Chemical Characteristics of Essential Oil from Five Basil Cultivars Grown Hydroponically in a Controlled Environment Using the Nutrient Film Technique

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Abstract

Controlled environment studies were initiated to obtain chemical species and amounts of the essential oil content of Cinnamon, Italian Large Leaf, Osmin Purple, Red Rubin, and Sweet Dani basil grown hydroponically using the nutrient film technique (NFT). Seeds were sown in moist arcillite and transplanted into growth troughs (0.15 x 0.15 x 1.2 m) after 14-d in reach-in growth chambers, and nutrients continuously supplied by a half-Hoagland solution. Growth conditions included 300±25-µmol m^{-2} s^{-1} photosynthetic photon flux (PPF); 16/8 h light period, 25 ºC and RH of 70±10 %. Chemical composition of the components of essential oils was determined by GC/MS. Sixteen were found and 10 identified including 1, 8-cineole, linalool, neral, chavicol, geranial, and eugenol. Minor components included α-terpineol, methyl chavicol, methyl eugenol, and γ-cadinene. Based on cluster analysis on six major components, the cultivars were classified into three chemotypes: "linalool and eugenol", "neral and geranial", and "linalool and chavicol".

Key words: *Ocimum basilicum*, chemical characteristics; water culture, nutrient solution

Introduction

Basil (*Ocimum basilicum*), also called common or sweet basil is native to the tropical regions of Africa (Splittstoesser, 1990). It has been traditionally used as a culinary herb, fragrance raw material, medicinal plant, and insect-control agent. Although the whole herb is often used, the most important product of basil is its essential oil, which is widely, used in the food, cosmetic, pharmaceutical, and aromatherapy industries. The chemical characteristics and functional properties of essential oils have been the subject of many studies. The components of essential oil mainly include phenylpropanoid derived compounds (methyl chavicol, chavicol, eugenol, methyl eugenol) and terpenoids (cineole, camphor, linalool, citral). Researchers have confirmed the antimicrobial, insecticidal and antifungal properties of the essential oil of basil (Bagamboula et al., 2004; Sacchetti et al., 2004; Wan et al., 1998). The essential oils of basil were demonstrated to possess antioxidant property (Lee and Shibamoto, 2002; Lee et al., 2005).

There are many cultivars within the *Ocimum basilicum* group and the essential oil composition is different among cultivars. Based on this difference, the oil extracted from different cultivars has been classified into several chemotypes: European, Egyptian, Reunion, Bulgarian and Java basil oils (Simon et al., 1990). Other classifications have also been reported including the Tropical chemotype and chemotypes from North Africa and the former USSR (Vernin et al., 1984). In addition to the cultivar, growth conditions, agronomic techniques, and post-harvest processing methods can also significantly affect the composition of essential oil. Johnson et al., (1997) reported that UV-light could induce a change of essential oil composition. Environmental factors, such as soil and temperature under which the plants were grown, appear to significantly affect the oil composition (Gil and Randhawa, 1996; Grayer et al., 1996; Rakic and Johnson, 2002).

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The essential oil composition has shown differences even within the same genotype when plants were grown in the greenhouse vs. field-grown (Grayer et al., 1996). Both air- and freeze-drying were found to alter the oil composition (Bowes and Zheljazkov., 2004; Grayer et al., 1996).The National Aeronautics and Space Administration (NASA) has supported research with higher plants over several decades, to evaluate their potential for use in a bioregenerative life support system (BLSS) to revitalize the air, produce food, and potable water, while removing carbon dioxide (Tibbitts and Alford, 1982; Millard and Ward, 1966; Myers, 1954; Wheeler, 2001). Basil is among several crops that have been recommended for use in such a bioregenerative system due to its unique flavor and food usage history. Emphasis is being placed on food crop systems addressing near-term missions such as to the International Space Station (ISS).

Conventionally, basils are planted in soil, and most microgravity experiments utilize some form of solid matrix (Mortley et al. 2008) to stabilize the water and deliver nutrients. And, while the chemical properties of essential oil have been the subject of many studies, most focused on basil grown conventionally. There have been few published studies on the chemical characteristics of essential oil from basil grown hydroponically and few using NFT exclusively. For instance, Succop and Newman (1998) grew fresh-market basil using hydroponic rockwool slab culture, hydroponic perlite raised beds or hydroponic peat/perlite/compost bag culture with either organic or conventional fertilizers. They reported that yield response varied depending on which fertilizer was used. Yields were greater in the perlite and bag mix, while organic fertilizer yields were lower for plants grown in the rockwool system and fertilized conventionally.

Likewise, Kreder and Markhart, III (1999) grew basil in continuous flow solution culture or in pots containing a well-irrigated potting mix. Their findings indicated that growth rate was more rapid and more harvestable leaves were produced among plants grown in solution culture compared to the media-grown plants.
If basil and other culinary herbs are to be utilized in the diets for space travelers, baseline controlled environment and cultivar selection studies are needed to increase our general horticultural, nutritional, and physiological knowledge of the crop. Our objective was to obtain baseline chemical characteristic (i.e. chemical species and amounts) of the essential oil of five cultivars of basil grown hydroponically in a controlled environment using NFT.

1. Materials and Methods

2.1 Starting Transplants. Basil seeds were planted in moist Arcillite in TLC-PRO Trays transplant flats (TLC Polyform, Plymouth, Minn.), and covered with approximately 0.6 cm of the medium. Flats were placed in a growth chamber with a constant 25ºC, a 16/8 h light period and a relative humidity of 70±5%. Flats were moistened at approximately 3-d intervals with deionized water and seedlings were grown for approximately two weeks.

2.1.1 Planting. Before transplanting, seedlings of uniform growth (height, number of true leaves etc) of each cultivar were removed from its respective transplant tray and roots were gently rinsed in running tap water to remove adhering media particles. Four seedlings of each cultivar were transplanted into each of two rectangular NFT growth channels (0.15 x 0.15 x 1.2 m), in each growth chamber through small openings 10 cm apart in a PVC-1 flat plate assembly.

2.1.2 Nutrient Solution. A half strength-Hoagland's nutrient solution (Hoagland and Arnon, 1950) with NO_3-N as the sole source of nitrogen was used. The solution was replenished once per week based on electrical conductivity (EC) by adding deionized water to volume after which EC was adjusted to 1200 μS^{-1} with a concentrated Hoagland stock. Solution pH was adjusted to 5.8 at each replenishment with 1 M HNO_3. The solution was supplied to the plants in each channel from 30.4-liter reservoirs with in-line pumps (Little Giant Pump Co., Oklahoma City, OK). Growth channels were on a 1% slope to facilitate return of the nutrient solution to the reservoir by gravity flow.

2.1.3 Growth Chamber Conditions. Growth chamber conditions included a relative humidity of 70$\pm$10%. The photosynthetic photon flux (PPF) at the top of the plant canopy (approximately 20 cm above the plants) averaged 300$\pm$25-µmol m^{-2} s^{-1} and was provided by cool white fluorescent lamps. Light period was 16/8 h with a constant 25ºC and CO_2 was at ambient levels (400 µmol mol^{-1}).

2.1.4 Harvest and Sample Preparation. Leaves were harvested four times at 3-week intervals beginning 30-d after transplanting and composited for analysis.

Fresh samples (30-g) were placed in plastic bags and stored at -80ºC for subsequent essential oil analysis. The samples were prepared in a 250 ml flask by combining frozen tissue with 100 ml distilled water. This mixture was hydrodistilled, and the distillate extracted into methylene chloride. In a 250 ml flask, 10 g of leaves and 100 ml distilled water were combined. This mixture was hydrodistilled until 40 ml of distillate were recovered. The distillate was extracted twice with 10 ml of methylene chloride each time in a 150 ml separatory funnel. The methylene chloride was pooled, and 3 ml methylene chloride phase were transferred to a vial containing 1 ml of anhydrous Na_2SO_4 and exposed for about 30 minutes. After exposure, the methylene chloride phase was transferred to a 1.5 ml vial for subsequent GC/MS analysis (Julian and Simon, 2002).

2.1.5 GC-MS Analysis. Analysis was carried out on a Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Shimadzu GCMS-QP2010 mass spectrometer and Restek XTI-5 capillary column (30 m×0.25mm, 0.25 μm film thickness). For electron ionization, 70 eV was used. The injection mode was split with a split ratio of 10. The injection volume was 1 μL and an injection port temperature of 230C. Helium, the carrier gas, was at a column flow rate of 1.0 ml/min. Oven temperature was held initially at 60C for 2 min, and then ramped at 5 ºC/min, up to a final temperature of 220C, and held for 1 min. Ion source temperature was 200C and m/z range was 35.0-500.0. Identification of components was based on a comparison of retention time and mass spectra with those of authentic standards. These standards and chemical abstracts service numbers were 1,8-cineole (470-82-6), linalool (78-70-6), citral (5392-40-5), eugenol (97-53-0), thymol (89-83-8), and methyl chavicol (140-67-0; Sigma, St. Louis, MO)]. Components without standards were identified by matching their mass spectra with those of the National Institute of Standards and Technology (NIST) library and by comparing their relative retention times with previous studies (Lee et al., 2002; Juliani and Simon, 2002). The proportions of individual components were calculated based on the area percentage of total ion current (TIC) signal. Each analysis was done in duplicate.

2.1.6 Statistical Analysis. Experiments were conducted in a randomized complete block design with three replications (three runs) in time. Four reach-in growth chambers (Environmental Growth Chambers, Chagrin Falls, OH) were used, and each experiment lasted approximately 86 days. The data of six major components, 1, 8-cineole, linalool, neral, chavicol, geranial, and eugenol were chosen and a similarity distance matrix was calculated. Clustering of cultivars was obtained by hierarchical clustering (Ward, 1963). Clustering and correlation were performed using SPSS (SPSS for Windows, Version 10.0.5. 1999, SPSS Inc., Chicago, IL).

3. Results and Discussion

Based on the chromatogram of the five cultivars (Fig 1), sixteen components were found in the essential oil and 10 were identified. In previous studies, Marotti et al., (1996) and Lee et al., (2002), identified 40 and 86 components, respectively, while other studies reported fewer components than the current study (Bowes and Zheljazkov., 2004). The composition of essential oil of the five cultivars is shown in Table 1. The major components were 1, 8-cineole, linalool, neral, chavicol, geranial, and eugenol. The combination of these compounds comprised over 80% of the essential oil content of all five cultivars. Other minor components included α-terpineol, methyl chavicol, methyl eugenol, and γ-cadinene. Eugenol was the only component found in all cultivars. The essential oil composition appeared to be different among cultivars.

The six major components were used for the cluster analysis to identify possible chemotypes of basil. Three clusters were identified (Fig. 2), therefore, the five cultivars were classified into three chemotypes. The first chemotype included 'Italian Large Leaf', 'Red Rubin', and 'Osmin Purple'. They could be ascribed to "linalool and eugenol" type. Linalool and eugenol were the two components with the highest concentration ranging from 15 % to 76 %. In contrast, the concentration of neral, genieral, and chavicol were low and ranged from negligible to 2 %. Within this group, the oil composition of cvs Red Rubin and Osmin Purple were very similar. The second chemotype included Cinnamon, and could be ascribed to "linalool and chavicol" type. It had the highest concentration of linalool (53%) followed by chavicol (34 %), but was low in eugenol (8 %) and little or no neral or genieral. The third chemotype included Sweet Dani and could be ascribed to "neral and genieral" type. It had high concentrations of neral and genieral (46-52 %), but low levels of eugeol and chavicol and very little 1, 8-cineole and linalool.

In previous studies, Grayer et al. (1996) analyzed the essential oil composition of 16 accessions of basil and identified five chemotypes: (a) linalool as the major compound; (b) methyl chavicol as the major component; (c) a mixture of linalool and methyl chavicol as the two major constituents; (d) a mixture of linalool and eugenol; and (e) a mixture of methyl chavicol and methyl eugenol. Lawrence (1992) recognized four major chemotypes: 1) methyl chavicol-rich, 2) linalool-rich, 3) methyl eugenol-rich, and 4) methyl cinnamate-rich. In a study by Marotii et al., (1996) the "linalool", "linalool and methyl chavicol", "linalool and eugenol", and "elemene, carene and α-humulene" chemotypes were reported. Grayer et al., (1996) found the neral and genieral chemotype in an *O. basilicum* x *O. americanum*, hybrid but not in sweet basil itself. Juliani and Simon (2002) also found the neral and genieral chemotype in Sweet Dani. If these chemotype classifications are followed, two chemotypes found in this study, "linalool and eugenol" and "neral and genieral", were reported by previous studies. The chemotype of "linalool and chavicol" was not reported previously.

Since growth conditions and agronomic techniques could significantly affect the composition of essential oil, growing plants in NFT in controlled environments could also impact the oil composition compared with field-grown plants. In spite of the fact that essential oil composition can vary based on factors stated above and no comparative field studies were done, we felt that it was useful to compare our data (Table 1) with previously published data involving the same cultivar (Julaini and Simon, 2002; Lachowicz et al., 1997). One common finding was that the relative concentration of eugenol in all five cultivars was higher than reported previously. The oil composition of 'Sweet Dani' was similar to that reported by Juliani and Simon (2002). The correlation analysis (data not shown) indicated that correlation coefficient between the two sets of data was 0.983. These results suggest that the essential oil composition of 'Sweet Dani' was similar regardless of whether plants were grown in NFT or a soil-based medium. In contrast, the oil composition of 'Cinnamon', 'Italian Large Leaf', 'Osmin Purple', and 'Red Rubin' was different from that reported by Juliani and Simon (2002) and Lachowicz et al., (1997). For example the concentration of linalool and eugenol in 'Cinnamon' was significantly higher than that reported by Juliani and Simon (2002) and Lachowicz et al., (1997). While Juliani and Simon (2002) and Lachowicz et al., (1997) found methyl cinnamate in 'Cinnamon' with a relative concentration ranging between 28-45% and no chavicol, we found none. However, we found chavicol which comprised 40 % of the oil.

In addition, we found that linalool and eugenol were the two major oil components in 'Italian Large Leaf' while Juliani and Simon (2002) reported that linalool and methyl chavicol were the two major components. For 'Osmin Purple' and 'Red Rubin', we found that eugenol was the component with the highest concentration followed by linalool, while the opposite was reported previously (Juliani and Simon, 2002). Both linalool and eugenol are synthesized in plants through two separate biosynthetic pathways. Linalool synthesis follows the pathway of terpenoids and is synthesized from mevalonic acid via geranyl pyrophosphate, while that of eugenol follows the pathway of phenylpropanoid pathway and is synthesized from shikimic acid via the precursor of phenylalanine and cinnamic acid (Buchanan et al., 2002). It is probable that there could have been an alteration of the reaction rate between different biosynthetic pathways in 'Osmin Purple' and 'Red Rubin' because of the environment and media in which they were grown. Growing the plants under hydroponic conditions could have increased the synthesis of phenylpropanoid-type oil and lower the terpenoids-type oil component in some basil cultivars.

In conclusion, five cultivars of basil were grown hydroponically using NFT. Sixteen components were found in the essential oil and 10 were identified. The major components found in this study were 1, 8-cineole, linalool, neral, chavicol, geranial, and eugenol. The five cultivars could be classified into three chemotypes, two of which, "linalool and eugenol" and "neral and genieral", were reported in previous studies. The chemotype of "linalool and chavicol" was not previously reported.

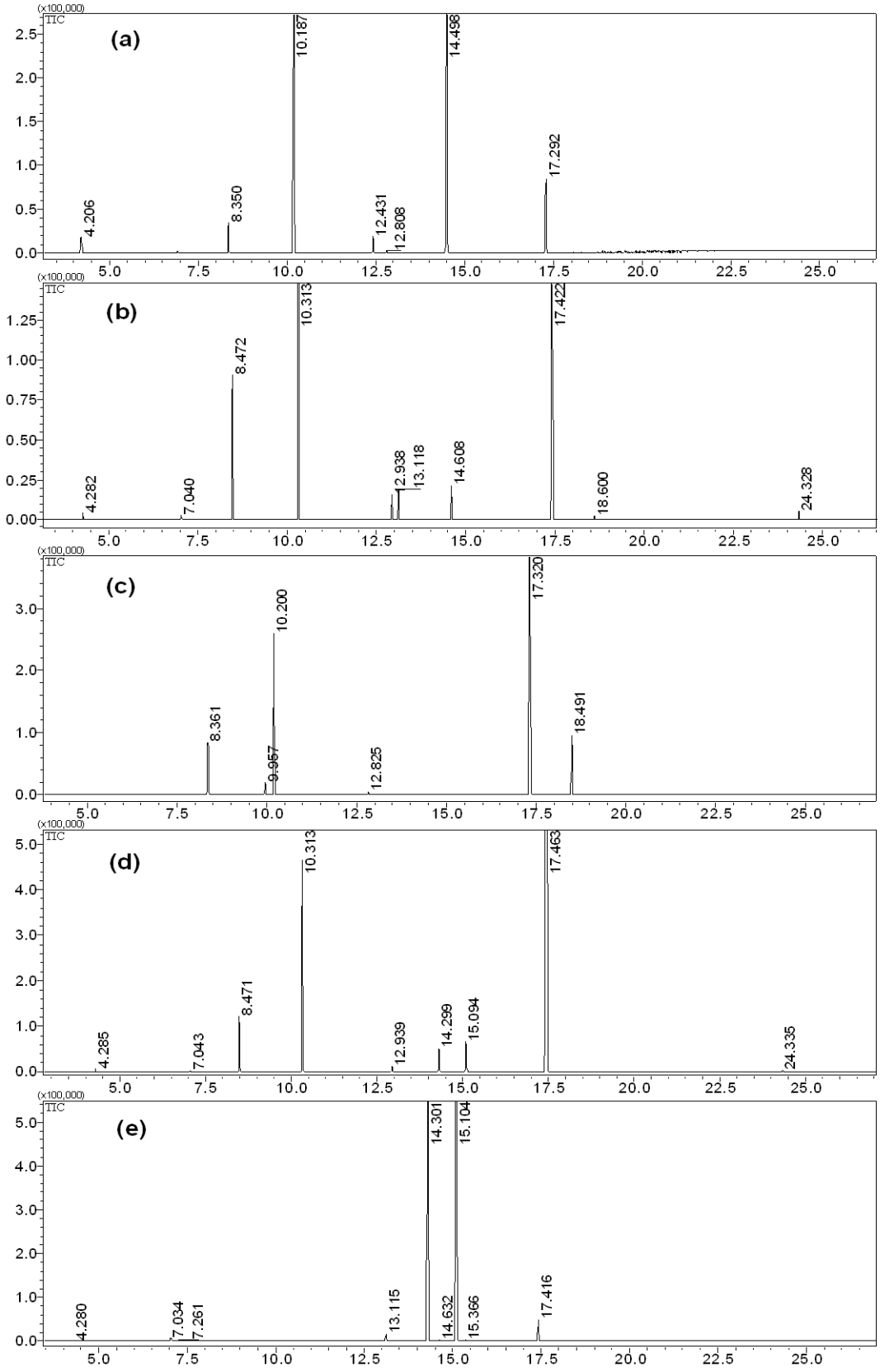

Fig. 1. The total ion current (TIC) chromatogram of essential oil from five basil cultivars. (a) Cinnamon, (b) Italian Large Leaf, (c) Osmin Purple, (d) Red Rubin, and (e) Sweet Dani, grown hydroponically using the nutrient film technique.

Table 1. Essential oil composition (%) of five basil cultivars grown hydroponically using the nutrient film technique.

	Cultivar				
	Cinnamon	Italian Large Leaf	Osmin Purple	Red Rubin	Sweet Dani
Components of Essential oils					
unknown component1	1.66	0.14	-	0.07	0.04
unknown component2	-[y]	0.26	-	0.06	0.28
unknown component3	-	-	-	-	0.15
1,8-cineole	2.13	8.79	6.10	4.18	-
- unknown component 4	-	-	1.47	-	
- linalool	53.12	53.48	21.03	15.69	
- unknown component5	1.49	-	-	-	-
α-terpineol	0.14	1.46	0.27	0.43	-
methyl chavicol	-	2.50	-	-	0.70
neral	-	-	-	1.21	45.60
chavicol	33.39	2.04	-	-	0.03
geranial	-	-	-	2.06	51.83
unknown component6	-	-	-	-	0.05
eugenol	8.10	30.37	66.53	76.25	1.34
methyl eugenol	-	0.15	4.61	-	-
γ-cadinene	-	0.83	-	0.09	-

[z]Values represent the mean from two independent analyses.

[y]Not detected

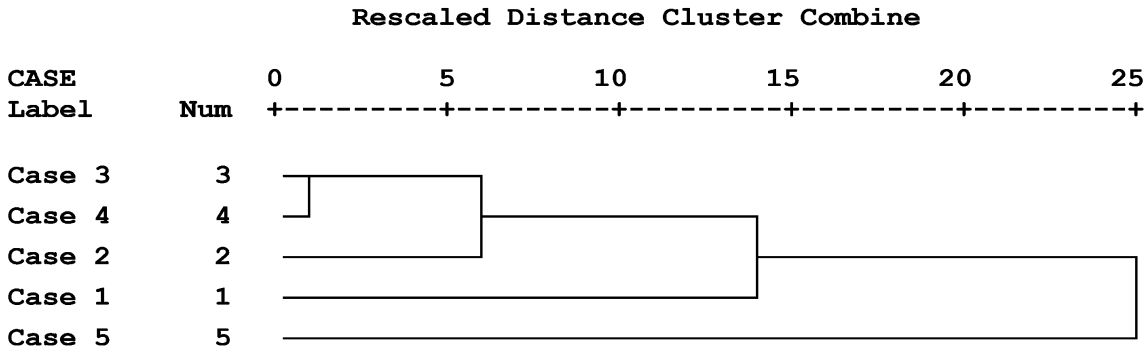

Fig. 2. Dendrogram showing clustering of five basil cultivars grown hydroponically using the nutrient film technique. The data were standardized with Z- score and the dendrogram was drawn according to the method of Ward, (1963). Case 1=Cinnamon, Case 2=Italian Large Leaf, Case 3=Osmin Purple, Case 4-Red Rubin, Case 5=Sweet Dani.

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