

REVIEWS

Antimicrobial Properties of Basil and Its Possible Application in Food Packaging

PANUWAT SUPPAKUL,[†] JOSEPH MILTZ,^{*,‡} KEES SONNEVELD,[†] AND
 STEPHEN W. BIGGER[§]

Centre for Packaging, Transportation and Storage, Victoria University, P.O. Box 14428, Melbourne City Mail Centre, Melbourne, 8001, Australia, Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa, 32000, Israel, and School of Molecular Sciences, Victoria University, P.O. Box 14428, Melbourne City Mail Centre, Melbourne, 8001, Australia

Basil (*Ocimum basilicum* L.) is a popular culinary herb, and its essential oils have been used extensively for many years in food products, perfumery, and dental and oral products. Basil essential oils and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast, and mold. The present paper reviews primarily the topic of basil essential oils with regards to their chemical composition, their effect on microorganisms, the test methods for antimicrobial activity determination, and their possible future use in food preservation or as the active (antimicrobial), slow release, component of an active package.

Keywords: *Ocimum basilicum*; basil; antimicrobial activity; antimicrobial packaging

INTRODUCTION

Basil is one of the oldest spices belonging to the *Ocimum* genus and to the Lamiaceae (Labiatae) family. The botanical nomenclature of the *Ocimum basilicum* L. varieties from which the different types of basil oil are distilled is complicated. The reason for this complexity stems from the fact that botanists have assigned several designations to the same varieties and, in some instances, have confused some varieties with forms of other species (1). The *Ocimum* genus contains approximately 30 species of herbs and shrubs from the tropical and subtropical regions of Asia, Africa, and Central and South America. However, the major place of diversity appears to be in Africa (2). This genus is characterized by a great variability in its morphology and chemotypes (3). The ease of its cross-pollination contributes to a myriad of subspecies, varieties, and forms (1).

Basil is a popular culinary herb, and its essential oils have been used extensively for many years in the flavoring of confectionary and baked goods, condiments (e.g., ketchups, tomato pastes, chili sauces, pickles, and vinegars), sausages and meats, salad dressings, nonalcoholic beverages, ice cream, and ices. Basil oil has also found a wide application in perfumery, as well as in dental and oral products (1). In addition, because

the public nowadays prefers natural over synthetic direct or indirect food additives (4), naturally derived antimicrobial agents such as basil are becoming increasingly more important in antimicrobial packaging as they present a perceived lower risk to the consumers. Antimicrobial packaging is part of the broader area of "Active Packaging", which has become, in the past decade, one of the major areas of research in food packaging (5). In view of their possible future use in food preservation, this paper reviews the basil essential oils in regards to their chemical composition, their effect on microorganisms, and their potential use in antimicrobial packaging for food preservation.

There are several types of basil oil traded commercially. These oils are conventionally extracted by steam distillation from leaves and flowering tops. An alternative to the conventional steam distillation method is carbon dioxide (CO₂) extraction, under liquid or supercritical conditions.

Numerous investigations on basil essential oils have been reported in the scientific literature. These include studies of (i) taxonomy (2, 6, 7), (ii) chemistry (8–12), and (iii) antimicrobial activity (13–16).

COMPOSITION OF BASIL EXTRACTS

Cultivar and Chemotaxonomic Classification. Most commercial basil cultivars available on the market belong to the *O. basilicum* L. species. Darrah (17) classified the *O. basilicum* cultivars into seven types: (i) tall, slender types, including the sweet basil group; (ii) large-leafed, robust types, which include "Lettuce Leaf" also called "Italian" basil; (iii) dwarf types, which

* To whom correspondence should be addressed. Tel: 972 48 292451. Fax: 972 48 293603. E-mail: jmiltz@tx.technion.ac.il.

[†] Centre for Packaging, Transportation and Storage, Victoria University.

[‡] Technion-Israel Institute of Technology.

[§] School of Molecular Sciences, Victoria University.

Table 1. Chemotaxonomic Classification of *O. Basilicum* L. Based on Geographical Origins^a

chemotype	major constituent	country of origin
European	linalool, methyl chavicol	France, Italy, Egypt, Hungary, South Africa, U.S.A.
reunion	methyl chavicol	Comoro Islands, Malagasy Republic, Thailand, Vietnam, Seychelles
tropical	methyl cinnamate	Bulgaria, India, Guatemala, Pakistan
java	eugenol	Indonesia, North Africa, Russia

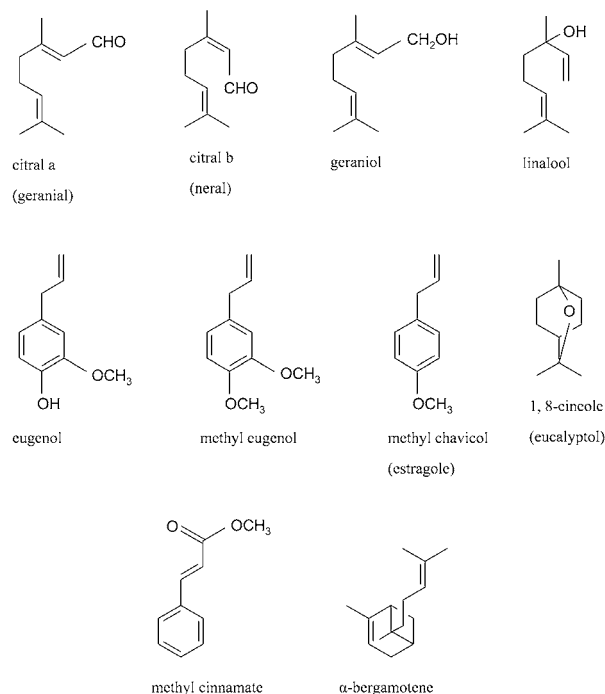
^a Adapted from references 6, 18, and 19.**Table 2.** Chemotaxonomic Classification of *O. Basilicum* L. Based on the USDA Germplasm Collection^a

PI no. or cultivar name	major constituent	country of origin
175793	linalool	Turkey
368699	linalool, 1,8-cineole	Yugoslavia
358465	linalool, geraniol	Yugoslavia
174285	linalool, methyl chavicol	Turkey
190100	methyl chavicol, linalool	Iran
253157	methyl chavicol, citral	Iran
170579	methyl cinnamate, methyl chavicol, linalool	Iran
Purdue selection	methyl eugenol	Thailand

^a Adapted from reference 18.**Table 3.** Major Constituents of *O. Basilicum* L.

proportion	major constituent	ref
almost equal	linalool, eugenol	6
	methyl chavicol, methyl eugenol	6
	linalool, methyl cinnamate	20
various quantities	linalool, eugenol	12, 21
	linalool, <i>trans</i> - α -bergamotene	12
	methyl cinnamate, linalool, 1,8-cineole	11
	methyl chavicol, 1,8-cineole	11
	methyl chavicol, linalool, geraniol	6
	methyl chavicol, citral	18

are short and small-leaved, such as "Bush" basil; (iv) compact types such as *O. basilicum* var. *thyriflora*, commonly termed "Thai" basil; (v) *Purpurascens*, the purple-colored basil types, with conventional sweet basil flavor; (vi) purple types such as "Dark Opal", a possible hybrid between *O. basilicum* and *Ocimum forskolei* with lobed leaves and a sweet basil plus clovelike aroma; and (vii) *Citriodorum* types including lemon-flavored basils. The essential oils derived from *O. basilicum* L. have been traditionally classified into four distinct chemotypes with many subtypes (Table 1), based on the biosynthetic pathways that produce the principal components in the oil (3). There is an enormous variation in composition of basil essential oils. The different chemotypes contain various proportions of allyl-phenol derivatives, like methyl chavicol (estragole), eugenol, and methyl eugenol as well as linalool, a monoterpene alcohol. Simon et al. (18) have classified chemotaxonomically the selected *Ocimum* species including *basilicum* (Table 2). Other basil essential oils have been reported to contain two or more major constituents in almost equal proportions or in various quantities as shown in Table 3. Clearly, these oils cannot be readily classified using the conventional system of chemotypes.

**Figure 1.** Chemical structures of major constituents found in *O. basilicum* L.

It has been therefore suggested that the profiles of the essential oils of basil are to be classified on the basis of all of the main components, even if there are only three or four major volatile compounds present (4). The chemical structures of the major constituents of basil are shown in Figure 1. The taxonomy of basil is also complicated due to the existence of numerous botanical varieties and cultivar names within the species that may not differ significantly in morphology (18). Paton and Putievsky (7) proposed a system of standardized descriptions that includes volatile oils. This should allow easier communication and identification of the different forms of *O. basilicum*. Investigations to revise the genus are underway at the Royal Botanical Garden, Kew, London (2), and at Delaware State University (22).

Compositional Variation. The chemical analysis of essential oils derived from *O. basilicum* L. has been the subject of many studies with varying results from country to country (Table 4). The variation in the chemical composition of basil essential oils is thought to be due to polymorphism in *O. basilicum* L., which in turn is caused by interspecific hybridization (7). In an early paper, Lawrence et al. (8) listed a total of 75 chemical constituents in *O. basilicum* L. obtained from Thailand. They identified, for the first time, *trans*-ocimene oxide, germacrene D, caryophyllene oxide, and τ -cadinol. Later, over 100 constituents were separated in various basil essential oils and 10 new compounds were identified (29). Hasegawa et al. (21) characterized the components of the essential oils produced from nine different cultivars of *O. basilicum* L. and oils from a local cultivar found in the Philippines. More than 130 compounds were identified in the latter cultivar, 32 of which were found in basil oil for the first time. Simon et al. (22) reported a comparative evaluation of North American commercially available *Ocimum* sp. cultivars, including *basilicum*, as shown in Table 5.

Enantiomeric Consideration. Ravid et al. (30) determined the amount and the enantiomeric composition of linalool in the essential oils of seven chemotypes of *O. basilicum* L., in the oils of *Ocimum sanctum* L., *Ocimum gratissimum* L., and

Table 4. Compositional Variation in *O. Basilicum* L.

country of origin	chemical composition (% w/w)	ref
Benin		12
"methyl chavicol"	methyl chavicol (> 65)	
"methyl chavicol–linalool"	methyl chavicol (55), linalool (20–30)	
"linalool–eugenol"	linalool (42–45), eugenol (15), with or without <i>trans</i> - α -bergamotene (6–15)	
Brazil		11
"linalool"	linalool (49.73)	
"1,8-cineole"	1,8-cineole (22)	
"methyl chavicol"	methyl chavicol (47)	
"methyl cinnamate"	methyl-(<i>E</i>)-cinnamate (65.5)	
Cameroon	linalool (50.8), eugenol (13.5), limonene (10.4), 1,8-cineole (3.1)	9
Cuba	methyl chavicol (66.75), 1,8-cineole (5.44)	23
	linalool (4.95), α -bisabolene (3.60)	
	(<i>E</i>)- α -bergamotene (2.96)	
Germany	methyl chavicol (86.1)	24
Italy		19
"linalool"	linalool (70), 1,8-cineole (13)	
"linalool–methyl chavicol"	linalool (41–60), methyl chavicol (18–41)	
	1,8-cineole (2–6)	
"linalool–eugenol"	linalool (61–76), eugenol (4), 1,8-cineole (1–11)	
Italy		19
"eugenol", low linalool	eugenol (2.22–3.89), linalool (60.76–64.14)	
"eugenol", high linalool	eugenol (trace–1.99), linalool (69.06–76.20)	
Mongolia	methyl chavicol (52), linalool (23.8), τ -cadinol (4.4)	25
Republic of Guinea	linalool (69), eugenol (10), <i>trans</i> - α -bergamotene (3), thymol (2)	10
Somalia	dihydrotagetone (> 80)	26
Thailand	methyl chavicol and α -humulene (88.2)	8
Togo		27
"Reunion"	methyl chavicol (84–89)	
"European"	linalool (41), methyl chavicol (22)	
Turkey	linalool (17–24), methyl-(<i>E</i>)-cinnamate (12–16), 1,8-cineole (7–13), τ -cadinol (5–7)	28

Table 5. Comparative Evaluation of North American Commercially Available *O. Basilicum* L. Cultivars^a

cultivar	color leaf	color flower	oil yield ^b % v/dw	major compds ^c (%)
Anise	green–purple	light pink	0.62	L, 56; MC, 12
Cinnamon	green	pink	0.94	L, 47; MCM, 30
Dark Opal	purple	pink	1.08	L, 80; 1,8-C, 12
Fino Verde	green	white	0.50	L, 48; MC, 7
Genovese	green	white	0.90	L, 77; 1,8-C, 12
Green Ruffles	light green	white	0.55	L, 33; 1,8-C, 18
Holy Sacred Red	purple	pink	0.83	L, 77; 1,8-C, 14
Italian Large Leaf	green	white	0.83	L, 65; MC, 18
Lettuce Leaf	green	white	0.78	L, 60; MC, 29
Licorice	green–purple	pink	0.43	L, 58; MC, 13
Mammoth	green	white	0.77	L, 60; MC, 32
Napoletano	green	white	0.89	L, 66; MC, 10
Opal	purple	pink	0.91	L, 80; 1,8-C, 13
Osmin Purple	purple	pink	0.66	L, 77; 1,8-C, 15
Purple Ruffles	purple	bright purple	0.49	L, 55; 1,8-C, 20; MC, 6; ME, 9
Red Rubin Purple Leaf	purple	pink	0.74	L, 70; 1,8-C, 9; MC, 10
Sweet	green	white	0.84	L, 69; 1,8-C, 11; MC, 13
Sweet Fine	green	white	0.98	L, 86; 1,8-C, 6
Sweet Thai	green	pink	0.40	L, 6; MC, 60
Thai (Companion Plants)	green	pink	0.75	L, 12; MC, 65
Thai (Richters)	green	pink	0.52	MC, 90
Thai (Rupp Seeds)	green	white	0.25	L, 15; MC, 13

^a Plant density of 12 000 plants ha⁻¹. ^b Oil yield % volume per dry weight. ^c L, linalool; MC, methyl chavicol; MCM, methyl cinnamate; 1,8-C, 1,8-cineole; ME, methyl eugenol. Adapted from reference 22.

Ocimum canum Sims., originating from Thailand, and in commercial basil oils. The linalool isolated from cultivars of *O. basilicum* L., from various origins and from commercial basil oils, consisted of (*R*)-(–)-linalool and was optically pure in most cases. On the other hand, (*S*)-(+)-linalool was the main enantiomer in essential oils of *O. sanctum* L. and *O. canum* Sims. Consequently, the enantiomeric differentiation of linalool may be useful in interspecific taxonomy in the genus *Ocimum*.

Tateo et al. (31) studied the quantity and the enantiomeric composition of camphor in the essential oils of two types of *O. basilicum* L. from Italy. In regards to *O. basilicum* L. essential

oils having a camphor content below 1%, a chiral analysis showed that the camphor (isolated from the so-called "Genuese type" containing mainly linalool (47–52% w/w) and a trace of methyl chavicol) consisted of (*R*)-(+)-camphor and was optically pure in this type. For the so-called "Neapolitan type" (containing primarily linalool (47–63% w/w) and 9–12% w/w methyl chavicol), (*R*)-(+)-camphor was the main enantiomer with a content of above 94%. It appears that the enantiomeric composition of camphor is a useful parameter in assessing the genuineness and is used as a characterizing element of various botanical genuses.

Other Factors Affecting Composition. A number of factors were found to affect the composition of basil extracts: (i) the harvest season and plant phenological stages including vegetative, early blooming, full blooming, and seeding. For instance, *O. basilicum* L. cv. Genovese Gigante, the basil cultivar used most in the production of typical Italian pesto sauce, contains linalool as its main component at the beginning of the flowering stage but methyl eugenol and eugenol become significant between 4 and 6 weeks after sowing (32). (ii) The extraction method affects the composition of the extracts. Thus, it is claimed that the yields are highest using supercritical CO₂ followed by liquid CO₂ and then water (20, 33). However, these claims oppose the results obtained by Ehlers et al. (34). The extraction method of basil also affects the chemical composition of the major volatiles in the extract. A hydrodistilled material contains a significantly larger proportion of the lower boiling point hydrocarbons such as pinene, myrcene, terpinene, limonene, and oxygenated terpenes including 1,8-cineole, fenchone, and camphor. On the other hand, the CO₂ extracts contain a large number of unidentified high boiling point constituents with retention times, measured by GC, between those of geraniol (33.5 min) and eugenol (43.7 min), which are either present in small amounts or are undetectable in the hydrodistilled oil. As far as the chemical composition is concerned, there appears to be no major benefits in using supercritical CO₂ over liquid CO₂ for extracting basil; actually, the production costs for extraction with liquid CO₂ are lower. However, sensory evaluation has shown that the hydrodistilled oil and the liquid CO₂ extracts (obtained with a one stage separator under controlled conditions) were quite different (20, 33). (iii) Different milling techniques might induce modifications in the composition of the vegetable matrix and may have an adverse effect on the content of thermally labile compounds, such as linalool, methyl chavicol, and methyl cinnamate (35). (iv) Freeze-drying of basil leaves may affect the concentration of linalool (6). (v) Supplementary UV-B treatment of glasshouse-grown sweet basil may affect the levels of most major volatiles such as the phenyl-propanoids (eugenol, methyl eugenol) and the terpenoids (notably linalool, 1,8-cineole, and *trans*- β -ocimen) (36). (vi) The wavelengths of light reflected from colored mulches can affect leaf size, aroma, and concentrations of soluble phenolics in sweet basil. Plants grown over yellow and green mulches contain significantly higher levels of aroma compounds (including linalool and eugenol) than those grown over white and blue covers (37). (vii) The content of methyl chavicol and methyl-(*E*)-cinnamate is usually higher in field-grown basil than in greenhouse plants; in contrast, linalool content falls in all field-grown basil as compared to greenhouse plants (11).

TESTING METHODS OF THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

Different methods of antimicrobial activity determination of essential oils (or essential oil components) affect the results. Numerous studies on the antimicrobial activity of essential basil oil and its principal constituents have been reported. However, it is difficult to compare the results of these studies because of substantial variations in the basil essential oils, test microorganisms, and test methods. There is a need for the development and validation of standard methods to accurately determine the efficacy of essential oils or oil components and to compare the published data in different studies.

Principal Methods. Generally, these techniques provide preliminary information on the potential usefulness of the tested compound. The techniques represented in this category include

the following: (i) diffusion methods—the agar diffusion test has probably been the most widely used in the past of the end point tests. It has been often referred to as the paper disk assay. However, this description is probably too narrow (38). Many variations in the test exist, including the use of cylinders, cups, wells, ditch plates, and agar overlays (39, 40). A zone of “no growth” around the disk defines the extent of antimicrobial activity. The size of this zone depends on the rates of diffusion and cell growth (39). Results of these tests are qualitative. Microorganisms are generally termed susceptible, intermediate, or resistant—depending upon the diameter of the inhibitory zone. Quantitative results are adequate with a high degree of standardization, but better methods are available. (ii) Dilution methods—broth and agar dilution methods are widely used to determine the MIC of oil-based compounds. The MIC is defined as the lowest concentration of the compound that inhibits growth of a microorganism after a specified incubation period (41). (iii) Microatmosphere method—this method allows the determination of the antimicrobial activity of essential oils or essential oil components in the vapor phase. In this method, the agent diffuses toward the agar in an inverted Petri dish (9) or a vial (42). Microbial growth is monitored through visible growth detection in the case of an inverted Petri dish. In the case of a vial, this growth is monitored by the presence, at equilibrium in the headspace, of metabolic carbon dioxide produced by the microorganism.

Limitation of the Test Methods. The antimicrobial activity of essential oils can be demonstrated by numerous methods. Methods involving agar media such as the paper disk diffusion assay, the agar well diffusion assay, and the agar dilution assay are used most frequently. Because of the low water solubility of essential oils, emulsifiers such as Tween 20 (polyoxyethylene-2-sorbitan monolaurate), Tween 80 (polysorbate 80), and Triton \times 100 or solvents such as ethanol are often used to enhance the solubility of hydrophobic compounds in both the solid and the liquid media. However, emulsifiers as well as solvents have attracted some criticism concerning their direct action on microorganisms and their possible effect on the antimicrobial activity of essential oils. Emulsifiers are claimed to assist in the penetration of antimicrobial agents into the bacterial cell wall and membrane (16, 43). The quantity of an emulsifying agent can also affect the results. For example, lipophilic molecules, including the components of the essential oil of basil, may become soluble within the micelles formed by nonionic surfactants such as Tween 20 and Tween 80 and thereby partition out from the aqueous phase of the suspension (44). Kazmi and Mitchell (45) claimed that antimicrobial agents solubilized within micelles do not contribute to the antimicrobial activity, as they do not come in direct contact with the target microorganisms. Remmal et al. (46) found that MICs vary with the kind of emulsifying agent used. They confirmed the fact that solvents and emulsifiers often used in antimicrobial studies decrease the antimicrobial activity of essential oils.

RESULTS ON THE ANTIMICROBIAL ACTIVITY OF BASIL OILS

The published data on the antibacterial activity of the basil essential oils and their constituents are larger than that on their antifungal activity. Unfortunately, the published data on the former subject are very difficult to compare. The chemical composition of basil essential oils and extracts is known to vary with the local climatic and environmental conditions (11). Thus, samples of basil essential oil may have the same common name even when they are composed of different subspecies of *O.*

Table 6. Range of Concentrations of Basil Essential Oils Reported to Inhibit the Growth of Microorganisms

concentration	test condition and inhibition type	ref
bacteria		
10 mg/mL	liquid medium, MIC	48
1250–5000 µg/mL	liquid medium, MIC	49
200 µL/mL	liquid medium, viable cell count	50
0.05–0.1 mL/mL	liquid medium, MIC	51
>2–0.5 mL/mL	solid medium, MIC, visible growth	16
5–100 µL/mL	liquid medium, viable cell count	52
fungi		
1–1.5 µL/mL	liquid medium, mycelial growth	53
5000 µg/mL	liquid medium, MIC, mycelial growth	49
15.6–31.2 µg/mL	liquid medium, MIC, mycelial growth	54
yeast		
6.25 mg/mL	liquid medium, MIC	48
1250 µg/mL	liquid medium, MIC	49
0.5 mL/mL	solid medium, MIC, visible growth	16

Table 7. Range of Concentrations of the Principal Constituents of Basil Essential Oils Reported to Inhibit the Growth of Microorganisms

concentration	test condition and inhibition type	ref
bacteria		
500–750 µg/mL	E ^a , solid medium, visible growth	55
10–20 µg/mL	E, liquid medium, MIC	56
>1000 µg/mL	L, solid medium, visible growth	55
2.5 mg/mL	L, liquid medium, MIC	48
>13.3–3.33 µL/mL	L, liquid medium, MIC	57
>20–10 µL/mL	L, liquid medium, MIC	58
1.25–20 µL/mL	MC, liquid medium, MIC	58
fungi		
125 µg/mL	E, liquid medium, mycelial growth	59
62.5–125 µg/mL	E, liquid medium, toxin production	59
200 µg/mL	E, liquid medium, mycelial growth	60
6.25 mg/mL	L, liquid medium, MIC	48
>5–0.2 µL/mL	L, liquid medium, MIC	57
125 µg/mL	MC, liquid medium, mycelial growth	42
1–2.5 µL	MC, vapor phase in vial, CO ₂ production (42)	
yeast		
6.25 mg/mL	L, liquid medium, MIC	48
0.2 µL/mL	L, liquid medium, MIC	57

^aE, eugenol; L, linalool; MC, methyl chavicol.

basilicum L. (1, 3). The selection of test microorganisms, the way of exposure of the microorganisms to basil essential oils, and the method used to evaluate their antimicrobial activity all vary among the different publications (40, 46, 47).

Thus, the contradictory conclusions reached in the early studies on the antimicrobial activity of basil essential oils are not surprising. Poor solubility and high volatility often preclude the application of traditional antimicrobial assays, such as agar diffusion or zone of inhibition tests. As a result, agar or broth dilution methods using variable concentrations against a variety of target species are often used (39, 41). The findings of some studies are summarized in Tables 6 and 7.

Studies Involving Diffusion Methods. Lahariya and Rao (61) studied the antimicrobial effectiveness of the essential oil of *O. basilicum* tested in vitro against 10 different microorganisms. They found that this essential oil was more active than the reference, streptomycin, in inhibiting the growth of *Bacillus pumilus*, but it had no activity against *Bacillus mycoides*, *Pseudomonas mangiferae indica*, *Staphylococcus albus*, and *Vibrio cholerae*. The oil was found to be most effective against *Bacillus anthracis* and less effective against *Bacillus substalis* and *Salmonella paratyphi*. In addition, it had certain activity

against all of the tested fungi, including *Microsporum gypseum*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Penicillium liliacinum* but was less active than the reference, griseofulvin.

Reuveni et al. (62) investigated the fungistatic activity of essential oils from *O. basilicum* chemotypes against *Fusarium oxysporum* f. sp. *Vasinfestum* and *Rhizopus nigricans*. They reported that both European and Reunion chemotypes showed 100% inhibition of *R. nigricans* whereas the local selection type (in Israel) exhibited 96.4% inhibition of this strain. European, Reunion, and local selection types exhibited 46.8, 2.6, and 65.4% inhibition of *F. oxysporum*, respectively. Conner and Beuchat (63) determined the effects of plant essential oils on the growth of 13 food spoilage and industrial yeasts using a standard zone of inhibition test on YMPG agar. The basil essential oil inhibited only slightly the growth of *Kloeckera apiculata*, but there was no inhibition of *Candida lipolytica*, *Debaryomyces hansenii*, and *Saccharomyces cerevisiae*. This result is in agreement with the findings of Ozcan and Erkmen (64), who used a dilution method, but it is in contradiction with the findings of Meena and Sethi (65) and Lachowicz et al. (66). This discrepancy might be due to different chemotypes of sweet basil. However, further work is required to explain this contradiction.

Prasad et al. (13) studied the antimicrobial activity of essential oils of *O. basilicum* (French), *O. basilicum* (Indian), and *O. basilicum* (Niazbo), which are rich in linalool, methyl chavicol, and methyl cinnamate, respectively, against 11 Gram-positive and seven Gram-negative bacteria. They found that these oils were more effective against Gram-positive than against Gram-negative bacteria. For example, all of the Gram-positive bacteria *Bacillus sacharolyticus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thurengiensis*, *Micrococcus glutamicus*, and *Sarcina lutea* were inhibited by each of these Basil essential oils. However, the Gram-negative strain *Salmonella weltevreden* only was suppressed by all of the oils. Prasad et al. (13) also found that methyl cinnamate type basil essential oil inhibited all of the 13 tested fungi and only *Histoplasma capsulatum* grew in the presence of linalool type basil oil. *Candida albicans*, *H. capsulatum*, and *Sporotrichum schenckii* were found to be resistant to methyl chavicol type basil oil. Sinha and Gulati (14) found that each of these basil essential oils was also effective against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, and *Proteus vulgaris*. All basil essential oils showed also an antifungal effect on *C. albicans* and *S. schenckii*, with methyl chavicol type basil essential oil being highly effective. This effect has been predominantly associated with the main constituents, linalool and methyl chavicol.

Deans and Ritchie (67) screened 50 plant essential oils (including basil) for their antibacterial properties against 25 genera of bacteria by using the agar diffusion technique. They found that most of the bacteria, including *Aeromonas hydrophila*, *B. subtilis*, *Brevibacterium linens*, *Brocothrix thermosphacta*, *Erwinia carotovora*, *E. coli*, *Lueconostoc cremoris*, *S. aureus*, *Streptococcus faecalis*, and *Yersinia enterocolitica*, show a reasonably broad sensitivity to undiluted basil essential oil. Gangrade et al. (68) examined the antibacterial properties of the linalool and the methyl cinnamate types of the essential oils of *O. basilicum*, in the pure state and at four dilutions (1:10, 1:100, 1:1000, and 1:10000) prepared with DMSO against four major bacterial species. They found that both essential oils had an inhibitory activity against *S. aureus* and *E. coli* at all dilutions. The dilution of either oil with DMSO beyond 1:1000 resulted in no inhibition against *Streptococcus pyogenes*. Basil

essential oil also showed an inhibition against *Bacillus cereus*, *Lactobacillus acidophilus*, *A. niger*, and *S. cerevisiae*, as determined by the paper disk agar diffusion method, both at ambient temperature and at 37 °C (65). These results were expanded and supported by Aboul Ela et al. (48) and Elgayyar et al. (69), who showed that basil essential oil has antibacterial and antifungal activity against *S. aureus*, *E. coli*, and *A. niger*.

Baratta et al. (24) reported that the methyl chavicol type of basil essential oil showed a significant activity against the growth of *S. aureus* food poisoning organisms. They also reported that *B. subtilis*, *B. thermosphacta*, *E. carotovora*, *B. linens*, and *P. aeruginosa* were resistant to undiluted basil essential oil. These findings contradict those obtained by Dean and Ritchie (67) and Lachowicz et al. (66), presumably because of the different chemotypes of basil essential oil used in the two studies. In two publications, Lis-Balchin and Deans (70) and Lis-Balchin et al. (15) described the relationship between the bioactivity and the chemical composition of commercial essential oils, including that of the methyl chavicol type basil essential oil. The authors reported that a strong bioactivity was observed when the major component was eugenol and a less pronounced one when the main constituents were geraniol, citronellol, and linalool. Methyl chavicol has not shown a strong antimicrobial activity. The findings of Lis-Balchin et al. (15) contradict those of Baratta et al. (24), Reuveni et al. (62), and Sinha and Gulati (14).

Essential oils extracted by hydrodistillation from five different varieties of *O. basilicum* L. plants (Anise, Bush, Cinnamon, Dark Opal, and a commercial sample of dried basil) in Australia were examined by the agar well diffusion method for their antimicrobial activity against a wide range of food-borne Gram-positive and Gram-negative bacteria, yeasts, and molds. All five essential oils of basil showed antimicrobial activity against 20 out of 24 tested microorganisms including *A. hydrophila*, *B. cereus*, *B. subtilis*, *B. thermosphacta*, *E. coli*, *Lactobacillus plantarum*, *Listeria monocytogenes*, *Mucor piriformis*, *Penicillium candidum*, *Penicillium expansum*, *S. cerevisiae*, *Salmonella typhimurium*, *S. aureus*, *Candida colliculosa*, *Candida formata*, *Candida humicola*, and *Zygosaccharomyces bailli*. In addition, the spectrum of antimicrobial activity did not vary greatly between oils from the different varieties of basil, except for *Enterococcus faecalis* that was found to be resistant to Cinnamon basil oil but sensitive to the other four basil oils. *Pseudomonas* species were found to be resistant to all of the tested oils (66). Nascimento et al. (56) found *P. aeruginosa* to be susceptible to basil essential oil (containing linalool, methyl chavicol, and eugenol). Rai et al. (54) examined the antifungal activity of the essential oils of 10 plant species (including *O. basilicum*), grown in Chhindwara, India, against five *Fusarium* species. They found that the essential oils of basil (methyl cinnamate-rich type) were active against all *Fusarium* species and especially active against *Fusarium acuminatum*, *Fusarium solani*, *Fusarium pallidoroseum*, and *Fusarium chlamydosporum*.

The antimicrobial activity of the individual principle constituents of basil essential oil (linalool, methyl chavicol, eugenol, and methyl cinnamate) was also studied. Knobloch et al. (71) evaluated the antimicrobial activity of essential oil components against Gram-negative bacteria (e.g., *Enterobacter aerogenes* and *P. vulgaris*), Gram-positive bacteria (e.g., *S. aureus* and *B. subtilis*), and fungi (e.g., *Aspergillus flavus*, *A. niger*, *A. ochraceus*, and *P. expansum*). They found that linalool, with its high water solubility, had a significant antimicrobial activity as compared to cinnamaldehyde, citral, geraniol, eugenol, and

menthol whereas methyl chavicol, with its lower water solubility, had a low antimicrobial activity. The solubility in water of essential oil constituents is directly related to their ability to penetrate the cell walls of a bacterium or fungus. Thus, the antimicrobial activity of essential oils is due to their solubility in the phospholipid bilayer of cell membranes (71). It was also reported that the antibacterial activities of monoterpene alcohols (including linalool, nerol, citronellol, and geraniol) are more effective than their antifungal activity.

Meena and Sethi (65) found that eugenol has an inhibitory effect against *A. niger*, *L. acidophilus*, and *S. cerevisiae*. Kim et al. (43) studied the antibacterial activity of some essential oil components (including linalool and eugenol) against five food-borne pathogens (*E. coli*, *E. coli* O157: H7, *S. typhimurium*, *L. monocytogenes*, and *Vibrio vulnificus*). They found that eugenol showed a dose-related increase in the zone of inhibition against the five strains, whereas linalool exhibited a similar effect against all tested strains except for *L. monocytogenes*. Linalool inhibited the growth of *L. monocytogenes*, but the difference in the zone size between the test concentrations (5, 10, 15, and 20% v/v) was not significant.

Pattnaik et al. (57) studied the antibacterial properties of the aromatic constituents of essential oils. The results of the disk diffusion assays showed that linalool was the most effective compound and retarded 17 out of 18 bacterial strains (only VR-6, a *Pseudomonas*, is resistant), followed by cineole, geraniol, menthol, and citral. They also found that the MIC values of the essential oils were usually lower than those of their constituents. One possible reason for this result could be the synergistic action of the constituents in the oils. Mazzanti et al. (72) found that linalool was the active compound that completely inhibited the growth of all yeasts (seven strains of *C. albicans*, *Candida krusei*, and *Candida tropicalis*), *S. aureus* and *E. coli*. Authentic pure linalool showed a similar antibacterial spectrum to that of basil essential oils. However, pure methyl chavicol exhibited a much narrower antibacterial spectrum, with an activity against only eight out of the 24 strains of organism tested (66). This result is in contradiction with the findings of Wan et al. (58), although the same parameters and the same experimental technique were used. A possible explanation might be batch to batch variations (70) or a difference in sources of compounds.

Scora and Scora (73) investigated the fungicidal effect of volatile compounds, with the main basil essential oil components, against three *Penicillium* species. It is known that phenolic compounds such as carvacrol, thymol, and eugenol possess a major fungicidal effect. Etherified compounds such as anethole, methyl chavicol, and safrole exhibit less fungicidal action while monoterpene hydrocarbons such as limonene and β -myrcene have almost no effect. As noted by Knobloch et al. (71), the variation in the fungicidal action of essential oil components seems to rely on their water solubility and lipophilic properties (i.e., their ability to penetrate the chitin-based cell walls of fungal hyphae). Specific functional groups and the interference of membrane-associated enzyme proteins may also affect the results (71, 74).

Recently, Dorman and Deans (75) reported on the antibacterial activity of 21 plant volatile oil components (including eugenol and linalool) against 25 bacterial strains by the agar well diffusion technique. Eugenol exhibited the widest spectrum of activity against 24 out of 25 bacteria, except for *L. cremoris*, followed by linalool (against 23 strains, except *L. cremoris* and *P. aeruginosa*). These results contradict those obtained by Lachowicz et al. (66) and Wan et al. (58) who used the same technique and found that linalool inhibited *L. cremoris*. The

components with phenolic structures, including eugenol, were highly active against the test microorganisms. Members of this class are known as either bacteriocidal or bacteriostatic agents, depending on the concentration (76). These components are strongly active despite their relatively low solubility in water (43, 71, 77). Alcohols are known to possess bacteriocidal rather than bacteriostatic activity against vegetative cells. The tertiary alcohol, linalool, is active against the test microorganisms, potentially acting as either a protein denaturing agent (76) or as a solvent dehydrating agent. Knobloch et al. (71) demonstrated the relationship between water solubility of terpenoids and their antimicrobial activity on whole cells. The solubility of essential oils and their terpenoid compounds in water should therefore be taken into consideration when studying the action of these compounds on the membrane-catalyzed functions within the cell wall that acts as a physical barrier.

Studies Involving Dilution Methods. *Agar Dilution.* Dube et al. (53) studied the antifungal activity of the essential oil of *O. basilicum* by an agar dilution method. They showed that the essential oils of basil at a concentration of 1.5 mL/L completely suppress the mycelial growth of 22 species of fungi, including the mycotoxin-producing strains of *A. flavus* and *Aspergillus paralyticus*. In addition, the lethal dose of the oil was found to be four times less than that of Agrozim, Bavistin, and Emison and six times less than Sulfex and Celphos. The oil of *O. basilicum* is evidently a potent mycotoxic agent endowed with the ability to kill aflatoxin-producing strains. Therefore, this oil is more effective and preferable, being natural, over synthetic fungicides.

Hammer et al. (16) studied the antimicrobial activity of a large number of essential oils and other plant extracts (including basil essential oils) against a diverse range of organisms using either the agar dilution or the broth microdilution method. The MICs of basil essential oils obtained by the agar dilution method ranged from 0.5 to >2.0% v/v. The essential oils of basil inhibited all tested organisms at concentrations below 2.0% v/v except for *E. faecalis*, *P. aeruginosa*, and *Serratia marcescens*. Kurita et al. (74) examined the antifungal activity of 47 kinds of essential oils and several related compounds against seven fungi. The results suggest that secondary alcohols (e.g., 2-octanol, L-menthol, borneol) and tertiary alcohols (e.g., linalool) possess a markedly lower antifungal activity as compared to primary alcohols such as cinnamyl alcohol, geraniol, and citronellol. The antifungal activity of eugenol (4-allyl-guaiacol), a phenolic compound, was found to be 8–10 times higher than that of guaiacol (*o*-methoxyphenol) and 3–4 times higher than that of creosol (4-methylguaiacol). From the molecular structure, it is clear that the addition of alkyl or alkenyl group(s) to the benzene ring of either phenol or guaiacol enhances the antimicrobial activity. The activity of these phenolic compounds appeared to depend on the size of the added alkyl or alkenyl group, where the larger the size of the alkyl or alkenyl group, the stronger the antimicrobial activity (71, 74, 76). Because alkyl or alkenyl groups are hydrophobic, these results indicate that a hydrophobicity above a minimum extent is required for phenolic compounds to show a potent antimicrobial effect. Reuveni et al. (62) studied the percentage of inhibition of principle constituents of basil on *R. nigricans* and *F. oxysporum*. They found that both linalool and methyl chavicol had the highest percentage of inhibition (100%) against *R. nigricans* while a value of 38.1% only was found for eugenol. The reverse was found for *F. oxysporum* where the percentage inhibition of eugenol was highest (100%), while for linalool and methyl chavicol the values were only 26.4 and 30.3%, respectively. In

addition, the antimicrobial activities of basil essential oils of different chemotypes were predominantly related to their main components (62, 78). This is in agreement with the results of Pattnaik et al. (57) working with citral, a major antifungal component in lemongrass. The study by Karapinar and Aktug (79) on the inhibition of food-borne pathogens by four spice components showed that eugenol is the most effective inhibitor against *S. typhimurium*, *S. aureus*, and *Vibrio parahaemolyticus*. Moleyar and Narasimham (55) studied the antibacterial activity of 15 essential oil components against the food-borne pathogens: *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., and *Enterobacter* sp., using an agar plate technique. Cinnamic aldehyde was found to be the most active compound, followed by citral, geraniol, eugenol, and menthol. Linalool was found to exhibit only a slight antibacterial activity.

Broth Dilution. Different researchers used the broth dilution method reporting the following results: Hitokoto et al. (59) claimed that basil leaves showed a complete inhibition of ochratoxin A, in the production of *A. ochraceus*, and a partial inhibition of the growth and toxin production of *A. flavus* and *Aspergillus versicolor* and the growth of *A. ochraceus*. Basilio and Basilio (80) investigated the inhibitory effects of some spice essential oils, including the essential oil of basil (*O. basilicum*), on *A. ochraceus* growth and ochratoxin A production. They reported that at a level of 1000 ppm, only basil affected the fungal growth and the production of ochratoxin A up to 7 days but permitted mold growth afterward. This is in agreement with the work of Hitokoto et al. (59). Lis-Balchin et al. (15) studied the antifungal activity of the methyl chavicol type of basil essential oil against three fungi and found that the oil exhibited 94, 76, and 71% of inhibition on *A. niger*, *A. ochraceus*, and *Fusarium culmorum*, respectively. These results are in agreement with those of Baratta et al. (24) who worked with the agar well diffusion method and found that methyl chavicol type basil essential oil shows 93.1% inhibition on *A. niger*. Amvam Zollo et al. (9) concluded from the MIC results of the oil, as determined by the broth microdilution method after 7 days of incubation, that *O. basilicum* essential oil has an important antifungal activity. The oil was fungicidal against *C. albicans* and *A. flavus* at 5000 ppm, but it was not fungistatic on *Cryptococcus neoformans* up to 1250 ppm. Recently, Ozcan and Erkmen (64) studied the antifungal activity of basil essential oil collected in Turkey. They found the oil to be ineffective on *S. cerevisiae*, *A. niger*, and *Rhizopus oryzae*, contrary to the findings of Dube et al. (53), Meena and Sethi (65), and Prasad et al. (13). This contradiction might be due to the different chemotype of sweet basil or due to different test methods. Smith-Palmer et al. (51) examined the antimicrobial properties of 21 plant essential oils and two essences (including basil essential oil) against five predominant food-borne pathogens: *Campylobacter jejuni*, *Salmonella enteritidis*, *L. monocytogenes*, *S. aureus*, and *E. coli*. The results of bacteriocidal and bacteriostatic concentrations showed that the two Gram-positive bacteria, *S. aureus* and *L. monocytogenes*, are more sensitive to inhibition by plant essential oils than the three Gram-negative bacteria. This is in agreement with the results of Prasad et al. (13). Thus, in general, lower bacteriostatic and bacteriocidal concentrations are required for basil essential oil against *S. aureus* and *L. monocytogenes*. It is not completely clear why Gram-negative bacteria should be less susceptible, but it may be associated with the outer membrane of Gram-negative bacteria that endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (81). Fyfe et al. (50) studied the inhibition of *L. monocytogenes* and *S. enteritidis* by combina-

tions of plant essential oils with either benzoic acid or methylparaben (ester of *p*-hydroxybenzoic acid). This work highlighted the fact that the essential oil of basil at 0.2% v/v in the broth is a potent inhibitor of both strains where cells are undetectable (<10 colony forming units (cfu)/mL) at 4, 8, 24, and 48 h. Even after 1 h only of exposure, there were only 3.4 and 1.4 log cfu/mL of the cultures *L. monocytogenes* and *S. enteritidis*, respectively. Fyfe et al. (50) suggested that the properties of basil essential oil should be determined in both a broth and a food system. Lachowicz et al. (66) reported that a synergistic antibacterial effect was found when a combination of 5% w/v of sodium chloride (NaCl) and 0.1% v/v Anise basil essential oil in MRS broth (pH 6.2) was used. This system completely suppressed the growth of *Lactobacillus curvatus* up to 99 h (which was the time for growth detection) as compared to the Anise basil essential oil (51.4 h) or 5% NaCl (28.3 h) alone. The work of Mejlholm and Dalgaard (82) showed that 0.1% v/v basil essential oil resulted in over 85% reduction in the growth rate (RGR) of *Photobacterium phosphoreum* in a liquid medium at 2 and 15 °C.

Koga et al. (52) studied the bacteriocidal activity of basil and sage essential oils against a range of bacteria, including *V. parahaemolyticus*, by viable count determination. Using this method, they were able to compare the bacteriocidal activity in both the exponential and the stationary growth phases. Their findings show that Gram-positive bacteria exhibit higher resistance to basil essential oil than Gram-negative bacteria. *S. aureus*, *M. luteus*, and *B. subtilis* show very high resistance to the essential oil of basil. The viability of these three strains treated with 1% v/v this essential oil was above 90%. *L. monocytogenes* and *B. cereus* were more sensitive to basil essential oil than other Gram-positive bacteria. Nearly all Gram-negative bacteria exhibited high sensitivity to basil essential oil. In particular, *Vibrio* species and *Aerobacter hydrophila* had very high sensitivities to this oil. The viability of these strains treated with 0.01% v/v basil essential oil ranged from 0.014 to 3.64% (52). There is a partial disparity between the results of Koga et al. (52) and those of Elgayyar et al. (69), Prasad et al. (13), and Smith-Palmer et al. (51).

According to Mahmoud (83), the antifungal action and antiaflatoxigenic properties of certain essential oil constituents (including linalool and eugenol) could be determined on a toxigenic strain of *A. flavus*. Similarly, myrcene, ocimene, δ -3-carene, and linalool appeared to cause slight enhancement of both growth and aflatoxin production. Initially, no growth or aflatoxin production in the presence of eugenol for up to 8 days was observed. This can be attributed to the presence of the aromatic moiety and the phenolic-hydroxy group of eugenol; the latter is known to be reactive and forms hydrogen bonds with active sites on target enzymes (78). After 8 days, there was a poor vegetative growth, accompanied by remarkably high concentrations of aflatoxin. This observation is in accordance with the observation made by Basilico and Basilico (80), despite the different species tested.

Kim et al. (43) used a liquid culture assay and found that eugenol possesses a potent inhibitory/bacteriocidal activity against the five bacterial strains: *E. coli*, *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes*, and *V. vulnificus*, followed by linalool. Wan et al. (58) determined later the effect of BSL and BMC on the growth of *A. hydrophila* and *P. fluorescens*. The effect of BMC (0.1 and 1% v/v) on resting cells (10^5 cfu/mL) of *A. hydrophila* and *P. fluorescens* in saline (0.9% w/v NaCl) was also determined after treatment at 20 °C for 10 min. The addition of either 0.1 or 1% v/v BMC caused a decrease in the

viable count of *A. hydrophila* to levels below the detection limit (<1 cfu/mL). BMC at 0.1% v/v showed no effect while at the level of 1% it was bacteriocidal also to *P. fluorescens* resting cells.

Studies Involving Microatmosphere Method. Caccioni et al. (42) studied the antifungal activity of natural volatile compounds (including methyl chavicol) by monitoring their vapor pressures. Methyl chavicol appeared to be active against *P. expansum* and *Botrytis cinerea*, when added to the liquid substrate or when introduced directly into the headspace. In the latter case, it was active at much lower doses. Methyl chavicol was less effective in the vapor phase than hexanal at the same temperature and concentration. At the same dose in the headspace, hexanal induced fungistasis at a level approximately 10-fold higher than that of methyl chavicol.

The vapor pressure, at a given temperature, of a specific volatile molecule in a biological system can be used as an indirect measure of its "actual hydrophobicity". It is inversely related to its capacity to form links with the sheath of water molecules surrounding its polar groups (42, 84). The reason for it is that the tendency of a molecule to pass into the vapor phase is associated with the level of its interaction with water and various solutes (42, 85). At a constant concentration and temperature, the higher the vapor pressure, the lower the steric hindrance, due to the linked water molecules, and the higher is the hydrophobicity (42). However, the ability of a potentially active molecule to interact with the hydrophobic cell membranes can be regarded as a result of its intrinsic hydrophobicity, which increases with the hydrocarbon chain length and/or with the presence of double bonds (42, 71) and with its "actual hydrophobicity" (84). Thus, because of its higher volatility, hexanal has proven to be biologically more active than methyl chavicol, even though methyl chavicol is more hydrophobic (42).

FOOD PRESERVATION

Arora et al. (86) found that oranges coated with an emulsion containing an essential oil (including basil essential oil) or a volatile compound (*Citrus reticulata* Blanco) had a longer shelf life than uncoated ones. Oranges treated with geraniol were rendered almost completely free from blue mold decay ($\geq 95\%$). Other treatments, in a decreasing order of effectiveness, were found to be mentha and basil essential oils. Montes-Belmont and Carvajal (87) showed that basil oils cause a total inhibition of fungal (*A. flavus*) development on maize kernels. The optimal dosage for protection of maize was 5% (v/v) with hexane as the solvent. In addition, no phytotoxic effect on germination and corn growth was detected with this oil.

Ismail et al. (88) studied the efficacy of the immersion of raw poultry in herb decoctions (including basil decoction) on the reduction of the population of *Yarrowia lipolytica*, predominant yeasts believed to play an important role in the spoilage of raw poultry. They found that a significant reduction in the populations of *Y. lipolytica* occurred when the yeast was inoculated into 100% basil, marjoram, sage, or thyme decoctions but not in 100% oregano or rosemary decoctions, kept at 5 °C for 24 h. Further studies included only the treatment of chicken wings with sage or thyme decoctions. It was found that 100% sage or thyme decoctions significantly decreased the populations of *Y. lipolytica* but did not control its growth during storage at 5 °C for up to 9 days.

Lock and Board (89) examined the effect of acidulants and oils on the autosterilization of homemade mayonnaise. They found that the death rate of *S. enteritidis*, a major cause of human salmonellosis, differed among the various oils. Olive oil with

garlic or basil showed the fastest rate of death of *S. enteritidis*, followed by soya, grape seed, rape seed, groundnut, sunflower, hazelnut, and a blended olive oil.

Wan et al. (58) studied the effect of washing fresh lettuce with methyl chavicol on the survival of natural flora. They found that the effectiveness of washing the lettuce with 0.1 and 1% (v/v) methyl chavicol derived from basil in regards to the total viable count and the presumptive counts of *Pseudomonas*, *Aeromonas*, and *Enterobacteriaceae* was comparable to that of washing the lettuce with a 125 ppm chlorine solution. Because chlorine-based washing systems may produce harmful byproducts (like chloramines and trihalomethanes), this result indicates that methyl chavicol, from basil, could offer a natural alternative to washing of selected fresh salad produce and replace (or minimize) the need for chlorine solutions containing chlorine concentrations of up to 200–300 ppm (90). Wan et al. (58) suggested enhancing fresh salad preservation by delivering essential oils to the product in the washing solution of the vegetables. Avina-Bustillos et al. (91) suggested incorporating these oils into an edible coating whereas Nicholson (4) mentioned a food packaging film containing these oils. Such uses of essential oils would depend on cost considerations and on the original odor and flavor of the oils and their suitability for the type of final product (58).

Lachowicz et al. (20, 66) assessed the antimicrobial effect of basil essential oil (Anise variety) on the growth of *L. curvatus* and *S. cerevisiae* in a tomato juice medium. They found that the growth of these microorganisms was completely inhibited by 0.1 and 1.0% (v/v) anise basil oil containing 44% linalool and 27% methyl chavicol.

THE FUTURE

Basil essential oil and its principal constituents are not widely used as food preservatives. The published data show that these compounds have a potential use in food preservation, especially in conjunction with technologies of antimicrobial packages for food products. Further research on the antimicrobial activity of basil essential oil and its main components and a better understanding of the mode of action are required in order to evaluate its usefulness in the shelf life extension of packaged foods such as bakery, meat, poultry, seafood, and cheeses. An additional challenge is in the area of odor/flavor transfer from packages containing natural plant extracts to the packaged foods. Thus, research is needed to determine whether natural plant extracts could act as an antimicrobial agent, as an odor/flavor enhancer in packaged foods, and as a component in antimicrobial packages.

ABBREVIATIONS USED

BMC, basil methyl chavicol; BSL, basil sweet linalool; DMSO, dimethyl sulfoxide; GC, gas chromatography; GC-MS, gas chromatography–mass spectrometry; MIC, minimum inhibitory concentration; MLC, minimum lethal concentration; MRS, man rogosa sharpe; RGR, reduction in growth rate; YMPG, yeast–malt extract peptone glucose.

LITERATURE CITED

- (1) Guenther, E. *The Essential Oils*; Krieger Publishing Company: Malabar, FL, 1975; Vol. III.
- (2) Paton, A. A synopsis of *Ocimum* L. (Labiatae) in Africa. *Kew Bull.* **1992**, *47*, 403–435.

- (3) Lawrence, B. M. A further examination of the variation of *Ocimum basilicum* L. In *Flavors and Fragrances: A World Perspective*; Lawrence, B. M., Mookherjee, B. D., Willis, B. J., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1988; pp 161–170.
- (4) Nicholson, M. D. The role of natural antimicrobials in food/packaging biopreservation. *J. Plast. Film Sheeting* **1998**, *14*, 234–241.
- (5) Miltz, J.; Passy, N.; Mannheim, C. H. Trends and applications of active packaging systems. In *Food and Food Packaging Materials-Chemical Interactions*; Ackerman, P., Jagerstad, M., Ohlsson, T., Eds.; Royal Society of Chemistry: Cambridge, 1995; pp 201–210.
- (6) Grayer, R. J.; Kite, G. C.; Goldstone, F. J.; Bryan, S. E.; Paton, A.; Putievsky, E. Intraspecific taxonomy and essential oil chemotypes in sweet basil *Ocimum basilicum*. *Phytochemistry* **1996**, *43*, 1033–1039.
- (7) Paton, A.; Putievsky, E. Taxonomic problems and cytotoxic relationships between and within varieties of *Ocimum basilicum* and related species (*Labiatae*). *Kew Bull.* **1996**, *51*, 509–524.
- (8) Lawrence, B. M.; Hogg, J. W.; Terhune, S. J.; Pichitakul, N. Essential oils and their constituents. IX. The oils of *Ocimum basilicum* from Thailand. *Flavour Ind.* **1972**, *3*, 47–49.
- (9) Amvam Zollo, P. H.; Biyiti, L.; Tchoumboungang, F.; Menut, C.; Lamaty, G.; Bouchet, P. Aromatic plants of tropical central africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. *Flavour Fragrance J.* **1998**, *13*, 107–114.
- (10) Keita, S. M.; Vincent, C.; Schmit, J.-P.; Belanger, A. Essential oil composition of *Ocimum basilicum* L., *O. gratissimum* L. and *O. suave* L. in the Republic of Guinea. *Flavour Fragrance J.* **2000**, *15*, 339–341.
- (11) Vieira, R. F.; Simon, J. E. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. *Econ. Bot.* **2000**, *54*, 207–216.
- (12) Yayi, E.; Moudachirou, M.; Chalchat, J. C. Chemotyping of three *Ocimum* species from Benin: *O. basilicum*, *O. canum* and *O. gratissimum*. *J. Essent. Oil Res.* **2001**, *13*, 13–17.
- (13) Prasad, G.; Kumar, A.; Singh, A. K.; Bhattacharya, A. K.; Singh, K.; Sharma, V. D. Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. *Fitoterapia* **1986**, *57*, 429–432.
- (14) Sinha, G. K.; Gulati, B. C. Antibacterial and antifungal study of some essential oils and some of their constituents. *Indian Perfum.* **1990**, *34*, 126–129.
- (15) Lis-Balchin, M.; Deans, S. G.; Eaglesham, E. Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Fragrance J.* **1998**, *13*, 98–104.
- (16) Hammer, K. A.; Carson, C. F.; Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985–990.
- (17) Darrah, H. H. *The Cultivated Basils*; Buckeye Printing: Independence, MO, 1980.
- (18) Simon, J. E.; Quinn, J.; Murray, R. G. Basil: a source of essential oils. In *Advances in New Crops*; Janick, J., Simon, J. E., Eds.; Timber Press: Portland, OR, 1990; pp 484–489.
- (19) Marotti, M.; Piccaglia, R.; Giovanelli, E. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. *J. Agric. Food Chem.* **1996**, *44*, 3926–3929.
- (20) Lachowicz, K. J.; Jones, G. P.; Briggs, D. R.; Bienvenu, F. E.; Palmer, M. V.; Mishra, V.; Hunter, M. Characteristics of plants and plant extracts from five varieties of basil (*Ocimum basilicum* L.) grown in Australia. *J. Agric. Food Chem.* **1997**, *45*, 2660–2665.
- (21) Hasegawa, Y.; Tajima, K.; Toi, N.; Sugimura, Y. Characteristic components found in the essential oil of *Ocimum basilicum* L. *Flavour Fragrance J.* **1997**, *12*, 195–200.

- (22) Simon, J. E.; Morales, M. R.; Phippen, W. B.; Vieira, R. F.; Hao, Z. Basil: a source of aroma compounds and a popular culinary and ornamental herb. In *Perspectives on New Crops and New Uses*; Janick, J., Ed.; ASHS Press: Alexandria, VA, 1999; pp 499–505.
- (23) Pino, J.; Rosado, A.; Goire, I.; Roncal, E.; Garcia, I. Analysis of the essential oil from cuban basil (*Ocimum basilicum* L.). *Die Nahrung* **1993**, *37*, 501–504.
- (24) Baratta, M. T.; Dorman, H. J. D.; Deans, S. G.; Figueiredo, A. C.; Baroso, J. G.; Ruberto, G. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour Fragrance J.* **1998**, *13*, 235–244.
- (25) Shartar, S.; Altantsetseg, S. Essential oil composition of some plants cultivated in Mongolian climate. *J. Essent. Oil Res.* **2000**, *12*, 745–750.
- (26) Ruberto, G.; Spadaro, A.; Piattelli, M.; Piozzi, F.; Passannanti, S. Volatile flavour components of *Ocimum basilicum* var. *hispidum* (Lam.) Chiov. *Flavour Fragrance J.* **1991**, *6*, 225–227.
- (27) Sanda, K.; Koba, K.; Nambo, P.; Gaset, A. Chemical investigation of *Ocimum* species growing in Togo. *Flavour Fragrance J.* **1998**, *13*, 226–232.
- (28) Ozek, T.; Beis, S. H.; Demircakmak, B.; Baser, K. H. C. Composition of the essential oil of *Ocimum basilicum* L. cultivated in Turkey. *J. Essent. Oil Res.* **1995**, *7*, 203–205.
- (29) Vernin, G.; Metzger, J.; Fraisse, D.; Suon, K.-N.; Scharff, C. Analysis of basil oils by GC-MS data bank. *Perfum. Flavor.* **1984**, *9*, 71–80, 83–86.
- (30) Ravid, U.; Putievsky, E.; Katzir, I.; Lewinsohn, E. Enantiomeric composition of linalool in the essential oils of *Ocimum* species and in commercial basil oils. *Flavour Fragrance J.* **1997**, *12*, 293–296.
- (31) Tateo, F.; Bononi, M.; De Dominicis, E.; Fumagalli, V. Update on enantiomeric composition of (1R)-(+)- and (1S)-(-)-camphor in essential oils by enantioselective gas chromatography. *Anal. Commun.* **1999**, *36*, 149–151.
- (32) Miele, M.; Dondero, R.; Ciarallo, G.; Mazzei, M. Methyl Eugenol in *Ocimum basilicum* L. Cv Genovese Gigante. *J. Agric. Food Chem.* **2001**, *49*, 517–521.
- (33) Lachowicz, K. J.; Jones, G. P.; Briggs, D. R.; Bienvenu, F. E.; Palmer, M. V.; Ting, S. S. T.; Hunter, M. Characteristics of essential oil from basil (*Ocimum basilicum* L.) grown in Australia. *J. Agric. Food Chem.* **1996**, *44*, 877–881.
- (34) Ehlers, D.; Nguyen, T.; Quirin, K.-W.; Gerard, D. Untersuchung von Basilikumölen-superkritische CO₂-Extrakte und Wasserdampfdestillate. *Dtsch. Lebensm.-Rundsch.* **2001**, *97*, 245–250.
- (35) Reverchon, E.; Donsi, G.; Pota, F. Extraction of essential oils using supercritical CO₂: Effect of some process and pre-process parameters. *Ital. J. Food Sci.* **1992**, *4*, 187–194.
- (36) Johnson, C. B.; Kirby, J.; Naxakis, G.; Pearson, S. Substantial UV-B-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). *Phytochemistry* **1999**, *51*, 507–510.
- (37) Loughrin, J. H.; Kasperbauer, M. J. Light reflected from colored mulches affects aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves. *J. Agric. Food Chem.* **2001**, *49*, 1331–1335.
- (38) Davidson, P. M.; Parish, M. E. Methods for testing the efficacy of food antimicrobials. *Food Technol.* **1989**, *43*, 148–155.
- (39) Barry, A. L. Procedure for testing antimicrobial agents in agar media: Theoretical considerations. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Larian, V., Ed.; Williams and Wilkins: Baltimore, MD, 1986; p 1.
- (40) Zaika, L. L. Spices and herbs: Their antimicrobial activity and its determination. *J. Food Saf.* **1988**, *9*, 97–118.
- (41) Parish, M. E.; Davidson, P. M. Method for evaluation. In *Antimicrobials in Foods*, 2nd ed.; Davidson, P. M., Brannen, A. L., Eds.; Marcel Dekker: New York, 1993; pp 597–615.
- (42) Caccioni, D. R. L.; Gardini, F.; Lanciotti, R.; Guerzoni, M. E. Antifungal activity of natural volatile compounds in relation to their vapour pressure. *Sci. Aliments* **1997**, *17*, 21–34.
- (43) Kim, J.; Marshall, M. R.; Wei, C. Antibacterial activity of some essential oil components against five foodborne pathogens. *J. Agric. Food Chem.* **1995**, *43*, 2839–2845.
- (44) Schmolka, I. R. The synergistic effects of nonionic surfactants upon cation germicidal agents. *J. Soc. Cosmet. Chem.* **1973**, *24*, 577–592.
- (45) Kazmi, S. J. A.; Mitchell, A. G. Preservation of solubilised emulsion systems. II. Theoretical development of capacity and its role in antimicrobial activity of chlorocresol in cetamicrogrol-stabilised systems. *J. Pharm. Sci.* **1978**, *67*, 1266–1271.
- (46) Remmal, A.; Bouchikhi, T.; Rhayour, K.; Ettayebi, M. Improved method for the determination of antimicrobial activity of essential oils in agar medium. *J. Essent. Oil Res.* **1993**, *5*, 179–184.
- (47) Hulin, V.; Mathot, A. G.; Mafart, P.; Dufosse, L. Revue-Les propriétés anti-microbiennes des huiles essentielles et composés d'arômes. *Sci. Aliments* **1998**, *18*, 563–582.
- (48) Aboul Ela, M. A.; El-Shaar, N. S.; Ghanem, N. B. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. *Pharmazie* **1996**, *51*, 993–994.
- (49) Ndounga, M.; Ouamba, J. M. Antibacterial and antifungal activities of essential oils of *Ocimum gratissimum* and *O. basilicum* from Congo. *Fitoterapia* **1997**, *68*, 190–191.
- (50) Fyfe, L.; Armstrong, F.; Stewart, J. Inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations. *Int. J. Antimicrob. Agents* **1998**, *9*, 195–199.
- (51) Smith-Palmer, A.; Stewart, J.; Fyfe, L. Antimicrobial properties of plant essential oils and essences against five important foodborne pathogens. *Lett. Appl. Microbiol.* **1998**, *26*, 118–122.
- (52) Koga, T.; Hirota, N.; Takumi, K. Bactericidal activities of essential oils of basil and sage against a range of bacteria and the effect of these essential oils on *Vibrio parahaemolyticus*. *Microbiol. Res.* **1999**, *154*, 267–273.
- (53) Dube, S.; Upadhyay, P. D.; Tripathi, S. C. Antifungal, physicochemical, and insect-repelling activity of the essential oil of *Ocimum basilicum*. *Can. J. Bot.* **1989**, *67*, 2085–2087.
- (54) Rai, M. K.; Qureshi, S.; Pandey, A. K. *In vitro* susceptibility of opportunistic *Fusarium* spp. to essential oils. *Mycoses* **1999**, *42*, 97–101.
- (55) Moleyar, V.; Narasimham, P. Antibacterial activity of essential oil components. *Int. J. Food Microbiol.* **1992**, *16*, 337–342.
- (56) Nascimento, G. G. F.; Locatelli, J.; Freitas, P. C.; Silva, G. L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.* **2000**, *31*, 247–256.
- (57) Pattnaik, S.; Subramanyam, V. R.; Bapaji, M.; Kole, C. R. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios* **1997**, *89*, 39–46.
- (58) Wan, J.; Wilcock, A.; Coventry, M. J. The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. *J. Appl. Microbiol.* **1998**, *84*, 152–158.
- (59) Hitokoto, H.; Morozumi, S.; Wauke, T.; Sakai, S.; Kurata, H. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Appl. Environ. Microbiol.* **1980**, *39*, 818–822.
- (60) Moleyar, V.; Narasimham, P. Antifungal activity of some essential oil components. *Food Microbiol.* **1986**, *3*, 331–336.
- (61) Lahariya, A. K.; Rao, J. T. *In Vitro* antimicrobial studies of the essential oils of *Cyperus scariosus* and *Ocimum basilicum*. *Indian Drugs* **1979**, *16*, 150–152.
- (62) Reuveni, R.; Fleisher, A.; Putievsky, E. Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes. *Phytopath. Z.* **1984**, *110*, 20–22.
- (63) Conner, D. E.; Beuchat, L. R. Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Sci.* **1984**, *49*, 429–434.
- (64) Ozcan, M.; Erkmen, O. Antimicrobial activity of the essential oils of Turkish plant spices. *Eur. Food Res. Technol.* **2001**, *212*, 658–660.

- (65) Meena, M. R.; Sethi, V. Antimicrobial activity of essential oils from spices. *J. Food Sci. Technol.* **1994**, *31*, 68–70.
- (66) Lachowicz, K. J.; Jones, G. P.; Briggs, D. R.; Bienvenu, F. E.; Wan, J.; Wilcock, A.; Coventry, M. J. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. *Let. Appl. Microbiol.* **1998**, *26*, 209–214.
- (67) Deans, S. G.; Ritchie, G. Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.* **1987**, *5*, 165–180.
- (68) Gangrade, S. K.; Shrivastava, R. D.; Sharma, O. P.; Moghe, M. N.; Trivedi, K. C. Evaluation of antibacterial properties of essential oils of *Ocimum* species. *Indian Perfum.* **1989**, *33*, 130–136.
- (69) Elgayyar, M.; Draughon, F. A.; Golden, D. A.; Mount, J. R. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. Food Prot.* **2001**, *64*, 1019–1024.
- (70) Lis-Balchin, M.; Deans, S. G. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Microbiol.* **1997**, *82*, 759–762.
- (71) Knobloch, K.; Pauli, A.; Iberl, B.; Weigand, H.; Weis, N. Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res.* **1989**, *1*, 118–119.
- (72) Mazzanti, G.; Battinelli, L.; Salvatore, G. Antimicrobial properties of the linalool-rich essential oil of *Hyssopus officinalis* L. var *decumbens* (Lamiaceae). *Flavour Fragrance J.* **1998**, *13*, 289–294.
- (73) Scora, K. M.; Scora, R. W. Effect of volatiles on mycelium growth of *Penicillium digitatum*, *P. italicum*, and *P. ulaiense*. *J. Basic Microbiol.* **1998**, *38*, 405–413.
- (74) Kurita, N.; Miyaji, M.; Kurane, R.; Takahara, Y. Antifungal activity of components of essential oils. *Agric. Biol. Chem.* **1981**, *45*, 945–952.
- (75) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316.
- (76) Pelczar, M. J.; Chan, E. C. S.; Krieg, N. R. Control of microorganism: Chemical agents. In *Microbiology: Concepts and Applications*; McGraw-Hill: New York, 1993; pp 221–241.
- (77) Suresh, P.; Ingle, V. K.; Vijayalakshmi, V. Antibacterial activity of eugenol in comparison with other antibiotics. *J. Food Sci. Technol.* **1992**, *29*, 254–256.
- (78) Farag, R. S.; Daw, Z. Y.; Hewedi, F. M.; EL-Baroty, G. S. A. Antimicrobial activity of some Egyptian spice essential oils. *J. Food Prot.* **1989**, *52*, 665–667.
- (79) Karapinar, M.; Aktug, S. E. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. *Int. J. Food Microbiol.* **1987**, *4*, 161–166.
- (80) Basilico, M. Z.; Basilico, J. C. Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin A production. *Let. Appl. Microbiol.* **1999**, *29*, 238–241.
- (81) Nikaido, H.; Vaara, M. Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.* **1985**, *49*, 1–32.
- (82) Mejlholm, O.; Dalgaard, P. Antimicrobial effect of essential oils on the seafood spoilage microorganism *Photobacterium phosphoreum* in liquid media and fish products. *Let. Appl. Microbiol.* **2002**, *34*, 27–31.
- (83) Mahmoud, A.-L. E. Antifungal action and anti-aflatoxigenic properties of some essential oil constituents. *Let. Appl. Microbiol.* **1994**, *19*, 110–113.
- (84) Guerzoni, M. E.; Nicoli, M. C.; Massini, R.; Lerici, C. R. Ethanol vapour pressure as a control factor during alcoholic fermentation. *World J. Microbiol. Biotechnol.* **1997**, *13*, 11–16.
- (85) Gardini, F.; Lanciotti, R.; Caccioni, D. R. L.; Guerzoni, M. E. Antifungal activity of hexanal as dependent on its vapor pressure. *J. Agric. Food Chem.* **1997**, *45*, 4297–4302.
- (86) Arora, R.; Pandey, G. N. The application of essential oils and their isolates for blue mould decay control in *Citrus reticulata* Blanco. *J. Food Sci. Technol.* **1977**, *14*, 14–16.
- (87) Montes-Belmont, R.; Carvajal, M. Control of *Aspergillus flavus* in maize with plant essential oils and their components. *J. Food Prot.* **1998**, *61*, 616–619.
- (88) Ismail, S. A. S.; Deak, T.; Abd El-Rahman, H. A.; Yassien, M. A. M.; Beuchat, L. R. Effectiveness of immersion treatments with acids, trisodium phosphate, and herb decoctions in reducing populations of *Yarrowia lipolytica* and naturally occurring aerobic microorganisms on raw chicken. *Int. J. Food Microbiol.* **2001**, *64*, 13–19.
- (89) Lock, J. L.; Board, R. G. The influence of acidulants and oils on autosterilization of homemade mayonnaise. *Food Res. Int.* **1996**, *28*, 569–572.
- (90) Beuchat, L. R. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* **1996**, *59*, 204–216.
- (91) Avina-Bustillos, R. J.; Cisneros-Zevallos, L. A.; Krochta, J. M.; Saltveit, M. E. Optimization of edible coatings on minimally processed carrots using response surface methodology. *Am. Soc. Agric. Eng.* **1993**, *36*, 801–805.

Received for review October 14, 2002. Revised manuscript received February 14, 2003. Accepted February 19, 2003. On behalf of the Royal Thai Government, P.S. gratefully acknowledges the Australian Agency for International Development (AusAID) for providing financial support. This work was partially supported by a fund for the promotion of research at the Technion-Israel Institute of Technology. J.M. expresses his thanks and appreciation for this support.

JF021038T