J. Natn. Sci. Coun. Sri Lanka 1982 **10** (1) : 143—147

Effect of Plant Maturity on Oil Composition of Two Mint Varieties Recently Introduced to Sri Lanka

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(*Date of receipt* : 11 *March* 1981)
(*Date of acceptance* : 15 *April* 1982)

Abstract : Oil from two different varieties of mints designated Menthol variety (tentatively identified as *Mentha arvensis* var. *piperascens*) and Menthone variety (tentatively identified as F_1 hybrid between *Mentha arvensis* var. *piperascens* and *Mentha rotundifolia*) harvested in three different stages of maturity were analysed by GLC for their chemical composition. The effect of age was more marked in the Menthol variety than in the Menthone variety. The amount of menthol in the Menthol variety was extremely low at two month stage and increased to its highest level at three month stage (blooming stage). This was almost equal to the decrease in the menthone content of the same variety during this period. In the Menthone variety there was hardly any difference in the content of menthone, but showed a slight increase in the content of menthol with the plant maturity.

1. Introduction

The changes of the quantitative chemical composition of the essential oil in most of the essential oil bearing crops with their plant maturity have been reported by many workers.[4,5] There are also reports on the changes of the oil composition of plants belonging to the genus *Mentha* itself with plant maturity.[3] Therefore a study was carried out to find the changes in the chemical composition of two varieties of more recently introduced to Sri Lanka, with their plant maturity. These two varieties were introduced to Sri Lanka by Lever Brothers (Ceylon) Limited and can be found in their spice garden at Veyangoda.

2. Materials and Methods

Oil distilled from the two introduced mint varieties designated Menthol variety (tentatively identified as *Mentha arvensis* var. *piperascens*) and Menthone variety tentatively identified as F_1 hybrid between *Mentha arvensis* var. *piperascens* and *Mentha rotundifolia*), which were grown at the University farm, Peradeniya, were used in this study.

Transplanting of suckers in the field were carried out in June. The suckers were 3 to 4 weeks old when the transplanting was carried out. Plants were harvested at three different stages of maturity, namely, two, three and four months after transplanting. In each case plants were harvested from three separate plots (per variety per harvest) which served as 3 replicates in this experiment. Three months after transplanting stage coincided with the full bloom stage and also gave the highest herbage yield in this experiment. Before distillation the harvested material was dried in the shade till it was about one-third of its fresh weight. Then the semi-dried herb was chopped and 800g of withered herb was well packed into the flask and water was added sufficiently to cover the charge. Oil was obtained by steam distillation procedure using a laboratory type still made of glass. Period of distillation was prolonged up to three hours, to ensure the maximum distillation of oil.[2]

After oil distillation, the oil samples were stored in tightly stoppered bottles under refrigeration until the Gas-Liquid chromatography (GLC), by which the different constituents and their exact amounts in the oil were studied. GLC analyses were carried out using a Varian - 1700 instrument equipped with flame ionization detector. Analysis was carried out with 10% Carbowax 20M column, 3m long and 3mm diameter, held isothermal at 60°C for 2 minutes, programmed at 2°C per minute to 140°C and then 4°C per minute to 160°C. The injector and detector temperatures were 200°C and 220°C respectively and sample size was 0.7 μl. Tentative peak identification was made by comparing retention data with master chart. The technique of peak enrichment was employed to confirm the identification.

3. Results

The age of harvest had an effect on the quantitative chemical composition of oil in mints. This effect was more marked in the Menthol variety than in the Menthone variety. There was a striking difference in the content of menthol of Menthol variety between the different ages of harvest namely two, three and four months after transplanting. The amount of the major component menthol in this variety was extremely low (as low as 0.53%) at two month stage, increased to its highest level 77% at the three month stage and again declined to a low value of about 69.9% at four month stage (Table 1). At the same time, the amount of menthone in this variety (Menthol variety) was extremely high (as high as 81%) at the two month stage, decreased to its lower level of 4% at three month stage and this low level was maintained at four month stage (Table 1). There was hardly any difference in the content of major component menthone in Menthone variety, between the different ages. The amount of menthone was maintained at its high level of 72-76%. However, the menthol content of oil in this variety was low (0.56%) at two month stage, increased to a moderate level (4.9%) at three month stage and this high level was maintained at four month stage (Table 1).

TABLE 1. Chemical Composition of Oil extracted from Plants harvested in different Stages of Maturity

Compound	Menthol variety			Menthone variety		
	2 months	3 months	4 months	2 months	3 months	4 months
1. α-Pinene	0.05	0.09	0.91	1.32	1.14	0.63
2. Camphene	trace	trace	trace	0.03	—	trace
3. β-Pinene 4. Unidentified	0.08	0.22	1.22	1.46	1.40	1.22
5. α-Phellandrene	0.16	0.17	0.04	0.87	0.72	0.63
6. α-Terpenene	—	—	—	—	—	—
7. Limonene	1.37	1.12	1.86	7.49	4.48	5.06
8. π-Terpenene	trace	trace	trace	trace	trace	trace
9. ρ-Cymene	trace	—	trace	0.17	trace	trace
10. Unidentified	trace	trace	trace	trace	trace	trace
11. 3-Hexenol	trace	—	0.06	0.10	0.20	0.33
12. Unidentified	—	—	—	trace	trace	—
13. 3-Octanol	1.03	0.78	0.72	1.18	1.08	1.03
14. Thujone	—	—	—	—	—	—
15. Menthone	81.44	4.78	6.78	73.55	72.17	76.78
16. Iso-menthone	8.27	2.69	2.51	7.35	7.20	4.02
17. Unidentified	trace	—	—	trace	trace	trace
18. Linalool	0.40	0.26	0.18	0.23	0.20	0.22
19. Unidentified	trace	5.74	—	trace	0.71	1.00
20. Menthyl acetate	0.29		3.28	0.32		trace
21. Piperitone oxide	1.90	3.33	9.36	1.66	1.58	1.79
22. β-Caryophylene	trace	trace	trace	trace	trace	trace
23. Menthol	0.55	77.65	69.96	0.86	4.95	4.27
24. Pulegone	0.29	0.54	1.05	0.25	0.52	0.35
25. Carvone	0.84	0.58	0.44	0.53	0.58	0.39
26. Piperitone	4.05	2.07	1.65	2.75	3.26	2.69
* Total monoterpene hydrocarbons	1.66	1.60	4.23	11.34	7.74	7.54

* α-Pinene + camphene + β-pinene + ϖ-phellandrene + α-terpene + Limonene + π-terpinene + β-cymene.

There was also remarkable differences in the contents of some of the minor components of oil namely total monoterpene hydrocarbons (Table 1), iso-menthone, piperitone oxide and piperitone between the different stages of the crop. In the Menthol variety there was no difference in the amount of total monoterpene hydrocarbons between the two month stage and three month stage while it increased to a moderate level (4.2%) at four month stage. In the Menthone variety the amount of total monoterpene hydrocarbons was very high at two month stage (11.3%) and declined to 7% at both three and four month stages. The amount of iso-menthone of Menthol variety was high (8%) at two month stage and showed a decline (to 2.6%) at both three and four month stages whereas in Menthone variety it was high (7%) at two month and three month stages and showed a decline (to 4%) at four month stage.

The content of piperitone oxide in oil of Menthol variety was low (1.9 %) at two month stage, increased to 3 % at three month stage and further increased to a high level (to 9 %) at four month stage, whereas its content did not show any appreciable difference between the different ages in Menthone variety. The amount of piperitone in oil of Menthol variety was moderate (4 %) at two month stage and showed a decreasing tendency with age, while it did not show any appreciable difference between the ages in Menthone variety.

4. Discussion

The effect of age of plant at the time of harvest on the quantitative chemical composition of mint oil was in agreement with the reports of Parry[4], Viramani and Datta[5] and Murray, Fass and Marble[3] who indicated that harvesting stage of the plant had an influence on the quantity and quality of the essential oil in most of the essential oil bearing crops. These changes in the amount of chemical constituents may be mainly attributed to the flowering physiology of mint since at three months age flowering commenced in both varieties of mints. It was observed that the effect of age was highly remarkable on the menthol content. In fact, the menthol content in Menthol variety rose from trace amount to its very high level of 77 % when the plants reached flowering stage (three months). The increase in the menthol content from the early vegetative phase (two months) to 'full bloom' stage (three months) was almost equal to the decrease in the menthone content of oil during this period. This could be explained on the basis that the ketone menthone was the first product of biogenesis of mintoil and the conversion of this ketone to the alcohol menthol was completed when the plants reached the flowering stage. A similar observation have been reported by Datta and Viramani[1] who showed that the menthol content in mint plants increased while the percentage of menthone decreased with increasing age of plants.

Thus it becomes important to harvest the crop at three month stage in Menthol variety in order to obtain the maximum yield of menthol. In the case of Menthone variety where the major component was menthone the time of harvest did not have any appreciable effect on the menthone content of oil. The level of menthone throughout its growing period in this variety was generally high and it may be attributed to its genetic constitution. Thus the stage of harvest need not be an important criterion for the composition of oil in Menthone variety. However, even in this variety it would be desirable to take harvest at three month stage because it was found that the maximum oil yield could be obtained at this stage.

The remarkable changes in the content of some of the minor components of oil may also be attributed to the flowering physiology of the crop. It was noticed that the monoterpene hydrocarbons, iso-menthone and piperitone decreased in their amounts with age. Since these components of mint oil are commercially undesirable,

harvesting at three months stage would maintain these levels low. It was also observed that the content of piperitone oxide increased remarkably with age from 1.9% to 9%. However, the levels of these constituents were comparatively lower at three month stage (3%) than that of four month stage. Therefore harvest after three month stage should be avoided in mint cultivation.

5. Conclusion

The effect of age was highly remarkable in the Menthol variety. The content of menthol, in Menthol variety was extremely low (0.53%) at two month stage and increased to its highest level (77%) at the three month stage and again declined to a low value of about 69.9% at four month stage. Although there was hardly any difference in the content of menthone in Menthone variety, the menthol content of oil in this variety too showed a slight change between the different ages. Since these changes were remarkable during three month stage or at the blooming stage, these changes in the amount of chemical constituents may be mainly attributed to the flowering physiology of mints.

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