by steam distillation. Furfural and furfuryl alcohol, which are major precursor of heterocyclic flavor compounds, were isolated satisfactorily by Porapak Q. Porapak Q demonstrated a simple method to recover volatile organic chemicals from brewed coffees. It is useful to obtained relatively high quantities of volatile chemicals for various studies, including sensory, antioxidant and other biological activities.

Keywords: Coffee volatiles; GC/MS; Porapak Q; Solid-phase extraction

Introduction

Since invention of gas chromatography (GC) in 1950s, analysis of volatile chemicals in foods and beverages, in particular offee, have been conducted for many years and tremendous numbers of volatile chemicals are reported [1-4]. On the other hand, major drawback of GC is that the samples must be in an organic solvent. HPLC, particularly, interfaced to a mass spectrometer, can analyze aqueous samples directly. However, resolution of volatile chemicals by HPLC is considerably lower than by GC. Therefore, GC has still been used commonly for studies on volatile chemicals. On the other hand, a sample preparation plays an important role in analytical studies by GC. One of the most popular sample preparation methods for volatile studies is a headspace sampling, which has also applied to the analysis of brewed coffee volatile chemicals [5-7]. Recently, the headspace method has become a main stream of sample collections for volatile chemicals because the significant development of the computer library of chemicals, which gives sufficient information of mass spectra and GC retention indices for identification. However, the amounts collected by this method are extremely low-just sufficient for GC analysis. It should be noted that the conventional method for sample collection is still important because some chemicals may not be recovered by the headspace method. Therefore, conventional solid phase extraction methods have been still used commonly to collect volatile chemicals from foods and beverages. In particular, solid phase Porapak Q is widely used to concentrate volatile chemicals from water and aqueous samples obtained from foods and beverages, such as tomato [8], alcoholic beverage, Awamori [9] and other alcoholic beverages [10]. In the present study, conventional method for volatile collections was conducted for analysis of brewed coffee volatile chemicals.

Materials and Method

Materials

Porapak Q™ porous polymer adsorbent (50 - 80 mesh) was bought from Sigma-Aldrich Inc. (St. Louis, MO). A 10 cm x 2.2 cm i.d. glass cartridge was filled with the Porapak Q (6.5g) and used to extract volatile chemicals from brewed coffees. The used Porapak Q cartridges were washed with ethyl ether (20 ml) twice, methanol (20 ml) twice, water/methanol (50/50, 20 ml) three times and methanol (20 ml) twice in series and then used for additional coffee extractions. Commercial ground roasted coffees, Arabica Colombian supreme coffee beans were purchased from a local market in Davis, California. All solvents were from the reliable chemical companies. Standard volatile chemicals were gift from Takata Koryo Co. Ltd., Hyogo, Japan.

Collection of Volatile Chemicals with Porapak Q Cartridge and Steam Distillation from Brewed Coffee

Roasted ground coffee (40g) was brewed with 400 ml of deionized water using a Mr. Coffee NCX-20 model coffee maker (Sunbeam Product, Inc., Boca Raton, FL). A brewed coffee sample (200 ml) was place in a Porapak Q cartridge prepared above. Water was drained off under a reduced pressure for 3 min and then the cartridge was washed with 30 ml water three times. The volatile chemicals trapped on Porapak Q were eluted with 30 mL of ethyl ether twice. The eluates were combined. The ethyl ether sample solution (60 ml) was dried over anhydrous sodium sulfate for overnight. After 5 ml of 10% ethyl ether solution of 3-heptanol added as a GC internal standard, the sodium sulfate was filtered off. The filtrated was condensed to 1.0 ml with purging a purified nitrogen stream. A brewed coffee sample (200 ml) was steam distilled for approximately 30 min. After addition of 20 ml saturated sodium chloride solution, the distillate (apx. 50 mL) was extracted with 30 ml potion of ethyl ether three times using a separatory funnel. The combined extracts was dried over anhydrous sodium sulfate for overnight. After sodium sulfate was filtered off, the extract was condensed to 1.0 ml purging with a purified nitrogen stream, and then 5 ml of 10% ethyl acetate solution of 3-heptanol was added as a GC internal standard. The samples were analyzed by GC and GC/mass spectrometry (GC/MS). The experiment repeated six times.

Recovery efficiency tests of standard typical coffee volatile chemicals from porapak Q

Standard volatile chemicals (200 mg each) were spiked into 200 ml distilled water. The standard chemicals used were 2,5-dimethylpyrazine, furfural, furfuryl alcohol, 2-hydroxy-3-methylcyclobent-2-ene-1-one, maltol, and 3-pyridinol. These spiked chemicals were recovered with the exactly same procedures used for the coffee extracts described above. The experiments were repeated three times.

Qualitative and quantitative analyses of coffee volatile chemicals

An Agilent 5890 series II gas chromatograph equipped with a 30m x 0.25 mm i.d. DBWAX and a flame ionization detector (FID) was used for routine quantitative analysis. The initial column temperature was 50ºC and programed to 200ºC at 3ºC/min. A linear helium carrier-gas flow rate was 35 cm/sec. Split ratio was 20/1. Injector and detector temperatures were 250ºC. The concentration of each volatile chemical was calculated using a previously reported GC internal standard method [11]. The standard calibration curve for the volatile chemicals identified was prepared using five standard ethyl acetate solutions of 3-heptanol (0.1, 0.2, 0.3,0.4, and 0.5 µg/ml) R^2-0.9984. This curve was used for all chemicals identified to estimate their concentration. Qualitative analysis of coffee volatile chemicals in the extracts from Porapak Q and steam distillation was performed with the same GC interfaced to Agilent 5971A mass selective detector at MS ionization voltage of 70 eV. The GC conditions were exactly same as the ones described above. Identification of GC components was conducted by comparison with the Kovats GC retention index (KI) and by the MS fragmentation pattern of each component compared with those of authentic compounds.

Results and Discussion

Table 1 shows the recovery efficiencies of representative coffee volatile chemicals from Porapak Q. The all standard chemicals tested recovered over 90% except 3-pyridinol in first elution. Therefore, routine experiments conducted using just first eluate. The retention

time of chemicals on Porapak Q is depend on partition between the surface of solid-phase (Porapak Q) and solvent (water or ethyl ether) [12]. Water has an extremely short retention time on Porapak Q. Therefore, it is ideal to isolate or concentrate organic chemicals from a water sample. As table 1 shows, all volatile chemicals identified in coffee brew except 3-pyridinol were separated from water satisfactory in the present study. Figure 1 shows the gas chromatograms of volatile chemicals recovered by Porapak Q and steam distillation from a brewed coffee. The two chromatograms look similar. Almost all chemicals appear in the both chromatograms. Slight differences are observed in amount recovered. It is reasonable because isolation of chemicals by a solid-phase steam distillation based on the partition and boiling point, respectively. Roughly, steam distillation recovered more high boiling chemicals than Porapak Q did, whereas steam distillation recovered less low boiling point chemicals than Porapak Q did. On the other hand, two gas chromatograms of high boiling point range look almost same, which is important to evaluate a sensory nature of food flavors. Table 2 shows volatile chemicals identified in extracts obtained by Porapak Q and steam distillation. Values are mean ± SD (n = 6). Use of Porapak Q for recovery of organic volatile chemicals from aqueous or alcoholic samples is useful because water and ethanol has significantly short retention time compared with volatile organic chemicals on Porapak Q [13]. In the case of 3-pyridinol, it forms zwitterionic form, which has extremely long retention time on solid-phase [14]. Consequently, it was not recovered sufficiently in a first elutate. Moreover, it was not recovered by the steam distillation because it is an ionic form in water. 3-Pyridinol was reported as one of the antioxidants in an instant coffee [15]. Also, maltol was not recovered by a steam distillation. Maltol is a naturally occurrence chemical and also formed by sugar degradation [16]. In the case of coffee, the content of sugar in green coffee beans reduces significantly by roasting, suggesting that sugar in green coffee beans undergo degradation to form maltol. Subsequently maltol undergoes to produce many flavor chemicals with anime compounds, such as protein, by Maillard reaction [17]. Maltol itself has a caramel-like odor [18]. It also possesses a fruity-strawberry aroma in dilute solution [19]. These natures suggest that maltol has an important role in brewed coffee flavors. Also, other sugar degradation products, furfural (0.603 mg/ml from Porapak Q and 1.503 mg/ml from steam distillation) and furfuryl alcohol (6.723 mg/ml from Porapak Q and 14.128 mg/ml from steam distillation), were recovered by relatively high quantities. Furfural possesses sweet and caramel-like flavor and taste. Furfuryl alcohol has a caramel-coffee-like taste and odor. It has been used in imitation coffee flavor for chocolate, fruit and nut complexes [20]. Both furfural and furfuryl alcohol were reportedly undergo Maillard reaction with amine compounds to give various heterocyclic flavor compounds as well as maltol does [17]. In the present study, heterocyclic compounds identified were 7 pyrroles, 2 pyridines, 11 pyrazines, 9 furans in brewed coffee. They have been reported in coffee previously [3]. Among these chemicals, heterocyclic compounds have been received much attention not only as flavor chemicals but also antioxidants found in coffees [21].

	pt Q eluate number		
	1	**2**	**3**
2,5-Dimethylpyrazine	100.0 ± 3.1	0.0	0.0
Furfural	100.0 ± 4.8	0.0	0.0
Furfuryl alcohol	99.5 ± 3.6	0.5 ± 1.0	0.0
2-Hydroxy-3-methylcyclpent-2-en-l-one	93.1 ± 2.3	6.9 ± 1.2	0.0
Maltol	100.0 ± 4.2	0.0	0.0
3-Pyridinol	67.9 ± 7.6	32.1 ± 5.2	0.0

Table 1: *Results of recovery efficiency tests on selective volatile coffee chemicals (%).*

Chemicals	KI	Porapak Q	Amount (mg/mL) SD
Alcohols, aldehydes, and ketones			
Pentane-2,3-dione	1065	0.117 ± 0.011	0.268 ± 0.054
2-Methyl-2-pentenal	1169	trace	trace
3-Penten-2-ol	1360	0.052 ± 0.001	0.113 ± 0.008
1-(acetyloxy)-2-Propanone	1477	0.556 ± 0.020	0.537 ± 0.040
2-Methyl-3-pentanone	1541	0.018 ± 0.004	0.230 ± 0.016
3,4-Dimethyl-2-hydroxycyclopent-2-en-1-one	1804	0.251 ± 0.009	0.172 ± 0.005
2-Hydroxy-3-methylcyclopent-2-en-1-one	1840	0.443 ± 0.024	0.086 ± 0.004
3-Ethyl-2-hydroxycyclopent-2-en-1-one	1904	0.226 ± 0.020	0.077 ± 0.002
Pyrroles			
1-Methyl-1H-pyrrole	1148	trace	0.067 ± 0.011
Pyrrole	1520	0.110 ± 0.008	0.287 ± 0.033
1-Methyl-1H-pyrrole-2-carboxaldehyde	1622	0.091 ± 0.002	0.192 ± 0.007
1-(methyl-1H-pyrrole-2-yl)-Ethanone	1660	0.047 ± 0.003	0.096 ± 0.004
1-(1H-pyrrole-2-yl)-Ethanone	1979	0.695 ± 0.039	0.202 ± 0.007
1H-Pyrrole-2-carboxaldehyde	2029	0.389 ± 0.027	0.122 ± 0.003
1-Methyl-1H-pyrrole-2-carboxaldehyde	2110	0.263 ± 0.021	0.055 ± 0.006
Pyridines			
Pyridine	1198	0.635 ± 0.065	0.479 ± 0.064
3-Pyridinol	2432	0.344 ± 0.038	ND
Pyrazines			
Pyrazine	1221	ND	0.159 ± 0.022
2-Methylpyrazine	1279	0.502 ± 0.028	1.224 ± 0.131
2,5-Dimethylpyrazine	1336	0.226 ± 0.004	0.508 ± 0.031
2,6-Dimethylpyrazine	1343	0.236 ± 0.005	0 .534 ± 0.038
2-Ethylpyrazine	1346	0.101 ± 0.002	0.234 ± 0.017
2,3-Dimethylpyrazine	1352	0.038 ± 0.001	0.106 ± 0.006
2-Ethyl-6-methylpyrazine	1396	0.068 ± 0.001	0.168 ± 0.008
2-Ethyl-5-methylpyrazine	1406	0.059 ± 0.001	0.128 ± 0.006
2,3,5-Trimethylpyrazine	1418	0.119 ± 0.003	0.208 ± 0.018
1-Acetylpyrazine	1630	0.168 ± 0.020	0.229 ± 0.011
1-(6-methyl-2-pyrazinyl)-1-Ethanone	1696	0.067 ± 0.002	0.081 ± 0.009
Furans			
Dihydro-2-methyl-3(2H)-furanone	1274	0.221 ± 0.015	0.598 ± 0.049
Furfural	1470	0.603 ± 0.026	1.503 ± 0.125

Citation: Takayuki Shibamoto., *et al.* "Application of Porapak Q Column for a Sample Preparation of Volatile Chemicals from Brewed Coffee". *EC Nutrition* 14.10 (2019): 880-886.

Furfuryl formate	1503	0.094 ± 0.001	0.068 ± 0.005
1-(2-furanyl)-Ethanone	1510	0.369 ± 0.007	0.620 ± 0.037
Furfuryl acetate	1546	0.620 ± 0.013	0.662 ± 0.036
5-Methyl-2-furfural	1579	0.748 ± 0.019	1.591 ± 0.088
Furfuryl alcohol	1675	6.723 ± 0.382	14.128 ± 0.753
2,5-Dimethyl 4-hydroxy-3-(2H)-furanone	2050	0.355 ± 0.021	ND
1-(3-methylphenyl)-Ethanone	2199	0.525 ± 0.008	0.471 ± 0.053
Acids			
Acetic acid	1458	ND	1.009 ± 0.053
3-Methyl butanoic acid	1680	0.551 ± 0.017	0.892 ± 0.031
Phenols			
2-Methoxy phenol	1866	0.180 ± 0.007	0.249 ± 0.012
Phenol	2012	0.342 ± 0.015	0.322 ± 0.012
4-Ethyl 2-methoxyphenol	2035	0.078 ± 0.006	0.081 ± 0.004
Miscellaneous compounds			
Butyl hydroxytoluene	1915	0.235 ± 0.014	0.234 ± 0.034
Maltol	1976	0.435 ± 0.072	ND

Table 2: Chemicals identified in extracts from brewed coffee by Porapack Q and steam distillation (SD).
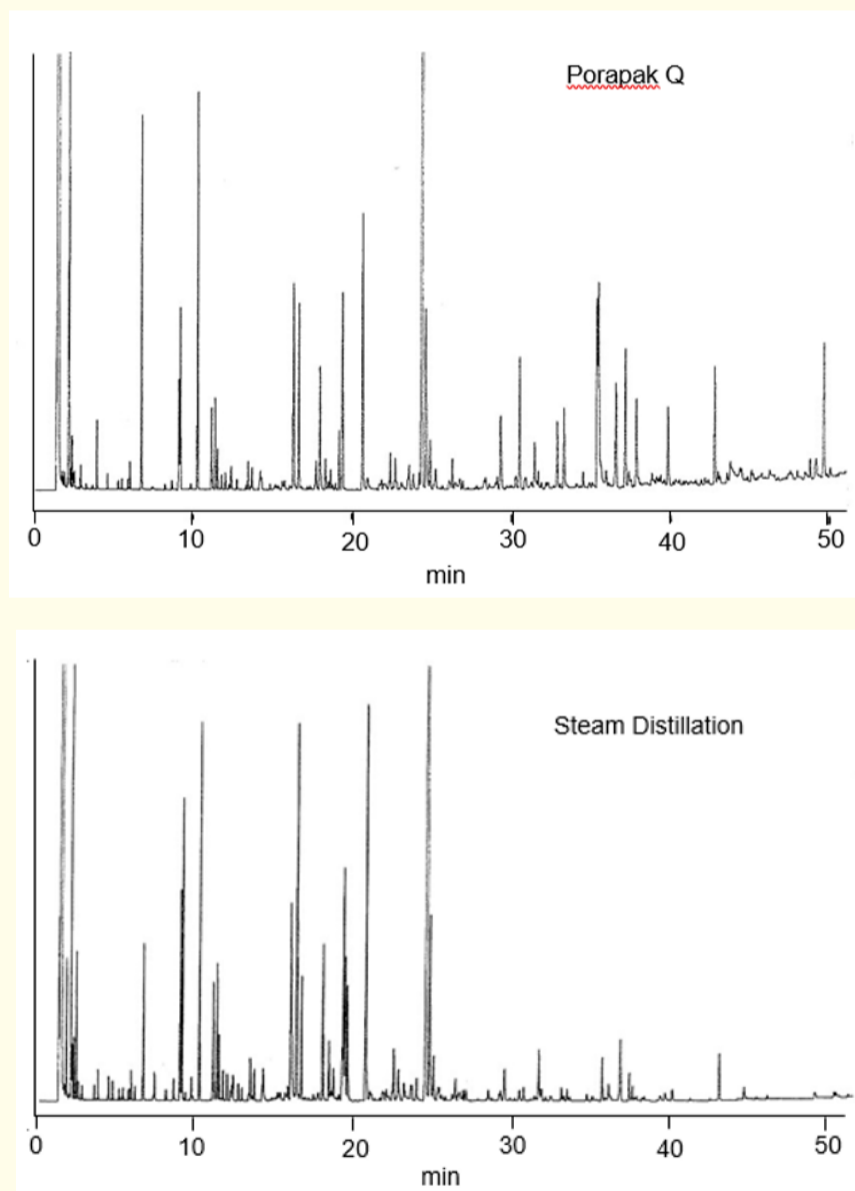*Trace: amount less than 0.01 mg/mL; ND: not detected.*

Figure 1: Gas chromatograms of volatile chemicals recovered by Porapak Q and steam distillation from a brewed coffee.

Conclusion

As mentioned above, the mainstream of the collection of volatile chemicals from foods and beverages is headspace methods. one drawback of this method is that amounts of samples collected are extremely low. However, some researches on volatile flavor chemicals, such as sensory evaluations and applications to analytical apparatus (NMR, IR, etc.) for identification of unknowns, require relatively high quantities of samples. A Porapak Q column demonstrated satisfactory recovery of volatile chemicals from a coffee brew in the present study. This method is simple and has a high repeatability.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Shibamoto T., *et al*. "Application of HPLC for evaluation of coffee flavor quality". In: The Quality of Food and Beverages. Charalambous G, Ed., York New, Academic Press 193 (1981): 312-334.

2. Shibamoto T. "An overview of coffee aroma and flavor chemistry". In: Proceedings of the fourteenth international conference on coffee science. San Francisco 991(1992): 196107-116.

3. Flament I and Bessiere-Thomas Y. "Coffee Flavor Chemistry". New York. Wiley (2002).

4. Amaral MSS., *et al*. "Ionic liquid capillary columns for analysis of multi-component volatiles by gas chromatography-mass spectrometry: performance, selectivity, activity andretention indices". *Analytical and Bioanalytical Chemistry* 410.19 (2018): 4615-4632.

5. Shimoda M and Shibamoto T. "Isolation and identification of headspace volatiles from brewed coffee with an on-column GC/MS Method". *Journal of Agricultural and Food Chemistry* 38.3 (1990): 802-804.

6. Akiyama M., *et al*. "Analysis of volatile compounds released during the grinding of roasted coffee beans using solid-phase microextraction". *Journal of Analytical Chemistry* 51.7 (2003): 1961-1969.

7. Fujioka K and Shibamoto T. "Improved malonaldehyde assay using headspace solid-phase microextraction and its application to the measurement of the antioxidant activity of phytochemicals". *Journal of Agricultural and Food Chemistry* 53.12 (2005): 4708-4713.

8. Maneerat C., *et al*. "Application of the Porapak Q extraction method for tomato flavor volatile analysis". *Journal of Agricultural and Food Chemistry* 50.12 (2002): 3401-3404.

9. Tamamura, T., *et al*. "Analysis of volatile compounds in Awamori using solid phase extraction". *Nippon Shokuhin Kagaku Kogaku Kaishi* 48.3 (2001): 202-209.

10. Ferreira V., *et al*. "A comparative study of the ability of different solvents and absorbents to extract aroma compounds from alcoholic beverages". *Journal of Chromatographic Sciences* 38.11 (2000): 469-476.

11. Ettre LS. "Interpretation of analytical results". In: The practice of gas chromatography; Ettre S, Zlatkis A, Eds. New York, Interscience Publishers (1967): 402-440.

12. Prosen H and Zupancic-Kralj L. "Solid-phase microextraction". *Trends in Analytical Chemistry* 18.4 (1999): 272-274.

13. Zenkevich IG. "Calculation of retention indexes of low-boiling organic compounds of different chemical nature on the polymer sorbent Porapak Q". *Journal of Analytical Chemistry* 53 (1998): 816-828.

14. Huq F and Yu JQ. "Molecular modeling analysis: "Why is 2-hydroxypyridine soluble in water but not 3-hydroxypyridine". *Journal of Molecular Modeling* 8.3 (2002): 81-86.

15. Kurakane S and Igarshi K. "A radical scavenging compound, 3-pyriidinol, in instant coffee and its hepatoprotective activity". *Food Science and Technology Research* 12.2 (2006): 148-151.

16. Hodge J. "Origin of flavor in foods nonenzymatic browning reactions". In: Chemistry and Physiology of Flavors; Schultz HW, Day EA, Libbey LM, Eds.; Westport, AVI Pub. Com. 231 (1967): 465-491.

17. Shibamoto T. "(Formation of selected heterocyclic flavor chemicals in beverages". In: Melton L Shahidi F Varelis P (Eds.) Encyclopedia of Food Chemistry 2, London, 234 Elsevier, (2019): 363-373.

18. O'Neil MJ. "The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge". *London, Royal Society of Chemistry* (2013): 1061.

19. Larranaga MD., *et al*. "Hawley's Condensed Chemical Dictionary" 16th Edition. Hoboken, John Wiley & Sons, Inc., (2016): 859.

20. Arctander S. "Perfume and Flavor Chemicals". Montclair, Published by the author (1969).

21. Yanagimoto K., *et al*. "Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard Reaction". *Journal of Agricultural and Food Chemistry* 50.19 (2002): 5480-5484.

Volume 14 Issue 10 October 2019