Analysis and Potential End-user Applications of Steam Distilled Essential Oils of *Ocimum basilicum* L. grown in Trinidad, West Indies

Sharad Maharaj, David R. McGaw, Ivan Chang Yen

Abstract— Steam distilled essential oils of *Ocimum basilicum L. var Genovese*, grown in the Maracas Valley, Trinidad, were obtained at different stages of plant maturity. The overall oil yields were measured together with the percentage compositions for seven components - a combination of both major and minor components. Maximum yields were obtained at the late flowering stage with the component compositions varying between stages. Based on these results, potential end-user applications were identified for essential oil obtained from each maturity stage. Following these investigations, natural air-drying studies were undertaken with flowering plants. Thus steam distilled essential oils were obtained from plants that were naturally air-dried for 24, 48 and 72 hours, respectively. Yields and compositions were less than those from freshly harvested material, demonstrating that some of the essential oils are evaporated off during the drying process. The overall and relative percentage compositions of these essential oils were then determined to assess their potential for consumer products.

Index Terms— Ocimum basilicum, maturity stage, essential oil, applications.

1 INTRODUCTION

Basil essential oils have a wide range of applications in the flavour and fragrance industry. Depending on the specie, the essential oils may be used as seasoning, particularly in Italian delicacies, or, in the formulation of aromatic scents.

The essential oils are rich in anti-oxidants [6], [10], [11] and contain significant amounts of sesquiterpenes - components that have been shown to support the immune system, act as vasodilators and provide pain relieving effects [3].

Previous investigations determined the time of harvesting by identifying the maturity stage that produced the maximum oil yield, this being the flowering stage [1], [2]. A recent study also showed that the essential oil yield and its antioxidant activity were enhanced when the soil was innoculated with *Trichoderma harzianum* and its metabolites [4].

While steam distillation is the traditional method used for extracting the essential oil, compound-specific extraction of polyphenols and antioxidants can be achieved by way of solvent extraction techniques that use specific solvent mixture ratios [13].

It is expected that the chemical composition of steam distilled basil essential oils would vary at different maturity stages, this being supported by the studies conducted with the *Ocimum ciliatum* specie [7]. However, studies into the potential applications based on these differences have apparently not been carried out for *Ocimum basilicum L.* var Genovese - a popular specie cultivated in Trinidad. Investigations were carried out to address this issue and also to assess the effect of natural air-drying on the final essential oil composition and their subsequent applicability for commercial use.

- *Sharad Maharaj E-mail: Sharad.Maharaj@sta.uwi.edu*
- *David McGaw E-mail: drmcgaw@gmail.com*
- *Ivan Chang Yen E-mail: ichangyen@gmail.com*

2 EXPERIMENTAL

2.1 Raw material supply - *Ocimum basilicum L. var* Genovese

Basil seedlings, obtained from a nursery were transplanted into prepared soil after having reached a height between 7.5 to 10 cm. The plants were grown and tended to in the Maracas Valley, Trinidad.

2.2 Procedure

Steam distillation extractions (SDE) were carried out using the apparatus shown in Figure 1. All steam distillations runs were done in duplicate to allow sample means to be determined.

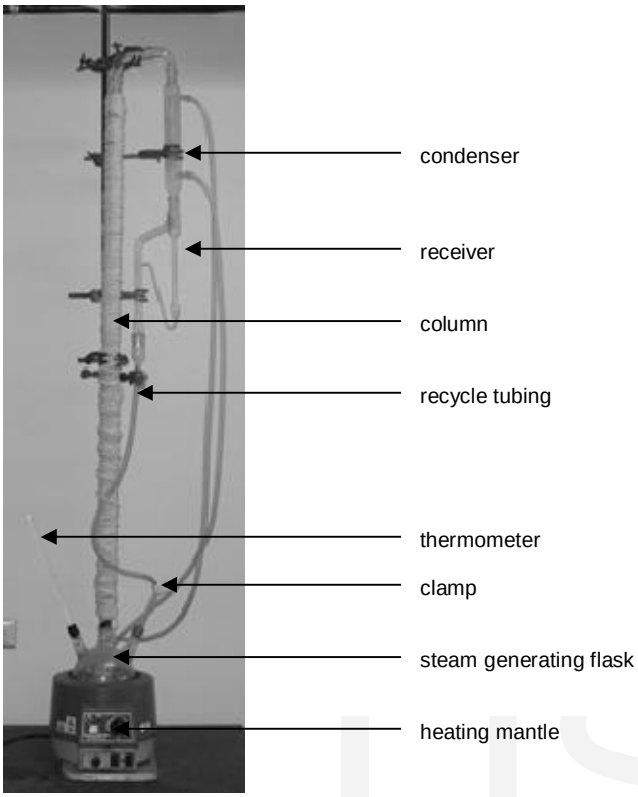

Fig. 1. The SDE Apparatus

2.3 Maturity stage investigations

SDE was carried out for *Ocimum basilicum L. var Genovese* (basil) plants at different maturity stages, as follows:

1) Prior to flowering (3.5 weeks after transplanting)
2) Just flowering (6 weeks after transplanting, just starting to flower)
3) Full flowering (9 weeks after transplanting, but just before seed formation)
4) Re growth that had reached the flowering stage (6 weeks after first cutting)

Leaves and flowers of each sample were separated from their stems and each subjected to SDE to obtain the essential oils at different stages of maturity. The moisture contents of the fresh materials were determined by Dean-Stark distillation and used to correct oil yields to dry weight basis (DWB).

2.4 Drying Investigations

Since highest essential oil yields were obtained from plants 9 weeks after flowering i.e. Stage 3, a subsequent batch of basil, picked at this stage of maturity was air dried in the laboratory for 24, 48 and 72 hours respectively at temperatures ranging from 30°C during the day, to 24°C at night. The moisture content of each sample was determined using the Dean-Stark method, to allow essential oil yields to be corrected to its respective dry matter content. Each dried sample was steam distilled in duplicate and the oils analysed by GC-MS.

2.5 Gas-Chromatography Mass-Spectroscopy (GCMC) Analysis

The essential oils were analysed by GC-MS to determine the percentage composition of the following components: α-pinene, α-terpinene, eucalyptol, linalool, geraniol, eugenol, and geranyl-acetate. A calibration mixture of standard components (Sigma-Aldrich, USA) was prepared by weighing about 1mL of each component sequentially into a pre-weighed vial and determining their respective masses by difference. Portions of this calibration mixture were placed in small vials, tightly sealed with PTFE-lined caps and stored at -15°C for subsequent analyses. This enabled a single standard mix to be used for all analyses. This standard mixture was first used to optimise the separation of the components by capillary GC, by varying the temperature programs to allow as complete separation as possible, in the shortest possible time. The essential oils were analysed using an Agilent Technologies 6890N network GC system. A DB1-MS capillary (30m x 0.22mm id, 0.25 µm film thickness) was operated in 25:1 spilt injection mode for 1 µL sample injections. The optimized column temperature program used was as follows:

70°C, hold 7 minutes; 70-76°C at 3oC/min; 76-88°C at 0.5°C/min; 88-97°C at 30°C/min; 97-125°C at 1°C/min; 125°C at 10°C/min to 220°C, hold until all peaks eluted. Total analysis time was 73.5 minutes. A chromatogram of the standard mixture is shown in Figure 2.

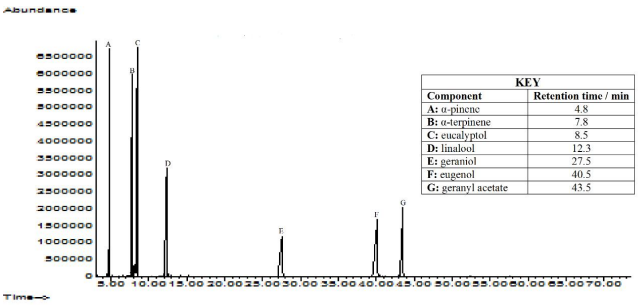

Fig. 2. Chromatogram of the Standard Mixture

The standard mixture was analysed by GC-MS, to identify by comparison with database references, using a match >90% as proof of identification. Each component was quantified using its response factor, based on the respective peak area and standard mass per injection. Once the individual component mass corresponding to each identified peak was calculated, their respective mass and % composition in a sample was determined, relative to the volume (1µL) of sample injected into the GC column. These results were then compared with results obtained by Hasegawa [5], Ozcan and Chalchat [8] and

Ozcan and Chalchat [9] who reported the oil composition for the same specie of basil used for this study.

An alternative way of visualizing the changes is by analysing the relative percentage composition of each essential oil sample. This is a weight % composition representing the relative proportions of the seven investigated components in a sample and allowed the essential oils to be evaluated for their commercial end-user values.

3 Results & Discussion

3.1 Relation Between Basil Maturity Stage and Essential Oil Composition

Table 1 shows average yields of oil (± % deviation about the means), obtained from fresh basil leaves and flowers (moisture content ~ 87 wt %) from bench-scale SDE at various stages of maturity. Each maturity stage produced different oil yields with the highest - 2.75% (DWB) - being produced at the flowering stage, 9 weeks after transplanting.

TABLE 1

PERCENTAGE YIELD OF ESSENTIAL OIL OBTAINED FROM EACH MATURITY STAGE (DRY WEIGHT BASIS – DWB)

MATURITY STAGE OBTAINED	AVERAGE PERCENTAGE OIL YIELD (DWB) [± DEVIATION]
PRIOR TO FLOWERING	0.57 ± 0.01
FLOWERING (JUST STARTING TO FLOWER)	0.48 ± 0.01
FLOWERING (9 WEEKS AFTER PLANTING)	2.75 ± 0.08
RE-GROWTH	1.89 ± 0.18

Table 2 shows the chemical compositions of the essential oils obtained at different maturity stages; the last three columns in this table compare the composition of essential oil with those already reported for the same type of basil used in this study [5], [8], [9].

TABLE 2

PERCENTAGE COMPOSITION OF ESSENTIAL OIL OBTAINED FROM PLANTS AT DIFFERENT MATURITY STAGES

COMPONENTS	PERCENTAGE COMPOSITION						
	PRIOR TO FLOWERING	FLOWERING (6 WEEKS AFTER PLANTING)	FLOWERING (9 WEEKS AFTER PLANTING)	RE-GROWTH	REPORTED RESULTS [5]	REPORTED RESULTS [9]	REPORTED RESULTS [8]
α-PINENE	*n.d.	0.038	0.469	0.266	0.210	0.002	0.1
α-TERPINENE	*n.d.	0.397	4.927	3.260	0.010	-	-
EUCALYPTOL	0.187	0.670	8.327	5.510	4.720	0.001	3.2
LINALOOL	0.485	2.884	37.797	29.146	41.560	0.003	17.2
GERANIOL	0.010	0.072	2.124	0.962	0.170	0.259	-
EUGENOL	0.084	1.941	22.659	13.153	14.810	-	-
GERANYL ACETATE	*n.d.	0.087	0.886	0.278	2.280	0.088	1.4

*n.d.: Not detected (value < 0.005 wt %);
-: not reported

The percentage compositions of the essential oils obtained at each maturity stage, showed consistent trends in components with stage of maturity. All eight components monitored increased from early to late stages of growth, but decreased with re-growth after trimming, with the two major components, namely linalool and eugenol, reaching their maximal concentrations of 37.8 and 22.7% respectively, at 9 weeks after transplanting. Comparison with essential oil composition reported by Hasegawa, et al. [5], Ozcan and Chalchat [8] and Ozcan and Chalchat [9] showed that the results of Hasegawa, et al. [5] more closely matched our composition from the 9-week flowering phase than the other phases, with linalool, eugenol and eucalyptol being in highest concentrations.
One-way ANOVA of the results showed that the 4 maturity stages produced significantly different ($p<.05$) α-pinene, α-terpinene, eucalyptol, eugenol concentrations, while the linalool, geraniol and geranyl acetate concentrations remained similar.

3.2 Drying investigations and the influence of drying time on oil quality

Fresh plant material was found to have a moisture content of 87% which subsequently decreased to 78.7% after 24 hours, 50.4% after 48 hours and 39.7% after 72 hours' exposure to laboratory conditions, respectively.

To allow for direct comparison of the different moisture contents, assessment was based on the average yield of oil obtained on a dry weight basis (DWB) and the average percentage composition of the essential oils from duplicate runs. The results are presented in Tables 3 and 4, respectively.

TABLE 3

PERCENTAGE OIL YIELDS WITH DRYING TIMES (DWB)

CONDITION OF RAW MATERIAL	AVERAGE PERCENTAGE OIL YIELD (DWB) [± % DEVIATION]
DRIED FOR 24 HOURS	1.44 ± 0.08
DRIED FOR 48 HOURS	0.77 ± 0.03
DRIED FOR 72 HOURS	1.09 ± 0.03

TABLE 4

CHEMICAL COMPOSITIONS WITH DRYING TIMES

COMPONENTS	PERCENTAGE COMPOSITION		
	DRIED 24 HRS	DRIED 48 HRS	DRIED 72 HRS
α-PINENE	0.531	0.679	1.210
α-TERPINENE	3.348	5.016	7.124
EUCALYPTOL	5.658	8.476	12.040
LINALOOL	19.887	26.648	32.032
GERANIOL	0.490	0.533	1.058
EUGENOL	4.853	14.992	10.014
GERANYL ACETATE	0.134	0.292	0.430

Whereas the maturity stage affects the oil yield and composition, the effect of drying on these parameters also needs to be considered, especially when field trials are considered.
Table 3 shows that drying for 24 hours results in the highest average oil yield of dried material, 1.44 ± 0.08%, which is near-

ly 50% less than yields from fresh, 9-week flowering material (Table 1), clearly emphasizing the losses in essential oil yield on drying.

However, compared to the undried material, as drying times were increased, the concentrations of all components generally increased, apart from eugenol, which peaked after 48 hours' drying, then decreased after 72 hours. The composition of the essential oils obtained after 72 hours drying differed markedly and were also close to the results reported by Hasegawa, et al. [5], while the 24-hour dried material (with highest oil yield), was roughly similar to the fresh basil regrowth after trimming (Table 2). This demonstrates how significantly natural air-dying can influence the quality of the essential oil.

3.3 End-user application of SDE Essential Oils based on the findings of the investigations

The investigations undertaken show that the maturity stages of the basil plants, as well as drying of the material prior to SDE can influence the composition and potential end-use of the essential oils obtained. The relative compositions of the essential oils at each maturity stage (Table 5) were examined in collaboration with an established local natural-products developer (*Cher Mere® Herbal Personal Care Products, Trinidad & Tobago*) to assess the potential end-user application of the essential oils produced in this project.

TABLE 5

AVERAGE RELATIVE PERCENTAGE COMPOSITIONS AT EACH MATURITY STAGE

COMPONENTS	AVERAGE RELATIVE PERCENTAGE COMPOSITION			
	PRIOR TO FLOWERING	FLOWERING (6 WEEKS AFTER PLANTING)	FLOWERING (9 WEEKS AFTER PLANTING)	RE-GROWTH
α-PINENE	0.000	0.617	0.595	0.167
α-TERPINENE	0.000	6.543	6.301	5.629
EUCALYPTOL	24.531	11.058	10.649	9.514
LINALOOL	63.115	47.817	48.440	59.279
GERANIOL	1.334	1.152	2.655	5.275
EUGENOL	11.020	31.373	30.250	20.039
GERANYL ACETATE	0.000	1.440	1.109	0.098

At the stage prior to flowering, the essential oils had the highest relative percentage composition of eucalyptol and linalool, while that of eugenol was lowest. Since eucalyptol is used in muscle rubs to reduce muscular inflammation, the use basil essential oil at this maturity stage may be feasible for such applications. The low relative percentage composition of eugenol was also a determining factor, since at high concentrations, eugenol is a skin irritant.

At the flowering stage, the relative percentage composition of eucalyptol and linalool decreased while that of α-pinene, α-terpinene, eugenol and geranyl acetate increased, making these oils favourable for the formulation of perfumes or in aromatherapy. Since the relative compositions shown in Table 5 do not vary by much for up to 3 weeks after the start of flowering, the flowering stage is considered the best stage for harvesting to obtain essential oils consistent in quality for these purposes.

A similar conclusion was arrived at when the results from the drying investigations (Table 6) were considered.

TABLE 6

AVERAGE RELATIVE PERCENTAGE COMPOSITIONS FROM DRYING INVESTIGATIONS

COMPONENTS	AVERAGE RELATIVE PERCENTAGE COMPOSITION		
	DRIED 24 HOURS	DRIED 48 HOURS	DRIED 72 HOURS
α-PINENE	1.496	1.196	2.018
α-TERPINENE	9.651	8.822	11.207
EUCALYPTOL	16.310	14.909	18.940
LINALOOL	56.871	46.964	49.878
GERANIOL	1.446	0.950	1.632
EUGENOL	13.868	26.626	15.590
GERANYL ACETATE	0.359	0.535	0.734

Depending on the preferences of the end-user, the oils can be extracted after one day from harvesting the flowering plants or between 2 and 3 days after naturally air drying the plants.

4 CONCLUSIONS

There are significant differences between the oil compositions obtained from basil plants at different stages of maturity and the time of harvesting would depend on the end-user application of the essential oil. For medicinal purposes, such as in the manufacture of muscle-rubs, the plants should be steam distilled at the stage prior to flowering. Where the Essential Oils are to be used for the formulation of perfumes and fragrances for aromatherapy, harvesting should take place at the flowering stage, but before seeds are formed.

Drying before SDE can have a favourable effect on the essential oil composition, as reported by Yousif [12] where drying was found to promote favourable conversions of oil components that enhance the quality of the oil for fragrance formulations.

REFERENCES

[1] J. Davis, "North Carolina Basil Production Guide," *Public document, Raleigh. AG-477.: North Carolina Cooperative Extension Service, N.C. State University*. http://www.ces.ncsu.edu/depts/hort/hil/hil-125.html. 1995.

[2] A. Fleisher, "Essential Oils from 2 varieties of Ocimum basilicum L. grown in Israel." *J. Sci. Food Agric*. vol. 32, pp. 1119-1122, 1981.

[3] E. Gaydou, R. Faure, J. Bianchini, G. Lamaty, O. Rakotonirainy, and R. Randriamiharisoa, "Sesquiterpene Composition of Basil Oil. Assignment of the 1H and 13C NMR spectra of β-elemene with two-dimensional NMR," *J. Agric. Food Chem*. vol. 37 no. 4, pp. 1032-1037, 1989.

[4] R. Gupta, A. Singh, R. Kanaujia, S. Kushwaha, and R. Pandey, "Trichoderma harzianum ThU and Its Metabolites Underscore Alteration in Essential Oils of Ocimum basilicum and Ocimum sanctum." *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, pp. 1-9, 2016.

[5] Y. Hasegawa, K. Tajima, N. Toi, and Y. Sugimura, "Characteristic Components found in Essential Oil of Ocimum basilicum L.," *Flavour*

Frag. J. vol. 12, no. 3, pp. 195-200, 1997.

[6] C. Jayasinghe, N. Gotoh, T. Aoki, and S. Wada, "Phenolics Composition and Antioxidant Activity of Sweet Basil (Ocimum basilicum L.)," *J. Agric. Food Chem.* vol. 51, no. 15, pp. 4442-4449, 2003.

[7] M. Moghaddam, A.G. Pirbalouti, L. Mehdizadeh, and M.R. Pirmoradi, "Changes in composition and essential oil yield of Ocimum ciliatum at different phenological stages," *Eur. Food Res. Technol*. vol. 240, no. 1, pp. 199-204, 2015.

[8] M. Ozcan, and J-.C. Chalchat, "Essential oil composition of a new chemotype of Basil (Ocimum basilicum L.) cultivating in Turkey," J. Essential oil Bearing Plants. vol. 7, no. 2, doi:10.1080/0972-060X.2004.10643383, pp. 155-159, 2004.

[9] M. Ozcan, and J.-C. Chalchat, "Essential Oil Composition of Ocimum basilicum L. and Ocimum minimum L. in Turkey," *Czech J. Food Sci*. vol. 20, no. 6, pp. 223–228, 2002.

[10] J.E. Simon, and W. Phippen, "Anthocyanins in Basil (Ocimum basilicum L.)," *J. Agric. Food Chem*. vol. 45, no. 5, pp. 1734-1738, 1998.

[11] J.M. Vivanco, J. Javanmardi, A. Khalighi, A. Kashi, and H. P. Bais, "Chemical Characterization of Basil (Ocimum basilicum L.) Found in Local Accessions and Used in Traditional Medicines in Iran," *J. Agric. Food Chem.* vol. 50, no. 21, pp. 5878-5883, 2002.

[12] A. Yousif, C. Scaman, T. Durance, and B. Girard, "Flavour Volatiles and Physical Properties of Vacuum-Microwave and Air-Dried Sweet Basil (Ocimum basilicum L.)," *J. Agric. Food Chem*. vol. 47 no. 11, pp. 4777-4781, 1999.

[13] U. Złotek, S. Mikulska, M. Nagajek, and M. Świeca, "The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts," *Saudi J. Biol. Sci.* vol. 23 no. 5, pp. 628-633, 2016.