ORIGINAL ARTICLE

Variation of lipid and fatty acid compositions in Thai Perilla seeds grown at different locations

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Abstract

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Variation of lipid and fatty acid compositions in Thai Perilla seeds grown at different locations
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Perilla or Nga-Kee-Mon (*Perilla frutescens*) seed has long been known as a rich source of α-linolenic acid (18:3, n-3). It is widely cultivated throughout Thailand. However, there are no data on the variation of lipid and fatty acid compositions among crops from different regions. The aim of this study was to examine the compositions of lipids and fatty acids in Thai perilla seed grown at different locations. Two different perilla seeds were harvested from Maehongsorn and Chiang Mai districts, and one commercial perilla was purchased from local market. Seeds were ground, lipid was extracted with chloroform: methanol (2:1, v/v) and its composition determined by Iatroscan (TLC/FID). Fatty acid composition was analyzed with GLC using standard methods. Lipid content was between 34-36% (w/w). Triacylglycerol was a predominant lipid in perilla seed (97% of total lipids), and a minor component was phytosterol (3% of total lipids). The ratio of

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saturates: monounsaturates: polyunsaturates was approximately 1: 1: 8. Most predominant fatty acid was α-linolenic acid (18:3, n-3) (55-60% of total fatty acid). Seeds from Maehongsorn district had the highest concentration of α-linolenic acid, and commercial perilla had the lowest (P<0.05). Other two predominant fatty acids were linoleic acid (18:2, n-6) (18-22% of total fatty acid) and oleic acid (18:1) (11-13% of total fatty acid). The results showed that the compositions of lipids and fatty acids in Thai perilla seeds varied significantly among samples from different locations.

Key words : perilla oil, lipid composition, fatty acid composition, α-linolenic acid, growing location, *Perilla frutescens*

It has long been known that dietary fat (lipids) intake is associated with numerous chronic diseases, such as cardiovascular disease and diabetes due to an increased serum LDL-cholesterol levels (Hu *et al*., 1999; Iso *et al*., 2001) and insulin resistance (Salmeron *et al*., 2001; Meyer *et al*., 2001). It is, therefore, important to search for good sources of oil that would have beneficial health effects. Perilla or Nga-Kee-Mon (*Perilla frutescens*) seed has been known as a rich source of α-linolenic acid (ALA, 18:3, n-3), accounting for approximately 60% of total fatty acids (Gunstone *et al*., 1994).

ALA was found in plants, animals, zoo-plankton, phytoplankton and marine species (Li *et al*., 2002). The most predominant n-3 poly-unsaturated fatty acid (PUFA) in terrestrial plants is ALA (Sinclair *et al*., 2002). ALA is the parent fatty acid of the long-chain n-3 PUFA and is an essential fatty acid for mammals because they do not possess the ability to insert double bonds in 18- carbon PUFA between the methyl end and the middle of the molecule (Sinclair *et al*., 2002).

A number of epidemiological and experimental studies have reported the beneficial effects of perilla oil on health (Li, 2003). Dietary sources of ALA, such as perilla seed oil may have the capacity to inhibit the generation of leucocytes in patients with asthma (Okamoto *et al*., 2000). This study also reported that dietary supplementation of perilla seed oil significantly decreased serum levels of cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and phospholipids. In addition, a study on the rats fed with diets high in linoleic acid (LA, 18:2, n-6) from safflower oil and ALA from perilla oil reported that ALA had more potent serum cholesterol-lowering ability than LA both in short and long-feeding terms (Ihara *et al*., 1998). ALA contained in perilla oil is less atherogenic than oleic acid (18:1) and LA (Sadi *et al*., 1996). Since perilla seed oil is known to have health benefits, it was of interest to examine whether growing conditions has any effect on fatty acid and lipid compositions of perilla seed oil.

Materials and Methods

Samples

Perilla seeds were harvested from Maehongsorn (PM) and Chiangmai (PC) districts. These two seed samples and one commercial perilla (PR) seed sample purchased from local market were used in this study. Before extraction of lipids the seed samples were ground with a mortar and pestle.

Lipid analysis

Approximately 2 g of ground seed was used for lipid extraction with 20 mL of chloroform-methanol (2:1, v/v) containing 10 mg/L of butylated hydroxytoluene (BHT) and 0.2 mg/mL of tricosanoic acid (C23:0, Sigma, USA) as internal standard (Folch *et al*., 1957). The extracted lipids were separated and identified with MK-6s Iatroscan TLC/FID (Laboratories Inc. Japan). The fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by saponification using 3 mL of H_2SO_4 in methanol (0.9 mol/L) and 1 mL of toluene. The FAMEs were separated by capillary gas chromatography using a 60 m x 0.25 mm (I.D.) fused silica bonded phase column (BPX70, SGE,

Table 1. Lipid content of perilla seed oils grown at different locations.

Perilla populations	Lipid content (g/100g)
Maehongson	34.62±0.97 [b]
Changmai	37.14±0.95 [a]
Commercial	34.43±0.83 [b]

Data presented as Mean ± Standard Deviation.
Numbers on the same column with differing superscripts are significantly different at P≤0.05.

Table 2. Lipid composition of perilla seed oils analyzed with TLC/FID.

Seeds	CE	TAG[ns]	DAG	FFA	PTS[ns]
Maehongson	nd	96.48±0.27	nd	nd	3.52±0.27
Changmai	nd	97.18±0.52	nd	nd	2.82±0.52
Commercial	nd	97.02±0.49	nd	nd	2.98±0.49

CE = Cholesterol ester, TAG = Triacylglycerol, DAG = Diacylglycerol, FFA = Free fatty acids, PTS = Phytosterol
ns = no-significant difference, nd = not detected

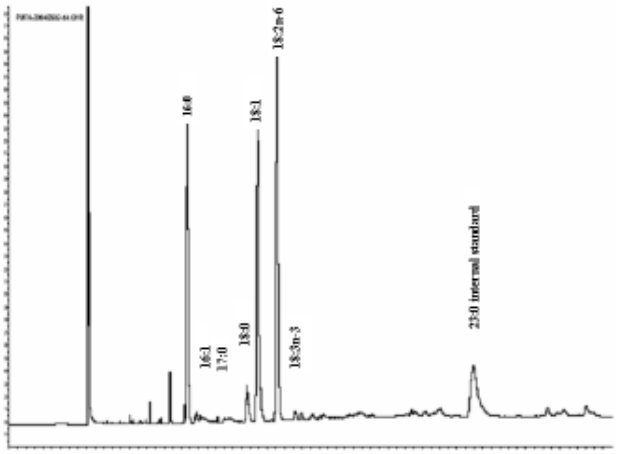

Figure 1. Chromatogram showing fatty acid composition of perilla seed analyzed with GC.

Melbourne, Australia) (Li *et al*., 1998). The column oven was programmed from 125ºC for 3 min to 220ºC at 8ºC/min, using helium as carrier gas with a flow rate of 43 cm/s. Fatty acids were identified by comparison with standard mixtures of fatty acid methyl esters and the results calculated using response factors derived from chromatograph standard of known position (Li *et al*., 1998) (Nu-Chek-Prep, Elysian, MN, USA).

Results and Discussion

Total lipid content of perilla seed ranged from 34 to 37% (g/100g) (Table 1). A previous

study by Longvah *et al*., (1999) from India reported the content of 50%. Triacylglycerol (TAG) was the most predominant lipid in perilla seed (97% of total lipids), and phytosterols (3% of total lipids) was the other lipid in this oil (Table 2). It appears that this is the first time the lipid composition of the perilla seeds is reported. Fatty acid composition of the perilla seed oil is presented in Figure 1. The ratio of saturates: monounsaturates: polyunsaturates was approximately 1:1:8 in the present study, while Longvah *et al*., (1999) from India reported the ratio of 1.25:1.25:7.5, and Kim and Choi (2004) from Korea reported the ratio of 1:1.5:7.5.

The predominant saturated fatty acid (SFA) in this oil was C 16:0 (ranging from 6.5 to 7.3%). The other two minor fatty acids were C18:0 (ranging from 2.6 to 3.3%) and C17:0 (ranging from 0.03 to 0.16%). C18:1 (ranging from 11.4 to 12.6%) and C16:1 (ranging from 0.08 to 0.17%) were the two main monounsaturated fatty acids (MUFA) in perilla seed oils. Two essential fatty acids, LA and ALA were the significant fatty acids in terms of quality and quantity of perilla seed oils. Most predominant fatty acid was ALA (55-60% of total fatty acid) ($P<0.05$). These results were similar to those reported in previous studies on perilla seed where ALA content of the seed was about 58 to 60 % (Ihara *et al*., 1998; Gunstones *et al*., 1994). In plants, ALA is also found high in green leaves (mainly in glycolipids) and in certain seed and nut oils such as rapeseed, flaxseed and chia seed, walnuts and some beans (Sinclair *et al*., 2002). Another PUFA in the analyzed samples was LA, ranging from 18 to 22%. The ratio of n-6:n-3 ranged from 0.3 to 0.4, which has been suggested to have a beneficial effect on human health (Kim *et al*., 2000).

The present study revealed that lipid composition of three perilla samples grown at different locations in Thailand did not differ significantly. However, fatty acid composition of these samples varied significantly. Total amount of SFA was significantly higher in PR than in PC. PR also had significantly higher level of MUFA, especially oleic acid (18:1). On the other hand, PC contained significantly higher amount of PUFA than PR.

Table 3. Fatty acid composition of perilla seed oils grown at different locations.

Seeds	SFA				MUFA			PUFA		
	16:0	17:0	18:0	Total	16:1	18:1	Total	18:2 n-6	18:3 n-3	Total
Maehongson	6.86±0.08^{ab}	0.03±0.01^{b}	3.16±0.17^{ab}	10.05±0.26^{ab}	0.11±0.01^{b}	11.55±0.27^{b}	11.66±0.29^{b}	18.45±0.07^{b}	59.84±0.54^{a}	78.29±0.61^{ab}
Changmai	6.54±0.30^{b}	0.16±0.05^{a}	2.67±0.18^{b}	9.37±0.53^{b}	0.17±0.01^{a}	11.41±0.23^{b}	11.58±0.24^{b}	22.09±0.07^{a}	56.96±0.73^{b}	79.05±0.80^{a}
Commercial	7.33±0.42^{a}	0.09±0.03^{b}	3.32±0.35^{a}	10.74±0.79^{a}	0.08±0.03^{b}	12.66±0.69^{a}	12.74±0.71^{a}	22.26±0.24^{a}	54.26±1.41^{c}	76.52±1.65^{b}

Data presented as Mean ± SD. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids and PUFA = polyunsaturated fatty acids. Numbers on the same column with differing superscripts are significantly different at $P\leq0.05$

This result indicated that the amount of ALA in perilla seed oil varied significantly among the three samples. Since the genotypes or cultivars used in the three samples were unknown (may be different) it is hard to suggest that the differences observed are due to genotype or environment or their interaction.

Conclusion

Lipid content and composition, and fatty acid composition of perilla seed varied considerably among the samples from different locations. ALA is a most predominant fatty acid in terms of quantity and quality in perilla seed. The ratios of SFA: MUFA:PUFA and n-3:n-6 in perilla seed oil are desirable with respect to health benefits.

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