JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Harvest Time and Drying Method on Biomass Production, Essential Oil Yield, and Quality of Peppermint (*Mentha* × *piperita* L.)

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In the period from 2000 to 2002, studies on peppermint (*Mentha* × *piperita*) herb and essential oil (EO) production have been conducted at Planteforsk, Apelsvoll Research Centre Div. Kise in Norway. The trials were aimed at finding the optimal harvest date and suitable drying methods to maximize EO yield and to obtain a desirable oil quality. Peppermint plants from the first production year (2000 and 2001) and the second production year (2002) were harvested during flowering at three developmental stages (early, full, and late bloom). Biomass and leaf production were recorded, and the water content of the plant material was detected after the application of different drying methods: instantaneous drying at 30, 50, and 70 °C and prewilting (ground drying) for 1 or 5 days followed by final drying at 30 °C. Finally, plant samples were transferred to The Plant Biocentre at NTNU, Trondheim, Norway, for hydrodistillation and gas chromatography−mass spectrometry (GC−MS) analyses of the EOs. Peppermint oil yield increased from early to full bloom and late bloom (average of all years and drying methods except for 50 and 70 °C: 2.95, 4.13 and 4.20 L/daa, respectively) as an effect of biomass production and leaf growth. The flavor-impact compounds, menthol and menthone, reached their optimum at full bloom (43−54 and 12−30%, respectively). Prewilting led to slight decreased EO levels after 1 day (7.7%) and 5 days of ground drying (1.5%) and no EO quality changes, compared to direct drying at 30 °C. The plant weight (H_2O content) was drastically decreased to the average under 80 and 45% in all years, thus reducing the energy supply and costs for the necessary final drying step.

KEYWORDS: ***Mentha* × *piperita* L.; ground drying; developmental stage; essential oil; menthol; menthone**

INTRODUCTION

Besides supercritical fluid extraction, microwave-assisted extraction, and solvent extraction for the production of flavor and aroma extracts (*1*, *2*), genuine essential oils (EOs) are produced by distillation or pressing of plant raw materials. Direct distillation after harvesting is important to ensure optimized yield and EO quality and, simultaneously, to reduce drying costs (*3*−*5*). The transfer of cut plant material into a combined harvester−distillation tank is widely applied in large-scale production, where the tank is directly connected to the steam generator when filled. However, when fresh processing is not practicable, different drying methods from natural air drying at ambient temperatures to fully automated systems are being applied for the instantaneous reduction of water content to stop enzymatic and other metabolic processes. Long-distance transport and time prior to distillation might reduce oil content and quality because of a high water content, thus leading to a warming up of the plant mass. Short seasons and harsh climatic conditions in the Nordic countries additionally limit the cultivation of aromatic plants. The optimal developmental stage of the plant material at harvest, drying, and distillation conditions are therefore crucial for a successful EO production.

The monoterpene accumulation in peppermint leaves has been shown to be restricted to leaves not older than 3 weeks with low catabolic losses (<1% of the total pool) after maximal leaf expansion (*6*−*8*). Simultaneously, numerous studies conclude with that both EO yield and compositional changes in mint species highly depend on plant ontogenesis, favoring the flowering stage as the optimal time point for the highest oil yield and desirable menthol contents (*9*−*15*). In general, the EO yield is highly correlated with the biomass (i.e., number of leaves per area), while quality oils with high menthol and, simultaneously, low menthone levels are determined by the ratio of young to aged leaves when harvesting at a given time point.

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10.1021/jf047998s CCC: $30.25
Published on Web 04/16/2005

Because production and labor costs in Norway are quite high when compared with important world producers of peppermint EO such as India, China, and the U.S.A., we were interested in finding simple and inexpensive drying techniques for the conservation of the accumulated oil and, simultaneously, its quality with regard to pharmacopeial requirements (*16*).

The temperature is the main parameter in controlling the rate of drying mint herbs (*17*, *18*), and different techniques such as blanching prior to final processing (*18*, *19*), infrared drying (*20*), and freeze drying (*21*, *22*) have been applied. Ambient temperature and temperatures below 50 °C best meet EO quality requirements (*17*, *22*, *23*). In contrast, drying under day light might readily lead to brownish and unacceptable leaves, but because the focus in the present project was on EO production, the visual quality of peppermint herb was of minor importance. The study was therefore aimed at investigating the effect of (a) harvest time during flowering and (b) drying methods such as prewilting of cut plant material (ground drying) and different drying temperatures in peppermint production (*Mentha* × *piperita* L.) with regard to biomass production, EO yield, and quality.

MATERIALS AND METHODS

Field trials with peppermint (*Mentha* × *piperita*) focusing on five different drying methods, were conducted at Planteforsk, Apelsvoll Research Centre Div. Kise, in the period from 2000 to 2002, in cooperation with The Plant Biocentre at NTNU, Trondheim, Norway, where chemical analyses were carried out. The effect of harvest date, plant developmental stage, and drying regime on the parameters, total biomass and leaf production, water content, EO yield, and quality regarding the distribution of valuable oil components, was recorded.

Plant Material and Cultivation. Runners from three clones grown in Norway (*24*) were planted in plug trays for rooting prior to field establishment at Planteforsk, Apelsvoll Research Centre, Div. Kise (Hedmark county) in 2000 and 2001. The clones were planted separately in three parallels with 75 cm space between and 25 cm within row space. Each parallel (plot) covered an area of 4.5 m^2, comprising 24 sample plants. The plants were fertilized with 80 kg of 15-4-12/daa, i.e., 12 kg of N, 3.2 kg of P, and 9.6 kg of K per daa. Directly after planting in May 2000, the plants were covered with 1−2 cm algae fibers (Pronova Biopolymer as, Haugesund, Norway) to supress weed growth. Because of harsh conditions in the winter period 2000−2001, the plant survival rate was low and a new field was established in May 2001. The trial field was fertilized with 80 kg of 15-4-12/daa prior to planting on brown plastic mulch. This field was harvested both in the first and second production year (2001 and 2002), whereas plant material from the first trial field was only harvested in 2000.

Harvest Regime and Drying Techniques. Plant material from each plot was harvested at three different stages of flower development (early, full, and late bloom) in all trial years (3 replicates): 2000, Aug 21th, Sep 21th, and Oct 2nd; 2001, Aug 17th, Sep 13th, and Sep 25th; and 2002, Aug 15th, Aug 26th, and Sep 13th. Plants were cut 10 cm above ground for the instantaneous recording of total plant weight, leaf weight, and later, dry matter, while about 50% of the cut plants were left on the field for prewilting (ground drying) for 1 and 5 days, respectively. A total of 500 g of plant material (f.w.) was transferred to chamber drying at different temperatures until weight stabilization (30, 50, and 70 °C), while plant material from ground drying was finally dried in a chamber at 30 °C.

Hydrodistillation of EO. Peppermint leaves and flowers were separated from the stems prior to distillation and analysis at The Plant Biocentre, NTNU, Trondheim, Norway. The plant material was coarsely crushed and subjected to hydrodistillation in a modified Clevenger-type apparatus consisting of a 500 mL distillation bottle, a 3 mL graduated receiver, and a jacketed-coil condenser. A total of 20 g of dried plant material and 250 mL of H_2O were used, and the distillation was carried out for 1.5 h after the mixture had reached the boiling point. The GC samples were prepared by diluting 10 μL of oil in 1 mL of ethanol in brown autosampler flasks and stored at 4 °C prior to analysis.

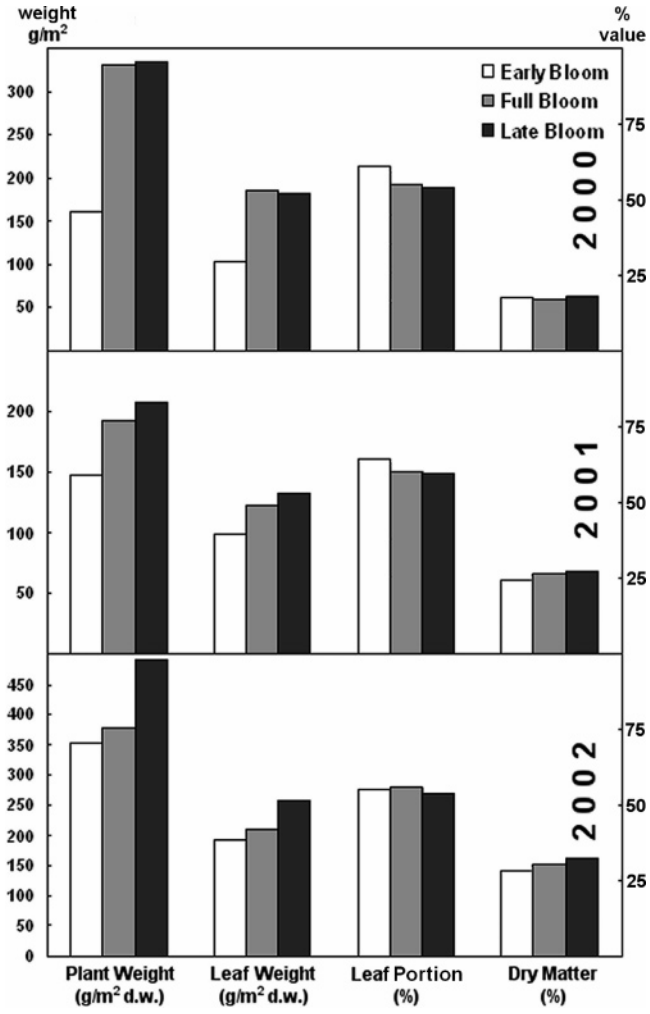

Figure 1. Biomass (plant and leaf weight; g/m^2), leaf portion (in % of total plant weight), and total dry matter (%) of *Mentha* × *piperita* plants harvested at different developmental stages (early, full, and late bloom) in trial years 2000, 2001, and 2002.

The EO content of the dried plant material (mL/100 g) and the EO area yield (L/daa) based on the recorded leaf weight per area (d.w.) were calculated.

Gas Chromatography−Mass Spectrometry Analysis (GC−MS). A Varian Star 3400 CX gas chromatograph coupled with a Varian Saturn 3 mass spectrometer was used for GC−MS analysis. The GC was equipped with a Chrompack CP Wax 52CB capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness), the flow of the carrier gas He (12 psi) was held at 50 mL/min (injector) and 30 cm/s (column). The injector temperature was 220 °C (split injection; 1 μL), and the GC temperature program was 60−220 °C at a rate of 2.0 °C/min and held at 220 °C for 5 min.

The MS detector was set at 170 °C, and a mass range of m/z 40−300 was recorded. All mass spectra were acquired in EI mode. All EO constituents were tentatively identified by the use of a combination of mass spectrum database search (IMS Terpene Library, 1989, and NIST MS Database, 1998), relative retention indices, and comparison of mass spectra from published data. Quantitative analysis (in %) was performed by peak area normalization measurements (TIC = total ion current).

Statistical Analyses. Data from plant growth recording, water content, and EO yield were subjected to statistical analysis by pairwise T-testing ($p = 0.05$).

Table 1. Variation of Plant Weight (f.w.) and Water Content in 100 kg of Plant Raw Material of *Mentha* × *piperita* Subjected to Instantaneous Drying in a Chamber (30 °C) and after Ground Drying for 1 or 5 days Followed by Final Drying at 30 °C

plant growth stage	early bloom			full bloom			late bloom		
ground drying (number of days)	plant weight (kg f.w.)	H_2O content (%)	H_2O content (liter)	plant weight (kg f.w.)	H_2O content (%)	H_2O content (liter)	plant weight kg f.w.	H_2O content (%)	H_2O content (liter)
field I, year 2000[a]									
directly at 30 °C	100	82	82	100	82	82	100	82	82
1 day + 30 °C	86	79	68	82	78	64	75	76	57
5 days + 30 °C	47	62	29	47	62	29	35	48	17
field II, year 2001[a]									
directly at 30 °C	100	75	75	100	73	73	100	73	73
1 day + 30 °C	*b*	*b*	*b*	120[c]	78[c]	93[c]	68	60	41
5 days + 30 °C	38	34	13	79[c]	66[c]	52[c]	37	27	10
field II, year 2002[d]									
directly at 30 °C	100	72	72	100	70	70	100	69	69
1 day + 30 °C	46	39	18	60	50	30	66	53	35
5 days + 30 °C	32	12	4	41	27	11	39	20	8

[a] First production year. [b] No sampling. [c] Rainy weather after harvest. [d] Second production year.

RESULTS AND DISCUSSION

Both total plant weight and leaf weight (d.w.) were distinctly increased ($p = 0.0327$) from early to full bloom in all years (see **Figure 1**), although the harvest dates differed greatly from year to year. In general, highest plant weights were recorded in 2002 (second production year). In contrast, the leaf portion of the total biomass was decreased on average from 63 to 57% (no significant differences) during the harvest season. The total dry matter only slightly changed from 24 to 26%, expressed as the water content loss when drying plant raw material (f.w.) at 30 °C to stableness (see **Table 1**). Plant weight and water content were naturally highly correlated (an average of 0.975), when comparing common chamber drying at 30 °C with prewilting followed by chamber drying at 30 °C. With the exception of plant samples harvested at full bloom in 2001, which were exposed to rain fall, the plant weight (f.w.) was distinctly decreased to the average under 80 and 45% (all years) of the original weight, when applying ground drying for 1 or 5 days. Water contents were reduced by an average of 25 and 56 L/100 kg f.w. (all years; data not shown) under these drying regimes. Dependent upon the varying climatic conditions (data not shown), the drying effect of prewilting was much more efficient after 5 days in 2002 compared to the results in 2000. Only small portions of the harvested material were totally dried. However, this did not lead to a loss of EO by damaging/crushing of the oil-containing leaves through picking up and transport.

The EO content and EO area yield is presented in **Table 2** showing no significant differences between the recorded oil content and the plant developmental stage. Peppermint oil yield increased from early to full bloom and late bloom (average of all years and drying methods except for 50 and 70 °C: 2.95, 4.13, and 4.20 L/daa, respectively) as an effect of biomass production and leaf growth, showing significant differences between the early and full bloom ($p = 0.0154$), and the late bloom stage ($p = 0.0337$) as observed in earlier Norwegian investigations (*11*, *25*). In accordance with results on *M. arvensis* conducted by Srivastava and co-workers (*26*), the parameter EO yield was positively correlated with biomass ($R^2 = 0.928$) and leaf production ($R^2 = 0.917$) in all years. Although the relative leaf portion decreased from early to late bloom (see **Figure 1**), the totally increased biomass compensated for a potential EO loss, thus favoring harvesting at the full or late bloom stage for optimal yield. In general, drying temperatures at 50 and 70 °C significantly reduced oil content and EO yield

Table 2. Effect of Plant Developmental Stage at Harvest and Drying Method on EO Content (mL/100 g d.w.) and EO Yield (L/daa) of *Mentha* × *piperita* in the Trial Period 2000–2002[a]

plant growth stage	early bloom		full bloom		late bloom	
ground drying (days) + drying temperature (°C)	EO content (mL/100 g)	EO yield (L/daa)	EO content (mL/100 g)	EO yield (L/daa)	EO content (mL/100 g)	EO yield (L/daa)
field I, year 2000[b]						
0 + 30 °C	2.54	2.64	2.59	4.82	2.67	4.89
0 + 50 °C	1.63	1.70	1.34	2.49	0.25	0.46
0 + 70 °C	0.13	0.14	0.11	0.20	0.11	0.20
1 day + 30 °C	2.50	2.60	2.59	4.82	2.30	4.21
5 days + 30 °C	2.50	2.60	2.67	4.97	2.59	4.74
field II, year 2001[b]						
0 + 30 °C	1.96	1.94	2.04	2.51	1.63	2.15
0 + 50 °C	0.14	0.14	0.29	0.36	0.17	0.22
0 + 70 °C	0.10	0.10	0.10	0.12	0.17	0.22
1 day + 30 °C	1.63	1.61	1.67[c]	2.05[c]	1.50	1.98
5 days + 30 °C	2.21	2.19	1.92[c]	2.36[c]	2.04	2.69
field II, year 2002[d]						
0 + 30 °C	2.45	4.70	2.53	5.29	2.32	5.94
0 + 50 °C	0.25	0.48	0.40	0.84	0.45	1.15
0 + 70 °C	0.05	0.10	0.05	0.10	0.25	0.64
1 day + 30 °C	2.27	4.36	2.33	4.87	2.23	5.71
5 days + 30 °C	2.02	3.88	2.63	5.50	2.13	5.45

[a] Drying of plant raw material was carried out directly after harvest (drying chamber at 30, 50, or 70 °C) or primary ground drying (1 or 5 days) followed by drying in a chamber (30 °C). [b] First production year. [c] Rainy weather after harvest. [d] Second production year.

Table 3. Variations in Menthol and Menthone Amounts (Peak Area %) in *Mentha* × *piperita* EO as an Effect of Plant Developmental Stage at Harvest (Early, Full, and Late Bloom) and Different Drying Methods (Drying Chamber at 30, 50, or 70 °C, or Primary Ground Drying by 1 or 5 Days Followed by Drying in a Chamber at 30 °C)[a]

plant growth stage	early bloom			full bloom			late bloom		
ground drying (days) + drying temperature (°C)	menthol (%)	menthone (%)	others (%)	menthol (%)	menthone (%)	others (%)	menthol (%)	menthone (%)	others (%)
field I, year 2000[b]									
0 + 30 °C	34.26	40.68	25.06	43.11	30.26	26.63	52.65	20.30	27.05
0 + 50 °C	36.54	37.70	25.76	47.36	27.62	25.02	43.53	19.40	37.07
0 + 70 °C	31.81	34.83	33.36	34.70	33.67	31.63	43.34	24.40	32.26
1 day + 30 °C	35.50	39.01	25.49	44.96	28.73	26.31	52.91	19.53	27.56
5 days + 30 °C	38.63	36.04	25.33	44.71	28.14	27.15	53.51	19.31	27.18
field II, year 2001[b]									
0 + 30 °C	40.27	28.19	31.54	51.13	12.57	36.30	56.46	10.75	32.79
0 + 50 °C	37.61	22.71	39.68	54.76	8.77	36.47	56.56	7.21	36.23
0 + 70 °C	39.25	16.68	44.07	48.73	9.44	41.83	52.14	7.73	40.13
1 day + 30 °C	39.28	30.46	30.26	51.67[c]	13.87[c]	34.46[c]	52.99	12.32	34.69
5 days + 30 °C	39.14	28.54	32.32	53.76[c]	11.92[c]	34.32[c]	54.82	11.23	33.95
field II, year 2002[d]									
0 + 30 °C	46.31	20.99	32.70	51.08	14.53	34.39	60.15	4.71	35.14
0 + 50 °C	53.11	14.51	32.38	65.70	9.64	24.66	65.72	4.16	30.12
0 + 70 °C	49.52	22.26	28.22	54.63	18.70	26.67	64.29	5.90	29.81
1 day + 30 °C	48.16	21.98	29.86	50.33	14.49	35.18	57.54	6.61	35.85
5 days + 30 °C	47.64	21.75	30.61	52.84	16.17	30.99	57.78	5.30	36.92

[a] Compounds were tentatively identified by MS database search. [b] First production year. [c] Rainy weather after harvest. [d] Second production year.

to unacceptable amounts as observed by Blanco and co-workers (*27*). Drying at 30 °C and ground drying were the most favorable methods for EO preserving, resulting in quite similar and acceptable EO levels. This is in accordance with studies of other mint species emphasizing the application of ambient or temperatures up to 40 °C for the drying of mint species (*21*) to minimize the heating of the plant material (*17*). Average data independent of trial year and harvest date showed that prewilting by 1 and 5 days on average reduced the EO yield (7.7 and 1.5%, respectively) compared to direct drying at 30 °C, thus indicating that a longer period of ground drying had a slight better EO preserving effect.

The variation in the menthol−menthone ratio in the direction of increasing menthol and vice versa menthone levels in *Mentha*

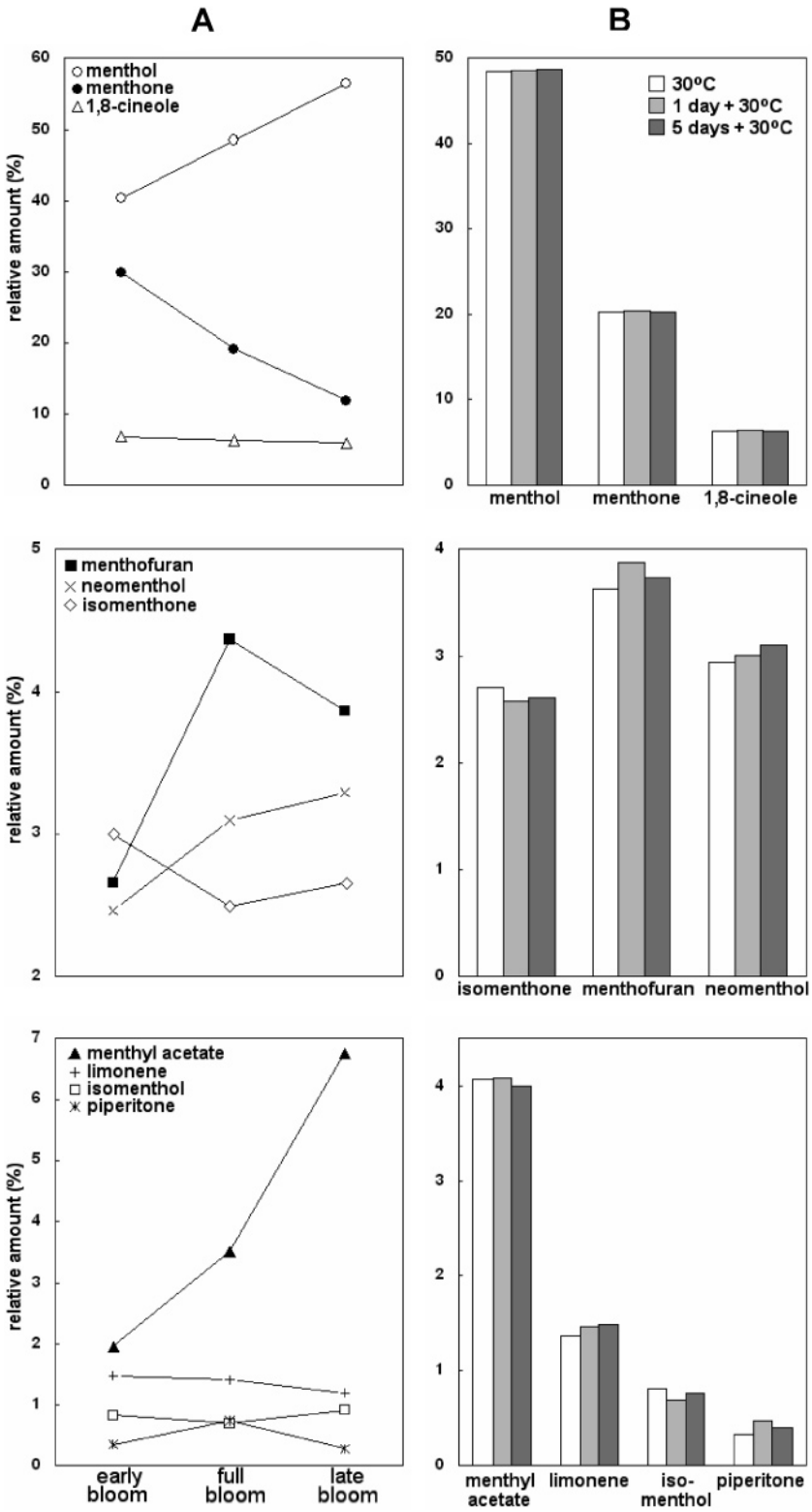

Figure 2. Variation of the quality-impact compounds of *Mentha* × *piperita* EO when (A) harvesting at different plant developmental stages (early, full, and late bloom) and (B) applying different drying methods before distillation (instantaneously drying in a chamber at 30 °C and 1 or 5 days ground drying followed by chamber drying at 30 °C). Data represent average values from 3 trial years (2000−2002). Compounds were tentatively identified by MS database search.

species as an effect of plant development and aging, has been reported previously (*9*, *12*−*15*, *25*), and could also be observed in our study (see **Table 3** and **Figure 3**). Moreover, related structures such as menthol, neomenthol, and menthyl acetate showed increasing levels from early to late bloom (see **Figure 2A**), whereas the ketone levels decreased (menthone and isomenthone, from early to full bloom). Simultaneously, the levels of limonene decreased slightly as already described in leaf studies on peppermint by Brun and co-workers (*28*), because this monoterpene is a key compound in the biosynthesis of menthol (*6*, *7*). As reported earlier from morphological studies (*13*, *14*), menthofuran levels were highest in full bloom because

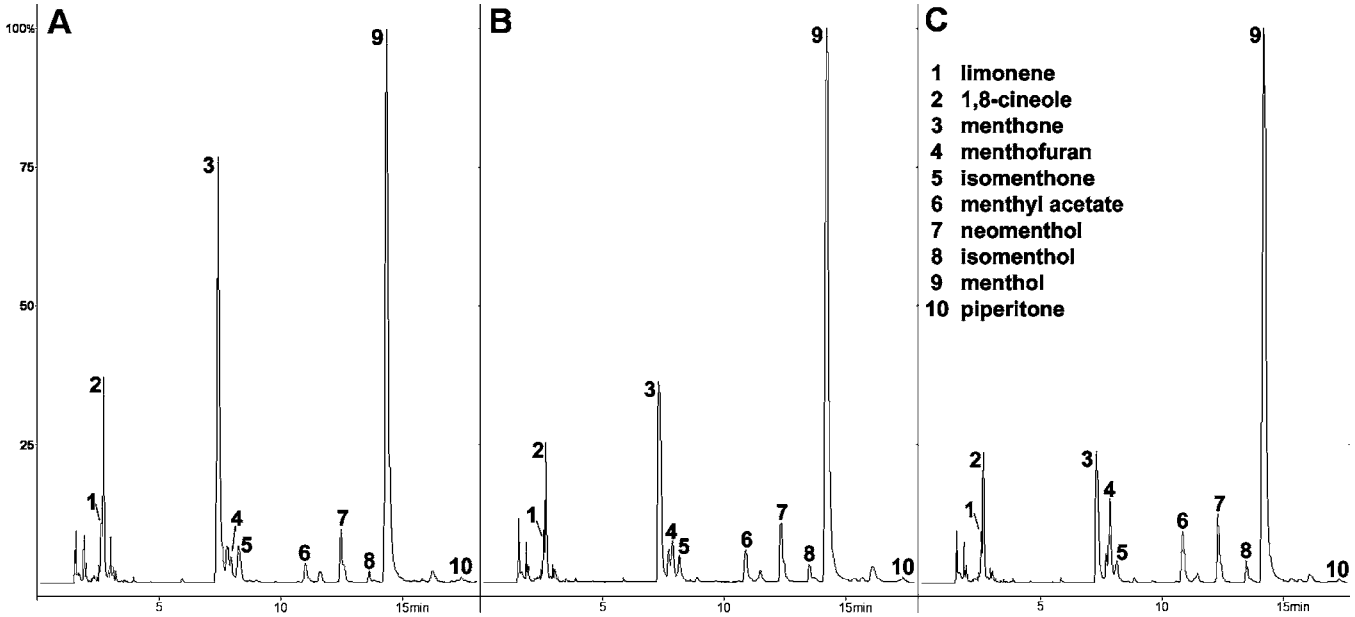

Figure 3. Example GC–MS chromatograms and EO compositions of *Mentha* × *piperita* emphasizing the impact of the plant developmental stage on the menthol/menthone ratio. (A) Early bloom, instantaneously drying at 30 °C. (B) Full bloom, 1 day ground drying, final at 30 °C. (C) Late bloom, 5 day ground drying, final at 30 °C. Compounds were tentatively identified by MS database search.

of the relatively higher flower portion in the plant raw material and decreased in the end of the flowering period. No significant changes could be observed when analyzing EO from samples dried at 30 °C or pretreated by ground drying for 1 or 5 days (see **Figure 2B**). In contrast to results obtained by Böttcher and co-authors, who reported EO compositional changes based on the physiological postharvest response of peppermint (*29*), no obvious changes of quality-impact compounds (menthol, menthone, menthyl acetate, and menthofurane) could be found in our study. Only the menthol and neomenthol levels seemed to be slightly increased (early and full bloom), which might be explained as an effect of postharvest biosynthetical changes.

Key elements of peppermint EO quality are described in the monograph of the European Pharmacopeia (*16*). The peppermint samples (drying at 30 °C and ground drying) showed superior EO composition in full bloom with high menthol concentrations between 43 and 54% and, simultaneously, menthone levels ranging from 12 to 30% (see **Table 3** and **Figure 3**). Detected amounts of menthol were also acceptable when harvesting at an earlier stage, while oil samples from late bloom partly showed quite high concentrations (>60%; requirement Eur.Ph.4: 30−50%), which one might expect to find in mint species such as *Mentha arvensis* (*30*) and *Mentha sachalinensis* (*9*, *11*). The menthol metabolism in the typical long-day plant peppermint also has to be seen on the background of the photoperiodic reaction. Biotron studies by Fahlén and co-authors have shown that the biosynthesis of menthol is favored under long-day conditions through flower initiation and development (*31*). Field experiments conducted in Norway concluded with similar results regarding day length and menthol concentrations above 40% (*22*, *25*, *32*), in contrast to studies on other mint species (*M. arvensis*, *M. spicata*, and *M. cardiaca*) cultivated in India (*33*). Other flavor-impact compounds within the *p*-menthane group (isomenthone, menthyl acetate, and menthofuran) and the monoterpenes, limonene and 1,8-cineole, were detected within the pharmacopeial quality range (*16*) at full bloom, while the undesirable ketones pulegone and carvone were only measured at trace levels.

In conclusion, plant harvesting should be carried out at full bloom to obtain the highest EO yield with simultaneously desirable EO quality. Ground drying (prewilting) of cut plant material might be suitable to conserve EO content and yield where the distillation of fresh plant material is not practicable. Because of reduced transport weight and energy supply for the final drying step, ground drying represents a promising procedure for the preprocessing of plant raw material for EO production to reduce drying costs and should be further investigated.

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Received for review November 30, 2004. Revised manuscript received March 22, 2005. Accepted March 27, 2005. The financial funding for the project "Grunnlag for lokal foredling og produktutvikling med norske urter som råvare" [*Regional Breeding and Product Development Based on Norwegian Aromatic Plants*] by the Norwegian Industrial and Regional Development Fund (SND) and research allocations from the National Agricultural Agreement is gratefully acknowledged.

JF047998S