

CHEMICAL PROFILING AND EXTRACTION  
PROCESSING OF BASIL (*OCIMUM L.*) CULTIVARS  
GROWN IN OKLAHOMA

By

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CHEMICAL PROFILING AND EXTRACTION PROCESSING OF BASIL (*OCIMUM*  
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## CHAPTER I

### INTRODUCTION

The *Ocimum* genus consists of sixty five species possessing dramatically different chemical compositions (Paton et al., 1999). To understand taxonomy of this genus, a combined analysis of essential oil composition, morphological characteristics, and molecular markers may be necessary. This would provide accurate identification and communication among the producers and marketing/business people and perhaps provide an infrastructure for the production and marketing of the extracts. Beside genetic diversity and extraction methods, many other factors such as environment and stage of growth also affect the essential oil yield/composition. The effects of these factors on the oil yield/composition are species dependent. Metabolic sequences involved in oil production within special glands (trichomes) remains controversial, reflecting the variation in chemical composition of these extracts from *Ocimum* species. Extracts from *Ocimum* species will continue to find multiple uses in the industry, in particular due to their flavoring, aromatic, biological and antioxidant properties.

#### **Classification of *Ocimum* and Economic Uses**

**Taxonomic Classification:** *Ocimum* is a member of the Labiatae family having a square stem, opposite and decussate leaves with two distinctive lips. It belongs to tribe *Ocimeae* that has declinate stamens. The stamens lie over the lower (anterior) lip of the corolla rather than ascending under the upper lip (Paton et al., 1999). *Ocimum* includes annual and perennial herbs,



as well as shrubs endemic to the tropical and subtropical regions of Asia, Africa and Central South America (Darrah, 1998).

The taxonomy of *Ocimum* is complicated due to its proficiency in cross-pollination which has spawned numerous breeding and selection efforts by man. No generic classification can communicate the variation of *Ocimum* through its geographical range and indicate delimitation of this genus from related genera. Swofford (1993) did parsimony analysis and chose a representative sample of 20 taxa to indicate the variation in *Ocimum*, *Becium*, *Erythrochlamys*, and *Orthosiphon* subgenus *Nautochilus*. Within subsect. they only differ in habit, leaf shape and characters which are variable phylogenetically. He represented sections and subsections with their diagnosing characters as follows:

1. Sect. *Ocimum* subsect. *Ocimum*. It contains 7 species and is suggested in the analysis by *O.americanum* (Syn. *O.canum*), differing in only leaf morphology and habit. Bract persistence, calyx throat open in fruit, throat with a hairy annulus and pollen with angled muri of primary reticulum are the key features.
2. Sect. *Ocimum* subsect. *Gratissima*. It contains 6 species represented by variations of *O. gratissimum*, *O. jamessi*, and *O.cufodontii*. Bract persistence, calyx throat closed in fruit and pollen with rounded muri of primary reticulum are the key features.
3. Sect. *Ocimum* subsect. *Hiantia* (*Becium*). It contains 35 species and is represented by variations of *B. fimbriatum*, *B. frimbriatum*, *B. grandiflorum*, and *B. irvinei* with some morphological variation among them. Caducous bract, calyx throat open or compressed in fruit, glabrous throat and pollen with rounded or angled muri of primary reticulum are the key features.

4. Sect. *Ocimum* subsect. *Hierocymum*. It contains 11 species and is represented by variations of *O.lamiifolium* Benth. and *O. tenuiflorum* (Syn. *O.sanctum*) L. Basally pubescent posterior statements and equal anther thecae are the keys to this subsect.
5. Sect. *Ocimum* subsect. *Gymnocimum*. It contains 8 species and is recognized by *O. campechianum* Mill. Glabrous posterior statements and unequal anther thecae are the keys to this subsect.

Pushpangadan and Bradu (1995) did another infrageneric classification used mostly for economic and industrial purposes:

1. *O. basilicum* includes annuals and perennials with black, ellipsoid, strongly mucilaginous seeds. *O. basilicum* has only section *Ocimum* subsection *Ocimum* which has 7 species.
2. *O. sanctum* contains perennial shrubs with brown globule non-mucilaginous seeds. *O. sanctum* has the rest of the genus which contains 144 species.

Although they acknowledged more than 150 species in this genus, most of their taxa were done based on morphology and color that could depend on environmental conditions. Later, Paton et al. (1999) reduced this number to 65 while suggesting the other ascriptions should be considered synonyms or false.

Lawrence (1993) and Grayer et al. (1996) proposed chemotype classification based on volatile oil (aromatic portion) composition. In this classification they separated basil chemotypes on the basis of the major aromatic compound, whose relative percentage was 20-50% of the total oil. This percentage composition of the essential oil was based on gas chromatographic analysis. This method was problematic since commonly one plant includes 2 or more major compounds in

nearly equal amounts. Thus, it would be more suitable to consider overall % of the major compounds which are unaffected by environmental conditions at a defined growth stage.

Genetic tools based on DNA analysis have shown some promise for taxonomic studies. As an example, Labra et al. (2004) used molecular markers of DNA polymorphism to explain uncertain attributes combined with morphological characterization and chemotype classification. They found that genomic similarity does not always reflect similarity or difference in output traits, such as oil composition/ agronomic traits: they characterized two cultivars which had unlike oil compositions, but they were genetically similar. Their findings indicate that chemotype classification based on the essential oil composition should only be used for practical purposes and not for taxonomy. Morphological characters would provide primary classification, but DNA genotyping offers capability to classify accessions independent of environmental factors. They concluded that the taxonomic analysis of morphological traits and molecular markers, combined with essential oil composition, shows the optimum method to verify taxonomy of the *Ocimum* genus.

**Economic Uses:** The Sect. *Ocimum* subsect. *Ocimum* is economically the most important taxon. In this section *O. basilicum*, *O. americanum* and their hybrid *O. x citriodorum* are the most commonly used species for oil production and pot herbs. They are also used as a culinary condiment and for insect control (Paton et al., 1999). *O. basilicum* is used as a sedative and anticonvulsant in Spain, Mexico and Brazil (Di Stasi et al., 2002). *O. kilimandscharicum* is grown for camphor production, and *O. forskolei* is used for traditional medicine. Hybridizing leads to the species being very morphologically and chemically variable and indistinguishable varieties. The cultivars and trade names, such as ‘Cinnamon’, are useful to communicate some

characteristics such as scent, leaf shape and texture, but a standardization in the descriptive terms for varieties is needed for the section *Ocimum*.

Within the sect. *Ocimum* subsect. *Gratissima*, *O. gratissimum* is important economically and used as medicine and insecticide. *O. lamiifolium* and *O. obovatum* are used as traditional medicine in Ethiopia. Within subgenus *Gymnocimum*, *O. tenuiflorum* L. (*sanctum*) was found to have sedative and anticonvulsant effects. Also, it is used as medicines to treat central nervous system diseases in the tropical part of the world as well as for oil production and as a pot herb (Freire, 2006). *O. campechianum* also has applications as medicine and pot herb.

### Composition

The fresh leaves and flowers of common basil species grown in Bangladesh, *O. basilicum* L. var. *purpurascens*, *O. sanctum* L. and *O. americanum* L., contained, 0.38%, 0.26-0.52% and 0.47-0.93 % essential oil (ml 100g fresh weight<sup>-1</sup>), respectively (Koba et al., 2009). Unseparated dried leaves, stems and flower tops of *O. basilicum* L. (sweet basil) and *O. sanctum* L. grown in Mississippi contained 0.40-0.75 and 0.50-0.72% essential oil (ml 100g fresh weight<sup>-1</sup>), respectively (Zhejzkov et al., 2008a). The dried leaves and flowers of *O. basilicum* L. species and *O. kilimandscharicum* grown in Africa contained 0.5-4.05% and 3.13% (ml 100g fresh weight<sup>-1</sup>), respectively with a major compound as camphor (50% of detected compounds; Lopez et al., 2008; Table 1). Fresh leaves of *O. gratissimum* L. contained 3.2-4.1% essential oil (ml 100g fresh weight<sup>-1</sup>) and major compound as linalool (50% of detected compounds) (Hiltunen, 1999). The composition of dried leaves and flower tops of sweet basil grown in Japan was given as: 0.08% essential oil, 14% protein, 61% carbohydrates, high concentrations vitamins A and C, rosmarinic acid, and a flavanoid called xanthomicrol (Leung and Foster 1996).

## Composition of Essential oil

Essential oil composition has been studied extensively for chemotaxonomic purposes by many researchers (Lawrence et al.; 1997, Chien 1988; Ngassoum et al., 2004; Kothari et al., 2005 a and b; Tchoumboungang et al., 2006; Telci et al., 2006). Up to present, around 140 chemicals of the oil have been discovered (Hiltunen, 1999). These included more than 30 monoterpenes, around 30 sesquiterpenes, 20 carboxylic acids, 11 aliphatic aldehydes, 6 aliphatic alcohols, and 20 phenolic compounds. The basil oils mainly consist of oxygenated monoterpenes (Fig. 1) and phenylpropane derivatives (Fig. 2). They were also characterized by containing monoterpene hydrocarbons (Fig. 1). Limonene, myrcene, p-cymene and  $\gamma$ -terpinene occur in the oil in minor amounts. However, ocimenes,  $\gamma$ -terpinene and p-cymene of those monoterpene hydrocarbons were exceptions with significant quantities measured by means of gas chromatography in the oil of *O. gratissimum* (Paton and Putievsky, 1996). Also, some other species in this genus have different compositions than the others. For example, *O. basilicum* var. *hispidum* contains a unique acyclic monoterpene ketone, dihydrotageton as a major compound (Paton et al., 1999). The common compounds found in the essential oils of *Ocimum* species are summarized in appendix Table A.1 and further discussed in Appendix A.

## Plant segment

Charles et al., (1990) reported that for *Ocimum micranthum* Wild. yield of essential oil (ml 100g fresh weight<sup>-1</sup>) varied by plant part:  $1.54 \pm 0.12$  for leaves,  $0.63 \pm 0.08$  for flowers, and  $0.08 \pm 0.01$  for stems. They concluded that the concentration of essential oil glands in plant parts was species-dependent. Werker et al. (1985) found that the oil yield was significantly higher in flowers than in leaves of *O. vulgarare* and agreed with that statement. In *O. basilicum* oil, linalool was 73.9%, 48.2 %, and 30.4% of detected compounds in the oils from flowers, leaves

and stems, respectively (Charles et al., 1990). Methyl chavicol was 11.5 %, 31.6%, and 12.6 % of detected compounds in the oils from flowers, leaves and stems, respectively. Stem oil contained more sesquiterpenes than the oil from other plant parts. In spice basil (*O. gratissimum*), the flowers, leaves and stems contributed 19%, 80% and 1% of total oil yield, respectively (Kothari et al., 2005). The leaf oil had higher eugenol content but lower (E)- $\beta$ -ocimene content compared to the other plant segment oils. Chalchat and Ozcan (2008) also determined that compositions of the oils from flowers, leaves and stems of *O. basilicum* were variable. Methyl chavicol was the major compound of flower (58% of detected compounds) and leaves (53% of detected compounds). Dillapidole was the major compound for stems (50% of detected compounds).

### **Biosynthesis of Essential oil Components**

Essential oil components are secondary metabolites, which function *in planta* in different ways such as defense against fungi and attraction of pollinating insects. They are produced and stored in surface glandular trichomes (glands) distributed through the aerial parts of basil plants and most concentrated on the leaves and floral structures (Iijima et al., 2004). These special cells are sequestered to protect the plant from its own toxicity. The glandular trichome cells are attractive model systems for studying the biosynthesis of essential oil compounds in plants. Damage to gland cells results in evaporation of volatile compounds, such as eugenol, chavicol, methyl chavicol and methyl eugenol. These are the major phenylpropenes derived from phenylalanine, which is also a precursor in general phenylpropanoid metabolism. The various steps of biosynthesis for many compounds predominant in basils have been complicated to

identify entirely (Iijima et al., 2004). Koeuduka et al. (2006) studied the biosynthesis pathways of eugenol and isoeugenol from *O. basilicum*. They showed that these compounds are produced from substituted phenylpropanol and accumulated in the peltate glandular trichomes (glands) of the leaves of basil plants. They also suggested these glands have an NADPH-dependent reductase enzyme which uses coniferyl acetate to produce eugenol. However, Gang et al. (2002) proposed the cinnamic acid metabolism employing specific acyltransferases and hydroxylases instead of the reductase enzyme, was responsible for production of eugenol, chavicol, methyl chavicol and methyl eugenol from phenylalanine. Deschamps et al. (2006) found that phenylpropene composition and content depended on developmental stage; methyl chavicol decreased as the leaf matured, and its content was correlated with chavicol O-methyltransferase and eugenol O-methyltransferase activities.

Unlike phenylpropenes, the production of terpenes from basil is well known. There are two pathways for the synthesis of terpenes in basil: 1) Mevalonate (MVA) pathway, occurring in the cytosols of the glandular trichomes, uses Acetyl-CoA as the precursor to synthesize sesquiterpenes and sterols. 2) Methylerythriol 4-phosphate (MEP) pathway, localized in plastids of the glandular trichomes, uses pyruvate to produce monoterpenes, diterpenes, and carotenoids. Iijima et al. (2004) examined the molecular mechanisms underlying the differences in terpene contents from different basil chemotypes. The total amount of terpenes were positively related to total levels of terpene synthase activities in the glandular trichomes but negatively related to phenylpropanoid accumulation for all chemotypes examined.

### **The Other Important Chemicals**

**Flavonoids:** *O. basilicum* extracts obtained with 80% methanol include flavonoid glycosides (0.6-1.1 g 100g dry wt<sup>-1</sup>) and also flavonoid aglycones (Paton et al., 1999).

Navadensin and salvigenin from *O. canum* and xanthomicrol, eriodictyol, eriodictyol-7 glucoside and vicenin-2 from *O. basilicum* have been isolated (Paton et al., 1999). Vieira et al. (2003) suggested that these flavonoids would be more useful than the essential oils to establish chemotypes among basil species, since they are often unique characteristics of certain species. Vieira et al. (2001) studied the chemical profiling of *O. americanum* using flavonoids. They found that there were significant differences among the species of *O. americanum*, *O. x citriodoram*, *O. basilicum*, and *O. minimum* in total flavonoid concentration and in their navadensin/salvigenin ratio, which, on the whole, were not correlated with morphological characters. Grayer et al. (2004) concluded that it would be possible to identify most of the *Ocimum* species (*O. americanum*, *O. x citriodorum*, and *O. basilicum*) from the flavonoids since each species had its own typical profile. Nguyen et al. (2011) also found that level of anthocyanin, another type of flavonoid, varied among the cultivars as ‘Dark Opal’ exhibiting significantly higher levels than ‘Sweet Thai’ and ‘Genovese’.

**Phenolic acids, triterpenes, sterols, tannins and polyphenols:** Rosmarinic acid, gallic acid, vanilic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillin, caffeic acid and chlorogenic acid were isolated phenolic acids from leaves of *O. sanctum* (Paton et al., 1999). Ursolic acid and oleanolic acids as triterpenes (0.2% dry wt.) were also reported in *O. sanctum* (Balanehru and Nagarajan, 1994). In addition to that, Tarchoune et al. (2009) also found rosmarinic, gentisic and caffeic acids as main phenolic acids of fresh leaves of ‘Fine’ and ‘Genovese’ varieties of *Ocimum basilicum* L. from Tunisia extracted with chloroform/methanol. Also, Javanmardi et al. (2002) detected rosmarinic acid as a major phenolic acid in the local accessions of *O. basilicum* L. grown in Iran. However, Kwee and Niemeyer (2011) found that ‘Ararat’, ‘Blue Spice’, ‘Bush’, ‘Envigor’, ‘Gecofure’, ‘Nufar’, ‘Osmin’, ‘Siam Queen Thai’, and



'Sweet Salad' cultivars had chicoric acid as a major phenolic acid which existed at levels 5-10 times higher than rosmarinic acid in 'Nufar', 'Osmin', and 'Siam Queen Thai'. Also, Lee and Scagel (2009) found chicoric acid, which is a main phenolic in *Echinacea* genus and known for antioxidant activities, in 'Thai' basil stems. Chlorogenic acid, known antioxidant was found as a major phenolic acid in *Ocimum basilicum* cv. Genova (Sgherri et al., 2010). B-Sitosterol was the most common sterol reported in *O. basilicum* (Paton et al., 1999). *O. basilicum* also contained significant amount of tannins and polyphenols (2.2-2.3% dry wt.) (Paton et al., 1999).

### **Biological and Antioxidant Activities of Basil**

Basil is used as a fragrance ingredient in cosmeceuticals, a spice in food products, an antioxidant in food preservation, an alternative medicine, and in aromatic therapy. Its wide range of biological and antioxidant activities have formed the basis of these many applications. These activities will be described briefly below.

Antimicrobial activity of sweet basil oil has been reported against *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *Mycoderma* sp. *Aspergillus niger*, *Bacillus cereus*, *Staphylococcus aureus*, *E. coli*, *Candida albicans*, *E. coli*, *Candida albicans*, *Corynebacterium* sp., and *Penicillium italicum* (Paton et al., 1999). Eugenol was also indicated to have an inhibitory effect on *Bacillus subtilis*, *Salmonella enteridis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *E.coli*. In general, sweet basil oil showed higher activity against gram positive bacteria than gram negative bacteria. Likewise, *O. kilimandscharicum* oil showed higher activity against gram positive bacteria than gram negative bacteria (Paton et al., 1999).

Thymol and carvacrol exhibited inhibition of *Shigella* sp. (Bagamboula et al., 2004). The oil of *O. gratissimum* leaves inhibited *B. cerus*, *B. subtilis*, *C. glutamicum*, *S.aureus*, *E. coli* and *E. faecalis* (Ngassoum et al., 2003). The oil of *O. basilicum* var. *citratum* from Thailand showed inhibition against *Salmonella* spp., *Escherichai coli* 0157, and *Camylobacter jejunii* (Bagamboula et al., 2004). The oils of *O. selloi* and *O. canum* also exhibited inhibition against *Staphylococcus aureus* and *E. coli* (Nascimento et al., 2011).

The oil of *O. basilicum* showed antifungal activity against *Trichophyton mentagrophytes*, *T. rubrum*, *T. verrucosum*, *T. rubrum* and *Epidermophyton floccosum*(Paton et al., 1999). Also, eugenol, caryophyllene, 1-8 cineole, thymol and linalool components exhibited antifungal activities. A food grade basil extract, obtained from methyl chavicol chemotype of *O. basilicum*, inhibited the growth of *Fusarium oxysporum*, *F. proliferatum* and *F. verticillioides* as an alternative preservative for food (Kocic-Tanackov et al., 2011).

Insecticidal activity is another important bioactivity of basil species. In warm countries basil has been traditionally used as an insect repellent. The extract of *O. basilicum* from Czech Republic showed toxicity against *Spodoptera littooralis* (Mediterranean Brocade) (Pavela, 2004). The oil of the same specie from Turkey had repellency against *Culex pipiens* (Northern House Mosquito) (Erler et al., 2006). Besides these biological activities, basil also exhibits many other activities, such as anti-inflammatory, immunomodulatic, adaptonic, anticarcinogenic and antioxidant. Recently, with respect to food production, the antioxidant activity of basil has become one of the most important ones since it is natural, considered safer and more functional than synthetic antioxidants. Flavonoids, phenolic acids, and phenolic diterpenes in the basil extract contribute to antioxidant property. Basil showed higher antioxidant effectiveness than mint, oregano, parsley, garlic, BHA (butylhydroxyanisol) and BHT (butylhydroxytoluene), if

added to sunflower oil at a level 5g 250g oil<sup>-1</sup> (Paton et al., 1999).  $\beta$ -carotene, tocopherol, eugenol, isoeugenol, linalool, and flavonoid components showed promising antioxidant properties. Lee et al. (2005) also found that eugenol exhibited antioxidant property. Basil leaves added to food concentrates /chocolate inhibited peroxide formation and decomposed peroxides previously formed (Paton et al., 1999).

## **Conclusions**

*Ocimum* species, one of the most commonly used herbs, are the source of a wide variety of extracts. Since these extracts possess many biological activities and antioxidant properties, they would be a natural replacement for synthetic chemical additives in many products including foods, cosmetics and medicines. However, taxonomy of this genus with lots of varieties, hybrids and selections is complicated. A combined analysis of essential oil composition, morphological characteristics, and molecular markers should provide the optimum method to verify taxonomy of this genus. This would provide accurate identification of these species and clear communication in the extraction market. Comprehensive studies including correlative environmental, taxonomic, structural, and chemical analysis are necessary to develop clear identification and an infrastructure for the production and commercial sale of basil extracts.

## **OBJECTIVES AND SCOPE**

Research on basil essential oil content and composition has been limited to the northern and southeastern United States (Zheljazkov et al., 2008a) or locally grown cultivars in other countries (Javanmardi et al., 2002). A substantial body of evidence indicates that basil chemical

accumulation is strongly influenced by production environment (Zheljazkov et al., 2008b). One aim of this study was to substantiate production potential and chemical profile of a large number of basil cultivars grown in Oklahoma. Maintenance of basil quality after harvest is complicated by its susceptibility to chilling injury (Lange and Cameron, 1994) and by negative impacts of drying at temperatures exceeding 40°C (Yousif et al., 1999). Another aim of this study was to examine the impact of drying temperatures on basil quality, as a prerequisite step for herb stabilization prior to extraction. Unlike other commercial extraction processes, ambient temperature extraction (ATE) with propane offers the advantage of the recovery of the compounds obtained with little thermal degradation (Yang et al., 2007). In many cases, the herb raffinate retains a high level of flavor and is shelf stable when dry. For herbs, ATE extraction parameters are usually optimized to only partially extract herb compounds and somewhat selectively remove compounds including lower molecular weight degradation products which lead to off flavors (Kanamangala et al., 1999). The final aim of this study is to characterize an ATE process for basil which would result in high quality edible raffinate products as well as valuable extract. The objectives of this study were:

1. To develop an aromatic compounds of common interest chemical profile library for basil cultivars with potential production in OK.
2. To evaluate changes in chemical profile of basil cultivars affected by year, time of harvest within a given year and harvest frequency as well as the effects of drying methods and postharvest handling on chemical profiling of the basil cultivars.
3. To evaluate the effect of ATE process on chemical profiles, which would result in a high quality dry feed stock (raffinate) as well as valuable extract with many uses.

## Reference

Bagamboula, C.F., M. Uyttendaele, and J. Debevere. 2004. Inhibitory effect of thyme and basil essential oils, carvacol, thymol, estragol, linalool, and p-cymene towards *Shigella sonnei* and *S.flexneri*. Food Microbiology 2:33-42.

Balanehru, S. and B. Nagarajan. 1994. Triterpenes intervene in adriamycin-induced histamine release in rodents. Med. Sci. Res. 22:357-359. Bariatux, O., H. Richard, J. Touche, and M. Derbesy. 1992. Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flav. Fragr. J. 7: 267-271.

Chalchat, J.C. and M.M. Ozcan. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. Food Chemistry 110(2): 501-503.

Charles, D.J., J.E. Simon, and K.V. Wood. 1990. Essential constituents of *Ocimum micranthum* Willd. J. Agric. Food Chem. 38:120-122.

Chien, M.J. 1988. A computer database of essential oils. In: Flavors and fragrances. Lawrence BW. Elsevier Science Publishers BV, Amsterdam. 65p.

Darrah, H.H. 1998. The cultivated Basil. Buckeye Printing. Independence, MO. 68 p.

Deschamps, C., D. Gang, and N. Dudareva. 2006. Developmental regulation of phenylpropanoid biosynthesis in leaves and glandular trichomes of basil (*Ocimum basilicum* L.) International Journal of Plant Sciences. 167(3): 447-454

Di Stasi, L.C., G.P. Oliveira, M. A. Carvalhaes, Jr. M. Oueiroz, O.S. Tien, S.H. Kakinami and M.S. Reis. 2002. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 73:69-91.

Erler, F., I. Ulug, and B. Yalcinkaya. 2006. Repellent activity of five essential oils against *Culex pipines*. Fitoterapia 77:491-494.

Freire, C.M., M. O. Marques, and M. Costa. 2006. Effects of seasonal variation on the central nervous system activity of *O. gratissimum* L. essential oil. J. of Ethnopharmacology 105:161-166.

Gang, D.G., T. Beuerle, P. Ullmann, D. Werck-Reichhart, and E. Pichersky. 2002. Differential production of meta hydroxylated phenylpropanoids in sweet basil peltate glandular trichomes and leaves is controlled by the activities of specific acyltransferases and hydroxylases. Plant physiology 130:1536-1544.

Grayer, R.J., G. C. Kite, F.J. Goldstone, S.E. Bryan, A. Paton., and E. Putievsky. 1996. Intraspecific taxonomy and essential oil chemotype in sweet basil, *Ocimum basilicum*. Phytochemistry 43:1033-1039.

Grayer, R.J., R.F. Vieira, and A.M. Price. 2004. Characterization of cultivars within species of *Ocimum* by exudate flavonoid profiles. Biochemical Systematic and Ecology 32(10): 901-913.

Hiltunen, R. and Y. Holm. 1999. Essential oil of *Ocimum*. In Basil The genus *Ocimum*. Hardwood Academic Publishers. Amsterdam The Netherlands. Pg 67.

Iijima, Y., R. Davidovich-Rikanati, E. Fridman, D.R. Gang, E. Bar, E. Lewinsohn, and E. Pichersky. 2004. The biochemical and molecular basis for divergent patterns in biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant physiology* 136: 3724-3736.

Javanmardi, J., A. Khalighi, A. Kashi, H. P. Bais, and J.M. Vivanco. 2002. Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. *J. Agric. Food Chem.* 50(21):5878-5883.

Kanamangala, R.V., N.O. Maness, and M.W. Smith. 1999. Reduced lipid pecans: Chemical alterations and implications for quality maintenance during storage. *J. Amer. Soc. Hort. Sci.* 124: 389-398.

Koba, K., P.W. Poutouli, and C. Raynaud. 2009. Chemical composition and antimicrobial properties of different basil essential oils chemotypes from Togo. *Bangladesh Journal of Pharmacology.* 4 (1): 1-8.

Kocic-Tanackov, S.G. Dimic, J. Levic, I. Tanackov, and D. Tuco. 2011. Antifungal activities of basil (*Ocimum basilicum* L.) extract on *Fusarium* species. *African Journal of Biotechnology.* 10 (50): 10188-10195.

Koeduka, T., E. Fridman, D.R. Gang, D.G. Vassao, B.L. Jackson, C.M. Kish, I. Orlava, S.M. Spassova, N.G. Lewis, J.P. Noel, T.J. Baiga, N. Dudareva and E. Pichersky. 2006.

Eugenol and isoeugenol, characteristics aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. PNAS 103:10128-10133.

Kothari, S.K., A.K. Bhattacharya, S. Ramesh, S.N. Garg and S.P.S. Khanuja. 2005 a. Volatile constituents in oil from different plant parts of methyl eugenol-rich *O.sanctum* grown in South India. J. Essential Oil Res. 17: 656-658.

Kothari, S.K., A.K. Bhattacharya, K. Singh, S.I. Ramesh, and E.V.S. Rao. 2005 b. Pre-Flowering of *Ocimum gratissimum* for higher essential oil and eugenol yields under semi-arid tropics. J. Essential Oil Res. 17:212-216.

Kwee, E.M. and E.D. Niemeyer. 2011. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. Food Chemistry 128:1044-1050.

Labra, M., M. Miele, B. Ledda, F. Grassi, M. Mazzei and F. Sala. 2004. Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant science 167:725-731.

Lange, D. D. and A. C. Cameron. 1994. Postharvest shelf-life of sweet basil (*Ocimum basilicum* ). Hortscience. 29(2): 102-103.

Lawrence, B.M. 1993. Labiatae oils: Mother nature's chemical factory. In: Essential oils. Carol Stream, IL: Allured Publishing p 188-206.

Lawrence, B.M. 1997. Progress in essential oils. Perfumer & Flavorist 22: p 57-74.



Lee, S.K., Umamo, T. Shibamoto and K. Lee. 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food chemistry 91:131-137.

Lee, J. and C.F. Scagel. 2009. Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. Food chemistry 115(2):650-656.

Leung, A.Y. and S. Foster. 1996. Encyclopedia of Common Natural Ingredients used in foods, drugs, and cosmetics, 2<sup>nd</sup> Ed. John Wiley & Sons. New York, USA.

Lopez, M.D., M.J. Jordan and M.J. Pascual-Villalobos. 2008. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. J. of Stored Products Res. 44( 3): 273-278.

Nascimento, J.C., C.A. Barbosa, and V.F. Paula. 2011. Chemical composition and antimicrobial activity of essential oils of *Ocimum canum* Sims. and *Ocimum selloi* Benth. Anais da Academia Brasileira de Ciencias 83(3):787-799.

Ngassoum, M.B., H. Ousmaila, L.T.Ngamo, P.M. Maponmetsem, L. Jirovetz and G. Buchbauer. 2003. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. Journal of Food Composition and Analysis 17: 197-204.

Ngassoum, M.B., J.J. Essia-Ngang, L.N. Tatsadjieu, L. Jirovetz, G. Buchbauer and O. Adjoudji. 2003. Antimicrobial study of essential oils of *Ocimum gratissimum* leaves and *Zanthoxylum xanthoxyloides* fruits from Cameroon. Fitoterapia 74:284-287

Nguyen, P.M., E.M. Kwee, and E.D. Niemeyer. 2011. Potassium alters the antioxidant capacity and phenolic concentration of basil (*Ocimum basilicum* L.) leaves. Food Chemistry.123: 1253-1241.

Paton, A. and E. Putievsky. 1996. Taxonomic problems and cytotoxic relationships between and within varieties of *Ocimum basilicum* and related species. Kew bulletin 51 p 509-524.

Paton, A., R.M. Harley, and M.M. Harley. 1999. *Ocimum*. Medicinal and Aromatic Plants. Vol. 11. Amsterdam: Harwood Academic. 166p.

Pavela, R. 2004. Insecticidal activity of certain medicinal plants. Fitoterapia 75: 745-749.

Pushpangadan, P. and B.L. Bradu. 1995. Advances in Horticulture. In: Medicinal and aromatic plants. vol.11. New Delhi: Malhotra publishing House p 627-657.

Sgherri, C., S. Cecconami, and C. Pinzino. 2010. Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil. Food Chemistry 123:416-422.

Swofford, D.L. 1993. Phylogenetic analysis using parsimony (PAUP), version 3.1. Illinois Natural History Survey Champaign.

Tchoumboungang, F., P.V. Amvam Zollo, F. Avlessi, G.A. Alitonou, D.K. Sohounhloue, J.M. Oumba, E. Dagne, and C. Menut. 2006. Variability in the chemical compositions of the essential oils of five *Ocimum* species from tropical African area. J. Essential Oil Res. 18: 194-199.

Tarchoune, I., A. Incerti, and M. Lachaal. 2009. Relations between antioxidant activity and salinity in basil (*Ocimum basilicum* Mill.). *Agrochimica*. 53(1): 56-64.

Telci, I., E. Bayram, G. Yilmaz, and B. Avci. 2006. Variability in essential oil composition of Turkish basils. *Biochemical Systematics and Ecology* 34: 489-497.

Vieira, R.F., R.J. Grayer, and A.J. Paton. 2003. Chemical profiling of *Ocimum americanum* using external flavonoids. *Phytochemistry* 63:555-567.

Werker, E., E. Putievsky, and U. Ravid. 1985. The essential oils and glandular hairs in different chemotypes of *Ocimum vulgarare* L. *Ann. Bot.* 55:793-801.

Yang, Y., B. Kayan, N. Bozer, B. Pate, C. Baker, and A.M. Gizir. 2007. Terpene degradation and extraction from basil and oregano leaves using subcritical water. *Journal of chromatography A*. 1152 (1-2): 262.

Yousif, A.N., C.H. Scaman, T.D. Durance, and B. Girard. 1999. Flavor volatiles and physical properties of vacuum-microwaves and air-dried sweet basil (*Ocimum basilicum*). *J. Agr. Food Chem.* 47:4777-4781.

Zheljazkov, V.D., A. Callahan, and C.L. Cantrell. 2008a. Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *J. Agr. Food Chem.* 56:(1): 241-245.

Zheljazkov, V.D., C.L. Cantrell, and W.B. Evans. 2008b. Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. *Hortscience*. 43(3): 737-741.

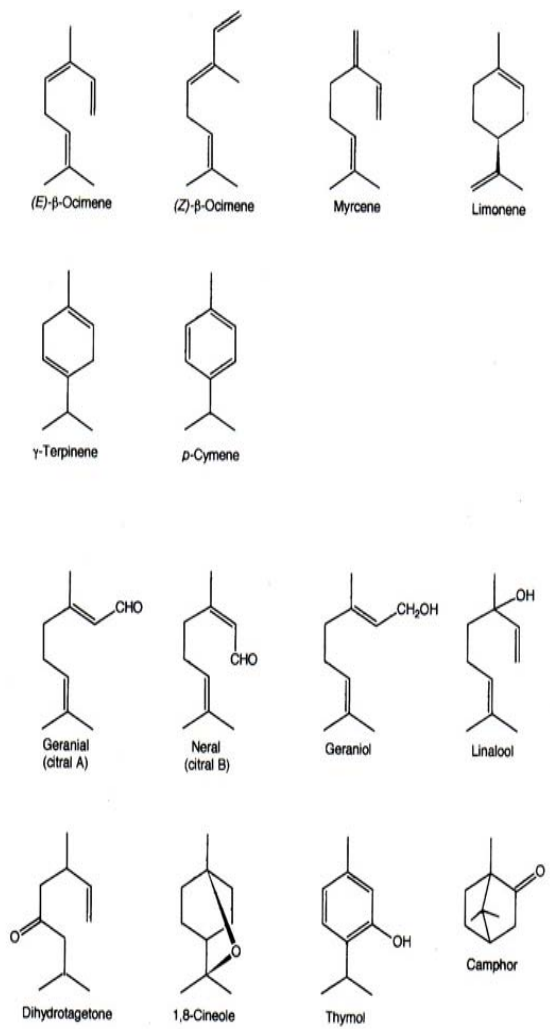


Figure 1. Monoterpene hydrocarbons and oxygenated monoterpenes found in the essential oils of *Ocimum* species, adapted from Paton et al. (1999).

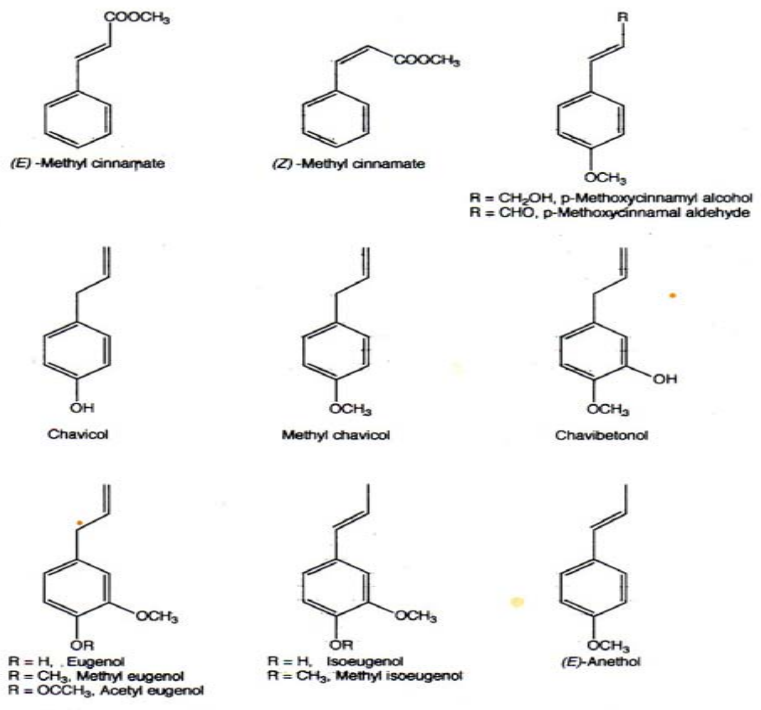


Figure 2. Phenylpropane derivatives found in essential oils of *Ocimum* species, adapted from Paton et al. (1999).

## CHAPTER II

### FACTORS INFLUENCING CHEMICAL PROFILES OF BASIL (*OCIMUM SP. L.*) CULTIVARS GROWN IN OKLAHOMA

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#### Abstract

Production potential and chemical profiles for a large number of basil cultivars grown in Oklahoma were documented, as influenced by cultivar, production year, harvest timing within the year, harvest frequency and postharvest treatment. Throughout the duration of this work from 2005 to 2011, foliage harvests were intermittently conducted from sixteen cultivars prior to full flowering starting in early July and ending in late September at three to four week intervals and between 10 am and 2pm. During 2011, harvest frequency was varied on strict 2 week or 3 week intervals for five of the cultivars previously studied. Samples were typically

dried at 40°C after harvest. Dried samples were ground, extracted with hexane and aromatic chemical components were identified and quantified using gas chromatography. Based on chemotype, the cultivars could be separated into three categories; the pesto-type basils, such as ‘Genovese’, ‘Napolitano’ and ‘Italian Large Leaf’, featuring linalool and eugenol; the spice-type basils, such as ‘Ethiopian Mint’, ‘Blue Spice’ and ‘Sweet Thai’ featuring eugenol and methyl chavicol; the citrus flavored basils, such as, ‘Mrs. Burns’ Lemon’ and ‘Lime’, featuring geranial and neral. The absolute herb yields, impact chemical concentrations, and total phytochemical production of basil cultivars were affected by the year, harvest time within a year and the harvest frequency. Spice type basils were more prone to exhibit alterations in concentrations of impact chemicals, lipid content and fatty acid composition than the pesto and citrus types. Drying at 40°C and short time storage higher than 10 °C, especially for cultivars including eugenol/linalool as major chemicals, was recommended to avoid excessive changes in aroma and aromatic intensity. All cultivars exhibited substantial content of lipid from 3.8 to 8.8%, containing main fatty acids of linolenic (44.17-55.07%) and linoleic acid (12.82-22.36%), which is desirable for nutritional quality and potential industrial uses, but undesirable for oxidative stability of the dry basil.

### **Introduction**

Basil (*Ocimum* sp.) chemical profiles are affected by many factors, such as climatic conditions, harvesting time, development stage, storage/postharvest treatments, drying method and other environmental factors. Sweet basil (*O. basilicum*) is a warm climate plant and the optimum temperature for its growth is 25°C (Chang et al., 2005). At this temperature the oil contents and leaf size are greatest. Basil grown at 25°C had higher eugenol and cis-ocimene contents, while plants grown at 15°C had more camphor and trans-β-farnesene content. Rakic

and Johnson (2002) studied the effect of climatic conditions on sweet basil leaf oil composition. They reported that the monoterpene (limonene, myrcene, p-cymene and  $\gamma$ -terpinene) content was higher in summer and autumn than in winter and spring, reflecting a shortage of the precursor isopentenyl pyrophosphate (IPP) for monoterpene synthesis; sesquiterpenes ( $\beta$ -elemene,  $\delta$ -elemene,  $\alpha$ -humulene and isocaryophyllene) remained constant and phenylpropanoids (methyl chavicol, eugenol and methyl eugenol) varied inconsistently throughout the year. They also indicated that monoterpene and phenylpropanoid content increased during exposure to UV-B but sesquiterpene content was not affected. Silva et al. (1999) observed a diurnal impact on eugenol content in the essential leaf oil of *O. gratissimum*. Eugenol constituted 98% of the detected compounds in the leaf oil at 12 noon, but it was only 11% of detected compounds in the essential leaf oil at 5pm. In a later study Silva et al. (2004) found that eugenol content in the leaf oil of *O. micranthum* Wild. was 97% of the detected compounds at 6am and 84% of detected compounds at 6pm. These findings could indicate a diurnal effect on eugenol accumulation and the optimum time for collection of plants for extraction. Similarly, Chang et al. (2008) found day length altered the growth and chemical content of *O. basilicum*. Linalool and eugenol increased with longer day length while methyl eugenol decreased. However, no difference was observed regarding the content of 1,8-cineole. Belkamel et al. (2008) compared the essential oil yields and compositions of 'Great Green', 'Crimson', and 'Fine Dwarf Green' varieties of *O. basilicum* grown in Morocco at five successive harvests from July to December. They indicated 'Fine Dwarf Green' had the highest oil yield and though the same compounds were present at each harvest, their concentrations varied in each harvest. May et al. (2008) also investigated essential oil yield and composition at eight successive harvests made from June 25<sup>th</sup> of 2005 to April 18<sup>th</sup> of 2006 in Brazil. The basil plants (*O. basilicum* L.) were harvested every 42 days. The



essential oil yield increased until the fourth harvest then started to slightly decrease. Linalool and camphor contents increased up to the fourth harvest and then decreased. However, eugenol and cineole contents increased with successive harvests.

Silva et al. (2004) studied optimum stage of development for harvesting *O. micranthum* Wild. The essential leaf oil composition differed from vegetative, commencement of flowering and end of reproductive stages. In the vegetative stage, beside eugenol (72.9% of detected compounds), elemicin was also a major compound (21.6% of detected compounds). However, by the commencement of flowering and end of reproductive stages, elemicin disappeared. Eugenol increased from vegetative stage (72.9% of detected compounds) to end of reproductive stage (94.0% of detected compounds). The essential leaf oils from this species were analyzed by Charles et al. (1990) from two different locations in Brazil which differed substantially in climate. They found that in the Amazon region with high humidity and low solar light irradiance, the oil contained more  $\beta$ -elemene than  $\beta$ -caryophyllene and eugenol; in a semiarid region with low humidity and high solar light irradiance the oil contained more eugenol than  $\beta$ -caryophyllene and elemicin. In the semi arid region of India, Kothari et al. (2005) studied the oil of *O. gratissimum* from pre-flowering and post-flowering growth stages. The total oil yield and eugenol content from pre-flowering were higher compared to the later growth stages.

Soil fertility is another factor studied for its effect on oil quality/quantity (yield). In French basil, both organic and inorganic fertilizer [six different combinations of organic manure and inorganic fertilizers (nitrogen-phosphorus-potassium)] increased methyl chavicol and linalool content (Anwar et al., 2005). Addition of fertilizer increased the total essential oil yield (l/ha) from 66.49 l/ha for the control (no fertilizer) to 88.57 to 121.3 l/ha for the fertilized plants. The combination of Vermicompost (organic manure) at 5 t/ha and inorganic fertilizer NPK

50:25:25 kg/ha performed best regarding to herb yield (16.85 t/ha), dry matter yield (4.05 t/ha), essential oil yield (121.3 l/ha) and oil content (0.72%). This combination also resulted in the highest methyl chavicol and linalool content (78.69 and 19.60% of detected compounds), which were main components of the oil. Nurzynska-Wierdak et al. (2011) studied yield of basil cultivars ('Kasia', 'Wala' and 'Opal') affected by foliar feeding with nitrogen (0.5% urea) until complete wetting of leaf blade surface compared with control (no foliar fertilizer/only water). Nitrogen fertilization and plant watering was applied four times in 10-day intervals, starting from the initial stage of vegetation growth and finishing two weeks before harvest. They found this application increased the weight and yield of fresh herb compared with control plants. Mean fresh weight increased about 14% compared with control treatments. Klimankova et al. (2008) studied the effect of organic crop culture on aroma profiles of five basil (*O. basilicum*) cultivars compared to conventionally grown basils. They found only one difference in the chemical profile of the cultivars grown in these two different conditions; organically grown 'Cinamonette' had lower relative concentration of methyl chavicol and cinnamote compared to its conventionally grown counterpart. Zheljzakov et al. (2008) studied the effect of N and S rate and location on biomass production, oil content and oil yield of *O. basilicum* grown in Mississippi. They found that location, N rate and their interaction were significant on basil dry herb yield (594-2406 kg ha<sup>-1</sup>). Oil content was affected by the location and varied from 0.64 to 0.80%. The oil yields were highest for the treatment between 50-60 kg N ha<sup>-1</sup>. S treatment rate increased oil yield linearly. Location and N rate (maximum at 60 kg N ha<sup>-1</sup>) affected the yields of major oil compounds, which were linalool, eugenol, bornyl acetate, and eucalyptol; S rate increased only eucalyptol yield at all locations.

Storage/postharvest treatments also affect the composition of basil extracts. Lange and Cameron (1994) found that the best storage temperature for freshly harvested greenhouse sweet basil was 15 °C. The shelf life of basil, packed in low-density polyethylene film covered with black plastic material, was 12 days at that temperature. They assumed that this material did not lose a significant amount of essential oil during extended storage, though they did not do chemical analysis of the basil. The method of drying can significantly influence the basil essential oil content and quality. Yousif et al. (1999) showed that air-dried and fresh samples from *O. basilicum* had similar volatile compositions and concentrations. However, they had different compositions and concentrations of volatiles from the samples dried with vacuum-microwave. Air-dried samples had lower oil yield than the samples dried with vacuum-microwave due to loss of volatiles during air-drying. Vacuum-microwave dehydrated basil samples yielded more volatiles than fresh basil due to chemical reactions (hydrolysis of nonvolatile conjugates/glycosides) during the volatiles extraction in the purge and trap apparatus. Bowes and Zheljzkov (2004) studied the effects of air and freeze-drying on the compositions and contents of oils of *O. basilicum* and *O. sanctum*. Both air-drying and freeze-drying changed the composition of the essential oil from *O. basilicum* and *O. sanctum*. Unlike freeze-drying, air-drying eliminated carene component of the oil. Yousif et al. (1999) indicated that freeze-drying changed the oil compositions and contents of *O. basilicum* significantly. For example, the linalool content in freeze-dried plants was 15% less than in fresh samples. Diaz-Maroto et al. (2004) investigated effects of air drying at 23°C and freeze-drying as well as oven drying at 45°C on the composition and yield of oil of *O. basilicum*. They found that the total oil yield decreased significantly during oven drying and freeze drying but was not affected vs. fresh oil yield by air drying at 23°C. The linalool content decreased significantly due to drying with all three methods.

Eugenol content remained relatively constant, only slightly decreasing during oven and freeze-drying. They concluded that air-drying at 23°C was the best drying method for retaining oil quality and quantity.

Drying enhances preservation and reduces shipping weight but it could result in undesirable changes in composition. Drying temperatures lower than 40°C have been recommended for basil (Diaz-Maroto et al., 2004). However, there is a lack of information on changes of phytochemical concentrations of basil cultivars during storage above chilling temperature and air drying, which would be alternative conservation methods prior to extraction. The aims of this study were to document production potential and chemical profile of a large number of basil cultivars grown in Oklahoma, to evaluate changes in chemical profile affected by year and time of harvest within a given year and to evaluate the effects of drying methods and postharvest handling on chemical profiling of the basil cultivars, which would be considered as potential new extraction/cash crops grown in Oklahoma.

## **Materials and Methods**

### **Chemicals**

The following standards were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A) for analysis of volatiles in purity of >95%: Heptanol,  $\alpha$ -pinene, camphene,  $\beta$ -pinene, myrcene, limonene, cineole, ocimene,  $\delta$ -terpinene, fenchone, terpinolene, linalool, camphor, borneol,  $\alpha$ -terpineol, methyl chavicol, citral (geranial and neral), geraniol, bornyl acetate, thymol, carvacrol, eugenol, methyl cinnamate, methyl eugenol,  $\beta$ -caryophyllene, humulene, and thujone. For the analysis of fatty acids, standards in purity of 99% obtained from Sigma-Aldrich (St.

Louis, MO, U.S.A) were: Palmitic acid, palmitoleic acid, heptodecanoic acid, cis-10-heptadecanoic acid, oleic acid, linoleic acid and linolenic acid. Also, n-hexane, ethyl ether, methanol and tertiary butanol were purchased from Fisher Scientific (FairLawn, NJ) and methyl acetate (99%) and acetyl chloride (99%) were purchased from EM Science (Gibbstan, NJ) and Sigma-Aldrich (St. Louis, MO, U.S.A).

### **Plant Materials and Growth Conditions**

Basil transplants were grown in a greenhouse at the Oklahoma State University Horticulture Research Greenhouse Facility in Stillwater. Cultivars were seeded in 200 cell Speedling trays (Speedling Inc., Sun City, FL), filled with SunGro Redi-earth Plug and Seeding mix (SunGro Horticulture Canada Ltd., Vancouver, Canada). After emergence, basil plants were watered and fertilized to as needed, and thinned to one plant per cell. Seedlings were transplanted into field plots after reaching the 3-5 leaf stage at the Oklahoma Vegetable Research Station near Bixby. Transplanting was done with a two row cone type transplanter (Holland Transplanter Co., Holland, MI) in mid-to-late April in 2008, 2009, 2010 and 2011 with between row spacing of 91 cm and plants spaced approximately 38 cm apart within the row. In 2005, 2006 and 2007, initial plantings were direct seeded with selected cultivars using a Monosem vacuum planter (A.T.I. Inc., Lenexa, KS) with between row spacing of 91 cm and seeding rate of approximately 2 seed per cm. Plots were arranged in a randomized complete block design with four replications. The soil at the experimental site was Severn very fine sandy loam [coarse-silty, mixed (calcareous) thermic Typic Udifluvents] with 0.8% organic matter.

Two row plots were 61 m (two hundred feet), 15 m (fifty feet) and 12 m (forty feet) in 2005, 2006 and 2007, respectively. Plots were 11 m (thirty seven feet) in 2009 and 23 m

(seventy five feet) in 2010 as well as 2011. No herbicide was applied to the plots, and they were watered as needed from overhead sprinklers with approximately 1.3 cm (½ inch) water per application. Plots were fertilized before planting with 56 kg ha<sup>-1</sup> N (50 lb ac<sup>-1</sup>N) using urea. A top dress application of an additional 34 kg ha<sup>-1</sup> N (30 lb ac<sup>-1</sup> N) was applied as urea after a month and again following each harvest. Urea was water incorporated from dry soil surface within 24 h of application. Harvests were conducted prior to full flowering starting in early July and ending in late September at three to four week intervals and between 10 am and 2 pm. During the 2011 season harvest frequency was varied on strict 2 week or 3 week intervals. Photographs for basil cultivars ('Genovese', 'Mrs. Burns' Lemon', 'Sweet Thai', 'Cinnamon', 'Blue Spice') harvested at 2 week interval and 3 week intervals on 9 August 2011 before harvest and after harvest are shown in Appendix B.

Harvest was with a Kincaid self propelled greens harvester (Haven, KS). Cutting height was 28 cm (11 inches) for all cultivars except for 'Sweet Thai' (*Ocimum basilicum* L.), cut at 15 cm (6 inches). Total harvested weight was recorded. Around 6 kg of sample taken from all four replications from each cultivar was pre-cooled and then transported on ice to Stillwater. Yields were adjusted for plant density. All samples were held at 4°C overnight before sample processing and drying; Cultivars used in 2005 were 'Genovese', 'Italian Large Leaf', 'Sweet Thai', 'Mrs. Burns' Lemon' (*Ocimum basilicum citriodora* L.) acquired from Johnny's Seed Co. (Winslow, ME) and 'Ethiopian Mint' (*Ocimum basilicum* L.) from a privately acquired line, which were direct seeded. In 2006, the same cultivars were used except that 'Lime' (*Ocimum americanum*) replaced 'Sweet Thai'. In 2007, seeds of 'Genovese', 'Italian Large Leaf', 'Sweet Thai', 'Mrs. Burns' Lemon', 'Red Rubin' (*Ocimum basilicum* L.) and 'Cinnamon' (*Ocimum basilicum* L.) from Johnny's Seed Co. (Winslow, ME) as well as 'Ethiopian Mint' from a

privately acquired line were established by direct seeding. In 2008, in addition to the cultivars planted in 2005, ‘Thai Magic’ (*Ocimum basilicum* L.), ‘Napolitano’ (*Ocimum basilicum* L.), ‘Nufar’ (*Ocimum basilicum* L.) and ‘Cinnamon’ acquired from Johnny’s Seed Co. (Winslow, ME) were transplanted from the greenhouse in mid April. In 2009, in addition to the cultivars planted in 2005, ‘Blue Spice’ (*Ocimum basilicum* x *Ocimum americanum*), ‘Bush basil’ (*Ocimum basilicum minimum*), ‘Holy Red and Green’ (*Ocimum sanctum* L.), ‘Sweet Danni’ (*Ocimum* x *citriodorum*), ‘Napolitano’, ‘Aromata’ (*Ocimum basilicum* L.) and ‘Cinnamon’ were also transplanted. In 2010, ‘Genovese’, ‘Napolitano’, ‘Sweet Thai’, ‘Mrs. Burns’ Lemon’, ‘Blue Spice’, ‘Cinnamon’ obtained from Johnny’s Seeds (Winslow, ME), and seeds of ‘Purple’ basil (*Ocimum basilicum* L.) and ‘Ethiopian Mint’ obtained from a privately acquired lines were established by transplanting. In 2011, ‘Genovese’, ‘Sweet Thai’, ‘Mrs. Burns’ Lemon’, ‘Blue Spice’ and ‘Cinnamon’ obtained from Johnny’s Seeds (Winslow, ME) were established by transplanting. Throughout the duration of this work sixteen different basil cultivars were used.

### **Post harvest handling and sample preparation**

For the 2005 storage experiment, half of the samples (approximately 3 kg) were stored in the dark at 10°C for one week before processing while the other half considered control samples (approximately 3 kg) were processed immediately without storage. All samples were examined for damage and any sample damaged by harvester or otherwise injured or showing signs of discoloration or dehydration, in addition to extraneous material, was discarded. Sound plant material was double washed and spin dried to remove excess water using a greens spinner (model AD92, Dynamic, Oxnard, CA). Samples ( $\approx$ 1kg each) were then placed into cheesecloth and securely tied prior to forced air drying. Moisture content was determined by drying in an

oven at 70°C, and the moisture content (MC%) was calculated on a dry weight basis as follows:

$$MC\% = (Fresh\ weight - Dry\ weight / Fresh\ weight) * 100$$

Samples were dried for extraction in a forced air drier (Proctor and Schwartz, Inc., model P070146, Horsham, PA) at 40°C until equilibrium weight was achieved. In 2005 additional basil samples (three 1 kg samples from each harvest) were also dried at 60°C to equilibrium weight. Cheese cloth bags were turned every 12 h to assure uniform drying. This took about 60 h for samples dried at 40°C and 36 h for samples dried at 60°C. Equilibrium moisture content was evaluated by drying approximately 30 g subsample at 70°C as previously described and the remainder of the sample was weighed, placed into a labeled freezer bag, capped with nitrogen and securely sealed before placing into a -20°C freezer.

### **Chemical Analysis of Samples**

**Volatiles:** After bringing dry herb samples in the freezer bags to room temperature, a representative whole basil sample (mixture of stem, flowers and leaves) (≈15g) from each bag was ground using a UDY cyclone mill (UDY Corporation, Boulder, CO) to pass a 1 mm screen. After mixing to assure sample uniformity, ground samples (approximately 0.5 g) were weighed in triplicate into tared vials (2 dram; 7.4 ml volume) and 100 µl of thujone or δ-terpinene (1000 nmole) (Sigma-Aldrich Corp., St. Louis, MO) in hexane was added to the dry sample as an extraction internal standard. Extraction was carried out using 4 ml of hexane with stirring for 20 min, and samples were centrifuged in a Speed Vac apparatus (SVC-100H Savant Instrument Inc., Farmingdale N.Y.) at 3,000 g for 20 min. Supernatants were transferred into 2 dram vials and re-centrifuged for 20 min to remove any transferred ground sample. This step was repeated 2-3 times until supernatants free of solids were transferred into tarred 2 dram vials. Then, each



vial with extract was weighed, and their weights were recorded to calculate volume of recovered supernatants for extraction recovery calculations. The moisture contents of the ground samples were determined as follows and used as a correction of analytical results to a dry weight basis. Randomly selected ground samples (approximately 0.5 g) were weighed in triplicate into three tarred aluminum tins. Moisture content was determined by drying in an oven at 70°C and evaluated as previously described for fresh samples.

A gas chromatograph (Varian Star 3400 Cx, Varian Inc., Walnut Creek; CA) equipped with FID detector was used to analyze the chemical components of basil. Injector temperature was 40°C and detector temperature was 300°C. Separations were carried out on a DB-5 fused silica capillary column (30m x 0.25 mm, 0.25 µm film thickness; J and W Scientific Inc., Rancho Cordova, CA) with He carrier gas at a linear flow velocity of 20 cm/sec. Oven temperature was maintained at 55°C for 2 min, raised at 2°C/min up to 75°C, then raised at 1°C/min up to 95°C, then raised at 2°C/min up to 160°C and finally raised at 20°C/min to 280°C and held for 10 min. Injector temperature was 40°C during sample injection, then raised at 100°C/min to 290°C and held for 5 min. Just prior to injection of 1 µl of sample, samples were prepared by mixing 975 µl of sample with 25 µl of 2-heptanol (250 nmole) (Sigma-Aldrich Corp., St. Louis, MO) used as analytical internal standard for GC analysis. Sample components were identified by coelution with authentic standards and quantified using 2-heptanol as internal standard. Extraction recovery was estimated using either thujone or δ-terpinene as internal standard.

**Lipids:** Sample (0.5 g) ground and mixed as previously described for aromatic analysis was weighed in triplicate into 2 dram vials. Extraction was carried out using 4 ml of ethyl ether with stirring for 20 min, and samples were centrifuged in a Speed Vac apparatus at 3,000 g for 20 min. These steps were repeated two more times to extract the samples a total of three times.

Supernatants were transferred into clean 2 dram vials and re-centrifuged for 20 min to remove any transferred ground sample and the supernatant was filtered by using a 0.45 micron membrane (Alltech Associates Inc., Nylon 66) and transferred into a tarred 2 dram vial. Ethyl ether was then evaporated *in vacuo* using a Speed Vac apparatus, leaving the lipids in the vials. Lipid concentration (g lipid 100 g sample<sup>-1</sup>) was determined gravimetrically to the nearest mg.

For preparation of fatty acid methyl esters (FAME), 1 mg of extracted lipid was weighed into a vial containing 600 nmoles of heptadecanoic acid (HDA), added as internal standard. Two hundred  $\mu$ l methanolic HCL (3% HCL in methanol) and 50  $\mu$ l methyl acetate (as a water scavenger) was added to each vial before samples were incubated for 1.5 h at 90 °C. Vials were then cooled to room temperature and tertiary butanol was added before drying under a stream of nitrogen gas to coevaporate with HCL. Seven hundred  $\mu$ l of hexane was then added to dissolve the samples before injecting onto a gas chromatograph.

FAME's were analyzed using a Tracor model 540 gas chromatograph (Tracor Instruments, Austin, TX), equipped with a split injection port (split ratio of 50:1) and FID detector. FAME's were separated using a DB 23 fused silica capillary column (30 x 0.25 mm, 0.25  $\mu$ m film thickness; J and W Scientific Inc., Rancho Cordova, CA). Helium was carrier gas at a linear flow velocity of 20 cm/sec. The injector temperature was 275 °C and detector temperature was 300 °C. Initial column temperature was 50 °C for 2 min. The temperature was raised from 50 °C to 180 °C at 10 °C/min, and hold at 180 °C for 5 min, and a second temperature raised from 180 °C to 240 °C at 5 °C/min and hold at 240 °C for a final 5 min period. Peaks were identified by co-elution with authentic standards and quantified relative to HDA as an internal standard.

## Statistical Analysis

Analysis of variance (ANOVA) and mean separation procedures were performed to determine effects of cultivar, storage and harvest season on chemical profiles using the Statistical Analysis System (SAS Institute, Cary, NC) and data were analyzed with significance's at  $P \leq 0.05$ . Also, the MIXED procedure and mean separation procedure were used to analyze the differences in harvest yields during season for basil cultivars, percentage of separated flowers, leaves and stems of basil cultivars during season, and trends of impact compounds over the course of the harvest season as well as among years and between harvest frequencies (2 week vs. 3 week) using the Statistical Analysis System (SAS Institute, Cary, NC) and data were analyzed with significance's at  $P \leq 0.05$ .

## Results

Basil harvests were conducted at varying frequencies during most years of this study. However, in 2008 and 2010 harvests were conducted at approximately the same frequency. Cumulative air dry yields ( $\text{kg ha}^{-1}$ ) were less variable among basil cultivars harvested in 2008 than in 2010 (Table 1). 'Cinnamon', 'Sweet Thai', and 'Genovese' cumulative air dry yields were not different among years; 'Ethiopian Mint' and 'Mrs. Burns' Lemon' cumulative yields were highest in 2010 and 'Napolitano' was highest in 2008. 'Mrs. Burns' Lemon' had consistently high cumulative air dry yields in both seasons. All basil cultivars yielded less at the early season (early-July) harvest than at the other cultivars. They tended to have the highest yields during the two middle harvests and then declined or remained unchanged at the last harvest.

Concentration of certain aromatic chemicals ( $\text{mg kg}^{-1}$ ) in 16 basil cultivars ('Blue Spice', 'Cinnamon', 'Ethiopian Mint', 'Sweet Thai', 'Thai Magic', 'Spicy Globe', 'Holy', 'Nufar', 'Aroma', 'Genovese', 'Italian Large Leaf', 'Napolitano', 'Red Rubin', 'Purple', 'Mrs. Burns' Lemon' and 'Lime'), a description of their chemical profiles, were obtained from representative harvests during early to late August over six seasons/years (2005-2011) and are presented in Table 2. Basil cultivars were classified based on their typical use or aroma characteristic: The spice type basil cultivars were represented by 'Blue Spice', 'Cinnamon', 'Ethiopian Mint', 'Sweet Thai', 'Thai Magic', 'Spicy Globe' and 'Holy' and were characterized by one or two major impact chemicals. 'Sweet Thai' contained predominantly methyl chavicol (93%) as one major chemical, attributing anise-clove flavor. 'Ethiopian Mint' and 'Blue Spice' featured eugenol as 86 and 88% of total concentration of aromatic chemicals, imparting clove-like flavor and smell. 'Holy basil' included 31% beta-caryophyllene and 31% methyl eugenol as dual major chemicals, attributing clover-carnation flavor. Methyl cinnamate was a major chemical featured in 'Cinnamon' (68%) and 'Spicy Globe' (88%) spice type basil cultivars, attributing sweet cinnamon flavor.

The pesto-type basil cultivars included linalool and eugenol as major impact chemicals (>20% of total aromatic chemical concentration) and were represented by 'Aroma', 'Genovese', 'Italian Large Leaf', 'Napolitano', 'Red Rubin' and 'Purple'. Another pesto-type basil, 'Nufar' included methyl chavicol instead of eugenol as an impact chemical (28% of total concentration of impact chemicals). Methyl chavicol was also a major chemical (23%) for 'Italian Large Leaf'.

Citrus type basil cultivars ('Mrs. Burns' Lemon' and 'Lime') featured geraniol and nerol as major chemicals, which accounted for around 29% and 27% of total concentration of aromatic chemicals in 'Mrs. Burns' Lemon' and around 47% and 44% of those in 'Lime'. Those chemicals contributed to their tangy citrus flavors.

Annual phytochemical production ( $\text{g ha}^{-1}$ ) of basil cultivars harvested during 2005 were documented prior to and after one week of storage at  $10^\circ\text{C}$  (Table 3). These cultivars exhibited chemical profiles which were true to type shown in Table 3 with ‘Genovese’ and ‘Italian Large Leaf’ classified as pesto type, ‘Ethiopian Mint’ and ‘Sweet Thai’ classified as spice type and ‘Mrs. Burns’ Lemon’ classified as citrus type. A relative ranking for the total annual phytochemical production ( $\text{g ha}^{-1}$ ) was ‘Ethiopian Mint’ > ‘Mrs. Burns’ Lemon’ > ‘Sweet Thai’ > ‘Genovese’ > ‘Italian Large Leaf’ (Table 3). ‘Ethiopian Mint’ samples kept in storage for one week at  $10^\circ\text{C}$  prior to drying yielded less total phytochemicals than the control samples primarily due to a coincident loss of eugenol in storage (Table 3). Eugenol also seemed to be labile in stored samples for ‘Genovese’. In the stored ‘Italian Large Leaf’ samples, linalool apparently increased.

Besides storage, drying temperature also affected total concentration of impact compounds as well as individual chemical concentrations depending on the cultivar. Basils harvested in 2009 and dried at  $60^\circ\text{C}$  contained less total concentration of impact chemicals compared to those dried at  $40^\circ\text{C}$  (Fig. 3). In most cases, the impact chemicals themselves also decreased in concentration in samples dried at the higher temperature. Some notable exceptions were linalool in ‘Mrs. Burns’ Lemon’ which increased in the  $60^\circ\text{C}$  dried samples, and linalool and methyl chavicol in ‘Italian Large Leaf’, which were not impacted by drying temperature.

The concentration of impact chemicals in basil cultivars varied from one year to the next and is presented for spice type basils (Fig. 4), pesto-type basils (Fig.5) and citrus-type basils (Fig. 6). Major chemicals in spice type basil cultivars were eugenol in ‘Blue Spice’ and ‘Ethiopian Mint’, methyl cinnamate and linalool in ‘Cinnamon’ and methyl chavicol in ‘Sweet Thai’ (Table 2, Fig. 4). Total phytochemical concentration was different in all three years for

'Blue Spice', 'Cinnamon' and 'Sweet Thai'. Eugenol represented >78% of total conc. of chemicals in both 'Ethiopian Mint' and 'Blue Spice' in all the years. There appeared to be a notable difference between these cultivars in yearly stability of eugenol production. Whereas eugenol concentration differed in all three years presented for 'Blue Spice', it was consistent over two years (2005 and 2010) assayed for 'Ethiopian Mint' (Fig. 4). While methyl cinnamate concentration in 'Cinnamon' basil was different in all three years, linalool differed in one of the three years (Fig. 4).

The most consistent impact chemicals in pesto type basils were linalool and eugenol; two of the three pesto type basil cultivars also contained methyl chavicol (Fig. 5). Two of the pesto type basil cultivars appeared to produce consistent total concentrations of the aromatic chemicals analyzed; 'Napolitano' total chemical concentration did not change in all three years analyzed, and 'Italian Large Leaf' total chemical concentration changed in only one of three years analyzed. Eugenol was consistently produced across three years in 'Napolitano' and was different in only one of three years in 'Italian Large Leaf' and 'Genovese' (Fig. 5). Likewise linalool and methyl chavicol also differed in only one of the three years in 'Napolitano' and 'Italian Large Leaf'. 'Genovese' was the most inconsistent producer of aromatic chemicals across years for the pesto type basils; total phytochemicals were produced at different concentrations in all three years in which they were analyzed.

The citrus type cultivar ('Mrs. Burns' Lemon') differed in total concentration of chemicals in one of the three years studied (Fig. 6) and contained linalool, geranial and neral as impact compounds. In all years geranial and neral were major impact chemicals; in two of the three years they were present in essentially equivalent concentrations, and they were not

produced in consistent quantities across the three years. Linalool concentration differed in one of the three years studied.

While concentration of impact chemicals can be used to express their prevalence in harvested basil, the total weight of harvest must also be factored to evaluate a cultivar's potential for total chemical production. A comparison of the concentration data presented for pesto-type basil in fig. 5 to data provided in fig. 7 converted to g chemical ha<sup>-1</sup> serves as an example. While total chemical and eugenol concentration were highest for 'Genovese' in 2011 (Fig. 5), their contents were one of the lowest in 2011 (Fig. 7) due to reduced basil herb yield for 'Genovese' during that year. For the same reason the apparent consistency of concentration of impact and total chemicals observed in the first and third year for 'Italian Large Leaf' was not observed when data were converted to a content basis (Fig. 7). Spice type and citrus type basil impact chemical content also reflected differences from concentration data depending on total herb yield (data not shown).

Basil impact chemicals not only changed depending on the year of production but also depending on time within the year in which the herb was harvested. The concentration of impact chemicals in basil cultivars harvested in early July (first harvest), early and late August (mid season harvests) and late-September (last harvest) varied from one harvest time to the next and is presented for spice type basil (Fig. 8-11), pesto-type basil (Fig. 12) and citrus-type basil (Fig. 13). 'Blue Spice' (Fig. 8) and 'Ethiopian Mint' (Fig. 9) predominantly contained eugenol at all harvest times and across all years. For both cultivars in three of the four years shown (2005, 2008 and 2011) the individual impact chemicals as well as total chemical concentrations tended to be highest at the middle to later harvests and then declined or remained the same at the last harvest (Fig. 8 and 9). However, in 2010 both cultivars produced highest eugenol and total

chemicals at the early harvest, and then they declined at the middle or last harvest. It should be noted that herb yield for the first harvest is typically lowest for all basil cultivars (Table 1) so the apparent high concentrations during the first harvest in 2010 would not necessarily represent high chemical production potential.

‘Sweet Thai’ contained methyl chavicol as one impact chemical, and there was no consistent trend in terms of its concentration across the season in any given year (Fig. 10). Unlike ‘Blue Spice’, ‘Ethiopian Mint’ and ‘Sweet Thai’, ‘Cinnamon’ was a spice type basil which contained multiple impact chemicals rather than one major chemical (Fig. 11). Methyl cinnamate was the predominant impact chemical in all harvests, followed by linalool and eugenol. The increase or decrease of those chemicals did not appear to be strongly correlated, and there was no clear trend in concentration over the season across three years. It is significant to note the high early season total phytochemical concentration in 2010, as was seen for ‘Blue Spice’ (Fig. 8) and ‘Ethiopian Mint’ (Fig. 9).

‘Genovese’ pesto type basil contained predominantly eugenol and linalool and in most cases eugenol was present in higher concentration than linalool (Fig. 12). In two of the three years shown (2005 and 2008), eugenol, linalool and total chemicals decreased in concentration at the middle harvest and was highest at the final harvest; however, in 2011 this trend was reversed with highest concentrations at the middle harvests. ‘Mrs. Burns’ Lemon’, citrus type basil, yielded major compounds geranial, neral and linalool. Geranial and neral were present in almost identical concentrations across the harvest season in two of three years shown (Fig. 13). Linalool concentration did not appear to be strongly correlated to that of geranial/neral in ‘Mrs. Burns’ Lemon’. In two of the three years (2005 and 2011), highest total phytochemical concentration in ‘Mrs. Burns’ Lemon’ was at the final harvest in the season.



Basil impact chemical concentrations also changed depending on the frequency of herb harvest. The concentration of impact compounds in basil cultivars harvested every 2 weeks vs. every 3 weeks is presented for the early August harvest ('Blue Spice', 'Cinnamon', 'Genovese', 'Mrs. Burns' Lemon' and 'Sweet Thai') in 2011 (Fig. 14). The total concentrations of chemicals were higher in all basil cultivars harvested at 2 weeks vs. 3 weeks except for 'Mrs. Burns' Lemon', in which frequency of harvest had no effect on impact chemical concentration (Fig. 14). Methyl chavicol, eugenol and methyl cinnamate impact chemicals were higher for harvests at 2 week frequencies, linalool did not change consistently between the cultivars ('Cinnamon', 'Genovese' and 'Mrs. Burns' Lemon') or between harvest frequencies. Geraniol did not show any change between harvest frequencies while nerol was higher at 3 week harvest frequency versus 2 week in 'Mrs. Burns' Lemon'.

Harvest frequency and time of harvest within the season also affected percentage of lipids (w/w) and fatty acid composition (wt %) of lipids in basil cultivars harvested at 2 week vs. 3 week harvest intervals in 2011. At 2 week harvest interval, lipid percentage (w/w) in 'Cinnamon', 'Mrs. Burns' Lemon' and 'Sweet Thai' substantially increased from early July to early August but it only slightly increased for pesto type 'Genovese' (Table 4). However, at the 3 week harvest interval lipid percentage (w/w) in spice type 'Sweet Thai' and 'Cinnamon' exhibited different trend between the two harvests; lipid percentage (w/w) in 'Sweet Thai' decreased at early August and was unchanged for 'Cinnamon'. Lipid percentage in 'Genovese' was also unchanged between harvests at 3 week intervals while it increased by early August in citrus type 'Mrs. Burns' Lemon'.

Fatty acid composition (wt%) of lipid extracts for basil cultivars harvested at intervals of 2 weeks and 3 weeks at the early-August harvest in 2011 are presented in Table 5. Linolenic

(44.17-55.07%) and linoleic acids (12.82-22.36%) were the major fatty acids in the lipids of all the cultivars (Table 5). An apparent decrease in linolenic and coincident increase in oleic acids in ‘Cinnamon’ and ‘Sweet Thai’ were more pronounced than the other cultivars harvested at 3 week vs. 2 weeks intervals (Table 5).

Harvest frequency and time of harvest within the season also affected percentage of flowers, leaves and stems of basil cultivars (w/w) harvested at intervals of 2 weeks and 3 weeks in 2011. Stems were more prominent components of harvested basil at early harvests regardless of harvest interval; they declined as components of harvest by the last harvest in all cultivars at both harvest frequencies (Table 6). In all cultivars except ‘Mrs. Burns’ Lemon’, leaves increased as components of total yield as the harvest season progressed. For ‘Sweet Thai’, leaf percentage was highest at the final harvest, ‘Genovese’ exhibited consistently high leaf yield after the first harvest and ‘Cinnamon’ exhibited moderately high leaf yield by the final two harvests at two week harvest frequency and by the last harvest at three week harvest frequency. In all cultivars except ‘Genovese’ and ‘Mrs. Burns’ Lemon’, flower percentages were highest at the middle harvest and then declined or remained unchanged at the last harvest. Flowers were most prevalent in ‘Mrs. Burns’ Lemon’ by the final harvest.

## **Discussion**

Previous work assessing potential herb yield for various basil cultivars have included both once-over total plant yield (Marotti et al., 1996; Elementi et al., 2006; Zheljzakov et al., 2008 a; Wogiatzi et al., 2011) and multiple harvests from the same plants over the course of the season (Rakic and Johnson, 2002; May et al., 2008; Singh et al., 2010). We chose the later

option in order to more closely simulate a commercial production system and to integrate a means to document seasonal trends in herb and phytochemical yield across multiple years. In the multiple harvest system some leaf-bearing growth is retained below the cutting floor to contribute photosynthates and energy for herb re-growth and successional yield occurs from shoots regenerated from woody stems below the cutting floor; cultivars with greater potential to produce a large number of stems from which re-growth can occur appear to enjoy greater yield potential than cultivars which grow more erect and produce fewer woody stems below the cutting floor (Singh et al., 2010). We observed a more compact growth habit for ‘Napolitano’ and ‘Cinnamon’ which may have contributed to their lower yields in 2010 (Table 1). Wogiatzi et al. (2011) noted that ‘Genovese’ out-yielded ‘Italian Large Leaf’ in Greece. We noted that ‘Italian Large Leaf’ had an upright growth habit similar to ‘Napolitano’, providing further evidence that herb yield potential may be favored by greater stem production below the cutting floor.

The relative ranking for herb yield potential remained essentially the same but the magnitude in difference between cultivars differed. Five of the cultivars could not be separated in herb yield and only ‘Mrs. Burns’ Lemon’ and ‘Genovese’ exhibited higher yield than ‘Cinnamon’ basil in 2008 but there was a clear separation of the cultivars into four herb yield categories in 2010 with yield rankings of ‘Mrs. Burns’ Lemon’ > ‘Genovese’, ‘Ethiopian Mint’ and ‘Sweet Thai’ > ‘Cinnamon’ > ‘Napolitano’. Lachowicz et al. (1997) detected differences in morphological features and growing characteristics of five basil cultivars, which are ‘Reunion’, ‘Anise’, ‘Cinnamon’, ‘Dark Opal’ and ‘Bush’, as well as in air dry yields of two of those basil cultivars (‘Reunion’ and ‘Anise’) between years. There were no yield differences between years for the other basil cultivars.

Basil characterization in terms of chemical content, or chemotype, has been suggested as means to describe genetic similarity or dissimilarity among cultivars (Lachowicz et al., 1997; Elementi et al., 2006; Kacar et al., 2009). In our study, we characterized sixteen basil cultivars into three broad groups of spice types (mostly containing one and rarely two dominant chemicals), pesto types (mixture of linalool and eugenol and sometimes methyl chavicol as predominant chemicals) and citrus types (geranial and neral as predominant chemicals) (Table 2). Within these broad groupings some sub-groups became apparent. Within spice type basil 'Ethiopian Mint' and 'Blue Spice' basil appeared morphologically similar and both contained eugenol as a predominant chemical. 'Sweet Thai' and 'Thai Magic' were notably similar morphologically with leaf shape and size typical of Thai type basil, yet their major chemical contents were quite dissimilar with 'Sweet Thai' containing predominantly methyl chavicol and 'Thai Magic' containing predominantly methyl eugenol. 'Holy Basil' was morphologically dissimilar to 'Thai Magic' and was a different species (*Ocimum sanctum* L. vs. *Ocimum basilicum* L.), but it also included predominantly methyl eugenol. 'Cinnamon' and 'Spicy Globe' were notably morphologically dissimilar yet both contained methyl cinnamate as a predominant chemical. Within pesto types linalool, methyl chavicol and eugenol predominated in 'Italian Large Leaf' and 'Napolitano' (both types exhibited an erect growth habit); linalool and methyl chavicol predominated in 'Nufar' only; linalool and eugenol predominated in 'Aroma', 'Genovese', 'Red Rubin' and 'Purple'. Our findings are in agreement with those reported by Marrotti et al. (1996) who also detected linalool and eugenol predominantly in 'Genovese' and mostly linalool, methyl chavicol and eugenol in 'Napolitano' grown in Italy. However, 'Purple' cultivar grown in Iran contained predominantly linalool whereas ours contained eugenol in addition to linalool. Within spice type basil, 'Cinnamon' grown in Australia also predominantly

included methyl cinnamate and to a lesser extent linalool (Lachowicz et al., 1997). ‘Reunion’ cultivar grown in Australia had >80% methyl chavicol as major chemical (Lachowicz et al., 1997), which is similar to ‘Sweet Thai’ in our study. *O. sanctum* L., which is similar to our ‘Holy basil’ cultivar, was also recorded as methyl eugenol chemotype (>50% of total detected compounds) grown in South India (Kothari et al., 2005). However, *O. sanctum* L grown in Poland contained 1, 8-cineole as a major chemical (Kicel et al., 2005), attributing effect of location or environmental conditions on composition (Zheljazkov et al., 2008 a). A citrus type Lemon basil (*Ocimum basilicum citriodora* L.) grown in Turkey contained geranial and neral as predominant chemicals, similar to our results (Tansi and Sengul, 2000).

After harvest short-term or long-term storage of basil could be anticipated to store the herb prior to further use. Short-term refrigerated storage was reported for a period of 2 days prior to further processing (Amodio et al., 2005); basil is chilling sensitive and could be stored fresh at not less than 10 °C to prevent visual deterioration of leaf tissues for 12 days (Lange and Cameron, 1994). Very little is known relative to phytochemical changes associated with refrigerated storage of the fresh herb, so a small study was conducted to evaluate phytochemical changes before and after storage for one week at 10 °C. Our results indicated that phytochemicals were mostly stable to storage under these conditions, with the exception of eugenol which was particularly lost during storage of ‘Ethiopian Mint’ and ‘Genovese’ basil (Table 3). Eugenol loss in ‘Ethiopian Mint’ was severe enough to cause a reduction in total phytochemicals. In contrast to our results, Da Silva et al. (2005) found an increase in eugenol concentration after storage of fresh *O. basilicum* L. plants at 10 °C for 9 days. However, essential oil content decreased in these stored samples. In ‘Italian Large Leaf’ linalool concentration increased with storage at 10 °C both in terms of absolute amount and percentage of

total phytochemicals (Table 3). Da Silva et al. (2005) also documented an increase in linalool concentration following storage of *O. basilicum* L. plants at 10 °C for 9 days and attributed the increase to increased stability due to glycoside reaction with linalool.

Herb drying has been used for centuries to stabilize various herbs for long-term storage (Yousif et al., 1999). Too high drying temperature has been documented as the most critical feature causing basil tissue discoloration (Barbieri et al., 2004) as well as phytochemical loss (Diaz-Maroto et al., 2004). Our comparison of basil drying at 60 °C versus 40 °C revealed across the board reduction in total phytochemicals at the higher drying temperature, for cultivars representing citrus type ('Mrs. Burns' Lemon'), spice type ('Sweet Thai') and pesto type ('Italian Large Leaf') basil (Fig. 3). Individual impact chemicals responded differently to drying; geraniol, nerol and eugenol were substantially reduced in concentration by drying at 60 °C versus 40 °C, methyl chavicol was slightly reduced or unchanged and linalool was unchanged or slightly increased in concentration. The apparent lability of eugenol to both refrigerated storage as well as increased drying temperature suggests that cultivars containing substantial quantities of this chemical should be handled with care to maintain phytochemical content. Likewise, Baritoux et al. (1992) found that after drying (45°C for 12h) and during storing (3-7 months at room temperature) eugenol concentration in 'Grand Vert' basil (*Ocimum basilicum* L.) decreased, but linalool concentration increased, which would cause changes in aroma of the dried basil product.

Methyl chavicol appeared to be less labile to higher temperature drying (Fig. 3) and did not change after one week of refrigerated storage (Table 3) and thus cultivars featuring this chemical may be expected to be less sensitive to phytochemical loss due to handling abuse. The apparent increase or lack of change in concentration of linalool in basil dried at the higher

temperature was in agreement with the study of Soares et al. (2007) and may have been due to a similar glycoside stabilization phenomenon cited by Da Silva et al. (2005). Since linalool was not present in basil as a single predominant chemical but rather as part of a two or three chemical mix of impact chemicals (Table 2), its relative stability may cause changes in aroma of mishandled basil in addition to a general decrease in aromatic intensity due to loss of other phytochemicals.

Yearly variation in total phytochemical concentration appeared to be more pronounced for the spice type basil than for the pesto or citrus types that we studied. Three of four spice type cultivars ('Blue Spice', 'Cinnamon' and 'Sweet Thai') exhibited different total phytochemical and major impact chemical concentrations in all three years while 'Ethiopian Mint' differed in only one of three years which were documented (Fig. 4). Since spice type basil typically produce one major impact chemical (Table 3) which typifies their aroma and flavor, their flavor intensity may be expected to change depending on the year of production. Similarly to spice type basil, Lachowicz et al. (1997) also detected that 'Anise' cultivar was prone to have yearly alterations in composition between 1994 and 1995; methyl chavicol, methyl cinnamate and linalool concentrations in the oil of 'Anise' changed between years. However, Da Silva et al. (2005) reported that plants of *Ocimum basilicum* L. did not exhibit different concentrations of major compounds (linalool and eugenol) between two years, which is similar to our results for pesto types.

Although all basil types produced many of the impact chemicals, such as eugenol, linalool, methyl chavicol and methyl cinnamate, at high concentrations during the early harvest (especially in 2010; Fig. 8, 9, 11, 12 and 13), they also typically produce less herb yield at the early harvest (Table 1). Therefore, the phytochemical content would be less than indicated by

concentration data, and higher total phytochemical yields might be expected from later harvests (Fig. 5 and 7). Within season alterations in phytochemical concentration were dependent on the cultivar studied. Rakic and Johnson (2002) found there was no consistent seasonal pattern of eugenol and other phenylpropanoid compounds in basil (*Ocimum* sp.) varieties (sweet basil and sweet bush basil types) grown in Greece. There was no clear trend in seasonal eugenol production between years for ‘Blue Spice’ and ‘Ethiopian Mint’; their similarity in eugenol as a component of total phytochemical regardless of year (Fig. 4) or time of harvest during the year (Fig. 8 and 9) suggested no interaction between genotypes with year or harvest within a given year, perhaps due to genetic and origin similarities. There was also no clear trend in seasonal eugenol production between years for ‘Italian Large Leaf’ and ‘Genovese’ (Fig. 5). The relative instability of eugenol to storage and drying, combined with its inconsistency in production across years and across harvests within years suggests that cultivars exhibiting high eugenol may exhibit strong flavor and flavor intensity variability.

Changing harvest frequency caused more variation in total phytochemical concentration for the spice and pesto type basil than for the citrus type that we studied. ‘Mrs. Burns’ Lemon’ produced more flowers as a component of harvested herb than the other cultivars (Table 6), and was in a full flowering state at both harvest frequencies but the other cultivars were prior to full flowering stages (Appendix B). The lack of change in phytochemical concentration for the citrus type (‘Mrs. Burns’ Lemon’) may have been caused by rapid and profuse flowering at both 2 weeks and 3 weeks since flowers are known to have substantially high linalool, geraniol and nerol (major chemicals in ‘Mrs. Burns’ Lemon’) concentrations than the other parts (leaf and stems) of basil plants (Charles et al., 1990; Tansi and Sengul, 2000). Changes in linalool concentrations between harvest frequencies of 2 weeks or 3 weeks were dependent on basil



cultivars. However, phenolic chemicals, such as methyl chavicol and eugenol, decreased in all basil cultivars at 3 week versus 2 week harvest frequencies (Fig. 14). Gill and Randhawa (1996) found that at 50 percent flowering methyl chavicol concentration was higher and linalool concentration was lower than full flowering stage in the oil of French basil (*Ocimum basilicum* L.). However, Singn et al. (2010) reported that linalool content in oil of Indian basil ‘Vikar Sudha’ cultivar (*Ocimum basilicum* L.), which is different from French basil morphologically and chemotypically, was not significantly influenced by harvesting state of development (40, 60, 80 and 100 days after transplanting). These results would also support our finding that linalool did not change consistently between the cultivars (‘Cinnamon’, ‘Genovese’ and ‘Mrs. Burns’ Lemon’) or between harvest frequencies.

At both harvest frequencies, the similar lipid content in pesto type ‘Genovese’ basil between harvests may have been due to less variation in percentages of flowers, leaves and stems at successive harvests compared to the other cultivars (Table 6). Our results agreed with the literature (Nour et al., 2009; Wongsheree et al., 2009) that extracted lipid of the basil cultivars contained linolenic and linoleic acids as the major fatty acids (Table 5). The changes in fatty acid composition (wt%) of total lipids in spice type ‘Sweet Thai’ and ‘Cinnamon’ between harvest frequencies of two versus three weeks may also be related to changes in proportions of flowers, leaves and stems between plants harvested on two versus three-week intervals due to different fatty acid compositions of those parts. However, further studies are needed to assess the fatty acid compositions of flower, leaf and stem parts of the basil plant to prove that.

The absolute herb yields, impact chemical concentrations, and total phytochemical production of basil cultivars depended on the year, time of harvest within a given year and the frequency of harvests. Although the relative ranking for herb yield potential and cultivar

chemical profile remained essentially the same among the cultivars, spice type basil which typically produced one and in some cases two impact chemicals were more prone to exhibit fluctuations in concentrations of those chemicals and in extractable lipids and oil fatty acid composition than the other types. The spice-type basil may exhibit less stability in phytochemical yield over different years or growing locations as compared to pesto- or citrus-types. Similarly, Wogiatzi et al. (2011) also found that pesto type basil ('Italian Large Leaf' and 'Genovese') showed more stability in phytochemical yield over different years compared to narrow leaf cultivars ('Finissimo Verde a Palla' and 'Larosa Emenuale Sementi') grown in Greece. Zheljaskov et al. (2008 b) also detected that sweet basil 'German' cultivar (*Ocimum basilicum* L.) appeared to exhibit more stability in phytochemical yield in different locations in Mississippi than 'Holy' basil (*Ocimum sanctum* L.).

Our data shows that eugenol-rich spice and pesto-type basil may be less stable to short-term refrigerated storage (Table 3) or elevated drying temperatures (Fig. 3) than other types of basil and thus should be either utilized fresh or stabilized by low temperature drying soon after harvest. Linalool in cultivars appeared to be more stable to storage and drying practices, and may be expected to remain more stable to handling abuse; since other chemicals may be less stable to loss, the characteristic aroma and flavor of mishandled basil may be expected to change, as opposed to a decrease in aroma intensity alone (Baritoux et al., 1992). Drying at 40° C and careful handling of the cultivars with eugenol/linalool as a major chemical during short/long term storage may be particularly important. Cultivars representing spice, pesto and citrus basil types all exhibited substantial contents of extractable lipid (3.75-8.83%) which consisted of linolenic (44.17-55.07% of fatty acids) and linoleic acid (12.82-22.36% of fatty acids) primarily. These polyunsaturated fatty acids are desirable for nutrition properties

(Kinsella et al., 1990) and potential industrial uses, such as a drying oil (Nour et al., 2009), but they are also subject to rapid oxidative breakdown and formation of stale flavor overtones during storage of dry basil (Yang et al., 2007).

## Reference

Amodio, M.L., G. Peri, and G. Colelli. 2005. Effects of atmosphere composition on postharvest quality of fresh basil leaf (*Ocimum basilicum* L.). *Act Horticulture* 682: 731-736.

Anwar, M., D.D. Patrai, S. Chan, K. Alephs, A. Naqvi, and S.P.S. Khanuja. 2005. Effect of organic manures and inorganic fertilizer on growth, herb, and oil yield, nutrient accumulation, and oil quality of French basil. *Communications in soil science and plant analysis* 36:1737-1746.

Barbieri, S., M. Elustondo, and M. Urbicain. 2004. Retention of aroma compounds in basil dried with low pressure superheated steam. *J. of Food Engineering* 65 (1): 109-115

Bariatux, O., H. Richard, J. Touche and M. Derbesy. 1992. Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. *Flav. Fragr. J.* 7: 267-271.

Belkamel, A., J. Bammi, and V. Janneot. 2008. Evaluation of the biomass and analysis of the essential oil of three varieties of basil (*Ocimum basilicum* L.) cultivated in Morocco. *Acta Botanica Gallica* 155 (4): 467-476.

Bowes, K.M. and V.D. Zheljackov. 2004. Factors affecting yields and essential oil quality of *Ocimum sanctum* and *Ocimum basilicum* cultivars. *J. Amer. Soc. Hort. Sci.* 129(6): 789-794.

Chang, X.M., P.G. Alderson, and C.J. Wright. 2005. Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum* L.). J. of Hort. Sci. & Biotech. 80(5): 593-598.

Chang, X.M., P. G. Alderson, and C.J. Wright. 2008. Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. Environmental and Experimental Botany 63(3): 216-223.

Charles, D.J., J.E. Simon, and K.V. Wood. 1990. Essential constituents of *Ocimum micranthum* Willd. J. Agric. Food Chem. 38:120-122.

Da Silva, F., R.H.S. Santos, and N.J. de Andrade. 2005. Basil conservation affected by cropping season, harvest time and storage period. Pesq. Agropec. Bras. 40(4): 323-328

Deschamps, C., D. Gang and N. Dudareva. 2006. Developmental regulation of phenylpropanoid biosynthesis in leaves and glandular trichomes of basil (*Ocimum basilicum* L.) International Journal of Plant Sciences. 167(3): 447-454

Diaz-Maroto, M.C. Paloma, E.S. Castro, L.M.A. Vinas, and M.S. Perez. 2004. Changes produced in the aroma compounds and structural integrity of basil (*Ocimum basilicum*) during drying. J. Sci. Food and Agr. 15:54-58.

Elementi, S., R. Neri, and L.F.D. Antuona. 2006. Biodiversity and selection of “European” Basil (*Ocimum basilicum* L.) types. Acta Horticulture. 723:99-104.

Gill, B.S. and G.S. Randhawa. 1995. Effect of different transplanting dates and harvesting stages on the quality of French basil oil. J. of Herbs, Spices and Medicinal Plants 4(3):35-42.

Gupta, S.C. 1996. Variation in herbage yield, oil yield and major component of various *Ocimum* species/varieties (chemotypes) harvested at different stages of maturity. *J. Essential Oil Research* 8:275-279.

Kacar, O., E. Goksu, and N. Ozkan. 2009. Agronomic Properties and Essential Oil Composition of Basil Varieties of Landraces (*Ocimum basilicum* L.) in Turkey. *Asian J. of Chemistry* 21(4): 3151-3160.

Kicel, A., A. Kurowska, and D. Kalemba. 2005. Composition of the essential oil of *Ocimum sanctum* L. grown in Poland during vegetation. *J. Essential Oil Res.* 17:217-219.

Kinsella, J.E., B. Lokesh, and R.A. Stone. 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *The American journal of clinical nutrition* 52 (1): 1–28.

Klimankova, E., K. Holadova, J. Hajslova, T. Cajka, J. Poustka, and M. Koudela. 2008. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. *Food Chemistry* 107:464-472.

Kothari, S.K., A.K. Bhattacharya, K. Singh, S.I. Ramesh, and E.V.S. Rao. 2005. Pre-Flowering of *Ocimum gratissimum* for higher essential oil and eugenol yields under semi-arid tropics. *J. Essential Oil Res.* 17:212-216.

Lachowicz, K.J., G.P. Jones, D.R. Briggs, F.E. Bienvenu, M.V. Palmer, V. Mishra, and M.M. Hunter. 1997. Characteristics of plants and plant extracts from five varieties of basil (*Ocimum basilicum* L.) grown in Australia. *J. Agric. Food Chem.* 45:2660-2665.

- Lange, D. D. and A. C. Cameron. 1994. Postharvest shelf-life of sweet basil (*Ocimum basilicum*). Hortscience. 29(2): 102-103.
- Marotti, M., R. Piccaglia and E. Giovannelli. 1996. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J. Agric. Food Chem. 44:3926-3929.
- May, A., O. A., N. B. Bovi, L. E. S. Maia, R. C. Z. Barata, E. M. R Souza, A. R. A. Moraes and M. Q. Pinheiro. 2008. Basil plants growth and essential oil yield in a production system with successive cuts. Bragantia. 67(2): 1-7.
- Nour, A. H., S. A. Elhoussein and N. A. Osman. 2009. Characterization and chemical composition of the fixed oil of fourteen basil (*Ocimum basilicum* L.) accessions grown in Sudan. J. of Chemical Technology. 1(2): 52-58.
- Nurzynska-Wierdak R., B. Borowski and K. Dzida. 2011. Yield and chemical composition of basil herb depending on cultivar and foliar feeding with nitrogen. Acta Scientiarum Polonorum. 10(1): 207-219.
- Rakic, Z. and C.B. Johnson. 2002. Influence of environmental factors on the composition of the essential oil of *Ocimum basilicum*-Sweet basil. Breeding research on aromatic and medicinal plants. 9:157-169.
- Signh, S., M. Singh, A. K. Signh, A. Kalra, A. Yadav and D. D. Patra. 2010. Enhancing productivity of Indian basil (*Ocimum basilicum* L.) through harvest management under rainfed conditions of subtropical north Indian plains. Industrial Crops and Products 32:601-606.

Silva, M. G., A.A. Craveiro, F.J. Abreu Matos, M.I.L. Machado, and J.W. Alencar. 1999. Chemical variation during daytime of constituents of the essential oil of *Ocimum gratissimum* leaves. *Fitoterapia* 70:32-34.

Silva, M.G., F.J. A. Matos, M.I.L. Machado, and F.O. Silva. 2004. Essential oil Composition of the leaves of *Ocimum micranthum* Willd. *J. Essent. Oil Res.* 16:189-190.

Soares, R.D., M.A. Chaves, and A.A. da Silva. 2007. Influence of drying temperature and air velocity related to essential oil and linalool contents of the basil (*Ocimum basilicum* L.). *Ciencia E Agrotecnologia* 31(4): 1108-1113.

Tansi, S. and N. Sengul. 2000. First cultivation trials of lemon basil (*Ocimum basilicum* var. *citriodorum*) in Turkey. *Pakistan Journal of Biological Sciences* 3(3):395-397.

Vassao, D.G., D.R. Gang, T. Koeduka, B. Jackson, E. Pichersky, L.B. Davin, and N.G. Lewis. Chavicol formation in sweet basil (*Ocimum basilicum*): cleavage of an esterified C9 hydroxyl group with NAD(P)H-dependent reduction. *International Journal of Plant Sciences* 167(10): 547-554

Wogiatzi, E., A. Papachatzis, H. Kalorizou, C. Adamantia, and N. Chouliaras. 2011. Evaluation of essential oil yield and chemical components of selected basil cultivars. *Biotechnol & Biotechnol. Eq.* 25(3):2525-2527.

Wongsheree, T., S. Ketsa, and W.G. van Doorn. 2009. The relationship between chilling injury and membrane damage in lemon basil (*Ocimum x citriodorum*) leaves. *Postharvest Biology and Technology* 51:91-96.



Yang, Y., B. Kayan, N. Bozer, B. Pate, C. Baker, and A. M. Gizir. 2007. Terpene degradation and extraction from basil and oregano leaves using subcritical water. *Journal of chromatography A* 1152 (1-2): 262.

Yousif, A.N., C.H. Scaman, T.D. Durance, and B. Girard. 1999. Flavor volatiles and physical properties of vacuum-microwaves and air-dried sweet basil (*Ocimum basilicum*). *J. Agr. Food Chem.* 47:4777-4781.

Zheljazkov, V.D., A. Callahan, and C.L. Cantrell. 2008 a. Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *J. Agr. Food Chem.* 56:(1): 241-245.

Zheljazkov, V.D., C.L. Cantrell, and W.B. Evans. 2008 b. Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. *Hortscience* 43(3): 737-741.

Table 1. Air dry yields (kg ha<sup>-1</sup>) of selected basil cultivars harvested at selected intervals during 2008 and 2010.<sup>z</sup>

Season	Harvest time <sup>y</sup>	Cinnamon	Ethiopian Mint	Sweet Thai	Mrs. Burns' Lemon	Genovese	Napolitano
<b>2008</b>	Early-July	127d <sup>x</sup>	116c	204b	455c	539c	207d
	Early-Agust	1557a	1283a	1287a	1663a	1288a	1365a
	Late-August	328c	644b	484b	758b	739b	735c
	Late-Sept	687b	819b	1134a	578b	781b	952b
	<b>Cumulative:</b>	<b>2700b<sup>w</sup></b>	<b>2863ab<sup>†v</sup></b>	<b>3109ab</b>	<b>3454a<sup>†</sup></b>	<b>3348a</b>	<b>3259ab<sup>†</sup></b>
<b>2010</b>	Early-July	350c	339d	361b	723d	438d	127d
	Early-Agust	944a	1437a	1085a	1832a	990b	915a
	Late-August	908a	483c	1139a	1770b	1339a	879b
	Late-Sept	413b	1379b	1078a	1287c	627c	224c
	<b>Cumulative:</b>	<b>2617c</b>	<b>3640b<sup>†</sup></b>	<b>3663b</b>	<b>5613a<sup>†</sup></b>	<b>3394b</b>	<b>2144d<sup>†</sup></b>

<sup>z</sup> Yields from fresh plant material were converted to air dry weights based on moisture loss during drying of a subsample at 40°C to an equilibrium moisture content of approximately 10 percent.

<sup>y</sup> Cultivars were harvested at 28 cm (11 inches) except 'Sweet Thai' which was harvested at 15 cm (6 inches) above ground level. All successive harvests were from re-growth at the same harvest heights.

<sup>x</sup> Means of four replications followed by the same letter within columns of the same year do not differ significantly according to student t test (P<.05).

<sup>w</sup> Means of four replications of the cumulative yields for each cultivar within year followed by the same letter do not differ significantly according to student t test (P<.05).

<sup>†v</sup> Means of four replications of the cumulative yields of the cultivars followed by a cross harvested in 2008 are different from cultivars harvested in 2010 according to student t test (P<0.05).

Table 2. Concentration of impact chemicals (mg kg<sup>-1</sup>) in basil cultivars ('Blue Spice'<sup>z</sup>, 'Cinnamon'<sup>y</sup>, 'Ethiopian mint'<sup>x</sup>, 'Sweet Thai'<sup>w</sup>, 'Thai Magic'<sup>v</sup>, 'Spice Globe'<sup>z</sup>, 'Holly'<sup>z</sup>, 'Nufar'<sup>v</sup>, 'Aroma'<sup>z</sup>, 'Genovese'<sup>u</sup>, 'Italian Large Leaf'<sup>t</sup>, 'Napolitano'<sup>s</sup>, 'Red Rubin'<sup>r</sup>, 'Purple'<sup>z</sup>, 'Mrs. Burns' Lemon'<sup>q</sup> and 'Lime'<sup>p</sup>.) from representative harvests during six seasons/years (2005-2011).

Type: Impact Chemical <sup>n</sup>	Spice							Pesto						Citrus		
	Blue Spice	Cinnamon	Ethiopian Mint	Sweet Thai	Thai Magic	Spicy Globe	Holy	Nufar	Aroma	Genovese	Italian Large Leaf	Napolitano	Red Rubin	Purple	Mrs. Burns' Lemon	Lime
cineole	4	18	144	48	43	5	0	22	0	61	99	14	0	50	4	2
linalool	131	<b>512</b>	1	6	404	122	0	<b>302</b>	<b>285</b>	<b>483</b>	<b>595</b>	<b>291</b>	<b>454</b>	<b>459</b>	368	60
methyl chavicol	107	5	170	<b>2533</b>	3	28	8	<b>165</b>	0	12	<b>313</b>	103	15	6	5	4
geranial	0	0	0	0	0	0	0	0	0	0	2	0	0	0	<b>613</b>	<b>1267</b>
neral	0	0	0	0	0	0	0	0	0	0	3	0	0	0	<b>589</b>	<b>1166</b>
eugenol	<b>2541</b>	282	<b>4449</b>	9	494	48	244	17	<b>352</b>	<b>745</b>	<b>318</b>	<b>313</b>	<b>191</b>	<b>630</b>	13	0
methyl cinnamate	2	<b>1922</b>	3	0	4	<b>1689</b>	3	30	2	0	2	1	3	0	3	0
methyl eugenol	39	10	91	41	<b>1861</b>	1	<b>1312</b>	12	7	0	7	4	0	5	184	0
β-caryophyllene	67	21	39	14	41	5	<b>715</b>	7	0	5	13	3	21	20	85	152
humulene	49	50	140	67	26	10	38	41	8	13	18	14	4	23	55	22
<b>TOTAL:</b>	<b>2940</b>	<b>2820</b>	<b>5038</b>	<b>2719</b>	<b>2892</b>	<b>1907</b>	<b>2320</b>	<b>597</b>	<b>655</b>	<b>1319</b>	<b>1355</b>	<b>744</b>	<b>688</b>	<b>1193</b>	<b>2149</b>	<b>2673</b>

<sup>z</sup> The representative harvests chosen were done in 13-August 2009.

<sup>y</sup> The representative harvests chosen were done in 3-August 2010.

<sup>x</sup> The representative harvests chosen were done in 13-August 2009 and 3-August 2010. The average values of concentrations of impact chemicals are presented.

<sup>w</sup> The representative harvests chosen were done in 3-August 2010 and 9-August 2011. The average values of concentrations of impact chemicals are presented.

<sup>v</sup> The representative harvests chosen were done in 5-August 2008.

<sup>u</sup> The representative harvest chosen were done in 2-August 2005 and 31- August 2006. The average values of concentrations of impact chemicals are presented.

<sup>t</sup> The representative harvests chosen were done in 31- August 2006 and 23-August 2007. The average values of concentrations of impact chemicals are presented.

<sup>s</sup> The representative harvests chosen were done in 5-August 2008, 13- August 2009 and 3-August 2010. The average values of concentrations of impact chemicals are presented.

<sup>r</sup> The representative harvests chosen were done in 23-August 2007.

<sup>q</sup> The representative harvests chosen were done in 2-August 2005, 31- August 2006 and 3-August 2010. The average values of concentrations of impact chemicals are presented.

<sup>p</sup> The representative harvests chosen were done in 31- August 2006.

<sup>n</sup> Mean of three replications. Each replication involved three injections.

Table 3. Annual phytochemical production (g ha<sup>-1</sup>) of basil cultivars (stored<sup>z</sup> vs. nonstored) harvested during 2005.<sup>y</sup>

Impact chemical	Ethiopian Mint		Genovese		Italian Large Leaf		Mrs. Burns' Lemon		Sweet Thai	
	Nonstored	Stored	Nonstored	Stored	Nonstored	Stored	Nonstored	Stored	Nonstored	Stored
cineole	1851b <sup>x</sup>	2973a	612a	576a	403a	459a	41a	36a	428a	367a
linalool	31a	26a	4559a	4651a	2912b	4172a	3284a	3519a	31a	31a
methyl chavicol	3070a	3494a	26a	20a	2264a	2489a	36a	36a	10863a	9308a
geranial	ND <sup>w</sup>	ND	ND	ND	ND	ND	4723a	5131a	ND	ND
neral	316	ND	ND	ND	5a	10a	4712a	4922a	ND	ND
eugenol	26408a	17717b	5819a	3565b	1556a	1280a	66a	46a	46a	31a
β-caryophyllene	612a	750a	51a	41a	26a	36a	995a	1071a	87a	71a
humulene	760a	862a	138a	97a	82a	92a	179a	168a	332a	260a
<b>Total:</b>	<b>33048a</b>	<b>25826b</b>	<b>11205a</b>	<b>8951a</b>	<b>7247a</b>	<b>8537a</b>	<b>14035a</b>	<b>14928a</b>	<b>11786a</b>	<b>10078a</b>

<sup>z</sup>The subsamples were stored above chilling temperature (around 10°C) for one week.

<sup>y</sup>Annual phytochemical production (g ha<sup>-1</sup>) was calculated as sum of contents of impact compounds from 5 successive harvests during 2005, determined by GC analysis.

<sup>x</sup>Mean of three replications. Each replication involved three injections. Means within cultivar followed by the same letter within rows do not differ significantly between storage treatments according to student t test (P<0.05).

<sup>w</sup>Not detected during gas chromatography analysis.

Table 4. Lipid yield (%) of basil cultivars (w/w) harvested at frequencies of 2 weeks and 3 weeks during early July and early August in 2011.

Cultivar	2 week		3 week	
	Early July	Early August	Early July	Early August
Cinnamon	4.73b <sup>z</sup>	8.83a	7.34a	6.71a
Genovese	4.74a	6.30a	6.09a	5.02a
Mrs. Burns' Lemon	3.75b	6.20a	5.96b	8.66a
Sweet Thai	3.79b	7.60a	8.0a	5.80b

<sup>z</sup> Means of three replications followed by the same letter in the same harvest interval within cultivar do not differ significantly according to student t test (P<0.05).

Table 5. Fatty acid composition (wt%) of total lipids in basil cultivars harvested at frequencies of 2 weeks vs. 3 weeks from early August in 2011. Values represent mean of three replications. Each replication involved three injections.

Harvest intervals	2 Week				3 Week			
	Cinnamon	Genovese	Mrs. Burns'	Sweet Thai	Cinnamon	Genovese	Mrs. Burns'	Sweet Thai
<b>Fatty acid</b>			<b>Lemon</b>				<b>Lemon</b>	
Palmitic acid	16.69	15.77	15.62	15.47	13.32	10.91	16.43	8.42
Palmitoleic acid	0.78	0.82	0.47	0.60	2.34	2.02	0.66	4.32
Cis-10-Heptadecenoic acid	1.41	2.39	1.88	1.30	3.36	0.59	0.44	2.48
Stearic acid	3.96	2.99	4.88	3.68	3.93	4.78	3.43	7.34
Oleic acid	3.96	1.35	3.57	3.92	14.60	5.54	4.36	20.45
Linoleic acid	21.38	19.35	22.03	20.68	21.93	21.09	22.36	12.82
Linolenic acid	51.82	57.32	51.56	54.35	40.53	55.07	52.33	44.17

Table 6. Yield of flowers, leaves and stems of air dry basil cultivars (%) in herb harvested at frequencies of 2 weeks and 3 weeks during early July, early August, late August and late September in 2011.

Cultivar	Part	2 Week				3 week			
		Early July	Early August	Late August	Late Sept	Early July	Early August	Late August	Late Sept
Cinnamon	flowers	31b <sup>z</sup>	37a	32ab	26c	44a	31b	42a	39a
	leaves	31b	35b	45a	45a	21c	36ab	28bc	41a
	stems	39a	29b	23c	29b	35a	33ab	30bc	20d
Genovese	flowers	28a	21ab	20b	33a	32a	20b	20b	29a
	leaves	38b	59a	62a	56a	35b	57a	56a	59a
	stems	34a	20b	18b	10c	33a	23b	24b	12c
Mrs. Burns'	flowers	38c	51b	49b	69a	43bc	38c	50b	61a
Lemon	leaves	17a	18a	21a	14a	18ab	23a	18ab	14b
	stems	45a	32b	29b	17c	38a	39a	32b	25c
Sweet Thai	flowers	24b	31b	36ab	38a	31b	24b	42a	27b
	leaves	34b	38b	38b	47a	24c	39b	27c	45a
	stems	42a	31b	26c	15d	46a	34b	31b	28b

<sup>z</sup> Means of three replications ( $\approx 100$  g air dry sample per replication) followed by the same letter within rows and within the same harvest interval do not differ significantly according to student t test ( $P < 0.05$ ).

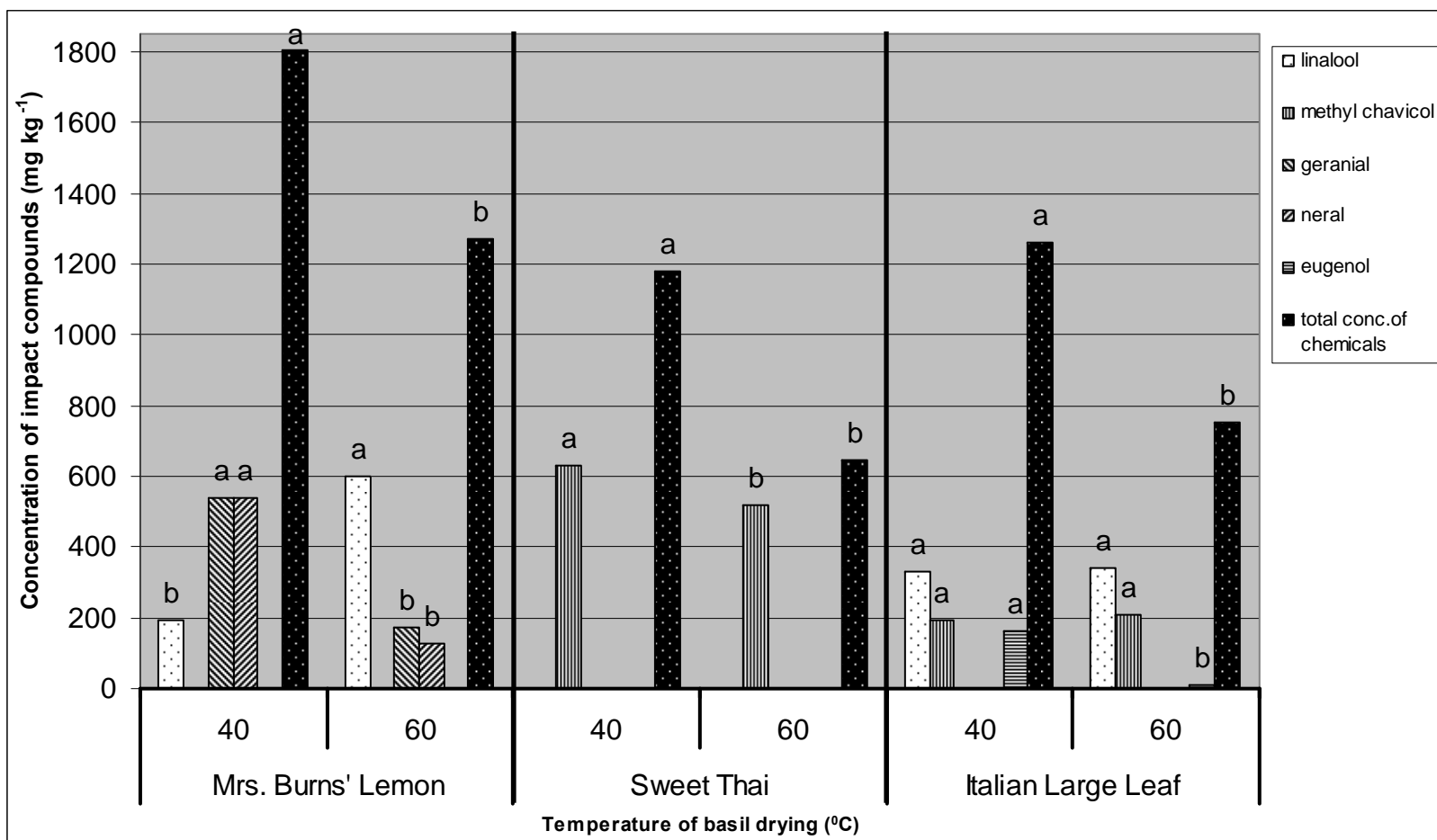


Fig. 3. Effect of temperature of drying on concentration of impact compounds in basil cultivars harvested in 2009. The letter above each bar shows differences between 40°C and 60°C in the same cultivar and phytochemical according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.



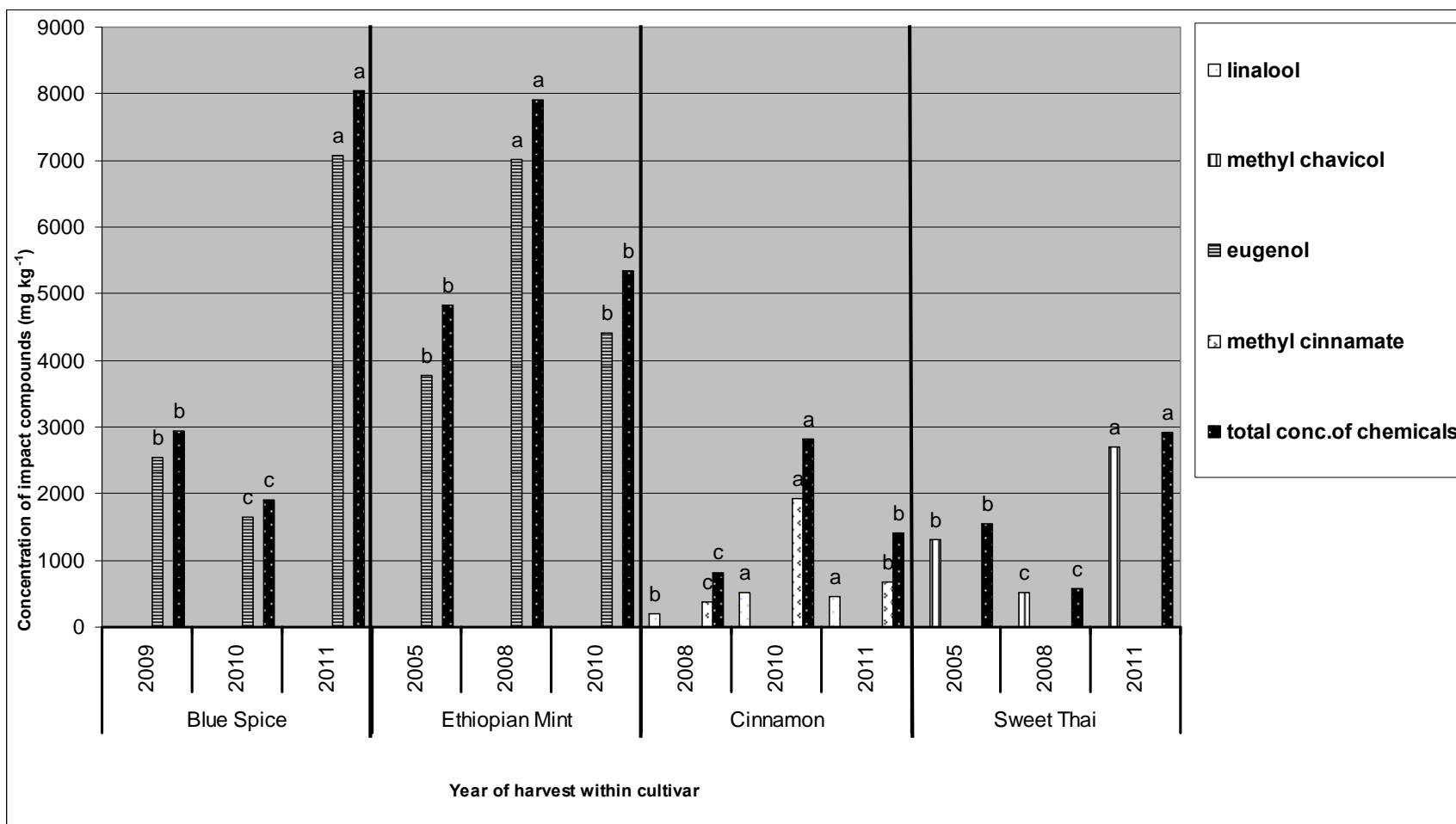


Fig. 4. Effect of harvest year on concentration of impact compounds in spice type basil cultivars harvested at early August. The letter above each bar shows differences among years in the same cultivar and phytochemical according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

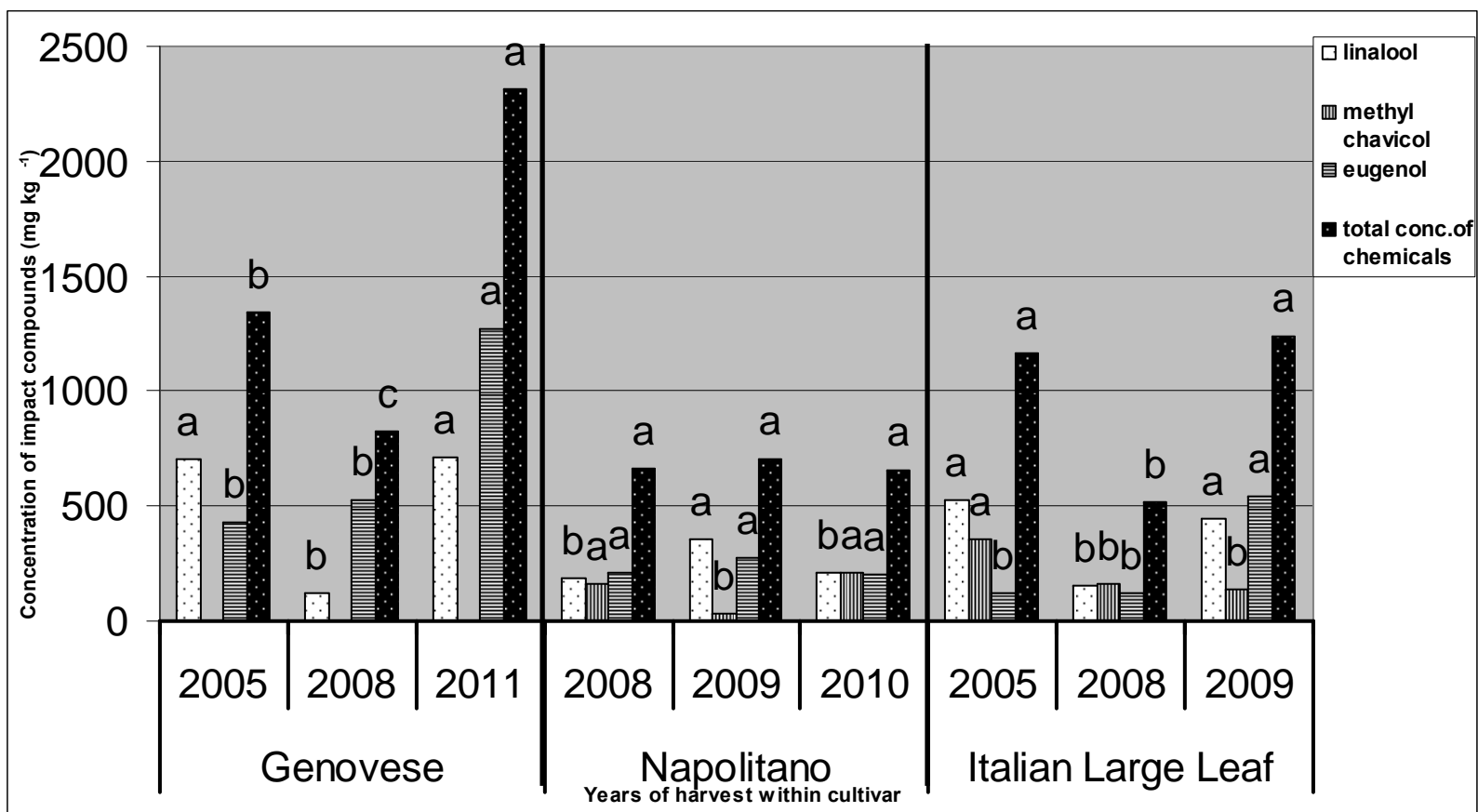


Fig. 5. Effect of harvest year on concentration of impact compounds in pesto type basil cultivars harvested at early August. The letter above each bar shows differences among years in the same cultivar and phytochemical according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

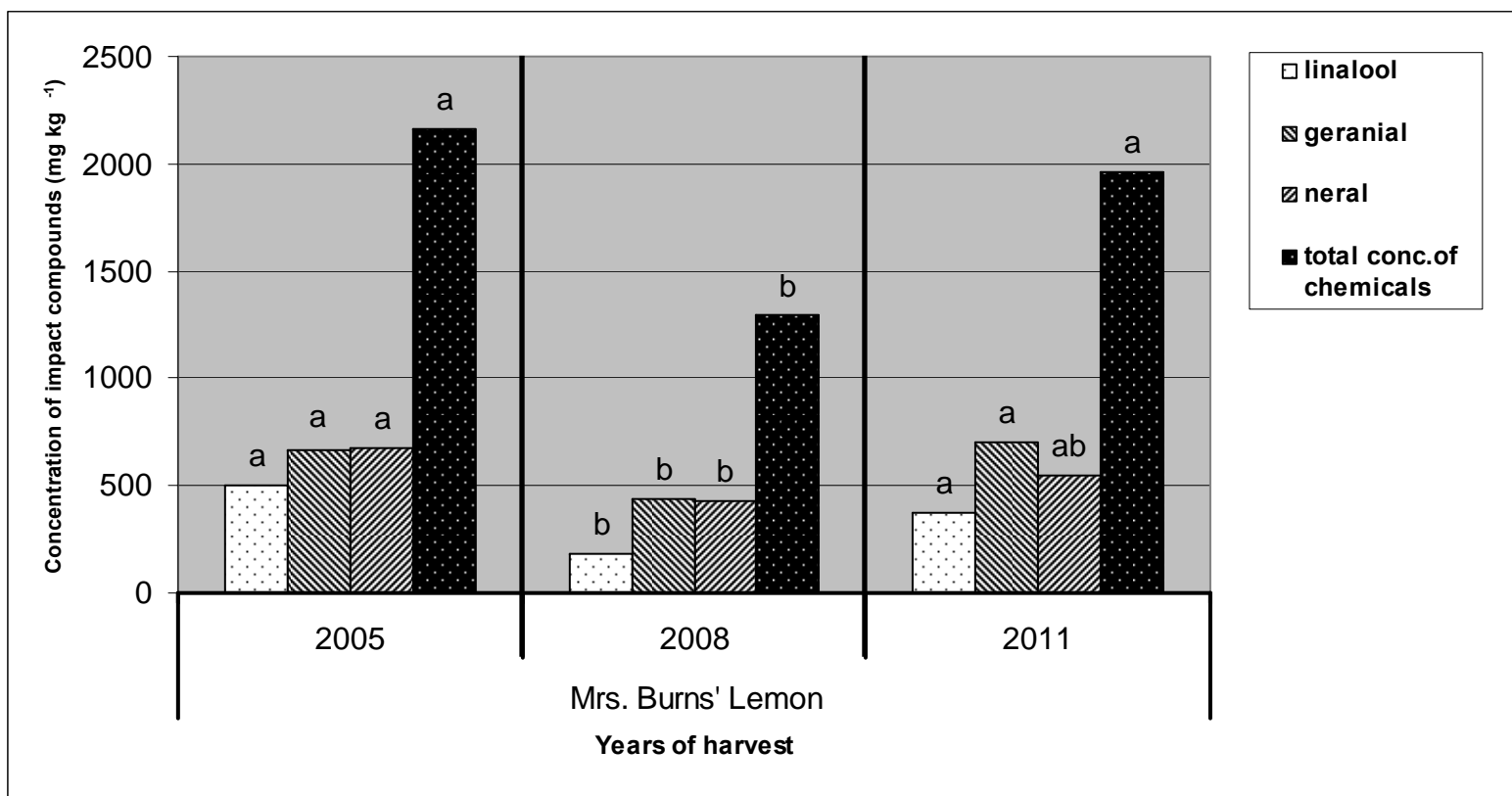


Fig. 6. Effect of harvest year on concentration of impact compounds in citrus type basil cultivar harvested at early August. The letter above each bar shows differences among years within each phytochemical according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

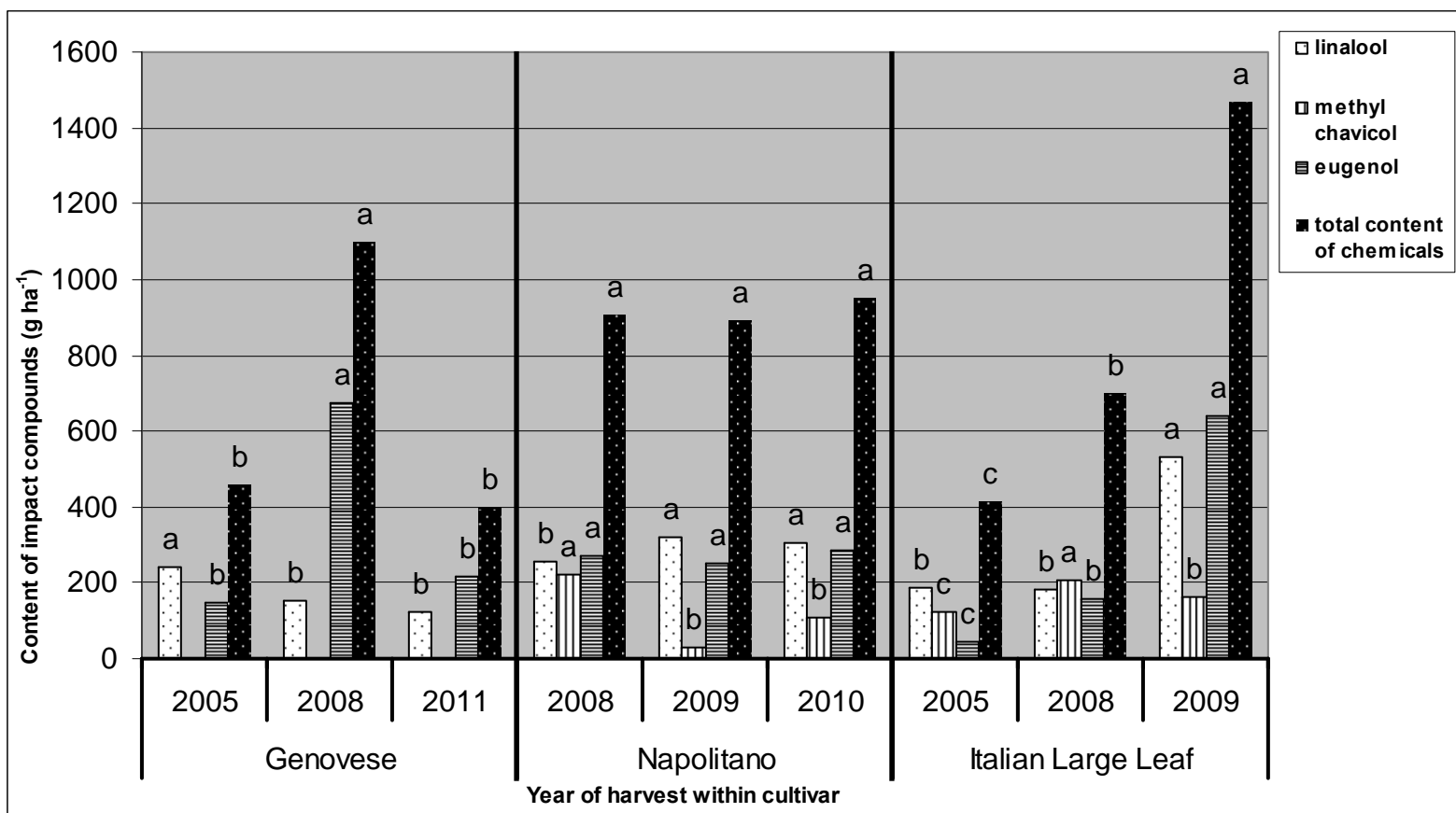


Fig. 7. Differences in impact compound contents of pesto type basil cultivars harvested at early August in three years. The letter above each bar shows differences among years in the same cultivar and phytochemical according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

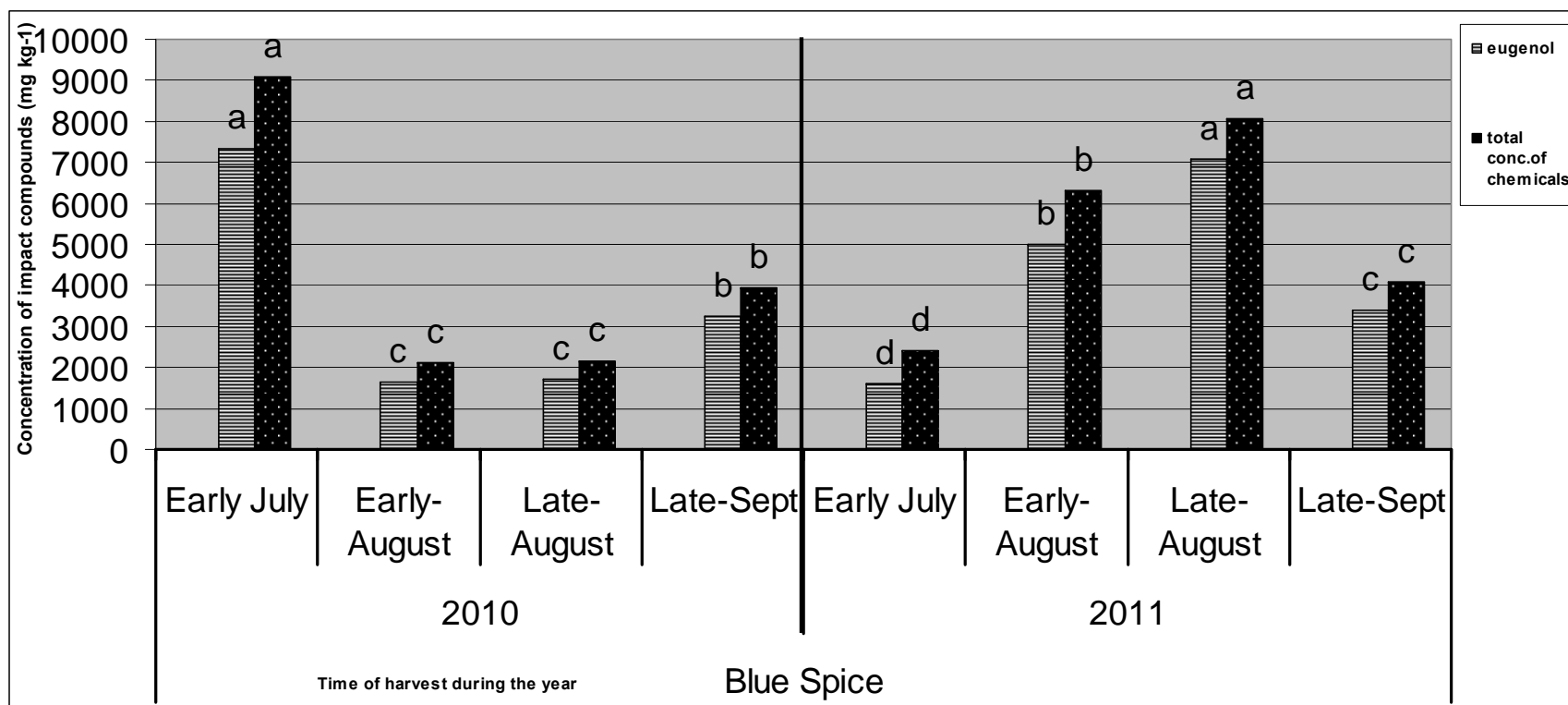


Fig. 8. Differences in impact compound concentrations of ‘Blue Spice’ basil harvested during early July, early August, late August, and late September in 2010 and 2011. The letter above each bar shows differences within each phytochemical and among different times of harvest in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

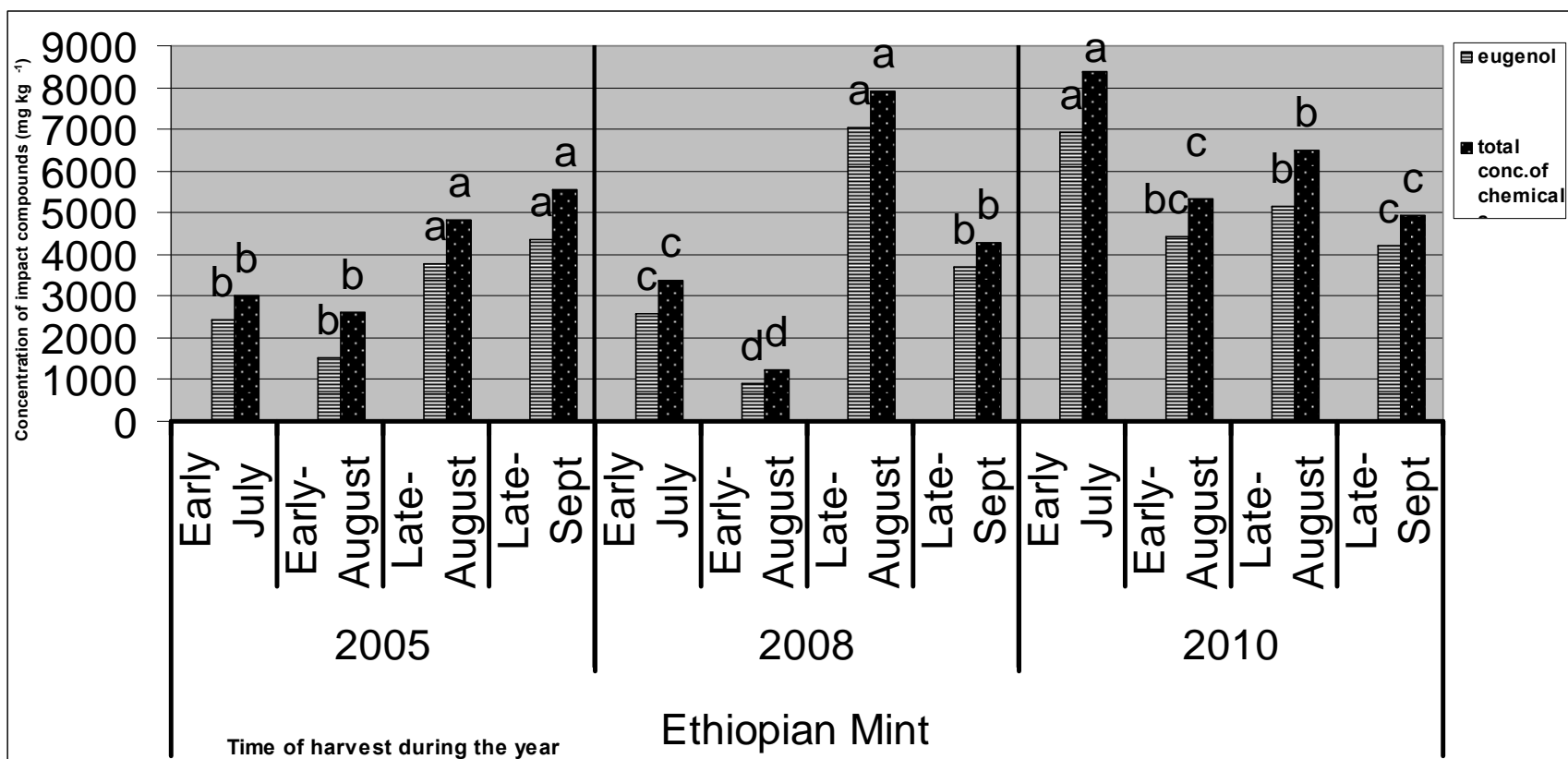


Fig. 9. Differences in impact compound concentrations of ‘Ethiopian Mint’ basil harvested during early July, early August, late August and late September in 2005, 2008 and 2010. The letter above each bar shows differences within each phytochemical and among different times of harvest in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

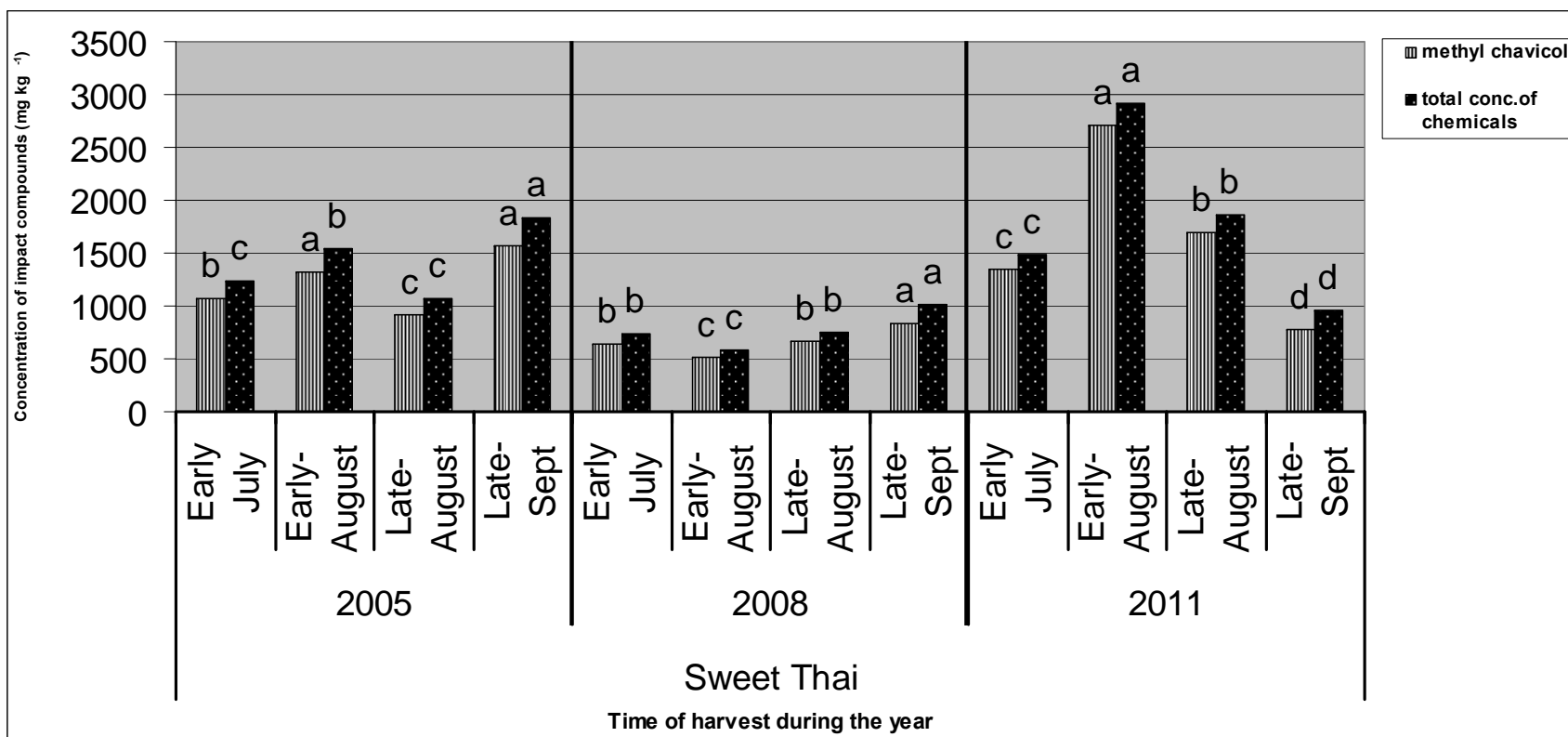


Fig. 10. Differences in impact compound concentrations of ‘Sweet Thai’ basil harvested during early July, early August, late August and late September in 2005, 2008 and 2011. The letter above each bar shows differences within each phytochemical and among different times of year in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

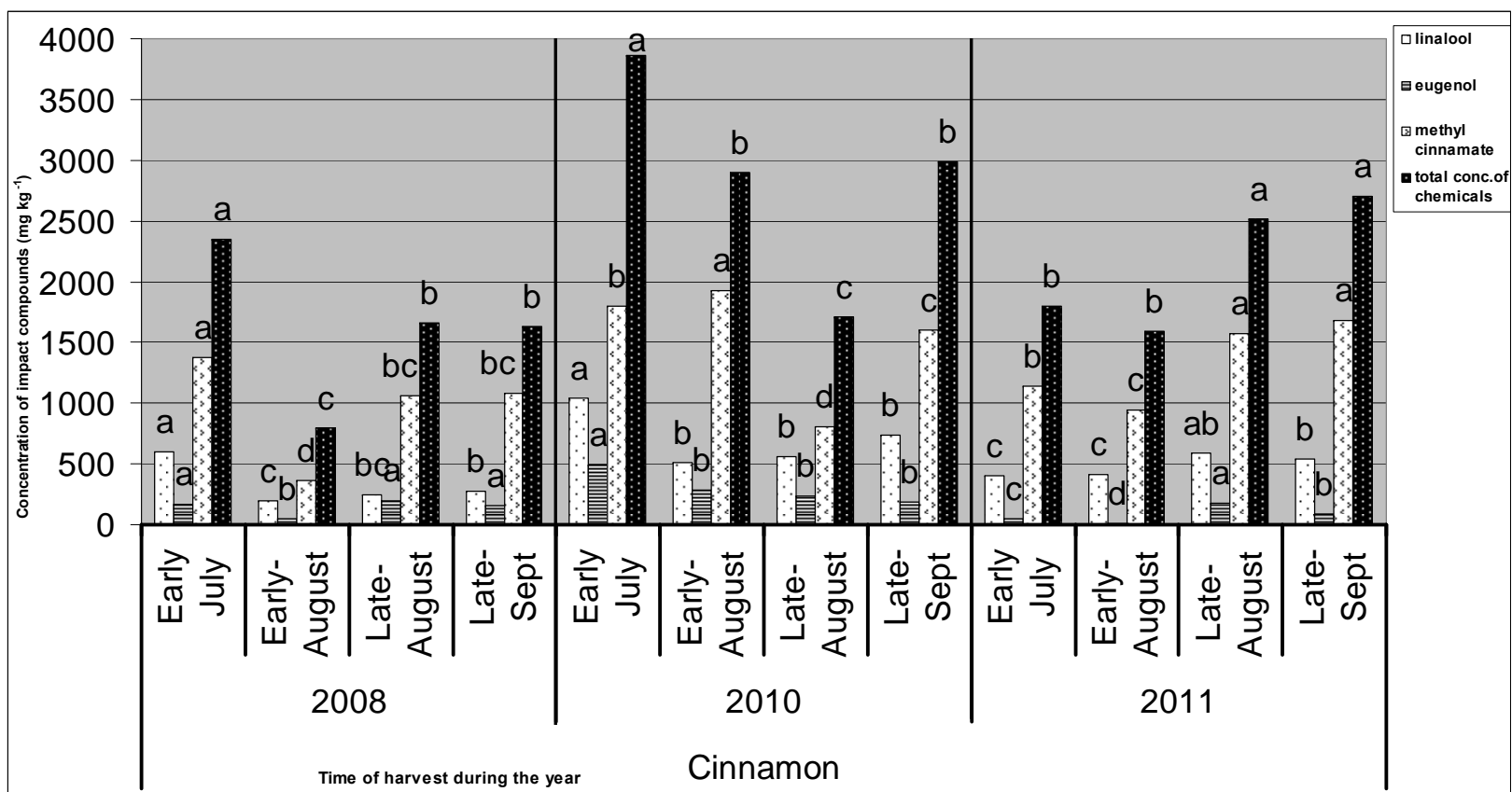


Fig. 11. Differences in impact compound concentrations of ‘Cinnamon’ basil harvested during early July, early August, late August and late September in 2008, 2010 and 2011. The letter above each bar shows differences within each phytochemical and among different times of year in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.



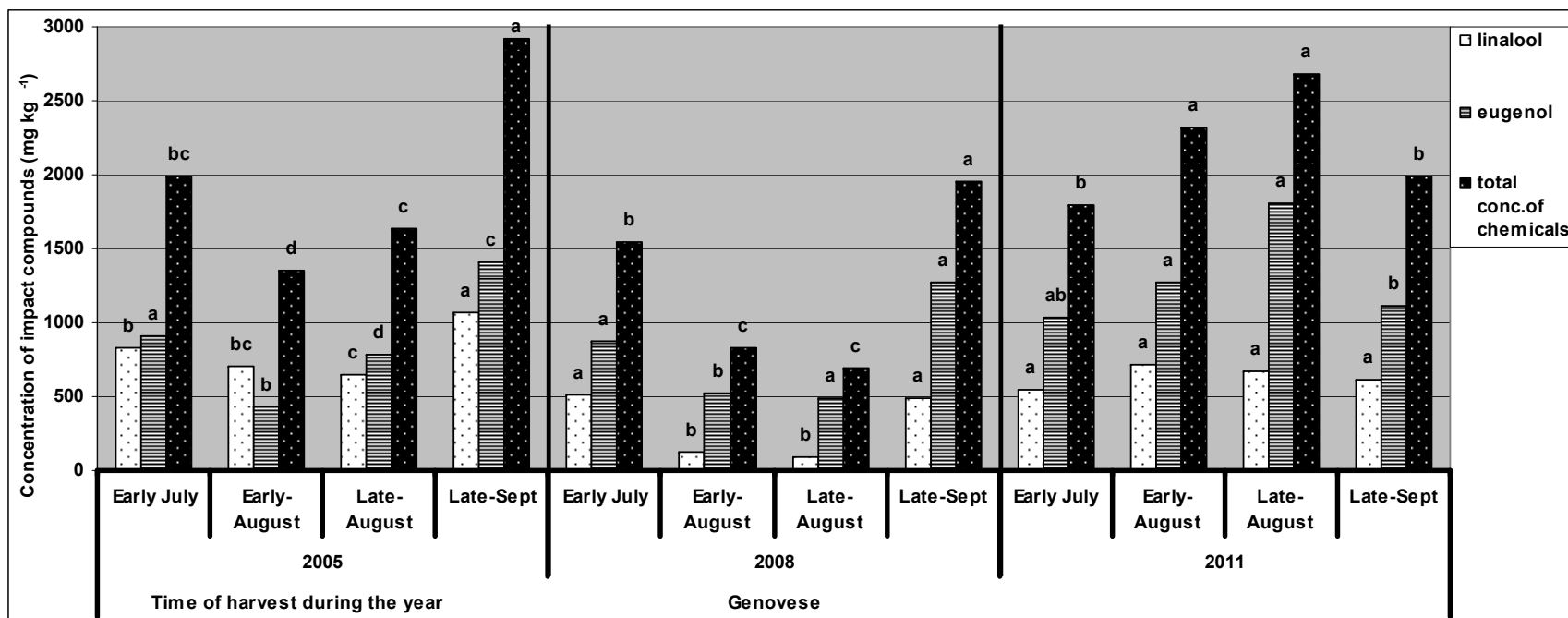


Fig. 12. Differences in impact compound concentrations of ‘Genovese’ basil harvested during early July, early August, late August and late September in 2005, 2008 and 2011. The letter above each bar shows differences within each phytochemical and among different times of year in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

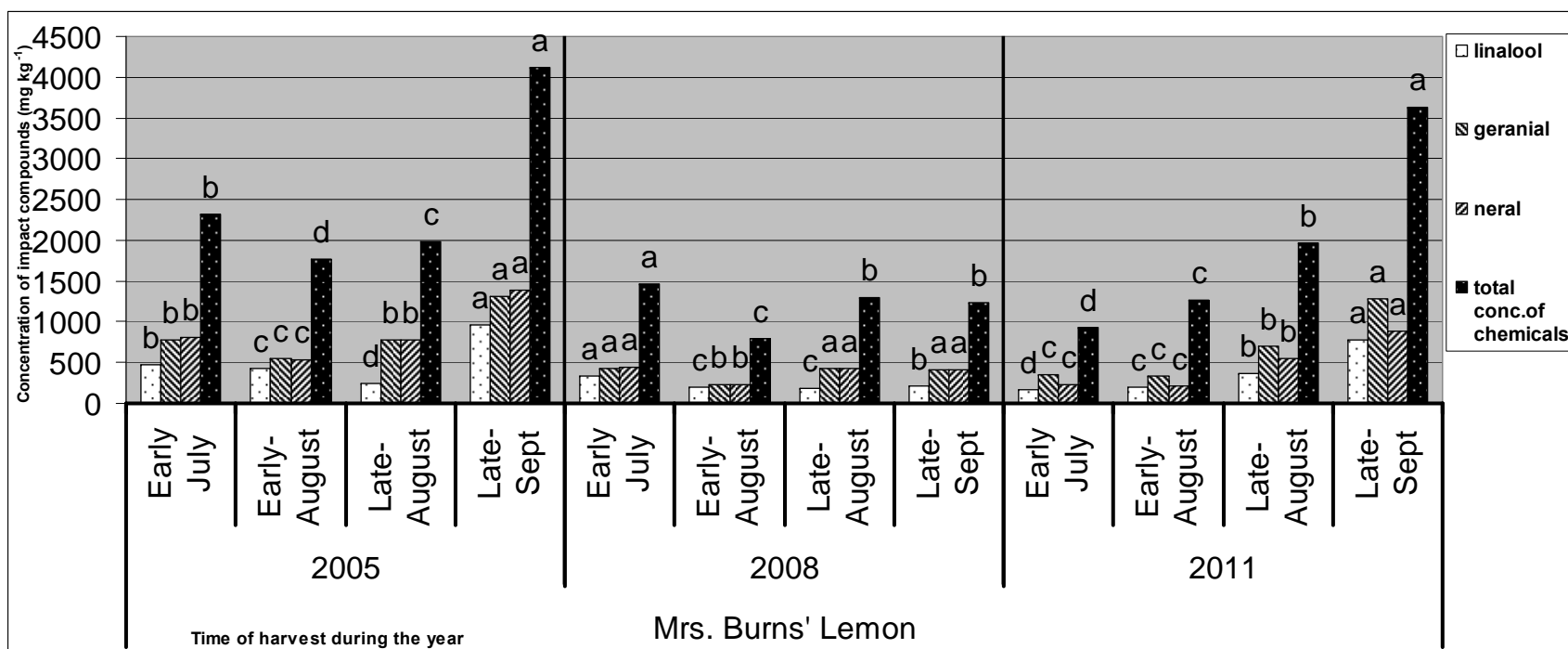


Fig. 13. Differences in impact compound concentrations of ‘Mrs. Burns’ Lemon’ basil harvested during early July, early August, late August and late September in 2005, 2008 and 2011. The letter above each bar shows differences within each phytochemical and among different times of year in the same year of harvest according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

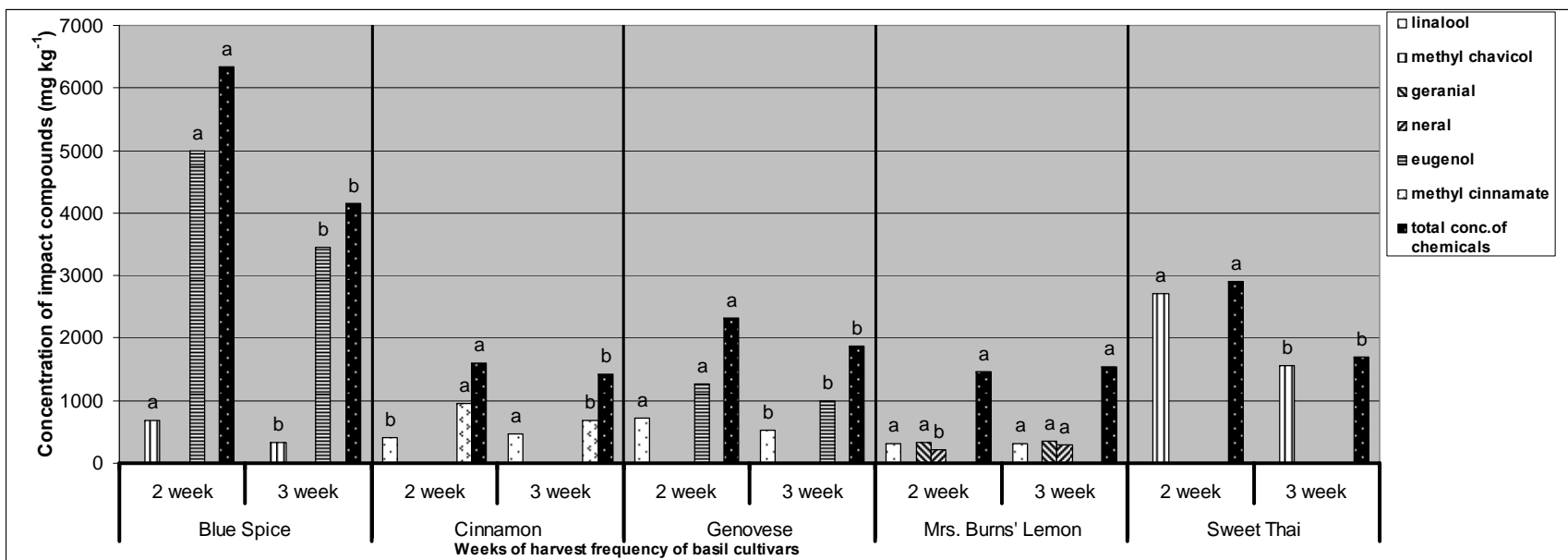


Fig. 14. Effect of harvest frequencies on impact compound concentrations in basil cultivars harvested at 2 week vs. 3 week frequencies from the early August harvest in 2011. The letter above each bar shows differences within each phytochemical between 2 weeks and 3 weeks harvest frequencies in the same cultivar according to student t test ( $P < 0.05$ ). Values represent mean of three replications. Each replication involved three injections.

## CHAPTER III

### EXTRACTION PROCESSING OF BASIL (*OCIMUM* L.) CULTIVARS GROWN IN OKLAHOMA BY MEANS OF PROPANE

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#### Abstract

The effect of an ambient temperature extraction process (ATE) with propane as extraction solvent on aromatic chemical extraction of six basil cultivars, ‘Napolitano’ (*Ocimum basilicum* L.; pesto type, linalool/methyl chavicol/eugenol rich), ‘Genovese’ (*Ocimum basilicum* L.; pesto type, linalool/eugenol rich), ‘Cinnamon’ (*Ocimum basilicum* L.; spice type, methyl cinnamate rich), ‘Sweet Thai’ (*Ocimum basilicum* L.; spice type, methyl chavicol rich), ‘Blue Spice’ (*Ocimum basilicum* x *Ocimum americanum*; spice type, eugenol rich) and ‘Mrs. Burns’ Lemon’ (*Ocimum basilicum citriodora* L.; citrus type, geranial and neral rich), as well as color property of the dry basil products from ‘Sweet Thai’ (flower, leaf and stems) were evaluated. After harvest, samples were dried at 40°C prior to separation into flowers, leaf and stem

components. Extractions were then conducted using a custom built continuous flow propane extractor at ambient temperature (21-27°C) and low pressure (1.1-1.9 MPa). Dried samples were ground before extracting with hexane while extracts were solubilized into hexane to identify and quantify aromatic chemical components using gas chromatography. Tristimulus  $L^*$ ,  $a^*$  and  $b^*$  values were used to calculate chroma, hue angle and browning index (BI) color attributes of unextracted 'Sweet Thai' and extracted parts. All of the extracts had a dark green-yellowish color and were enriched in aromatic chemicals typical of the starting material, such as lemon, cinnamon, spicy, clove or sweet pesto. The dry feedstocks of the ATE process after the extraction (raffinates) from leaf and/or flowers and stems of those cultivars appeared to be useable since their relative chemical profiles were similar to control samples with the same ratio of major chemicals resulting in no apparent change in flavor profile. Color of the separated controls and raffinates from 'Sweet Thai' was similar to a previously published commercial sample. Propane extraction appears to be feasible for dry basils. Cultivars differed in extraction kinetics, probably due to prevalence of trichome type for the cultivar. For 'Sweet Thai', extraction duration of 30 min should lead to optimum extraction of aromatic chemicals. However, extraction durations for other cultivars should be studied to optimize their propane extraction process.

## **Introduction**

Basils (*Ocimum* sp.) belong to the *Lamiaceae* family and are made up of several species. They are diverse in aroma and flavor and the herb may be used fresh or after drying to flavor foods, or aromatic compounds may be extracted and used for food flavoring and preservation,

pharmaceuticals, cosmetics and as a source of natural pesticides (Paton et al., 1999). Basil chemical profiles vary dramatically and can be classified according to dominant or impact chemical as (1) methyl chavicol-rich, (2) linalool-rich, (3) methyl eugenol-rich, (4) methyl cinnamate-rich, etc. (Lawrence, 1988) or in accordance with market use as sweet basils, spice basils, citrus basils, etc. The great diversity of chemicals making up the typical aroma of each individual basil, which are monoterpene hydrocarbons (limonene, myrcene, ocimenes,  $\gamma$ -terpinene, and p-cymene), oxygenated monoterpenes (linalool, camphor, 1,8-cineole, citral, citronellal, geraniol, thymol, geranial and neral), sesquiterpenes ( $\alpha$ -amorphene, bicycloelemen, bicyclogermagrene, cis-  $\alpha$ -bergamotene, trans-  $\alpha$ -bergamotene,  $\epsilon$ -bulgarene,  $\beta$ -bourbonene,  $\alpha$ -cadinene,  $\gamma$ -cadinene,  $\delta$ -cadinene, T-cadinol, calamene, caryophyllene oxide,  $\beta$ -cedrene, 2-epi- $\alpha$ -cedrene,  $\beta$ -cubenene,  $\beta$ -elemene,  $\delta$ -elemene,  $\alpha$ -farnesene, germacrene,  $\alpha$ -guaiene,  $\alpha$ -humulene, isocaryophyllene,  $\alpha$ -selinene,  $\beta$ -selinene,  $\beta$ -bisabolene,  $\beta$ -cadinene,  $\beta$ -caryophyllene,  $\beta$ -farnesene, germacrene,  $\gamma$ -muurolene,  $\alpha$ -gurjunene, scapanene,  $\alpha$ -elemene, and  $\alpha$ -bisobolene) and phenylpropanoids [methyl chavicol (estragol), methyl cinnamate, eugenol and methyl eugenol] (Paton et al., 1999), complicates their extraction and stability during extraction as well as during storage.

The most typical means of extraction of volatile compounds has been via distillation, with the distilled product termed as an “essential oil”. Volatile basil oils have been traditionally obtained with large scale commercial equipment via hydrodistillation (HD) or steam distillation (SD) to distill the oil from flowering tops and leaves. Common total oil yields were from 0.2% to 1.0% and maximum at 1.7% (ml 100g fresh weight<sup>-1</sup>) (Paton et al., 1999). The distillation was completed by 1.5 h using fresh material at above 100°C.

To characterize the essential oils from relatively small samples of basil tissues, Bicchi et al. (1983) developed a micro-scale apparatus for SD in which 1 g of basil (*O. basilicum* L.) material was extracted for 1h. This small sample size was useful for individual plant samplings but presented a challenge in drawing conclusions for a basil plant population. Charles and Simon (1990) employed SD as well as HD and solvent extraction to develop a simple, rapid and reliable technique for determination of essential oil yields from a larger sample of *O. basilicum*. The samples were oven dried at 30°C for one week and then 75 g of the dried sample was used for each extraction. For HD the material was placed in distilled water and distilled for 1 h at around 100°C. For SD steam was passed through the material for 1.5 h. Essential oil was recovered from steam by distillation and separation. Solvent extraction was carried out using hexane. They found that the oil yield from SD was significantly higher than those from HD and solvent extraction. The relative concentrations of major compounds were similar for both HD and SD, but the total amounts were higher for SD. These two methods offered high yields and efficient recovery of essential oil constituents compared to solvent extraction. HD was a more rapid and practical technique when steam was not available. During solvent extraction, losses of compounds due to thermal breakdown or co-evaporation during removal of the hexane solvent resulted in lower yield compared to the other methods. The distillation methods had one drawback, which was the change in the chemical structures of these extracts by means of hydrolysis, oxidation and trans-esterification of compounds through hydrolytic or thermal effects (Diaz-Maroto et al., 2002). Lucchesi et al. (2004) employed solvent-free microwave extraction (SFME) of the oil from basil (*O. basilicum* L.) as compared to conventional HD. They found the oil obtained by means of SFME for 30 min was richer in respect to percentages of valuable oxygenated compounds, such as eugenol and linalool, than from HD for 1.5 h. Linalool

increased from 25% to 39% by applying HD, but eugenol decreased from 43% to 11% by applying HD as compared to SFME. Shorter extraction times, energy saving and less CO<sub>2</sub> emission made SFME an attractive alternative for the extraction of volatile oil

Supercritical extraction (SCE) with CO<sub>2</sub> as solvent has become an alternative extraction technique. The dissolving powers of supercritical CO<sub>2</sub> can be adjusted by changing pressure and temperature conditions applied. Diaz-Maroto et al. (2002) recommended using range of 40-50°C temperature and 120 bar pressure for extraction of essential oils. They reported that SCE produced less standard deviation in oil yield and minimum thermal degradation compared to SD. Also, Gainar et al. (2002) suggested 40°C and 100 bar as optimum extraction conditions for the oil from dried leaves of basil (*O. basilicum*). They found that these SCE conditions resulted in an oil with higher content of oxygenated monoterpenes (11.7% of total detected compounds) and lower content of hydrocarbon monoterpenes (0.3% of total detected compounds) compared to the oil extracted by HD which contained 6.1% oxygenated monoterpenes and 1.6% hydrocarbon monoterpenes. The oil yields for SCE and HD were 0.65 and 0.84%, respectively. In the oil, methyl chavicol (estragol) was the major compound for both methods (74.7 and 80.2%, respectively). The odor of SCE oil was more similar to the basil feed stock than that of the HD oil (Gainar et al., 2002).

SCE produces extracts with similar properties to essential oils. Leal et al. (2006) employed SCE to extract *O. gratissimum* samples. They found that the highest yield (1.79%) was obtained at 40°C and 200 bar. Eugenol (35-60%) and β-selinene (11.5-14.1%) were the major compounds in the extract and 1, 8 cineole, trans-caryophyllene and α-selinene were detected in minimal amounts. Leal et al. (2008) also employed SCE using H<sub>2</sub>O as cosolvent. At 10 MPa and 30°C the total yield was 2% (g oil 100 g<sup>-1</sup> dry herb). Eugenol, germacrene-D,



cadinol, phytol, and neophytadiene were the major compounds. Menaker et al. (2004) applied ethanol as cosolvent in SCE at the conditions of 17.2-25.5 MPa at 45°C. At 17.2 MPa 3.8±0.4% total yield was achieved with linalool being a major compound (>20% of detected compounds) in the extract. However, at 25.5 MPa the yield was 4.4±0.4 % and linalool content decreased proportionally in the extract. Also, moisture content of natural materials affected extraction yield since a high amount of water would inhibit contact between supercritical CO<sub>2</sub> and lipid regions for SCE of oil (Kiran et al., 2000). Compaction of the material bed due to the high pressure can inhibit the flow of extract with CO<sub>2</sub>. However, changing the direction of flow or flow rate can alleviate the problem. Besides all these factors, extraction time, amount of solvent, solvent ratio (amount of solvent per unit of time to amount of solid), and extract removal (precipitation) conditions (temperature and pressure) from solvent would be the parameters determining the extraction yield/recovery and composition (Kiran et al., 2000). Although SCE is a powerful means to extract lipids from basil, it has the disadvantage of high operational complexity besides employing high pressures.

Compressed or liquefied propane can dissolve volatile and nonvolatile lipophilic substances at low temperatures. Due to its high volatility (boiling point is below -40°C), solvent removal of propane by vaporization can be achieved using mildly elevated temperatures. This causes less thermal deterioration of the compounds as compared to HD or SD and results in greater retention of chemicals in the extracts following solvent vaporization.

The pressures applied in the propane extraction are around one to two orders of magnitude lower than when using CO<sub>2</sub>, which can exceed 300 bars. Unlike subcritical/supercritical CO<sub>2</sub>, subcritical propane provides a high solvent capacity for lipophilic compounds at relatively low extraction pressure and results in faster extraction (Heidlas, 1994).

Liquefied propane becomes economically more attractive than SCE for many applications (Heidlas, 1994). Exposure of the feedstock and extract to milder processing temperatures and pressures results in less flavor modification. Organoleptic tests in roasted cocoa indicated that the typical aroma was conserved by employing propane for fat reduction applications (Heidlas, 1994).

Propane extracted at least 2 times more mass of *M. grandiflora* than supercritical CO<sub>2</sub> because propane had two times higher density than CO<sub>2</sub> at the experimental conditions (40-50°C temperature) (Castaneda-Acosta et al., 1995). Supercritical CO<sub>2</sub> provided extract with significantly less chlorophyll compared to propane, which was indicated by less yellow color of its extracts compared to propane extract. Liquefied propane rapidly and efficiently extracted oleoresin from cardamom seeds; lower ratio of propane:seeds was required for complete extraction compared to CO<sub>2</sub> (Hamdan et al., 2008). The β-Carotene content of extracts from propane was significantly higher than that from CO<sub>2</sub>. The maximum yield for propane was 7.24 g extract/100 g seed, being significantly higher than the 6.65 g extract/100 g seed for CO<sub>2</sub> at 55 °C, and 30MPa (4,351 psi). For paprika, propane extraction was more efficient than supercritical CO<sub>2</sub> extraction in the extraction of carotenoids and tocopherols but not capsaicinoids which it hardly solvated (Gnayfeed et al., 2001).

Non-toxicity, mild process conditions, high selectivity for lipophilic compounds, noncorrosiveness, recoverability and stability make propane an ideal solvent for agricultural commodities especially for applications requiring maintenance of flavor and functionality of food or pharmaceutical ingredients. Although propane has an explosion hazard, it has a narrow range of flammability compared to the other petroleum solvents, reducing danger of fire possibility (National Propane Gas Association, 2004).

In many cases, since many lipophilic breakdown products are co-extracted from the feedstock, ambient temperature extraction (ATE) with propane maintains functionality of dried herbs, retains a high level of flavor and is shelf stable. For herbs/vegetables, ATE extraction parameters can be optimized to only partially extract compounds and somewhat selectively remove compounds including lower molecular weight degradation products which lead to off flavors (Kanamangala et al., 1999). The aim of this study was to evaluate the effect of ATE process on chemical profiles as well as color property of the dry basil products from various cultivars, which would result in a high quality dry feed stock (raffinate) as well as valuable extract with many uses.

## **Materials and Methods**

### **Chemicals**

The following standards were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A) for analysis of volatiles in purity of >95%: Heptanol,  $\alpha$ -pinene, camphene,  $\beta$ -pinene, myrcene, limonene, cineole, ocimene,  $\delta$ -terpinene, fenchone, terpinolene, linalool, camphor, borneol,  $\alpha$ -terpineol, methyl chavicol, citral (geranial and neral), geraniol, bornyl acetate, thymol, carvacrol, eugenol, methyl cinnamate, methyl eugenol,  $\beta$ -caryophyllene, humulene, and thujone. N-hexane was purchased from Fisher Scientific (FairLawn, NJ). Instrument grade propane was obtained from AirGas (Radnor, PA).

## Plant Materials

Basil transplants were grown in a greenhouse at the Oklahoma State University Horticulture Research Greenhouse Facility in Stillwater. Cultivars acquired from Johnny's Seed Co. (Winslow, ME) were seeded in 200 cell Speedling trays (Speedling Inc., Sun City, FL), filled with SunGro Redi-earth Plug and Seeding mix (SunGro Horticulture Canada Ltd., Vancouver, Canada). After emergence, basil plants were watered and fertilized to maintain optimum growth, and thinned to one plant per cell. Seedlings were transplanted into field plots after reaching the 3-5 leaf stage at the Oklahoma Vegetable Research Station near Bixby. Transplanting was done with a two row cone type transplanter (Holland Transplanter Co., Holland, MI) in mid-to-late April in 2009, 2010 and 2011 with between row spacing of 91 cm and plants spaced approximately 38 cm apart within the row. Plots were arranged in a randomized complete block design with four replications. The soil at the experimental site was Severn very fine sandy loam (coarse-silty, mixed [calcareous], thermic Typic Udifluvents) with 0.8% organic matter.

Plots were 11 m (thirty seven feet) in 2009 and 23 m (seventy five feet) in 2010 and in 2011. No herbicide was applied to the plots, and they were watered as needed from overhead sprinklers with approximately 1.27 cm ( $\frac{1}{2}$  inch) water per application. Plots were fertilized before planting with 56 kg ha<sup>-1</sup> N (50 lb ac<sup>-1</sup> N) using urea. A top dress application of an additional 34 kg ha<sup>-1</sup> N (30 lb ac<sup>-1</sup> N) was applied as urea after a month and again following each harvest. Urea was water incorporated from dry soil surface within 24 h of application. Harvests were conducted prior to full flowering starting in early July and ending in late September at three to four week intervals and between 10 am and 2pm. During 2011, harvest frequency was varied on strict 2 week or 3 week intervals.

Cultivars used in 2009 were ‘Napolitano’ (*Ocimum basilicum* L.; pesto type, linalool/methyl chavicol/eugenol rich), ‘Cinnamon’ (*Ocimum basilicum* L.; spice type, methyl cinnamate rich), and ‘Mrs. Burns’ Lemon’ (*Ocimum basilicum citriodora* L.; citrus type, geraniol and neral rich), acquired from Johnny’s Seed Co. (Winslow, ME). In 2010, ‘Genovese’ (*Ocimum basilicum* L.; pesto type, linalool/eugenol rich), ‘Sweet Thai’ (*Ocimum basilicum* L.; spice type, methyl chavicol rich), and ‘Blue Spice’ (*Ocimum basilicum* x *Ocimum americanum*; spice type, eugenol rich) were used. In 2011 ‘Sweet Thai’ was used.

Harvest was done using a Kincaid self propelled greens harvester (Haven, KS). Cutting height was 28 cm (11 inches) for all varieties except for ‘Sweet Thai’, cut at 15 cm (6 inches). Precooled samples (mixture from four replications) were transported on ice to Stillwater. All samples were held at 4°C overnight before sample processing and drying. All samples were examined for damage, and any damaged sample by harvester or otherwise injured or showing signs of discoloration or dehydration, in addition to extraneous material, was discarded. Sound plant material was double washed and spin dried to remove excess water using a greens washer (model AD92, Dynamic, Oxnard, CA). Samples (≈1kg each) were then placed into cheesecloth and securely tied prior to a forced air drying in a forced air drier (Proctor and Schwartz, Inc., model P070146, Horsham, PA) at 40°C temperature until equilibrium weight was achieved. Moisture content of fresh samples and equilibrium moisture content of forced air dried samples was determined by drying in an oven at 70°C, and the moisture content (MC%) was calculated on a dry weight basis as follows:  $MC\% = (Fresh\ weight - Dry\ weight / Fresh\ weight) * 100$ . The remainder of the samples were weighed, placed into a labeled freezer bag, capped with nitrogen and securely sealed before placing into a -20°C freezer.

## **Extraction**

Dried samples were taken from -20 °C storage and allowed to thaw and equilibrate to room temperature before opening the freezer bag. The dried samples were crunched inside the freezer bag to separate stems from leaves and flowers, and then the plant material was hand separated into leaves/flower buds and stems. Then, by sieving through a kitchen stainless steel mesh strainer (#10), leaves were separated from flower buds.

The percentage of the leaf, flower buds and stem in the whole plant were calculated for each species. Prior to extraction, stems were ground to a course powder with a Vitamix (Vitamix Corp., Cleveland, OH) grinder. Stems were ground in three to five short pulses (5 to 10 sec each) to produce a consistent particle size (0.425 - 0.5 mm).

Extractions were conducted using a custom built continuous flow propane extractor (Eden Labs, Columbus, OH). The extraction system consisted of an eleven liter propane storage vessel, a five liter extraction vessel, two 7.4 liter primary and secondary separator vessels, and a vapor pump (Air driven gas booster pump, Model 5LG-TS-4, Hydraulics International Inc. Chatsworth, California U.S.). Samples ( $\approx$ 1 kg) were weighed into non-bleached cotton pull string bags and loaded inside the 5 liter extraction vessel. After sealing the vessel air was removed under vacuum to at least 68 kPa (20 inches) of vacuum and extractions were initiated with liquid propane at a flow rate of 0.64 lpm (0.17 gpm) for 20 min at temperature between 21° and 27°C and pressure between 1.1 and 1.9 MPa (140-265 psig). Propane was continuously recovered, and the extract fraction was separated by propane vaporization in separator vessels heated to 32° to 35°C. The extract and the raffinate were weighed to determine extraction percent, based on extract weight and on raffinate weight loss. Finally, they were stored at -20°C

until performing chemical analysis. To determine the effect of extraction time on extract yield and concentrations of volatiles (mg/kg), an extraction time course experiment from 10 min to 40 min or 60 min was conducted at 10 min intervals for separated parts (leaves, flowers and stems) of 'Sweet Thai'.

### **Chemical Analysis**

Representative sub samples ( $\approx 15$ g) from separated basil parts prior to and after ATE were evaluated for moisture content and employed for chemical analysis. After bringing samples to room temperature, they were ground using an UDY cyclone mill (UDY Corporation, Boulder, CO) to pass a 1 mm screen. After mixing to assure sample uniformity, ground samples (approximately 0.5 g) were weighed in triplicate into tared vials (2 dram; 7.4 ml volume) and 100  $\mu$ l of thujone or  $\delta$ -terpinene (1000 nmole) (Sigma-Aldrich Corp., St. Louis, MO) was added as an extraction internal standard. Extraction was carried out using 4 ml of hexane with stirring for 20 min, and samples were centrifuged in a Speed Vac apparatus (SVC-100H Savant Instrument Inc., Farmingdale N.Y.) at 3,000 g for 20 min. Supernatants were transferred into 2 dram vials and then re-centrifuged for 20 min to remove any transferred ground sample. This step was repeated 2-3 times until supernatants free of solids were transferred into tared 2 dram vials. Then, each vial with liquid was weighed, and their weights were recorded to calculate volume of supernatants and then concentration of each component in a sample.

For analysis of basil propane extracts, the samples were also brought to room temperature. After mixing to assure sample uniformity, approximately 0.05 g extract was weighed into a tared centrifuge tube (50 ml, Fisher Scientific, FairLawn, NJ). 100  $\mu$ l of thujone or  $\delta$ -terpinene (1000 nmole) (Sigma-Aldrich Corp., St. Louis, MO) was added as an extraction internal standard. The

extract was solubilized using 10 ml of hexane, and samples were centrifuged in a Fisher Centrifuge (Fisher Scientific, FairLawn, NJ) for 20 min. Supernatants were transferred into 2 dram vials and then re-centrifuged for 20 min to remove any foreign material in the sample. This step was repeated around 2-3 times until supernatants free of solids which were transferred into tared centrifuge tube. Then, each tube with liquid was weighed, and their weights were recorded to calculate volume of supernatants and then concentration of each component in a sample.

The moisture contents of the ground samples were determined and used as a correction of result of the concentration of each component in a sample to a dry weight basis. Randomly selected ground samples (approximately 0.5 g) were weighed in triplicate into three tared tins. Moisture content was determined by drying in an oven at 70°C and evaluated as previously described for fresh samples.

A gas chromatograph (Varian Star 3400 Cx, Varian Inc., Walnut Creek; CA) equipped with FID detector was used to analyze the chemical components of basil control, raffinate and extracts. Injector temperature was 40°C and detector temperature was 300°C. Separations were carried out on a DB-5 fused silica capillary column (30m x 0.25 mm, 0.25 µm film thickness; J and W Scientific Inc., Rancho Cordova, CA) with He carrier gas at a linear flow velocity of 20 cm/sec. Oven temperature was maintained at 55°C for 2 min, raised at 2°C/min up to 75°C, then raised at 1°C/min up to 95°C, then immediately raised at 2°C/min up to 160°C and finally raised at 20°C/min up to 280°C and held for 10 min. Injector temperature was 40°C during sample injection, then immediately raised at 100°C/min up to 290°C and held for 5 min. Just prior to injection of 1 µl of sample, samples were prepared by mixing 975 µl of sample with 25 µl of 2-heptanol (250 nmole) (Sigma-Aldrich Corp., St. Louis, MO) used as analytical internal standard.



One  $\mu\text{l}$  of sample was injected for GC analysis. Sample components were identified by coelution with authentic standards and quantified using 2-heptanol as internal standard. Extraction recovery was estimated using thujone or  $\delta$ -terpinene as internal standard. The same procedures above were also followed for the chemical analysis of the basil extracts.

### **Color Analysis**

To determine the effect of propane extraction on color, control samples and samples extracted for 20 min of separated leaf, flower and stem parts of 'Sweet Thai' were subjected to a colorimetric analysis. Five replicates of randomly selected 1 g of each treatment were crunched to produce a uniform particle size (0.85-1.18 mm). Tristimulus  $L^*$ ,  $a^*$  and  $b^*$  values were obtained with a Minalto Chroma meter CR-300 (Minolta Co., Ltd., Japan) and then the readings were averaged. The results were used to calculate chroma, hue angle and browning index (BI) by the formulas as mentioned below:

$$\text{Chroma} = (a^2 + b^2)^{0.5}$$

$$\text{Hue Angle} = \tan^{-1}\left(\frac{b}{a}\right) + 180$$

$$\text{BI} = \frac{[100(x - 0.31)]}{0.17}$$

$$\text{where } x = \frac{(a + 1.75L)}{(5.645L + a - 3.012b)}$$

## Results and Discussion

In this study, we chose to include six cultivars of basil (*Ocimum* sp.) assessing the effect of a propane ATE process on aromatic chemical extraction. These six cultivars could be grouped into three types (citrus, spice and pesto) according to their flavor or uses: a citrus type ‘Mrs. Burns’ Lemon’ basil contained geraniol, neral and linalool as predominant chemicals; spice types ‘Sweet Thai’, ‘Blue Spice’ and ‘Cinnamon’ were morphologically different and their major chemicals were dissimilar with ‘Sweet Thai’ containing predominantly methyl chavicol, ‘Blue Spice’ containing eugenol and ‘Cinnamon’ containing predominantly methyl cinnamate and linalool to a lesser extent; within pesto types linalool, methyl chavicol and eugenol predominated in ‘Napolitano’, and linalool and eugenol predominated in ‘Genovese’.

The major volatile chemicals mentioned above are produced and stored in various types of glandular trichomes (glands) distributed through the aerial parts of basil plants and concentrated mostly on the leaves and floral structures (Iijima et al., 2004). These six cultivars studied also exhibited different types of glands: We observed that ‘Blue Spice contained hair type glands present through all the aerial parts including stems; citrus type ‘Mrs. Burns’ Lemon’ had elongated capitate type trichomes (Handilou et al., 1991); and ‘Italian Large Leaf’, similar to our ‘Genovese’ cultivar, had short peltate type trichomes concentrated on mostly flowers and leaf (Handilou et al., 1991). During extraction the glands may rupture to release their contents; leaf and flower tissues contain more trichomes (Iijima et al., 2004) and would be expected to yield more extractable volatiles, especially if trichomes containing these chemicals are easily ruptured.

Examination of aromatic profiles of extracts of ‘Mrs. Burns’ Lemon’, ‘Genovese’ and ‘Cinnamon’ basil cultivars, obtained via propane extraction of combined flowers and leaves (20 min duration), using GC analysis revealed that all the extracts included monoterpene compounds, such as  $\beta$ -pinene, myrcene, limonene and ocimene, and sesquiterpenes, such as  $\beta$ -caryophyllene and humulene, in minor amounts (Table 7). All of them also had variety of phenylpropanoid compounds, such as camphor, methyl chavicol, eugenol, methyl cinnamate and methyl eugenol; methyl cinnamate predominated in ‘Cinnamon’, and eugenol predominated in ‘Genovese’. Oxygenated monoterpenes were also included in the extracts: cineole was featured in both ‘Mrs. Burns’ Lemon’ and ‘Genovese’ extracts; geranial, geraniol and neral were included in only ‘Mrs. Burns’ Lemon’ extract; and linalool was an impact chemical in all of them (>20% of total chemical concentrations).

Total concentration of all these chemicals appeared to be higher in ‘Genovese’ and ‘Cinnamon’ than ‘Mrs. Burns’ Lemon’ in the extract (table 7), attributed to presence of major phenylpropanoid compounds in ‘Genovese’ and ‘Cinnamon’ and their substantial removal with propane in those two cultivars. Methyl chavicol and methyl cinnamate were more readily extracted than eugenol and the other major oxygenated compounds (Table 8). Linalool was significantly reduced in concentration in all cultivars except ‘Genovese’ (Table 8). The sesquiterpenes  $\beta$ -caryophyllene and humulene were slightly extracted with propane in those three type cultivars;  $\beta$ -caryophyllene was significantly reduced by extraction in ‘Mrs. Burns’ Lemon’ only, (Table 8), which resulted in a notable enrichment in the extract (Table 7).

Chemical profiles of ‘Cinnamon’ and ‘Mrs. Burns’ Lemon’ extracts were enriched in linalool (Table 7) and linalool was significantly reduced in the raffinates after propane extraction (Table 8). More than two times increase in linalool content was also documented in the extract

of cardamom seed obtained via propane extraction at the conditions of 5 MPa and 25°C compared to SCE extraction at similar conditions (Hamdan et al., 2008), which is in agreement with our results for those two cultivars. A significant reduction in linalool concentration was not observed in ‘Genovese’, although the extract contained a substantial concentration of linalool (Table 7 and 8). A lower proportion of flowers in ‘Genovese’ (Table 9), known to have higher concentration of linalool than the other parts of basil plants (Charles et al., 1990; Munoz-Acevedo et al., 2010), may have contributed to lower extraction especially since trichomes of leaves in ‘Genovese’ more likely more peltate and thus more difficult to rupture/extract than those of flowers (Werker, 2000) (Table 9). Our ‘Genovese’ extract contained similar amount of linalool and higher amount of eugenol compared to the basil (*Ocimum basilicum* L.) extract obtained via SD (Hasegawa et al., 1997). At similar conditions of extraction, SCE provided similar amounts of linalool to SD in basil (*Ocimum basilicum* L.) (Diaz-Maroto et al., 2002).

Previous studies assessing comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) showed substantial differences between their compositions (Charles et al., 1990; Tansi and Sengul, 2000). In this study we determined aromatic profiles of propane extracts of separated flowers, leaves and stems of ‘Blue Spice’ and ‘Sweet Thai’ basil cultivars (Table 10). The relative chemical profiles of the leaf, flower and stem extracts within each cultivar had the same major chemicals; ‘Blue Spice’ leaf and flower extracts exhibited higher concentrations of eugenol and methyl chavicol while the stem extract had mostly eugenol, and all ‘Sweet Thai’ extracts included methyl chavicol as only major chemical. Stem extracts contained significantly lower total chemicals compared to leaves and flowers in both cultivars (Table 10). Cultivars differed in relative ranking for total extracted chemicals in leaves and

flowers with 'Blue Spice' yielding greater total chemicals from leaves and 'Sweet Thai' yielding greater total chemicals from flowers in two years.

Chalchat and Ozcan (2008) also found methyl chavicol predominated in the essential oils of the 'Purple' basil (*Ocimum basilicum* L.) flowers and leaves grown in Turkey (58% and 53% of total identified compounds, respectively) but methyl chavicol was only 16% of all identified compounds in the essential oil of the stem. Hasegawa et al. (1997) also detected that methyl chavicol was a major chemical (51-83% of total identified compounds) in the essential oils obtained by steam distillation of leaf and stem of four local basil cultivars (PK-1, 2, 3 and 4), (*Ocimum basilicum* L.) grown in Philippines. These oils had powerful spicy odor and dark green color similar to our propane extracts from 'Sweet Thai' cultivar. Similar to 'Blue Spice', leaf extract of clove basil (*Ocimum gratissimum* L.), grown in Brazil, obtained by SCE included eugenol as a major chemical (41-47%) (Leal et al., 2006). Also, the essential oil from leaf and flower of spice basil (*Ocimum gratissimum* L.) grown in India contained 63% and 56% eugenol of total identified compounds, respectively (Kothari et al., 2005), which agreed with our results.

The differences in total concentrations of impact chemicals among plant parts in extracts from both cultivars with ranking of leaves > flowers > stems for 'Blue Spice' and flowers > leaves > stems for 'Sweet Thai' (Table 10) was probably due to higher concentrations in the respective plant parts in the dry herb (Table 11). 'Blue Spice' leaf extract included the highest concentrations of all the individual chemicals except  $\beta$ -caryophyllene, but 'Sweet Thai' flower extract included the highest concentrations of all individual chemicals except  $\beta$ -caryophyllene and humulene (Table 10). A greater degree of reduction of chemicals in stems from dry herb for the raffinates was probably due to our particle size reduction; while stems were ground to a coarse powder, leaves and flowers were left intact prior to extraction. The major impact

chemical eugenol in 'Blue Spice' was more readily extracted than from 'Genovese' (Table 8 and 11). We noted a substantial presence of hair-like trichomes in 'Blue Spice' with highest prevalence in leaf. These trichomes should have been readily extractable and perhaps more easily ruptured than the peltate trichomes of 'Genovese'. Since 'Genovese' contained different trichome structure, the observed difference in chemical extractability may have been due to greater tissue resistance due to differences in tissue and trichome structure.

Extractions were carried out for 10, 20, 30, 40, 50 and 60 min for separated leaves and 10, 20, 30 and 40 min for separated flowers and stems of 'Sweet Thai'. Total aromatic chemical recoveries in extracts from the plant parts are presented in figure 15, and individual chemical recoveries are shown in table 12. Extraction time and total concentration of the chemicals were most highly correlated for stems ( $R=0.978$ ) followed by flowers ( $R=0.859$ ) and leaves ( $R=0.564$ ). The constant extraction rate period, the falling extraction rate period and diffusion controlled extraction rate period, typical for extraction course of natural materials (Wan and Wakelyn, 1997), were observed for all the parts (Fig. 15). During the constant extraction rate period, disruptions of trichomes by extraction solution take place in a constant rate and most of the essential oil is easily extracted from those structures. In the second period, the oil tied to membranes of trichomes is extracted and at the last period diffusion through the stretched membranes takes place at a steady state (Stamenic et al., 2008).

The kinetics of SCE depended on the preparation of the material and its morphology (Wan and Wakelyn, 1997). Although those periods mentioned above were seen for all the parts, they can be more clearly observed in the phenomenon of flower and stem extraction probably due to more homogenous nature of the ground stem material and smaller size of flowers compared to the leaves. The slight S shape of the extraction curve of the leaf was similar to the S

shapes of the yield (%) curves for SCE extractions of leaf of Lamiaceae family species, such as basil, rosemary, marjoram and pennyroyal studied (Zizovic et al., 2005). Those S shapes were due to period of low essential oil concentrations in the extractor vessel before peltate gland cracking and aromatic chemical removal by supercritical CO<sub>2</sub> (Stamenic et al., 2008). Standard deviations of total concentration of chemicals in flowers vs. extraction time seemed to be higher than those in the other parts of 'Sweet Thai' (Fig. 15). This deviation may be contributed to heterogeneous structure of the original flower material (control) which includes petal, sepal and pedicel parts.

In leaves there was a significant increase in total chemical yield after 10 min of extraction; total chemicals remained constant after 20 min extraction (Table 12). All leaf individual chemicals followed the same trend except linalool and eugenol which required 30 min of extraction to reach maximum concentration. Flowers and stems required a longer extraction duration to reach their maximum total aromatic chemical yield than leaves. Instead of reaching equilibrium concentration by 20 min extraction the flowers and stems didn't reach equilibrium until 30 min (Table 12). Methyl chavicol was the major aromatic chemical in extracts from all plant parts and at all extraction durations; Cineole was also a predominant component of all extracts, mirroring their concentrations in the dry herb (Table 11). While our results indicated that similar extract chemical profiles could be expected during extraction progress, Mukhopadhyay (2000) found that compositions of SCE with CO<sub>2</sub> extracts from flowers and leaf of sage (*Salvia officinalis*) varied with time; sesquiterpenes were present in higher concentrations in later fractions. Also, Daood (1999) recorded that considerable quantity of pigment, such as carotenoids, was lost during SCE with CO<sub>2</sub> process as a function of degradation.

Since linalool and the major phenylpropanoid compounds are the main contributors to aroma in basil, the propane extracts retained more of the sensory attributes like SCE extracts

(Diaz-Maroto et al., 2002). As a result, all of our extracts from six different cultivars appeared to preserve their original flavors, such as lemon, cinnamon, spicy, clove or sweet pesto. Our observation in leaves indicating that linalool and eugenol may require more time to reach equilibrium concentration in extracts indicates that for consistent flavor the leaves should be extracted for 30 min, even though total aromatic chemicals had reached equilibrium by 20 min (Table 12). Propane extraction is also known to solubilize high amount of lipid as well as pigments (Heidlas, 1994); the propane extraction yielded maximum content of chlorophylls and pheophytins at 5MPa and 25°C in cardamom extract (Hamdan et al., 2008). Similarly, all extracts obtained from ATE process with propane from six cultivars had dark green-yellowish colors.

The dry feedstocks of the ATE process after the extraction (raffinates) are useable products from basil herb whose properties were also documented. The relative chemical profiles of the raffinates from leaf and/or flowers of ‘Mrs. Burns’ Lemon’, ‘Napolitano’, ‘Cinnamon’, ‘Genovese’, ‘Blue Spice’ and ‘Sweet Thai’ as well as stems of ‘Blue Spice’, ‘Sweet Thai’ and ‘Genovese’ were not substantially different from the control samples since the major impact chemicals % were similar after the extraction (Table 8 and 11), indicating no changes in flavor. Since total chemical concentration was usually reduced by extraction (Table 8 and 11), flavor intensity would be expected to decrease in extracted basil.

Color is a physical property and the first quality parameter evaluated by the consumer for dry herbs. The final color measurement of the dried product can be used as quality indicator to evaluate deterioration caused by processing (Demirhan and Ozbek, 2009). In this study, we documented color attributes of the control vs. raffinate samples from leaf, flower and coarsely ground stem parts of ‘Sweet Thai’ cultivar grown in 2010 and 2011, and compared our results to



a commercial basil leaf sample obtained from Yousif et al. 1999 (Table 13). Only L\* value of the leaf control sample from season 2010 was higher than L\* value of the leaf raffinate sample which would indicate less brightness and more browning in the raffinate. There were no differences in a\* or b\* values between the control and raffinate samples of all the parts which means they had the same degree of greenness and yellowness due to no significant degradation of chlorophyll and carotenoid pigments during ATE process (Demirhan and Ozbek, 2009). No differences in chroma or hue angle values between the control and raffinate samples supported this result. A decrease in BI for stem after extraction was observed probably due to removal of browning products by means of ATE process with propane.

Color attributes of the leaf samples before or after ATE process (Table 13) were also compared to commercial basil leaf sample obtained from Yousif et al. (1999). Our control leaf sample from 2010 had similar L\* value to commercial basil leaf sample. All the stem samples had higher L\* values than the commercial basil sample indicating brighter and less browning in our samples. All samples had higher a\* values than the commercial basil sample indicating they were greener than the commercial one. The b\* values did not appear to be substantially different from b\* value of the commercial sample perhaps indicating no significant decomposition of carotenoids pigments during ATE process. Our chroma values were comparable to the commercial sample value. Hue angles in our samples were equal or higher than the commercial sample indicating they were greener. BI values were lower than the commercial sample resulting from less formation of brown pigments from enzymatic or nonenzymatic reactions due to ambient conditions of ATE process and removal of the lower molecular degradation products by means of propane.

Propane extraction appears to be feasible for dry basil. Compared to SCE with CO<sub>2</sub>, the propane process may result in more extract aromatic chemical consistency regardless of duration of extraction for most plant parts. Our data indicates that cultivars differ in extraction kinetics, which may be due to prevalence of trichome type for the cultivar. For 'Sweet Thai', an extraction duration of 30 min should result in optimum extraction of aromatic chemicals; extraction durations for other cultivars should be determined to optimize their propane extraction process.

## Reference

Bicchi, A., D.G.M. Nano, and C. Frattini. 1983. Improved method for the analysis of small amounts of essential analysis by microdistillation followed by capillary gas chromatography. *J. Chrom.* 279:409-416.

Castaneda-Acosta, J., A.W. Cain, N.H. Fischer, and F.C. Knopf. 1995. Extraction of Bioactive Sesquiterpene Lactones from *Magnolia grandiflora* Using Supercritical Carbon Dioxide and Near-Critical Propane. *J. Agric. Food Chem.* 43(1): 63-68.

Chalchat, J.C. and M.M. Ozcan. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. *Food Chemistry.* 110(2): 501-503.

Charles, D.J. and J.E. Simon. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *J. Amer. Soc. Hort. Sci.* 115(3): 458-462.

Demirhan, E. and B. Ozbek. 2009. Color change kinetics of microwave-dried basil. 2009. *Drying Technology.* 27:156-166.

Diaz-Maroto, M.C., M.S. Perez-Coello, and M. Dolares Cabezudo. 2002. Supercritical carbon dioxide extraction of volatiles from spices. *J. of Chrom.* 947(1): 23-29.

Gainar, L., R. Vilcu, and M. Mocan. 2002. Supercritical fluid extraction of basil essential oil. Influence of the process parameters and chemical characterization of extracts. *Revue Roumaine de Chimie.* 47(2): 29-32.

Gnayfeed, M.H., H.G. Daood, V. Illes, and P.A. Biacs. 2001. Supercritical CO<sub>2</sub> and subcritical propane extraction of pungent paprika and quantification of carotenoids, tocopherols and capsaicinoids. *J. Agric. Food Chem.*49:2761-66.

Hamdan, S., H.G. Daood, M. Toth-Markus, and V. Illes. 2008. Extraction of cardamom oil by supercritical CO<sub>2</sub> and subcritical propane. *J. of Supercritical Fluids* 44:25-30.

Handilou, E., S. Kokkini, A.M. Bosabalidis, and J.M. Bessière. 1991. Glandular trichomes and essential oil constituents of *Calaminta menthifolia* (Lamiaceae). *Plant Systematics and Evolution* 177: 17-26.

Hasegawe, Y., K. Tajima, N. Toi, and Y. Sugimura. 1997. Characteristic components found in the essential oil of *Ocimum basilicum* L. *Flav. Fragn. J.* 12:195-200.

Heidlas, J. 1994. De-oiling of lecithins by near-critical fluid extraction. *Agro-Food industry Hi-Tech.* January/9-11.

Iijima, Y., R. Davidovich-Rikanati, E. Fridman, D.R. Gang, E. Bar, E. Lewinsohn, and E. Pichersky. 2004. The biochemical and molecular basis for divergent patterns in biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant physiology* 136: 3724-3736.

Kanamangala, R.V., Maness N.O., and M.W. Smith. 1999. Reduced lipid pecans: Chemical alterations and implications for quality maintenance during storage. *J. Amer. Soc. Hort. Sci.*124: 389-398.

Kiran, E., P.G. Debenedetti, and C..J. Peters. 1998. Supercritical fluids. *NATO Science Series.*Vol.366. Kluwer Academic Publishers. The Netherlands.

Kothari, S.K., A.K. Bhattacharya, K. Singh, S.I. Ramesh, and E.V.S. Rao. 2005. Pre-Flowering of *Ocimum gratissimum* for higher essential oil and eugenol yields under semi-arid tropics. *J. Essential Oil Res.* 17:212-216.

Lawrence, B.M. 1988. A further examination of the variation of *Ocimum basilicum* L.. In B.M. Lawrence, B.D. Mookerjee and B.J. Willis (Eds.), *Flavors and Fragrances: A world perspective*, Elsevier, Amsterdam, pp. 161-170.

Leal, P.F., F. Chaves, L.C. Ming, A.J. Petenate, and M.A. Meireles. 2006. Global yields, chemical compositions and antioxidant activities of clove basil (*O. gratissimum*) extracts obtained by supercritical fluid extraction. *J. of Food Process Engineering* 29: 547-559.

Leal, P.F., N.B. Maia, and Q.A.C. Carmello. 2008. Sweet Basil (*Ocimum basilicum*) Extracts Obtained by Supercritical Fluid Extraction (SFE): Global Yields, Chemical Composition, Antioxidant Activity, and Estimation of the Cost of Manufacturing. *Food and Bioprocess Technology* 1(4): 326-338.

Lucchesi, M.E., F. Chemat, and J. Smadja. 2004. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *J. Chromatography A.* 1043(2): 323-327.

Mabe, G.D., G.M. Foco, E.A. Brignole, and S.B. Bottini. 1998. Extraction of Rosa mosquata oil with dense fluids. *Acta Horticulturae* 503:37-44.

Mukhopadhyay, M. 2000. *Natural extracts using supercritical CO<sub>2</sub>*. CRS press LLC: Boca Raton, FL. pp. 338.

- Munoz-Acevedo, A., E.E. Stashenko, V.V. Kouznetsov, and J.R. Martinez. 2011. Differentiation of leaf and flower extracts of basil (*Ocimum* sp.) varieties grown in Colombia. *Jeobp*. 14(4): 387-395.
- National Propane Gas Association. 2004. General U.S. Industry Statistics and Characteristics of Propane.
- Paton, A., R.M., Harley, and M.M. Harley. 1999. *Ocimum*. Medicinal and Aromatic Plants. Vol. 11. Amsterdam: Harwood Academic. 166p
- Stamenic, A., I. Zizovic, A. Orlovic, and D. Skala. 2008. Mathematical modeling of essential oil SFE on the micro-scale-Classification of plant material. *J. of Supercritical Fluids* 46: 285-292.
- Wan, P.J and P.J. Wakelyn. 1997. Technology and solvents for extracting oilseeds and nonpetroleum oils. AOCS Press. Champaign, IL. 353p
- Yousif, A.N., C.H. Scaman, T.D. Durance, and B. Girard. 1999. Flavor volatiles and physical properties of vacuum-microwaves and air-dried sweet basil (*Ocimum basilicum*). *J. Agr. Food Chem.* 47:4777-4781.
- Zizovic, I., M. Stamenic, A. Orlovic, and D. Skala. 2005. Supercritical carbondioxide essential oil extraction of Lamiaceae family species: Mathematical modeling on the micro-scale and process optimization. *Chemical Engineering Science* 60: 6747-6756.
- Werker, E. 2000. Trichome diversity and development. *Advances in Botanical Research Incorporating Advances in Plant Pathology* 31:1-35

Table 7. Aromatic profiles of extracts of basil cultivars, ‘Mrs. Burns’ Lemon’ from citrus type, ‘Genovese’ from pesto type and ‘Cinnamon’ from spice type, obtained via propane extraction of combined flowers and leaf parts.

Impact chemical	Mrs. Burns' Lemon	Genovese	Cinnamon
	(mg kg <sup>-1</sup> )		
<b>β-pinene</b>	523	53	701
myrcene	157	50	0
limonene	510	0	828
cineole	484	1836	0
ocimene	79	143	122
fenchone	318	46	0
terpinolene	0	66	0
<b>linalool</b>	<b>49701<sup>z</sup></b>	<b>70371</b>	<b>50467</b>
camphor	135	860	244
borneol	0	662	1101
α-terpineol	6060	2185	945
methyl chavicol	1243	520	6436
geranial	<b>13497</b>	0	0
geraniol	2699	0	0
neral	<b>13258</b>	0	0
bornyl acetate	0	968	775
thymol	0	74	0
carvacrol	449	118	998
eugenol	595	<b>62324</b>	<b>21017</b>
methyl cinnamate	536	112	<b>64156</b>
methyl eugenol	1374	226	1194
β-caryophyllene	8655	122	934
humulene	1929	1235	954
<b>Total:</b>	102202	141972	149126

<sup>z</sup>Chemicals in bold type have been noted as impact chemicals for these cultivars (representing >20% of total aromatic chemicals in the dry herb). Values represent mean of three replications. Each replication involved three injections.

Table 8. Effect of propane extraction on chemical profiles of the basil cultivars, ‘Mrs. Burns’ Lemon’ from citrus type, ‘Napolitano’ and ‘Genovese’ from pesto type and ‘Cinnamon’ from spice type grown in 2009.

Impact chemical <sup>z</sup>	Mrs. Burns' Lemon leaves and flowers		Napolitano leaves and flowers		Genevose leaves and flowers		Cinnamon leaves and flowers	
	Control	Raffinate <sup>y</sup>	Control	Raffinate	Control	Raffinate	Control	Raffinate
	(mg kg <sup>-1</sup> )							
<b>cineole</b>	10 a <sup>x</sup>	8 a	14 a	16 a	185 a	93 b	9 a	16 a
<b>linalool</b>	<b>874<sup>w</sup></b>	<b>574</b>	<b>967</b>	<b>583</b>	<b>1125</b>	<b>941</b>	<b>1074</b>	<b>656</b>
<b>methyl chavicol</b>	8 a	4 a	<b>835</b> a	<b>400</b> b	41 a	30 a	303 a	162 b
<b>gerenial</b>	<b>1254</b> a	<b>953</b> b	ND <sup>w</sup>	ND	ND	ND	ND	ND
<b>neral</b>	<b>1247</b> a	<b>999</b> a	ND	ND	ND	ND	ND	ND
<b>eugenol</b>	6 a	7 a	233 a	188 a	<b>1377</b> a	<b>1645</b> a	474 a	366 a
<b>methyl cinnamate</b>	105 a	22 b	6 a	4 a	4 a	2 a	<b>1878</b> a	<b>919</b> b
<b>β-caryophyllene</b>	230 a	152 b	9 a	2 a	9 a	10 a	23 a	14 a
<b>humulene</b>	72 a	46 a	18 a	14 a	13 a	25 a	46 a	21 a
<b>Total:</b>	3805 a	2763 b	2083 a	1212 b	2754 a	2747 a	3807 a	2154 b

<sup>z</sup> Mean of three replications. Each replication involved three injections.

<sup>y</sup> Dry feed stock obtained after extraction of control samples.

<sup>x</sup> Means followed by the same letter in the same cultivar within rows do not differ significantly according to pair t test (P<0.05).

<sup>w</sup> Chemicals in bold type have been noted as impact chemicals for these cultivars (representing >20% of total aromatic chemicals in the dry herb).

<sup>y</sup> Not detected during gas chromatography analysis.



Table 9. Yield of flowers, leaves and stems of air dry basil cultivars (%) in herb harvested in 2010.

Part	Genovese	Blue Spice	Sweet Thai
flowers	9c <sup>z</sup>	25b	24a
leaves	59a	50a	42a
stems	32b	25b	33a

<sup>z</sup>Means of thirty five, thirty nine and thirty replications (approximately 200g air dry whole sample per each) of ‘Genovese’, ‘Blue Spice’ and ‘Sweet Thai’, respectively, followed by the same letter in the same cultivar within columns do not differ significantly according to t test ( $P < 0.05$ ).

Table 10. Aromatic profiles of extracts of basil cultivars, ‘Blue Spice and ‘Sweet Thai’, obtained via propane extraction.

Impact chemical in extract <sup>z</sup>	Blue Spice harvested in 2010			Sweet Thai harvested in 2010			Sweet Thai harvested in 2011		
	leaf	flower	stem	leaf	flower	stem	leaf	flower	stem
	(mg kg <sup>-1</sup> )								
cineole	19379	12968	1930	5336	9544	2126	5795	13768	5352
	a <sup>y</sup>	b	c	b	a	c	b	a	b
linalool	1275	1094	441	76	326	93	559	1193	534
	a	b	c	b	a	b	b	a	b
methyl chavicol	<b>52445<sup>x</sup></b>	<b>34323</b>	1157	<b>61005</b>	<b>95206</b>	<b>29128</b>	<b>62271</b>	<b>135177</b>	<b>45900</b>
	a	b	c	b	a	c	b	a	c
eugenol	<b>139723</b>	<b>100478</b>	<b>44161</b>	273	0	0	450	648	490
	a	b	c	a	b	b	a	a	a
β-caryophyllene	205	1564	954	348	2615	3288	392	173	145
	c	a	b	c	b	a	a	b	b
humulene	13995	10152	5632	1072	1268	2921	768	1176	1101
	a	b	c	b	b	a	b	a	a
<b>Total:</b>	<b>244824</b>	<b>171178</b>	<b>66113</b>	<b>73305</b>	<b>115415</b>	<b>38882</b>	<b>75239</b>	<b>170697</b>	<b>60223</b>
	<b>a</b>	<b>b</b>	<b>c</b>	<b>b</b>	<b>a</b>	<b>c</b>	<b>b</b>	<b>a</b>	<b>c</b>

<sup>z</sup> Mean of three replications. Each replication involved three injections.

<sup>y</sup> Means followed by the same letter in the same cultivar within rows do not differ significantly according to t test (P<0.05).

<sup>x</sup> Chemicals in bold type have been noted as impact chemicals for these cultivars (representing >20% of total aromatic chemicals in the dry herb).

Table 11. Effect of propane extraction on chemical profiles of parts (leaf, flower and stem) of the basil plants from cultivars of ‘Blue Spice’ and ‘Sweet Thai’ grown in 2010 vs. 2011.

Impact chemical <sup>z</sup>	Blue Spice harvested in 2010						Sweet Thai harvested in 2010						Sweet Thai harvested in 2011						
	leaf		flower		stem		leaf		flower		stem		leaf		flower		stem		
	Control	Raffinate <sup>y</sup>	Control	Raffinate	Control	Raffinate	Control	Raffinate	Control	Raffinate	Control	Raffinate	Control	Raffinate	Control	Raffinate	Control	Raffinate	
	(mg kg <sup>-1</sup> )																		
cineole	258	343	204	334	7	3	200	171	316	210	31	16	125	132	236	151	24	3	
	a <sup>x</sup>	a	a	a	a	a	a	a	a	b	a	b	a	a	a	a	a	a	b
linalool	14	20	14	31	ND <sup>x</sup>	ND	13	12	42	6	ND	ND	13	9	42	4	ND	ND	
	a	a	a	a			a	a	a	b			a	a	a	b			
methyl chavicol	816	901	682	748	38	59	<b>2390<sup>w</sup></b>	<b>1970</b>	<b>3237</b>	<b>2197</b>	<b>154</b>	<b>19</b>	<b>1071</b>	<b>952</b>	<b>2198</b>	<b>583</b>	<b>136</b>	<b>27</b>	
	a	a	a	a	a	a	a	b	a	b	a	b	a	b	a	b	a	b	
eugenol	<b>4413</b>	<b>3786</b>	<b>3546</b>	<b>2974</b>	<b>173</b>	<b>43</b>	12	9	9	13	1	ND	8	3	19	3	2	ND	
	a	b	a	b	a	b	a	a	a	a	a		a	a	a	b			
β-caryophyllene	375	ND	156	126	ND	ND	39	28	52	25	2	6	14	12	36	17	11	2	
			a	a			a	a	a	b	a	a	a	a	a	a	a	b	
humulene	481	339	312	263	12	2	96	84	129	82	11	5	26	30	60	23	5	1	
	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	b	a	a	
<b>Total:</b>	<b>6357</b>	<b>5388</b>	<b>4914</b>	<b>4476</b>	<b>231</b>	<b>107</b>	<b>2981</b>	<b>2485</b>	<b>4290</b>	<b>2816</b>	<b>213</b>	<b>47</b>	<b>1408</b>	<b>1302</b>	<b>2997</b>	<b>953</b>	<b>199</b>	<b>49</b>	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	

<sup>z</sup> Mean of three replications. Each replication involved three injections.

<sup>y</sup> Dry feed stock obtained after extraction of control samples.

<sup>x</sup> Means followed by the same letter in the same parts of cultivar within rows do not differ significantly according to pair t test (P<0.05).

<sup>w</sup> Not detected during gas chromatography analysis.

<sup>v</sup> Chemicals in bold type have been noted as impact chemicals for these cultivars (representing >20% of total aromatic chemicals in the dry herb).

Table 12. Effect of propane extraction time on chemical profiles of the leaf, flower and stem extracts of ‘Sweet Thai’ basil cultivar.

Part Impact chemical in extract <sup>z</sup>	leaf						flower				stem			
	Extraction time (min)						Extraction time (min)				Extraction time (min)			
	10	20	30	40	50	60	10	20	30	40	10	20	30	40
conc. (mg kg <sup>-1</sup> )														
cineole	4640	6902	6638	6169	6410	6085	5006	9544	12023	12102	2033	2126	3756	5206
	b <sup>y</sup>	a	a	a	a	a	b	a	a	a	c	c	b	a
linalool	159	165	136	411	311	278	267	326	328	216	99	93	282	337
	b	b	b	a	a	a	a	a	a	a	b	b	a	a
camphor	783	1220	1505	1263	1519	1550	1304	1648	1833	1400	454	662	1354	1553
	b	a	a	a	a	a	b	a	a	b	b	b	a	a
<b>methyl chavicol</b>	<b>42915<sup>x</sup></b>	<b>72708</b>	<b>76700</b>	<b>73955</b>	<b>73913</b>	<b>69661</b>	<b>86749</b>	<b>102813</b>	<b>101899</b>	<b>114220</b>	<b>23037</b>	<b>29128</b>	<b>48170</b>	<b>56209</b>
	b	a	a	a	a	a	b	a	a	a	b	b	a	a
eugenol	171	146	194	586	614	452	ND <sup>x</sup>	ND	ND	ND	ND	ND	ND	ND
	b	b	b	a	a	a								
<b>β-caryophyllene</b>	329	2844	2785	2317	2998	2154	2073	2615	2929	2485	3082	3288	3890	3855
	b	a	a	a	a	a	b	a	a	a	b	b	a	a
humulene	700	1301	1205	1182	1245	1052	760	1268	1295	1100	2836	2921	3376	2918
	b	a	a	a	a	a	b	a	a	a	a	a	a	a
<b>Total</b>	<b>52653</b>	<b>85428</b>	<b>89994</b>	<b>87567</b>	<b>88440</b>	<b>86710</b>	<b>100192</b>	<b>115415</b>	<b>126155</b>	<b>135169</b>	<b>32256</b>	<b>38882</b>	<b>62273</b>	<b>69740</b>
	b	a	a	a	a	a	b	ab	a	a	b	b	a	a

<sup>z</sup>Mean of three replications. Each replication involved three injections.

<sup>y</sup>Means followed by the same letter in the same part within rows do not differ significantly according to t test (P<0.05).

<sup>x</sup>Chemicals in bold type have been noted as impact chemicals for these cultivars (representing >20% of total aromatic chemicals in the dry herb).

<sup>w</sup>Not detected during gas chromatography analysis.

Table 13. Color parameter values (L\*, a\*, b\*, chroma, hue angle and browning index (BI)) of parts (leaf, flower and stem) of the basil plants from cultivar of ‘Sweet Thai’ grown in 2010 and 2011 vs. the commercial basil leaf sample.

Season	Part	Sample	L*	a*	b*	chroma	hue angle	BI
2010	leaf	control	38.27† <sup>z</sup>	-4.62	15.47	16.14	178.72	40.24
		raffinate <sup>y</sup>	35.92	-3.94	13.87	13.88	178.47	38.00
	flower	control	35.80	-2.39	13.56	13.77	178.60	40.79
		raffinate	32.70	-2.22	11.66	11.87	178.62	37.37
	stem	control	45.00	-0.36	17.47	17.47	178.45	47.00†
		raffinate	44.01	-1.04	15.39	15.83	178.50	40.64
2011	leaf	control	34.96	-2.49	11.10	11.38	178.65	31.51
		raffinate	29.02	-1.38	9.06	9.15	178.61	34.22
	flower	control	31.11	-1.81	10.19	10.35	178.60	35.82
		raffinate	32.49	-2.46	12.19	12.44	178.63	39.53
	stem	control	41.79	-1.60	14.67	14.76	178.54	38.98
		raffinate	43.13	-1.73	14.82	14.92	178.55	37.74
	leaf	commercial <sup>x</sup>	38.90	-0.32	17.63	17.64	178.45	57.52

<sup>z</sup>†Means of L\*, a\* or b\* in the same parts of cultivar within columns in the same season are different according to pair t test (P<0.05).

Values represent mean of three replications.

<sup>y</sup>Dry feed stock obtained after extraction of control samples.

<sup>x</sup>Data obtained by Yousif et al. (1999).

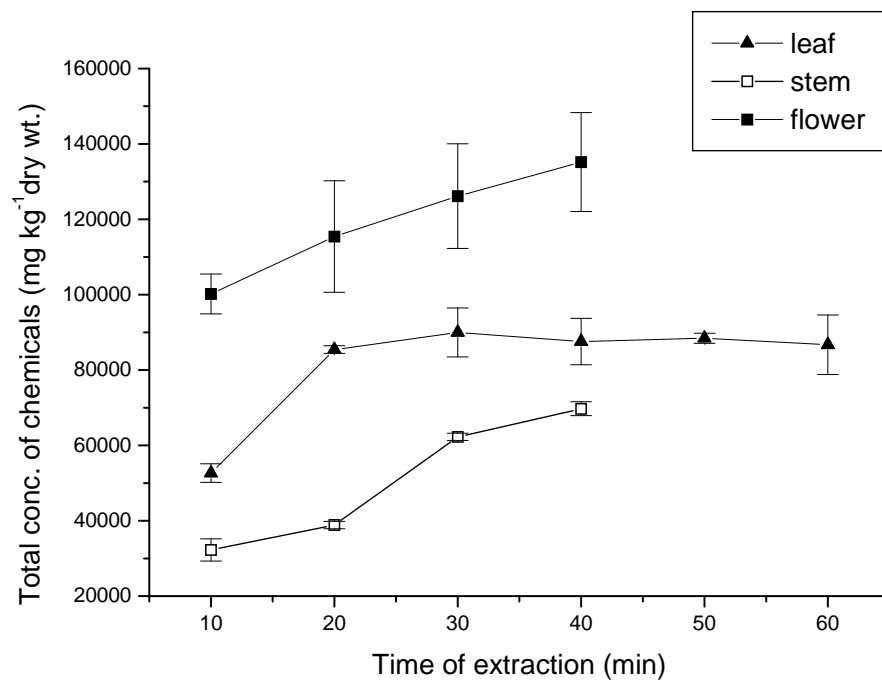


Fig. 15. Total concentration of chemicals in extract as a function of time in leaf, stem and flower extracts of ‘Sweet Thai’ basil cultivar. Error bars represent standard deviations from mean of nine injections done during GC analysis from three randomly selected 0.05 g extract subsamples from the parts of the cultivar.

**APPENDIX A: The common compounds found in the essential oils and history and problems of chemotype classification of *Ocimum* species based on the essential Oil compositions**

## The common compounds found in the essential oils of *Ocimum* species

**1) Monoterpene Hydrocarbons (Figure 1):** The major monoterpene hydrocarbons are reported as limonene, myrcene, p-cymene,  $\gamma$ -terpinene, and ocimens. Their total amount in the oil usually ranges from traces to a few percent of detected compounds in the oil. The average percentage is usually less than 1% of detected compounds in the oil. However, they may exist as a major compound and co-exist with other compounds as a major compound in some species (Table A.1) (Paton et al., 1999). For example, the oils of *O. gratissimum* from Cameroon were rich in p-cymene and  $\gamma$ -terpinene as % of detected compounds: 21.1% and 21.9% (Table A.1), respectively (Tchoumboungang et al., 2006). While the oil of *O. canum* leaves grown in Cameroon had 41.5% limonene of detected compounds (limonene type) (Table 1), that of the flowers had only 5.7% (Ngassoum et al., 2003).

**2) Oxygenated Monoterpenes as Main Constituents (Figure 1):** Linalool, camphor, 1,8-cineole, citral, citronellal, geraniol and thymol, the most common compounds and key compounds in the chemotaxonomy, exist in many species, such as *O. basilicum* (Sweet or European basil), *O. canum* (Hairy or African basil) (Gupta and Tawa, 1997), *O. gratissimum* (Tchoumboungang et al., 2006) and *O. sanctum* (Holy or Indian basil) oil (Hegnauer, 1966) up to 90% of detected compounds (Table A.1). They may also co-exist with other compounds as a major compound (Table A.1). For example, in the oils from three cultivars of *O. basilicum* (Crimson, Great Green and Fine Dwarf Green) grown in Morocco, 1,8-Cineole was the main compound with linalool and elemene (Belkamel, 2008) (Table A.1). However, in the oil of *O.*



*basilicum* grown in Turkey 1,8-Cineole existed only less than 10% of detected compounds (Akgul, 1981). Among them, citral, citronellal and geraniol are acyclic monoterpenes that generally exist together. Citral is the mixture of two acyclic monoterpene aldehydes, geranial and neral.

**3) Sesquiterpenes:** In general, sesquiterpenes exist as a trace compound among detected compounds in the oil of *O. basilicum*. Paton et al. (1999) reported the following trace compounds in the oil of *O. basilicum*:  $\alpha$ -amorphene, bicyloelemen, bicyclogermagrene, cis-  $\alpha$ -bergamotene, trans-  $\alpha$ -bergamotene,  $\epsilon$ -bulgarene,  $\beta$ -bourbonene,  $\alpha$ -cadinene,  $\gamma$ -cadinene,  $\delta$ -cadinene, T-cadinol, calamine, caryophyllene oxide,  $\beta$ -cedrene, 2-epi- $\alpha$ -cedrene,  $\beta$ -cubenene,  $\beta$ -elemene,  $\delta$ -elemene,  $\alpha$ -farnesene, germacrene,  $\alpha$ -guaiene,  $\alpha$ -humulene, isocaryophyllene,  $\alpha$ -selinene,  $\beta$ -selinene,  $\beta$ -bisabolene,  $\beta$ -cadinene,  $\beta$ -caryophyllene,  $\beta$ -farnesene, germacrene,  $\gamma$ -muurolene,  $\alpha$ -gurjunene, scapanene,  $\alpha$ -elemene,  $\alpha$ -bisobolene, and  $\beta$ -caryophyllene. Also,  $\beta$ -elemene (34.6 % of the sesquiterpene fraction) and  $\beta$ -caryophyllene (18.0% of the sesquiterpene fraction) were the main fractions of the sesquiterpenes. However, sesquiterpene chemotypes were detected from other *Ocimum* species (Kicel et al., 2005, Hussain et al.,2008) (Table A.1).

**4) Phenylpropene Derivatives as Main Constituents (Figure 2):** Methyl chavicol (estragol), methyl cinnamate, eugenol and methyl eugenol, the major types of those derivatives, would also exist as a major compound alone or with other compounds, such as linalool (Shatar and Altantssetseg, 2000; Sajjadi, 2006) and citral (Telci et al., 2006) in many basil species (Table A.1) .

## **History and Problems of Chemotype Classification of Basil Species Based on the Essential Oil Compositions**

Although Gunther (1949) classified *O. basilicum* into four type according to oil compositions based on their major compounds (European type, Reunion type, Methyl cinnamate, Eugenol type) (Table A.1), Lawrence (1992) differentiated them into four chemotypes based on the analysis of 200 types of basil oils for either mevalonic acid pathway or shikimic acid pathway alone or dual of those pathways: 1) methyl chavicol rich 2) linalool rich 3) methyl eugenol rich 4) methyl cinnamate rich. Pushpangadan and Bradu (1995) added eugenol rich type to this classification latter. Lawrence (1992) also critiqued chemotype classification done while cultivars containing at least two major compounds since the essential oil composition could be different before and after drying. Likewise, Baritoux et al. (1992) found that after drying (45°C for 12h) and during storing (3-7 months at room temperature) the content of methyl chavicol and eugenol (mg/100g dry wt. of plant material) in the oil decreased, but the linalool and 1,8 -cineole content (mg/100g dry wt. of plant material) increased. The loss in total oil yield content (mg/100g dry wt. of plant material) was 66% after 7 months of storage at room temperature. Also, they noted that essential oil loss was due to evaporation and the increase in oxygenated monoterpenes was due to hydrolysis of monoterpene glucosides during distillation. Grayer et al. (1996) also reported that the distilled oil of the fresh leaves of the *O. basilicum* cultivar represented a methyl chavicol type, but the distilled oil of the dried leaves represented a linalool type. Da Silva et al. (2005) also found a linear decrease in essential oil content (g/kg of dry wt.) and change in composition during 3-9 days of storage in cold chamber at 10°C. They determined that eugenol and linalool content (of total peak area) in the oil decreased during storage. Carvalho Filho et al. (2006) also noted that the concentration of linalool increased from

45.2% to 86.8% of detected compounds after five days of drying at 40°C. Soares et al. (2007) also noted the greatest essential oil content (%v/fresh wt.) was obtained in the drying process with 40°C and 1.9m/s of air velocity, but the greatest linalool concentration (ppm) in the oil was obtained with 54.4°C and 1.9m/s.

Later, Zheljzakov et al. (2008) divided 38 *O. basilicum* accessions grown in Mississippi on the basis of the oil compositions into seven groups (Table A.1). They concluded that the presence of these various chemotypes supplies the opportunity for the production of basil for the essential oil markets or individual compounds like eugenol, linalool, methyl chavicol, methyl cinnamate, or methyl eugenol. Lopez (2008) classified five *O. basilicum* varieties obtained from the US National Plant Germplasm system but grown in Spain into three chemotypes (Table A.1). They reported the differences in the oil compositions of *O. basilicum* varieties were sometimes related to plant morphological characteristics, but seasonal variations also would have an effect. The French Standards of Normalization AFNOR (2000) (Adam et al., 2009) described two chemotypes of the species based on the composition of the essential oils commercialized: The first chemotype was typical European chemotype but found in Brazil and Cameroon also. The second type or Reunion type was present in Madaskar, Toga, Benin, Egypt, Mongolia, Germany, Brazil and Thailand. Methyl cinnamate or tropical type was found in Fiji, Brazil, India Philippines and French Polynesia recently (Adam et al., 2009). In Northeast India camphor rich type was also recorded (Adam et al., 2009). Abduelrahman et al. (2009) analyzed 19 accessions of *O. basilicum* grown in Sudan. They found the broad chemical variability among the compositions of the oils from those accessions, classified into seven groups based on their major compounds (>50% of the detected compounds) (Table A.1). They concluded that this classification was necessary if international marketing was considered. Kacar et al. (2009)

analyzed four varieties and six landraces grown in different regions in Turkey. They detected five chemotypes of *O. basilicum* (Table A.1) and reported that seeds of the same origin produced different chemotypes if cultivated under different conditions in Turkey.

Vieira et al. (2001) classified two morphological varieties of *O. gratissimum* grown in the US but obtained from Brazil and Russia (var. *gratissimum* and var. *macrophyllum*) into three chemotypes (eugenol, thymol, and geraniol). However, Freire et al. (2006) found only either eugenol or eugenol and 1,8-cineole chemotype depending on the season grown in Brazil. The oil of *O. sanctum* (Holy basil) has either phenolic constituents, like eugenol, thymol, methyl eugenol, detected in India and Thailand (>70% of the detected compounds/total peak area), and methyl chavicol, discovered in Australia (87% of the detected compounds), or sesquiterpene alcohols, like humulene and elemene as major oil constituents (Kothari et al., 2005) (Table A. 1). Also, cv. 'Local' of *O. sanctum* grown in Mississippi had eugenol chemotype depending on harvest time (5-42% of the detected compounds) (Zheljazkov et al., 2008). However,  $\alpha$ -humulene and elemene were the major constituents of the oil from cv. 'Local' of *O. sanctum* grown in Canada (Bowes and Zheljazkov, 2004). The chemical compositions of the other species have not been studied extensively, meaning that chemical diversity at the level of terpenoids and phenylpropanes is not clear cut between other basil species.

## Reference

- Abduelrahman, A.H.N., S.A. Elhoussein, N.A. Osman, and A.H. Nour. 2009. Morphological variability and chemical composition of essential oils from nineteen varieties of basil (*O. basilicum*) growing in Sudan. *Int. J.Chem. Tech.* 1(1):1-10.
- Adam, F., I.Vahirua-Lechat, and E. Deslandes. 2009. Aromatic Plants of French Polynesia. III. Constituents of the Essential Oil of Leaves of *Ocimum basilicum* L. *J. Essential Oil Res.* 21 (3): 237-240.
- Bariatux, O., H. Richard, J. Touche, and M. Derbesy. 1992. Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. *Flav. Fragr. J.* 7: 267-271.
- Belkamel, A., J. Bammi, and V. Janneot. 2008. Evaluation of the biomass and analyst of the essential oil of three varieties of basil (*Ocimum basilicum* L.) cultivated in Morocco. *Acta Botanica Gallica* 155 (4): 467-476.
- Bowes, K. M. and V. D. Zheljjackov. 2004. Factors affecting yields and essential oil quality of *Ocimum sanctum* and *Ocimum basilicum* cultivars. *J. Amer. Soc. Hort. Sci.* 129(6): 789-794.
- Carvalho Filho, J.L., A.F. Blank, P.B. Alves, P.A.D. Ehlert, A.S. Melo, S.C.H. Cavalcanti, M.F. Arrigoni-Blank, R. Silva-Mann R. 2006. Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil. *Rev. Bras. Farmacogn.* 16(1): 1-8

Da Silva, F., R.H.S. Santos, and N.J. de Andrade. 2005. Basil conservation affected by cropping season, harvest time and storage period. *Pesq. Agropec. Bras.* 40(4): 323-328

Freire, C.M., M.O. Marques, and M. Costa. 2006. Effects of seasonal variation on the central nervous system activity of *O. gratissimum* L. essential oil. *J. of Ethnopharmacology* 105:161-166.

Grayer, R.J., G.C. Kite, F.J. Goldstone, S.E. Bryan, A. Paton., and E. Putievsky. 1996. Intraspecific taxonomy and essential oil chemotype in sweet basil, *Ocimum basilicum*. *Phytochemistry* 43:1033-1039.

Gubta, S. and A. Tawa. 1997. Chemical composition of an improved hybrid strain of *Ocimum canum* Sims. *J. Essential Oil Res.* 9: 375-377.

Gunther, E. 1949. *The essential oils*. Van Nostrand Company. New York. 3:151pg.

Hasegawa, Y., K. Tajima, N. Toi, and Y. Sugimura. 1997. Characteristic components found in the essential oil of *Ocimum basilicum* L. *Flav. Fragn. J.* 12:195-200.

Hegnauer, R. 1966. *Chemotaxonomie der Pflanzen*, Band 4. Birkhauser verlag, Basel und Stuttgart, Germany.

Hiltunen, R. and Y. Holm. 1999. Essential oil of *Ocimum*. In *Basil The genus Ocimum*. Hardwood Academic Publishers. Amsterdam The Netherlands. Pg 67.

Hussain, A.I., F. Anwar, and S.T.H. Sherazi. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depend on seasonal variations. *Food Chemistry* 108(3): 986-995.

Kacar, O., E. Goksu, and N. Ozkan. 2009. Agronomic Properties and Essential Oil Composition of Basil Varieties of Landraces (*Ocimum basilicum* L.) in Turkey. *Asian J. of Chemistry* 21(4): 3151-3160.

Kicel, A., A. Kurowska, and D. Kalemba. 2005. Composition of the essential oil of *Ocimum sanctum* L. grown in Poland during vegetation. *J. Essential Oil Res.* 17:217-219.

Klimankova, E., K. Holadova, J. Hajslova, T. Cajka, J. Poustka, and M. Koudela. 2008. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. *Food Chemistry* 107:464-472.

Kothari, S.K., A.K. Bhattacharya, S. Ramesh, S.N. Garg, and S.P.S. Khanuja. 2005. Volatile constituents in oil from different plant parts of methyl eugenol-rich *O. sanctum* grown in South India. *J. Essential Oil Res.* 17: 656-658.

Lawrence, B.M. 1992. Progress in essential oils. *Perfumer & Flavorist* 17: p 45-56.

Lopez, M.D., M.J. Jordan, and M.J. Pascual-Villalobos. 2008. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. *J. of Stored Products Res.* 44( 3): 273-278.

Mukherjee, M. 2007. The basils - A review. *Plant Archives.* 7(2): 473-483.

Ngassoum, M.B., H. Ousmaila, L.T. Ngamo, P.M. Maponmetsem, L. Jirovetz, and G. Buchbauer. 2003. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. *Journal of Food Composition and analysis* 17: 197-204.

Paton, A., R.M. Harley, and M.M. Harley. 1999. *Ocimum*. Medicinal and Aromatic Plants. Vol. 11. Amsterdam: Harwood Academic. 166p.

Pushpangadan, P. and B.L. Bradu. 1995. Advances in Horticulture. In: Medicinal and aromatic plants. vol.11. New Delhi: Malhotra publishing House p 627-657.

Sajjadi, S.E. 2006. Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. Daru 14(3):128-130.

Shatar, S. and S. Altantssetseg. 2000. Essential oil composition of some plants cultivated in Mongolian Climate. J. Essential Oil Res. 12:745-750.

Soares, R.D., M.A. Chaves, and A.A. da Silva. 2007. Influence of drying temperature and air velocity related to essential oil and linalool contents of the basil (*Ocimum basilicum* L.). Ciencia E Agrotecnologia 31(4): 1108-1113.

Sobti, S.N., P. Pushpangadan, and S.N. Atal. 1982. Cultivation and utilization of aromatic plants. Perfumery and Cosmetic 69:564-568.

Tchoumboungang, F., P.V. Amvam Zollo, F. Avlessi, G.A. Alitonou, D.K. Sohounhloue, J.M. Oumba, E. Dagne, and C. Menut. 2006. Variability in the chemical compositions of the essential oils of five *Ocimum* species from tropical African area. J. Essential Oil Res. 18: 194-199.

Telci, I., E. Bayram, G. Yilmaz, and B. Avci. 2006. Variability in essential oil composition of Turkish basils. Biochemical Systematics and Ecology 34: 489-497.

Vieira, R.F., R.J. Grayer, and A. Paton. 2001. Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. Biochemical Systematic and Ecology 29(3): 287-304.



Zheljazkov, V. D., A. Callahan, and C.L. Cantrell. 2008. Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. J. Agr. Food Chem. 56:(1): 241-245.

Table A.1. Chemotypes of common basil species based on chemical composition of the oil.

Taxonomic classification	Species name	Chemotypes/Principal constituent(s) <sup>z</sup>	Source
Sect. <i>Ocimum</i> subsect. <i>Ocimum</i>	<i>O. basilicum</i>	methyl chavicol <sup>y</sup> , linalool <sup>x</sup>	Gunther, 1949, Sajjadi, 2006, Lopez, 2008, Zhejzakov et al., 2008 Adam et al., 2009, Mukherjee, 2007.
		methyl chavicol <sup>y</sup> , camphor <sup>x</sup>	Gunther, 1949.
		methyl chavicol <sup>y</sup> , linalool <sup>x</sup> , methyl cinnamate <sup>y</sup>	Gunther, 1949.
		eugenol <sup>y</sup>	Gunther, 1949.
		linalool <sup>x</sup>	Lawrence, 1992, Zhejzakov et al., 2008, Abdulrahman et al., 2009, Kacar et al., 2009. Tchoumboungang et al., 2006, Telci et al., 2006, Hussain et al., 2008, Klimankova et al., 2008. Shatar and Altantssetseg, 2000, Mukherjee, 2007 .
		methyl chavicol <sup>y</sup>	Lawrence, 1992, Zhejzakov et al., 2008, Abdulrahman et al., 2009, Kacar et al., 2009. Shatar and Altantssetseg, 2000, Tchoumboungang et al., 2006, Mukherjee, 2007.
		methyl cinnamate <sup>y</sup>	Lawrence, 1992, Hasegawa et al., 1997, Telci et al., 2006.
		methyl eugenol <sup>y</sup>	Telci et al., 2006.
		methyl chavicol <sup>y</sup> , citral <sup>x</sup>	Sajjadi, 2006, Telci et al., 2006.
		linalool <sup>x</sup> , eugenol <sup>y</sup>	Zhejzakov et al., 2008, Abdulrahman et al., 2009.
		methyl eugenol <sup>y</sup> , linalool <sup>x</sup>	Zhejzakov et al., 2008, Kacar et al., 2009.
		methyl cinnamate <sup>y</sup> , linalool <sup>x</sup>	Zhejzakov et al., 2008, Abdulrahman et al., 2009, Telci et al., 2006.
		bergamotene <sup>w</sup>	Zhejzakov et al., 2008
		geraniol <sup>x</sup>	Abdulrahman et al., 2009.
		linalool <sup>x</sup> , geraniol <sup>x</sup>	Abdulrahman et al., 2009.
		camphor <sup>x</sup>	Hegnauer, 1966, Gupta, 1994.
		1,8-Cineole <sup>x</sup> , linalool <sup>x</sup> , elemene <sup>w</sup>	Belkamel, 2008.
		geraniol <sup>x</sup> , linalool <sup>x</sup> , eugenol <sup>y</sup>	Sobti and Pushpangadan, 1982.
		geraniol <sup>x</sup>	Sobti and Pushpangadan, 1982.
		$\beta$ -caryophyllene <sup>w</sup> , $\alpha$ -bergamotene <sup>w</sup> , gemacrene <sup>w</sup>	Hussain et al., 2008.
$\gamma$ -cadinene <sup>w</sup> , and bicyclogermacrene <sup>w</sup>			
Sect. <i>Ocimum</i> subsect. <i>Ocimum</i>	<i>O. basilicum</i> var. <i>minimum</i> L.	linalool <sup>x</sup>	Kacar et al., 2009.

Table A.1. Chemotypes of common basil species based on chemical composition of the oil (continued).

Taxonomic classification	Species name	Chemotypes/Principal constituent(s) <sup>z</sup>	Source
Sect. <i>Ocimum</i> subsect. <i>Gratissimum</i> <i>O. gratissimum</i>		thymol <sup>z</sup>	Hiltunen and Holm, 1999, Vieira et al., 2001, Agnani et al., 2005, Tchoumboungang et al., 2006, Kothari et al., 2005.
		citral <sup>z</sup>	Hiltunen and Holm, 1999
		methyl cinnamate <sup>y</sup>	Hiltunen and Holm, 1999
		linalool <sup>z</sup>	Hiltunen and Holm, 1999
		eugenol <sup>z</sup>	Hiltunen and Holm, 1999, Kothari et al., 2005, Vieira et al., 2001, Freire et al., 2006, Tchoumboungang et al., 2006.
		p-cymene <sup>y</sup>	Hiltunen and Holm, 1999, Kothari et al., 2005
		geraniol <sup>z</sup>	Kothari et al., 2005, Vieira et al., 2001.
		eugenol <sup>z</sup> , 1,8-cineole <sup>x</sup>	Freire et al., 2006.
		p-cymene <sup>y</sup> , $\gamma$ -terpinene <sup>y</sup>	Tchoumboungang et al., 2006.
		camphor <sup>x</sup>	Hegnauer, 1966.
Sect. <i>Ocimum</i> subsect. <i>Hierocymu</i> <i>O. sanctum</i>		methyl eugenol <sup>z</sup>	Kothari et al., 2005.
		eugenol <sup>z</sup>	Zheljazkov et al., 2008
		$\alpha$ -humulene <sup>w</sup> , elemene <sup>w</sup>	Bowes and Zheljazkov, 2004
		linalool <sup>z</sup>	Hegnauer, 1966.
		1,8-cineole <sup>x</sup>	Kicel et al., 2005.
		$\beta$ -bisabolene <sup>w</sup>	Kicel et al., 2005.
Sect. <i>Ocimum</i> subsect. <i>Ocimum</i> <i>O. canum</i>		limonene <sup>y</sup>	Ngassoum et al., 2003
		$\beta$ -ocimene <sup>y</sup>	Tchoumboungang et al., 2006.
		linalool <sup>z</sup>	Gupta and Tawa, 1997
		camphor <sup>x</sup>	Hegnauer, 1966.
		citral <sup>z</sup>	Hegnauer, 1966.
		linalool <sup>z</sup> , geraniol <sup>z</sup>	Ngassoum et al., 2003
		methyl cinnamate <sup>y</sup>	Hiltunen and Holm, 1999

<sup>z</sup> Content >20% of the total identified compounds in the oil based on gas chromatographic analysis.

<sup>y</sup> Phenylpropene derivatives.

<sup>x</sup> Oxygenated monoterpenes.

<sup>w</sup> Sesquiterpenes.

<sup>v</sup> Monoterpene hydrocarbons.

**APPENDIX B: Basil cultivars ('Genovese', 'Mrs. Burns' Lemon', 'Sweet Thai', 'Cinnamon', 'Blue Spice') harvested at 2 week and 3 week intervals on August 9 2011 before and after harvest.**

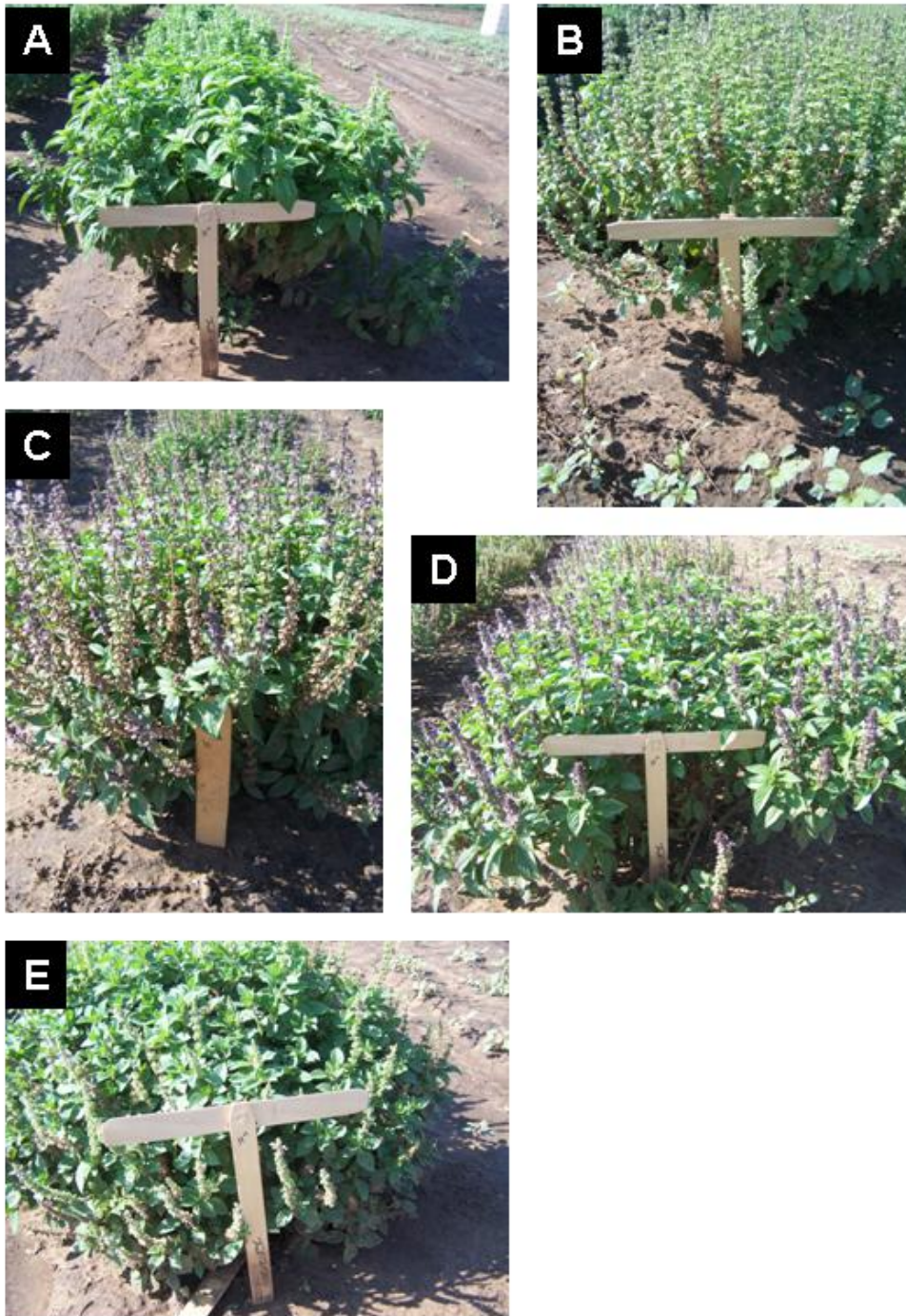


Figure. B.1. Basil cultivars ['Genovese'(A), 'Mrs. Burns' Lemon'(B), 'Sweet Thai'(C), 'Cinnamon'(D), 'Blue Spice'(E)] harvested at 2 week interval on August 9 2011 before harvest.

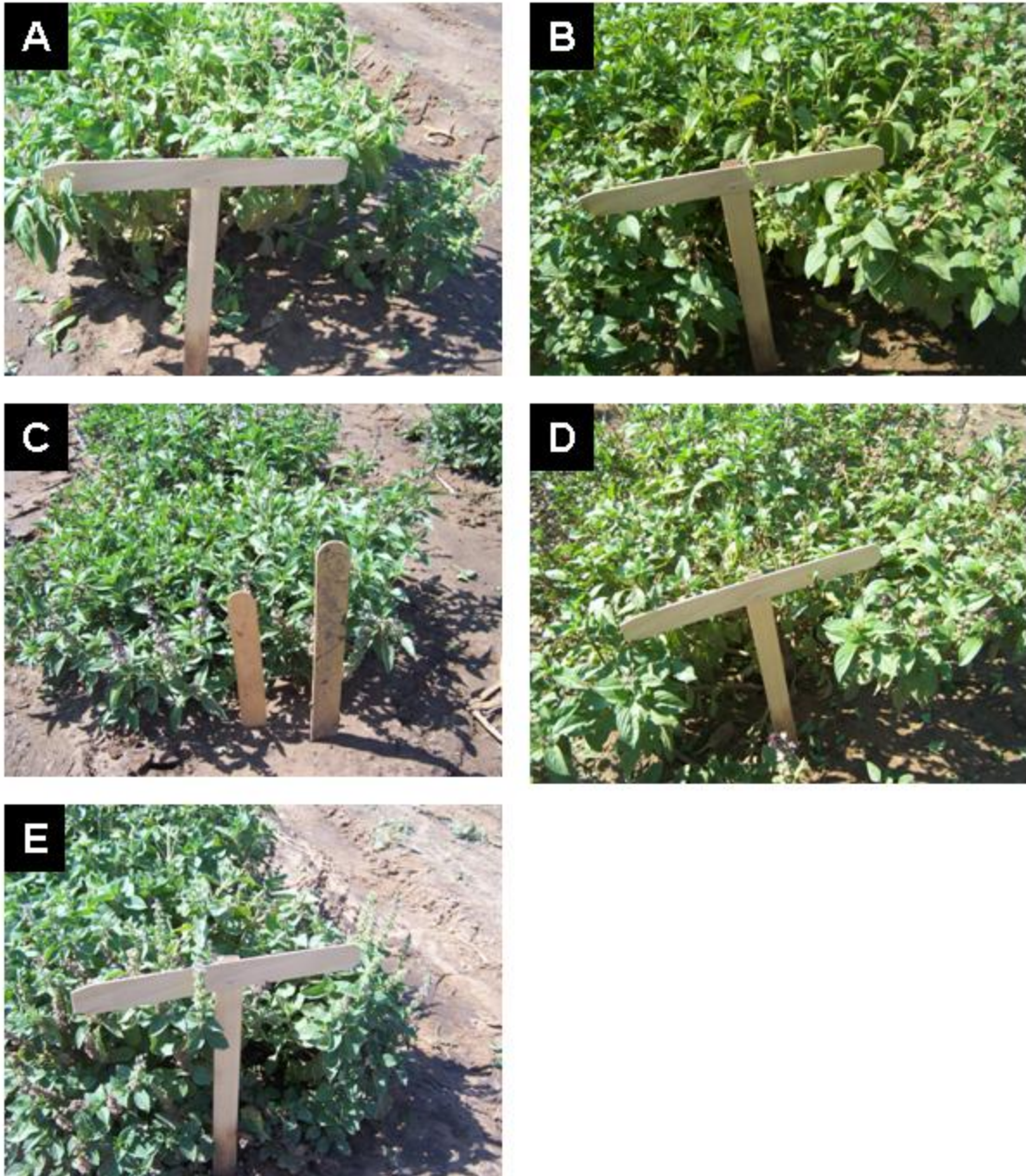


Figure B.2. Basil cultivars [‘Genovese’(A), ‘Mrs. Burns’ Lemon’(B), ‘Sweet Thai’(C), ‘Cinnamon’(D), ‘Blue Spice’(E)] harvested at 2 week interval on August 9 2011 after harvest.

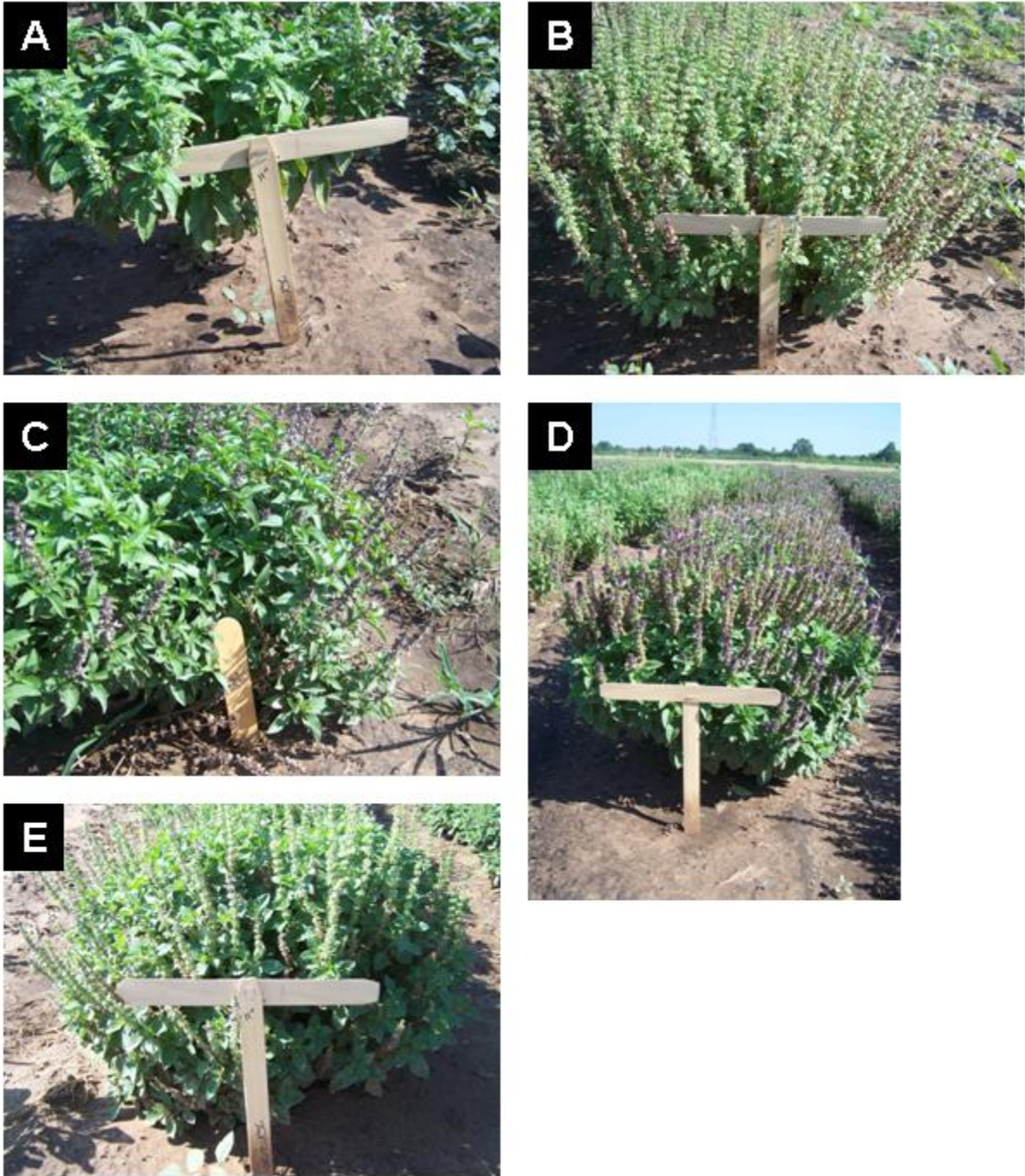


Figure. B.3. Basil cultivars [‘Genovese’(A), ‘Mrs. Burns’ Lemon’(B), ‘Sweet Thai’(C), ‘Cinnamon’(D), ‘Blue Spice’(E)] harvested at 3 week interval on August 9 2011 before harvest.

VITA

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Doctor of Philosophy

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**Scope and Method of Study:** The objectives for this study were to develop a chemical profile library for basil cultivars with potential production in Oklahoma while optimizing handling conditions for their dehydration processing as well as to evaluate and optimize ambient temperature extraction process (ATE) with propane for dehydrated basil. From 2005 to 2011, foliage harvests were intermittently conducted from sixteen cultivars prior to excessive flowering starting in early July and ending in late September at three to four week intervals, but in 2011 harvest frequency was varied on strict 2 week or 3 week intervals for five cultivars. Samples were typically dried at 40°C after harvest. For ATE, dried samples were separated into flowers, leaves and stems. Extractions were then conducted using a custom built continuous flow propane extractor at ambient temperature (21-27°C) and low pressure (1.1-1.9 MPa). Dried samples were ground before extracting with hexane while extracts were solubilized into hexane to identify and quantify aromatic chemical components using gas chromatography. Tristimulus L\*, a\* and b\* values were used to calculate chroma, hue angle and browning index (BI) color attributes of 'Sweet Thai' components.

**Findings and Conclusions:** Based on chemotype, the cultivars could be separated into three categories; the pesto-type basil, such as 'Genovese', 'Napolitano' and 'Italian Large Leaf', featuring linalool and eugenol, the spice-type basil, such as 'Ethiopian Mint', 'Blue Spice' and 'Sweet Thai' featuring eugenol and methyl chavicol and the citrus flavored basil, such as, 'Mrs. Burns' Lemon' and 'Lime', featuring geraniol and neral. The absolute herb yields, impact chemical concentrations, and total phytochemical production of basil cultivars were affected by the year, time of harvest within a year and the frequency of harvests. Spice type basil was more prone to exhibit changes in concentrations of impact chemicals than the pesto and citrus types. Drying at 40° C and careful handling, especially for cultivars including eugenol/linalool as major chemicals, was suggested to avoid excessive changes in aroma and aromatic intensity. Propane extraction appears to be feasible for dry basil. Cultivars differed in extraction kinetics, probably due to prevalence of trichome type for the cultivar. For 'Sweet Thai', extraction duration of 30 min should result in optimum extraction of aromatic chemicals, but extraction durations for other cultivars should be studied to optimize their process.

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