Headspace Hanging Drop Liquid Phase Microextraction and Gas Chromatography-Mass Spectrometry for the Analysis of Flavors from Clove Buds

Mi-Jin Jung, Yeon-Jae Shin, Se-Yeon Oh, Nam-Sun Kim, Kun Kim, and Dong-Sun Lee*

*Department of Chemistry, Seoul Women's University, Seoul 139-774, Korea. *E-mail: dslee@swu.ac.kr*
Received November 8, 2005

A novel sample pretreatment technique, headspace hanging drop liquid phase microextraction (HS-LPME) was studied and applied to the determination of flavors from solid clove buds by gas chromatography-mass spectrometry (GC-MS). Several parameters affecting on HS-LPME such as organic solvent drop volume, extraction time, extraction temperature and phase ratio were investigated. 1-Octanol was selected as the extracting solvent, drop size was fixed to 0.6 μL. 60 min extraction time at 25 °C was chosen. HS-LPME has the good efficiency demonstrated by the higher partition equilibrium constant (K_{lh}) values and concentration factor (CF) values. The limits of detection (LOD) were 1.5-3.2 ng. The amounts of eugenol, β-caryophyllene and eugenol acetate from the clove bud sample were 1.90 mg/g, 1.47 mg/g and 7.0 mg/g, respectively. This hanging drop based method is a simple, fast and easy sample enrichment technique using minimal solvent. HS-LPME is an alternative sample preparation method for the analysis of volatile aroma compounds by GC-MS.

Key Words : Headspace hanging drop liquid phase microextraction (HS-LPME), Gas chromatography-mass spectrometry (GC-MS), Sample pretreatment, Clove flavor

Introduction

Sample preparation step is a very important and necessary part of most analytical procedure. The main purpose of sample preparation is to transfer the analyte from matrix into a suitable form that is clean-up, concentrated, and compatible with analytical system.[1] The choice of appropriate sample preparation method greatly influences reliability and efficiency of the analysis.

Extraction of volatile aromatic compounds from solid plants is generally performed by steam distillation, solvent extraction, enfleurage, maceration, expression and supercritical fluid extraction.[2,3] Pressurized hot water at temperature lower than critical temperature was also used for the extraction of volatile compounds from samples such as clove.[4] The continuous research for better sample preparation procedure has led to new methods.

In analytical scale, the sampling techniques such as purge-and-trap, membrane-based extraction and headspace solid phase microextraction (HS-SPME) are so excellent that they are successfully employed in the analysis of natural aroma compounds.[5-9] SPME has the main advantage for achieving the process of extraction, concentration and sample introduction in one step. This technique has been a powerful alternative to the traditional extraction techniques, and used for the preparation of gaseous, liquid and solid samples.[10]

Recently, a novel sample preparation method of liquid phase microextraction (LPME) using a drop of solvent suspended from the tip of a syringe needle has been developed.[11-13] This method has also been called solvent microextraction, single drop microextraction, or liquid-liquid microextraction. Liu and Dasgupta[11] were the first to report a novel drop-in-drop system where a microdrop of a water-immiscible organic solvent (~1.3 μL), suspended in a larger aqueous drop, extracted sodium dodecyl sulphate ion pairs. At the same time, Jeannot and Cantwell[12] introduced a new solvent microextraction technique, where a microdrop (8.0 μL) of organic solvent containing a fixed amount of internal standard was left suspended at the end of a Teflon rod immersed in a stirred aqueous solution containing 4-methylacetophenone. He and Lee[13] first introduced the term "liquid phase microextraction (LPME)". They investigated the extraction of 1,2,3-trichlorobenzene by using two different modes of solvent microextraction. The first one, called static LPME, consisted of 1 μL drop suspended at the tip of a microsyringe needle immersed in an unstirred aqueous solution. The second one, called dynamic LPME, used the microsyringe as a separatory funnel and featured the repeated movement of the syringe plunger. De Jager and Andrews[14] reported preliminary work on solvent microextraction for the analysis of 11 organochlorine pesticides. They used 2 μL drop suspended at the end of a microsyringe needle, immersed in a stirred aqueous solution. Liu and Lee[15] recently introduced a new approach to single-drop microextraction, which was termed continuous-flow microextraction. According to this report, extraction was performed in ~0.5 mL glass chamber. Psillakis and Kalogerakis[16] applied solvent microextraction to the analysis of 11 nitroaromatic explosives. They used 1 μL drop suspended at the end of a microsyringe needle tip, immersed in a stirred aqueous solution. De Jager and Andrews[17] used the same drop-based method for the analysis of cocaine metabolites in urine samples.

More recently, headspace liquid phase microextraction (HS-LPME)[18-23] has been evaluated as an alternative to HS-SPME. HS-LPME is similar to traditional headspace sampling in that volatile compounds are collected from the vapors above the sample matrix, thus avoiding interferences from the sample matrix. In HS-LPME, the fiber used in SPME is

replaced by a liquid microdrop. This technique is very inexpensive when compared to the sorbent-based techniques, because the drop is completely renewable at negligible cost. This method enables to combine extraction, enrichment, clean-up, and sample introduction into a single step prior to the chromatographic process. Since only a few microliters of solvent are used, there is minimal waste or exposure to toxic organic solvents. LPME approach is mainly focused on aqueous sample, however, there is little reports dealing with the use of HS-LPME for the extraction and analysis of volatile aromas from solid materials of plant origin.[24,25]

In the present study, the use of HS-LPME is expanded to the field of natural plants in the form of solid. HS-LPME is applied for the determination of flavors from solid clove bud (*Syzygium aromaticum*) of the family myrtaceae by GC-MS. The major constituent of clove essential oils is eugenol follwed by β-caryophyllene.[26,27] Cloves are known to have antiseptic and antioxidative properties. They are widely used in medicine for promoting circulation in the lungs and the stomach. Their use as a preservative in pickles and spiced dishes is well documented. In China, cloves are also used as a mild anaesthetic for toothache. Cigarettes named *kretek* flavored with cloves are extremely popular and nearly every (male) Indonesian enjoys them.

The objective of this study is the evaluation of HS-LPME as a novel sampling technique for solid samples such as clove buds. Parameters that have been considered for HS-LPME were investigated. Efficiency of HS-LPME was evaluated by using the partition equilibrium constant, concentration factor.

Experimental Section

Plant material and reagents. The dried clove buds were purchased from the local market. All chromatographic grade solvents such as 1-octanol (> 99%) and *n*-hexane (> 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Working standards of eugenol (> 98.0%), β-caryophyllene (> 80%) and eugenol acetate (> 98%) were purchased from Tokyo Kasei (Nihonbashi, Tokyo, Japan).

Headspace liquid phase microextraction (HS-LPME). The dried clove buds were coarsely ground to powders prior to analysis, and then they were directly used without any further treatments. These clove buds' powders (2 g each) were obtained in 25 mL crimp top vial. For the investigation of the influence of phase ratio, aliquots (about 20 mg) of each working standards (liquid) was obtained in 2 mL, 15 mL, and 25 mL vials, respectively. The vial was immediately sealed with polytetrafluoroethlyene (PTFE)-silicon septum and an aluminum cap, and stored at room temperature (25 °C) for 60 min to equilibrate between sample matrix and headspace. The conventional 10 μL microsyringe (Hamilton #701, Reno, NV, USA) was rinsed with 1-octanol at least 10 times. After the uptake of 0.6 μL of 1-octanol, the needle was used to pierce the vial septum, and the syringe was clamped into place such that the tip of the needle was located in a consistent position in the headspace, as shown in Figure 1. Then 0.6 μL extraction solvent was extruded out of the needle and kept a single hanging drop at the needle tip to expose in the headspace for 60 min. When the extraction was finished, the drop was retracted back into the microsyringe and injected directly into the GC inlet.

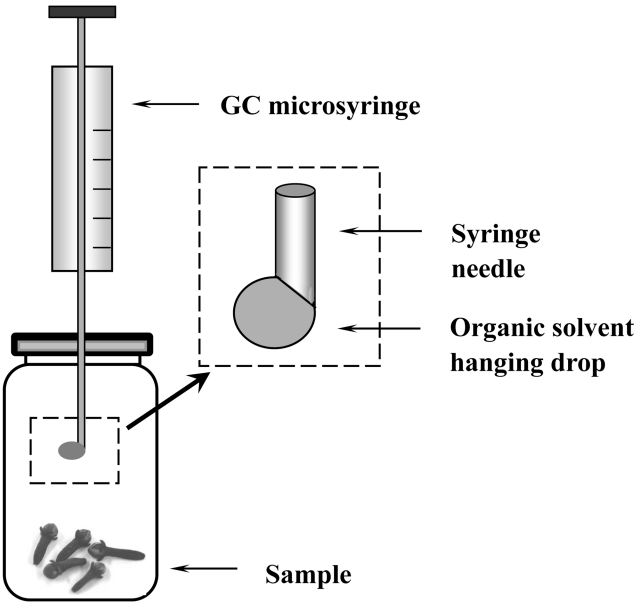

Figure 1. Schematic diagram of headspace hanging drop liquid phase micro-extraction (HS-LPME).

Gas chromatography (GC). A HP 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with flame ionization detector (FID) was used for all analysis. The analytical column was a cross-linked 5% phenyl polydimethylsiloxane (Rtx-5MS, Restek, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) column. The column oven temperature was held at 50 °C for 3 min and then programmed to 250 °C at a rate of 5 °C/min, and held at the final temperature for 10 min. The GC conditions were as follows: injector temperature: 250 °C; FID temperature: 250 °C; nitrogen carrier gas, 1 mL/min; hydrogen, 30 mL/min; air, 300 mL/min. The inlet was operated in a splitless mode.

Gas chromatography-mass spectrometry (GC-MS). A Trace GC 2000 and a GC-Q Plus ion trap MS^n (Thermoquest-Finnigan, Austin, TX, US) with electron impact ionization (EI) mode were used for identification of dried clove buds' flavors. Chromatographic separation was performed on a cross-linked 5% phenyl polydimethylsiloxane (SPB-5, Supelco, 30 m × 0.32 mm i.d. × 0.25 μm film thickness) column. The oven temperature was programmed to 50 °C (3 min) - 5 °C/min - 250 °C (10 min). Flow rate of He (99.9995 %) carrier gas was 1.0 mL/min, a split injection with a ratio of 1 : 10 was used, and the sample volume injected was 0.6 μL. The EI mass spectrometer was operated as follows: transfer line, 275 °C; ionization voltage, 70 eV; ion source temperature, 200 °C; mass range of scan mode, 50.0-500.0 unit. The major compounds of clove flavors were identified by comparing the retention time and comparison of the

obtained mass spectra of the relevant chromatographic peaks with those of authentic standards and with spectra of both the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Wiley (Wiley, New York, NY, USA) library.

Results and Discussions

Solvent selection. The choice of solvent for HS-LPME should be considered from physical properties of a low vapor pressure and high boiling point, in order to reduce vaporization of the solvent drop during the extraction process. In general, for LPME, the choice of organic solvent should be based on a comparison of selectivity, extraction efficiency, incident of drop loss, drop dissolution rate, and level of toxicity.[18] Also, it should have fewer impurities, which interfered with the determination of sample matrix, under very low concentration. Moreover, there is also a limit in detecting analytes when using a GC system due to the solvent peak, which may obscure early-eluting analytes.

In this study, 1-octanol (b.p. 194.4 °C), cyclohexane (b.p. 80.7 °C), *n*-decane (b.p. 174 °C), and *n*-hexadecane (b.p. 287 °C) were investigated to choose suitable solvents for HS-LPME. A single droplet of cyclohexane or *n*-decane was relatively easier to evaporate and to lose in a headspace within 1 min during extraction process. *n*-Hexadecane has ideal properties such as very low vapor pressure (0.1906 Pa, at 25 °C), and higher boiling point (287 °C). The primary shortcoming of *n*-hexadecane is that this solvent is strongly retained in a GC column.[25] Therefore, 1-octanol was selected as the extracting solvent because of its very low vapor pressure (9.3326 Pa, at 25 °C), good solubility of a large number of organic compounds, and the availability of 1-octanol-water partition coefficients (log $K_{o/w}$) for a large number of organic compounds.

Organic solvent drop volume. The influence of organic solvent drop volume on HS-LPME optimization was investigated in the range of 0.2-0.8 μL under the following conditions: amount of standards (liquid), 20 mg each in 2 mL vial; extraction time, 60 min; extraction temperature, 25 °C. As shown in Figure 2, the peak areas for analytes, especially for β-caryophyllene, increased with 1-octanol drop volume in the range of 0.2-0.6 μL and then showed steady signals. In contrast, for eugenol and eugenol acetate which have relatively higher boiling points and smaller log $K_{o/w}$ values than those of β-caryophyllene as shown in Table 1, the significant mass transfer of β-caryophyllene is probably attributable to a significant peak area.

When the organic solvent drop volume was over 1 μL, the 1-octanol drop became too unstable to be hung at the needle tip and occasionally fell down from the end of the microsyringe needle. Moreover, larger injection volume of organic solvent brought out huge band broadening. To avoid these drawbacks, drop size was fixed to 0.6 μL for further investigation.

Constant volume of liquid phase of 1-octanol should be taken in employing the experimental system of HS-LPME.

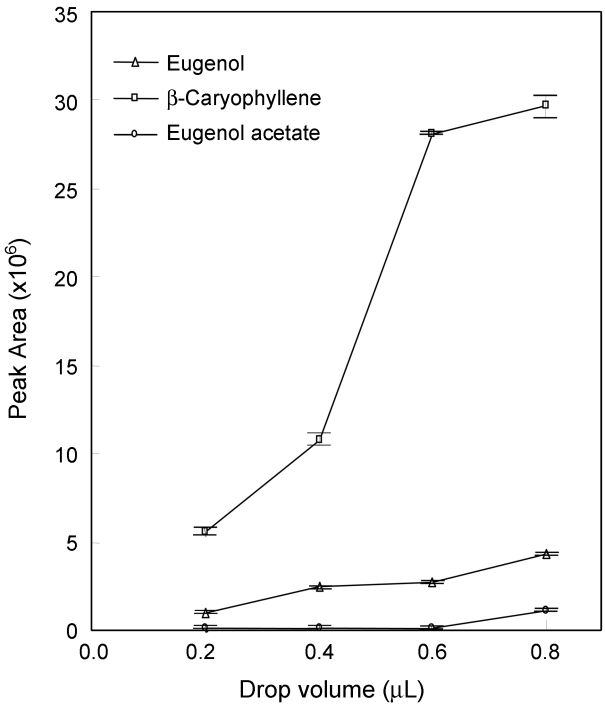

Figure 2. Effect of drop volume in HS-LPME on the analytical signal: amount of standards (liquid), 20 mg ea.; extraction time, 60 min; extraction temperature, 25 °C; 2 mL vial.

Considering the vapor pressure of liquid phase of 1-octanol (9.3326 Pa), evaporation of the 1-octanol drop may be negligible. Under the given experimental conditions, the number of moles of organic solvents needed to saturate the headspace can be estimated by using perfect gas equation of state: $PV = nRT$, where P is vapor pressure of 1-octanol (9.3326 Pa, at 25 °C), V is headspace volume occupied (2×10^{-6} m^3 for 2 mL vial, 15×10^{-6} m^3 for 15 mL vial and 25×10^{-6} m^3 for 25 mL vial), R is gas constant (8.3145 J K^{-1} mol^{-1}) and T is temperature (298 K). The numbers of moles (n) in the state of vapor pressure saturated was estimated to 7.53×10^{-10} mol for 2 mL vial, 5.65×10^{-10} mol for 15 mL vial and 9.42×10^{-9} mol for 25 mL vial, respectively. On the other hand, the number of moles (n) of 0.6 μL 1-octanol is estimated to 3.81×10^{-6} mol by the following calculation: (6×10^{-4} mL) × (0.827 g·mL^{-1}) / (130.23 g·mol^{-1}). It is found that the number of moles of vapor needed to saturate the headspace are significantly less than moles of 0.6 μL volume of 1-octanol hanging drop.

Extraction time. The effect of extraction time on HS-

Table 1. Physical properties of major aroma compounds from clove buds

Compound	Molecular weight	Boiling point (°C)	Log $K_{o/w}$	Vapor pressure (Pa, 25 °C)
Eugenol	164.20	255	2.27	3.0126
β-Caryophyllene	204.36	129	6.30	N/A
Eugenol acetate	206.24	281	3.06	0.4252

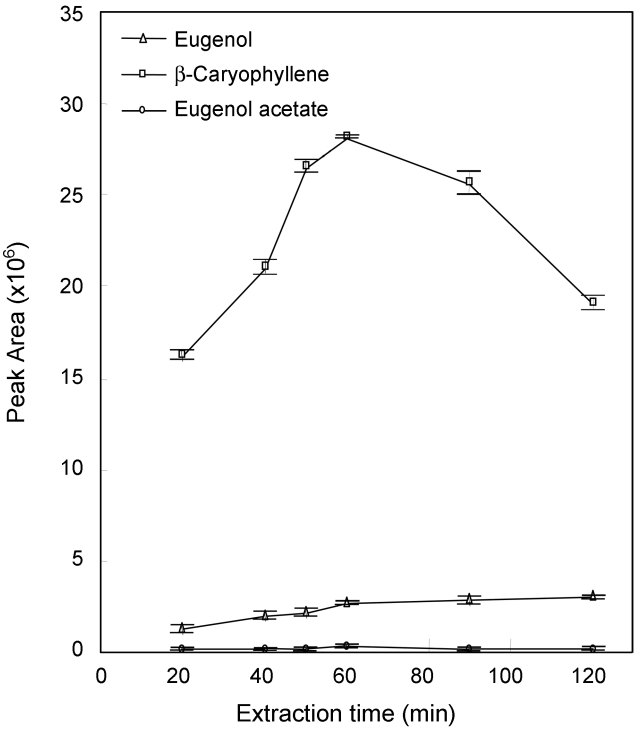

Figure 3. Effect of extraction time in HS-LPME on the analytical signal: amount of standards (liquid); 20 mg ea.; solvent volume 0.6 μL; extraction temperature 25 °C; 2 mL vial.

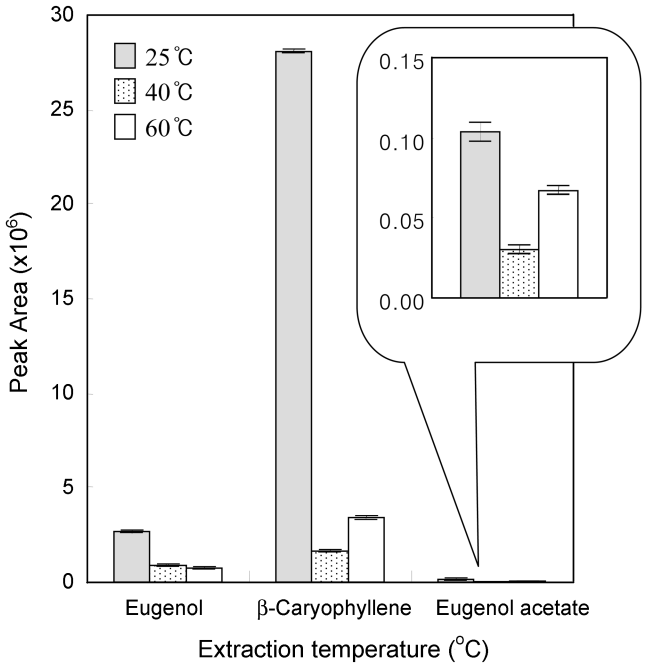

Figure 4. Effect of extraction temperature on HS-LPME: amount of standards (liquid), 20 mg ea.; extraction time, 60 min; solvent volume, 0.6 μL; 2 mL vial.

LPME was examined by monitoring the variation of the FID signal (peak area) with exposure time in the range of 20-120 min at room temperature (25 °C). The 0.6 μL 1-octanol drop was exposed to headspace over 20 mg each of working standards (liquid) in 2 mL vial for every 20 min from 20 min to 120 min at 25 °C. Figure 3 shows that peak areas increased with the extraction time until 60 min. After 60 min, no dramatic increase of peak area of eugenol and eugenol acetate was observed with additional extraction time, however, the peak areas of β-caryophyllene were decreased.

The amount of analyte transferred into the hanging drop is expected to increase with increasing its exposure time to the headspace of the sample vial. However, HS-LPME as well as HS-SPME is a partial extraction process depending on equilibrium rather than exhaustive extraction.[28] The amount of analyte extracted at a given time depends on the mass transfer of analyte from sample phase to the organic solvent phase of hanging drop through the headspace. Thus, the amount of analyte transferred into the hanging drop reaches its maximum when this equilibrium is established. Normally, the exposure time for establishing equilibrium was selected as the extraction time. In addition, drop depletion increased with extension of extraction time.[29] Figure 3 shows that the equilibrium is reached after about 60 min. Therefore, 60 min extraction time was chosen for further studies.

Extraction temperature. It is important to recognize that the effect of temperature on the mass transfer between gaseous phase and the organic solvent phase, because a partition coefficient between these two phases depends on temperature. The effect of the extraction temperature on the HS-LPME was investigated at 25, 40 and 60 °C, respectively. In this investigation, other conditions were set the same as mentioned above. The peak areas of eugenol and β-caryophyllene obtained at 25 °C were higher than those at 40 °C or 60 °C, as shown in Figure 4. This result suggests that if a higher temperature was maintained in HS-LPME using 0.6 μL drop volume, some of analytes dissolved in a hanging drop solvent were re-evaporated and returned into headspace. In the extraction process of the dried clove bud sample, depletion of organic solvent volume was also observed and more thermally degraded materials were observed when the temperature was elevated. In this study, the lower temperature is preferred to prevent degradation of thermally labile components and to observe flavor composition emitted from clove at ambient temperature. Consequently, all other investigations were carried out at 25 °C.

Effect of phase ratio between headspace volume and sample volume. The amount of analyte, n, present at equilibrium in the headspace over samples is described by the following equation[20,30]:

$$n = (K_{hs}V_h\, C_oV_s)/(K_{hs}V_h + V_s) \quad (1)$$

where K_{hs} is the headspace-sample distribution constant, C_o the initial concentration of the analyte, V_s and V_h the volumes of the sample and the headspace, respectively.

The Eq. (1) can be rearranged to the following new Eq. (2), Eq. (3) and Eq. (4) which involve the phase ratio (β) defined as headspace volume to sample volume. The n is correlated in terms of β by the relation of the forms:

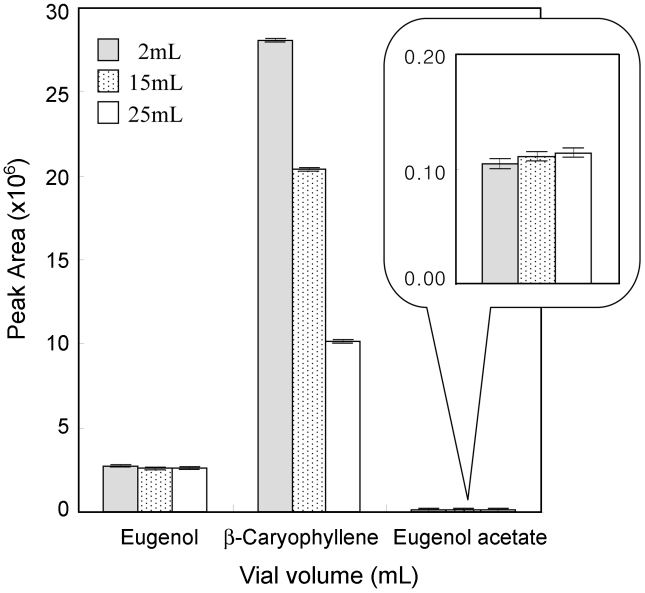

Figure 5. Effect of vial volume on HS-LPME: amount of standards (liquid), 20 mg ea.; extraction time, 60 min; solvent volume, 0.6 μL; extraction temperature, 25 °C.

$$n = C_o/\{(\beta/V_h) + (1/K_{hs}V_h)\} \quad (2)$$

$$n = C_o K_{hs}V_h / (\beta K_{hs} + 1) \quad (3)$$

$$1/n = (1/C_oV_h)\beta + (1/C_oV_hK_{hs}) \quad (4)$$

The influence of β on peak area was investigated by three different volumes of vial (2, 15, 25 mL) under the same experimental conditions as mentioned above. The relative peak areas obtained for 20 mg each standard (liquid) with different headspace volume are shown in Figure 5. As shown in Table 2 and Figure 5, the 2 mL vial has the smallest phase ratio, but it showed similar intensity of eugenol and eugenol acetate or relatively larger peak area of β-caryophyllene than those of 15 mL and 25 mL vials.

Extraction efficiency of HS-LPME. Partition coefficient (K) and concentration factors (CF) were measured to evaluate the extraction efficiency of HS-LPME. Both of them are widely used in evaluation of the relative extraction efficiency for a given analyte. In HS-LPME, the overall partition coefficient (K) is given by the equation[5,9]:

$$K = K_{hs} \cdot K_{lh} \quad (5)$$

where K_{hs} is defined as the analyte partition coefficient between the headspace gaseous phase and sample matrix; K_{lh} is the analyte partition coefficient between hanging drop microsolvent and the headspace gaseous phase; and K is the overall partition coefficient. Since K_{hs} is constant, under standardized equilibrium condition, K_{hs} can be eliminated from the above equation. Thus, the K_{lh} may be written as the following form :

$$K_{lh} = (A_lV_h) / (A_hV_l) \quad (6)$$

where A_l refers to the peak area of analyte on hanging drop microsolvent; V_h is the volume (5 mL) of the gas sample injected by static headspace GC using gas tight syringe (5.0 mL, Hamilton, # 1005, Reno, NV, USA); A_h is the peak area of analyte in the headspace; and V_l is the volume (0.6 μL) of the hanging drop solvent. The experimental K_{lh} values for characteristic components of clove flavors are summarized in Table 3.

The CF was also measured for the evaluation of extraction efficiency. The CF is defined as the ratio between the analyte areas obtained by HS-LPME with selected solvent drop and the corresponding area obtained by static headspace:

$$CF = A_l/A_h \quad (7)$$

As summarized in Table 3, the experimental K_{lh} values and relative CF values for major compounds of clove flavors exceed approximately 10^6 order and 10-710, respectively. Compounds with high K_{lh} values tend to partition more easily into the hanging drop from gaseous phase of headspace, and have relatively higher responses and lower limits of detection. It has been found in this study that HS-LPME has the good efficiency having the higher K_{lh} values and CF values.

Limit of detection and reproducibility. Based on a signal-to-noise ratio (S/N) of 3, the limits of detection (LOD) were 1.5 ng for eugenol, 2.7 ng and 3.2 ng for β-caryophyllene and eugenol acetate, respectively (Table 4). And the reproducibility of HS-LPME procedure was investigated on ten replicate samples under the optimized conditions listed above. Under the optimized conditions, the reproducibility of the proposed HS-LPME method for peak areas was 0.6% for eugenol, 0.3% and 5.7% for β-Caryophyllene and eugenol acetate, respectively.

Table 3. Partition coefficient (K_{lh}) between solvent drop and headspace gas phase, and concentration factors (CF)

Compound	K_{lh}	CF
Eugenol	5.93×10^6	712
β-Caryophyllene	2.77×10^6	332
Eugenol acetate	1.0×10^5	12

Table 2. The calculation of phase ratio

Compound	Phase ratio (β) = headspace volume/sample volume		
	2 mL vial	15 mL vial	25 mL vial
Eugenol	105.5	796.9	1330.5
β-Caryophyllene	89.8	680.8	1133.4
Eugenol acetate	107.1	809.8	1349.8

Table 4. Limits of detection (LOD) and reproducibility using HS-HDME

Compound	LOD (ng)	Reproducibility (%)
Eugenol	1.5	0.6
β-Caryophyllene	2.7	0.3
Eugenol acetate	3.2	5.7

Table 5. Characteristic mass spectral ions of volatile compounds identified in clove fragrances using 5% phenylpoly(dimethylsiloxane) column (SPB-5, Supelco, 30 m × 0.32 mm i.d. × 0.25 mm film thickness)

Compound	M_r	t_R	Base Peak m/z (100%)	Characteristic mass spectral ions (EI)
Methyl Salicylate	152	10.6	63	92(53.9), 53(44.8), 62(32.5), 64(24.8), 120(21.1), 65(20.8), 61(18.6), 51(15.2)
β-Cubebene	204	15.0	91	105(92.6), 204(54.7), 77(50.1), 81(48.6), 119(47.0), 79(44.8), 55(27.8), 65(26.0)
Eugenol	164	15.8	164	91(34.6), 77(26.6), 165(25.8), 103(24.0), 131(21.3), 51(15.0)
β-Caryophyllene	204	17.0	91	105(48.6), 79(47.2), 77(46.5), 93(27.7)
α-Caryophyllene	204	17.7	91	78(91.2), 77(75.7), 67(60.1), 93(53.2), 53(41.4), 80(35.6), 51(32.1)
Eugenol acetate	206	19.8	51	77(53.8), 164(41.3), 65(36.1), 63(34.9), 55(32.3), 103(21.9)

Application of HS-LPME for the analysis of clove sample. Eugenol, β-caryophyllene and eugenol acetate were identified by GC-MS with EI mode as major components of clove flavors. Characteristic mass spectral ions of volatile compounds from clove buds are listed in Table 5.

The amounts of eugenol, β-caryophyllene and eugenol acetate from the clove bud sample were 1.90 mg/g (± 2.4% RSD), 1.47 mg/g (± 6.3% RSD) and 7.0 mg/g (± 4.9% RSD), respectively.

Conclusion

HS-LPME has been shown to be an effective headspace technique for the volatile aroma compounds. HS-LPME coupled with GC-FID or GC-MS was successfully applied to determine eugenol, β-caryophyllene and eugenol acetate from the solid clove bud samples. Solvent selection, organic solvent drop volume, extraction time, extraction temperature and phase ratio should be considered to optimize this sampling technique. HS-LPME has the good efficiency demonstrated by the higher K_{lh} values and CF values. This hanging drop based method is a simple, fast and easy sample enrichment technique using minimal solvent. It can be concluded that HS-LPME is a novel sample preparation technique, which offers an attractive alternative to traditional and recently developed extraction techniques for the analysis of natural aromas.

Acknowledgements. This study was supported by Bahrom Grant from Seoul Women's University (2005).

References

1. Ulrich, S. *J. Chromatogr. A* **2000**, *902*, 167.
2. Kim, H. J.; Kim, K.; Kim, N. S.; Lee, D. S. *J. Chromatogr. A* **2000**, *902*, 389.
3. Kim, N. S.; Lee, D. S. *J. Chromatogr. A* **2002**, *982*, 31.
4. Rovio, S.; Hartonen, K.; Holm, Y.; Hiltunen, R.; Riekkola, M.-L. *Flavour Fragr. J.* **1999**, *14*, 399.
5. Kim, N. S.; Lee, D. S. *J. Sepa. Sci.* **2004**, *27*, 96.
6. Yoo, Z. W.; Kim, N. S.; Lee, D. S. *Bull. Korean Chem. Soc.* **2004**, *25*(2), 271.
7. Lee, S. N.; Kim, N. S.; Lee, D. S. *Anal. Bioanal. Chem.* **2003**, *377*, 749.
8. Lord, H.; Pawliszyn, J. *J. Chromatogr. A* **2000**, *885*, 153.
9. Bicchi, C.; Drigo, S.; Rubiolo, P. *J. Chromatogr. A* **2000**, *892*, 469.
10. Peñalver, A.; Pocurull, E.; Borrull, F.; Marcé, R. M. *Trends Anal. Chem.* **1999**, *18*, 557.
11. Liu, H.; Dasgupta, P. K. *Anal. Chem.* **1996**, *68*, 1817.
12. Jeannot, M. A.; Cantwell, F. F. *Anal. Chem.* **1996**, *68*, 2236.
13. He, Y.; Lee, H. K. *Anal. Chem.* **1997**, *69*, 4634.
14. de Jager, L. S.; Andrews, A. R. J. *Chromatographia* **1999**, *50*, 733.
15. Liu, W.; Lee, H. K. *Anal. Chem.* **2000**, *72*, 4462.
16. Psillakis, E.; Kalogerakis, N. *J. Chromatogr. A* **2001**, *907*, 211.
17. de Jager, L. S.; Andrews, A. R. J. *J. Chromatogr. A* **2001**, *911*, 97.
18. Wood, D. C.; Miller, J. M.; Christ, I. *LCGC North America* **2004**, *22*, 516.
19. Flórez Menéndez, J. C.; Fernández Sánchez, M. L.; Sánchez Uría, J. E.; Fernández Martínez, E.; Sanz-Medel, A. *Anal. Chim. Acta* **2000**, *415*, 9.
20. Zhao, R.; Lao, W.; Xu, X. *Talanta* **2004**, *62*, 751.
21. Kokosa, J. M.; Przyjazny, A. *J. Chromatogr. A* **2003**, *983*, 205.
22. Psillakis, E.; Kalogerakis, N. *Trends Anal. Chem.* **2002**, *21*, 54.
23. Psillakis, E.; Kalogerakis, N. *Trends Anal. Chem.* **2003**, *22*, 565.
24. Kim, N. S.; Yoo, Z. W.; Lee, S. N.; Lee, D. S. *Bull. Korean Chem. Soc.* **2005**, *26*, 1996.
25. Kim, N. S.; Yoo, Z. W.; Lee, S. N.; Lee, D. S. *Proceedings of the 7th Asian Conference on Analytical Sciences (ASIANALYSIS VII)*, Hong Kong Baptist Univ.: Hong Kong, **2004**, July, 28-31.
26. Raina, V. K.; Srivastava, S. K.; Aggarwal, K. K.; Syamasundar, K. V.; Kumar, S. *Flavour Fragr. J.* **2001**, *16*, 334.
27. Mandrioli, R.; Musenga, A.; Ferranti, A.; Lasaponara, S. S.; Fanali, S.; Raggi, M. A. *J. Sepa. Sci.* **2005**, *28*, 966.
28. Yamini, Y.; Hojjati, M.; Haji-Hosseini, M.; Shamsipur, M. *Talanta* **2004**, *62*, 265.
29. Zhao, L.; Lee, H. K. *J. Chromatogr. A* **2001**, *919*, 381.
30. Przyjazny, A.; Kokosa, J. M. *J. Chromatogr. A* **2002**, *977*, 143.