

SPICES AND HERBS: THEIR ANTIMICROBIAL ACTIVITY AND ITS DETERMINATION¹

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ABSTRACT

Throughout the years numerous investigations concerning the inhibition of microorganisms by spices, herbs, their extracts, essential oils and various constituents have been reported. Many of these materials possess significant antimicrobial activity, which in many cases is due primarily to a particular constituent. Interpretation and comparison of results of various studies is complicated by variations in the methodology used for the determination of antimicrobial activity. The antimicrobial activity varies depending on the microorganism, the spice or herb and the test medium. These and other factors are examined in the light of their effect on the outcome of the test method.

INTRODUCTION

Many of the spices and herbs used today were known to the people of the ancient cultures throughout the world, and they were valued for their preservative and medicinal powers besides their flavor and odor qualities. How the ancients obtained their knowledge we do not know, but modern research has shown that many of their ideas are valid. Scientific experiments on the antimicrobial properties of spices, herbs and their components have been documented in the late 19th Century and interest continues to the present (Corran and Edgar 1933; Fabian *et al.* 1939; Webb and Tanner 1945; Dold and Knapp 1948; Maruzzella and Freundlich 1959; Beuchat 1976; Zaika and Kissinger 1979; Hitokoto *et al.* 1980; Mabrouk and El-Shayeb 1980; Shelef *et al.* 1980; Azzouz and Bullerman 1982; Ueda *et al.* 1982; Zaika *et al.* 1983; Deibel and Banwart 1984). It was also recognized quite early that the antiseptic power of spices and herbs resides in the essential oils and in some cases can be attributed

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to major components of the oils. Most of the published reports deal with the effect of the essential oils of spices and herbs on a variety of microorganisms (Collier and Nitta 1930; Blum and Fabian 1943; Kellner and Kober 1954; Maruzzella and Henry 1958; Maruzzella and Liquori 1958; Tiwari *et al.* 1983; Conner and Beuchat 1984; Galli *et al.* 1985; Thompson and Cannon 1986). A review of literature on antimicrobial activity of essential oils for the period 1960-1976 has been published by Koedam (1977). Reviews of earlier work on spices may be found in the work of Rippetoe and Wise (1912); Collier and Nitta (1930); Fabian *et al.* (1939); Boyle (1955) and Steudel (1971).

Among the earliest reports is that of Chamberland (1887), who tested over 100 essential oils. He found that cinnamon oil vapor was lethal to spores of *Bacillus anthracis* and that in contact with the test medium other oils, notably oregano oil, were also active. Interest in these botanicals was undoubtedly prompted by the desire to find antiseptic and disinfectant substances from natural sources, the idea being that these would be safer than synthetic antimicrobials. This consideration remains important today, particularly among researchers in developing countries.

A laboratory study on the effect of spices on food preservation was first made by Hoffman and Evans (1911), although they stated that "it is a matter of common knowledge" that spices have a role in the preservation of food. Hoffman and Evans (1911) found that cinnamon, mustard and clove were useful in preserving apple sauce. Allspice and nutmeg had some preservative powers, while ginger, black pepper and cayenne pepper had no activity. They used pure cultures of yeasts and bacteria but relied on chance inoculation of molds. They attributed the antimicrobial activity of spices to their essential oils and showed that cinnamaldehyde and eugenol were more inhibitory than benzoic acid.

Although in laboratory experiments many spices and herbs were shown to possess significant antimicrobial properties, nevertheless it soon became evident that at the levels normally used for flavoring food these materials were not very effective as preservatives (Bachmann 1916). However, under some circumstances, a spice or its active component could be used in low concentrations to control microorganisms in foods without impairing flavor (Hoffman and Evans 1911; Bachmann 1916; Bullerman 1974). Recently Shelef (1983) reviewed the antimicrobial activity of spices in microbiological growth media and in food systems.

Antimicrobial Activity

The question, which spices and herbs are most inhibitory?, is not easily answered and depends on a number of factors. The numerous experiments that have been carried out over the years indicate that many spices, herbs and their constituents possess at least some antimicrobial activity. Examination of the available literature suggests that spices and herbs may be grouped according to their antimicrobial activity as shown in Table 1. Cinnamon, clove and mustard are mentioned most often as being

TABLE 1.
ANTIMICROBIAL ACTIVITY OF SPICES AND HERBS

<u>Inhibitory Effect</u>			
Strong:	Cinnamon		
	Clove		
	Mustard		
Medium:	Allspice	Coriander	Rosemary
	Bay Leaf	Cumin	Sage
	Caraway	Oregano	Thyme
Weak:	Black Pepper		
	Red Pepper		
	Ginger		

the strongest inhibitors for a variety of microorganisms. Significant inhibitory properties have been reported for allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme. On the other hand, red pepper, ginger and black pepper possess little if any antimicrobial properties. If one includes garlic among spices and herbs, it also has been reported to strongly inhibit a variety of microorganisms (Dold and Knapp 1948; Azzouz and Bullerman 1982; Graham and Graham 1987). Important flavor constituents of spices and herbs have also been shown to possess antimicrobial activity. Examples of these are given in Table 2.

Although a considerable volume of data is available, it has been difficult to obtain any quantitative estimate of the antimicrobial effect of a given spice or its component. At least part of the difficulty is due to the large variety of test methods that have been used. Attempts have been made to quantitatively express the antimicrobial properties of essential oils and their components, such as in terms of the "phenol coefficient" proposed by Martindale (1910) and used for a time by others. The "phenol coefficient" is a ratio of the minimum concentration of phenol to the minimum concentration of test substance that will destroy a specific microorganism. Martindale (1910) found that oregano, thyme, cinnamon and clove oils and their constituents thymol, carvacrol and cinnamic aldehyde were much more active than phenol.

TABLE 2.
ANTIMICROBIAL CONSTITUENTS OF SPICES AND HERBS

Compound	Occurrence	Antimicrobial Activity, ^a Lit. Ref.
Allicin	garlic	- 4
Allyl isothiocyanate	mustard	- 1
Anethole	anise, star anise, fennel	- 5
Carvacrol	oregano, thyme	- 1, 6
Carvone	caraway, dill	- 6
1,8-Cineole	sage, rosemary, laurel, cardamom	- 6
Cinnamaldehyde	cinnamon, cassia	- 1, 3, 7
p-Cymene	cumin, thyme, oregano	- 6
Eugenol	clove, allspice, cinnamon	- 1, 3, 5, 6, 7
Limonene	celery seed, caraway, dill	- 6
Linalool	coriander, sage, rosemary, basil	- 6
Thymol	thyme, oregano	- 2, 5, 6

^a(1) Blum and Fabian 1943; (2) Buchanan and Shepherd 1981; (3) Bullerman *et al.* 1977; (4) Cavallito and Bailey 1950; (5) Hitokoto *et al.* 1980; (6) Kellner and Kober 1955; (7) Ueda *et al.* 1982.

TABLE 3.
FACTORS INFLUENCING THE ANTIMICROBIAL ACTIVITY OF SPICES

Test Medium:

- 1) Water
- 2) Microbiological nutrient medium
 - Liquid
 - Semisolid—agar plates
- 3) Food or beverage

Spice or its Component:

- 1) Method of Addition to Test Medium
 - Added as is
 - Sterilized with test medium
 - Added as extract (aqueous, alcoholic)
 - Applied to agar plate
- 2) Concentration Tested
- 3) Variation in Composition
 - Geographic origin
 - Climate
 - Processing
 - Varietal differences

Microorganism:

- 1) Spore or vegetative cell
- 2) Strain difference
- 3) Inoculum size

Determination of Antimicrobial Activity

To evaluate the reported data, it is necessary to consider the methods used to test for antimicrobial activity. Variations in three main factors can affect the outcome of the test method (Table 3): the test medium, the spice or its component and the microorganism. Each factor is subject to a great deal of variation.

Test Medium. The test medium can be water, a microbiological nutrient medium as either liquid or semisolid agar, or a food or beverage. This factor is very important since the survival or growth of a microorganism is highly dependent on the

medium to which it is exposed. Generally, the antimicrobial activity of spices is lower in food systems than in microbiological media. The active components are generally only slightly soluble in aqueous systems. In foods, particularly in those containing high levels of lipids, partition effects become important.

Spice. For the spice or its component, one must consider the method of its application to the test medium, the concentration being tested and variation in composition. Ground spices, as well as their extracts or infusions, may be added directly to the sterile test medium, or they may be sterilized with the medium. The essential oils or their solutions may be mixed with the test medium or applied to agar plates by means of a paper disk or in a cup. If the spice is added to the test medium without subsequent sterilization, sterile spice must be used. Commercial spices may harbor a variety of microorganisms or their spores, even though the counts may be relatively low. Sterile spices are available commercially or may be sterilized by means of ethylene oxide or irradiation. Autoclaving spices with the medium may result in loss of active components. Essential oils are generally sterile. Possible differences in the composition of the active components should be considered. These may be due to geographic origin, climate, processing or varietal differences.

Microorganism. The microorganism being tested may be used in the form of vegetative cells or spores. It may be mixed with the test medium or applied to the surface of agar plates. Sensitivity to a particular test substance may be dependent on the strain of the microorganism used. Inoculum size is also important in determining the test results.

Interpretation of test results with fungi may present problems in that, unlike bacteria where one deals with single-cell organisms, fungi in their filamentous form are multicellular organisms capable of differentiation. Recently, Plempel *et al.* (1987) reviewed the problems associated with methods of testing antifungal activity.

Test Methods

The following are some examples of test methods that have been used to assess the antimicrobial activity of spices, herbs and their constituents.

To test for antimicrobial activity in a liquid medium, serial dilutions of a spice or its component are prepared in a nutrient medium which are then inoculated with the test microorganism. After a period of incubation at a suitable temperature, the number of surviving microorganisms is determined. The antimicrobial activity of the spice may be expressed in terms of maximum dilution at which microbial growth is inhibited. For example, Collier and Nitta (1930) tested various essential oils against several species of bacteria (Table 4). Oils of cardamom, cinnamon, mustard, clove and thyme were active at high dilutions, cinnamon oil being the most inhibitory. Oils of ginger and black pepper had very little antibacterial activity even at dilutions of one to 100. Gonococci were the most sensitive, while *Escherichia coli* was quite resistant to the essential oils.

TABLE 4.
EFFECT OF ESSENTIAL OILS ON BACTERIA IN LIQUID MEDIUM

Essential Oil	Dilution					Vibrio nasik
	Streptococci	Staphylococcus aureus	Gonococci	Escherichia coli		
Ginger	- ^a	-	150	-	200	
Cardamom	800	100	2,400	100	800	
Black pepper	100	-	300	-	100	
Cinnamon	32,000	1,600	1,600	4,000	800	
Mustard	40,000	400	48,000	800	8,000	
Clove	4,000	800	15,000	800	1,600	
Cumin	400	800	3,000	-	1,600	
Anise	200	-	3,000	-	1,600	
Sage	200	-	1,200	-	1,600	
Thyme	4,000	1,600	3,000	400	800	

- a = Not active at a dilution of 1 to 100.
From: Collier and Nitta (1930).

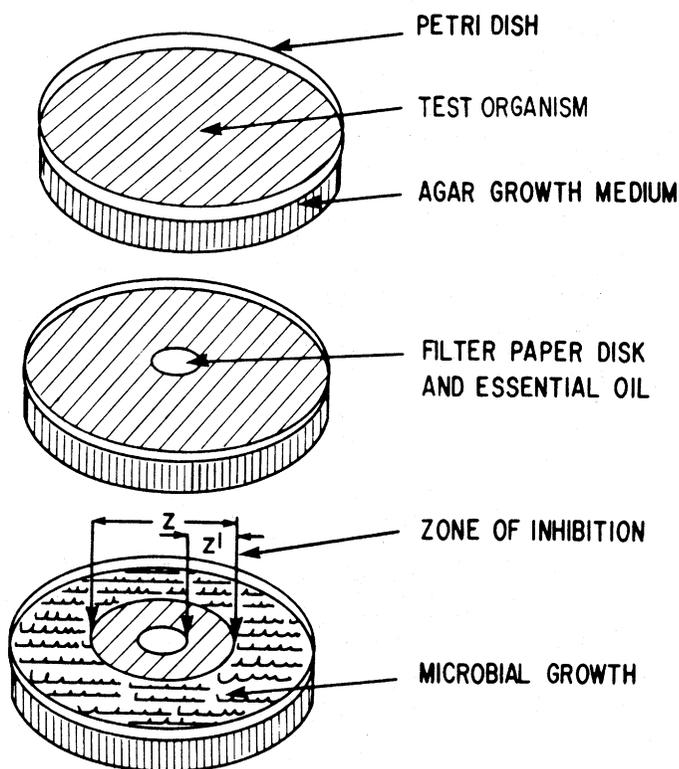


FIG. 1. DETERMINATION OF THE "ZONE OF INHIBITION" BY THE FILTER PAPER DISK DIFFUSION METHOD

A commonly used method for the evaluation of essential oils involves the so-called "zone of inhibition" (Fig. 1). An essential oil is deposited on a small filter paper disk, usually 0.5 cm in diameter, which is then placed in the center of a Petri dish containing agar growth medium inoculated with the test microorganism. The plates are then incubated for a suitable period of time and observed for microbial growth. If an oil exerts antimicrobial activity, the microorganism will not grow in an area surrounding the filter paper disk. This clear area, or "zone of inhibition," is measured and recorded in mm. This is a simple, convenient and widely used method, but it suffers from the limitation that the inhibitory effect of the oil is dependent on its ability to diffuse through the agar medium. Also the vapor of the oil may exert its effect.

A similar technique may be used to measure the antimicrobial activity of essential oil vapor. The method involves placing the essential oil, which is in a small cup

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or on a filter paper disk, on the inside of a Petri dish cover and measuring the “zone of inhibition” after a suitable incubation of the growth medium inoculated with the test microorganism (Fig. 2).

In the 1950's and 1960's Maruzzella and co-workers used these techniques extensively to evaluate a large number of essential oils and perfumery materials for antibacterial and antifungal activity. As shown in Table 5, the sizes of the zones of inhibition vary considerably. Of the 10 oils tested against *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger* and *Penicillium digitatum*, oregano and thyme oils produced the largest zones of inhibition for the four organisms (Maruzzella and Liguori 1958).

Another method of testing for antimicrobial activity involves the use of a “double plate.” An agar plate is prepared (Fig. 3) in such a way that one half contains agar medium only and the other half contains agar medium plus spice. The test microorganism is then streaked across both sides of the plate and the plate is observed after incubation for a suitable period of time. Streak “a” shows no inhibition of growth,

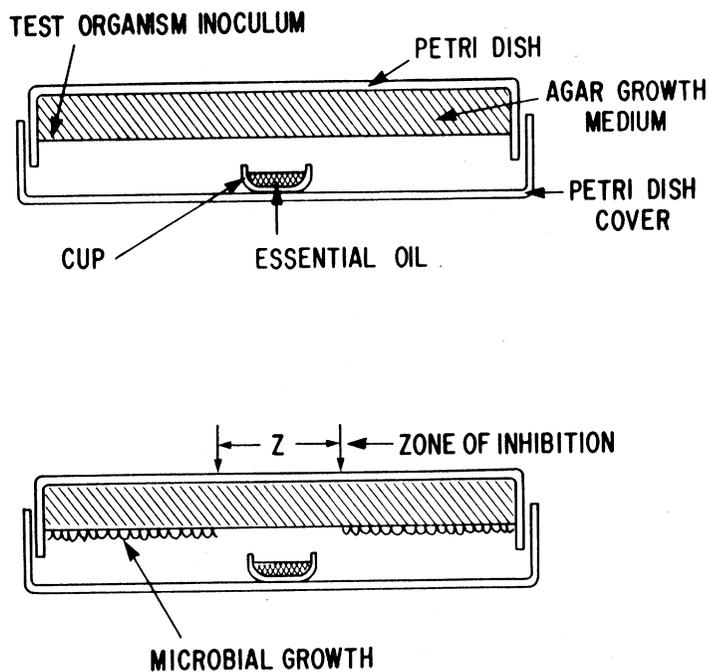


FIG. 2. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL VAPOR

TABLE 5.
ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS

Essential Oil	Zone of Inhibition, mm			
	Saccharomyces cerevisiae	Candida albicans	Aspergillus niger	Penicillium digitatum
Anise	0	2	13	15
Cardamom	4	1	7	4
Clove	5	3	10	9
Oregano	26	24	26	23
Ginger	0	0	0	0
Cinnamon	6	15	16	17
Marjoram	4	7	11	8
Coriander	7	5	21	14
Rosemary	7	2	3	6
Thyme	16	14	22	17

From: Maruzzella and Liquori (1958)

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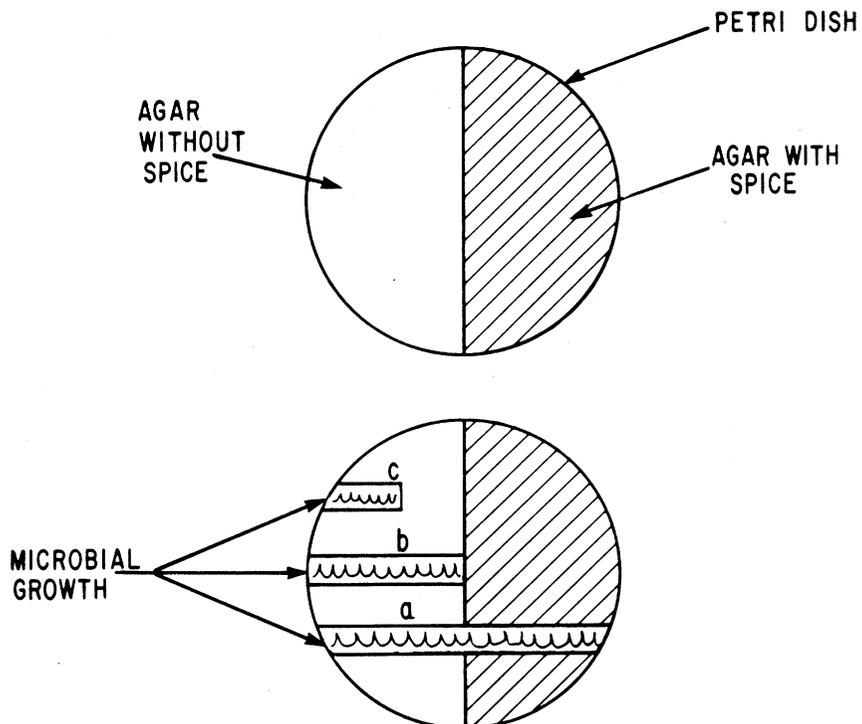


FIG. 3. DOUBLE PLATE METHOD FOR THE DETERMINATION OF ANTIMICROBIAL ACTIVITY OF SPICES

“b” shows inhibition due to contact with spice, and “c” shows inhibition due to diffusion of the active components to the unsiced side of the plate. Webb and Tanner (1945) applied this technique to determine the effect of spices and flavoring materials on growth of yeasts.

To evaluate the effect of spice concentration, a gradient plate (Fig. 4) may be used as was described by Shelef *et al.* (1980). Sterile nutrient agar containing 1% spice was poured into a Petri dish set on an incline so that a depth of 5 mm is obtained at one end and zero on the other. When the agar solidified, the dish was set horizontally and an equal volume of agar without spice was added. The test organism was then swabbed the length of the spice gradient and the plate incubated. The minimum inhibitory concentration may be calculated using the length of the growth zone.

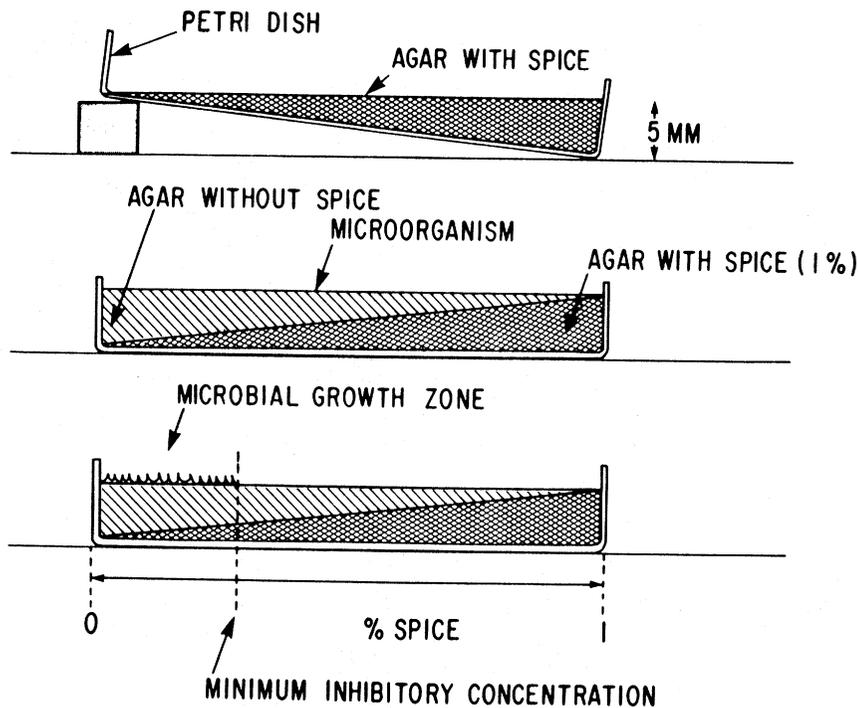


FIG. 4. GRADIENT PLATE METHOD FOR THE DETERMINATION OF ANTIMICROBIAL ACTIVITY OF SPICES

A rather elegant variation of the filter paper disk technique is the Agar Cup Method (Fig. 5), used by Blum and Fabian (1943). Sterile agar medium is poured into a Petri dish containing 0.5 mL of suspension of the test microorganism, and mixed. When the agar solidifies, a cup, 1 cm in diameter is cut out in the center. The base of the cup is sealed with a thin layer of agar, essential oil sample is added, the cup is covered with a sterile cover slip and sealed with agar. After incubation the zone of inhibition is measured. Thus, the effect of the oil vapor is avoided, and inhibition is due to diffusion of the inhibitory components through agar.

To evaluate antifungal activity, various modifications of the poisoned-food technique have been used. A simple poisoned-food system consists of agar medium mixed with the test substance which is then inoculated with the fungus and growth measured. Falck (1907), apparently the first to use agar as poisoned food, showed that the growth rate of a fungus on solid media in Petri dishes is linear with time. Recently, Thompson and Cannon (1986) used the poisoned food technique to screen 40 essential oils for inhibition of 20 fungi. Solutions of various concentrations of

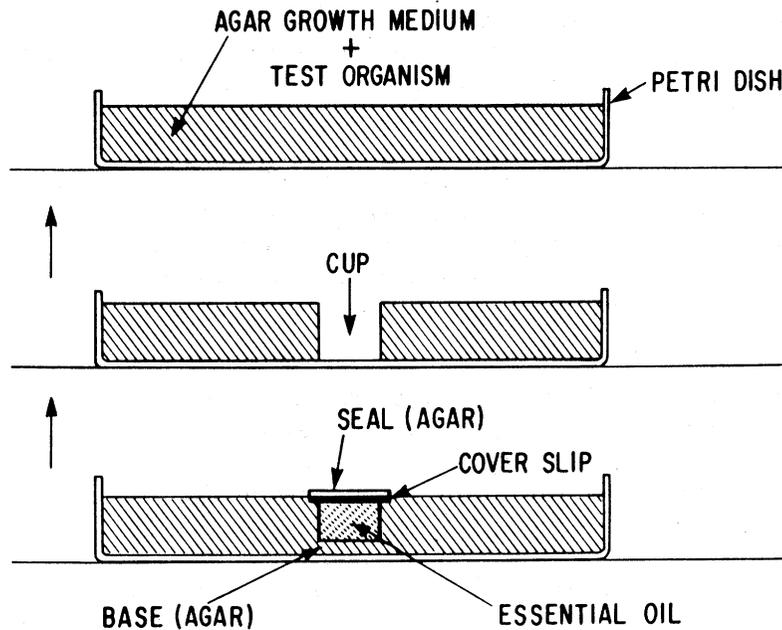


FIG. 5. AGAR CUP METHOD FOR THE DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

essential oil in 95% ethanol were added to 10 mL of sterilized potato dextrose agar medium and swirled to mix. A mycelial disk 6 mm in diameter was cut from the periphery of a 5-day old culture and was aseptically inoculated upside down in the center of the Petri plate. For the controls, 0.1 mL of sterilized water and 0.1 mL 95% ethanol were added to the agar medium in place of the essential oil. The plates were incubated and observed after two and five days. Radial linear growth was determined by measuring colony diameters at two positions on the fungal colony. Growth inhibition was calculated as: $100 - (\text{radial growth on essential oil-supplemented medium} \times 100) / \text{radial growth on control medium}$. Of the oils tested, those of bay, cinnamon bark, cinnamon leaf, clove, pimenta berry, pimenta leaf and thyme showed significant inhibitory effect.

A procedure for the determination of the effect of essential oils on fungal spore germination has been described (Thompson 1986). Germination was defined as the extension of a germ tube to a length equal to one-half the diameter of the spore. Germination was reported as percentage of spore population determined by microscopic count and evaluated on a solid agar medium in standard size Petri plates.

Agar media containing appropriate amounts of essential oil solutions in 95 % ethanol were prepared and a spore inoculum spread over the surface of the agar. After a short period of incubation (6-8 h), agar blocks were aseptically removed with a scalpel. The blocks were placed on microscope slides and examined under 100, 400 and 1000 x magnification. At least 400 spores were counted for each sample. Germination percentage was determined and compared with an appropriate control receiving a similar volume of 95 % ethanol. Three replicate plates per treatment were used and experiments were repeated two times. The data were evaluated statistically. Of the 40 oils tested (Thompson 1986), seven completely inhibited the majority of 22 fungal species tested at 50 and 100 ppm. These were bay, cinnamon bark, cinnamon leaf, clove bud, pimenta berry, pimenta leaf and thyme.

General Considerations

Determination of Germicidal Activity. If no growth is observed on the spice-containing medium, it should not be assumed that the spice has exerted a lethal effect on the test microorganism, which may have been merely inhibited. Spores may be less susceptible than vegetative cells. Also, after prolonged incubation, loss of the inhibiting substance through evaporation may occur and growth can take place. The microorganism should be subcultured into a medium free from the inhibiting agent to test for viability. Plempel *et al.* (1987) described a method for testing fungicidal activity, which involves culturing the mold on sterile cellophane membranes laid on agar medium in Petri dishes. The colonies are grown on a medium free from the test substance, then are transferred on their membranes to a medium containing the inhibitor. After a period of time, they are transferred to a medium free from the inhibitor. In this manner, fungicidal activity depending on exposure time may be observed.

Strain Differences. Comparison of results from different studies is complicated by the fact that different strains of a given microorganism may show quite different sensitivities to the spice being tested. Shelef *et al.* (1980) tested the growth of three strains of *Vibrio parahaemolyticus* on agar plates containing rosemary or sage. One strain was only slightly inhibited at the 0.5 % level of either herb; however, the other two strains did not grow on plates containing 0.3 or 0.5 % of either herb during the first 48 h of incubation. Growth occurred after one week on plates containing 0.3 %.

Brand/Variety of Spice. The antimicrobial effect of spices can vary significantly as a result of brand or varietal differences. Bachmann (1918) tested several brands each of ground clove, cinnamon and allspice against bacteria, yeast and fungi. Some brands of a spice were strongly inhibitory, while others showed little if any activity. Pataková and Chládek (1974) examined the inhibitory effect of essential oils from 7 different varieties of thyme against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Oils with a high content of thymol were strongly inhibitory, while those containing little or no thymol were practically inactive. *B. subtilis* was the most sensitive microorganism and *E. coli* the least sensitive.

Microbial Contamination, Spices as Substrates. Commercial spices may be contaminated with a variety of microorganisms (Pivnick 1980; Pafumi 1986) even though commercial treatment with ethylene oxide reduces the microbial populations considerably. Certain spices, such as black pepper, typically carry heavy loads of bacteria while others, such as clove, have relatively few (Julseth and Deibel 1974). This might be a reflection of the antimicrobial activity of the spices. Of particular interest to the food industry is the possibility that spices may contain aflatoxins, which are carcinogenic metabolites of the molds *Aspergillus flavus* and *Aspergillus parasiticus*. Much effort has been spent on screening spices and herbs for the presence of aflatoxins. Scott and Kennedy (1975) examined 132 samples of 15 spices and herbs for aflatoxins. Most of the samples were free from aflatoxins; however, several samples of coriander, ginger, nutmeg and turmeric contained aflatoxin in concentrations ranging from trace amounts to 45 $\mu\text{g}/\text{kg}$ in one sample of coriander. Scott and Kennedy (1973) also examined 24 samples of black and white peppers and 70 samples of capsicum peppers. Aflatoxin was not detected in black and white peppers, but was present in capsicum peppers at levels of up to 8 μg aflatoxin B_1/kg with a high incidence for several types such as cayenne and Indian Chili powder.

This led to the consideration that under suitable conditions of moisture and temperature, spices and herbs themselves may serve as substrates for microbial growth and toxin production. Table 6 shows a few examples of aflatoxin production by *Aspergillus* strains inoculated on sterilized rehydrated spices originally free from aflatoxins. Aflatoxin production by *A. parasiticus* was low on black and white peppers and somewhat higher on cumin and anise. Substantial amounts were produced by *A. flavus* strains on ginger, allspice, red pepper and white pepper. However, it should be noted that these strains were originally isolated from these spices. According to Llewellyn *et al.* (1981), *Aspergillus* strains differ in their ability to grow and produce aflatoxin on spices as substrates. Cinnamon and clove and possibly mustard were antimycotic, while sesame seed, ginger and rosemary supported high levels of aflatoxin production. In general, spices and herbs are poor substrates for mold growth and toxin production, but exceptions do exist.

A variety of spices and herbs, notably clove, cinnamon, mustard, allspice, garlic (Azzouz and Bullerman 1982) and thyme (Karapinar 1985) have been reported to inhibit *Aspergillus parasiticus* and *Aspergillus flavus*. The effect of these materials and their components have also been examined with respect to aflatoxin production. Buchanan and Shepherd (1981) reported that 500 $\mu\text{g}/\text{mL}$ of thymol, the active component of thyme, completely inhibited growth and aflatoxin production by *A. parasiticus* NRRL 2999 in a liquid medium while amounts as low as 10 $\mu\text{g}/\text{mL}$ gave partial inhibition. The same strain was inhibited by cinnamon and clove oils at levels above 250 ppm and by cinnamic aldehyde and eugenol at levels above 200 ppm (Bullerman *et al.* 1977). According to Morozumi (1978), 100 ppm of *o*-methoxycinnamaldehyde, isolated from cinnamon, completely inhibited *A. parasiticus* and *A. flavus*. It appears that inhibition of aflatoxin production by spices

TABLE 6.
SPICES AS SUBSTRATES FOR AFLATOXIN PRODUCTION

<u>Aspergillus</u> Strain	Spice	Aflatoxin B ₁ µg/g	Reference
<u>A. parasiticus</u>			
NRRL 2999	Black pepper	0.062	Madhyastha and Bhat (1984)
NRRL 2999	White pepper	0.044	
<u>A. parasiticus</u>			
NRRL 2999	Anise	2.80	Llewellyn et al. (1981b)
NRRL 2999	Cumin	0.32	
<u>A. flavus</u> Strains			
G4	Ginger	22	Flannigan and Hui (1976)
JP4	Allspice	176	
RP1	Red pepper	4	
WPI	White pepper	24	

and their components may be due to inhibition of growth; however, there is no agreement on this point and not enough data are available to satisfactorily resolve this question (Bullerman *et al.* 1977, Mabrouk and El-Shayeb 1980).

Stimulation of Microbial Metabolism by Spices. It should be noted that under favorable conditions spices and herbs can stimulate some metabolic activity in microorganisms. Reports indicate that lactic acid bacteria are relatively resistant to the inhibitory effects of spices (Salzer *et al.* 1977; Zaika and Kissinger 1979; Shelef *et al.* 1980).

Lactic acid bacteria are used as starter cultures in fermented meat products such as Lebanon bologna and pepperoni. They ferment added sugars to lactic acid, thus lowering the pH and inhibiting pathogenic microorganisms. These sausages typically are heavily spiced. In the course of experiments on meat fermentation by *Lactobacillus plantarum* and *Pediococcus acidilactici*, instead of possible inhibitory effects, we noted that acid production was stimulated in the presence of spices, and the effect increased with increasing spice concentration (Zaika *et al.* 1978). The stimulatory effect was not due to microbial contaminants in spices, since sterile spices produced the same effect. The effect of individual spices on the starter bacteria was examined using a liquid medium containing meat extract and sugars, designed to simulate sausage fermentation (Kissinger and Zaika 1978; Zaika and Kissinger 1979; Zaika *et al.* 1983). In most cases, the bacteria grew even in the presence of 12 g/L of spice and produced increased amounts of acid. Increasing concentrations of clove produced a pronounced inhibition of growth. At the same time, clove was the most stimulatory spice tested; as little as 0.5 g/L of clove resulted in a two-fold increase in acid production. Subsequent investigation (Zaika and Kissinger 1984) determined that the stimulatory factor of spices was found in the solvent-insoluble, acid-soluble fraction, and was shown to be manganese, an element known to be essential to lactic acid bacteria. Stimulatory activity of 0.1 N HCl extracts of spices increased with increasing manganese concentration. It was possible to observe the stimulatory effect because the growth medium used, meat extract, contained only low levels of manganese, insufficient for optimum acid production by lactic acid bacteria. Nes and Skjelkvale (1982) also noted that spices stimulated fermentation by *L. plantarum* strains; however, they noted that oleoresins of these spices had no effect. It has been reported that spices can stimulate other microbial species such as micrococci (Salzer *et al.* 1977) and yeast (Corran and Edgar 1933; Wright *et al.* 1954).

SUMMARY

This discussion attempted to present a general overview of the factors that are important in determining the antimicrobial properties of spices, herbs and their components. These may be summarized as follows:

1. A variety of methods have been reported for testing the antimicrobial activity of spices, herbs and their components.
2. The degree of observed microbial inhibition depends on the method employed to test for antimicrobial activity.
3. Microorganisms differ in their resistance to a given spice or herb.
4. A given microorganism differs in its resistance to various spices and herbs.
5. Bacteria are more resistant than fungi
6. The effect on spores may be different than that on vegetative cells.
7. Gram-negative bacteria are more resistant than gram-positive bacteria.
8. Effect of spice or herb may be inhibitory or germicidal.
9. Essential oils in contact with microorganisms in the test media possess greater germicidal powers than they do in their vaporous state.
10. Spices and herbs harbor microbial contaminants.
11. Spices and herbs may serve as substrates for microbial growth and toxin production.
12. Amounts of spices and herbs added to foods are generally too low to prevent spoilage by microorganisms.
13. Active components at low concentrations may interact synergistically with other factors (NaCl, acids, preservatives) to increase preservative effect.
14. Decreased antimicrobial activity of spices and herbs in food may be due to partitioning of active components into the lipid phase.
15. Nutrients present in spices and herbs may stimulate growth and/or biochemical activities of microorganisms.
16. The extent to which spices inhibit microbial growth depends on their source and processing.

REFERENCES

- AZZOUZ, M. A., and BULLERMAN, L. B. 1982. Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. *J. Food Prot.* *45*, 1298-1301.
- BACHMANN, F. M. 1918. The use of microorganisms to determine the preservative values of different brands of spices. *J. Ind. Eng. Chem.* *10*, 121-123.
- BACHMANN, F. M. 1916. Inhibiting action of certain spices on the growth of microorganisms. *J. Ind. Eng. Chem.* *8*, 620-623.
- BEUCHAT, L. R. 1976. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. *J. Food Sci.* *41*, 899-902.
- BLUM, H. B., and FABIAN, F. W. 1943. Spice oils and their components for controlling microbial surface growth. *Fruit Products J.* *22*, 326-329, 347.
- BOYLE, W. 1955. Spices and essential oils as preservatives. *Am. Perfum. Essent. Oil Rev.* *66*, 25-28.

- BUCHANAN, R. L. and SHEPHERD, A. J. 1981. Inhibition of *Aspergillus parasiticus* by thymol. J. Food Sci. 46, 976-977.
- BULLERMAN, L. B. 1974. Inhibition of aflatoxin production by cinnamon. J. Food Sci. 39, 1163-1165.
- BULLERMAN, L. B., LIEU, F. Y. and SEIER, S. A. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. 42, 1107-1109, 1116.
- CAVALLITO, C. J. and BAILEY, J. H. 1944. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66, 1950-1951.
- CHAMBERLAND, M. 1887. Les essences. Au point de vue de leurs propriétés antiseptiques. Ann. Inst. Pasteur, Paris 1, 153-164.
- COLLIER, W. A. and NITTA, Y. 1930. Über die Wirkung ätherischer Öle auf verschiedene Bakterienarten. Z. Hyg. Infektionskrankh. III, 301-312.
- CONNER, D. E. and BEUCHAT, L. R. 1984. Effects of essential oils from plants on growth of food spoilage yeasts. J. Food Sci. 49, 429-434.
- CORRAN, J. W. and EDGAR, S. H. 1933. Preservative action of spices and related compounds against yeast fermentation. J. Soc. Chem. Ind. 52, 149 T-152 T.
- DEIBEL, K. E. and BANWART, G. J. 1984. Effect of spices on *Campylobacter jejuni* at three temperatures. J. Food Saf. 6, 241-251.
- DOLD, H. and KNAPP, A. 1948. Über die antibakterielle Wirkung von Gewürzen. Z. Hyg. Infektionskrankh. 128, 696-706.
- FABIAN, F. W., KREHL, C. F. and LITTLE, N. W. 1939. The role of spices in pickled food spoilage. Food Res. 4, 269-286.
- FALCK, R. 1907. Cited by Horsfall, J. G. 1956. *Principles of Fungicidal Action*, pp. 4-27, Chronica Botanica Co. Waltham, Mass.
- FLANNIGAN, B. and HUI, S. C. 1976. The occurrence of aflatoxin-producing strains of *Aspergillus flavus* in the mould floras of ground spices. J. Appl. Bacteriol. 41, 411-418.
- GALLI, A., FRANZETTI, L. and BRIGUGLIO, D. 1985. Attività antimicrobica in vitro di oli essenziali ed estratti di spezie di uso alimentare. Ind. Aliment. 24, 463-466.
- GRAHAM, H. D. and GRAHAM, E. J. F. 1987. Inhibition of *Aspergillus parasiticus* growth and toxin production by garlic. J. Food Saf. 8, 101-108.
- HITOKOTO, H., MOROZUMI, S., WAUKE, T., SAKAI, S. and KURATA, H. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. Environ. Microbiol. 39, 818-822.
- HOFFMAN, C. and EVANS, A. C. 1911. The use of spices as preservatives. J. Ind. Eng. Chem. 3, 835-838.
- JULSETH, R. M. and DEIBEL, R. H. 1974. Microbial profile of selected spices and herbs at import. J. Milk Food Technol. 37, 414-419.

- KARAPINAR, M. 1985. The effect of citrus oils and some spices on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. *Int. J. Food Microbiol.* 2, 239-245.
- KELLNER, W. and KOBER, W. 1954. Möglichkeiten der Verwendung ätherischer Öle zur Raumdesinfektion. I. Die Wirkung gebräuchlicher ätherischer Öle auf Testkeime. *Arzneim. Forsch.* 4, 319-325.
- KELLNER, W. and KOBER, W. 1955. Möglichkeiten der Verwendung ätherischer Öle zur Raumdesinfektion. 2. Ermittlung der für die keimtötende Wirkung verantwortlichen Einzelbestandteile relativ unschädlicher ätherischer Öle. *Arzneim. Forsch.* 5, 224-229.
- KISSINGER, J. C. and ZAIKA, L. L. 1978. Effect of major spices in Lebanon bologna on acid production by starter culture organisms. *J. Food Prot.* 41, 429-431.
- KOEDAM, A. 1977. Antimikrobielle Wirksamkeit ätherischer Öle. Eine Literaturarbeit 1960-1976. *Riechst., Aromen, Kosmet.* 27, 6, 8-11; 27, 36-41.
- LLEWELLYN, G. C., BURKETT, M. L. and EADIE, T. 1981a. Potential mold growth, aflatoxin production, and antimycotic activity of selected natural spices and herbs. *J. Assoc. Off. Anal. Chem.* 64, 955-960.
- LLEWELLYN, G. C., DIXON, E. C., EADIE, T., DASHEK, W. V. and O'REAR, C. E. 1981b. Aflatoxin formation on whole and ground cumin and anise seeds. *J. Am. Oil Chem. Soc.* 58, 985A-988A.
- MABROUK, S. S. and EL-SHAYEB, N. M. A. 1980. Inhibition of aflatoxin formation by some spices. *Z. Lebensm. Unters. Forsch.* 171, 344-347.
- MADHYASTHA, M. S. and BHAT, R. V. 1984. *Aspergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. *Appl. Environ. Microbiol.* 48, 376-379.
- MARTINDALE, W. H. 1910. Essential oils in relation to their antiseptic powers as determined by their carbolic coefficients. *Perfum. Essent. Oil Rec.* 1, 266-274.
- MARUZZELLA, J. C. and FREUNDLICH, M. 1959. Antimicrobial substances from seeds. *J. Am. Pharm. Assoc.* 48, 356-358.
- MARUZZELLA, J. C. and HENRY, P. A. 1958. The *in vitro* antibacterial activity of essential oils and oil combinations. *J. Am. Pharm. Assoc.* 47, 294-296.
- MARUZZELLA, J. C. and LIGUORI, L. 1958. The *in vitro* antifungal activity of essential oils. *J. Am. Pharm. Assoc.* 47, 250-254.
- MOROZUMI, S. 1978. Isolation, purification and antibiotic activity of o-methoxycinnamaldehyde from cinnamon. *Appl. Environ. Microbiol.* 36, 577-583.
- NES, I. F. and SKJELKVALE, R. 1982. Effect of natural spices and oleoresins on *Lactobacillus plantarum* in the fermentation of dry sausage. *J. Food Sci.* 47, 1618-1621, 1625.
- PAFUMI, J. 1986. Assessment of the microbiological quality of spices and herbs. *J. Food Prot.* 49, 958-963.

- PATÁKOVÁ, D. and CHLÁDEK, M. 1974. Über die antibakterielle Aktivität von Thymian- und Quendelölen. *Pharmazie* 29, 140, 142.
- PIVNICK, H. 1980. Spices. In *Microbial Ecology of Foods*, Vol. II. Food Commodities. The International Commission on Microbiological Specifications for Foods, pp. 731-751, Academic Press, New York.
- PLEMPEL, M., BERG, D., BÜCHEL, K. H. and ABBINK, D. 1987. Test methods for antifungal agents—a critical review. *Mykosen* 30, 28-37.
- RIPPETOE, J. R. and WISE, L. E. 1912. The preservative action of essential oils. *J. Am. Pharm. Assoc.* 1, 1273-1282.
- SALZER, U. J., BRÖKER, U., KLIE, H. F. and LIEPE, H. U. 1977. Wirkung von Pfeffer und Pfefferinhaltsstoffen auf die Mikroflora von Wurstwaren. *Fleischwirtschaft* 57, 2011-2014, 2017-2021.
- SCOTT, P. M. and KENNEDY, B. P. C. 1975. The analysis of spices and herbs for aflatoxins. *Can. Inst. Food Sci. Technol. J.* 8, 124-125.
- SCOTT, P. M. and KENNEDY, B. P. C. 1973. Analysis and survey of ground black, white and capsicum peppers for aflatoxins. *J. Assoc. Off. Anal. Chem.* 56, 1452-1457.
- SHELEF, L. A. 1983. Antimicrobial effects of spices. *J. Food Safety* 6, 29-44.
- SHELEF, L. A., NAGLIK, O. A. and BOGEN, D. W. 1980. Sensitivity of some common food-borne bacteria to the spices sage, rosemary, and allspice. *J. Food Sci.* 45, 1042-1044.
- STEUDEL, H. 1971. Die antimikrobielle Wirksamkeit der ätherischen Öle und Pflanzenextrakte. *Seifen, Oele, Fette, Wachse* 97, 736-738.
- THOMPSON, D. P. 1986. Effect of essential oils on spore germination of *Rhizopus*, *Mucor* and *Aspergillus* species. *Mycologia* 78, 482-485.
- THOMPSON, D. P. and CANNON, C. 1986. Toxicity of essential oils on toxigenic and nontoxigenic fungi. *Bull. Environ. Contam. Toxicol.* 36, 527, 532.
- TIWARI, R., DIKSHIT, R. P., CHANDAN, N. C., SAXENA, A., GUPTA, K. G. and VADEHRA, D. E. 1983. Inhibition of growth and aflatoxin B₁ production of *Aspergillus parasiticus* by spice oils. *J. Food Sci. Technol. (Mysore)* 20, 131-132.
- UEDA, S., YAMASHITA, H. and KUWABARA, Y. 1982. Inhibition of *Clostridium botulinum* and *Bacillus* sp. by spices and flavoring compounds. *Nippon Shokuhin Kogyo Gakkaishi* 29, 389-392.
- WEBB, A. H. and TANNER, F. W. 1945. Effect of spices and flavoring materials on growth of yeasts. *Food Res.* 10, 273-282.
- WRIGHT, W. J., BICE, C. W. and FOGELBERG, J. M. 1954. The effect of spices on yeast fermentation. *Cereal Chem.* 31, 100-122.

- ZAICA, L. L. and KISSINGER, J. C. 1979. Effects of some spices on acid production by starter cultures. *J. Food Prot.* 42, 572-576.
- ZAICA, L. L. and KISSINGER, J. C. 1984. Fermentation enhancement by spices: Identification of active component. *J. Food Sci.* 49, 5-9.
- ZAICA, L. L., KISSINGER, J. C. and WASSERMAN, A. E. 1983. Inhibition of lactic acid bacteria by herbs. *J. Food Sci.* 48, 1455-1459.
- ZAICA, L. L., ZELL, T. E., PALUMBO, S. A. and SMITH, J. L. 1978. Effect of spices and salt on fermentation of Lebanon bologna-type sausage. *J. Food Sci.* 43, 186-189.