

## Characterization of Fennel Flavors by Solid Phase Trapping-Solvent Extraction and Gas Chromatography-Mass Spectrometry

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Headspace solid phase trapping solvent extraction (HS-SPTE) and GC-MS was applied for the characterization of volatile flavors from fennel, anise seed, star-anise, dill seed, fennel bean, and Ricard aperitif liquor. Tenax was used for HS-SPTE adsorption material. Recoveries, precision, linear dynamic ranges, and the limit of detection in the analytical method were validated. There were some similarities and distinct differences between fennel-like samples. The Korean and the Chinese fennels contained *trans*-anethole, (+)-limonene, anisaldehyde, methyl chavicol as major components. The volatile aroma components from star anise were characterised by rich *trans*-anethole, (+)-limonene, methyl chavicol, and anisaldehyde. Additionally, principal component analysis (PCA) has been used for characterizing or classifying eight different fennel-like samples according to origin or other features. A quite different pattern of dill seed was found due to the presence of apiol (dill).

**Key Words :** Headspace solid phase trapping solvent extraction (HS-SPTE), GC-MS, Fennel, Anethole

### Introduction

Fennel (*Foeniculum vulgare* Miller) is biennial or perennial herb of Umbelliferae up to 2 meters high, with feathery leaves and golden yellow flowers. The fruit (Foeniculi Fructus, Korean; so-hoe-hyang) of fennel is a dry seed from 4-9 mm long, half as wide or less, and grooved. Dried fennel seed is an aromatic, anise-flavored spice, brown or green in color, they slowly turn a dull gray as the seed ages.

Fennel seed is used as a flavoring for foods and beverages, and the essential oil from the seed and plant is used in condiments, creams, soaps, perfumes, cosmetic, and pharmaceuticals. Fennel fruits are aromatic, stimulant, and carminative, analgesic, anti-inflammatory, antispasmodic, diuretic, emmenagogue, expectorant, galactagogue, hallucinogenic, laxative, stimulant and stomachic. Fennel can relieve intestinal gas accumulations and gastro intestinal spasm.<sup>1,2</sup>

Fennel seeds are sometimes confused with the anise seed (Anisi Fructus, *Pimpinella anisum*), which is very similar in taste and appearance, though smaller. In this respect, its use resembled that of anise and dill (Anethi Fructus, *Anethum sowa* or *Anethum graveolens*). In Asia, anise is less known, as fennel and star anise (*Illicium verum*) being more easily available and more popular. Fennel and fennel oil are listed in the Korean Pharmacopoeia (KP VIII-2), whereas aniseed (*Pimpinella anisum*), anise oil (*Illicium verum* or *Pimpinella anisum*), and powdered aniseed are listed in the British Pharmacopoeia. Anise may substitute fennel in Northern Indian recipes, but it is a less suited substitute for star anise in Chinese foods. In China, fennel is found in the well-known five-spice powder, along with star anise and cinnam-

on. China and Vietnam are major producers of *trans*-anethole from the star anise, and are supplying a large proportion of the demand.

Volatile compounds of fennel seeds extracted by simultaneous distillation-extraction (SDE)<sup>3</sup> and supercritical fluid extraction (SFE) showed similar compositions, with *trans*-anethole, estragole, and fenchone as the main components.<sup>4</sup> All available methods in the analytical scale can be applied to the extraction of flavors of plant origin. However, isolation techniques by either steam distillation or solvent extraction have some shortcomings, namely losses of thermolabile compounds, low extraction efficiency, long extraction time, degradation of compounds and toxic solvent residue. To overcome these problems several approaches have been used to extract volatile flavor compounds of plant origin. Alternative to conventional techniques, supercritical fluid extraction, subcritical water extraction at temperatures between 100 °C and 374 °C and a pressure high enough to maintain the liquid state, and head space solid-phase micro-extraction (HS-SPME) are also reported.<sup>5-11</sup>

The purpose of the present study is to develop a simple, rapid, sensitive and reproducible procedure for the quantitative determination of flavor compounds in the fennel and fennel-like samples. The method in the present paper for the extraction and determination of flavor compounds in fennel and fennel-like samples is based on the headspace solid-phase trapping solvent extraction (HS-SPTE) and gas chromatography-mass spectrometry (GC/MS).<sup>6,7</sup> Additionally, principal component analysis (PCA) has been used for characterizing or classifying eight different fennel-like samples according to origin or other features.<sup>12-14</sup> This approach could be employed in pattern recognition problems and chemotyping to distinguish fennel seed, anise seed, star anise and dill seed, because these samples have similar patterns of flavor compositions.

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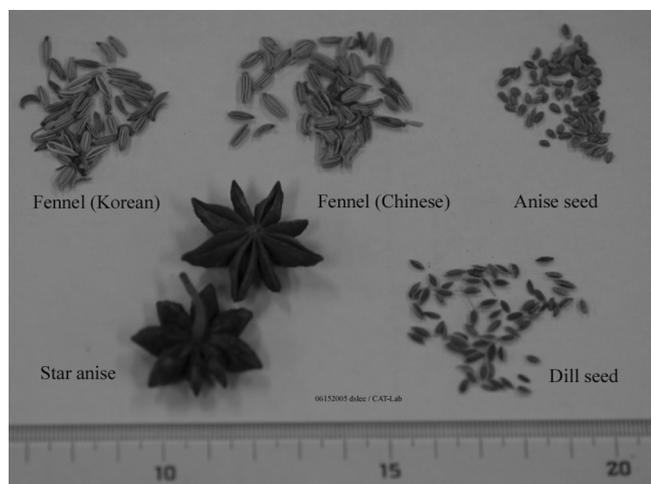
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## Experimental

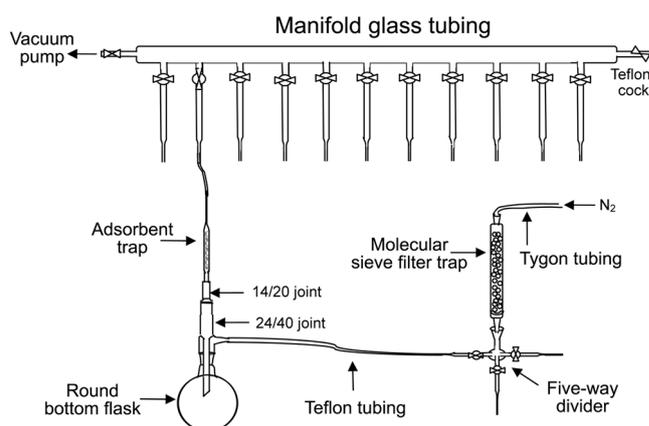
**Materials and reagents.** Eight fennel-like samples illustrated in Figure 1 were used in this study. Two different kinds of fennel (*Foeniculum vulgare*) samples were purchased from the local markets of Suncheon in Korea and Wuhan in China. The dried fruit samples of anise seed (*Pimpinella anisum*) and dill seed (*Anethum sowa*) were purchased from McCormick (Chicago, USA). Fennel bean (Hui Xiang Dou) is green bean steamed with fennel and salt, and purchased from the Xian Heng Jiu Dian (Shaoxing, Zhejiang, China). Star anise (*Illicium verum*) harvested in Guang-Xi of China was purchased from the local market of Wuhan (China) and fried star anise was commercial product from the Shanghai MeiFeng Food (China). Aperitif anise liquor (Ricard®) was 45% alcoholic liquor of anise seed and purchased from La Fee Verte (Marseille, France).

All working reference standards were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Tokyo Kasei (Nihonbashi, Tokyo, Japan). Organic solvents of chromatographic grade were purchased from Sigma-Aldrich.

**Headspace solid phase trapping solvent extraction (HS-SPTE).** Flavor compounds were collected from the fennel-like samples by using a HS-SPTE apparatus illustrated in Figure 2 designed in our laboratory.<sup>15,16</sup> Each sample (about 3 g) was filled in a 20 mL round flask, but different amounts were used for anise seed, star anise and fried star anise (50 mg each) and Aperitif anise liquor (10 mL) were used, respectively. Volatile flavor components were collected for 60 min at ambient temperature by use of the Pasteur pipet (0.565 cm i.d. × 15 cm length, Kimia Pajoooh Co. Ltd., Tehran, Iran) as a trap-housing which is packed with about 260 mg of 2,6-diphenylene-oxide polymer resin adsorbent (Tenax, particle size 250-177 μm, P/N 1-1982, Supelco, Bellefonte, PA, USA) and glass wool plugs, according to the procedure identical with that reported in our previous papers.<sup>16</sup> Prior to use, the Tenax trap was



**Figure 1.** Photographs of fennel seed, anise seed, star anise, and dill seed.



**Figure 2.** Scheme of solid-phase tapping-solvent extraction (SPTE) apparatus.

pre-rinsed with 2 mL of petroleum ether to remove impurities. The inlet of the Pasteur pipet was attached to the flask (20 mL) containing the fennel or fennel-like samples. An oil-free electric vacuum pump (Vacuubrand, Wertheim, Germany; diaphragm ME2 model, 2.4 m<sup>3</sup>/h) and a polytetrafluoroethylene (PTFE, Teflon) valve restrictor were connected with Tygon tubing to the outlet end of the trap via glass-manifold. A purified nitrogen gas (99.99%) flow *ca.* 400 mL/min was passed into a flask and out through the adsorbent trap under reduced pressure. After a run, the retained compounds were eluted by three extractions with 2 mL of petroleum ether. The elute was then further concentrated to a final volume of approximately 200 μL on water bath at 35 °C by using a Kuderna-Danish type device jointed with a Snyder column. Aliquots were analyzed by GC/MS.

**Gas chromatography-mass spectrometry (GC/MS).** GC/MS analyses were carried out by using a Trace GC 2000 and a GC-Q plus ion trap MS<sup>n</sup> (Thermoquest-Finnigan, Austin, TX, USA) with electron impact ionization mode. Chromatographic separations were performed on a fused silica cross-linked 6% cyanopropylphenyl-94%-dimethylsiloxane copolymer capillary column (DB-624, 30 m length × 0.25 mm i.d. × 1.4 μm film thickness, temperature limits -20 ~ 260 °C, J & W Scientific, Folsom, CA, USA). Flow rate of carrier gas (He, 99.9995%) was 1.0 mL/min. The injector temperature was 240 °C. A split injection with a ratio of 1:30 was used. The injected sample volume was 1 μL. The oven temperature program was 50 °C (3 min) - 5 °C/min - 220 °C (5 min). Transfer line temperature was 230 °C. The electron impact ionization mass spectrometer was operated as follows; ionization voltage, 70 eV; ion source temperature, 200 °C. The measuring mode was scanned from 50 to 350 mass ranges. The identity of volatile compounds was assigned by comparison of their Kovats retention indices (*I*), relative to the homologous series of *n*-alkane (C<sub>8</sub>-C<sub>20</sub>) and comparison of the obtained mass spectra of relevant chromatographic peaks with those of authentic standards and with corresponding spectra of the NIST and Wiley libraries.

**Analysis of enantiomers from fennel sample.** HP 5890

series II GC (Hewlett-Packard, Avondale, PA, USA) with flame ionization detector (FID) and a 30% heptakis (2,3-di-O-methyl-6-O-*t*-butyldimethyl-silyl)- $\beta$ -cyclodextrin column (Cyclosil-B, J&W, 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film thickness) were used to analyze enantiomers from fennel-like samples. The oven temperature program was 50 °C (3 min) - 5 °C/min - 220 °C (5 min); injector, 240 °C; detector, 250 °C; split ratio, 1:30; carrier gas flow rate of N<sub>2</sub>, 99.99%, 1 mL/min; air, 300 mL/min; hydrogen, 30 mL/min.

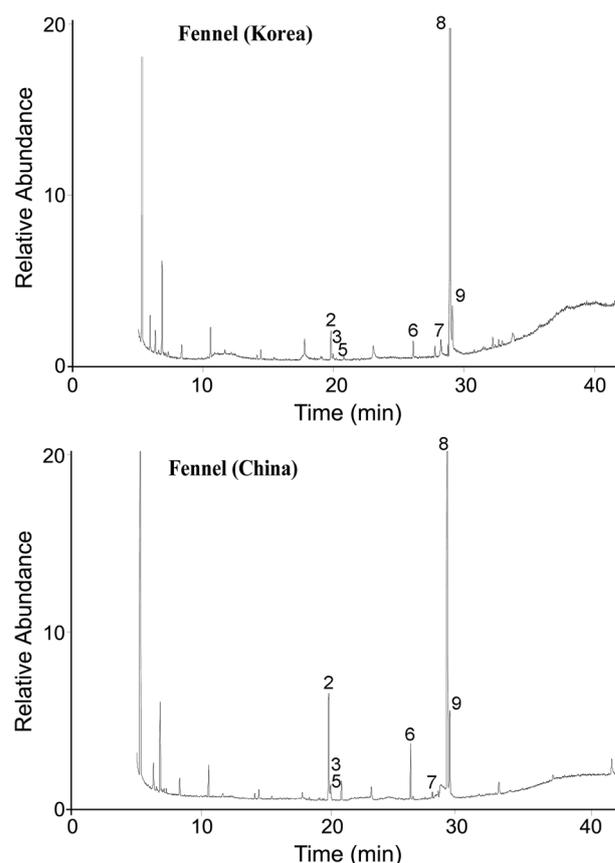
**Principal component analysis (PCA).** PCA was accomplished with multivariate statistical analysis program (MVSAP, version 4.0) software developed in our laboratory and pre-validated by using known values and data sets.<sup>12,14</sup> From a multivariate data matrix having  $p$  variables and  $n$  samples, principal component scores were computed by using MVSAP.

## Results and Discussion

**Identification of the chemical constituents in the fennel like samples by gas chromatography and mass spectrometry.** The total ion chromatograms (TIC) on a DB-624 column of the aroma components collected from fennel analyzed by HS-SPTE and GC-MS are shown in Figure 3.

Table 1 gives a list of 10 aroma components found for eight fennel-like samples. The retention indices as well as characteristic mass spectral ions of each peak are also given. The peak numbers in Figure 3 correspond to the numbers indicated in the first column of Table 1, in increasing order of retention time.

Comparison of aroma components found in fennels with those found in anise seed, star anise, dill seed, fennel bean, fried star anise, and Aperitif anise liquor is summarized in Table 2. Among the flavor compounds identified, *trans*-anethole was the primary component with relative percentile composition (normalized peak area %) of 78.07%-88.58% for fennel, 81.55% for anise seed, 95.71% for star anise, 88.76% for fried star anise, and 94.12% for Aperitif anise liquor. Fennel bean has similar flavor composition with fennels. Star anise and fried star anise contained *trans*-anethole, (+)-limonene, anisaldehyde, methyl chavicol,



**Figure 3.** Total ion chromatograms of flavors in fennel samples by SPTE-GC/MS.

carvone, phellandrene,  $\gamma$ -terpinene, and  $\beta$ -myrcene, whereas apiole (Dill), carvone and (+)-limonene were major components of dill seed investigated.

The enantiomeric analyses of limonene and carvone were undertaken using a cyclosil-B column with HS-SPTE and GC-FID. The enantiomer of limonene in the flavor emitted from Korean fennel seed was found in the (+) form. Chiral compounds from natural origins usually exist as one predominant optical isomer. Enantiomers show different odor properties. (+)-limonene was found to have an orange odor,

**Table 1.** Characteristic mass spectral ions of volatile flavor compounds identified from fennel-like samples

Peak No.	Compound	$t_R$ (min)	$I$	Mr	Base peak m/z	Characteristic mass spectral ions (EI) m/z (relative abundance %)
1	$\beta$ -Myrcene	18.20	1002	136	91	53(27), 69(30), 77(53), 91(100), 93(90), 107(5), 121(5), 136(7)
2	(+)-Limonene	19.88	1050	136	67	53(25), 67(100), 79(48), 93(46), 107(20), 121(17), 136(16)
3	<i>p</i> -Cymene	19.96	1052	134	119	65(4), 91(50), 119(100), 134(30)
4	$\alpha$ -Phellandrene	20.01	1054	136	91	51(5), 65(15), 77(65), 91(100), 93(60), 119(3), 136(15)
5	$\gamma$ -Terpinene	20.79	1076	136	91	50(15), 65(10), 77(50), 91(100), 93(65), 105(10), 121(15), 136(20)
6	Methyl chavicol	26.10	1245	148	148	50(40), 63(30), 77(45), 91(38), 105(32), 117(47), 113(25), 147(60), 148(100)
7	Carvone	28.22	1317	150	93	54(50), 65(10), 79(45), 91(80), 93(100), 108(60), 135(20), 151(50)
8	<i>trans</i> -Anethole	28.98	1345	148	148	51(11), 63(8), 77(24), 91(30), 115(22), 121(17), 133(12), 147(50), 148(100)
9	Anisaldehyde	29.10	1349	136	135	50(15), 63(20), 77(50), 92(12), 107(10), 135(100), 136(50)
10	Apiole (dill)	37.96	1686	222	222	51(11), 63(10), 77(20), 98(13), 106(13), 121(22), 149(30), 150(10), 177(50), 191(22), 207(40), 222(100)

$I$  = retention indices, Mr = relative mass

**Table 2.** Flavor compositions of fennel-like samples identified by SPTE and GC-MS

Peak No.	Compound	Fennel (Korea)	Fennel (China)	Anise seed	Star anise
1	$\beta$ -Myrcene	–	–	–	0.04 $\pm$ 4.24
2	(+)-Limonene	1.46 $\pm$ 6.30	6.17 $\pm$ 1.17	–	0.81 $\pm$ 2.00
3	<i>p</i> -Cymene	0.41 $\pm$ 5.70	0.69 $\pm$ 3.82	–	–
4	$\alpha$ -Phellandrene	–	–	–	0.07 $\pm$ 6.32
5	$\gamma$ -Terpinene	0.95 $\pm$ 8.86	1.01 $\pm$ 8.21	–	0.02 $\pm$ 6.03
6	Methyl chavicol	0.81 $\pm$ 6.10	2.98 $\pm$ 0.08	0.97 $\pm$ 1.08	0.31 $\pm$ 5.55
7	Carvone	1.23 $\pm$ 5.16	2.27 $\pm$ 5.05	–	0.12 $\pm$ 8.00
8	<i>trans</i> -Anethole	88.58 $\pm$ 1.07	78.07 $\pm$ 1.74	81.55 $\pm$ 0.01	95.71 $\pm$ 0.01
9	Anisaldehyde	2.87 $\pm$ 8.95	4.78 $\pm$ 1.36	12.10 $\pm$ 0.21	0.44 $\pm$ 3.20
10	Apiole (Dill)	–	–	–	–

Peak No.	Compound	Dill seed	Fennel bean	Fried star anise	Aperitif Anise liquor
1	$\beta$ -Myrcene	–	–	0.16 $\pm$ 1.00	–
2	(+)-Limonene	10.45 $\pm$ 3.80	20.83 $\pm$ 1.08	3.83 $\pm$ 4.20	–
3	<i>p</i> -Cymene	–	2.21 $\pm$ 1.98	–	–
4	$\alpha$ -Phellandrene	–	–	1.83 $\pm$ 3.02	–
5	$\gamma$ -Terpinene	–	0.84 $\pm$ 4.63	0.37 $\pm$ 0.06	–
6	Methyl chavicol	2.34 $\pm$ 1.70	0.63 $\pm$ 5.69	0.41 $\pm$ 0.51	0.78 $\pm$ 8.97
7	Carvone	24.26 $\pm$ 0.79	–	–	–
8	<i>trans</i> -Anethole	4.92 $\pm$ 2.44	11.51 $\pm$ 0.07	88.76 $\pm$ 0.08	94.12 $\pm$ 0.14
9	Anisaldehyde	0.97 $\pm$ 4.25	2.33 $\pm$ 3.61	2.44 $\pm$ 0.09	3.32 $\pm$ 1.95
10	Apiole (Dill)	39.20 $\pm$ 0.97	–	–	–

unit: normalized peak area (%)  $\pm$  RSD, –: Not detected

while (–)-limonene was found of terpenine type.<sup>17</sup> However, carvone enantiomers were not resolved under the given conditions.

**Validation of the analytical method of HS-STPE and GC/MS.** The accuracy of the analytical procedure was assessed by the absolute recovery study. A pair of test samples was prepared for analysis as follows: The first test sample is prepared by adding known amount of authentic standard to a known amount of sample specimen that contains the sought for analyte. The second one is the same known amount of unspiked sample specimen. Both of the test samples were analyzed by the experimental procedure. Triplicate measurements were made on all test samples. Percentile recovery was calculated by comparison of added and found amounts of analyte in spiked samples using the following equation.

$$\% \text{ Recovery} = [(A_{\text{o+s}} - A_{\text{o}}) / A_{\text{s}}] \times 100$$

where  $A_{\text{o+s}}$  is peak area for the sample matrix spiked with known amount of analyte;  $A_{\text{o}}$  is peak area for the analyte of the unspiked sample matrix;  $A_{\text{s}}$  is peak area for the known amount (same as the spiked amount) of authentic analyte standard.

The absolute recovery test of three major compounds was carried out. As shown in Table 3, about 45%–53% recoveries were obtained for *trans*-anethole, methyl chavicol, and anisaldehyde. This result means that no exhaustive extraction of three compounds could be obtained by HS-SPTE, because the headspace extraction is a technique of partial extraction.

The precision of the measurements was evaluated using

**Table 3** Recoveries of SPTE-GC/MS for three major flavor compounds from spiked anise seeds ( $n = 3$ )

Compound	Amount spiked (ng)	Recovery ( $\pm$ RSD)
<i>trans</i> -Anethole	50	45.95% ( $\pm$ 4.39)
Methyl chavicol	10	45.33% ( $\pm$ 17.38)
Anisaldehyde	10	53.42% ( $\pm$ 8.32)

the results obtained by repeated analysis of samples. The reproducibility of the SPTE-GC/MS for four major compounds as the relative standard deviation (RSD) of the mean amount and accuracy (%) is reported in Table 4. RSDs ( $n = 3$ ) range from 1.23 to 4.37 and accuracy (%) ranges from 1.55 to 7.30 for the mean amount.

Standard calibration curves for limonene, methyl chavicol, *trans*-anethole, and anisaldehyde are given in Table 5. As represented in Table 4 and 5, a good linearity peak area vs. concentration was observed in between picogram and nanogram scale, depending on the compound. This is lower than those observed by Shukui *et al.* in the microgram scale.<sup>18</sup> Despite this, the correlation coefficients ( $R^2$ ) value exceeds 0.97, and hence appears to be a good linearity.

At high concentrations, the curves deviate from linearity. Since analytical measurements must be made using the linear part of the curve, such linear dynamic ranges are suitable for the analysis of fennel-like sample without dilution.

The limit of detection (LOD) values based on a signal-to-noise ratio (S/N) of 3 were very low, ranging from 0.11 pg/ $\mu$ L for anisaldehyde to 1.09 pg/ $\mu$ L for methyl chavicol (Table 5).

**Application to quantitative analyses of real samples.**

**Table 4.** Reproducibility of the SPTE-GC/MS for four major compounds

Compounds	Real concentration ( $\mu\text{g/mL}$ )	Running #	Amount			
			Found ( $\mu\text{g/mL}$ )	Mean ( $\mu\text{g/mL}$ )	RSD (%)	Accuracy (%)
(+)-Limonene	40	1	38.75	39.38	1.48	1.55
		2	39.91			
		3	39.49			
Methyl chavicol	20	1	21.60	21.44	1.23	7.20
		2	21.58			
		3	21.13			
<i>trans</i> -Anethole	50	1	54.94	53.65	2.77	7.30
		2	52.02			
		3	53.98			
Anisaldehyde	15	1	16.09	15.45	4.37	3.00
		2	14.74			
		3	15.52			

**Table 5.** The limit of detection, dynamic range, and response factor for the determination of five major volatile flavor compounds in fennel-like samples using SPTE-GC/MS

Compounds	LOD ( $\text{pg}/\mu\text{L}$ )	Dynamic range	RF (Area/ng)	Eq. of calibration curve ( $R^2$ )
(+)-Limonene	0.77	0.77 pg - 80 ng	0.046	$y = 0.0461x - 0.1006$ (0.9980)
Methyl Carvicol	1.09	1.09 pg - 30 ng	0.088	$y = 0.0884x - 0.122$ (0.9888)
Carvone	0.69	0.69 pg - 90 ng	0.018	$y = 0.1825x - 0.631$ (0.9901)
<i>trans</i> -Anethol	0.59	0.59 pg - 150 ng	0.089	$y = 0.0888x - 0.2395$ (0.9919)
Anisaldehyde	0.11	0.11 pg - 40 ng	0.045	$y = 0.0448x - 0.225$ (0.9769)

The external method has been employed to determine the major flavor compounds in fennel-like samples. Quantitative data obtained by external calibration method is summarized in Table 6. There are some similarity and distinct differences among fennel-like samples. The Korean and the Chinese fennels contained *trans*-anethole, limonene, anisaldehyde, and methyl chavicol (estragole or *iso*-anethole). The volatile aroma component from star anise investigated was characterized by rich *trans*-anethole, limonene, anisaldehyde, carvone, and methyl chavicol. Several other plants emanate aroma similar to that of anise (*Pimpinella anisum*). To a lesser extent, dill (*Anethum sowa* or *Anethum graveolens*) also resemble anise, although their anise fragrance is not so much pure as the former mentioned plants are.

It can be seen that the volatile aroma composition of Aperitif anise liquor showed quite similar compositional pattern with anise seed. There are many applications of anise-flavored liquors in different Mediterranean countries: *Pastis* in France, *Raki* in Turkey, and *Ouzo* in Greece. In many cases, oil of anise is substituted by oil of star anise in these products, at least partially.

The amounts of secondary compounds like volatile aroma compounds are affected by genetic and geographical factors, climate, soil, and cultivation techniques. Other studies<sup>19</sup> have demonstrated, using steam distillation techniques, the presence of *trans*-anethole as the major compound of the essential oils of fennel and anise seed. Anise seed and star anise contain 1%-4% and about 5% volatile oil, respectively. According to the recent research by Gurbuz,<sup>20</sup> essential oil levels of Turkish anise varied from 1.3% to 3.7% and its

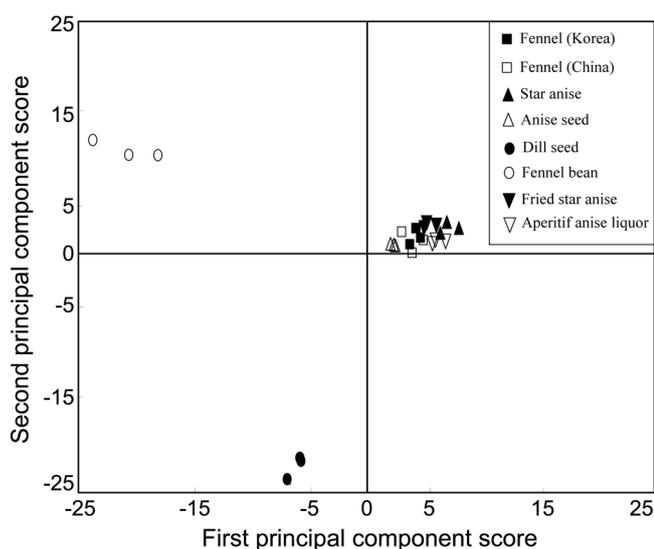
major component was *trans*-anethole ranging from 78.63% to 95.21%. Oil of anise or star anise has carminative and expectorant properties, but its major component (anethole) has been reported to be the cause of dermatitis in some people. Anethole has two isomers (*trans* and *cis*) with the *cis* isomer being 15-38 times more toxic to animals than the *trans* one, depending on species. The *trans*-anethole is more stable than the *cis* isomer. In natural resources, *trans* form is the major component, but only the trace amount of the *cis* isomer is present.<sup>21</sup> Anethole was formerly considered as an active estrogenic agent of the essential oil of anise. However, further research suggests that the active estrogenic compounds are polymers of anethole, such as dianethole and photoanethole.<sup>2</sup>

The pharmacologic effects of *trans*-anethole most often noted are reduction in motor activity, lowering of body temperature and hypnotic, analgesic, and anticonvulsant effects. *trans*-Anethole is largely used as a substrate for the synthesis of various pharmaceutical substances such as chloral, an anticonvulsant agent, diethyl stielbesterol and phenobarbital. The antioxidant activity of star anise oil is due to the high percentage of *trans*-anethole.<sup>22</sup> In addition, *trans*-anethole possesses muscle relaxant effect.<sup>23</sup> Anethole was evaluated for acceptable daily intake (ADI) by the joint FAO/WHO Expert Committee on Food Additives (FAO, 1968) in 1967 and given a conditional ADI of 0-1.25 mg/kg body weight.

At low concentrations, *trans*-anethole loses its licorice flavor and gives a sweet flavor. Therefore, very small amount of *trans*-anethole is used in many types of toothpaste to

**Table 6.** Quantitation of five major flavor compounds from fennel-like samples ( $n = 3$ )

Sample	(+)-Limonene (ng/g)	Methyl chavicol (ng/g)	Carvone (ng/g)	<i>trans</i> -Anethole (ng/g)	Anisaldehyde (ng/g)
Fennel Suncheon, Korea	1.26 ± 2.21	0.62 ± 3.08	2.34 ± 0.67	18.79 ± 2.39	2.46 ± 1.78
Fennel Wuhan, China	5.20 ± 0.40	1.60 ± 1.32	2.11 ± 2.27	30.73 ± 5.12	4.99 ± 3.77
Anise seed McCormick, USA	–	6.32 ± 1.07	–	1.82 (×10 <sup>2</sup> ) ± 3.36	1.64 (×10) ± 2.89
Star anise Wuhan, China	1.41 (×10 <sup>3</sup> ) ± 1.79	2.70 (×10 <sup>2</sup> ) ± 1.36	6.67 ± 4.23	1.61 (×10 <sup>4</sup> ) ± 0.91	1.54 (×10 <sup>2</sup> ) ± 4.81
Dill seed McCormick, USA	3.14 ± 3.57	0.83 ± 2.11	15.26 ± 1.26	4.33 ± 6.14	1.64 ± 4.00
Fennel bean Xianheng, Shaoxing, China	1.88 ± 2.98	0.59 ± 1.15	–	1.75 ± 2.09	1.40 ± 0.08
Fried star anise Shanghai MeiFeng Food, China	1.04 (×10) ± 1.09	7.25 ± 4.10	–	7.43 (×10 <sup>2</sup> ) ± 4.72	2.86(×10) ± 1.05
Aperitif anise liquor RICARD®, Marseille France	–	0.19 ± 0.72	–	1.80 (×10 <sup>2</sup> ) ± 1.12	3.15 ± 6.25

**Figure 4.** The PCA plot based on the normalized peak area (%) for flavor compositions of fennel-like samples by SPTE and GC-MS.

make them sweet without adding sugar.<sup>24</sup>

Minor components as well as major components found from botanical materials may be typical for some varieties and provoke a distinction of chemotypes or aroma characteristics. For example, in dill seed, four chemotypes are known, based on the presence of myristicin, or apiole (Dill), or both, or none of them.<sup>25</sup> Indian dill seed (*Anethum sowa*) contains dillapiole, while European dill seed (*Anethum graveolens*) does not contain apiole (Dill). According to Bailer *et al.*,<sup>26</sup> limonene and carvone are the major constituents of European dill seed as well as caraway essential oils. Some authors suggested that carvone/limonene ratio of the dill seed oil mirrors, with astonishing fidelity, the variations of the essential oil contents. Carvone has potential uses for inhibiting the growth of bacteria and some fungi, and for an insect repellent. The most important technical application of carvone is the use as a reversible suppressant of sprouting in stored potatoes and flower bulbs.

Dill seeds contain volatile oil with concentration varying from 1.2% to 7.7% according to geographical origin and seasons. The quality of dill oil can fluctuate greatly, depending on the percentage of seed oil, and the physiological maturity of the seeds used for oil. The time of harvest may

also be significant, because carvone is synthesized during the day from phellandrene breakdown. Dill herb oil is sometimes adulterated with terpenes from other sources. Both myristicin and apiole (dill) present in dill oil are effective natural insecticides. Myristicin is also known to be responsible for psychoactive and hallucinogenic properties in some other plants, and the content of apiole may be responsible for diuretic properties.

**Flavors pattern recognition by principal component analysis.** PCA was employed to provide an overview of capacity to distinguish fennel-like flavors based on GC/MS data sets as tabulated in Table 2 and 6. The plots in Figure 4 illustrated the first two principal components scores as a dimension reduction device. A PCA plot showed some important features of the data set such as a significantly different pattern and the presence of characteristic compounds in a sample. As shown in Figure 4 and Table 2, a quite different pattern of dill seed is found due to the presence of apiole (dill). Dill seed containing a different flavor composition can be easily distinguished from other fennel-like samples by the PCA plot. The star anise can be distinguished from other samples based on the very rich *trans*-anethole (Table 6). These results suggest that PCA is very useful for many aspects of spice flavor industry, including pattern recognition or primary evaluation of category similarity, detection of adulterants, and quality control.

## Conclusion

Headspace-solid phase trapping solvent extraction (HS-SPTE) is an effective analytical method for the volatile flavor compounds from fennel, anise seed, star-anise, dill seed, fennel bean, and Ricard aperitif liquor. It has the good linear dynamic range ( $R^2 > 0.97$ ), which was observed in between pictogram and nanogram scale, and low detection limit ( $LOD < 1.09 \text{ pg}/\mu\text{L}$ ). In addition, principal component analysis (PCA) was useful for the characterization or classification of fennel-like samples according to origin or other features. There are some similarity and distinct differences among fennel-like samples.

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