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Fatty Acid Content and Chemical Composition of Vegetative Parts of Perilla (*Perilla frutescens* L.) after Different Growth Lengths

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ABSTRACT

Perilla (*Perilla frutescens* L.) belonging to the Lamiaceae family, is an edible plant that is frequently used as one of the most popular garnishes and food colorants in some Asian countries and as part of popular and traditional Chinese herbal medicines. The objective of this study was to determine the Fatty Acid (FA) content, chemical composition and Gross Energy (GE) of the plant during the growth cycle. Herbage samples were collected four times at progressive morphological stages from 15 to 70 cm of plant height. The FA profiles in the plant were characterised by three dominant FAs: palmitic acid (C16:0), linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3), which ranged from 8.5-9.7%, 11.5-12.1% and 52.0-55.5% of the total FA, respectively. The FA pattern in the whole plant during growth only differed for palmitoleic acid (C16:1) and stearic acid (C18:0). The evolution of the whole perilla plant quality during growth was characterised by a progressive increase in the neutral and acid detergent fibre contents, while the crude protein content decreased from the first month after sowing to the last stage. Organic matter and GE were higher at the last stage than at the other stages. The first summer cut of perilla, whose fat fraction is rich in polyunsaturated fatty acids, should be harvested at around two months after sowing, since its nutritional quality deteriorates when cutting is delayed. Further studies are necessary to determine the changes in the other chemical constituents of perilla plant during the growth cycle.

Key words: *Perilla frutescens*, lipid, crude protein, fibrous fraction, gross energy

INTRODUCTION

Perilla (*Perilla frutescens*), belonging to the Lamiaceae family, is an edible plant that is frequently used as one of the most popular garnishes and food colorants in some Asian countries such as Japan and China (Peng *et al.*, 2005). The leaves of perilla are used as a garnish for raw fish in Japan. It is believed that *Perilla* is used not only as a flavor but also as an antidote to food poisoning (Kurita and Koike, 1982). Intact leaves are also used as condiments or flavoring agents in various Korean foods (Shin and Kim, 1994). The leaves as well as the seeds of perilla are part of popular and traditional Chinese herbal medicines, which are prescribed for colds and coughs and to promote digestion (Duke, 1988). Dried red perilla leaves are also used as soyou in Chinese herbal medicine and it is one of the components of saibokuto, which is used to treat bronchial asthma (Ueda *et al.*, 2002).

Longvah and Deosthale (1998) have demonstrated that perilla seed is a potential source of food, that is rich in fat and protein of good quality, which be used in both human and animal nutrition. They also demonstrated that the potential of perilla seed protein can be increased by dehulling the seeds and then cooking them. Perilla seed is particularly used in India (Sharma *et al.*, 1989) and in Korea where the seeds are consumed as flavoring and nutritional sources in combination with

cereals or vegetables after roasting (Shin and Kim, 1994). Perilla seeds and oil are good source of α -linolenic acid (C18:3 n-3; ALA) and these and other aspects of their dietary value have been researched (Longvah and Deosthale, 1991). Perilla oil is widely used as a salad oil dressing or cooking medium (Shin and Kim, 1994).

Perilla has recently been introduced into Europe, Russia and USA as an oilseed crop (Nitta *et al.*, 2003).

Terpenoids, phenolics, flavonoids, cyanogenic glycosides and anthocyanins have been reported as the chemical constituents of perilla, but there has been no indication concerning the oral pharmacological effects of this plant. The oral administration of a perilla leaf extract to mice can inhibit the overproduction of the tumor necrosis factor- α (TNF- α) (Ueda and Yamazaki, 1997) and shows anti-inflammatory and anti-allergic activities (Ueda and Yamazaki, 2001). Rosmarinic acid (Okuda *et al.*, 1986) and ALA (Tsuyuki *et al.*, 1978) have been reported to be anti-inflammatory and anti-allergic substances and luteolin an anti-inflammatory (Ueda *et al.*, 2002) and antitumor promoting substance (Ueda *et al.*, 2003) in perilla leaves and seeds. Perilla leaves have shown to be detoxicant, antitussive, antibiotic and antipyretic (Liu *et al.*, 2000; Nakamura *et al.*, 1998) and are also utilized as a folk medicine to treat intestinal disorders and allergies, particularly in traditional Chinese medical practice (Nakazawa and Ohsawa, 2000).

Perilla extract appears to be a strong anti-inflammatory agent as it inhibits mast cell release of histamine (Simpol *et al.*, 1994), inhibits lipoxygenase activity (Yamamoto *et al.*, 1998) and is an antioxidant (Lamaison *et al.*, 1990; Frankel *et al.*, 1996; Tada *et al.*, 1996).

Among the vegetable oils that are good sources of linoleic acid (C18:2 n-6; LA), perilla seed oil has the highest ALA content (56%). The consumption of perilla oil has also been reported to improve learning ability, retinal function, the suppression of carcinogenesis, metastasis, thrombosis and allergies (Kinsella, 1991) and has shown potential beneficial effects to decrease the circulating levels of serum cholesterol and triglycerides without toxicity in a short term animal experiment (Longvah *et al.*, 2000). Many medical properties, including the antidermatophytic properties of perilla, have been reported (Honda *et al.*, 1984; Terao *et al.*, 1991; Hirose *et al.*, 1990; Duke, 1988).

The objective of this study was to determine the Fatty Acid (FA) profile, chemical composition and Gross Energy (GE) in perilla plants during the growth cycle.

MATERIALS AND METHODS

Plant material and environmental conditions: Perilla was obtained from the Manitoba Seed Expert of Manitoba Inc. (Winnipeg, Canada). The study was conducted in the Western Po Valley near Cuneo, Italy (latitude 44°N, longitude 7°E). The stands were seeded on 20 May 2006 and no irrigations or fertilisers were applied after sowing. Herbage samples were collected with edging shears (0.1 m cutting width) at four progressive morphological stages from vegetative (plant height 15 cm) to the early flowering stage (plant height 70 cm), on subplots of 2 m² randomly located in 3×8 m² plots with three replicates cut to a 1 to 2 cm stubble height. The sampling time ranged from June to July 2006. Sampling was not performed on rainy days and was carried out in the morning, only after the disappearance of dew.

Chemicals: The chemicals used in this study were obtained from Sigma Chemical Co. (St Louis, MO, United States) and from Merck (Darmstadt, Germany).

Fatty acid analysis: Fresh samples of the whole plants were immediately frozen, then freeze-dried and ground to pass a 1 mm screen. Lipid extraction was performed on freeze-dried samples according to Hara and Radin (1978), while the transesterification of the Fas was carried out according to Christie (1982), with the modifications described by Chouinard *et al.* (1999). The FA methyl esters were then determined by gas chromatography according to Peiretti *et al.* (2004).

Chemical analysis: Whole plant samples were immediately dried in a forced-draft oven to constant weight at 65°C to determine the Dry Matter (DM) content and were then air equilibrated, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass a 1 mm screen and stored for later analyses. Dried samples were analysed for Crude Protein (CP) and Ether Extract (EE) according to the methods of the Association of Official Analytical Chemists (AOAC, 1990), ash by ignition to 550°C, Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) without sodium sulfite and α -amylase, as described by Van Soest *et al.* (1991) expressed exclusive of residual ash. The GE was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

Statistical analysis: The variability in FA and the herbage chemical composition of the samples harvested at four stages of maturity were analysed by one-way Analysis of Variance (ANOVA) using the Statistical Package for Social Science (v 11.5, SPSS Inc., Chicago, Illinois, USA) to test the effect of the growth stage. When the values of F were significant (i.e., $p < 0.05$), the Ryan-Einot-Gabriel-Welsch range test (Hochberg and Tamhane, 1987) was used to detect any differences among the means.

RESULTS AND DISCUSSION

Fatty acid profile: The FA analyses disclosed quantitative differences between the plant stages (Table 1) and a high percentage of unknown FAs, which ranged from 24.0 to 18.3% of total FA. The FA profile was characterised by a high percentage of polyunsaturated fatty acids (PUFA), which made up from 64 to 68% of the total FA in the plant during the growth cycle. The FA profiles in the plant were characterised by three dominant FAs: palmitic acid (C16:0; PA), LA and ALA, which ranged from 8.5-9.7, 11.5-12.1 and 52.0-55.5% of the total FA, respectively. The FA pattern in the whole plant during growth only differed for the palmitoleic acid (C16:1) and stearic acid (SA, C18:0) contents.

The FA profile in the plant during growth differs from that of the oil in the seed, which has a similar FA composition to that of linseed oil and contains about 57-64% of ALA, 14-18% of LA,

Table 1: Fatty acid composition (% of total FA) of *Perilla frutescens* at four morphological stages

Plant height (cm)	Days after sowing	Fatty acid								
		C ₁₆	C _{16:1}	C ₁₈	C _{18:1n-9}	C _{18:1n-7}	C _{18:2n-6}	C _{18:3n-6}	C _{18:3n-3}	Unidentified
15	42	8.5	0.33 ^a	1.27 ^a	1.69	0.35	11.7	0.15	52.00	24.00
30	49	8.7	0.37 ^{ab}	1.15 ^a	1.58	0.32	12.1	0.49	55.50	19.80
40	56	8.7	0.34 ^a	1.25 ^a	1.81	0.20	11.5	2.58	54.30	19.30
70	104	9.7	0.41 ^b	1.66 ^b	1.63	0.27	11.8	1.84	54.30	18.30
SEM		0.2	0.01	0.06	0.05	0.03	0.2	0.43	0.73	0.91

Within a column, values with different letter differ ($p < 0.05$)

Table 2: Chemical composition (g kg⁻¹ DM) and gross energy (GE) of *Perilla frutescens* at four morphological stages and of the seed

Plant height (cm)	Days after sowing	Chemical composition							
		DM (g kg ⁻¹ FM)	OM	Ash	Crude protein	Ether extract	NDF	ADF	GE (MJ kg ⁻¹ DM)
15	42	138.3 ^b	844.7 ^a	155.3 ^a	158.0 ^b	40.0 ^a	378.0 ^a	251.3 ^a	16.2 ^a
30	49	102.3 ^a	856.7 ^a	143.3 ^a	206.0 ^a	37.5 ^a	418.0 ^b	298.0 ^b	16.3 ^a
40	56	100.7 ^a	853.7 ^a	146.3 ^a	130.0 ^c	37.0 ^a	432.7 ^b	303.3 ^b	16.0 ^a
70	104	171.0 ^c	911.7 ^b	88.3 ^b	81.7 ^d	22.2 ^b	547.7 ^c	415.7 ^c	17.1 ^b
SEM		8.8	8.1	8.1	13.8	2.8	19.2	18.7	1.5
Seed		952.7	962.2	37.8	238.5	430.4	296.8	225.6	28.0

Within a column, values with different letter differ (p<0.05)

12-15% of oleic acid (C18:1), 9% of PA and 4% of SA (Shin and Kim, 1994; Longvah *et al.*, 2000). Mink and Kim (1992) studied the change in lipid composition during maturation of perilla seed. They found that the content of ether-extractable lipids increased continuously as the seed matured while the content of triglyceride, an essential component of ether-extractable lipids, increased rapidly at the beginning of maturation and ranged from 61.4 to 68.2% in mature seeds (30 days after flowering). The glycolipids and phospholipids contents were reduced and the amount of the individual component of glyco- and phospholipids varied irregularly. Ichihara and Suda (2003) reported the profiles of lipid accumulation and changes in the FA composition in developing perilla seeds; they showed that lipids rapidly accumulated between 15 and 19 days after flowering. Dietary intake of perilla seed oil containing a large amount of ALA provides various health benefits (Kim *et al.*, 2007) such as the lowering of the plasma lipid level and the increase in eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) in the hepatic membranes of rats (Kim and Choi, 2001; Kim *et al.*, 2004).

Crop quality: The evolution of the perilla plant quality at the four different stages of development is reported in Table 2. The DM was highest at the last stage and the perilla plant was characterised by a progressive increase in the NDF and ADF contents, while the organic matter and GE was higher at the last stage than at the other stages. The CP content was highest at the second stage and then decreased with plant aging, while the EE content decreased during the growth cycle.

Perilla seeds are higher in DM, OM, CP and GE contents than the plant during the growth cycle, while the EE content was from twentyfold to tenfold more in the seed than in the plant during the growth cycle. The ash content was very low in the seeds, while the NDF and ADF contents of the seed were lower than those of the plant at all the studied stages.

To the best of the researcher's knowledge, no studies exist regarding the chemical composition of perilla plants during growth, but only researches on the chemical composition of the seeds (Longvah and Deosthale, 1991, 1998). *Perilla frutescens* has been evaluated for its nutrient composition and protein quality. It has been found to be a rich source of protein (17.0%) and fat (51.7%) (Longvah and Deosthale, 1991). The protein content of whole perilla seed has been reported to be in the range of 15.7-23.7% (Sharma *et al.*, 1989). Dehulling increases the protein content of perilla seed from 17 to 20%. The hull, which makes up 18% of the wholeseed, had 5% of protein. The defatted perilla wholeseed protein content is 36% and that of perilla kernel meal is 46% (Longvah and Deosthale, 1998).

CONCLUSION

From these results it may be concluded that the nutrient contents of perilla plant depends on the stage of maturity and in order to obtain an optimal compromise between yield and nutritional value, the crop should be harvested at the end of the second month after sowing, since the fibrous fractions and CP contents decrease and the nutritional quality deteriorates when cutting is delayed.

Further research is required to determine the evolution of the other chemical constituents of the perilla plant during the growth cycle.

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