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The effect of sowing date and growth stage on the essential oil composition of three types of parsley (*Petroselinum crispum*)

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Abstract: Essential oils obtained by simultaneous distillation–extraction (SDE) from leaves, petioles and roots of three types of parsley (turnip-rooted, plain leaf and curly leaf type), sown on three different dates, were analysed by GC-MS (gas chromatography–mass spectrometry) analysis. Parsley plants were found to produce mainly β-phellandrene, 1,3,8-*p*-menthatriene, α-,*p*-dimethylstyrene, myristicin, β-myrcene and apiole. In some cases α- and β-pinene were also found, whereas β-elemene was detected, especially in the curly leaf type. The growth stage, plant tissue and date of sowing, as well as the climate conditions, all had a significant effect on the essential oil composition by altering the ratio of the above substances.

Keywords: essential oils; 'Hamburg' parsley; parsley; *Petroselinum crispum*; GC-MS analysis

INTRODUCTION

Parsley (*Petroselinum crispum* (Mill) Nym) is a biennial plant, which is cultivated widely as an annual. The three main types of parsley are the plain leaf type (ssp *neapolitanum,* Danert) and the curly leaf type (ssp *crispum*), which are cultivated for their foliage, and the turnip-rooted or 'Hamburg' type (ssp. *tuberosum*), primarily grown for its roots. In addition to their use as a fresh or dried herb, parsley leaves (also seeds) contain essential oils that can be used in perfumes, creams and soaps. Moreover, parsley possesses medicinal properties, first-mentioned by the ancient Greeks.[1]

The strong flavor of parsley is derived from its oil content. Simon *et al*[2] and Simon and Quinn[3] studied the essential oils of 104 accessions from the USDA Plant Introduction Station and identified 1,3,8-*p*-menthatriene, myristicin, β-phellandrene and myrcene as the principal components. The first three of these compounds, together with apiole and 1-methyl-4-isopropenylbenzene, formed the main constituents of the essential oil of a desert parsley,[4] whereas β-phellandrene, 1,3,8-*p*-menthatriene, α-*p*-dimethylstyrene (*p*-cymenene) and terpinolene constituted the primary components of the plain leaf type.[5] Although the distillation technique was shown to affect the essential oil composition of plain leaf parsley,[6] sniff tests showed that 1,3,8-*p*-menthatriene was responsible for the characteristic parsley aroma.

In a systematic study of the potent odorants of curly leaf parsley essential oils by aroma extract dilution analysis (AEDA), Jung *et al*[7] did not find β-phellandrene, 1-methyl-4-isopropenylbenzene and apiole to be significant contributors to parsley aroma, as previously reported by Macleod *et al*.[8] More recently, Masanetz and Grosch,[4] using gas chromatography/olfactometry-head space (GC/OH), reported that the aroma of curly leaf parsley was derived from a mixture of seven constituents, including myrcene, 1,3,8-*p*-menthatriene and myristicin. In the absence of myrcene and 1,3,8-*p*-menthatriene, there was a loss of the parsley-like character of the aroma. Pino *et al*[9] found myristicin and apiole to be the principal components of the essential oils of the plain leaf type, followed by β-phellandrene and 1,3,8-*p*-menthatriene, while Lopez *et al*[10] identified 1,3,8-*p*-menthatriene as the most abundant component followed by β-phellandrene and apiole.

It is evident from the above that the essential oil composition of parsley differs between species and with the climate conditions. Moreover, it is likely that the essential oil content may vary with the part of the plant under analysis, as well as with the stage of plant development and the cultivation season. In the present paper, after SDE, GC-MS was used to analyse the essential oils of the aerial part and roots of three types of parsley at two growth stages following sowing on two different dates. Especially for the aerial part, the petioles and leaves were analysed separately instead of being analysed as a whole. The aim of the study was to determine the effect of these factors,

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as well as the effect of Greek climate conditions, on the composition of the essential oils using the same analytical procedure.

MATERIALS AND METHODS

Plant material

Parsley seeds of the three types ((a) *Petroselinum crispum* (Mill) Nym ssp *neapolitanum* Danert cv plain-leafed (G Fytotechniki), (b) *P crispum* ssp *crispum* cv curly-leafed (G Fytotechniki) and (c) *P crispum* ssp *tuberosum* (Bernh) Crov cv Fakir (Bejo Holland)) were sown at a depth of 0.5–1 cm in germination trays containing a commercial peat substrate (KTS2, Klasmann-Deilman Gmbh). On reaching an adequate size for handling (5–6 cm height), seedlings were transplanted to 10 l plastic pots containing peat and sand in a ratio of 2:1 (v/v). Five seedlings were transplanted to each pot. The plants were grown under natural light in a glasshouse without heating during the winter and transferred outdoors during spring.

Two sowings were carried out: 27 September 2000 and 15 December 2000. Irrigation and fertilizer application (one application of 170 ppm NH_4NO_3) was carried out by hand as deemed necessary and plants were harvested at two stages: (1) after the formation of six to eight leaves (20 December 2000 and 21 March 2001 for the first and second sowing respectively), and (2) a month after the first harvest.

After harvest, the plants were separated into the aerial part and the root system and dried at room temperature ($25 \pm 3\,^{\circ}C$) for 30 days, after which the aerial part was further separated into leaves and petioles. The leaves and petioles were chopped by hand into very fine pieces, and the roots, because of their woody structure, were blended.

Isolation of the essential oils

Essential oils were isolated by SDE using a Lickens–Nickerson apparatus for organic solvents lighter than water. The extracting solvent was pentane. Plant tissue (5 g) and 3 ml of pentane were used per treatment. The distillation period was 1 h. All pentane extracts were stored at $<4\,^{\circ}C$ until their analysis by GC-MS.

GC-MS analysis conditions

The analysis of the essential oils was performed using a Hewlett Packard (Waldbronn, Germany) 5890 II GC, equipped with a HP-5MS (crosslinked 5% PH ME siloxane) capillary column (30 m, 0.25 mm id, 0.25 μm film thickness) and a mass spectrometer 5972 (Hewlett Packard, Waldbronn, Germany) as detector. The carrier gas was helium, at a rate of 1 ml min^{-1}. Column temperature was initially kept for 3 min at $50\,^{\circ}C$; then gradually increased to $200\,^{\circ}C$ at $4\,^{\circ}C\,min^{-1}$, and held for 5 min. For GC-MS detection an electron ionization system was used with ionization energy of 70 eV. Injector and detector (MS transfer line) temperatures were set at 220 and $290\,^{\circ}C$, respectively. Samples (1 μl) of the pentane extracts were injected manually and splitless.

The identification of components was based on comparison of their GC retention times and mass spectra with authentic standards (when possible). The tentative identification of compounds was carried out by comparison of their mass spectra with spectral data from the Mass Spectra Library NBS75K library data of the GC-MS system and literature data.[11] Relative percentage amounts of the components were calculated from the TIC (total ion chromatogram) by the computer. Oil yields were not measured.

RESULTS

Nine major components were detected in the essential oils of most samples. The quantities of these compounds within the various plant organs, expressed as percentages of the total oil and in relation to harvest stage, are given in Tables 1–3. A typical TIC of the essential oil of parsley leaves is shown in Fig 1. Because of the relative slow growth of the curly leaf type, plants were not sampled at the first stage of the second sowing.

In turnip-rooted parsley, the principal components of the essential oils of the leaves at the first growth stage were β-phellandrene and 1,3,8-*p*-menthatriene, with β-myrcene and α-*p*-dimethylstyrene also being found in relatively high concentrations (Table 1). A similar pattern was observed at the second (mature) growth stage, but with a large increase in the concentration of myristicin. Comparing the relative concentrations of the components for the two sowing dates, important differences were found for β-phellandrene (39.0–22.0%) and 1,3,8-*p*-menthatriene (17.4–45.7%) at the first growth stage and between 1,3,8-*p*-menthatriene (15.7–29.0%) and myristicin (27.2–4.6%) at the second stage.

The composition of the essential oils of the petioles was similar to that of the leaves, with β-phellandrene, 1,3,8-*p*-menthatriene and β-myrcene forming the major constituents, followed by myristicin and α-*p*-dimethylstyrene (Table 1). Both the growth stage and the time of sowing affected the relative amounts of 1,3,8-*p*-menthatriene and myristicin in the petioles.

In contrast, the essential oils profile of the roots of turnip-rooted parsley was quite different from that of the leaves and petioles and varied according to the sowing date and stage of sampling. Apiole formed a major constituent at both growth stages of both sowings (Table 1). However, β-pinene, β-phellandrene and myristicin, which formed important constituents of the roots at both harvest stages of the first sowing, were either absent or of minor importance in roots of the second sowing. Roots of the second sowing showed major differences in composition between the two harvest stages. Thus α-pinene, β-myrcene and myristicin, which were important constituents of the first growth stage, were not detected in the second stage, whereas

Table 1. Composition of the essential oils of turnip-rooted parsley for two growth stages and three plant parts

	First sowing						Second sowing					
	Leaves		Petioles		Roots		Leaves		Petioles		Roots	
Compounds (% of essential oil)	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]
α-Pinene (1)[a]	3.1	5.2	6.6	3.5	tr[d]	tr	1.8	4.8	4.6	9.2	13.9	—
β-Pinene (2)	1.6	—	4.3	2.6	20.7	30.6	1.1	1.6	3.5	3.0	1.2	12.0
β-Myrcene (3)	12.4	13.8	20.6	17.2	tr	5.1	9.5	17.3	16.1	21.7	19.1	—
β-Phellandrene (4)	39.0	26.5	31.4	24.8	26.8	17.4	22.0	27.4	23.0	27.1	1.6	—
α-*p*-Dimethylstyrene (5)	11.6	11.7	11.1	7.4	tr	tr	12.7	12.7	12.2	10.0	—	—
1,3,8-*p*-Menthatriene (6)	17.4	15.7	4.3	37.4	tr	tr	45.7	29.0	25.3	9.0	—	11.3
β-Elemene (7)	—	—	—	—	—	—	—	—	—	—	—	—
Myristicin (8)	3.5	27.2	11.1	0.8	19.6	15.8	1.6	4.6	11.1	16.0	34.3	tr
Apiole (9)	—	—	0.4	tr	32.8	20.3	—	tr	—	0.4	25.3	70.1

[a] Numbers in parentheses refer to the elution order of the compounds.
[b] The plants had six to eight leaves when first harvested.
[c] This growth stage refers to plants one month after the first harvest.
[d] tr, trace.

Table 2. Composition of the essential oils of plain leaf parsley for two growth stages and three plant parts

	First sowing						Second sowing					
	Leaves		Petioles		Roots		Leaves		Petioles		Roots	
Compounds (% of essential oil)	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]
α-Pinene (1)[a]	2.7	4.8	4.7	3.4	—	—	1.5	5.9	1.6	2.4	—	—
β-Pinene (2)	1.0	0	0	1.6	—	2.0	0.5	2.6	0.7	1.6	25.1	1.7
β-Myrcene (3)	5.1	6.4	19.1	10.6	—	—	4.6	4.6	8.5	6.9	tr[d]	—
β-Phellandrene (4)	30.2	51.9	48.7	39.9	10.1	—	23.2	16.8	19.6	24.1	36.9	tr
α-,*p*-Dimethylstyrene (5)	14.4	6.9	19.9	10.0	—	1.8	18.5	8.6	15.8	8.3	32.6	tr
1,3,8-*p*-Menthatriene (6)	28.8	27.4	6.4	25.8	89.9	—	46.7	14.0	23.7	33.6	5.4	0
Elemene (7)	—	—	—	—	—	—	—	—	—	—	—	—
Myristicin (8)	5.8	tr	—	2.0	tr	6.6	0.7	21.7	18.0	16.1	—	35.9
Apiole (9)	2.1	tr	—	1.0	tr	85.0	—	17.8	1.5	1.8	—	51.8

[a] Numbers in parentheses refer to the elution order of the compounds.
[b] The plants had six to eight leaves when first harvested.
[c] This growth stage refers to plants one month after the first harvest.
[d] tr, trace.

Table 3. Composition of the essential oils of curly leaf parsley for two growth stages and three plant parts

	First sowing						Second sowing					
	Leaves		Petioles		Roots		Leaves		Petioles		Roots	
Compounds (% of essential oil)	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]	First stage[b]	Second stage[c]
α-Pinene (1)[a]	1.3	tr[d]	4.2	tr	—	—	—	tr	—	tr	—	—
β-Pinene (2)	0.5	tr	1.9	tr	tr	4.8	—	tr	—	tr	—	1.9
β-Myrcene (3)	5.4	2.4	9.7	9.5	tr	—	—	6.3	—	6.4	—	—
β-Phellandrene (4)	40.6	13.2	31.6	25.6	5.0	tr	—	24.3	—	20.9	—	—
α-*p*-Dimethylstyrene (5)	7.0	3.9	8.1	14.2	tr	—	—	8.1	—	10.0	—	—
1,3,8-*p*-Menthatriene (6)	11.8	2.1	11.7	8.1	tr	tr	—	3.4	—	8.8	—	—
Elemene (7)	2.2	2.5	1.6	3.6	9.7	1.6	—	3.3	—	2.0	—	tr
Myristicin (8)	25.2	75.8	21.6	36.3	10.8	41.3	—	37.4	—	28.7	—	4.8
Apiole (9)	0.5	—	1.0	tr	34.2	29.7	—	17.1	—	19.9	—	83.2

[a] Numbers in parentheses refer to the elution order of the compounds.
[b] The plants had six to eight leaves when first-harvested.
[c] This growth stage refers to plants one month after the first harvest.
[d] tr, trace.

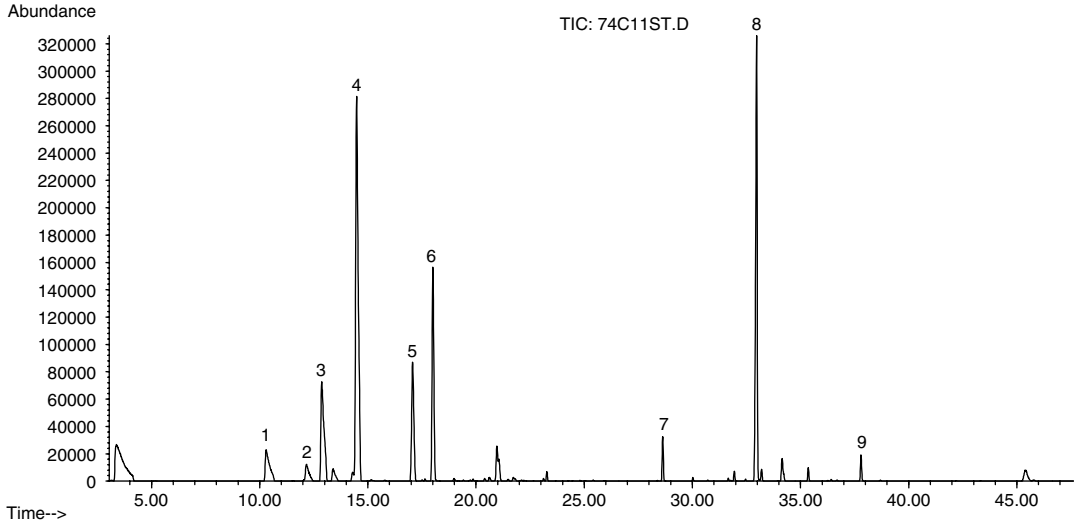

Figure 1. A typical TIC of essential oils of curly leaf parley analysed on an HP-5MS column (for the identification of peaks see the tables).

1,3,8-*p*-menthatriene, present in the second stage, was not detected in the first stage (Table 1).

The major components of the essential oils of the leaves of the plain leaf type harvested at both growth stages were β-phellandrene and 1,3,8-*p*-menthatriene, with α-*p*-dimethylstyrene also forming a significant constituent at the first stage (Table 2). The two sowing dates differed with respect to the concentrations of β-phellandrene, 1,3,8-*p*-menthatriene and myristicin at both growth stages, as well as apiole at the second stage.

Apart from a relative increase in β-myrcene content, the essential oil profiles of the petioles were similar to those of the leaves and, with the exception of 1,3,8-*p*-menthatriene (first sowing), they were not affected by growth stage (Table 2). On the contrary, there were differences between the two sowing dates, the most important being those of β-phellandrene, myristicin and 1,3,8-*p*-menthatriene. The essential oils of the roots of the plain leaf parsley differed both from those of the leaves and petioles and from those of the roots of the turnip-rooted parsley (Tables 1 and 2). There were also noticeable differences between the growth stages and sowing dates. Thus, at the first stage of the first sowing, the major components were β-phellandrene and 1,3,8-*p*-menthatriene, whereas, in the second sowing, β-pinene and α-*p*-dimethylstyrene were also detected in relatively high amounts. At the second stage of both sowings, the principal components were apiole and myristicin (Table 2).

The essential oils of the leaves of the curly leaf type were composed mostly of β-phellandrene and myristicin, followed by 1,3,8-*p*-menthatriene (first stage), and myristicin and β-phellandrene (second stage of both sowings), followed by apiole, β-myrcene and α-*p*-dimethylstyrene (second stage of the second sowing; Table 3). β-Elemene was detected only in curly leaf type, in leaves and petioles as well as in roots.

The essential oils of the petioles followed a similar pattern to those of the leaves, although less myristicin was detected in the petioles than the leaves (second stage) and apiole was not detected at this stage of the first sowing (Table 3).

Apiole was the main component of the essential oils of the roots of the curly leaf type at the first growth stage, followed by myristicin and β-elemene. At the second stage, apiole and myristicin were the major components, whereas β-elemene was reduced (Table 3).

DISCUSSION

The results of the present experiments show that the essential oil composition of parsley varies with the type of parsley, the tissue source, the stage of growth at harvest and the date of sowing.

The biggest differences, due to the plant tissue, were observed between the essential oils of the roots and the aerial organs. The composition of the oils of leaves and petioles was more similar, although some notable differences occurred, eg the relative concentrations of myristicin at the second stage of harvest of the curly leaf type (Table 3) and the 1,3,8-*p*-menthatriene content of the turnip-rooted type at the first stage (Table 1). Although β-phellandrene and 1,3,8-*p*-menthatriene were usually the major components of the leaves and petioles, myristicin, β-myrcene and α-*p*-dimethylstyrene were also found in relatively high concentrations. This result agrees with those of Simon and Quinn[3] who, while not distinguishing between leaf and petiole, identified 1,3,8-*p*-menthatriene, β-phellandrene, myristicin and myrcene as the major components of the essential oils of the aerial part of 104 accessions of parsley. In addition, the detection of apiole in the leaves and petioles of the plain leaf and curly leaf types supports the findings of Macleod *et al*[8] and Pino *et al*,[9] whereas the absence of this compound from the aerial organs of the turnip-rooted type, or the presence of β-elemene in the leaves and petioles of the curly leaf type, indicates that a source of difference between results in the literature could be the type of parsley investigated.

Previous reports on the essential oil composition of parsley refer to the aerial part of the plant as a whole, without analysing leaves and petioles separately, as has been done in the present study. Although the major components for both the leaves and petioles were generally the same, the ratio of these compounds as percentages of the total oil varied. Moreover, there was both an empirical and a quantitative change in essential oil composition between the two growth stages at which plants were harvested. This result agrees with those of Lopez *et al*[10] who analysed the essential oils of parsley at five different stages and concluded that the generation of compounds is time-dependent, so the composition of the essential oils has a different profile during the growth cycle, thus causing different aroma features. Moreover, the present results clearly indicate that the date of sowing (ie season of cultivation) has an appreciable effect on the essential oil composition of the plant tissues. Plants of the first sowing developed under conditions of relatively low temperatures, low light intensity and shorter days, whereas those of the second sowing developed under high temperatures, low light intensity and longer days.

To our knowledge, this is the first time that turnip-rooted parsley has been investigated for essential oils, whereas the roots of the other parsley types do not appear to have been examined to date. In addition this is the first time that plants of turnip-rooted parley have been cultivated in Greece. Turnip-rooted parsley, which is less well known in Western Europe than the leafy forms, produces a much larger root and thus offers a good source of tissue for extraction. Apiole, myristicin and β-phellandrene, regarded as important by Pino *et al*,[9] were also present in relatively large concentrations.

The growth stage at which the plants are harvested, as well as the sowing date, significantly affect the composition of the essential oils. Therefore, in aerial parts, there is a trend of β-phellandrene to decrease from first to second stage in the first sowing and increase from first to second stage of the second sowing. In addition, α-*p*-dimethylstyrene seems to be stable during the growth cycle of the plant, with minor changes, although 1,3,8-*p*-menthatriene seems to be unchanged in the first sowing and decreases from the first to second stage of the second sowing in leaves. Particularly for the curly-leaf type, β-phellandrene follows the same pattern, and myristicin increases from the first to the second stage for both sowings. Finally, apiole shows a rapid increase at the second stage of the second sowing. Trends of root essential oils are difficult to interpret because of their qualitative differences.

In conclusion, the effect of growth stage and sowing date is mainly observed in the relative concentration of each compound as a part of the total oil content of the leaves and petioles whereas, in roots, there are differences not only in the relative concentrations of the compounds but qualitative differences too.

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