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Evaluation of proximate composition, bioactive lignans and volatile composition of *Schisandra chinensis* fruits from Inje and Mungyeong, Republic of Korea

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ABSTRACT

The present study aimed to determine the proximate composition, mineral content, active ingredients (schisandrin, schisandrin A, gomisin A and gomisin N) and volatile composition of *Schinsandra chinensis* fruits collected from Inje and Mungyeong, two major cultivating areas in Republic of Korea. The bioactive ingredients were determined by high performance liquid chromatography (HPLC). The volatile composition of the supercritical carbon dioxide extracts (SFE) from the fruits of *S. chinensis* was determined by solid phase microextraction (SPME) - gas chromatography/mass spectrometry (GC/MS). The proximate composition was found to be higher in Mungyeong than Inje samples. The minerals such as Mn, Ca and P were higher in the samples obtained from Inje when compared with Mungyeong. The active ingredients such as schisandrin (5018.67 µg/g), gomisin N (4772.87 µg/g) and schisandrin A (717.18 µg/g) were found to be higher level in Mungyeong samples with the exception of gomisin A. The SPME–GC/MS analysis revealed the identification of 40 components from each place, representing 97.22% (Inje) and 96.81% (Mungyeong) of the SFE. Ylangane, α-himachalene, longipinene and italicene were detected as the major components in the SFE. In conclusion, Inje and Mungyeong are the suitable places to collect *S. chinensis* fruits with higher level of nutrient and chemical contents.

INTRODUCTION

The genus *Schisandra* belongs to the family of Schisandraceae and contains 23 deciduous vine species that are widely distributed in East Asia. In the genus, *Schisandra chinensis* (Turcz.) Baill. is an important traditional medicinal plant and mainly cultivated in northeastern regions of China, Japan, Korea and Russia (Sun *et al.*, 2010). This cash crop has been cultivated in alpine areas of Republic of Korea and the quality has been known to be determined by the climate of cultivating areas. The fruits of *S. chinensis* (Korean name - omija) are traditionally used for the treatment of various disorders such as cough, spontaneous sweating, palpitation, spermatorrhea, dyspnea, kidney disorders, mouth dryness, dysentery and amnesia (Wang *et al.*, 2008; Teng and Lee, 2014). The fruits contain various bioactive components including essential oil, organic acids, vitamins, lignans, terpenes, amino acids, polysaccharides, etc (Huang *et al.*, 2008; Stacchiotti *et al.*, 2009; Gao *et al.*, 2009; Lu and Chen, 2009). Among them, lignans and the essential oil with terpenes are the most bioactive ingredients in the fruits of *S. chinensis* (Wang *et al.*, 2008). Pharmacological studies have showed that the lignans exhibited various biological activities, including hepatoprotective, anti-cancer, antioxidant, cardioprotective, adaptogenic and central nervous system protecting activities etc. (Jiang *et al.*, 2005; Huang *et al.*, 2007; Hu *et al.*, 2012; 2013). Owing to the wide range of bioactive properties, the consumption of *S. chinensis* fruits has gained increasing popularity as dietary supplements.

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In addition to lignans, the volatile components from the fruit of *S. chinensis* were widely used in pharmaceuticals and cosmetic industries. The steam distillation or hydrodistillation is a conventional method widely used to isolate the volatile components from various medicinal and aromatic plant materials (Herrero *et al.*, 2010).

However, the conventional techniques have many disadvantages such as low extraction efficiency, longer extraction time and toxic solvent residue. Further, the essential oil quality is also highly affected due to the effect of the high temperatures (Fornari *et al.*, 2012).

Previously, some authors have reported the volatile composition of *S. chinensis* fruits obtained from steam distillation, supercritical fluid extraction (SFE), Soxhlet, microwave assisted extraction and headspace solid-phase micro-extraction (SPME) methods (Deng *et al.*, 2003; Li *et al.*, 2003; Wang *et al.*, 2008; Chen *et al.*, 2012; Teng and Lee, 2014). In recent times, SFE has attracted significant attention as an effective and environmentally friendly extraction technique to replace conventional methods (Capuzzo *et al.*, 2013). The SFE method has been developed significantly in the extraction of *S. chinensis* fruits, but most of the studies are related to the extraction of lignans (Wang *et al.*, 2008). Furthermore, SPME combined with GC/MS is a novel technique, solvent-free, rapid and very simple to extract and detect the volatile components (Delgado *et al.*, 2010). Hence, SPME-GC/MS was used in the present study to analyze the volatile composition of SFE from the fruits of *S. chinensis* .

According to the previous results, there were considerable qualitative and quantitative variations in the volatile composition from the fruits of *S. chinensis* and these variations might be influenced by various biotic and abiotic factors (Dhouioui *et al.*, 2016). In the previous studies, the samples were mainly collected from China in relation to volatile composition of *S. chinensis* .

In addition, Inje and Mungyeong are the two major cultivating areas of *S. chinensis* in Republic of Korea, but there has been no comparative study on the nutritional and chemical composition of this fruit. Further, there is no report on volatile composition of SFE of *S. chinensis* fruits using SPME-GC/MS. Therefore, the present study was designed with the aim of determining and comparing the proximate composition, mineral content, active ingredients and volatile composition of *S.chinensis* fruits collected from Inje (Gangwon-do) and Mungyeong (Gyeongsangbuk-do) in Republic of Korea.

MATERIALS AND METHODS

Materials

The matured and dried fruits of *S. chinensis* were procured from different locations of Inje (10 places) and Mungyeong (10 places) in Republic of Korea during February 2014 (Fig. 1). The lignans such as schisandrin, schisandrin A, gomisin A and gomisin N were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). All other chemicals and solvents used in the study were of analytical grade.

Fig. 1: Map of Inje and Mungyeong, the two major cultivating areas of *Schinsandra chinensis* fruits in Republic of Korea.

Analysis of proximate composition

In the proximate composition, moisture, crude protein, crude lipid, crude fiber and ash contents of *S. chinensis* fruit samples were determined by standard methods (AOAC, 2000). Moisture content of sample was determined using the direct drying method. The sample was dried in a hot air-oven set at 105ºC until constant weight of the sample was obtained. Protein content of the sample was determined according to the principle of Kjeldahl method. A conversion factor of 6.25 was used to convert the measured nitrogen content to protein content. Lipid content of sample was determined by using a Soxhlet extractor with diethyl ether as solvent. The crude fiber was determined by alternately digesting the dried, defatted sample in 1.25% HCl and 1.25% NaOH. Ash content of sample was determined using the dry ashing method. The sample was incinerated in a cold muffle furnace set at 550ºC until whitish/greyish ash was obtained.

Determination of mineral composition

The minerals such as iron (Fe), manganese (Mn), copper (Cu), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P) were determined in the fruit samples from Inje and Mungyeong. Ten milliliters of concentrated HNO_3 (70%) were added to 0.2 g of the fruit samples in a test tube. The dispersion was digested in a digestion block at 100ºC for 3 h. After cooling, the digested products were diluted to 40 ml with deionized water, and centrifuged at 5000 rpm for 10 min. The clear solution was used for mineral determination by an inductively coupled plasma-optical emission spectrometer(Integra XL, GBC Scientific, Australia). For P, absorbance was measured in a UV-visible spectrophotometer (HP 8453E, Hewlett-Packard

Co., USA) at 420 nm. The results are expressed as g/100 g dry weight of sample for each mineral element.

Quantitative determination of active components by HPLC

The dried and pulverized fruits of *S. chinensis* (0.3 g) were extracted with 25 ml of methanol in an ultrasonic bath for 30 min at room temperature and centrifuged for 10 min at 5,000 rpm. Then the supernatant was filtered through 0.45 µm membrane syringe filter and the filtrate was used for HPLC analysis.

The active components (lignans) were quantified by Shiseido NANOSPACE SI-2 HPLC system (Shiseido, Tokyo, Japan). The standard stock solutions of schisandrin, schisandrin A, gomisin A and gomisin N were prepared in methanol. Chromatographic separation was performed using a reversed-phase column Unison UK-C18 (4.6 mm x 150 mm x 3 µm) at a column temperature of 30ºC. The mobile phases used to elute were distilled water (A) and acetonitrile (B), with an isocratic condition (A, 40% and B, 60%) for 30 min. The injection volume of the sample was set at 10 µL, with a fixed flow rate of 1 mL/min, and the detector wavelength was set to 254 nm (Accela PDA, Thermo Fischer Scientific, Bremen, Germany).

Supercritical CO$_2$ extraction (SFE)

The SFE was performed by ISA-SCCO-S-050-500 (ILSHIN Autoclave Co. Ltd., Daejeon, Republic of Korea). A hundred grams of dried fruits (not-pulverized) were loaded into a stainless steel extraction vessel. And the CO$_2$ was pressurized with a high-pressure pump and then charged into the extraction column to desired pressure. Back pressure regulators are used to set the system pressure (in extractor and separator). To optimize the SFE conditions for *S. chinensis* fruits, the extraction was conducted at different pressures (200, 300 and 400 bar) and temperatures (40, 50, 60 and 70ºC). The CO$_2$ flow rate was maintained at 30 mL/min. Each extraction process was performed for 60 min and the yield of extract obtained from the different extraction conditions was expressed as percent of the dry weight of fruits.

Solid phase microextraction (SPME) conditions

SFE (1 g) obtained from the fruits of *S. chinensis* was introduced into SPME vial (20 mL). The SPME device coated (fused-silica fiber) with a 100 µm layer of polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was used for extraction of the volatiles and the vial was sealed with a silicone septum. The fiber was exposed in the SPME vial at 60ºC for 30 min and immediately introduced in the gas chromatography injector.

Gas chromatography/mass spectrometry (GC/MS) analysis

GC-MS analysis was performed with a Varian CP 3800 gas chromatography equipped with a VF-5 MS polydimethylsiloxane capillary column (30 × 0.25 mm x 0.25 µm) and a Varian 1200 L mass detector (Varian, CA, USA). Helium was used as a carrier gas at the rate of 1 mL/min. Oven temperature was kept at 50ºC for 5 min initially, and then raised with rate of 5ºC/min to 250ºC/min. The injected volume of essential oil was 10 µL with a split ratio of 1:10. The injector temperature was set at 250ºC. The mass spectra were recorded in the electrospray ionization mode at 70 eV in a scan range of 50 - 600 m/z. The components of essential oils were identified by comparing the retention indices of the GC peaks obtained using homologous series of n-alkanes (C$_8$-C$_{20}$) with those reported in literature (Adams, 2007). The mass spectra of the peaks were also matched with standards reported in literature and National Institute of Standards and Technology (NIST, 3.0) library.

RESULTS AND DISCUSSION

Proximate composition and mineral content of *Schisandra chinensis* fruits

In the proximate composition analysis, moisture, crude protein, lipid and fiber and ash contents of the fruit samples obtained from Inje and Mungyeong were determined. The mean value of proximate composition for the fruits of *S. chinensis* is shown in Table 1. The moisture value of the fruits from Inje and Mungyeong were 3.88% and 3.81%, respectively. The major proximate components in the fruit samples of Inje and Mungyeong were crude lipid (13.51% and 14.73%, respectively) and crude fiber (13.77% and 14.19%, respectively) followed by crude protein (9.50% and 10.58%, respectively). The fruit samples also registered considerable amount of ash content (Inje - 5.41% and Mungyeong - 6.11%). From the results, the fruit samples from Mungyeong possess higher level of proximate composition than Inje samples.

Table 1: Proximate composition and mineral content of fruits of *Schisandra chinensis* from two different places (Inje and Mungyeong).

Content	Inje	Munkyong
Proximate composition (%)		
Moisture	3.88 ± 0.79*	3.81 ± 0.87
Crude protein	9.50 ± 1.81	10.58 ± 0.78
Crude lipid	13.51 ± 1.38	14.73 ± 1.46
Ash	5.41 ± 1.76	6.11 ± 0.67
Crude fiber	13.77 ± 1.51	14.19 ± 1.23
Mineral content (mg/100 g)		
Iron (Fe)	13.43 ± 3.15	15.03 ± 3.93
Manganese (Mn)	6.49 ± 4.04	5.61 ± 3.26
Copper (Cu)	0.48 ± 0.10	0.48 ± 0.11
Calcium (Ca)	82.92 ± 17.95	78.46 ± 9.96
Potassium (K)	1085.82 ± 390.57	1246.53 ± 312.40
Magnesium (Mg)	124.71 ± 26.09	129.05 ± 27.94
Sodium (Na)	14.37 ± 1.26	14.71 ± 1.95
Phosphorus (P)	0.07 ± 0.09	0.05 ± 0.01

*Values are mean of three replicate determinations (n=10) ± standard deviation.

In general, a significant amount of ash content specified the presence of appreciable amounts of inorganic nutrients in the plant materials. The presence of substantial amount of lipids reveals the potential of this fruits to have dietary purposes with good nutritional qualities (Iqbal *et al.*, 2012). Further, the fruits also possess considerable amount of crude fiber and crude protein contents. The results of proximate composition indicated the fruits of *S. chinensis* possess a high nutritional value. Previously, Kim and Choi (2008) investigated the physicochemical and antioxidative properties of *S. chinensis* fruits. The fruits contained 57.5% of moisture, 18.8% of crude fat, 12.6% of carbohydrate, 11.1% of crude protein, 4.9% of ash and 5.4% of crude fiber. The authors also reported the amino acid contents of the fruits with the largest portion of glutamic acid 131.7 mg/100 g followed by 51.5% aspartic acid. In addition, the fruits contained 7 types of free sugar contents with the glucose and fructose were registered as the dominant sugars.

It is essential to understand the chemical composition of the fruits, especially the composition of minerals and other trace elements, because the mineral composition of foods has a fundamental role in the diet of human. Table 1 shows the mineral contents of *S. chinensis* fruit samples from Inje and Mungyeong. The mineral composition of *S. chinensis* fruit samples varied considerably between two different places. Among the macro-minerals, K was the most concentrated mineral in the fruits from both the places, Inje and Mungyeong (1085.82 and 1246.53 mg/100 g, respectively) followed by Mg (124.71 and 129.05 mg/100 g, respectively) and Ca (82.92 and 78.46 mg/100 g, respectively). Fe was the most concentrated micro-mineral in the fruits of Inje and Mungyeong (13.43 and 15.03 mg/100 g, respectively) followed by Mn (6.49 and 5.61 mg/100 g, respectively). Among the eight minerals analyzed, Fe, Cu, Mg and Na contents were found to be higher in the Mungyeong samples than Inje samples. These minerals are essential for the correct functioning of the human body.

The composition of minerals and other nutrients in the plant materialis mainly influenced by various biotic and abiotic factors. Hwang *et al.* (2015) studied the mineral contents of *S. chinensis* fruits from Korea and China. The mineral contents of *S. chinensis* fruits from China (100 g) were K (923.31 mg), Mg (83.91 mg), Ca (14.80 mg), Mn (6.19 mg), Fe (4.30 mg), Zn (1.12 mg), Na (2.20 mg) and Cu (0.30 mg). In addition, the authors reported that the contents of K and Zn were found to be significantly higher in Korean fruits than the Chinese fruits. Kim and Choi (2008) reported the presence of 10 minerals in the fruits with the highest levels of K (912.6 mg/100 g) and Ca (613.8 mg/100 g), followed by Al, Mg, Na and Mn. The present study also confirmed that the fruit samples comprised higher levels of K, Mg and Ca minerals. Further, these nutrients were found to be higher in the current study when compared to that of previous reports. Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins. Nour *et al.* (2014) stated that the nutrition with a high ratio of K/Na have been associated with a lower incidence of hypertension.

Active components

Previously, a number of studies have reported that the lignans from *S. chinensis* possess various pharmacological properties, such as hepatoprotective, antioxidant and anti-carcinogenic activities, and strong inhibitory effect on human immunodeficiency virus (Wang *et al.*, 2008; Choi *et al.*, 2006; Ip *et al.*, 1996; Lee and Kim *et al.*, 2010; Xie *et al.*, 2010). Further, those studies suggest that the bioactive lignans mainly comprised of dibenzocyclooctadiene skeletons with (S)- or (R)-biphenyl configurations. Until now, more than 40 lignans have been characterized from the different organs of *S. chinensis* (Kim *et al.*, 2010; Takimoto *et al.*, 2013).

In the present study, the most bioactive lignans such as schisandrin, schisandrin A, gomisin A and gomisin N were quantified by HPLC (Fig. 2). The fruits contained the highest amount of schisandrin followed by gomisin N, gomisin A and schisandrin A. When compared to the fruit samples of Inje, the contents of schisandrin (5018.67 µg/g), gomisin N (4772.87 µg/g) and schisandrin A (717.18 µg/g) were higher in the samples of Mungyeong. The level of Gomisin A was higher in Inje samples (1383.03 µg/g) than Mungyeong samples (1202.60 µg/g).

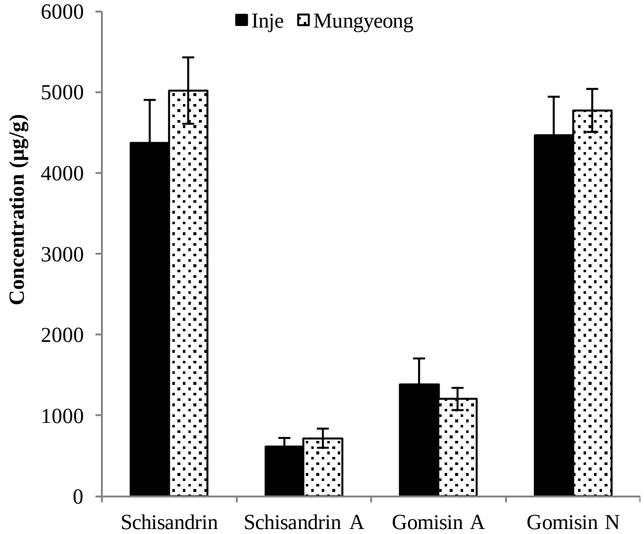

Fig. 2: Active ingredients in the fruits of *Schisandra chinensis* from two different places (Inje and Mungyeong). Values are mean of three replicate determinations (n=10) ± standard deviation.

Zhang *et al.* (2009) established a rapid and specific HPLC method for simultaneous determination of six major lignans in *S.chinensis* such as schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, γ-schisandrin and schisandrin C. The six lignans were successfully separated on a C18 column and the mobile phase consisted of acetonitrile and water with the detection wavelength of 225 nm. In addition, Hu *et al.* (2013) successfully validated the method to quantify 11 lignans (schisandrin, gomisin J, schisandrol B, angeloylgomisin H, gomisin G, schisantherin A, schisantherin B, deoxyschisandrin, γ-schisandrin, schisandrin B and schisandrin C) in *S. chinensis* by HPLC. Recently, a simplified sample extraction method using matrix solid phase dispersion followed by HPLC determination was established for the determination of five most abundant lignans, schisandrin, schisandrol B, schisantherin A, deoxyschisandrin and γ-schisandrin in *S. chinensis* (Zhang *et al.*, 2016). In the current study, schisandrin was the most abundant component in the fruits followed by gomisin N, gomisin A and schisandrin A. The contents of schisandrin, gomisin N and schisandrin A were found to be higher in Mungyeong samples than Inje samples. However, the concentration and distribution of these lignans in *S. chinensis* are mainly influenced by plant origins and harvest seasons. Among the various lignans, schisandrin is a prominent lignin with multiple pharmacological properties including antioxidant, anti-inflammatory, antitumor, sedative and hepatoprotective effects (Park *et al.*, 2011; Kang *et al.*, 2012; Zhang *et al.*, 2016). Previous studies have shown that the lignans, gomisin N and gomisin A, significantly inhibited the liver damage from toxic chemicals. Gomisin A has been shown to inhibit acetylcholinesterase activity and anti-hypertensive effect and improve scopolamine-induced memory impairment in mice. Gomisin A showed anti-inflammatory properties by potentially inhibiting the pro-inflammatory mediators through the down-regulation of receptor-interacting protein 2 and activation of nuclear factor-kappa B (Park *et al.*, 2012; Jeong *et al.*, 2014). Schisandrin B (gomisin N) enhanced the cytotoxic and pro-apoptotic potentials of doxorubicin (Li *et al.*, 2006; Kim *et al.*, 2010). Gomisin N remarkably inhibited the nitric oxide production in lipopolysaccharide-induced RAW 264.7 cells and reduced the mRNA expression and the secretion of pro-inflammatory mediators (Oh *et al.*, 2010). In addition, gomisin N exhibited antiproliferative properties against various cancer cell lines with the IC_{50} values of 10–70 μM (Min *et al.*, 2008). Furthermore, Giridharan *et al.* (2011; 2012) reported that the lignan, schisandrin B effectively prevent scopolamine-induced dementia and cisplatin-induced memory deficits in animal model. Schisandrin A is also an important bioactive lignan and is a strong antioxidant which possesses hepatoprotective and antitumor properties. It also showed positive effects on preventing memory impairment in mice (Hu *et al.*, 2012; Cheng *et al.*, 2013; Lu *et al.*, 2014).

Volatile composition of SFE

The SFE yield of *S. chinensis* fruits was significantly influenced by the extraction temperature and pressure. In the different pressures performed for the optimization of extraction condition, there were no yield at 200 and 300 bar. Further, the yield was increased with increase of temperature [50ºC (0.01%), 60 ºC (0.02%) and 70ºC (0.45%)]at constant pressure of 400 bar. At the constant temperature (70 ºC) and pressure (400 bar), the SFE yields of Inje and Mungyoeng samples were 0.42±0.13% and 0.45±0.13%, respectively. The results revealed that the pressure plays a vital role in the extraction yield of *S. chinensis* fruits when compared with the temperature. Previously, several studies have reported that the temperature and pressure played an important role on the yield of essential oils from various plant materials by SFE method (Ansari and Goodarznia, 2012; Ahmed *et al.*, 2012). Wang *et al.* (2008) obtained the SFE yield of 185.6 mg/g from the fruits of *S. chinensis* with the operating conditions of pressure, 25 MPa; temperature, 50ºC; carbon dioxide flow rate, 25 L/h; and extraction time, 3 h. In the present study, the SFE yield was very low, because the fruits samples were not pulverized as well as the extraction time was 1 hour. The volatile composition of the SFE from *S. chinensis* fruits was determined by SPME-GC/MS and the result is presented in Table 2.

Table 2: Volatile composition of supercritical carbon dioxide extract of *Schisandra chinensis* fruits from two different places (Inje and Mungyeong)

No.[a]	Components[b]	RI[c]	Area (%)	
			Inje	**Mung yeong**
Monoterpene hydrocarbons				
1	α-Pinene	939	0.27	0.27
2	Camphene	954	0.43	0.29
3	Sabinene	975	0.15	0.17
4	β-Pinene	979	0.17	0.19
5	β-Myrcene	990	0.36	0.26
6	2-Carene	1002	0.45	0.45
7	o-Cymene	1026	0.36	0.25
8	Limonene	1029	0.41	0.31
9	γ-Terpinene	1059	1.34	1.43
10	Terpinolene	1088	0.18	-
Oxygenated monoterpenes				
11	Citronellal	1153	0.09	0.10
12	Borneol	1169	0.34	0.14
13	Terpinen-4-ol	1177	0.25	0.18
14	Sabinene hydrate acetate	1221	0.66	0.21
15	Thymol methyl ether	1235	1.45	1.82
16	Bornyl acetate	1285	3.54	4.31
17	Myrtanyl acetate	1326	2.80	1.80
18	1,4-Dimethoxy-2-tert-butylbenzene	1365	1.38	1.55
Sesquiterpene hydrocarbons				
19	Cyclosativene	1371	0.54	0.30
20	Ylangene	1375	17.66	25.66
21	β-Cubebene	1388	1.69	2.90
22	β-Elemene	1390	1.44	1.93
23	Longipinene	1400	10.09	8.69
24	Italicene	1405	5.32	4.86
25	Caryophyllene	1419	2.97	2.66
26	Seychellene	1446	1.65	2.45
27	α-Himachalene	1451	20.70	18.03
28	β-Farnesene	1456	1.54	0.96
29	Acoradiene	1466	-	2.57
30	γ-Muurolene	1479	1.71	1.78
31	α-Calacorene	1545	0.40	0.15
Oxygenated sesquiterpenes				
32	Germacrene B	1561	0.60	0.90
33	Humulane-1,6-dien-3-ol	1611	1.68	0.24
34	Aromadendrene epoxide	1641	3.72	2.74
35	Cedr-8-en-15-ol	1644	3.88	-
36	Cubenol	1646	1.45	2.01
37	α-Cadinol	1654	0.78	0.29
38	Bisabolol	1685	1.94	1.43
39	Longipinocarvone	1775	1.21	0.58
40	Nootkatone	1806	-	0.22
41	(E,E)-Farnesyl acetate	1822	1.20	1.51
42	Methyl hinokiate	1865	0.42	0.22
			97.22	**96.81**

[a]In order of elution on VF-5ms. [b]Components identified based on mass spectra and retention indices. [c]RI, Retention indices reported in the literature. The SFE of fruits obtained from 10 sites of each place were pooled together and used for the SPME-GC/MS analysis.

A total of 42 components were identified in the SFE of fruits obtained from Inje and Mungyeong, accounting for 97.22 and 96.81%, respectively. In general, slight differences in the profiles of volatile components were detected among the fruit samples from two different places. Fig. 3 shows the percentage concentration of different chemical groups in the SFE of *S. chinensis* fruits from Inje and Mungyeong. The SFE were mainly represented by sesquiterpene hydrocarbons followed by oxygenated sesquiterpenes, oxygenated monoterpenes and monoterpene hydrocarbons. When comparing the SFE obtained from Inje and Mungyeong fruits, sesquiterpene hydrocarbons were found to be higher level in Mungyeong samples (72.94%) than Inje samples (65.71%). On the other hand, Inje samples contained higher concentration of oxygenated sesquiterpenes (16.88%) than Mungyeong samples.

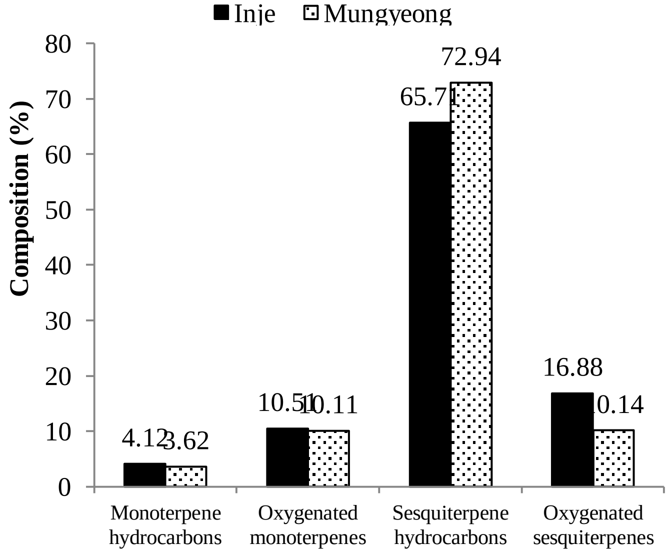

Fig. 3: Percentage composition of different chemical groups in the supercritical carbon dioxide extract of *Schisandra chinensis* fruits from two different places (Inje and Mungyeong).

Ylangene(17.66 and 25.66%), α-himachalene (20.70 and 18.03%), longipinene (10.09 and 8.69%), italicene (5.32 and 4.86%), bornyl acetate (3.54 and 4.31%), aromadendrene epoxide (3.72 and 2.74%)were recorded as the major components of the SFE (Inje and Mungyeong, respectively). In addition, considerable amount of cedr-8-en-15-ol (3.88%) and acoradiene (2.57%) were detected in the samples of Inje and Mungyeong, respectively. The major differences between the SFE of Inje and Mungyeong samples can be referred to ylangene, α-himachalene and longipinene. The SFE of Mungyoeng sample contained higher concentration of ylangene (25.66%) than Inje sample (17.66%). Whereas Inje sample registered higher level of α-himachalene (20.70%) and longipinene (10.09%) than Mungyoeng sample (18.03 and 8.69%, respectively).

According to the previous reports, sesquiterpene hydrocarbons and oxygenated sesquiterpenes are the main volatile components from the fruits of *S. chinensis* and responsible for its specific fragrance. The results of the present study also clearly showed that the fruits mainly composed of sesquiterpenes group of components with ylangene, α-himachalene, longipinene as the major components. In the previous report, 40 components were identified in the essential oil of *S. chinensis* fruits and the main components were ylangene (37.72%), β-himachalene (10.46%) and α-bergamotene (8.57%) (Chen *et al.*, 2012). In another study, Teng and Lee (2014) compared the simultaneous distillation extraction with Soxhlet and microwave assisted extraction methods. The results revealed that the major ingredients in the oil extracted by simultaneous distillation extraction were ylangene (15.01%), α-phellandrene (8.23%), β-himachalene (6.95%), and cuparene (6.74%). However, the oils obtained by other extraction methods mainly contained aromatics such as schisandrins and gomisin A.

Deng *et al.* (2003) compared the volatile composition of *S. chinensis* obtained from steam distillation and headspace SPME and identified 33 and 35 volatile compounds, respectively. Further, Li *et al.* (2003)investigated the essential oil composition of *S. chinensis* obtained from steam distillation and characterized 48 different volatile components from the oils.Wang *et al.* (2008) investigated the comparison of volatile composition from the fruits of *S. chinensis* obtained by SFE, steam distillation, Soxhlet extraction and ultrasound-assisted extraction and identified 37, 45, 27 and 37 compounds in the samples, respectively. Further, the authors stated that the SFE method shared 32 compounds in common with the other three methods. Among them, schisandrin (16.4%), methostenol (16.2%), and β-tocopherol (11.0%),α-ylangene (7.94%) 2-methyl-2-bornene (7.64%), 1-butyl-1,3,5,7-cyclooctatetraene (6.50%)and α-farnesene (5.58%)were the major components in the SFE.In the current study, totally 42 components (40 components from each place) were identified in the SFE of fruits. Ylangene, α-himachalene, longipinene, italicene, bornyl acetate and aromadendrene epoxide were the most abundant components in the SFE.

The composition of SFE of *S. chinensis* fruits is totally different from previous report and analysis methods might be responsible for these variations (Wang *et al.*, 2008). Moreover, this is the first report on the volatile composition of SFE of *S. chinensis* using SPME-GC/MS. The findings of the previous and the present studies clearly suggested that the volatile composition of *S. chinensis* fruits is mainly influenced by the extraction techniques, place of sample collection and environmental conditions (biotic and abiotic factors).

CONCLUSION

In the present investigation, the fruits of *S. chinensis* collected from both the places, Inje and Mungyoeng possess almost similar and appreciable levels of proximate composition, mineral contents, bioactive lignans and various volatile components. The considerable amount of nutrients and bioactive components in the *S. chinensis* fruits suggest that the fruits may have the potential for enriching ingredient for locally processed foods and can contribute to certain nutritional requirements in the human diet.

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