Influence of Harvesting Time on Chemical Composition of *Mentha piperita* L. Essential Oil

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Peppermint (*Mentha piperita* L.), a perennial of the Lamiaceae family, is a hybrid plant obtained by crossing horse mint with spearmint, itself a hybrid of *Mentha rotundifolia* and *Mentha longifolia*. Clones of this hybrid from Great Britain (Mitcham), when transplanted elsewhere, have produced derivatives such as Italo-Mitcham, Franco-Mitcham (violet-black stems and leaves, moderately vigorous, 40 to 60 cm in height, sparse foliage), Milly mint and Maine-et-Loire mint.

In addition, we find Hungarian peppermints (violet-black stems and leaves, strongly vigorous, 100 to 150 cm in height, dense foliage) and white mints (true green stems and leaves, moderately vigorous, 60 to 90 cm in height, dense foliage).

The origins of these plants have been addressed by Murray et al.[1] and by Jolivet.[2] Sacco and Scanneri[3,4] have studied their chemotaxonomic and karyological features.

Recent work on the essential oils of peppermint has been reported by Peyron and Rouzet[5] and by Lawrence.[6] Perrin and Colson[7] analyzed oils from various organs of peppermint (*Mentha piperita* L.)—leaves, flowers and pluricellular glands. Sacco[8] studied the genetic and chemical features of the genus *Mentha*. Emberger et al.[9] analyzed enantiomeric substances in mint oil and carried out a sensory evaluation. Vlahov et al.[10,11] identified and isolated a certain number of sesquiterpenes in oils of Bulgarian peppermint. More recently, Gilly et al.[12] studied the chemical composition of peppermint clones from Grasse and Digne.

Closer to our work, Bouverat-Bernier[13] studied the impact of frequency and date of harvest on yield and quality of Hungarian peppermint oil. After studying a Mitcham Milly mint and finding an increase in oil levels up to flowering and a fall thereafter, Bouverat-Bernier showed that:

- In early July, well before flowering, menthol and menthone levels are equivalent. Thereafter, menthol and menthyl acetate levels increased, while those of menthone decreased.
- Menthofuran was characteristic of the flowering period, though it also remained abundant throughout the year.
- Pulegone, present at the time of flowering, vanished afterward.
- Yields of oil were lower when a second harvest was cut; no pulegone was present and menthofuran levels were lowered.
- Mid-July to late August seems to be the peak period of oil synthesis. However, this period is mostly the time of flowering and shedding of base leaves.

For Hungarian peppermint, given the yields per hectare and the optimal menthol/menthone ratio, the best procedure appears to be a harvest in late June to early July and a second harvest after mid-August (before the first flower buds and the yellowing of the base leaves). The levels of menthone were always greater than those of menthol regardless of harvest time.

Experimental

Plant material: Ten peppermint cultivation sites (ten growers) were set up within a radius of 15 km around Le Mayet-de-Montagne, Allier, in central France (altitude 400 to 800 m). The individual surface areas ranged from 0.6 to 2.5 ha for an overall surface of approximately 10 ha. Planting density was 30,000 plants/ha.

The plant material was steam distilled on an industrial scale at Le Mayet-de-Montagne for one hour in a still with

Table I. Chemical composition of essential oil of *Mentha piperita* L. from Le Mayet-de-Montagne, Allier, France

Peak No.	Constitutent	Percentage
1	α-pinene	0.6
2	β-pinene	0.8
3	sabinene	0.5
4	myrcene	0.5
5	limonene	1.1
6	1,8-cineole	3.9
7	(Z)-3-hexenol	0.3
8	menthone	39.8
9 + 10	menthofuran + isomenthone	3.7
11	menthyl acetate	1.5
12	β-caryophyllene	1.3
13	neomenthol	5.1
14	pulegone	0.3
15	menthol	22.9

a volume of 2.5 or 6 m^3. Distillations produced a total of 310 kg of oil, for an average yield of 0.3% or 30 kg/ha.

At the same time, laboratory-scale extractions were performed. According to the amounts collected or available, we used either a 250-litre pilot extractor or modified Kaiser-Lang extractors with volumes ranging from 2 to 20 L to extract from 100 g to 4 kg of plant material. Average yield was 0.3%.

Analysis: The oils were analyzed by gas-phase chromatography using a DELSI 121 C apparatus[a] and a flame ionization detector. Temperature program was 5 min at 50°C, then 50°C to 210°C at 2°C/min. We used a WCOT 25 m x 0.3 mm fused silica column with CP WAX 51 stationary phase.[b] The compounds were identified by GC/MS using a VG 70 mass spectrometer.[c] Conditions were 70 eV ionization energy, WCOT fused silica column (50 m x 0.3 mm, CP WAX 51), temperature program 50°C to 230°C at 3°C/min. The carrier gas was helium.

Results and Discussion

Peppermint oil at first flowering: For a peppermint oil obtained at first flowering (mid-July 1989), we observed the following physical constants:

- n_D^{20} = 1.4638
- d_{20}^{20} = 0.903
- $[\alpha]_D^{20} = -23.3°$

The chromatogram of the oil is shown in Figure 1. The main constituents identified by GC/MS are given in Table I, with their percentages. The oil contains the high levels of menthone (40%) and menthol (23%) characteristic of oil obtained at the beginning of flowering.

Effect of harvest time: During 1989, we distilled and analyzed four batches of mint collected from four sites (four different growers) at different growth periods. Ten samples were taken between June 15 and October 30. The chemical composition of the oils from these different batches is given in Tables IIa and IIb.

[a]ILS, Lyon, France
[b]Chrompack, Les Ulis, France
[c]Micromass/Fisons, Altringsham, England

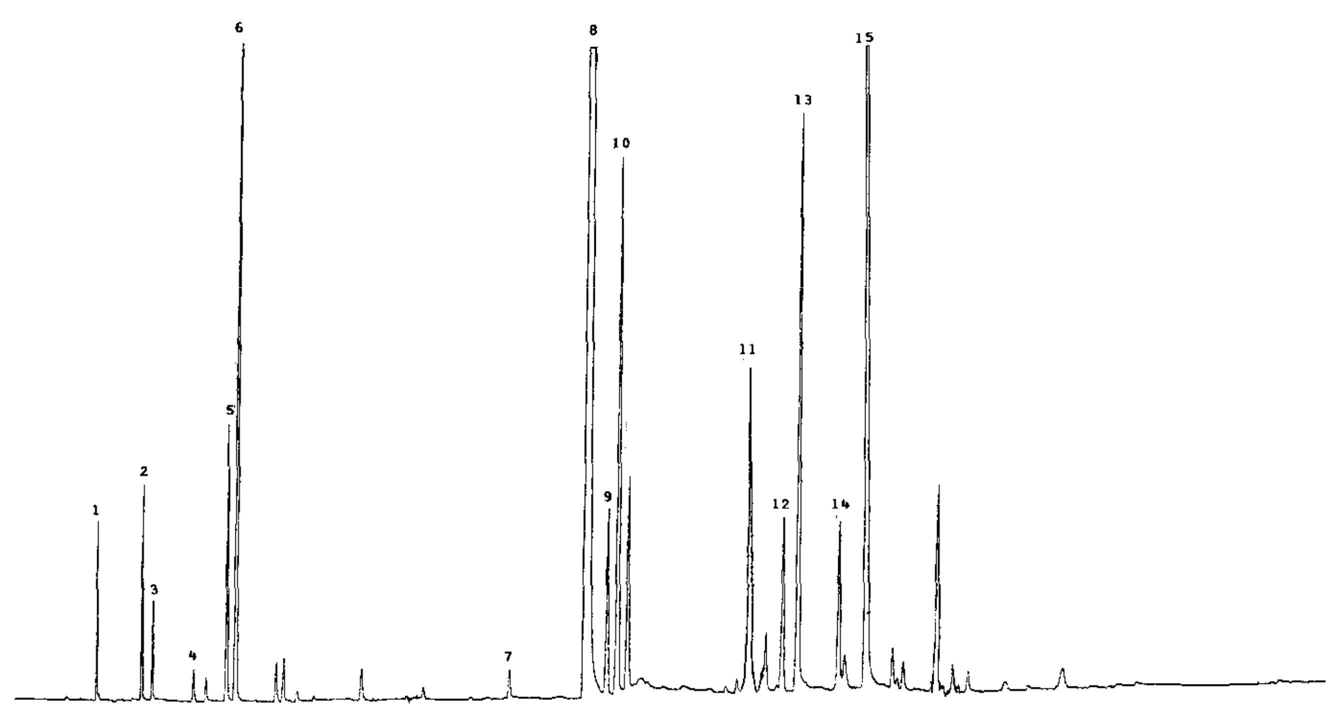

Figure 1. Chromatogram of the essential oil of *Mentha piperita* L. from Le Mayet-de-Montagne (France) (See Table I for identification of peaks)

Table IIa. Chemical composition (%) of peppermint (1989 production) according to harvesting time

Peak No.	Component	Grower 1 6/14	7/5	7/29	8/15	9/15	9/30	10/30	Grower 2 6/14	7/5	7/29	8/15	9/1	9/15	10/1	10/15	10/31
1	α-pinene	0.4	0.3	0.5	0.5	0.4	0.4	1.7	0.3	0.5	0.5	0.5	0.5	0.5	0.5	1.9	2.4
2	β-pinene	0.4	0.5	0.6	0.5	0.5	0.5	1.8	0.4	0.7	0.7	0.6	0.6	0.7	0.6	2.0	1.7
3	sabinene	0.2	0.3	0.3	0.3	0.3	0.2	0.4	0.2	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.6
4	myrcene	0.3	0.3	0.4	0.3	0.3	0.2	0.4	0.3	0.5	0.5	0.4	0.4	0.5	0.3	0.6	0.9
5	limonene	0.8	0.7	1.0	0.9	0.4	0.4	1.8	0.7	0.8	0.9	0.7	0.7	0.7	0.8	2.4	2.1
6	1,8-cineole	2.5	2.6	3.1	2.7	3.2	3.2	2.3	2.6	3.3	4.1	2.9	3.7	3.6	2.9	2.8	2.0
7	(Z)-3-hexenol	0.2	0.2	0.3	0.3	0.4	0.4	0.3	0.2	0.2	0.4	0.4	0.7	0.5	0.4	0.4	0.3
8	menthone	54.5	51.9	55.0	42.1	21.3	14.2	8.1	52.4	43.8	37.2	35.0	27.7	24.0	17.3	10.4	7.4
9	menthofuran	0.3	0.2	0.9	4.8	3.4	0.8	1.4	0.3	0.3	1.4	5.0	2.9	4.2	2.7	1.1	0.6
10	isomenthone	3.2	3.1	3.5	2.5	2.4	1.9	1.6	3.0	2.9	3.0	2.8	2.7	2.5	2.1	1.6	1.3
11	menthyl acetate	1.0	0.8	0.5	0.5	3.1	6.1	7.5	1.7	1.6	1.3	1.1	2.3	3.1	6.3	7.0	9.6
12	β-caryophyllene	1.7	2.3	2.1	1.4	1.2	1.1	1.0	2.0	2.4	1.8	1.2	1.2	1.2	0.6	1.0	0.8
13	neomenthol	2.9	3.2	1.9	2.2	5.4	5.5	5.6	3.8	3.8	3.5	3.0	3.5	4.8	5.4	8.7	6.9
14	pulegone	0.4	0.7	2.6	8.4	3.0	0.7	0.8	0.7	0.6	3.4	9.7	4.9	2.8	0.7	0.6	0.9
15	menthol	20.6	22.2	16.7	22.4	46.4	55.6	53.7	22.2	27.0	31.0	28.8	38.3	43.5	52.4	51.7	52.5
	menthone/ menthol	2.6	2.3	3.3	1.9	0.5	0.3	0.1	2.3	1.6	1.2	0.7	0.5	0.3	0.2	0.1	

Table IIb. Chemical composition (%) of peppermint (1989 production) according to harvesting time

Peak No.	Component	Grower 3 6/14	7/5	7/29	8/17	9/15	9/30	Grower 4 6/14	7/5	7/15	7/30	8/15	9/1	9/15	9/30	10/15	10/31
1	α-pinene	0.9	0.1	0.6	0.3	0.5	0.5	0.2	0.2	0.5	1.0	0.4	0.5	0.5	1.8	1.6	1.1
2	β-pinene	0.5	0.1	0.8	0.5	0.8	0.7	0.4	0.3	0.7	1.3	0.5	0.6	0.6	1.9	1.5	1.3
3	sabinene	0.3	0.1	0.4	0.3	0.3	0.3	0.2	0.2	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.2
4	myrcene	0.4	0.2	0.5	0.4	0.1	0.2	0.3	0.3	0.4	0.5	0.4	0.4	0.5	0.5	0.4	0.2
5	limonene	1.3	0.4	1.0	0.7	0.9	0.8	0.6	0.4	0.9	1.8	0.7	0.7	0.6	2.2	1.6	1.2
6	1,8-cineole	2.8	2.5	3.7	2.7	3.6	3.8	2.4	2.0	2.9	2.6	3.0	3.4	3.8	2.7	2.2	1.7
7	(Z)-3-hexenol	0.1	0.2	0.4	0.3	0.4	0.4	0.3	0.2	0.3	0.4	0.4	0.4	0.5	0.4	0.3	0.3
8	menthone	51.4	49.4	43.1	40.5	10.7	11.4	48.8	38.5	38.7	29.6	31.2	26.6	21.0	10.0	8.6	6.1
9	menthofuran	0.3	+	0.7	3.7	1.2	1.4	0.1	0.2	0.9	1.6	3.7	3.6	3.2	0.6	0.7	0.4
10	isomenthone	2.9	3.1	3.1	2.9	1.5	1.8	3.0	2.7	2.8	2.4	2.6	2.7	2.5	1.6	1.4	0.9
11	menthyl acetate	2.0	1.3	1.2	1.3	6.1	7.1	2.3	1.8	1.6	1.9	1.7	2.0	3.2	7.3	8.0	12.4
12	β-caryophyllene	1.5	2.3	1.7	1.5	1.5	1.3	2.1	3.0	2.1	2.1	1.5	1.9	1.5	1.5	1.3	1.1
13	neomenthol	3.3	2.9	3.5	3.2	5.0	5.3	4.1	4.4	5.0	5.1	4.5	5.0	5.3	6.6	6.7	7.1
14	pulegone	0.3	0.6	1.5	6.0	1.1	1.1	0.6	0.7	1.7	3.6	6.2	4.9	2.1	0.8	0.5	0.8
15	menthol	20.5	24.4	26.8	27.9	56.9	55.6	23.8	31.5	31.4	33.0	34.9	38.7	45.7	52.1	54.5	54.6
	menthone/ menthol	2.5	2.0	1.6	1.4	0.2	0.2	2.0	1.2	1.2	0.9	0.9	0.7	0.5	0.2	0.2	0.1

The seasonal time variations in the menthone, menthol, menthyl acetate, menthofuran and pulegone contents, as well as the menthone/menthol ratio, are plotted in Figures 2-6.

We observed:

- For the first time, an inversion of the menthone/menthol ratio during growth. The menthol concentration rose from approximately 20% in early July to more than 50%. The menthone/menthol ratio was close to unity by about August 15.
- Peak levels of menthofuran and pulegone during flowering.
- Low levels of menthyl acetate (approximately 1%) at the end of flowering, increasing (6-8%) up to the end of the growth cycle.

The variations in the chemical composition of these oils confirm the Mitcham character of this peppermint grown in this area of France.

The inversion of the menthone/menthol ratio occurs in mid-August at Le Mayet-de-Montagne, before flowering. In contrast, it occurs in the first fortnight of July in the Anjou region of France, after flowering. This may result from a lag in biosynthesis in relation to the growth stage due to the less friendly climate in Auvergne. In support of this explanation, we observe (Table III) that the date of the inversion varies with altitude; the greater the altitude, the

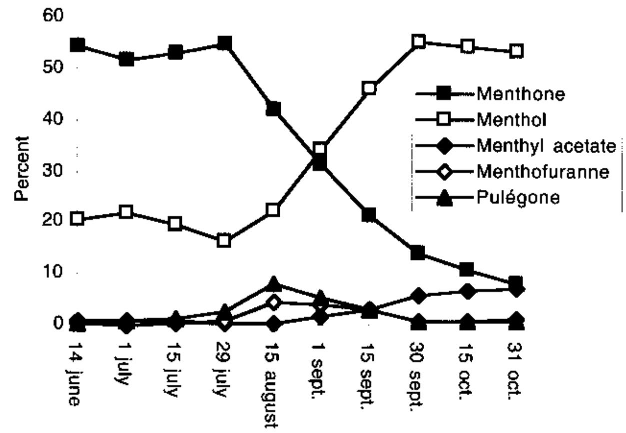

Figure 2. Variation of main components according to harvesting time (Grower 1; altitude 800 m)

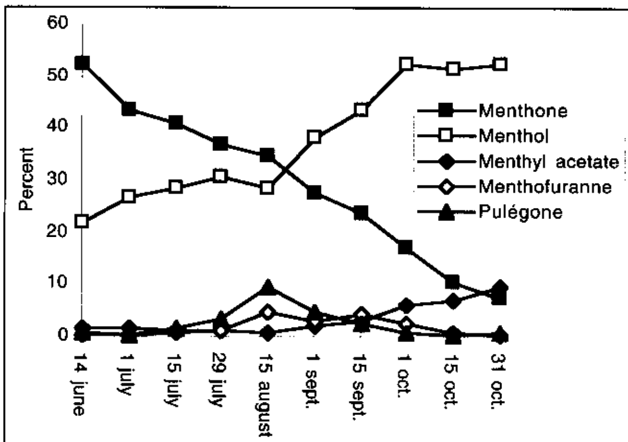

Figure 3. Variation of main components according to harvesting time (Grower 2; altitude 500 m)

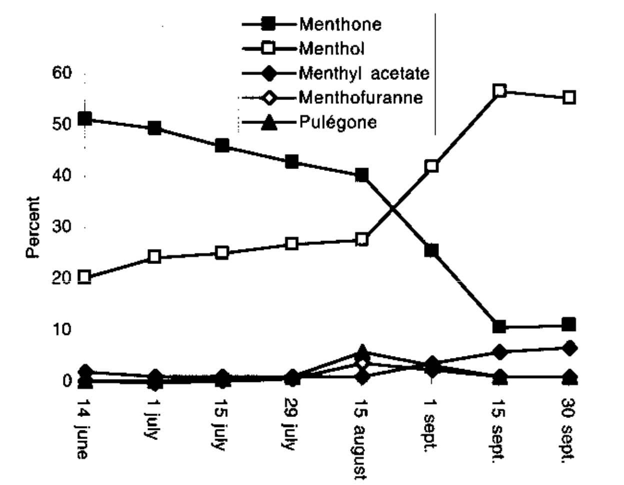

Figure 4. Variation of main components according to harvesting time (Grower 3; altitude 850 m)

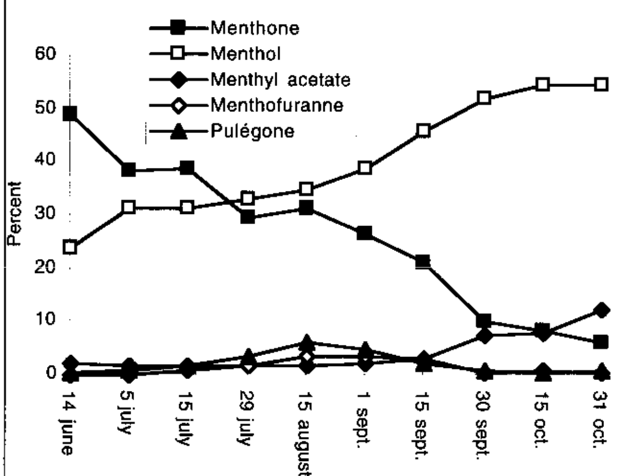

Figure 5. Variation of main components according to harvesting time (Grower 4; altitude 500 m)

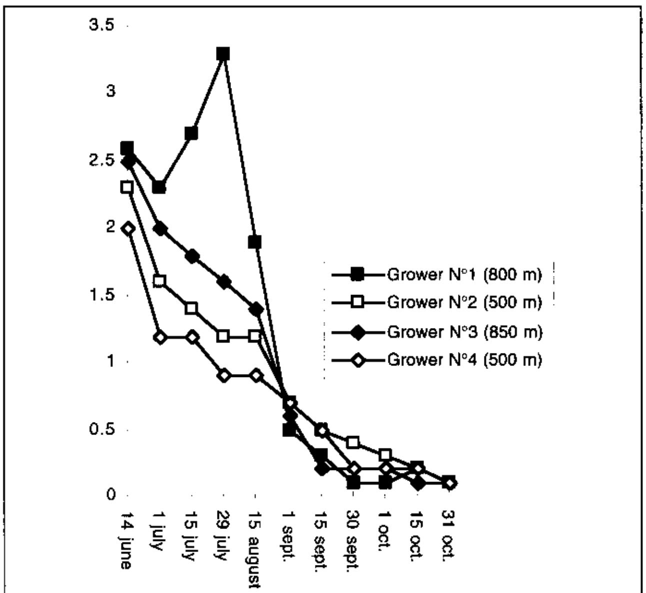

Figure 6. Variation of ratio menthone/menthol according to harvesting time

Table III. Effect of altitude on the menthone/menthol inversion date for peppermint oil from selected test sites near Le Mayet-de-Montagne

Grower	Altitude (m)	Menthone/menthol inversion
1	800	early September
2	500	mid August
3	850	late August
4	500	late July

Table IV. Analysis of main components (%) of peppermint from 1990 production

Grower / Component	1a*	1b*	2	2**	3a*	3b*	4	5a*	5b*	6	7	8	9a*	9b*
menthone	45.6	31.1	23.9	22.5	36.1	7.0	26.5	37.5	31.5	30.8	24.8	30.1	32.3	10.5
menthofuran	0.4	0.2	0.5	0.5	0.3	0.2	0.3	-	-	0.2	0.4	0.1	0.3	0.8
isomenthone	3.3	6.3	2.8	3.1	5.1	4.1	5.8	5.9	5.9	6.3	3.2	5.8	5.1	10.2
menthyl acetate	1.6	2.8	3.8	3.1	1.6	2.4	2.3	2.6	2.6	1.2	3.9	2.8	1.5	3.11
isomenthol	2.3	4.9	5.5	5.3	3.1	5.7	4.2	3.5	3.5	4.0	7.1	5.2	3.7	4.6
neomenthol	0.2	4.7	2.9	2.9	5.9	4.3	7.2	0.5	0.5	7.8	5.3	4.7	8.0	5.7
menthol	18.9	33.6	39.5	39.5	28.0	38.5	35.0	32.3	2.3	31.7	33.3	33.5	30.1	43.0
menthone/menthol	2.4	0.9	0.6	0.6	1.3	0.7	0.8	0.7	1.0	1.0	0.7	0.9	1.1	0.2

* For the same grower, two batches were sometimes supplied. These corresponded to two different plots.
** Predried

more intemperate the climate and the slower the growth. Hence, the later the inversion.

A consequence of this is that harvesting in July in Auvergne, just before flowering, gives an oil with more menthone than menthol, whereas harvesting in Anjou at the same date gives an oil with more menthol than menthone.

Although most peppermint producers in Auvergne currently harvest before the plant flowers, our findings show that they should harvest after the plant flowers in order to obtain the economic advantage of a menthol-rich oil. Also, the oil obtained at this period contains less menthofuran and pulegone, undesirable compounds which are abundant during flowering.

For 1990, the levels of the various components in the oils produced by Growers 1-9 are given in Table IV. Except for batch 9b, which was strongly Mitcham, these mints displayed varying degrees of Mitcham character.

In 1991, the main concern was monitoring the menthol/menthone levels in the oils supplied by the different growers (nine growers of mint planted in different years). Harvesting was at the start of flowering (early July to first fortnight of August). The results are given in Table V. As we observed in 1989 for the period July 2 to August 14, we obtained a higher level of menthone than of menthol, irrespective of the year of planting, and inversion was close.

Table V. Analysis of main components (%) of peppermint from 1991 production (first harvest)

Peak No.	Grower / Growing year / Harvesting time / Component	1 4th 7/2	9 2nd 7/9	8 1st 7/12	1 2nd 7/17	2 2nd 7/19	7 2nd 7/25	3 2nd 7/29	6 2nd 8/5	4 2nd 8/7	5 2nd 8/14
1	α-pinene	0.9	0.0	0.9	0.6	0.3	0.7	1.1	0.8	0.7	0.6
2	β-pinene	0.9	1.1	1.1	0.8	0.7	1.0	1.3	1.0	0.8	0.8
3	sabinene	0.3	0.8	0.7	0.4	0.3	0.6	0.8	0.7	0.5	0.4
4	myrcene	0.4	0.8	0.6	0.6	0.5	0.5	0.8	0.6	0.5	0.6
5	limonene	1.0	0.9	1.5	0.7	0.9	0.8	1.4	0.6	0.8	1.4
6	1,8-cineole	3.7	4.7	6.5	4.1	4.1	4.0	5.2	4.8	4.6	3.7
7	(Z)-3-hexenol	0.4	0.5	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3
8	menthone	55.2	50.1	55.4	47.4	54.4	54.0	45.0	43.0	45.3	42.9
9	menthofuran	0.2	0.6	3.3	0.1	0.6	1.3	1.0	0.7	0.3	1.9
10	isomenthone	3.9	3.8	4.1	3.7	4.0	4.1	3.6	3.6	3.4	3.5
11	menthyl acetate	1.1	1.2	1.0	1.6	1.3	1.2	1.9	1.8	1.0	1.5
12	β-caryophyllene	0.8	1.1	1.8	1.5	1.4	1.1	1.9	1.0	1.1	1.5
13	neomenthol	4.2	4.5	1.7	4.8	2.8	3.9	3.9	3.4	4.5	4.3
14	pulegone	0.3	0.3	1.4	0.4	1.6	2.0	1.4	0.3	2.1	4.2
15	menthol	16.9	22.4	13.8	24.5	18.4	20.0	26.6	26.8	21.2	22.8

Table VI. Predrying course for main components (%) of selected peppermint samples (Grower 4; predrying September 1989)

Peak No.	Predrying time (h) / Component	0	6	12	19	24	30	36	48
1	α-pinene	0.3	0.6	0.5	0.6	0.6	0.6	0.8	0.5
2	β-pinene	0.3	0.7	0.6	0.8	0.7	0.7	0.9	0.6
3	sabinene	0.2	0.4	0.4	0.4	0.4	0.4	0.5	0.3
4	myrcene	0.3	0.5	0.5	0.6	0.5	0.5	0.6	0.8
5	limonene	0.5	0.7	0.5	1.0	0.8	0.8	1.0	0.5
6	1,8-cineole	2.3	3.2	3.7	4.0	3.2	3.5	4.2	2.9
7	(Z)-3-hexenol	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.4
8	menthone	27.3	30.9	35.1	33.9	28.8	30.0	26.5	31.7
9	menthofuran	4.1	6.0	1.2	2.5	3.7	5.9	2.4	5.6
10	isomenthone	2.6	2.6	3.0	3.3	2.6	2.6	2.7	2.8
11	menthyl acetate	1.8	1.2	1.6	1.3	1.7	1.6	2.1	1.3
12	β-caryophyllene	1.5	1.2	1.5	1.1	1.2	1.2	1.3	1.2
13	neomenthol	4.2	3.5	4.2	3.8	3.3	3.9	3.8	3.7
14	pulegone	8.5	9.6	9.1	8.3	8.3	7.7	7.6	9.6
15	menthol	37.4	31.2	37.6	31.3	32.6	32.4	36.6	31.8
	menthone/menthol	0.7	1.0	0.9	1.1	0.9	0.9	0.7	1.0

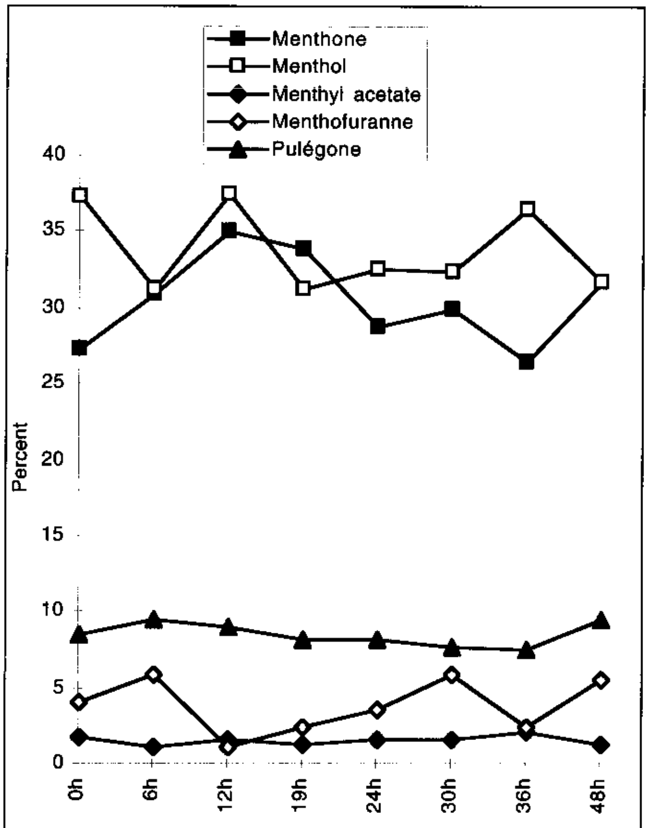

Figure 7. Variation of main components according to predrying time (Grower 4)

Effect of predrying: Trial predrying was carried out in 1989 by Grower 4. The time course of the main components (Table VI) was plotted against drying time (Figure 7). No appreciable changes in percentages of constituents were observed during the drying.

In 1990, a full-scale predrying study (Grower 2) confirmed the results of the first trial. The chemical composition of the essential oil obtained from dried plants was identical to that obtained from the undried plant (Table IV, Grower 2).

Conclusion

This study, specific to the growings at Le Mayet-de-Montagne for the production of oil of peppermint, shows that:

- The plants cultivated at Le Mayet-de-Montagne are Mitcham (Milly) type.
- Harvesting at the end of flowering affords an inversion of the menthone/menthol ratio, yielding an oil that is richer in menthol and therefore more valuable commercially.
- It is possible to harvest twice a year at the locations studied, thereby increasing the annual yield per hectare.
- Batches of differing qualities can be obtained, with a range of menthol/menthone ratios, according to harvest time.
- Predrying of peppermint herbage prior to distillation does not affect chemical composition, but allows steam distillation of greater amounts of plant material.

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