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Full Length Research Paper

Characterization of fatty acid composition from five perilla seed oils in China and its relationship to annual growth temperature

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Omega-3 fatty acids have been shown to reduce inflammation and may help prevent chronic diseases like heart disease and arthritis. Alpha-linolenic acid is a type of ω-3 fatty acids and has been reported with a remarkably high content (>50%) only in seeds of several plant species, for example, Perilla and *Linum usitatissimum* L. It is a valuable compound obtained from plants which can provide health benefits similar to those derived from fish oil. In this study, five seed samples from five varieties of the monotypic genus Perilla collected from five provinces in China were analyzed to determine their fatty acid composition. The seed oils were extracted using Soxhlet, and oil contents of these samples ranged from 33.25 to 42.58% (wt/wt). Fatty acid compositions were then analyzed using gas chromatography-mass spectrometry (GC-MS). Saturated, monounsaturated and polyunsaturated fatty acids accounted for 9.16 to 10.49, 13.78 to 20.93 and 63.12 to 73.45% of their totals, respectively, in which the content of the unsaturated fatty acids was over 90% on average. Moreover, α-linolenic acid was dominant (52.58 to 61.98%) in all the oil samples, and it comprised 65.67 to 68.37% of the total unsaturated fatty acids and 83.30 to 84.38% of the polyunsaturated acids, respectively. However, contents of the ω-6 linoleic acid in the five samples were only 10.54 to 15.87%, and the ratio of ω-6 to ω-3 fatty acids was as low as 0.2 to 0.26. The results indicate that Perilla oil is presently the best quality oil derived from plant sources and hence it could be developed into commercial products to serve as a valuable alternative vegetable oil. Furthermore, oil from seeds obtained in regions with lower average growth temperature has relatively higher percentage of unsaturated fatty acids.

Key words: Annual growth temperature, gas chromatography-mass spectrometry (GC-MS), ω-3 α-linolenic acid, ω-6 linoleic acid, Perilla, seed oil, variety.

INTRODUCTION

The genus Perilla belongs to the mint family (Lamiaceae). However, taxonomic nomenclature of Perilla is controversial (Park et al., 2008; Hu et al., 2010). Its recognized species vary from one (Li, 1974; Koezuka et al., 1985; Misra and Husain, 1987) to two (Pei, 1955) or four (Li, 1977). In China, Perilla is believed to have only one species (*Perilla frutescens* (L.) Britton) (Koezuka et al., 1985), which according to Hu et al. (2010) can be further divided into five varieties, namely (1) *P. frutescens* (Linn.) Britt. var. purpurascens (Hayata) H. W. Li., (2) *P. frutescens* (Linn.) Britt. var. crispa (Benth.) H. W. Li., (3) *P. frutescens* (Linn.) Britt. var. auriculato-dentata C. Y. Wu et Hsuan ex H. W. Li., (4) *P. frutescens* (Linn.) Britt. var. frutescens and (5) *P. frutescens* (Linn.) Britt. var. arguta (Benth.) Hand.-Mazz. In the present study, these five varieties are referred to as var. frutescens, var. arguta, var. acuta, var. auriculato-dentata and var. crispa, respectively. *P. frutescens* is an aromatic, bushy annual species cultivated as a commercial crop in East Asia particularly in China and Japan (Brenner, 1993; Lee et al., 2002). Because of its long history of cultivation, China

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is assumed to be the primary centre of its biodiversity (Li, 1969; Zeven and de Wet, 1982). Perilla is widely distributed in China, especially in provinces such as Heilongjiang, Liaoning, Shanxi, Ningxia, Gansu, Anhui, Hubei and Sichuan (Liu et al., 1996; Zhang et al., 2009). There are also wild distribution and sparse small scale plantations of Perilla in other provinces (Liu and Zhang, 1998; Hu et al., 2010). Perilla is also cultivated as one commercial oilseed crop in other countries like Japan and Korea (Choi et al., 1980; Brenner, 1993). Now, it has also been introduced to Europe, Russia and USA as an oilseed crop (Nitta et al., 2003).

Perilla plants have distinctive square stems and four stamens as with most species in the family Lamiaceae. However, the best diagnostic features of Perilla are the net-patterned testa of the nutlets as well as the typical minty odor of the crushed foliage (Miller, 1922; Tanaka, 1976; Brenner, 1993). Several analyses have reported that this characteristic odor is due to the various essential oil components which affect their nutritional and medical function and toxicity (Ito et al., 1999a, b, 2002). Perilla is listed in the Chinese Pharmacopoeia and has been used for centuries as a medicinal plant for asthma, influenza, cough, chronic bronchitis and vomiting (Ueda et al., 2002; Takano et al., 2004). It also helps to lower blood pressure and to reduce the risks of heart attack, breast and colon cancers, colitis, and rheumatoid arthritis (Connor, 2000; Okamoto et al., 2000).

Perilla seed oil contains a high percentage of unsaturated fatty acids which is consisted mainly of ω-3 α-linolenic (C18:3), ω-6 linoleic (C18:2) and ω-9 oleic (C18:1) acids (Park et al., 1981). Omega-3 fatty acids are often consumed by vegans for heart health (Calder, 2004; Lewis, 2008) and have largely been derived from flaxseed (Linum usitatissimum) in West Asia and Europe. Specifically, there has been increased interest on α-linolenic acid because a α-linolenic acid-rich diet is helpful in maintaining optimal health (Kim et al., 2004; Siriamornpun et al., 2006; Kim et al., 2007; Marangoni et al., 2007; Rao et al., 2008; Zatonski et al., 2008). For those who eat animal substances, fish oils can provide two nutrients called eicosapentaenoic acid (EPA; 20:5, ω-3) and docosahexaenoic acid (DHA; 22:6, ω-3) (Sayanova and Napier, 2004; Mbatia, 2011), which can be converted from α-linolenic acid (Ezaki et al., 1999; Davis and Kris-Etherton, 2003; Burdge and Calder, 2006). Above all, unlike fish oil, Perilla oil is considered safe because it has no mercury contamination risks (Mozaffarian and Rimm, 2006; Butler, 2009; Asif, 2011). Moreover, compared to flaxseed oil, Perilla oil contains an even higher proportion of α-linolenic acid.

The fatty acid composition and oil content in seeds depend on the plant species and factors such as degree of seed ripening and climatic conditions (Lee et al., 1986; Brenner, 1993; Lee and Ohnishi, 2001; Siriamornpun et al., 2006). In the present study, seeds of five varieties of Perilla were collected from five different provinces of China to determine their oil content, fatty acid composition and evaluate the effects of environmental factors on content and composition of the seed oil, especially the content of α–linolenic acid. It was determined whether that the α-linolenic acid content in these varieties can justify the potential commercial development of Perilla as a source of vegetable oil.

MATERIALS AND METHODS

Seeds and chemicals

Seed samples of the five Perilla varieties were collected in 2009 from Yunnan (var. auriculato-dentata), Sichuan (var. acuta), Guangxi (var. crispa), Hebei (var. frutescens) and Guizhou (var. arguta) provinces of China. The five collection sites had different average growth temperature. Authentication of the seed samples of Perilla was done based on Hu et al. (2010). All reagents, chemicals and solvents used were of analytical grade and were purchased from Beijing Lanyi Chemicals Co., Ltd (Beijing, China).

Preparation of seeds and oil extraction

The seeds were cleaned to remove impurities, stems and leaves. One thousand seeds of each sample were randomly selected and weighed to obtain their respective 'thousand seed weight'. Air-dried seeds were ground in FW100 high-speed universal mill (Taisite). Five gram of ground seed powder was refluxed with 150 ml petroleum ether for 10 h using Soxhlet (Sartorius). The refluxed mixture was then filtered to remove the seed residues. The solvent in the filtrate was removed by rotary evaporation, and the remaining oil was weighed and stored at -18℃ in the dark until use. The oil content of seeds was calculated on a dry weight basis. During extraction and evaporation process, samples were protected from light. Triplicate measurements were performed for each sample and the data were recorded as mean ± standard deviation (n = 3).

Determination of fatty acids composition

Fatty acids in the seed oils were converted to their corresponding methyl esters according to the AOCS method (AOCS, 1989). In brief, 0.4 g seed oil of each variety were dissolved in 2 ml 0.5% (w/v) sodium hydroxide in methanol (KOH-MeOH) in a 25 ml flask with a ground-glass stopper, then saponification was carried out at 60℃ for 15 min. After the solution was cooled to roo m temperature, 2 ml of 14% trifluoride in methanol (BF_3-MeOH) was added and esterification at 60℃ was performed for 5 min. The s olution was cooled again and 2 ml hexane (C_6H_{14}) and 2 ml saturated sodium chloride (NaCl) were then added to the samples. The fatty acid methyl ester (FAME) was recovered by centrifugation.

Gas chromatography - mass spectrometry (GC-MS) conditions

The fatty acid contents of the seed oil were determined by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of FAME was performed using a Voyager GC-MS coupled to Trace GC 2000 SERIES (ThermoQuest company in USA). Silex capillary column of VF-5 (0.25 µm×0.32 mm×30 m) from Varian (USA) was used for separation and UHP helium as carrier gas at a flow rate of 1.5 ml/min. Injection volume of each sample was 1 µl. The injection temperature was 250℃ while the interface temperatur e was 280℃.

The initial temperature of 50℃ was maintained fo r 1 min and

Table 1. Characteristics of the Perilla seeds and climatic conditions from where they were collected.

Variety name	Latitude	Altitude (m)	Mean annual growth temperature (℃)	Seed color	Thousand-seed weight (g)	Oil content* (wt %)
Frutescens	38°41'N (Hebei)	29	11.6	Grayish brown	4. 674^{aA}	33.25^{cC}
Arguta	27°7'N (Guizhou)	1045	15.2	Tan	1.186 ^{bB}	42.58^{aA}
Auriculato-dentata	23°42'N (Yunnan)	1230	16.9	Tan	2 .263^{bB}	41.65^{aA}
Acuta	30°48'N (Sichuan)	259	19	Tan	0.734 ^{cC}	39.78^{bB}
Crispa	23°51'N (Guangxi)	79	21.6	Tan	0.628 ^{cC}	39.62^{bB}

Note: 1 Percentage on a dry weight basis; 2 Different capitalized letters and small letters in the same column mean significant difference at 0.01 and 0.05 levels, respectively.

then ramped to 180℃ at a rate of 20℃/min. It was th en held for 5 min before the second ramp at a rate of 5℃/min to 220 ℃. After that the ramp at a rate of 10℃/min was used to raise the temperature until 290℃. The temperature was then held iso thermally for 15 min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 29 to 540 m/z.

Data analysis

Fatty acids were identified by comparing the gas chromatograph retention time of each peak with that of the authorized pure individual standard compounds. They were then quantified using the area under each peak. The data were processed using Xcalibur version 1.2 software (from Thermo Fischer Scientific, USA).

RESULTS AND DISCUSSION

Characterization of seeds from various geographical conditions

Five seed varieties from different region of China were collected and analyzed. The physical (weight and color) and chemical (oil contents) characteristics as well as the latitudes, altitudes and mean annual growth temperatures were summarized in Table 1. Seed colors of the five varieties were similar with four of them having tan surfaces while only one (var. frutescens) had a grayish brown color. Based on the annual growth temperature of the habitat, the five varieties have been divided into three groups. The first one had only one variety, var. frutescens, the second comprised of vars. auriculato-dentata and arguta, and the third consisted of vars. acuta and crispa (Table 1). The average annual growth temperature is 11.6, 16 and 20℃ for each group, respectively. Although, the habitat of group one had a lower altitude (29 m), its mean annual temperature (11.6℃) was the lowest. It mainly due to its appar ent high latitude (38°41'N). A relatively lower mean annual growth temperature of about 16℃ for the second group resu lted from comparatively higher altitudes, 1230 m (vars. auriculato-dentata) and 1045 m (arguta) respectively. The third group had the highest mean annual growth temperature of about 20℃. For var. acuta, this cou ld be due to its relatively lower altitude (259 m) as well as its higher latitude (30°48'N). On the other hand, the h igh growth temperature of var. crispa can be mainly attributed to its lower latitude (23°51'N) and to some extent the lower altitude (79 m) of its habitat.

Based on the thousand-seed weight of the samples, the five varieties could also be divided into three groups. The first group was made up of only one variety (var. frutescens). The second consisted of vars. auriculato-dentata and arguta, both of which had their thousand seed weights showing a significant difference (<0.01) to that of var. frutescens. The third included vars. acuta and crispa, and both of their thousand-seed weight showed a very significant difference (<0.01) to that of the var. frutescens. Considering the oil content of the seeds, three groups of varieties were also formulated. The first group comprised of var. frutescens only while the second consisted of vars. auriculato-dentata and arguta. Vars. acuta and crispa made up of the last group.

From the results shown in Table 1, it is clear that the five varieties originating from various habitats could be divided into the same three groups based on the mean annual growth temperature, the thousand-seed weight or the seed oil contents. The results imply that annual temperature has a great effect on the thousand seed weight. Generally, the higher the mean annual temperature, the lower the thousand seed weight, since seeds at habitats with high temperatures might require lesser energy reserves for survival after germination. The effect of annual temperature on seed oil contents was not obvious. This issue will be discussed further.

Fatty acid content of Perilla seed oil

Oil contents of the five seed samples ranged from 33.25 to 42.58% (Table 1). For all of them, nine different kinds of fatty acids were identified. Based on the number of fatty acid identified, the five varieties could be divided into two distinct groups. Three varieties, namely vars. acuta, auriculato-dentata and frutescens were in the first group which had seven fatty acids. They all lacked trans-10-

Table 2. Fatty acid compositions and their relative contents in seed oils of five Perilla. varieties.

Fatty acid name	Chemical structure	Varieties				
		Frutescens	Arguta	Auriculato-dentata	Acuta	Crispa
Myristic acid	14:0	0.37±0.1	0.23±0.1	0.16±0.0	0.31±0.1	0.21±0.1
Palmitic acid	16:0	7.23±0.1	6.26±0.2	6.02±0.2	5.68±0.1	6.31±0.0
Stearic acid	18:0	2.89±0.2	2.39±0.1	2.61±0.1	3.28±0 .0	2.77±0.1
Oleic acid	18:1 (ω-9)	20.77±0.1	14.82±0.2	15.75±0.2	13.54±0.2	12.86±0.2
Trans-10-octadecenoic acid	18:1	-	1.24±0.0	-	-	0.93±0.1
Linoleic acid	18:2 (ω-6)	10.54±0.0	13.05±0.1	11.47±0.1	15.87±0.2	14.18±0 .2
α-linolenic acid	18:3 (ω-3)	52.58±0.1	57.76±0.2	61.98±0.2	53.68±0.1	55.81±0 .1
Arachidic acid	20:0	-	0.28±0.0	0.41±0.1	-	0.22±0.1
Cis-11-eicosenoic acid	20:1	0.16±0.0	-	-	0.24±0.1	-

Note: 1 -, not detected; 2 Data are expressed as mean ±SD (n = 3).

Table 3. Saturated and unsaturated fatty acids in seed oils of five Perilla varieties.

Type and ratio of different fatty acid	Varieties				
	Frutescens	Arguta	auriculato-dentata	Acuta	Crispa
Saturated FA	10.49	9.16	9.18	9.27	9.29
Monounsaturated FA	20.93	16.06	15.75	13.78	13.79
Polyunsaturated FA	63.12	70.81	73.45	69.55	69.99
Total unsaturated FA	84.05	86.87	89.20	83.33	83.78
Saturated : monounsaturated : polyunsaturated	1: 2.00: 6.02	1: 1.75: 7.73	1: 1.72: 8.00 1: 1.69: 7.35	1:1.49: 7.50	1:1.48: 7.53
Saturated : unsaturated	1: 8.02	1: 9.48	1:9.72 1: 9.04	1:8.99	1:9.01
Monounsaturated : polyunsaturated	0.33	0.23	0.22	0.20	0.22

octadecenoic acid. Vars. acuta and frutescens both have no arachidic acid and var. auriculato-dentata has no cis-11-eicosenoic acid. The second group had two varieties, vars. crispa and arguta, both of which had eight various types of fatty acids, excluding only cis-11-eicosenoic acid (Table 2). The nine identified fatty acids included saturated, monounsaturated and polyunsaturated fatty acids which accounted for 9.16 to 10.49, 13.78 to 20.93 and 63.12 to 73.45% of their total fatty acids, respectively. In particular, total contents of unsaturated fatty acids were over 90% on average (Table 3). The saturated fatty acids identified include palmitic acid (C16:0; 5.68 to 7.23%), stearic acid (C18:0; 2.39 to 3.28%), arachidic acid (C20:0; 0.22 to 0.41%) and myristic acid (C14:0; 0.21 to 0.37%) in an order of decreasing quantity in the samples. Monosaturated ones were dominantly oleic acid together with rare amounts of both trans-10-octadecenoic acid and cis-11-eicosenoic acid. There were only two polyunsaturated fatty acids in the seed oil of the five varieties, namely the ω-6 linoleic acid and the ω-3 α-linolenic acid (Table 2).

The ratio of the saturated, monounsaturated and polyunsaturated fatty acids was 1:1.69:7.35 on average (Table 3). This result was comparable with those obtained by Kim and Choi (2004) in Korea (1:1.50:7.50) and Siriamornpun et al. (2006) in Thailand (1:1.19:7.76). Most interestingly, the ratios of saturated over unsaturated fatty acids in these three studies were nearly exact the same, 1:9 (1:9.04, 1:9, 1:8.95, respectively). It shows that the effects of environmental factors on fatty acid compositions are evidenced mainly by the relative ratios of monounsaturated over polyunsaturated fatty acids, with 0.24, 0.20, and 0.15 in China, Korea and Thailand, respectively.

Further characterization of unsaturated fatty acids in seed oils of the five Perilla varieties

Among the monounsaturated fatty acids, oleic acid

(C18:1) was the major one. Oleic acid is one of necessary components in diet. It has been shown that oleic acid is considered to be responsible for lowering levels of the low-density lipoprotein (LDL) cholesterol (Grundy, 1989). LDL is usually termed the bad cholesterol particles because current theory suggests that higher levels of LDL particles promote health problems and cardiovascular diseases as opposed to high-density lipoprotein (HDL) particles, which are often called good cholesterol or healthy cholesterol (Peskin et al., 2008). The highest amount of oleic acid was found in var. frutescens (20.77%) while the other samples contained 12.86 to 15.75%. cis-11-eicosenoic acid (C20:1 cis-11) was determined in var. acuta (0.24%) and var. frutescens (0.16%) samples. One trans-10-octadecenoic acid (C18:1 trans-10) was also found in var. crispa (0.93%) and var. arguta (1.24%).

The most predominant polyunsaturated fatty acid in terms of quality and quantity obtained in this study was α-linolenic acid, which ranged from 52.58 to 61.98% of the total fatty acids in seed oils of the five Perilla varieties, and it comprised 65.67 to 68.37% of the total unsaturated fatty acids and 83.30 to 84.38% of the polyunsaturated acids, respectively. This high proportion of α-linolenic acid in the seed oil outclassed those in most of other seed oil, such as olive, oil-tea, earthnut, corn, sunflower, sesame, and so on, which usually contains less than 1% of the total fatty acid in their seed oil (Rafalowski et al., 2008). The var. auriculato-dentata at latitude 23°4 2'N had the highest content while the one at 38°41'N had the lowest one. These results were similar to those reported in previous studies on Perilla seed where α-linolenic acid content of the seed was between 53 and 62% (Longvah and Deosthale, 1991; Shin and Kim, 1994; Gunstone et al., 1994; Siriamornpun et al., 2006; Peiretti, 2011). Polyunsaturated fatty acid have beneficial effects for both maintenance of normal health and prevention of chronic diseases by regulating lipid levels (Lauritzen et al., 2000; Mori et al., 2000), cardiovascular (Kris-Etherton et al., 2002) and immuno functions (Hwang, 2000). ω-3 fatty acid α-linolenic acid (C18:3) is one essential fatty acid and a member of polyunsaturated fatty acid in seed oils. It cannot be produced within the body and therefore must be acquired from sources such as Perilla seed oil. α-linolenic acid is a precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Animal studies suggest that α-linolenic acid-rich Perilla seed oil has ability to prevent atherosclerosis and chemical-induced cancer. It also improves immune and mental function (Kurowska et al., 2003). Kim and Choi (2005) indicated that feeding Perilla oil diets decreased postprandial plasma lipids in male Sprague-dawley rats. Dietary Perilla oil also exhibits a similar physiological activity to fish oil in modulating hepatic fatty acid oxidation (Kim et al., 2004). Chang (2008) also shown that dietary Perilla oil inhibits proinflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice.

Another polyunsaturated fatty acid in the analyzed samples was ω-6 linoleic acid (C18:2). It is also an essential fatty acid and its content ranged from 10.54 to 15.87% in the present study. Studies indicate that a high intake of ω-6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, characterized by increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time (Simopoulos, 1999). Today, more than 85% of the total dietary polyunsaturated fatty acid in developed country is ω-6 polyunsaturated fatty acid, mainly linoleic acid, a precursor of arachidonic acid, whereas the consumption of ω-3 polyunsaturated fatty acid has declined (Simopoulos, 2002). Since the consumption of ω-6 has been associated with childhood obesity, concerns have been raised (Ailhaud et al., 2006). However, animal studies have yielded conflicting results, with some studies demonstrating that a diet enriched in ω-6 polyunsaturated fatty acid decreases adipose tissue mass (Matsuo et al., 2002; Okuno et al., 1997), whereas others have showed that consumption of ω-6 polyunsaturated fatty acid is associated with an increased propensity for obesity (Cleary et al., 1999; Massiera et al., 2003) . Contents of the ω-6 linoleic acid in the five samples were only 10.54 to 15.87% (Table 2), which was far below those in seed oil from sunflower (≈55%), corn (≈49%) and soybean (≈46%), and was comparable to those in olive oil (≈11%) and tea oil (≈20%) (Sahari et al., 2004; Rafalowski et al., 2008). Furthermore, the ratio of ω-6 to ω-3 fatty acids in these oils was as low as 0.2 to 0.26. This indicates that Perilla oil has beneficial proportion of polyenoic fatty acids and has the potential to improve the ω-6/ω-3 ratio in diet. FAO (1994) recommends that the ratio of linoleic acid to α-linolenic acid in the diet should be between 5:1 and 10:1. Excessive amounts of ω-6 polyenoic acid and very high ω-6/ω-3 ratio promote the pathogenesis of many diseases, including cardiovascular disease, cancer and inflammatory and autoimmune diseases (Okuyama, 2001; Simopoulos, 2002; Griffin, 2008). Our results indicate that Perilla oil is one of the best oil derived from plant sources in terms of fatty acid composition. It could be developed into commercial products as a valuable alternative vegetable oil. Furthermore, oil from seeds obtained in regions with lower average growth temperature (for example, 16.05°C of vars. arguta and auriculato-dentata over 20.3°C of vars. acuta and crispa) have relatively higher percentage (88.04% in vars. arguta and auriculato-dentata over 83.56% in vars. acuta and crispa) of unsaturated fatty acids.

The previous results indicate that the 5 different Perilla varieties investigated here are significantly different based on their respective oil contents and fatty acids compositions. This could be accounted for by the possible differences in climate, latitude, soil and growth conditions, which could affect the chemical components and growth form of Perilla. The different natural evolution

of the different Perilla varieties, hence their genetic diversity could also have made them to adapt to the environment differently. These results might be significant for selection studies in order to better identify Perilla varieties for health diets, as well as in the introduction and breeding of Perilla L. The breeding of better varieties of Perilla could serve as a basic scientific reference for early realization of its large scale cultivation and its industrial production.

Conclusions

It is clear that Perilla seed oil was a great edible oil derived from plant resources: (1) the oil contains high proportion of unsaturated fatty acids. Saturated fatty acids accounted only for less than 10%. (2) Content of the ω-6 linoleic acid in the oil was much lower than those of the frequently-used edible oils, such as soybean and maize oils, and was comparable to those of the olive and tea oil. (3) Content of the ω-3 linolenic acid was the highest one among soybean, maize, sunflower, sesame, earthnut, walnut, olive and oil-tea, and even 10% higher than that of the flaxseed oil. Its ratio of ω-6/3 fatty acids was as low as 0.2 to 0.26, and this ratio of soybean and maize oils, for example, was as high as 100 and 60, respectively. It will be a great choice for us to reduce the high ratio of ω-6/3 fatty acids by adding a certain portion of Perilla oil into our frequently-used edible oil.

Oil contents of the five Perilla varieties are associated with annual growth temperature, which was mainly affected by the altitudes and latitudes at their habitats. The oil content is negatively associated with annual growth temperature and the ratio of saturated over unsaturated fatty acids is positively associated with annual growth temperature. There is no association between thousand-seed weight and the ratio between monounsaturated and polyunsaturated fatty acids.

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REFERENCES

Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P (2006). Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog. Lipid Res., 45(3): 203-236.

AOCS (1989). Official methods and recommended practices of the American Oil Chemists Society. 4th edn. Champaign: AOCS.

Asif M (2011). Health effects of omega-3,6,9 fatty acids: Perilla frutescens is a good example of plant oils. Orient. Pharm. Exp. Med., 11: 51-59.

Brenner DM (1993). Perilla: Botany, uses and genetic resources. In: New crops, (edited by J. Janick and J.E. Simon). Pp. 322-328. New York: Wiley.

Burdge GC, Calder PC (2006). Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. Nutr. Res. Rev., 19: 26-52.

Butler J (2009). The Fish Report: Why Public Health Policy Should Promote Plant Omega-3 in Preference to Fish Oils. Vegetarian and Vegan Foundation, Bristol.

Calder PC (2004). n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin. Sci., 107: 1-11.

Chang HH, Chen CS, Lin JY (2008). Dietary Perilla oil inhibits proinflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice. Lipids, 43: 499-506.

Connor WE (2000). Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr., 71: 171S-175S.

Choi IS, Son SY, Kwon OH (1980). Effect of seedling age and planting density on the yield and its component of Perilla (Ocymcides Var. Typica Makino) intercropped with tobacco or aftercropped. Korean Soc. Hort. Sci., 25: 68-75.

Cleary M, Phillips F, Morton R (1999). Genotype and diet effects in lean and obese Zucker rats fed either safflower or coconut oil diets. Proc. Soc. Exp. Biol. Med., 220: 153–161.

Davis BC, Kris-Etherton PM (2003). Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. Am. J. Clin. Nutr., 78: 640S-646S.

Ezaki O, Takahashi M, Shigematsu T, Shimamura K, Kimura J, Ezaki H, Gotoh T (1999). Long-term effects of dietary alpha-linolenic acid from Perilla oil on serum fatty acids composition and on the risk factors of coronary heart disease in Japanese elderly subjects. J. Nutr. Sci. Vitaminol., 45: 759-772.

FAO (1994). Food and Nutrition Paper, Fats and oils in human nutrition: Report of a joint expert consultation. No 57. FAO, Geneva.

Griffin BA (2008). How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the Optilip study. Curr. Opin. Lipidol., 19: 57-62.

Grundy SM (1989). Monounsatrated fatty acids and cholesterol metabolism: implication for dietary recommendations. J. Nutr., 119: 529-533.

Gunstone FD, Harwood J, Padley FB (1994). The Lipid Handbook (2nd ed.), Chapman and Hall, London.

Hu Y, Sun LW, Neo MC, Zhang YX, Wen CX, Xie XL, Liu YJ (2010). Primary identifications and palynological observations of Perilla L. in China. J. Syst. Evol., 48: 133-145.

Hwang D (2000). Fatty acids and immune responses a new perspective in searching for clues to mechanism. Ann. Rev. Nutr., 20: 431-456.

Ito M, Toyoda M, Honda G (1999a). Chemical composition of essential oil of Perilla frutescens. Nat. Med., 53: 32-36.

Ito M, Toyoda M, Honda G (1999b). Essential oil composition of hybrids and amphidiploids of Japanese wild Perilla. Nat. Med., 53: 118-122.

Ito M, Kiuchi F, Yang LL, Honda G (2002). A new type of essential oil from Perilla frutescens from Thailand. J. Essent. Oil Res., 14: 416-419.

Kim HK, Choi S, Choi H (2004). Suppression of hepatic fatty acid synthase by feeding α-linolenic acid rich Perilla oil lowers plasma triacyglycerol level in rats. J. Nutr. Biochem., 15: 485-492.

Kim HK, Choi H (2005). Stimulation of acyl-CoA oxidase by alinolenic acid-rich perilla oil lowers plasma triacylglycerol level in rats. Life Sci., 77: 1293–1306.

Kim KS, Park SH, Choung MG (2007). Nondestructive determination of oil content and fatty acid composition in Perilla seeds by near-infrared spectroscopy. J. Agric. Food Chem, 55: 1679-1685.

Koezuka Y, Honda G, Tabata M (1985). An intestinal propulsion promoting substance from Perilla frutescens and its mechanism of action. Planta Med., 6: 480-482.

Kris-Etherton PM, Harris WS, Appel LJ (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. Circulation, 106: 2747-2757.

Kurowska EM, Dresser GK, Deutsch L, Vachon D, Khalil W (2003). Bioavailability of omega-3 essential fatty acids from Perilla seed oil. Prostaglandins Leukot. Essent. Fatty Acids, 68: 207-212.

Lauritzen I, Blondeau N, Heurteaux C, Widmann C, Georges R, Michel

L (2000). Polyunsaturated fatty acids are potent neuroprotectors. E.M.B.O. J., 19: 1784-1793.
Lee JI, Han ED, Lee ST, Park HW (1986). Study on the evaluation of oil quality and the differences of fatty acids composition between varieties in Perilla (Perilla frutescens Britton var. japonica Hara). J. Kor. Breed., 18: 228-233.
Lee JK, Ohnishi O (2001). Geographical differentiation of morphological characters among Perilla crops and their weedy types in East Asia. Breed. Sci., 51: 247-255.
Lee JK, Nitta M, Kim NS, Park CH, Yoon KM, Shin YB, Ohnishi O (2002). Genetic diversity of Perilla and related weedy types in Korea determined by AFLP analyses. Crop Sci., 42: 2161-2166.
Lewis CJ (2008). Letter regarding dietary supplement health claim for omega-3 fatty acids and coronary heart disease, http://www.cfsan.fda.gov/~dms/ds-ltr11.htmland "Letter Regarding Dietary Supplement Health Claim for Omega-3 Fatty Acids and Coronary Heart Disease". U. S. Food and Drug Administration via Internet Archive.
Li HL (1969). The vegetable of ancient China. Econ. Bot., 23: 235-260.
Li XW (1974). Flora Reipublicae Popularis Sinicae. Beijing: Sci. Press, 65: 282-287
Li XW (1977). Perillinae. In: Flora Reipublicae Popularis Sinicae. Beijing: Sci. Press, 66: 282-287
Liu YX, Zhang WM, Qian XS (1996). Research and utilization of Perilla. Wild Plant Resour., 3: 24-27.
Liu YX, Zhang WM (1998). Classification and resources distribution of Perilla. Chin. Wild Plant Resour., 17: 1-4.
Longvah T, Deosthale YG (1991). Chemical and nutritional studies on Hanshi (Perilla frutescens), a traditional oilseed from Northeast India. J. Am. Oil Chem. Sci., 68: 781-784.
Marangoni F, Colombo C, Martiello A, Poli A, Paoletti R, Galli C (2007). Levels of the n-3 fatty acid eicosapentaenoic acid in addition to those of alpha linolenic acid are significantly raised in blood lipids by the intake of four walnuts a day in humans. Nutr. Metab. Cardiovasc. Dis., 17: 457-461.
Massiéra F, Saint-Marc P, Seydoux J, Murata T, Kobayashi T, Narumiya S, Guesnet P, Amri EZ, Négrel R, Ailhaud G (2003). Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern ? J. Lipid Res., 44: 271–279.
Matsuo T, Takeuchi H, Suzuki H, Suzuki M (2002). Body fat accumulation is greater in rats fed a beef tallow diet than in rats fed a safflower or soybean oil diet. Asia Pac. J. Clin. Nutr., 11: 302-308.
Mbatia BN (2011).Valorisation of fish waste biomass through recovery of nutritional lipids and biogas. Department of Biotechnology. Doctoral Thesis. Lund University, Sweden.
Miller W (1922). Perilla. In: The Standard Cyclopedia of Horticulture, (edited by L.H. Bailey), Pp, 2553. London, Macmillan.
Misra LN, Husain A (1987). The essential oils of Perilla ocimoides: A rich source of rosefuran. Planta Med., 53: 379-390.
Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, Beilin JL (2000). Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. Am. J. Clin. Nutr., 71: 1085-1094.
Mozaffarian D, Rimm EB (2006). Fish intake, contaminants, and human health: evaluating the risks and the benefits. J.A.M.A., 296: 1885-1899.
Nitta M, Lee JK, Ohnishi O (2003). Asian Perilla crops and their weedy forms: their cultivation, utilization and genetic relationships. Econ. Bot., 57: 245-253.
Okamoto M, Mitsunobu F, Ahida K, Mifune T, Hosaki Y, Tsugeno H, Harada S, Tanizaki Y (2000). Effects of dietary supplementation with n-3 fatty acids compared with n-6 fatty acids on bronchial asthma. Int. Med., 39: 107-111.
Okuno M, Kajiwara K, Imai S, Kobayashi T, Honma N, Maki T, Suruga K, Goda T, Takase S, Muto Y, Moriwaki H (1997). Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation. J. Nutr., 127: 1752-1757.Okuyama H (2001). High n−6 to n−3 ratio of dietary fatty acids rather than serum cholesterol as a major risk factor for coronary heart disease. Eur. J. Lipid Sci. Tech., 103: 418-422.
Park HS, Kim JG, Cho MJ (1981). Chemical composition of Perilla frutescens Britton var. crispa Decaisne cultivated in different areas of Korea: part 1. Characteristics of lipid and fatty acid composition (in Korean). J. Korean Agric. Chem. Soc., 24: 224-229.
Park YJ, Dixit A, Ma KH, Lee JK, Lee MH, Chung CS, Nitta M, Okuno K, Kim TS, Cho EG, Rao VR (2008). Evaluation of genetic diversity and relationships within an on-farm collection of Perilla frutescens (L.) Britt. using microsatellite markers. Genet. Resour. Crop Evolut., 55: 523-535.
Pei J (1955). Flora of Chinese medicinal herbs. Beijing: Sci. Press, 1: 44-45.
Peiretti PG (2011). Fatty acid content and chemical composition of vegetative parts of Perilla (Perilla frutescens L.) after different growth lengths. Res. J. Med. Plant, 5: 72-78.
Rafalowski R, Zegarska Z, Kuncewicz A, Borejszo Z (2008). Fatty acid composition, tocopherolsand ß-carotene content in Polish commercial Vegetable Oils. Pak. J. Nutr., 7: 278-282.
Rao S, Abdel-Reheem M, Bhella R, McCracken C, Hildebrand D (2008). Characteristics of High α-Linolenic Acid Accumulation in Seed Oils. Lipids, 43: 749-755.
Sayanova OV, Napier JA (2004). Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in transgenic plants. Phytochemistry, 65: 147-158.
Sahari MA, Ataii D, Hamedi M (2004). Characteristics of tea seed oil in comparison with sunflower and olive oils and its effect as a natural antioxidant. J. Am. Oil Chem. Soc., 81: 585-588.
Shin HS, Kim SW (1994). Lipid composition of Perilla seed. J. Am. Oil Chem. Soc., 71: 619-622.
Simopoulos AP (1999). Essential fatty acids in health and chronic disease[1,2]. Am. J. Clin. Nutr., 70: 560S-569S.
Simopoulos AP (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. World Rev. Nutr. Diet, 56: 365-379.
Siriamornpun S, Li D, Yang L, Suttajit S, Suttajit M (2006). Variation of lipid and fatty acid compositions in Thai Perilla seeds grown at different locations. J. Song Sci. Tech., 28: 17-21.
Takano H, Osakabe N, Sanbongi C, Yanagisawa R, Inoue K, Yasuda A, Natsume M, Baba S, Ichiishi E, Yoshikawa T (2004). Extract of Perilla frutescens enriched for rosmarinic acid inhibits seasonal allergic rhinoconjunctivitis in humans. Exp. Biol. Med., 229: 247-254.
Tanaka T (1976). Tanaka's cyclopedia of edible plants of the world. Keigaku Publishing Company, Tokyo.
Ueda H, Yamazaki C, Yamazaki M (2002). Luteolin as an antinflammatory and anti-allergic constituent of Perilla frutescens. Biol. Pharm. Bull., 25: 1197-1202.
Zatonski W, Campos H, Willett W (2008). Rapid declines in coronary heart disease mortality in Eastern Europe are associated with increased consumption of oils rich in alpha-linolenic acid. Eur. J. Epidemiol., 23: 3-10.
Zeven AC, de-Wet JMJ (1982). Dictionary of cultivated plants and their regions of diversity. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands. 34.
Zhang X, Wu W, Zheng YL, Chen L, Qianrong C (2009). Essential oil variations in different Perilla L. accessions: chemotaxonomic implications. Plant Syst. Evol., 281: 1-10.