Pancreatin and Intracellular Peptidases". *Agric. Biol. Chem.* **1982**, *46*, 465–472.
Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. S. "Investigation on Selenium in Wheat". *Phytochemistry* **1970**, *9*, 1181–1188.
Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. "Selenium: Biological Role as a Component of Glutathione Peroxidase". *Science (Washington, D.C.)* **1973**, *179*, 588–590.
Sakurai, H.; Tsuchiya, K. "A Tentative Recommendation for Maximum Daily Intake of Selenium". *Environ. Physiol. Biochem.* **1975**, *5*, 107–118.
Schwartz, K.; Foltz, C. M. "Selenium as an Internal Part of Factor 3 against Dietary Necrotic Liver Degeneration". *J. Am. Chem. Soc.* **1957**, *79*, 3292–3293.
Schwartz, K.; Foltz, C. M. "Factor 3 Activity of Selenium Compounds". *J. Biol. Chem.* **1958**, *233*, 245–251.
Sliwkowski, M. X.; Stadtman, T. C. "Incorporation and Distribution of Selenium into Thiolase from *Clostridium kluyveri*". *J. Biol. Chem.* **1985**, *260*, 3140–3144.
Thanh, H. V.; Shibasaki, K. "Major Proteins of Soybean Seeds. A Straightforward Fractionation and Their Characterization". *J. Agric. Food Chem.* **1976**, *24*, 1117–1122.
Thompson, J. N.; Scott, M. L. "Role of Selenium in the Nutrition of the Chick". *J. Nutr.* **1969**, *97*, 335–342.
Underwood, E. J. "Selenium". In *Trace Element in Human and Animal Nutrition*, 4th ed.; Academic: New York, 1977; pp 302–346.
Watkinson, J. H. "Fluorometric Determination of Selenium in Biological Material with 2,3-Diaminonaphthalene". *Anal. Chem.* **1966**, *38*, 92–97.
Yasumoto, K.; Suzuki, T.; Yoshida, M. "Selenomethionine in Soybean Proteins and Its Intestinal Absorption". *Daizu Tanpakushitsu Eiyo Kenkyuukai Kaishi (Nutr. Sci. Soy Protein Jpn.)* **1984**, *5*, 48–52.
Yasumoto, K.; Iwami, K.; Yoshida, M. "Nutritional Efficiency and Chemical Form of Selenium, an Essential Trace Element, Contained in Soybean Protein". *Diazu Tanpakushitu Eiyo Kenkyuukai Kaishi (Nutr. Sci. Soy Protein Jpn.)* **1983**, *4*, 35–40; *Selenium-Tellurium Abstracts* **1985**, *101*, 129289j.
Yoshida, M.; Yasumoto, K. "Selenium Contents of Rice Grown at Various Sites in Japan". *J. Food Com. Anal.* **1987**, *1*, 71–75.
Yoshida, M.; Yasumoto, K.; Iwami, K.; Tashiro, H. "Distribution of Selenium in Bovine Milk and Selenium-Deficiency in Rats Fed Casein-Based Diets, Monitoring by Lipid Peroxide Level and Glutathione Peroxidase Activity". *Agric. Biol. Chem.* **1981a**, *45*, 1681–1688.
Yoshida, M.; Iwami, K.; Yasumoto, K.; Iwai, K. "Bioavailability of Selenium Contained in Milk Casein, Soybean Proteins and Skipjack Strips". *Nippon Nogei Kagaku Kaishi (J. Jpn. Soc. Agric. Chem.)* **1981b**, *55*, 689–693.

Received for review May 1, 1987. Accepted December 9, 1987. This work was supported by a grant (No. 60480054) from the Ministry of Education, Science and Culture of Japan.

Characterization of Essential Oil of Parsley

James E. Simon* and James Quinn

A germplasm collection of parsley (*Petroselinum crispum*), consisting of 104 accessions from the USDA Plant Introduction Station including curly and flat leaf and Hamburg types, was greenhouse grown and the essential oil extracted from fresh leaves by water distillation and analyzed by GC and GC/MS for essential oil content and composition. The essential oil content ranged from 0.00 to 0.16% (v/fresh weight), and the constituents include α-pinene, β-pinene, myrcene, α-phellandrene, β-phellandrene, terpinene, terpinolene and 1-methyl-4-isopropenylbenzene, 1,3,8-*p*-menthatriene, thymol, myristicin, apiol, plus three unknowns, two of molecular weight 168 and one of 268. Individual accessions varied greatly in essential oil composition. In general, the major constituent was 1,3,8-*p*-menthatriene, followed by β-phellandrene, myristicin, and myrcene. Parsley accessions high in the specific constituents (as a percent of essential oil) 1,3,8-*p*-menthatriene (68%), myristicin (60%), β-phellandrene (33%), apiol (22%), myrcene (16%), terpinolene and 1-methyl-4-isopropenylbenzene (13%), and MW 268 dimer (10%) were identified. Thymol was detected in seven accessions, as 2% or less, and this is the first report of this compound in parsley leaf oil.

Parsley [*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill, Apiaceae] is native to Europe and Western Asia (Bailey and Bailey, 1976) and cultivated in the United States as an annual for its aromatic and attractive leaves. The two major types of parsley are the common or curly leaf parsley (var. crispum) and the flat leaf, Italian parsley (var. neapolitanum Danert) (Simon et al., 1984). A third lesser grown parsley type is the Hamburg or turnip-rooted parsley [var. tuberosum (Bernh.) Crov.], which is cultivated to a limited extent for its enlarged edible root.

Fresh, dried, and dehydrated leaves are used as a condiment, garnish, and flavoring ingredient. A fixed oil and an essential oil can be extracted from the leaves and seeds. The constituents of the fixed oil have been reported elsewhere (Balbaa et al., 1975; Constantinescu et al., 1972).

Department of Horticulture, Purdue University, West Lafayette, Indiana 47907.

The essential oil of parsley is used as a flavoring agent or fragrance in perfumes, soaps, and creams. The commercial essential oil of parsley is largely derived from the seed or the herb harvested at seed formation, prior to ripening (Heath, 1981). Parsley leaf oil, much more characteristic of parsley aroma, is not generally extracted and used because of the low essential oil yield.

The essential oil of parsley leaves has been previously reported (Garnero and Chretien-Bessiere, 1968; Kasting et al., 1972; Freeman et al., 1975; MacLeod et al., 1985). MacLeod (1985) identified 45 constituents including the following that constituted over 1% of the total peak area: α-pinene, β-pinene, myrcene, limonene, α-phellandrene, *p*-cymene, α-terpinolene, 1,3,8-*p*-menthatriene, 4-isopropenyl-1-methylbenzene α-terpineol, *p*-methylacetophenone, α-elemene, apiol, and myristicin.

Although the composition of essential oil is influenced by the plant genetic base and development and environmental conditions (Bernath, 1986), little information is

0021-8561/88/1436-0467$01.50/0

Table I. Composition of Essential Oil (Percent of Constituents) of the More Novel Accessions of Parsley from the USDA Plant Introduction Station

essential oil constituents	USDA accession no. (country of origin)						
	173010 (Turkey)	182266 (Turkey)	169596 (Turkey)	155306 (Arabia)	358080 (Yug)[a]	171732 (Turkey)	143480 (Iran)
α-pinene	1.5	2.3	0.7	1.1	0.1	0.4	0.6
β-pinene	1.0	1.6	0.4	0.8		0.2	0.5
myrcene	16.3	9.7	8.7	10.4	2.8	3.0	2.9
α-phellandrene	1.8	1.1	1.1	0.8	0.3	0.5	0.9
β-phellandrene	29.8	12.1	13.1	8.0	6.2	8.7	9.7
terpinene	0.9	0.1	0.1	0.1		0.7	0.2
terpinolene + 1-methyl-4-isopropenylbenzene	2.5	2.9	3.5	13.9	2.1	3.1	5.4
1,3,8-p-menthatriene	42.1	62.8	54.1	44.0	20.1	29.3	64.7
thymol				2.0			
MW 168				1.8			
MW 168				2.8			
myristicin	1.0		12.2	1.5	60.5	2.3	0.2
apiol					1.4	22.1	
MW 268 isomers	1.8	4.6	4.7	5.9	2.4	7.9	10.9

[a] Yug = Yugoslavia.

available on these factors in parsley. This present study, using the U.S. Department of Agriculture (USDA) parsley collection, was conducted to characterize genetic variability of the major essential oil constituents (greater than 1%).

MATERIALS AND METHODS

Plant Material and Growing Conditions. Seed samples of the parsley germplasm collection (104 accessions from the USDA Plant Introduction Station, Ames, IA) were obtained, seeded into flats, and placed in a misthouse Sept 23, 1985. Four weeks later four to five plants of each accession were transplanted to individual 15-cm plastic pots. The soil mix was 1 part each of sterilized topsoil, peat moss, and perlite supplemented with fertilizer. Four pots of each seed accession, each containing four to five plants per pot, were placed (completely randomized design) in a greenhouse (18 °C night and 24–27 °C day temperatures) with supplemental lighting by very high output fluorescent lights providing between 110 and 140 mmol m^{-2} s^{-1} as measured by a LI-COR LI-1776 quantum meter and LI-190SB sensor.

Harvest and Distillation. Two pots, each containing four to five plants, were harvested for essential oil analysis at each of two time periods (80–87 days and 90–94 days following transplanting). The total fresh chopped parsley from the two pots averaged 217 g (from 44 to 387 g). Plant material was placed immediately into a 1000-mL round-bottomed boiling flask with 400 mL of distilled-deionized water and the essential oil extracted by water distillation via a modified clevenger trap method (ASTA, 1968). The distillation period was 1 h and the essential oil content determined on a volume to fresh weight basis. The values for essential oil content of the two replications were averaged, and the samples were stored in silica vials with Teflon-sealed caps at 2 °C in the absence of light.

Gas Chromatography. Essential oil samples from each of the distillations were analyzed separately, and the relative peak area for individual constituents was averaged for each accession. Identification of essential oil constituents was based on retention time and the relative percentage determined with a Varian 3700 gas chromatograph equipped with FID and an electronic 4270 integrator. A fused silica capillary column (12 m × 0.2 mm (i.d.)) with an OV 101 (Varian, polydimethylsiloxane) bonded phase was used. Direct injection of 0.5 μl of essential oil sample with N_2 as a carrier gas (200:1 split vent ratio) and an oven temperatures held isothermal at 80 °C for 2 min and then programmed to increase at 3 °C/min to 220 °C gave complete elution of all peaks (sensitivity 10^{-9}). The injector and detector temperatures were 220 and 300 °C, respectively.

GC/Mass Spectroscopy Analysis. Pure compounds (α-pinene, β-pinene, myrcene, β-phellandrene, terpinene, terpinolene, thymol, myristicin, apiol) and essential oil constituents of the selected parsley accessions 171737, 22814, and 325873 (those exhibiting a range of major constituents) were verified by GC/MS and assisted in retention time determination. A Finnigan GC (9610) and MS (4000), hooked on-line to a Data General Nova/4 data processing system, used electron impact analysis.

The GC conditions were as follows: direct injection of 1.0 μL of sample diluted 10:1 with MeOH; fused silica column (30 m × 0.25 mm) with DB-1 bonded phase (polydimethylsiloxane) (J&W Scientific Inc.); He carrier gas with a column pressure of 10.5 psi and split vent of 40 mL/min; oven program of 80 °C at 2 min rising to 180 °C at 2 °C/min; injector temperature of 225 °C.

The MS conditions were as follows: ionization voltage of 70 eV; emission current of 40 μA; scan rate and range of 1 scan/s and 40–500, respectively; source temperature of 160 °C.

RESULTS

Essential Oil Constituents. Parsley accessions of differing chemotypes are listed in Table I, with 15 constituents listed in the order of their elution (12 identified, 3 unknown). All major constituents from earlier studies on parsley leaf oil were also identified except for the monoterpenes, p-cymene, limonene, and *cis*-β-ocimene; the sesquiterpene β-elemene; and p-methylacetophenone. The monoterpenes listed did not separate well from β-phellandrene under the analytical GC conditions employed. Terpinolene and 4-isopropenyl-1-methylbenzene separated only under the GC/MS conditions, allowing confirmation of both constituents at this peak. Thymol was detected in parsley leaf oil for the first time in seven parsley accessions (155306, 167035, 165048, 169599, 170616, 222814, and 368707) and confirmed in one essential oil sample (222814; Table II). The relative percentage was highest in 155306 (2.0%). Two isomers of molecular weight 268 eluted at 209 and 215 °C, which was a higher temperature than used by either Kasting et al. (1972) or MacLeod et al. (1985). These compounds eluted at a lower temperature when analyzed via GC/MS, probably reflecting a greater pressure drop across the column in the GC/MS system (Willard et al., 1981). The two isomers (MW 268) appear to be dimers resulting from a Diels–Alder reaction of 1,3,8-p-menthatriene (Figure 1).

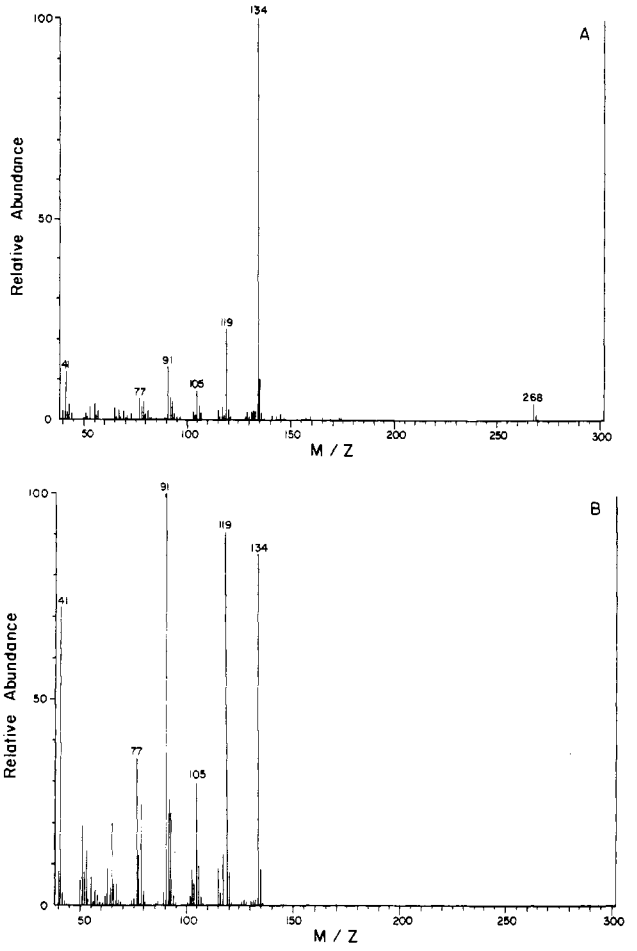

Figure 1. Comparison of the electron impact mass spectrum of the molecular weight 268 dimer (A) and 1,3,8-*p*-menthatriene (B).

Germplasm Evaluation. In Table II the parsley accession number, country of seed origin, essential oil content, oil content of the seven most predominant constituents, leaf type, size, and color are listed. Three accessions were of the Hamburg type, and only one curly leaf type parsley was found (325873). Various leaf sizes were observed, and the intensity of green ranged from light to dark green, with accession 180862 being purplish. Fourteen parsley accessions with long petioles, commercially desirable for the fresh market, were identified. The essential oil content ranged from 0.00 to 0.16% and averaged 0.07% on a fresh weight basis.

Individual parsley accessions varied greatly in essential oil composition. In general 1,3,8-*p*-menthatriene was identified as the major constituent, followed by β-phellandrene, myristicin, and myrcene. The relative percentages of the major constituents varied greatly in proportion to each other. A number of accessions had a balance of all major constituents (i.e., 173006, 177492, and 181891).

DISCUSSION

Essential Oil Constituents. The essential oil content was similar to previous studies reporting essential oil content between 0.04 to 0.15% on a fresh weight basis (Simon and Overly, 1986). Less efficient recovery of essential oil from some plant samples where less plant material was available for distillation may have occurred.

The major constituents of the parsley accessions evaluated were in agreement with previous reports indicating that 1,3,8-*p*-menthatriene, myristicin, apiol, β-phellandrene, myrcene, and 4-isopropyl-1-methylbenzene were among the most prevalent constituents in parsley leaf oil (Garnero and Chretien-Bessiere, 1968; Freeman et al., 1975; Kasting et al., 1972; MacLeod et al., 1985).

This is the first report of thymol and the molecular weight 268 dimer in parsley leaf oil. The detection of thymol was unexpected, but not surprising, given its low frequency of occurrence (less than 10% of total accessions screened) and presence in many essential oil bearing plant species. While thymol is of the same molecular formula as 2-*p*-tolylpropan-2-ol, first reported occurring naturally in plants by MacLeod et al. (1985), the mass spectra of the two compounds are distinctly different. The GC/MS peaks having a molecular weight of 268 were indicative of dimers of 1,3,8-*p*-menthatriene. Since the dimers have twice the mass of the monomer, this suggests the dimer might be a Diels–Alder type product. The mass spectrum of one of the dimers (which are identical) is given in Figure 1 for comparison with that of 1,3,8-*p*-menthatriene. This spectrum indicates the dimers undergo facile cleavage to the monomer, as evidenced by the base peak at m/z 134. Furthermore, the reduced intensity of the fragment ions at m/z 119 and 91 relative to that of 1,3,8-*p*-menthatriene is additional evidence the dimer results from more than simple hydrogen bonding. This can be understood in terms of the internal energy deposited within the dimer upon ionization. The majority of this energy is used to produce monomers, therefore leaving less energy for subsequent fragmentation like that observed for the monomer 1,3,8-*p*-menthatriene (i.e., base peak is m/z 91).

It is possible these MW 268 components could have been formed in either the distillation process or the gas chromatographic analysis if they were not naturally occurring in the parsley. If it is an artifact of the distillation process or GC analysis, the content of 1,3,8-*p*-menthatriene would be more predominant as a fragrance constituent in the plant. Due to the high temperature required for elution of this MW 268 dimer, it may have not been detected in previous studies (MacLeod et al., 1985) and could significantly alter parsley essential oil if arising in the commercial distillation process.

Flavoring Qualities. Garnero and Chretien-Bessiere (1968) first suggested that 1,3,8-*p*-menthatriene was important for the odor of parsley leaves, and Kasting et al. (1972) later added β-phellandrene and the unseparated terpinolene and 4-isopropenyl-1-methylbenzene to this category. Freeman et al. (1975) separated the latter two constituents and suggested 4-isopropenyl-1-methylbenzene as being responsible for parsley leaf aroma. In contrast, Vernon and Richard (1983) claimed β-phellandrene, 1,3,8-*p*-menthatriene, and myristicin were not characteristic of parsley aroma. Recently, MacLeod et al. (1985) agreed with Kasting et al. (1972) but concluded that the only constituent with a desirable parsley characteristic was apiol.

This lack of consensus is understandable given the results of our study, which shows significant variability in essential oil constituents. The characteristic odor of parsley will be influenced by the parsley accession chosen as a standard. If the constituents considered by earlier studies are important to the overall quality of parsley odor, then superior types could be selected by having a good balance of all the constituents.

Chemotypic Races and Distillation. Chemotypic races have been defined by Stahl and Jork (1964) for parsley fruit (seed) oils (myristicin, apiol, 2,3,4,5-tetramethoxyallylbenzene races), but little information is available on the chemotypic variation of parsley leaf essential oil. Under the same environmental conditions and developmental stage (rosette before bolting), chemotypes

Table II. Leaf Characteristics and Composition of Essential Oil from the Parsley Collection of the USDA Bureau of Foreign Plant Introductions

USDA accession no.	source country	leaf characteristics: type,[a] size[b]	color	EO[c] (% v/fr wt)	essential oil compn[d] (in % of constituents) 1	2	3	4	5	6	7
120991	Turkey	F, L	G	0.07	10.0	9.6	8.2	55.8	0.4		4.2
120999	Turkey	F, S	G	0.06	8.5	9.1	6.6	67.2			4.3
138930	Iran	F, M	G	0.07			NA				
140941	Iran	F, L	LG	0.06	6.4	33.2	3.0	40.0	7.3	0.1	2.3
143480	Iran	F, M	G	0.04	2.9	9.7	5.4	64.7	0.2		10.9
143481	Iran	F, L	LG	0.03			NA				
143482	Iran	F, L	LG	0.06	13.3	33.5	3.6	36.1	4.1	0.3	1.8
155306	Arabia	F, M	G	0.04	10.4	8.0	13.9	44.0	1.5		5.9
164934	Turkey	F, L	LG	0.11	7.1	9.5	3.2	50.1	22.6		2.7
165048	Turkey	F, M	DG	0.10	6.5	10.1	4.7	34.1	36.8	0.3	1.7
167035	Turkey	F, M	G	0.09	8.0	8.6	4.2	43.7	2.2		8.1
167080	Turkey	F, L	LG	0.06	8.2	13.8	4.9	55.0	1.0		9.6
167146	Turkey	F, L	LG	0.05	11.7	7.8	4.2	68.1	0.1		3.1
169591	Turkey	F, L	G	0.08	10.7	14.0	5.5	55.5	0.4		4.2
169593	Turkey	F, L	G	0.08	12.9	16.1	4.5	46.8	11.7		3.0
169596	Turkey	F, L*	G	0.08	8.7	13.1	3.5	54.1	12.2		4.7
169597	Turkey	F, S	G	0.06	10.6	11.5	5.9	62.8	0.1		3.5
169598	Turkey	F, L	G	0.06	13.0	26.7	3.1	46.6	1.4		3.0
169599	Turkey	F, XL*	LG	0.08	0.9	14.2	7.6	46.9	0.4	19.5	3.0
170030	Turkey	F, S	G	0.06	3.0	18.2	5.9	50.5	3.1	8.8	3.2
170615	Turkey	F, L	LG	0.03			NA				
170616	Turkey	F, M	DG	0.08	5.8	9.4	7.1	35.7	31.2	0.4	3.0
171730	Turkey	F, S	LG	0.06	3.8	10.3	3.0	45.9	30.5	0.1	2.7
171731	Turkey	F, M	G	0.10	8.0	12.0	8.5	52.9	11.0	0.3	3.9
171732	Turkey	F, M	LG	0.11	3.0	8.7	3.1	29.3	2.3	22.1	7.9
171733	Turkey	F, S	LG	0.06	2.4	18.1	3.8	49.7	0.2	14.4	5.2
171735	Turkey	F, S	G	NA	6.4	5.8	10.7	61.4			7.5
171736	Turkey	F, M	G	0.05	4.6	9.8	4.4	44.3	29.6	0.1	3.5
171737	Turkey	F, L	LG	0.10	2.5	22.7	4.5	51.6	0.2	9.9	2.3
171739	Turkey	F, S	DG	0.01			NA				
173005	Turkey	F, L	LG	0.06	14.5	15.6	4.8	51.6	0.6		4.9
173006	Turkey	F, M	LG	0.05	2.6	22.0	5.2	30.4	13.0	9.9	4.2
173008	Turkey	F, L*	G	0.02	8.4	10.9	5.5	63.9	0.6	–	4.7
173009	Turkey	F, L	G	0.04	8.1	15.1	5.8	48.4	11.4	0.1	4.8
173010	Turkey	F, L	G	0.03	16.3	29.8	2.5	42.1	1.0		1.8
173012	Turkey	F, L*	G	0.01			NA				
173014	Turkey	F, M	G	0.02			NA				
173015	Turkey	F, M	G	0.08	12.4	8.6	3.9	57.3	0.4	0.1	8.7
173758	Turkey	F, L	G	0.10	6.5	13.5	3.0	54.5	10.1		5.7
174290	Turkey	F, S	LG	0.04			NA				
174292	Turkey	F, M	DG	0.07			NA				
174294	Turkey	F, L	DG	0.03	6.4	12.7	5.1	57.2	0.7		10.5
175808	Turkey	F, L	DG	0.00			NA				
175810	Turkey	F, L	LG	0.03	12.4	20.3	4.1	54.0	0.4		3.1
176666	Turkey	F, L	DG	0.04	10.6	8.0	2.9	67.4	0.1		6.4
176668	Turkey	F, L	G	0.00			NA				
177024	Turkey	F, L	DG	0.06			NA				
177025	Turkey	F, L	G	0.04	12.2	11.3	3.4	64.6	0.1		3.8
177490	Turkey	F, L	DG	0.05	16.4	18.0	5.4	46.6	0.8		4.6
177492	Syria	F, L	LG	0.09	2.0	13.3	5.9	52.5	11.0	5.6	4.3
178995	Turkey	F, M	G	0.04	16.0	13.7	3.5	56.5	0.7		4.0
179396	Iraq	F, L*	LG	0.03	15.7	8.2	4.3	51.7	1.2		7.8
180862	Turkey	F, L	PG	0.00			NA				
181779	Syria	F, VL*	LG	0.05	12.2	12.8	3.8	57.1	3.1		3.5
181891	Syria	F, L*	LG	0.07	5.6	14.7	6.6	50.8	7.3	4.0	4.7
182266	Turkey	F, M	G	0.05	9.7	12.1	2.9	62.8			4.6
183702	Turkey	F, S	G	0.10	7.1	10.8	5.7	64.1	0.3		6.3
204599	Turkey	F, M	LG	0.03			NA				
222813	Iran	F, M	G	0.04	7.6	25.9	3.9	51.1	1.4		4.1
222814	Iran	F, L	DG	0.04	3.4	10.3	4.9	68.0	0.4		7.8
222815	Iran	F, L	G	0.03	13.6	23.7	4.2	48.1	1.5		3.1
223395	Iran	F, M	DG	0.09	8.3	19.2	2.7	42.3	19.4		2.5
226625	Iran	F, L	DG	0.00			NA				
251502	Iran	F, M	G	0.02			NA				
325873	USA-IL	VC, S	LG	0.06	3.3	18.3	3.5	47.5	19.5	0.1	2.9
344373	Turkey	F, M	G	0.07	11.9	10.4	4.5	56.3	0.8		7.4
344374	Turkey	F, L	G	0.06	8.2	10.2	9.7	57.0	0.8	0.1	6.0
358065	Yugoslavia	F, S	G	0.05	3.6	3.6	3.2	21.1	55.7	0.3	2.8
358066	Yugoslavia	F, L	DG	0.08	3.9	6.1	3.6	43.9	28.5	0.5	5.6
358067	Yugoslavia	F, M	G	0.07	4.5	10.6	3.6	22.3	52.9	0.5	2.3
358068	Yugoslavia	F, L	DG	0.16	3.7	8.6	1.9	35.2	27.2	0.1	3.1
358069	Yugoslavia	F, L	G	0.12	6.5	8.4	3.1	39.1	32.8	0.1	3.8
358070	Yugoslavia	F, L	DG	0.08	5.2	10.3	3.5	34.8	38.4	0.5	3.5

Table II (Continued)

USDA accession no.	source country	leaf characteristics: type,[a] size[b]	color	EO[c] (% v/fr wt)	essential oil compn[d] (in % of constituents) 1	2	3	4	5	6	7
358071	Yugoslavia	F, M	LG	0.06	4.4	4.0	3.8	29.9	46.6	0.5	3.5
358072	Yugoslavia	F, L	G	0.07	5.3	9.5	3.4	31.7	42.9	0.5	3.1
358073	Yugoslavia	F, L	G	0.02			NA				
358074	Yugoslavia	F, L	DG	0.08	5.2	15.9	4.6	44.4	18.6	0.3	5.1
358075	Yugoslavia	F, L	G	0.07	7.0	8.5	3.7	39.9	33.3	0.6	2.9
358076	Yugoslavia	F, L	DG	0.08	5.0	14.8	2.9	43.0	26.0	0.3	4.2
358077	Yugoslavia	F, L	G	0.08	9.3	13.6	2.3	39.7	28.3	0.1	3.1
358078	Yugoslavia	F, L	G	0.03			NA				
358079	Yugoslavia	F, VL	G	0.04	3.0	6.2	2.0	25.4	57.0	0.3	2.5
358080	Yugoslavia	F, L	DG	0.05	2.8	6.2	2.1	20.1	60.5	1.4	2.4
358082	Yugoslavia	F, L	G	0.06	5.2	6.7	5.5	58.2	15.6		5.7
358083	Yugloslavia	F, L*	G	0.06	8.5	12.0	3.1	42.5	25.3	0.1	3.8
358085	Yugoslavia	F, L*	G	0.06	11.2	5.7	3.8	37.7	17.7		5.6
358086	Yugoslavia	F, L*	DG	0.09	13.1	8.1	2.9	48.4	17.3	0.1	4.5
368701	Yugoslavia	F, L*	G	0.05	9.4	8.0	4.5	50.6	19.4		3.2
368703	Yugoslavia	F, M	LG	0.08			NA				
368705	Yugoslavia	F, L*	G	0.02			NA				
368706	Yugoslavia	F, L	G	0.07	5.2	7.2	2.6	33.9	43.7		3.7
368707	Yugoslavia	F, L	G	0.08	6.1	4.8	4.8	36.8	37.4	0.3	4.4
368708	Yugoslavia	F, L	G	0.07	9.0	6.8	5.5	45.9	23.1	0.1	3.6
370504	Yugoslavia	F, L	G	0.04	5.8	6.2	2.8	25.8	54.5	0.5	1.7
370505	Yugoslavia	F, D**	LG	0.00			NA				
370506	Yugoslavia	F, L	G	0.07	8.4	6.4	4.5	68.8	0.5		7.1
379416	Yugoslavia	F, VL**	DG	0.08	6.8	7.3	5.3	65.0	0.2		6.9
379417	Yugoslavia	F, VL	G	0.05	10.8	4.0	5.8	48.1	23.5	0.7	4.1
379418	Yugoslavia	F, L	DG	0.10	8.3	8.4	3.2	44.1	28.6	0.1	2.7
379420	Yugoslavia	F, D**	LG	0.00			NA				
379421	Yugoslavia	F, L	DG	0.11	5.8	9.3	1.9	30.7	25.9	0.3	7.6
379422	Yugoslavia	F, L*	G	0.06	8.6	9.5	3.7	41.9	28.7	0.2	2.4
379423	Yugoslavia	F, L	G	0.06	4.1	3.9	5.1	43.7	34.2	0.1	3.0
379424	Yugoslavia	F, L*	G	0.04	9.0	9.7	2.8	44.4	28.3	0.1	2.5

[a] Type: F = flat; C = curly; VC = very curly. [b] Size: S = small; M = medium; L = large; VL = very large; D = fine. Key *, long petiols; **, large taproot. [c] EO = essential oil content (percent volume/fresh weight). [d] Essential oil constituents: 1 = myrcene; 2 = β-phellandrene; 3 = terpinolene + 1-methyl-4-isopropenylbenzene; 4 = 1,3,8-*p*-menthatriene; 5 = myristicin; 6 = apiol; 7 = MW 268 isomers.

high in the following specific constituents (as a percent of the essential oil) were identified: 1,3,8-*p*-menthatriene (68%); myristicin (60%); β-phellandrene (33%); apiol (22%); myrcene (16%); terpinolene and 1-methyl-4-isopropenylbenzene (13%); MW 268 dimer (10%). Chemotypic groupings could not be made because of the large variation in individual constituents and range of essential oil composition in the parsley leaf among the accessions. Unique to the Apiaceae is 1,3,8-*p*-menthatriene (MacLeod et al., 1985), and the presence of this unusual natural plant product in parsley leaf oils strongly suggests the close phylogenic relationship among the parsley lines (Bell, 1980).

It has been suggested (Lawrence, 1982) that a high myristicin (i.e., 17%) content in parsley herb oil indicates a partial seed origin. This is in disagreement with our study and that of MacLeod et al. (1985), which reported 20% myristicin from parsley leaf oil, and probably that of Kasting et al. (1972), who reported myristicin as the largest constituent but failed to give the relative percentages. While the extraction procedure can substantially affect the relative amount of essential oil constituents obtained (Takeota et al., 1985), Kasting et al. (1972) found little difference in the extraction method in solvent versus steam distillation.

Our results demonstrate a rich diversity in essential oil constituents of parsley that suggest a genetic basis. This germplasm collection could serve as an important source of genetic material for plant breeding and selection based upon known comparative relationships among essential oil constituents. Knowledge of the chemical characteristics of an aromatic plant is critical for the improvement of essential oil content and composition (Bernath, 1986). Additional factors affecting parsley flavoring quality such as the fixed oils should also be considered in the selection for superior chemotypes and the development of new cultivars for the fresh and processed industry.

ACKNOWLEDGMENT

Published as Journal Paper No. 11,162 of Purdue University Agriculture Experiment Station, West Lafayette, IN. This work was supported in part by a grant from the Purdue University Agriculture Experiment Station (Specialty Crops Grant No. 014-1165-0000-65178). We acknowledge Dr. Mark Widrlechner, Regional Plant Introduction Station, Ames, IA, for providing seeds of the USDA parsley germplasm collection and the cooperation of Dr. Karl Wood, Department of Chemistry and Mass Spectrometry Laboratory, who assisted us in the GC/MS analysis.

Registry No. *p*-$MeC_6H_4C(Me)=CH_2$, 1195-32-0; α-pinene, 80-56-8; β-pinene, 127-91-3; myrcene, 123-35-3; α-phellandrene, 99-83-2; β-phellandrene, 555-10-2; terpinene, 8013-00-1; terpinolene, 586-62-9; 1,3,8-*p*-menthatriene, 18368-95-1; thymol, 89-83-8; myristicin, 607-91-0; apiol, 523-80-8.

LITERATURE CITED

ASTA *Official Analytical Methods of the American Spice Trade Association*; ASTA: Englewood Cliffs, NJ, 1968.

Bailey, L. H., Bailey, E. Z., Eds. *Hortus Third*; Macmillan: New York, 1976.

Balbaa, S. I.; Hilal, S. H.; Hagag, M. Y. *Egypt. J. Pharm. Sci.* **1975**, *16*(3), 383.

Bell, E. A. In *Secondary Plant Products*; Bell, E. A., Charlwood, B. V., Eds.; Springer-Verlag: New York, 1980; Vol. 8, p 11.

Bernath, J. In *Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology*; Craker, L. E., Simon, J. E., Eds.; Oryx Press: 1986; Vol. 1, p 185.

Constantinescu, E.; Popescu, M.; Denes, S. *Riv. Ital. Essenze, Profumi, Piante Off.* **1972**, *54*, 419.
Freeman, G. G.; Whenham, R. I.; Self, R.; Eagles, J. *J. Sci. Food Chem.* **1975**, *26*, 465.
Garnero, J.; Chretien-Bessiere, Y. *Fr. Ses Parfums* **1968**, *11*, 332.
Heath, H. *Source Book of Flavors*; AVI: Westport, CT, 1981.
Kasting, R.; Andersson, J.; Sydow, E. v. *Phytochemistry* **1972**, *11*, 2277.
Lawrence, B. M. *Perfum. Flavor.* **1981/1982**, *6*, 43.
MacLeod, A. J.; Snyder, C. H.; Subramanian, G. *Phytochemistry* **1985**, *24*(11), 2623.
Simon, J. E.; Overly, M. L. *Herb, Spice and Med. Plant Dig.* **1986**, *4*(1), 3 (Mass. Coop. Ext. Serv.: Amherst, MA).
Simon, J. E.; Craker, L. E.; Chadwick, A. *Herbs: An Indexed Bibliography, 1971–1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone*; Archon Books: Hamden, CT, 1984; p 75.
Stahl, E.; Jork, H. *Arch. Pharm. (Weinheim, Ger.)* **1964**, *297*, 273.
Takeota, G., Ebeler, S., Jennings, W., Bills, D. D., Mussinan, C. J., Eds. In *Characterization and Measurement of Flavor Compounds*; ACS Symposium Series 289; American Chemical Society: Washington, DC, 1985; p 95.
Vernon, F.; Richard, H. M. J. *Lebensm-Wiss. Technol.* **1983**, *16*(1), 32.
Willard, H. H.; Merritt, L. L.; Dean, J. A.; Settle, F. A. *Instrumental Methods of Analysis*, 6th ed.; Wadsworth: Belmont, CA, 1981; p 430.

Received for review June 26, 1987. Accepted December 7, 1987.

Nutritional and Antinutritional Factors of Green Leafy Vegetables

Kaushalya Gupta* and D. S. Wagle

On a moisture-free basis, mustard had the maximum crude protein (CP 29.82%), while spinach had the minimum CP (21.59%). Lipid content varied from 1.17 (spinach) to 3.73% (cauliflower). Crude fiber and total minerals varied from 7.20 to 13.95% and 12.54 to 26.16%, respectively. Chickpea had the highest amount of iron, copper, manganese, and calcium. Mustard had the maximum amount of phosphorus (1210.0 mg %) while spinach had the minimum amount (740.0 mg %). The maximum amounts of nitrate (5.35556%), saponin (2.45%), and oxalate (8.69%) were noted in spinach. No tryptic activity was detected in chickpea. A wide variation in amino acid profile was observed among green leafy vegetables. Green vegetables are good sources of minerals.

Leafy vegetable preparations include the raw salad, widely known all over the world, in partially or completely cooked or fried forms. Indian cuisine has a wide range of choice among the leafy vegetables. In most Indian households, the inclusion of a leafy vegetable preparation in daily diet is an accepted practice. These green leafy vegetables are inexpensive, are easily and quickly cooked, and are rich in several nutrients such as vitamins, minerals, proteins, etc. (Oke, 1966; Gopalan et al., 1971). Sigh et al. (1969) and Cheeke and Bronson (1980) have reported that green leaves are a good source of available calcium. The main problem in nutritional exploitation of green leafy vegetables is the presence of antinutritional and toxic principles. Amaranth, chenopodium, lettuce, spinach, etc., accumulate high concentrations of nitrate, oxalate, and saponin (Cheeke and Bronson, 1980; Olson et al., 1972; Pedersen and Wang, 1971; Fenwick and Oakenfull, 1983). Amaranth leaves were shown to be a potential plant source of nitrate and oxalate (Carlsson, 1975; Marshall et al., 1967; Teutonico and Knorr, 1985), and their nitrate content could reach 3.25%, causing fatal methemoglobinamea in cattle (Steyn, 1960; Clarke and Clarke, 1967; Fowler, 1967; Buck et al., 1973). Nitrate poisoning by amaranth was reported in livestock (Whitehead and Moxon, 1949). Reduced levels of vitamin A in liver, fatty degeneration of liver cells, and follicular atrophy were observed in rats fed amaranth dye (Galea et al., 1971; Shtenberg and Gavrilenko, 1972). Several authors have also indicated the presence of a specific trypsin inhibitor in alfalfa leaves (Kendall, 1951; Ramirej and Mitchell, 1960; Chang, et al., 1978). Kohler and Bickoff (1970) reported that green leaves were rich in protein, but their utilization was limited because of the presence of indigestible fiber. The presence of a large number of inexpensive edible green leafy vegetables, their abundance, and their attributive qualities create interest to study the nutritional value of selected green leafy vegetables.

Department of Chemistry and Biochemistry, Haryana Agricultural University, Hisar 125004, India.

Table I. Proximate Principles of Green Leafy Vegetables (Percent Dry-Weight Basis)[a]

vegetable	crude protein	ether extractive	crude fiber	minerals (ash)	sol ash
chickpea	22.61	1.30	13.95	14.55	11.87
chenopodium	28.60	2.55	7.20	21.05	20.41
spinach	21.59	1.17	9.61	26.16	24.84
mustard	29.82	1.68	11.99	17.49	15.85
cauliflower	23.65	3.73	9.23	12.54	11.94
F values	56.99	24.68	38.31	116.90	96.75

[a] Each value is the average of triplicate determinations

METHODS AND MATERIALS

Green leafy vegetables, viz. cauliflower (*Brassica oleracea*), chenopodium (*Chenopodium album*), chickpea (*Cicer arietinum*), mustard (*Brassica compestris*), and spinach (*Spinacea oleracea*), were collected from the Haryana Agricultural University Farm, washed under running tap water, sun-dried, ground to pass a 100-mesh sieve, and stored in colored air-tight containers until further analysis. Crude protein (CP), ether extractives (EE, lipid), crude fiber (CF), and ash (minerals) were estimated by the standard methods of the AOAC (1970). Calcium, magnesium, and phosphorus were estimated by the colorimetric methods of Trinder (1960), Neill and Neely (1956), and Fiske and Subharow (1925), respectively. Sodium and potassium were estimated by flame photometry after the sample was digested with a triacid mixture

0021-8561/88/1436-0472$01.50/0