Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 27 No. 1, 60-65, 2013
http://dx.doi.org/10.5806/AST.2014.27.1.60

Analysis of E,E-farnesol and squalene in makgeolli using stir bar sorptive extraction coupled with gas chromatography-mass spectrometry

Jaeho Ha★, You-Shin Shim, Yongsun Cho, Dongwon Seo, Hyewon Jang and Hyejin Jang

Food Analysis Center, Korea Food Research Institute, 1201-62 Anyangpangyo, Bundang, Seongnam, Gyeonggi 463-746, Korea

(Received January 3, 2014; Revised January 15, 2014; Accepted January 20, 2014)

SBSE-GCMS를 이용한 막걸리 중의 E,E-farnesol과 squalene분석법

하재호★ · 심유신 · 조용선 · 서동원 · 장혜원 · 장혜진

한국식품연구원 식품분석센터

(2014. 1. 3. 접수, 2014. 1. 15. 수정, 2014. 1. 20. 승인)

Abstract: The aim of this study was to establish an analytical method for the determination of E,E-farnesol and squalene in makgeolli, which is a traditional type of Korean fermented rice wine. E,E-farnesol and squalene in makgeolli were extracted using stir bar sorptive extraction (SBSE) coupled with gas chromatography-mass spectrometry. SBSE was found to be an effective method for analyzing the E,E-farnesol and squalene levels in makgeolli. The linear dynamic range of the SBSE method for detecting E,E-farnesol and squalene ranged from 0.5 to 200 ng/mL with R^2=0.9974 for E,E-farnesol and 100 to 50000 ng/mL with R^2=0.9982 for squalene. The limit of detection and the limit of quantification using the SBSE method were 0.1 and 0.5 ng/mL for E,E-farnesol and 15.0 and 40.0 ng/mL for squalene, respectively. The average recoveries obtained were, quantitatively, 101-107% for E,E-farnesol and 98-103% for squalene, respectively, supporting the accuracy of the SBSE-GCMS method.

요 약: 전통주류의 한가지인 막걸리 중의 기능성 성분인 E,E-farnesol과 squalene를 분석하는 방법을 확립하였다. 막걸리에 들어있는 E,E-farnesol과 squalene은 stir bar sorptive extraction (SBSE)와 GC-MS를 사용 분석하였다. SBSE방법은 막걸리 중의 E,E-farnesol과 squalene함량을 분석하는데 매우 효율적이었다. 이 두 성분을 SBSE법으로 분석하였을 때 E,E-farnesol은 0.5-200 ng/mL (R^2=0.9974) 범위에서squalene은 100-50000 ng/mL (R^2=0.9982)에서 직선성을 보였다. SBSE법으로 분석할 경우 E,E-farnesol과 squalene의 limit of detection (LOD)와 limit of quantification (LOQ)는 각각 0.1 과 0.5 ng/mL 및 15.0과 40.0 ng/mL 이었다. SBSE법에 의한 E,E-farnesol과 squalene의 회수율은 각각101-107%와 98-103%로 정확도가 매우 높았다.

Key words: makgeolli, E,E-farnesol, squalene, SBSE-GCMS

★ Corresponding author
Phone : +82-(0)31-7809127 Fax : +82-(0)31-780-9280
E-mail : jhkfri@gmail.com

1. Introduction

Makgeolli is a type of traditional rice wine in Korea. Makgeolli is made from the mash of rice flour or rice starch with nuruk, which is used for saccharification, and various yeasts, which are used as the fermenting agent. Makgeolli is composed of approximately 90% water, 6-8% ethanol, 2-4% sediment consisting of 1.5-2.0% protein, and 0.5-1.0% carbohydrates originating from the yeast and the fermented rice wine.[1-3] *Saccharomyces cerevisiae* has been known to play a role of makgeolli fermentation, and it can produce various functional compounds during the fermentation process. Among these compounds, E,E-farnesol has been found in makgeolli and reported for the first time by Ha *et al*.[4] using the SBSE method to extract the semi-volatile compounds. Many studies have reported that farnesol and the related isoprenoids, perillyl alcohol and geraniol, are natural compounds found in many fruits and aromatic plants. The isoprenoid alcohol farnesol is an effective inducer of cell cycle arrest and apoptosis in a variety of carcinoma cell types. In addition, farnesol has been reported to inhibit tumorigenesis in several animal models, suggesting that it functions as a chemo-preventative and an anti-tumor agent *in vivo*. A number of different biochemical and cellular processes have been implicated in the growth-inhibitory and apoptosis-inducing effects of farnesol.[5-9]

Conversely, triterpenoid squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is the main hydrocarbon in the non-saponifiable fraction of olive oil, occurring in high concentrations between 0.8 and 12 g/kg in virgin olive oil.[10] The richest source of squalene is the liver of the Aizame (dogfish) shark (Squalas spp) of the southern Pacific oceans of Australia.[11] Squalene is a potential oxidation inhibitor; it can protect cells against free radicals, strengthen the body's immune system and decrease the risk for various cancers.[12] Because E,E-farnesol and squalene were found in makgeolli in our previous work, we analyzed E,E-farnesol and squalene in makgeolli using SBSE coupled with thermal desorption gas chromatograph (TD GC)-quadrupole mass spectrometry. In this study, the analytical method for the determination of E,E-farnesol and squalene in makgeolli was optimized and validated.[13-15]

2. Experimental

2.1. Materials and reagents

Makgeolli was purchased at local retail stores in Gyeonggi province, Jeolla province, Chungcheong province, Busan, and Seoul, Korea. The ethanol content of Makgeoglli varied from 6% to 8% between the different bottles. The makgeolli samples were stored at 4 ºC prior to analysis. Analytical grade ethanol (99.8%, Sigma-Aldrich), E,E-farnesol (Sigma-Aldrich) and squalene (Sigma-Aldrich) were used. Ultra-pure water was obtained from a Milli Q system (Millipore, Bedford, MA, USA).

2.2. Stir bar sorptive extraction (SBSE)

Before extraction, the magnetic stir bars (poly-dimethylsiloxane (PDMS), 0.5 mm film thickness, 10 mm length, 24 μL, Gerstel, Müllheim a/d Ruhr, Germany) were cleaned at 250 ºC for 30 min in a nitrogen atmosphere. Then, 20 mL of makgeolli after 100 fold dilution with 6% ethanol was introduced in to a standard 20 mL vial that contained a magnetic stir bar. The extraction of volatile compounds, E,E-farnesol and squalene was performed at room temperature at a rotation speed of 450 rpm for 1 hour. The stir bar was thermally desorbed in an on-line Gerstel thermal desorption unit (TDU), which was connected to the cooled injection system-program temperature vaporizing injector (CIS-PTV). Firstly, the CIS with a glass liner filled with Tenax TA was set at -20 ºC. Then, the parameters of the TDU system containing a stir bar were programmed as follows: hold at 40 ºC for 0.2 min, increase at a rate of 600 ºC/min to 260 ºC and hold for 2 min. After the TDU cooled down to 40 ºC, the CIS started the following program: hold at -20 ºC for 0.2 min, increase at a rate of 12 ºC/sec to 250 ºC, and hold for 5 min.

2.3. Method validation

Calibration solutions with concentrations of 0.50,

2.00, 20.00, 100, and 200 ng/mL were prepared by diluting E,E-farnesol with ethanol, and solutions with concentrations of 100, 500, 5000, 10000, and 50000 ng/mL were prepared by diluting squalene standards with acetonitrile. The linear range of the proposed method was studied by determining the calibration curves for the concentrations of interest. The automatic injection was performed at the optimum condition. Recovery tests using the E,E-farnesol and squalene standards were carried out on the makgeolli samples. The makgeolli samples were fortified with a range of E,E-farnesol (10, 50, and 100 ng/mL) and squalene concentrations (500, 2500, and 5000 ng/mL), respectively. The recovery of E,E-farnesol and squalene using the standard addition method was calculated as follows:

$$\text{Recovery } \% = [(C_t - C_u)/C_a] \times 100$$

Here, C_t is the total concentration of E,E-farnesol and squalene measured, C_u is the concentration of E,E-farnesol and squalene present in the original makgeolli, and C_a is the concentration of pure E,E-farnesol and squalene added to the original makgeolli.

2.4. Chromatography

All analyses of E,E-farnesol and squalene were performed on an Agilent 6890 GC system coupled to an Agilent MD 5973 quadruple mass spectrometer. The compounds were separated using a 5% diphenyl-95% dimethyl siloxane fused-silica capillary column (HP-5MS, 30 m length, 0.25 μm i.d., 0.25 μm film thickness, Agilent Technologies, Middleburg, OI, USA). The carrier gas was helium with a flow rate of 1.1 mL/min, and the splitless mode was used. The injector temperature was set at 250 °C. The following column temperature program was used during the run: initial temperature of 40 °C (hold for 20 min), then increase to 150 °C at a rate of 3 °C/min, and finally increase to 280 °C at a rate of 10 °C/min (hold for 15 min). The transfer line, ion source and quadrupole (q) analyzer temperature were maintained at 280 °C, 230 °C, and 150 °C, respectively. In the full-scan mode, the electron ionization mass spectra in the range of 35-350 m/z were recorded at 70 eV electron energy. The mass spectra were obtained in full-scan mode and compared with the Wiley 275 mass spectral database (Agilent Technologies, Santa Clara, CA, USA). The data recording and instrument control were performed with the MSD Chemstation software (G1701CA; version C.00.00; Agilent Technologies, Santa Clara, CA, USA). Ions with masses of 69, 81, and 93 were selected for the determination and confirmation of E,E-farnesol, and 69, 95, 136 were selected for the determination of squalene.

2.5. Statistical analysis

The recovery experiments were performed, and the results are expressed as the mean ± standard deviation (SD).

3. Results and Discussion

3.1. Extraction of E,E-farnesol and squalene

The analysis of the volatile compounds present in liquors is sometimes inefficient because of their high water and alcohol content, which can disrupt the adsorption of volatile compounds on the surface of the porous material used as a DHS absorbent.[16-17] By far the most effective and preferable method for semi-volatile compound analysis of wine has been the SPME method. However, SBSE is substantially more sensitive than SPME, provided that the same coating material is applied. SBSE has been widely used in several types of applications, especially in thermal desorption (TD) systems coupled with

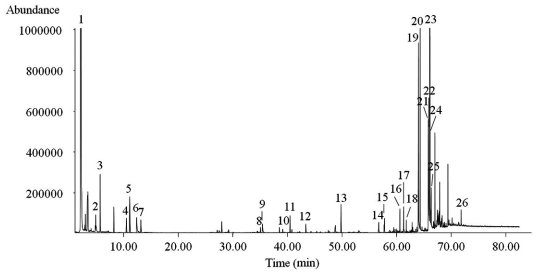

Fig. 1. The total ion chromatogram of the semi-volatile compounds isolated from makgeolli obtained by the SBSE-TD-GCMS

GC-MS analysis.[18-20] Compared with conventional techniques, SBSE has been shown to be an environmental friendly alternative due to its ease of use, high selectivity, high sensitivity and reproducibility, and short time requirement.[21-23] The total ion chromatogram of the semi-volatile compounds isolated from makgeolli obtained by the SBSE-TD-GCMS is shown in *Fig*. 1.

The relative abundance of several compounds, including ethanol, 3-methyl-1-butanol, and fatty acid ethyl esters, such as ethyl lactate, ethyl caproate, and ethyl caprylate, is presented in *Table* 1. E,E-farnesol and squalene were found in makgeolli for the first time, and their mass spectra are shown in *Fig*. 2. The complex biosynthesis of squalene has been studied

Table 1. Volatile and semi-volatile compounds in makgeolli obtained by SBSE-GCMS (n=2)

(Area count/10,000)

Peak No.	Compounds	Similarity	KI[1)]	Area count
1	ethanol	90	<700	110460
2	3-methyl butanol	86	727	2282
3	toluene	96	759	9221
4	ethyl benzene	96	849	3478
5	m-xylene	97	860	10828
6	1-butanol, 3-methyl-, acetate	90	872	4953
7	o-xylene	97	896	3521
8	nonanal	90	1102	1147
9	phenethyl alcohol	90	1111	2562
10	nonanol	90	1172	734
11	ethyl caprylate	97	1200	2828
12	acetic acid, phenylethyl ester	86	1256	1488
13	ethyl caprate	98	1390	4576
14	lauric acid	98	1589	2591
15	ethyl laurate	80	1597	1098
16	farnesol	81	1741	2407
17	myristic acid	99	1762	3395
18	ethyl myristate	95	1796	990
19	palmitic acid	98	1986	28114
20	ethyl palmitate	99	1988	19022
21	linoleic acid	98	2144	13856
22	oleic acid	99	2146	14262
23	ethyl linoleate	99	2165	25422
24	ethyl oleate	99	2180	17714
25	ethyl stearate	98	2195	2655
26	squalene	76	2847	1711

[1)]Kovat's index

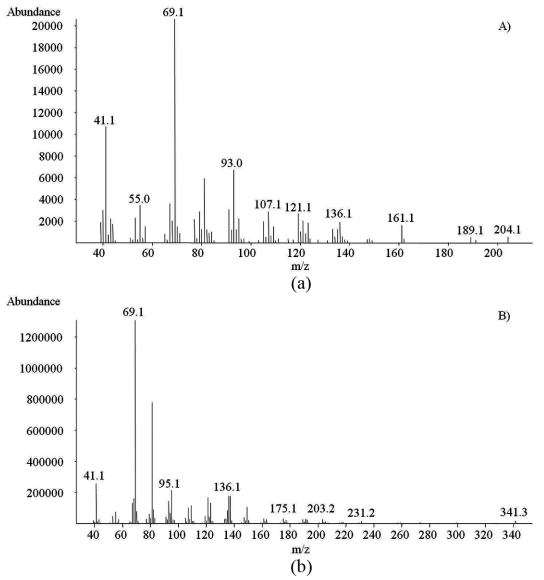

Fig. 2. Mass spectra of E,E-farnesol and squalene in makgeolli.

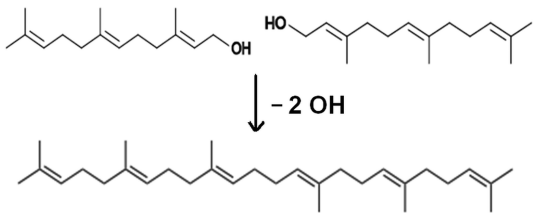

Fig. 3. The possible pathway of squalene formation from E,E-farnesol.

in remarkable detail.[24] It begins with the formation of the sesquiterpene alcohol farnesol, the pyrophosphate of which forms the presqualene alcohol pyrophosphate, which in turn is reduced to squalene by NADPH reductase after a series steps as illustrated in *Fig*. 3.[25] P. Bhattacharjee *et al.*[11] reported that the yield of squalene from *Saccharomyces cerevisiae* was found to be 41.16 μg/g, dry weight of yeast cells. It seemed that E,E-farnesol and squalene might be produced in makgeolli under anaerobic conditions by *Saccharomyces cerevisiae*.

3.2. Evaluation of the method performance

The SBSE method was used to determine the presence and concentration of E,E-farnesol and squalene in makgeolli. The results of the validation parameters of the SBSE method are listed in *Table* 2.

Table 2. Evaluation of the SBSE method performance

Analytes	Linear range, ng/mL	Linear Correl. (r^2)	LOD, ng/mL	LOQ, ng/mL	Amount added, ng/mL	Recovery[1] %
E,E-farnesol	0.5~200	0.9974	0.1	0.5	10	101 ± 5
					50	107 ± 4
					100	106 ± 3
Squalene	100~5000	0.9982	15.0	40.0	500	102 ± 5
					2500	98 ± 6
					5000	103 ± 4

[1]Values represent the mean of the triplicate analyses ± the standard deviation.

The linear range of E,E-farnesol and squalene levels detected by this method was from 0.5 to 200 ng/mL and from 100 to 50000 ng/mL with a correlation coefficient of 0.9974 and 0.9982, respectively. The limit of detection (LOD) and limit of quantification (LOQ) values were estimated at an SD/b ratio of 3 and 10, where SD and *b* stand for the standard deviation of the intercept and slope of regression line, respectively. The limits of detection of E,E-farnesol and squalene were 0.1 and 15.0 ng/mL, and the limits of quantification were 0.5 and 40.0 ng/mL[1], respectively. The recovery of E,E-farnesol from the 10, 50, and 100 ng/mL and the recovery of squalene from the 500, 2500, and 5000 ng/mL spiked makgeolli samples ranged from 101% to 107% and from 98% to 103%, respectively, indicating good accuracy of the SBSE-GCMS method (*Table* 2).

Table 3. Concentration[1] of E,E-farnesol and squalene in several makgeollis

Samples	E,E-farnesol, ng/mL	Squalene, ng/mL
1	92.3 ± 0.3	583.2 ± 0.7
2	73.6 ± 0.2	1205.5 ± 0.6
3	62.7 ± 0.6	1068.2 ± 0.3
4	41.4 ± 0.5	514.3 ± 0.6
5	44.9 ± 0.3	616.9 ± 0.5
6	103.2 ± 0.5	1919.7 ± 0.2
7	78.3 ± 0.8	572.4 ± 0.6
8	46.2 ± 0.6	794.3 ± 0.6
9	78.9 ± 0.4	1423.2 ± 0.8
10	73.2 ± 0.2	7431.1 ± 0.7
11	142.4 ± 0.4	1704.0 ± 0.2
12	33.6 ± 0.5	615.5 ± 0.3
13	68.2 ± 0.6	513.2 ± 0.3
14	71.7 ± 0.2	2756.7 ± 0.4
15	60.5 ± 0.2	562.7 ± 0.4
16	94.8 ± 0.6	3568.3 ± 0.3
Min[2]	33.6 ± 0.5	513.2 ± 0.3
Max[3]	142.4 ± 0.4	7431.1 ± 0.7
Average	72.6	1610.7

[1]Values represent the mean of the triplicate analyses ± the standard deviation.
[2]Maximum value.
[3]Minimum value.

3.3. Application of SBSE to makgeolli samples

To test the applicability of the SBSE method for the analysis of makgeolli samples, SBSE was used to determine E,E-farnesol and squalene in makgeolli. As shown in *Table* 3, the E,E-farnesol concentration in makgeolli ranged from 33.6 to 142.4 ng/mL, while the squalene concentration ranged from 513.2 to 7431.1 ng/mL. It seemed that the SBSE method was efficient and well suited for the analysis of E,E-farnesol and squalene in makgeolli and other liquors.

4. Conclusions

A sensitive, precise and effective SBSE method coupled with GC-MS was developed for the reliable analysis of E,E-farnesol and squalene in makgeolli, which is a traditional type of Korean fermented rice wine. E,E-farnesol and squalene in makgeolli were extracted using stir bar sorptive extraction (SBSE) coupled with gas chromatography-mass spectrometry. The SBSE method was found to be an effective method for analyzing the E,E-farnesol and squalene levels in makgeolli.

Acknowledgements

This research was supported by Korea Food Research Institute (ER136001) and we appreciate it.

References

1. J. Y. Kim and Y. H. Yi, *Korean J. Food Sci. Technol.*, **42**(6), 727-732 (2010).
2. J. H. Park, S. M. Bae, C. Yook and J. S. Kim, *Korean J. Food Sci. Technol.*, **36**(4), 609-615 (2004).
3. S. J. Lee and K. G. Lee, *J. Sci. Food Agr.*, **88**(4), 690-698 (2008).
4. J. Ha, Y. Wang, H. Jang, H. Seog and X. Chen, *Food Chem.*, **142**(1), 79-86 (2014).
5. J. H. Joo, G. Liao, J. B. Collins, S. F. Grissom and A. M. Jetten, *Cancer Res.*, **67**(16), 7929-7936 (2007).
6. S. Y. Lim and S. W. Park, *Yakhak Hoeji*, **50**(6), 372-380 (2006).
7. T. P. Ong, R. Heidor, A. de Conti, M. L. Z. Dagli and F. S. Moreno, *Carcinogenesis*, **27**(6), 1194-1203 (2006).
8. J. A. McAnally, M. Jung and H. Mo, *Cancer Lett.*, **202**(2), 181-192 (2003).
9. J. H. Joo and A. M. Jetten, *Cancer Lett.*, **287**(2), 123-135 (2010).
10. D. Grigoriadou, A. Androulaki, E. Psomiadou and M. Z. Tsimidou, *Food Chem.*, **105**(2), 675-680 (2007).
11. P. Bhattacharjee, V. B. Shukla, R. S. Singhal and P. R. Kulkarni, *World J. Microb. Biot.*, **17**(8), 811-816 (2001).
12. H. T. Lu, Y. Jiang and F. Chen, *J. Chromatogr. A*, **994**(1-2), 37-43 (2003).
13. J. Villén, G. P. Blanch, M. Ruiz del Castillo and M. Herraiz, *J. Agr. Food Chem.*, **46**(4), 1419-1422 (1998).
14. C. Samaniego-Sánchez, J. J. Quesada-Granados, H. López-García de la Serrana and M. C. López-Martínez, *J. Food Compos. Anal.*, **23**(7), 671-676 (2010).
15. N. Nenadis and M. Tsimidou, *J. Am. Oil Chem. Soc.*, **79**(3), 257-259 (2002).
16. M. Mestres, O. Busto and J. Guasch, *J. Chromatogr. A*, **881**(1-2), 569-581 (2000).
17. P. Chuenchomrat, A. Assavanig and S. Lertsiri, *ScienceAsia*, **34**, 199-206 (2008).
18. S. Insa, E. Anticó and V. Ferreira, *J. Chromatogr. A*, **1089**(1-2), 235-242 (2005).
19. R. F. Alves, A. M. D. Nascimento and JMF Nogueira. *Anal. Chim. Acta*, **546**(1), 11-21 (2005).
20. R. Perestrelo, J. M. F. Nogueira and J. S. Câmara, *Talanta*, **80**(2), 622-630 (2009).
21. R. Delgado, E. Durán, R. Castro, R. Natera and C. G. Barroso, *Anal. Chim. Acta*, **672**(1-2), 130-136 (2010).
22. D. J. Caven-Quantrill and A. J. Buglass, *J. Chromatogr. A*, **1218**(7), 875-881 (2011).
23. J. Marín, A. Zalacain, C. De Miguel, G. L. Alonso and M. R. Salinas, *J. Chromatogr. A*, **1098**(1-2), 1-6 (2005).
24. C. D. Poulter and H. C. Rilling, 'Biosynthesis of isoprenoid compounds', 1st Ed., Vol. 1, p455, John Wiley & Sons, Inc., New York, 1981.
25. M. Keller, D. Hafenbradl, K. O. Stetter, G. Teller, Y. Nakatani and G. Ourisson, *Angew. Chem. Int. Edit.*, **34**(17), 1898-1900 (1995).