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Fatty Acid Content in Perilla Cultivars and Commercial Oils Determined by GC Analysis

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Abstract – The content analysis of fatty acids in Perilla cultivars and commercial oils is conducted through gas chromatography with a flame ionization detector. Results show that Perilla cultivars, such as Deulsaem and Daesil, contain high amounts of α-linolenic acid (262.22 and 261.97 mg/g, respectively). Among commercial oils, Perilla oil contains a higher amount of α-linolenic acid (515.20 mg/g). Accordingly, α-linolenic acid is a major fatty acid of Perilla cultivars and oil. Therefore, Perilla cultivars could be used as a food supplement for nutritional and pharmaceutical purposes.
Keywords – *Perilla frutescens* var. *japonica*, α-Linolenic acid, Fatty acid, Gas chromatography

Introduction

Perilla (*Perilla frutescens*; Labiatae) is an annual herbaceous plant used for various purposes in Asia. In Korea, it has been used for food, medicinal, and industrial purposes. In China, Perilla is considered as a traditional medicinal herb used to treat various diseases, such as anxiety, tumor, intoxication, cough, allergy, bacteria, and fungal infections.[1-4] Previous studies have revealed that Perilla contains three major nutrients, namely lipid (44%), protein (17%), and carbohydrates (28%) and several fatty acids (FA). α-Linolenic acid (ALA) is more than 60% of the total FA in Perilla seeds.[5,6] ALA prevents colon cancer,[7] exhibits effects against Crohn's disease,[8] and affects learning and memory skills.[9] Linoleic acid shows anti-oxidant properties, prevents inflammation in the colon, and stimulates anti-tumorigenic proteins.[10–12] Oleic acid has anti-tumor activity,[13] while palmitic acid is related to diabetes mellitus and cardiovascular diseases.[14] Meanwhile, stearic acid exhibits antibacterial properties.[15]

In Asia, Perilla oil has been used as a garnish and food supplement. The oil is now being developed as a healthy functional food. Accordingly, Perilla oil has been the topic of some previous bodies of research. The oil has been found to lower serum lipids and Ovalbumin-specific lgG1 levels,[16] prevent brain damage by suppressing inflammation, and exert significant antidepressant-like effects.[17]

There are few reports on analysis of functional ingredients in Perilla cultivars. This study aims to determine the FA content in Perilla cultivars and commercial oils using gas chromatography (GC) with a flame ionization detector (FID).

Experimental

Plant materials – Perilla cultivars (i.e., Dami, Danjo, Deulsaem, Anyu, Yujin, Dayu, Yeupsil, Hyangim, and Hwahong) and oil (*P. frutescens* var. *japonica*) were contributed by the Southern Area Crop Science, Rural Development Administration, Miryang, Republic of Korea (2015). Commercial oils (i.e., nut, soybean, corn, olive, cooking, canola, and grape seed oils) were purchased from a local retailer in Anseong, Republic of Korea.

Sample preparation of Perilla cultivars for GC – Each Perilla cultivar seed (1 g) was ground and extracted with internal standard (1 mL).

Apparatus and chemicals – The samples were analyzed by Agilent 7890A GC (Agilent, CA, USA) equipped with a FID and a DB-Wax column (Agilent, 30 mm × 0.25 mm × 0.25 μm). The samples were stored in a FRS-300RWE

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Table 1. GC conditions for the ALA analysis

Instrument	GC model : Agilent 7890A (Agilent, CA, USA) Detector: FID (280 ºC, H_2 35 mL/min; air 350 mL/min; He 35 mL/min)
Column	DB-Wax (Agilent, 30 mm × 0.25 mm × 0.25 μm)
Injector temperature	250 ºC
Column temperature	50 C (1 min) → 200 ºC (5 min) → 230 ºC (20 min)
Split ratio	1:20
Separator temperature	250 ºC
Ion source temperature	200 ºC

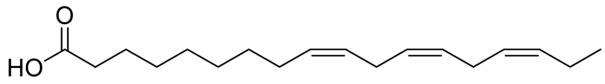

Fig. 1. ALA structure.

refrigerator (Dongbu Daewoo Electronics, Seoul, Republic of Korea) until use. The GC-FID methylation was conducted with sodium hydroxide and iodomethane purchased from DaeJung Chemicals and Metals Co., Ltd. (Siheung, Republic of Korea). Dimethyl sulfoxide (DMSO) was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan).

GC-FID methylation – Ground sodium hydroxide (20 mg) and iodomethane (0.1 mL) were added into DMSO (0.5 mL) with the samples, and then sonicated for 30 min at room temperature. CH_2Cl_2 and distilled water were added. Subsequently, the non-polar phase was obtained. The collected CH_2Cl_2 layer was evaporated by nitrogen gas.[18] The FA methyl ester was then prepared.

GC conditions – Accordingly, 1 μL (split ratio: 1:20) of FA methyl ester was injected into an Agilent 7890A autosampler (Agilent, CA, USA) at 250 ºC. The column was a DB-Wax (Agilent, 30 mm × 0.25 mm × 0.25 μm) in Agilent 7890A (Agilent, CA, USA) by FID. The column temperature was set to 50 ºC for 1 min. The analysis was started at 200 ºC. The temperature was increased at 25 ºC/min for 5 min. The column was set re-initiated at 230 ºC, then, increased at a rate of 3 ºC/min for 20 min. The injector and ion source temperatures were set at 250 ºC and 200 ºC, respectively. Table 1 shows the detailed conditions.

Result and Discussion

ALA (Fig. 1) is an essential fatty acid, with a structure of long chains that is a precursor of the eicosapentaenoic and docosahexaenoic acids. ALA is known as an omega-3 fatty acid.[19] The ALA analysis in ten Perilla cultivars was conducted using GC-FID. Table 2 shows the data. Among the Perilla cultivars, Deulsaem and Daesil contained higher contents of total FA, that is, 449.7 and 451.18 mg/g, respectively, as well as ALA contents of 262.22 and

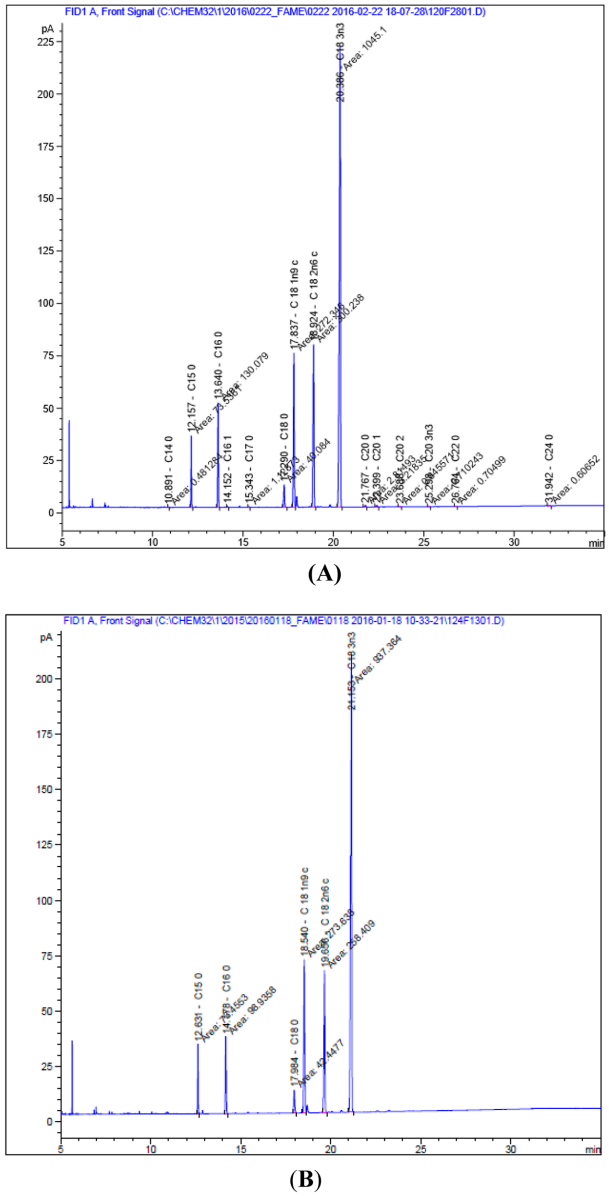

Fig. 2. GC chromatograms of Daesil (**A**) and Perilla oil (**B**).

261.97 mg/g, respectively. Fig. 2 shows the Deulsaem chromatogram. Shin and Kim (1994) revealed the composition of the total lipid and the major lipids in the

Table 2. FA contents from Perilla cultivars

Sample	FA (mg/g)					
	α-Linolenic acid	Linoleic acid	Oleic acid	Stearic acid	Palmitic acid	Total
Dami	188.07 ± 6.97	35.97 ± 2.41	51.83 ± 2.18	8.77 ± 0.38	22.44 ± 0.91	308.08 ± 12.85
Danjo	237.77 ± 5.16	64.99 ± 1.42	70.14 ± 1.80	12.06 ± 0.32	29.29 ± .68	415.25 ± 9.38
Deulsaem	262.22 ± 0.44	75.88 ± 1.50	72.22 ± 0.59	10.89 ± 0.15	28.49 ± 0.23	449.7 ± 2.91
Daesil	261.97 ± 3.70	76.69 ± 0.96	69.17 ± 0.67	10.09 ± 0.12	33.26 ± 0.20	451.18 ± 5.65
Anyu	242.38 ± 3.07	56.70 ± 1.88	69.99 ± 1.10	9.01 ± 0.09	28.11 ± 0.82	362.19 ± 6.96
Yujin	248.54 ± 4.26	62.71 ± 1.41	69.89 ± 1.41	8.81 ± 0.13	29.87 ± 0.57	419.82 ± 7.78
Dayu	249.06 ± 15.26	56.57 ± 3.29	57.94 ± 3.70	11.01 ± 0.69	29.29 ± 0.68	403.87 ± 23.62
Yupseol	244.51 ± 8.77	55.56 ± 1.65	54.12 ± 2.05	11.50 ± 0.40	30.76 ± 1.04	396.45 ± 3.91
Hyangim	208.84 ± 6.86	48.70 ± 1.59	53.28 ± 1.89	8.33 ± 0.31	24.30 ± 0.85	343.45 ± 11.5
Hwahong	189.08 ± 14.13	34.48 ± 2.28	42.99 ± 2.99	5.85 ± 0.39	21.29 ± 1.46	293.69 ± 21.25

Table 3. FA contents from commercial oils

Sample	FA (mg/g)					
	α-Linolenic acid	Linoleic acid	Oleic acid	Stearic acid	Palmitic acid	Total
Nut oil	33.19 ± 0.62	276.31 ± 5.40	456.32 ± 7.74	31.21 ± 0.53	78.10 ± 1.36	875.14 ± 15.65
Perilla oil	515.20 ± 1.92	141.16 ± 0.76	147.05 ± 1.34	23.08 ± 0.35	52.12 ± 0.45	878.61 ± 4.81
Soybean oil	55.27 ± 0.15	480.78 ± 1.45	205.03 ± 0.77	40.84 ± 0.21	102.48 ± 0.47	884.40 ± 3.06
Corn oil	8.45 ± 0.12	474.88 ± 3.09	294.09 ± 1.14	19.08 ± 0.12	105.93 ± 0.46	902.42 ± 4.93
Olive oil	5.03 ± 0.15	63.24 ± 0.69	693.67 ± 7.24	30.80 ± 0.36	127.45 ± 1.50	920.19 ± 9.94
Cooking oil	56.32 ± 1.72	260.31 ± 8.29	439.08 ± 14.75	22.19 ± 0.84	55.57 ± 2.05	833.47 ± 27.66
Canola oil	61.61 ± 1.70	165.86 ± 4.16	563.37 ± 13.94	17.76 ± 0.39	38.51 ± 0.95	847.11 ± 21.14
Grape seed oil	2.57 ± 0.07	592.96 ± 13.43	176.83 ± 2.45	38.98 ± 0.91	67.23 ± 1.14	878.57 ± 18.00

seeds of Perilla cultivars. Their conclusion was almost similar to ours. The major FAs were linolenic (61.1 – 64.0%), linoleic (14.3 – 17.0%), and oleic (13.2 – 14.9%) acids.[20] Lee *et al*. (1986) determined FA from seven Perilla cultivars. The FA content of oil from Perilla cultivars used ranged from 10% to 20% in linoleic acid and from 50% to 70% in linolenic acid content.[21] Choung (2005) also analyzed the seed and vegetables of Perilla varieties. They compared the characteristics of seed Perilla and vegetables Perilla varieties. The seed Perilla varieties had more total FA than the vegetables varieties. However, the difference in FA compositions was not observed.[22]

Additional analysis was conducted, wherein we compared the FA contents of Perilla and commercial oils. The content analysis of FAs from commercial oils was conducted using GC-FID. Table 3 shows the data. Among the commercial oils, olive and corn oils contain high amounts of total FAs (920.19 and 902.42 mg/g, respectively). However, the highest ALA amount was in Perilla oil (515.20 mg/g). Fig. 2 shows the chromatogram of Perilla oil. Sinclair and Gibson (1992) analyzed various oils, including Perilla, olive, fish, and safflower oils. They showed that Perilla oil contained 57% ALA and 13% linoleic acid,[23] which were almost similar to our results. According to Sargi et al. (2013), Chia and white Perilla seeds contained high ALA amounts (544.85 and 539.07 mg/g, respectively).[24] Kostik et al. (2013) investigated the FA compositions of coconut, corn, cottonseed, linseed, palm kernel, olive, soybean, sunflower, peanut, safflower, and canola varieties. Their results showed that the oleic acid composition in olive oil was 78.4%.[25] Ten Perilla cultivars were analyzed in this study. Among them, three Perilla cultivars (Dami, Hwahong, and Deulsaem) were analyzed for the first time.

In conclusion, our results revealed that Perilla oil and Deulsaem contained higher ALA amounts. These results could be used as a guideline in choosing Perilla cultivars and oils to be used as food supplement for nutritional and pharmaceutical purposes.

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