THE UNIVERSITY OF

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EFFECT OF SOME SELECTED SPICES ON THE GROWTH CHARACTERISTICS OF LACTIC ACID BACTERIA ISOLATED FROM PASTIRMA - A FERMENTED SAUSAGE INDIGENOUS TO IRAQ-

BY

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A C K N O W L E D G E M E N T S

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A B S T R A C T

An Iraqi fermented meat sausage, Pastirma, was formulated, processed and evaluated for sensory acceptance. Two lactic acid bacteria referred to as lactose fermenting (LF) and non lactose fermenting (NLF) were isolated from the sausage during curing. Growth, titratable acidity and pH development by these isolates was investigated in APT broth containing spices and spice mixtures used in the sausage formulation. Increasing concentrations of spice mixture (Ø-5%, w/v) progressively increased the time for maximum growth, titratable acid and pH development in both cultures. Final titratable acidity increased with increasing spice mixture concentration; increasing the temperature of incubation (15 to 30°C) did not increase the final titratable acidity values but did decrease the time necessary to reach them. Ten individual spices, representing the majority in the spice mixture were evaluated, in terms of their minimum inhibitory concentration (MIC) , with each isolate. The MIC values, which represent the minimum concentration of spice retarding pH development in growth media by Ø.5 units for 24 h indicated that clove, black pepper and nutmeg (Ø.3%, w/v) were the most effective spices with the LF culture while clove (Ø.3%, w/v) following by nutmeg and black pepper (Ø.5%, w/v) were the most effective spices with the NLF culture. Cardamom, cinnamon, coriander and cumin were the least effective with both cultures.

The antioxidant activity of the individual spices was highest with clove and lowest with cardamom and coriander. The

spice mixture showed low antioxidant activity. A correlation between antioxidant activity and antimicrobial acitivity could not be established.

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1. INTRODUCTION

Iraq, one of the developed countries of the middle east, has made large investments into the agricultural and industrial sectors over the past ten years. Despite the progress made in these areas, Iraq still imports many food commodities like processed, canned and frozen meats, cellophane packaged frankfurters, large bolognas and corned beef. This trade suggests that Iraq has the potential to develop and advance a food processing sector in the meat product area.

Pastirma is a spiced, salted sausage made by various ethnic people throughout Iraq. This product is made only in the winter season when the average ambient temperature is 15^{o}C. The productis consumed fresh or it can be stored as semidry sausage. Unfortunately there is no general ingredient formula for Pastirma; each community or local sausage maker has their own special formula. The basic understanding of the preservation principles for this product is lacking and technical and scientific reports are very few.

Pastirma sausage seems to be very similar to Lebanon bologna. This bologna is a semidry, fermented, all beef sausage which is smoked but not cooked; Pastirma sausage is neither smokednor cooked during processing. Lebanon bologna apparently originated amongst the Pennsylvanian Germans in the Lebanon Pennsylvania region of the United States. The bologna is fermented by lactic acid bacteria while the cured meat color is fixed by nitrite reduction through the activities of micrococci.

Controlled fermentation of meat products through the use of salt and spice formulations, such as seems to occur with the processing of Pastirma, is a logical commercial processing procedure to develop for Iraq. This procedure does not appear to require the expensive thermal processing or refrigeration processing technologies to develop stable meat products. This thesis was designed to develop an understanding of basic preservation principles that are operative in the Pastirma sausage. This study was also designed to determine the preservation effect of twelve spices commonly used in making Pastirma sausage. The basic assumption of this study was that the spice mixture used by the ethnic peoples of Iraq to make Pastirma sausage plays a major role in making the sausage a stable food product during the climatic conditions that exist in Iraq during the winter season.

The basic objectives of this study were as follows:

1. An acceptable Pastirma sausage formula had to be developed and approved by members of Winnipeg's Iraqi community.

2. The approved Pastirma sausage was evaluated to determine the basic preservation principles which gave the sausage its storage stability.

3. Typical fermentation organisms were isolated from the finished Pastirma sausage product and characterized.

4. The isolated bacterial cultures were evaluated for their growth and acid production in the presence of the spice mixture and in the presence of the individual spices. In addition the antioxidant properties of the spice mixture and the individual spices were evaluated.

2. LITERATURE REVIEW

2.1. Fermented Meat Sausage

Fermented meat sausages are generally produced as dry or semidry products although some are classified as being intermediate. Dry or Italian-type sausages contain between 30-40% moisture, are generally not smoked or heat processed and are eaten usually without cooking (Pederson, 1971). Genoa and Milano sausages, salamis and pepperoni are other examples of dry sausages (Jay, 1978; Raccach, 1980). Semidry sausages contain about 50 % moisture and are finished by heating to an internal temperature of 140-154^{o}F during smoking. Thuringer, cervelat, summer and Lebanon bologna are some examples of semidry sausages. "Summer sausage" refers to those traditionally of Northern European origin, made during colder months, stored, aged, and then eaten during summer months. They may be dry or semidry (Jay, 1978).

In the preparation of fermented sausage, curing mixtures which include glucose as a substrate for the fermenters, and nitrates and /or nitrites for color stabilization and seasonings agents (spice mixture) are added to ground meat. The meat (Deibel and Niven, 1957; Everson, 1971; Acton et al., 1972; Townsend and Davis, 1972; Klement et al., 1973; Wardlaw et al., 1973) is then stuffed into casings and incubated for varying time periods, during which fermentation occurs chiefly by lactic acid microoganisms present in the flora of the meat constituents and/or those introduced from equipment or starter culture addition (Everson, 1971; Townsend and Bard, 1978).

Traditional processes for the manufacture of fermented sausage require approximately 150 hours forfermentation and processing before drying (Acton et al., 1972). Following the incubation, the products are placed in drying rooms with a specified relative humidity for a specific time period. During drying these sausages lose 30% to 40% of their initial weight (Kramlich, 1978). The drying times and temperatures are based primarily on sausage diameter (Acton and Dick, 1976).

The temperature, relative humidity and air flow must be controlled so that drying proceeds properly. Air flow may vary from 15 to 20 changes per hour in the drying room. If drying is too slow the texture may be soft, the surface may discolor, and some molds or yeast may develop on the product. If drying is too fast, a surface crust develops and a brown or dark ring appears under the surface and at times marked ridges or invaginations occur at the sausage surface. The proper ratio of fat to lean is also important to give good conformation to the sausage (Kramlich et al., 1973). Townsend et al.(1975) reported that dry-type sausages having initial pH values of 5.5 and dried with high air velocity had poor internal cured meat color and texture and developed hollow centers, but not in those having pH values 5.5, 5.9, or 6.6 and dried with low air velocity.

Controlled use of starter cultures in meat products was introduced in a patent of Jenesen and Paddock (1940). Bacteria which have been proposed as starter cultures include Pediococcus cerevisiae (Deibel and Niven, 1957; Deibel et al., 1961) and a number of species of Lactobacillus (Jenesen, 1954). Starter cultures of Lactobacillus plantarum and Pediococcus

cerevisiae are currently available as a frozen concentrate (Anonymous., 1972; Everson et al., 1970; Everson, 1971); the latter organism is also availalble as a lyophilized starter.

Fermented sausages produced without use of starters have been found to contain large numbers of Lactobacillus such as Lactobacillus plantarum (Deibel et al., 1961). Smith and Palumbo (1973) found that when starter cultures were used in producing fermented sausages the final pH of the products ranged from 4.0 to 4.5, while those produced without starters ranged between 4.6 and 5.0. The pH of fermented sausage may actually increase by 0.1-0.2 units during long periods of drying due to uneven buffering produced by increases in amounts of basic compounds (Wardlaw et al., 1973).

2.2. Nomenclature of Spices and Herbs

Spices and herbs include any of a variety of aromatic vegetable and plant products used in cooking to season food and flavor beverages. When aromatic or fragrant vegetable products are of tropical origin, they are considered spices; when these vegetable products originate from temperate regions, they are considered herbs (Ayres et al.,1980; Rosengarten, 1969).

There are over seventy spices now officially recognized by the International Organization for Standardization (ISO). This organization represents the various spice producing, exporting, and importing countries. The ISO found it difficult to differentiate between spices and condiments, and therefore decided to keep both terms. According to the ISO (1972,1968) the term spice and condiment applies to natural vegetable products or

mixtures thereof, without any extraneous matter, that are used for flavoring , seasoning, and improving aroma to foods; the term applies to the product either in the whole or in the ground form. The nomenclature of spices and condiments, as now accepted, has been reviewed by the ISO member countries (Pruthi, 1980).

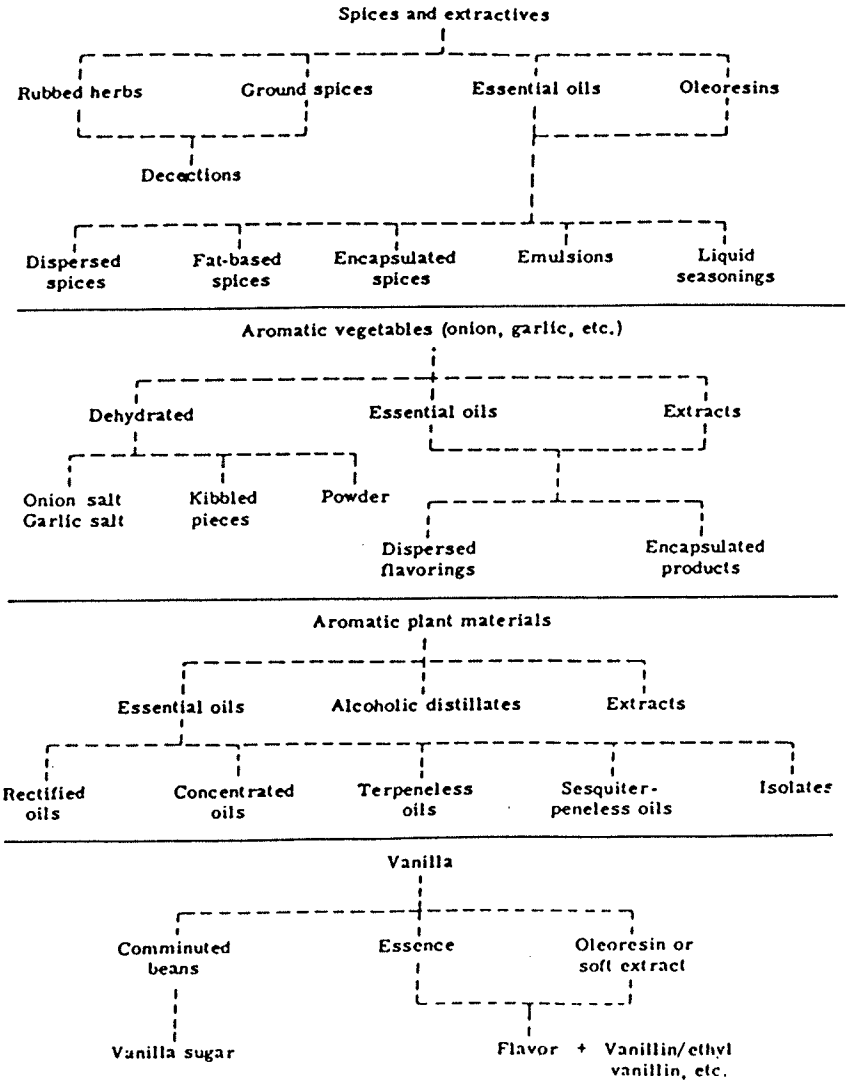
Spices and condiments originate from several parts of aromatic plants: fruits (capsicum, black pepper, cardamom) seeds (aniseed, caraway, celery, coriander, cumin, fennel, fenugreek, mustard) rhizomes or roots (ginger, turmeric) leaves (bay leaves, marjoram, parsley, sage, thyme) barks (cinnamon and cassia) floral parts (saffron, cloves, caper) bulbs (onion, garlic, shallot).

2.3. Products Technology

World demand has increased the need for processed spices and spice products and as such various specialized requirements of modern food processing have inspired the development of spice extractives, which may be liquid or dry.

The liquid spice extractions may be composed of extracted essential oils or oleoresins, depending on the nature of the subject spice (Fig. 1). As with ground spices the manufacturer blends the extractives to achieve the desired flavor, performance and consistency. There are several types of spice extractives: essential oils, oleoresins, essences, emulsions, decoctions, dispensed spices or dry soluble spices, instant spices, spray dried spices, encapsulated spices on salt or dextrosebases, oil-based or fat-based spices, liquid and dry seasonings.

Figure 1. A schematic plan of the manufacture of spice products and their interrelationship. Heath (1972).



2.3.1. Essential Oils

The aroma and flavoring properties of all spices have been attributed to their essential oil content. Essential oils are the volatile oils, aromatic or odorous, oily liquids obtained from plants or from parts thereof by a series of processes that include steam distillation, enzymatic action followed by steam distillation, or water and steam distillation. The aroma and characteristic organoleptic qualities of the spice are contained and concentrated within the essential oil fraction (Pruthi, 1980).

Most essential oils consist of mixtures of hydrocarbons (terpenes and sesquiterpenes) oxygenated compounds (alcohols, esters, aldehydes and ketones) and a small percentage of nonvolatile residues (paraffins and waxes) . The oxygenated compounds are more soluble in dilute alcohol and in general are more stable against oxidizing influences. The terpenes and sesquiterpenes, because of their unsaturated character, oxidize easily under the influence of air and light or under improper storage conditions. To improve the storage stabilities and other qualities of essential oils, spice manufacturers produce concentrated oils devoid of terpens and sesquiterpens. These oils are made by counter current extraction with polar and nonpolar solvents and by chromatographic separation (Pruthi, 1980). Although these distilled oils partially overcome problems of flavor variability, since they are reasonably constant in flavor character (Furia, 1980) and are used at a fixed rate, their acceptable flavor effects are nonetheless incomplete.

Essential oils lack many of the nonvolatile components that are present in freshly ground spices.

Among the natural products used to formulate flavor composition, essential oils are the most important, being the largest single category of flavoring substances currently available to the flavorist. The essential oils may be prepared or derived from specific parts of plants, including flowers (oil neroli), buds (cloves), seeds (coriander and anise), fruits (lime, lemon and orange), leaves (spearmint), twigs (petitgrain),bark (cinnamon) and roots (ginger)(Furia,1980; Pruthi, 1980).

2.3.2. Oleoresins

A closer approximation to the total spice flavor is an extremely concentrated , viscous, resinous extract obtained by solvent extraction which contains all the spice flavoring ingredients soluble in that particular organic solvent. The oleoresins are prepared from the ground dry spice by extraction with suitable volatile organic solvents or a mixture of solvents. As the solvent percolates through a bed of ground spice or herbs it removes not only the flavor constituents (mainly the essential oil), but also resins, gums and other materials that are miscible in the solvent. The solvent is then stripped for reuse, leaving behind a somewhat viscous material now called an oleoresin. Oleoresins differ from essential oils in that oleoresins contain many of the high boiling and nonvolatile constituents native to the spice or herb; these components have been removed from the corresponding essential oil. Certain spices

are extracted to produce oleoresins, which are not primarily intended for their flavor value, but rather for the intense coloring matter they contain. The oleoresins of paprika and turmeric are used to impart color in the manufacture of salad dressings and pickle products. Although oleoresins are normally thick, viscous and sometimes highly colored products, the majority impart less color to the finished product than the corresponding spices because of their low rate of addition to foods.

The oleoresins find favor in the food industry because they are more uniform, applicable and potent than the respective dry spice and are especially suited to high temperature use applications such as baking and frying. Their normal use in foods is 1/5 to 1/20 the amount of their corresponding dry spice. The resins and fatty oils contained within oleoresins act as natural fixatives for volatile essential oils. Oleoresins, because of the nature of their production are free from bacteria, molds and fungi; oleoresins do not support microbial growth (Furia, 1980; Pruthi, 1980).

2.4. Spice Microflora

Spices are known to contain a wide variety and number of microorganisms. These organisms represent commensal populations indigenous to the methods of cultivation and contaminants which gain access to spices during all phases of handling and processing.

2.4.1. Bacteria

Spices are known to contain varying populations of bacteria. In some cases their number may be so high, that the spice acts as a vector for food spoilage and poisoning (Powers et al.,1976; Goepfert et al.,1972; Krishnaswamy et al.,1971). In addition sporeformers can cause food spoilage when they survive the cooking process and multiply under favorable conditions (Beuchat et al., 1980; Powers et al., 1976). Bacterial counts for black pepper, paprika, ginger, mustard and celery seed often range from one to more than 100 million per gram (Christensen et al., 1967; Karlson and Gunderson, 1965). Total counts in excess of 500,000 per gram have been reported for bay leaves, curry powder, paprika, pepper, thyme, turmeric and celery flakes (Table 1; Karlson and Gunderson, 1965).

Escherichia coli, Citrobacter fruendii and species of Serratia,Klebsiella,Bacillus, Staphylococcus and Streptococcus represent some bacteria isolated from black and red pepper (Christensen et al.,1967). Shigellae and salmonellae have not been isolated from spices while occasionally coagulase-positive Staphylococcus aureus has been reported (Powers et al.,1975). The spore counts of many of the spices listed in Table 1 range from a few hundred to over a quarter million per gram; most anaerobic spore counts were well under a thousand per gram while anaerobic spores were randomly distributed amongst various bacterial species. Bacillus stearothermophilus and B. coagulans are usually present in black pepper, ginger and imported paprika. Spores of proteolytic and amylolytic organisms are numerous. B. cereus, which is some times pathogenic, is found

Table 1
Numbers of bacteria in spices.

Type of spice		*Number of microorganisms per gram of dry sample*				
		Total aerobes	*Coliforms*	*Yeasts & molds*	*Aerobic spores*	*Anaerobic spores*
Bay leaves		520,000	0	3300	9200	<2
Cloves		3000	0	5	<2	<2
Curry	1	>7,500,000	0	70	>240,000	>24,0000
	2	400,000	30	70	>110,000	430
Marjoram	1	370,000	0	18,000	54,000	>24,000
	2	160,000	0	5000	9	150
Paprika	1	>5,500,000	600	2300	>240,000	620
	2	6,500,000	80	30,000	2900	43
Pepper		>2,000,000	0	15	>240,000	>24,000
Sage		6800	0	10	7	1700
Thyme	1	1,900,000	0	11,000	160,000	>24,000
	2	1,300,000	200	8300	150	...
Turmeric	1	>6,700,000	0	0	<2	<2
	2	1,300,000	50	70	>110,000	430

Source: Karlson and Gunderson, 1965. Reprinted from Food Technology vol. 19, p. 86, 1965. Copyright © by Institute of Food Technologists.

in a high percentage of spices. Powers et al. (1976) reported that 53% of the spice samples analyzed contained B. cereus. Powers et al.(1975) also reported the presence of Clostridium perfringens in the spices analyzed. The organism was found in 4 out of 7 types of spices and constituted 15 % of the total microbial flora analyzed. The microflora of spices from 10 different lots was also shown to vary widely.

Masson (1978) carried out a bacteriological investigation of 50 retail spice samples. The result showed large variations in contamination; 34-44 % of the samples examined were contaminated by bacteria of fecal origin (coliforms, enterococci); C. perfringens and S. aureus were also identified as contaminants.

In another study performed by Baxter and Holzapfel (1982) the presence of high numbers of sporeformers was also reported. Many of these sporeformers were proteolytic and/or amylolytic. The total number of colony forming units (CFU) varied from several hundred to several million per gram, depending on the source, the manufacturing process, age and type of condiment. Exceptionally high contamination ($>10^6$CFU/g) was found in black pepper, coriander, paprika, mace, pimento and white pepper. No significant numbers of potentially pathogenic organisms were detected except for B. cereus, molds and faecal streptococci. The same authors confirmed the presence of B. cereus in 7 out of 19 samples from one source. Enterococci were isolated and identified from paprika, white pepper,black pepper, pimento, onion powder and salamita.

2.4.2. Molds

Spices have also been reported to harbor a large number of mold fragments and/or spores. Many mold species are commensals while some are introduced by insect infestation (Christensen et al.,1967; Christensen,1972). The mycological quality of some retail spices, especially pepper, is often quite poor, containing many genera and species of fungi. Most fungi are of the storage type, which develop after harvest when the relative humidity is not controlled during storage.

Samples of whole or ground black pepper from various sources yielded numerous species of Aspergilli which included A. glaucus, A. restrictus, A. ochraceous and A. flavus. In laboratory trials, both A. flavus and A. ochraceous produced mycotoxins. Other species of molds isolated from spices, included those of Penicillium, Spicaria, Scopulariopsis and Sporendonema (Ayres et al., 1980).

Wada et al. (1978) reported the presence of Aspergillus, Penicillium, Cladosporium, Mucor, Rhizopus, Chaetomium, Fusarium and Paecilomyces in garlic, red pepper, white and black pepper. Aflatoxin production was also observed on black pepper. In another study 525 mold strains were isolated from 8 samples of red pepper and from 3 samples of black pepper. Twenty-eight strains isolated from red pepper were found positive in producing aflatoxin B_1 while only 8 of the strains isolated from black pepper produced aflatoxin B_1; 16 of the strains isolated from red pepper also produced aflatoxin G_1 (Guergue and Ramirez, 1977).

Flannigan and Hui (1976) found that of the 20 different spices examined only cloves was free of molds (Tables 2 and 3). The remaining spices contained a variety of mold species of which A. glaucus and A. niger were the most common. A. flavus was found in 14 spices and 7 of 24 strains examined produced aflatoxins in vitro.

2.5. Sterilization

The bacteriological safety of spices may beeffectively achieved by several methods which include ethylene and propylene oxide treatment,microwaveand gammairradiation(Powers et al, 1975). Although these methods achieve reduced microbial numbers (less than 0.1% of the original counts by treatment with ethylene oxide (Ayres et al.,1980), they are limited by cost and the time required to completely destroy microoganisms and their effect on the resulting flavor and color of the treated spices. Furthermore, the amount of residues of ethylene and propylene oxide in spices is limited by food regulatory agencies. Gamma irradiation has been found to be more effective than ethylene oxide in reducing the bacterial population of spices. Recently, increasing the permitted usage level up to three megarads for spices, including dehydrated onions and garlic was announced by the Food and Drugs Administration (U.S.A.) (Anonymous, 1984).

Other conventional methods of sterilization are ineffective or destroy/alter spice properties. Ultraviolet light shows poor penetration into bulk spices and dry or wet heat causes charring and volatiles to dissipate, respectively. Steam sterilization, for example decreased pepper flavor potency by 10%

TABLE 2

Numbers of micro-organisms in ground spices

Spice	Propagules/g spice		
	Actinomycetes	Bacteria	Moulds
Aniseed	0	$1{\cdot}9 \times 10^5$	$9{\cdot}5 \times 10^3$
Black pepper	0	$5{\cdot}2 \times 10^7$	$6{\cdot}4 \times 10^5$
Cardamom	$2{\cdot}5 \times 10^3$	$7{\cdot}6 \times 10^6$	$1{\cdot}6 \times 10^3$
Caraway	0	$5{\cdot}2 \times 10^4$	$1{\cdot}5 \times 10^3$
Red pepper (cayenne)	0	$2{\cdot}2 \times 10^6$	$3{\cdot}9 \times 10^4$
Celery	0	$2{\cdot}6 \times 10^6$	$1{\cdot}2 \times 10^3$
Cinnamon	0	$3{\cdot}5 \times 10^5$	$8{\cdot}7 \times 10^4$
Cloves	$6{\cdot}0 \times 10^2$	$4{\cdot}3 \times 10^4$	0
Coriander	0	$3{\cdot}7 \times 10^6$	$1{\cdot}3 \times 10^5$
Cumin	0	$2{\cdot}1 \times 10^5$	$1{\cdot}5 \times 10^3$
Fennel	$2{\cdot}5 \times 10^3$	$1{\cdot}6 \times 10^5$	$6{\cdot}7 \times 10^3$
Fenugreek	$6{\cdot}5 \times 10^2$	$6{\cdot}2 \times 10^4$	$2{\cdot}5 \times 10^3$
Ginger	$1{\cdot}0 \times 10^2$	$8{\cdot}7 \times 10^6$	$1{\cdot}7 \times 10^3$
Jamaica pepper (allspice)	0	$6{\cdot}8 \times 10^6$	$7{\cdot}0 \times 10^4$
Mace	0	$6{\cdot}4 \times 10^4$	$8{\cdot}0 \times 10^2$
Mustard	0	$5{\cdot}8 \times 10^6$	$2{\cdot}7 \times 10^3$
Nutmeg	$4{\cdot}0 \times 10^3$	$1{\cdot}1 \times 10^5$	$6{\cdot}2 \times 10^4$
Paprika	0	$6{\cdot}6 \times 10^6$	$5{\cdot}5 \times 10^2$
Turmeric	0	$4{\cdot}8 \times 10^6$	$0{\cdot}5 \times 10^2$
White pepper	0	$6{\cdot}8 \times 10^4$	$6{\cdot}5 \times 10^4$
Mixtures			
Curry powders	0	$2{\cdot}0 \times 10^5$	$9{\cdot}2 \times 10^4$
Gharum masala	0	$2{\cdot}4 \times 10^6$	$4{\cdot}1 \times 10^4$
Mixed baking spice	0	$6{\cdot}8 \times 10^4$	$3{\cdot}2 \times 10^4$

From Flannigan and Hui (1976)

TABLE 3
The mould flora of ground spices

Spice	Main components of mould flora, as % of mould count												
	Absidia spp.	*Aspergillus candidus*	*A. flavus*	*A. fumigatus*	*A. glaucus* (group)	*A. nidulans*	*A. niger*	*A. tamarii*	*A. terreus*	*A. versicolor*	*Mucor pusillus*	*Penicillium* spp.	*Rhizopus* spp.
Aniseed	–	–	1	1	55	–	3	–	1	2	–	33	–
Black pepper	–	2	1	–	92	–	+	+	–	1	–	2	–
Cardamom	–	–	3	–	64	12	12	–	–	–	–	–	9
Caraway	7	–	–	–	81	–	3	–	–	–	3	–	–
Red pepper	–	–	4	–	69	1	17	–	–	1	1	1	1
Celery	4	–	–	–	79	–	4	–	–	–	9	–	–
Cinnamon	33	–	+	–	–	–	62	+	–	+	–	2	–
Cloves	–	–	–	–	–	–	–	–	–	–	–	–	–
Coriander	4	7	5	1	67	1	1	–	2	10	–	2	–
Cumin	–	–	–	–	62	7	7	–	7	–	–	17	–
Fennel	2	3	2	2	62	4	6	2	2	5	–	–	–
Fenugreek	–	2	–	–	16	6	8	–	2	–	2	60	2
Ginger	–	15	3	–	32	–	9	–	–	–	–	35	3
Jamaica pepper	3	+	1	–	9	–	80	–	–	1	–	6	–
Mace	12	–	–	–	88	–	–	–	–	–	–	–	–
Mustard	–	–	–	–	100	–	–	–	–	–	–	–	–
Nutmeg	–	–	4	–	70	–	12	8	2	–	–	3	–
Paprika	27	–	–	–	27	–	10	–	–	–	–	18	18
Turmeric	–	–	–	–	100	–	–	–	–	–	–	–	–
White pepper	–	16	7	8	2	13	11	2	3	12	–	21	–
Mixtures													
Curry powder	2	3	1	–	–	1	4	–	–	–	2	87	–
Gharum masala	5	25	5	–	–	2	7	–	5	1	–	48	2
Mixed baking spice	28	2	6	–	42	–	19	3	–	–	–	–	–
Number of samples contaminated/23	11	10	14	4	19	9	19	6	8	9	5	14	6

+, less than 1%; –, absent.

From Flannigan and Hui (1976)

when heated for 5 min at 5 to 15 p.s.i. (Yesair and Williams, 1942). Problems of microbiological contamination are best solved by the use of a combination of volatile oils and oleoresins since they are free of most microorganisms. Steam sterilization destroys bacteria and molds spores in the case of essential oils, while volatile solvents act in a similar fashion in the case of oleoresins (Furia, 1980).

2.6. Antimicrobial Effect of Spices

2.6.1. Bacteria

Spices have been known to elaborate antibacterial activity for many years and have been a staple ingredient in folk medicine. Garlic, one of the first spices to be investigated (Cavallito and Bailey, 1944) was found to contain an active substance, allicin, which was markedly bacteriostatic even at low concentrations. Subrahmanyan et al.(1957 a,b) observed that of the thirteen spices they studied (cardamom, ginger, mustard, chillies, cinnamon, cloves, aniseed, ajowan, cumin, onion, garlic, asafetida and black pepper) garlic was the most potent spice, exhibiting high antibacterial action to E. coli, A. aerogenes, S. aureus and S. sonnei. The concentrations of garlic required to inhibit Lactobacillus casei and Streptococcus faecalis were much higher than those required to inhibit E. coli, S. sonnei and S. aureus. Whereas the growth of E. coli, S. sonnei and S. aureus was markedly inhibited by 10 mg/ml; the growth of S. faecalis and L. casei was inhibited only at 20 mg/ml. Only S. aureus and S. sonnei were inhibited at 5 mg/ml garlic. Subrahmanyan et al.(1958) also found that garlic

considerably reduced the number of coliform, anaerobes and total counts in the ceca of rats fed with either a stock diet or a poor South Indian diet containing a high percentage of redgram dhal. The results indicated that the incorporation of garlic in the diet at moderate levels is likely to shift the balance of the microflora in the intestines in favor of lactic organisms, which generally have a positive effect on the absorption of minerals present in the diet.

Oregano and thyme (0.5 %) were found to be highly toxic to Vibrio parahaemolyticus in growth media when tested by Beuchat (1976). Ginger, red pepper, mustard, mace, cinnamon and cloves were evaluated as to their effects on the growth and acid production by a starter culture containing Lactobacillus plantarum and Pediococcus cerevisiae in a liquid medium. At 4, 8 and 12 g/l levels, all spices except cloves stimulated acid production by the starter bacteria, but did not stimulate an increase in bacterial population. Clove was inhibitory to the starter bacteria at and above the 4 g/l level, but low concentrations (0.5-2.0 g/l) stimulated acid production. High concentrations of cinnamon (8 and 12 g/l) delayed acid production but bacterial counts were similar to those of the control (Zaika and Kissinger, 1979).

The effects of garlic, ginger, welsh onion and red pepper on the growth of L. casei were studied by Park et al.(1980). Welsh onion stimulated the growth of L. casei at concentrations of up to 4% of the broth (TYG broth at 37° C) while garlic (1-5 %) and ginger(0.1-0.5 %) strongly inhibited the growth of L. casei even at the lowest concentration used; red

pepper slightly inhibited growth when added at 4 %. When garlic and ginger were heated at 90°C for 10 min, their inhibitory activity was completely removed.

Alcoholic extract of spices were prepared and tested for inhibition of Clostridium botulinum in culture media (Huhtanen, 1980). The author found that mace and achiote were the most inhibitory of 33 spices studied; bay leaf, white and black pepper and nutmeg, clove, oregano, turmeric, thyme and paprika were also effective. Studies performed by Garg et al. (1980) on the effect of garlic and ginger on in vitro gas production by selected intestinal clostridia, indicated that an increase in the concentration of ginger and garlic resulted in a parallel decrease in gas production by all clostridal species; between 30-60% gas reduction was noted with the use of 1% of these spices. Most of the reported studies on the antimicrobial activity of spices and herbs have involved pathogenic microorganisms and only a few reports involving lactic acid bacteria are available. These reports suggest that lactic acid bacteria are relatively resistant to the toxic effects of spices or their essential oils. Zaika et al.(1983) found that increasing concentrations (0.5-8 g/l) of oregano, rosemary, sage and thyme progressively delayed growth and acid production by Lactobacillus plantarum and Pediococcus acidilactici in a liquid medium. After the bacteriostatic activity was overcome, all four herbs strongly stimulated acid production. The relative inhibitory effect of the herbs toward both microorganisms was oregano > rosemary = sage > thyme. L. plantarum was more resistant than P. acidilactici to

the toxic effect of the herbs. Organisms from cultures exhibiting delayed fermentation in the presence of sublethal concentrations of herb when subcultured into fresh media containing identical herb concentrations, initiated fermentation without delay, indicating some development of resistance. Moreover, bacteria which had acquired a resistance to one herb were also resistant to the other three herbs.

Garlic extracts (3, 5 and 10 %) inhibited the growth of B. cereus on nutrient agar plates by 31.3, 58.2 and 100%, respectively (Saleem and Al-Delaimy, 1982). The sensitivity of 22 Gram-negative and 24 Gram-positive bacteria to sage, rosemary and allspice in growth media was studied. At concentrations up to 2%. Gram-positive bacteria were more sensitive than Gram-negative bacteria ; sage had the highest antibacterial activity, followed closely by rosemary . A combination of sage and rosemary enhanced the antibacterial effect (Shelef et al., 1980).

2.6.2. Molds

Various herbs and spices have been demonstrated to be mycostatic acting alone or synergistically with other spices. Frazier (1967) reported that large concentrations of cinnamon permitted mold growth but inhibited asexual spore production. Garlic extract was shown to exert the greatest antimycotic effect and while onion extract showed the least effect on molds, it completely inhibited spore germination from the sclerotia of Sclerotium cepivorum (Moubasher et al., 1970). Bullerman (1974) reported that cinnamon added to bread inhibited both mold growth and aflatoxin formation. Eugenol (125 ppm) , cinnamic aldehyde

(150 ppm), cinnamon oil (200-250 ppm) and clove oil (200-250 ppm) inhibited mold growth and toxin production of Aspergillus parsiticus (Bullerman et al., 1977). Hitokoto et al.(1977) reported that cinnamon inhibited growth of A. parasiticus , A. versicolor, A. ochraceus and Fusarium solani. O-methoxy cinnamaldehye, a spice component isolated and purified from powdered cinnamon, was shown to inhibit growth and toxin production of several mycotoxin producing fungi. The compound completely inhibited the growth of A. parasiticus and A. flavus at 100 ppm and A. ochraceus and A. versicolor at 200 ppm. Production of aflatoxin B_1, ochratoxin A and sterigmatocystin was inhibited by over 90%, when o-methoxy cinnamaldehyde was employed at 6.2, 25 and 50 ppm , respectively (Morozumi, 1978). The inhibitory effects of 29 commercial powdered spices on the growth and toxin production of three species of toxigenic Aspergillus were performed by Hitokoto et al.(1980). Of the 29 samples tested, cloves (3.5 mg/ml), staranise seeds (45.5 mg/ml) and thyme (133.4 mg/ml) completely inhibited the fungal growth, whereas most of the other spices inhibited only toxin production (Hitokoto et al.,1980). The effects of some spices on growth and aflatoxin formation by A. flavus were investigated by Souhair and El-Shayeb (1980). Black pepper, cinnamon, peppermint, cumin and ginger inhibited aflatoxin formation without affecting mycelial growth. Cloves completely inhibited mycelia growth and aflatoxin formation at a concentration greater than 0.1%. Black pepper and ginger (10 % w/w) decreased aflatoxin formation by 100%. Higher concentrations of cinnamon, mint, cumin and ginger stimulated mycelial growth. The antifungal effects of 16 herbs

and spices were tested, in vitro, using potato dextrose agar against seven mycotoxin-producing molds by Azzouz and Bullerman (1982). Cloves, cinnamon, mustard, allspice, garlic and oregano when incorporated into the agar at 2%, completely inhibited growth of all seven mycotoxigenic molds for various times up to 21 days. The remaining compounds either caused little or no inhibition. Combinations of different levels of potassium sorbate and cloves showed an enhanced or possible synergistic inhibitory effect on the growth of all molds tested, indicating the possibility of using spices and commercial antifungal agents together in small amounts to obtain antimycotic activity (Azzouz and Bullerman, 1982).

2.6.3. Yeasts

Spices are effective inhibitors of yeast growth under some conditions. Yeasts can readily adapt themselves to low concentrations of spices and oils and in some instances appear to be stimulated by them. Water infusions of ground cinnamon, cloves and allspice prevented growth of yeasts in broth cultures in a 1:50 concentration, while infusions (1:50 and 1:1000) of bay leaves, mustard, paprika ,ginger, black and red pepper and nutmeg showed no effect. Cinnamon completely inhibited one species of yeast in 1:1000 dilution. One-tenth percent by weight of ground cinnamon, cloves and allspice in dextrose agar markedly inhibited but did not prevent growth of the test yeasts. Nutmeg, bay leaves, paprika, ginger and peppers prevented yeast growth but only in concentrations approaching 10% (Webb and Tanner, 1945).

Novak and Fisher (1964) studied the antimycotic activity of several fatty acid derivatives including parsley seed oil against pathogenic yeasts and molds. Most of the compounds tested exhibited good antimycotic activity. Eugenol and vanillin were examined for their in vitro antifungal activity against Candida albicans and Cryptococcus neoformans. Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) were determined for each compound against 31 strains of C. albicans and 33 strains of C. neoformans. With eugenol, the mean MIC for C. albicans and C. neoformans was 625 and 293 ppm , respectively, while the mean MFC was 1209 and 521 ppm, respectively. Using vanillin, the mean MIC for C. albicans and C. neoformans was 1250 and 738 ppm, respectively, while the mean MFC was 5000 and 1761 ppm, respectively (Boonchird and Flegel, 1982).

2.7. Antimicrobial Effect of Essential Oils

Since the essential oils from spices contain greater concentrations of antimicrobial compounds, they are more effective than their counterpart whole or ground spice. In many cases, ground or whole spices harbor few microorganisms. It is believed that the relatively high essential oil fraction in garlic, cloves, mustard and allspice is responsible for their low microbial numbers (Frazier and Westhoff, 1978). The bactericidal efficiency of several Indian essential oils was found to be generally high against Gram-negative but very low against Gram-positive bacteria; no activity was found against acid-fast organisms (Bose et al., 1949,1950).

Webb and Tanner (1945) tested some essential oils against eight different kinds of yeast and found that the oilsof cinnamon, cloves, and mustard were germicidal in concentrations of 1%. Oils of anise, allspice, wintergreen and onion were germicidal in concentrations above 5% ; bay and ginger oils were in most instances bacteriostatic in all concentrations. Oils of cinnamon, cloves, allspice, almond and bay were germicidal when tested by agar difussion. Monilia candida and Mycoderma lactis survived contact with anise and wintergreen oils which were lethal to other organisms.

Maruzzella et al.(1960) reported that more than 100 essential oils exerted antimicrobial activity towards cellulose degrading fungi. Phytopathogenic bacteria such as E. carotovora, C. michiganense, P. glycinea, and P. striafaciens were also effected by various essential oils in concentrations ranging from 1/1000 to 1/10000. Beuchat (1976) reported that 100 ppm essential oil of oregano , thyme or sassafras was bactericidal to V. parahaemolyticus. Eugenol extracted from clove and thymol from thyme caused complete inhibition of the growth of both Aspergillus flavus and A. versicolor at 0.4 mg/ml or less. Anethol (an extract from staranise seeds) at a concentration of 2 mg/ml inhibited the growth of all strains (Hitokoto et al.,1980). Eugenol from cloves and vanillin, a major alcoholic extract of the vanilla bean, have long been used in certain medical applications and in flavoring; both essential oils have been reported to possess in vitro inhibitory properties against filamentous fungi (Boonchird

and Flegel, 1982).

In combination with Ø.1 % acetic acid and 3 % NaCl, perillaldehyde, citral, citronellol, geranoil, perillalcohol or cuminaldehyde at a concentration of Ø.5 mM, completely suppressed the growth of all air-borne microorganisms and cultured fungi. Cinnamaldehyde was approximately twice as potent as these components in this respect. In order to completely suppress the growth of all microorganisms over a period of one month, more than Ø.2 % acetic acid or more than 2.5% NaCl was required in the medium. The essential oil components in concentrations equal to or more than 1 mM permitted growth within several days (Kurita and Koike, 1982).

The essential oils of oregano, rosemary, sage and thyme have been shown to have antibacterial and antifungal activity. The essential oils of oregano and thyme were generally found to be the most inhibitory and were considered among the most active of the essential oils from a large number of tested spices and herbs (Zaika et al., 1983).

2.8. Antimicrobial Effect of Volatile Constituents of Spices

The antimicrobial activity of volatile compounds from nutmeg and coriander seeds were investigated by Katayama and Nagai (1959 a,b). The terpene fraction, one of the most volatile groups separated from nutmeg, was refractionated and components such as alpha-pipene,alpha-terpinol, linalool and geraniol were identified. Antibacterial activity with these components was exhibited towards B. subtilis, S. enteritidis, S. aureus, P. aeroginosa, P. morganii and E. coli. The same authors also

reported that coriander contained volatile constituents which exhibited antimicrobial effects. Among a number or spice based terpenes examined by Katayama and Nagai (1960) , carvanol, thymol, isoborneol, vanillin and salicyl aldehyde, in dilutions of 1:2000 or more, showed antibacterial activity against B. subtilis, S. entertidis, S. aureus, P. morganii and P. aeroginosa.

Farbood et al. (1976) reported that a spice extract from rosemary was active against S. aureus in culture media, but that Gram- negative bacteria were unaffected. Cinnamon contains 0.5 to 1.0 % volatile oil which is composed mainly of cinnamaldehyde (65 to 75 %) , eugenol (4 to 10 %), cinnamic acid and o-methhoxycinnamaldehyde. The latter was shown to have a strong inhibitory effect on the growth of dermatophytes such as Microsporum, Tichophyton and Epidermophyton (Morozumi, 1978). Bullerman et al. (1977) studied the inhibition of fungal growth and aflatoxin production by cinnamaldehyde and eugenol, and concluded that these compounds were the major antifungal substance in cinnamon oil. Shelef et al.(1984) demonstrated that the antimicrobial activity of sage increased with increase in the volatile oil fraction. Essential oils alone had limited bacterial inhibitory effect in broth and no effect in food; salt and water in the food increased bacterial sensitivity, while fat and protein decreased it.

2.9. Pro-microbial Activity of Spices

Oils of black and white peppers were reported to contain growth stimulants for yeast (Webb and Tanner, 1945). Kissinger

and Zaika (1978) described the effects of Lebanon bologna spice mixture and its major components (black pepper, allspice and nutmeg) on acid production in a liquid medium by Lactobacillus plantarum and Pediococcus cerevisiae. The spice mixture stimulated the growth of L. plantarum and P.cerevisiae when each organism was cultured singly. A mixture of nine spices, used in Lebanon bologna formulation, enhanced lactic acid fermentation in these sausages; sterile or nonsterile spices enhanced the fermentation to the same extent and similar stimulatory effects of spices were observed when sausages were fermented by natural flora or by the addition of starter cultures (mixture of L. plantarum and P. cerevisiae).

At 4, 8 and 12 g/l levels ginger, red pepper, mustard, mace and cinnamon stimulated acid production by the starter culture containing L. plantarum and P. cerevisiae in liquid medium but did not stimulate increases in bacterial population. Clove was inhibitory to the starter bacteria at and above 4 g/l level, but low concentrations (Ø.5-2.Ø g/l) stimulated acid production (Zaika and Kissinger, 1979).

Nes and Skjelkvale (1982) investigated the effect of natural spices and oleoresins on three commercial starter cultures of L. plantarum. Natural spices stimulated growth and enhanced the fermentation of glucose in liquid media. The same effect was not shown using oleoresins.

2.1Ø. Antioxidant Activity of Spices

The antioxidant activity of many spices has been recognized for many years (Maveety, 1938). In general the antioxygenic properties of spices resides with the volatile oil

fraction; a spice may exhibit a varying capacity to act as an antioxidant depending on the nature and concentration of its volatile oil fractions. Clove is generally recognized as one of the main spices exhibiting a high antioxidant activity. Cloves contain essential oils ranging from 15 to 20%, of which 85-92% is eugenol. Eugenol was shown to have an antioxidant activity equivalent to 14% of BHT activity (Cort, 1974). The antioxidant properties of several spices in different types of food were studied by Chipault et al.(1956). Cloves were shown to be a powerful antioxidant in oil-in- water emulsions and in ground pork. Chipault et al.(1952) tested seventy-eight samples of thirty-six spices for their antioxidant activity. All the ground spices, their petroleum ether-soluble fractions and their alcohol soluble fractions of spice were shown to have antioxidant activity in lard; rosemary and sage showed exceptionally high activity.

Chipault et al. (1955) demonstrated the antioxidant effect of spices in two-phase aqueous-fat systems. Cloves gave high antioxidant indices in three different emulsions. In lard, rosemary and sage were the best antioxidants of all the spices studied. In pie crusts, rosemary was superior to sage. In addition to cassia, cinnamon, allspice, turmeric and cloves, it was found that ginger, mace, nutmeg and oregano were also better than sage; black and white peppers were nearly equal to sage in protecting emulsions.

A comparison of the rosemary antioxidant with a mixture of BHA, BHT propyl gallate and citric acid used in animal fat and

vegetable oils was demonstrated by Chang et al. (1977). The authors found that the rosemary antioxidant was as effective as the synthetic antioxidant mixture in fat and was superior in oils. The antioxidant activity of the purified antioxidant prepared from sage was comparable to that of rosemary.

Clove, thyme, pimento, ginger, laurel and mace also showed relatively strong antioxidant activities when used in lard oil (Kihara and Inoue, 1962). Clove, pimento and ginger demonstrated antioxidant action in cookies. Cinnamon, ginger and cloves showed antioxidant activity in potato and sweet potato chips. Labiatic acid and flavonol which is found in some spices, such as cloves and oregano were able to inhibit rancidity in lard at different temperatures (Herrmann, 1962). Antioxidant activity has also been reported in onions and garlic (Lewis and Watts, 1958) and in vanillin (Bikoff, 1951).

In general, spices are more effective in a simple fat-water emulsion system than in dry lard, and more effective in dry lard than in baked pie crust (Chipault et al.,1952,1955).

2.10.1. Factors Affecting Antioxidant Activity

In order for an antioxidant to exhibit good activity, it must be in close contact with the fat or lipid phase in the food. Mattil and Black (1947) related the water solubility of an antioxidant to its ability to stabilize fats. Their results indicated that in certain products where the fat containing the antioxidant is in contact with water, if the solubility of the antioxidant in water is appreciable, it may be extracted from the fat and rendered ineffective. The antioxidant effectiveness of

spices is partly a function of their fat or water solubility particularily in food systems having a fat phase in intimate contact with an aqueous phase. Other factors, such as the physical state of the fat-water system, the kind of fat, the pH of the aqueous medium or the presence of other substances (for example, pro-oxidants, antioxidants, synergists) affecting the stability of fats, may markedly influence the antioxidant effects of spices in different foods (Chipault et al., 1956).

2.10.2. Mechanism of Inhibition by Phenolic Antioxidants

Although many herbs and spices exhibit antioxidant activity, it is not known whether this property contributes to its antimicrobial activity. The mechanisms and/or sites of action of these spice based phenolic antioxidants are unknown, however, several closely related compounds have been investigated. BHA, BHT and TBHQ are food grade, non spice based phenolic antioxidants. The mechanism of inhibition by these antioxidants has been reported to affect the function and composition of the cell membrane, the synthesis of DNA, RNA, protein and lipid, and the function of mitochondria (Raccach, 1984). Studies have shown that these phenolic antioxidants react with the cellular membrane impairing both function and integrity. The interaction of phenolic antioxidants with the cell membrane may cause intracellullar leakage. S. aureus treated with BHA (>100 ppm) was shown to lose UV (260 nm) absorbing material suggesting leakage of nucleotides (Degre and Sylvestre, 1983). Protoplasts made from the same bacteria were lysed at a concentration of 25 ppm BHA. The same phenomenon was observed with Pseudomonas fluorescens and

P. fragi when exposed to 100-200 ppm BHA. UV (280 nm) absorbing material (suggesting intracellular leakage of proteinaceous material) and previously incorporated ^{14}C-labeled compounds was also observed (Davidson, and Branen,1980). BHA treated *P. fluorescens* experienced a higher amount of intracellular leakage than BHA treated *P. fragi* and this may reflect the relatively higher sensitivity of the former microorganism to the phenolic antioxidant. According to Davidson and Branen (1980), intracellular leakage was observed before death took place. Degre and Sylvestre (1983) related the bactericidal activity of BHA to leakage of intracellular material.

Phenolic antioxidants may also induce changes in cellular lipids. BHA (100 ppm) was shown to increase the amount of phosphatidyl glycerol and reduced the amount of phosphatidyl ethanolamine in *P. fragi* but not in *P. fluorescens* . BHA (50 ppm) also caused a decline in the ratio of unsaturated to saturated fatty acids in *P. fluorescens* and an increase of that ratio in *P. fragi* (Davidson and Branen, 1980). It was postulated that these changes in fatty acids, probably took place to maintain a proper "fluid" state in the cellular membrane.

Phenolic antioxidants may depress the phase transition of biological membranes. BHT lowered the melting temperature of the fatty acyl chains of vesicles made of saturated phopholipids by 20^{o}C (Singer and Wan, 1977). These authors suggested that it would be necessary for a compound to contain a hydroxyl group in order to interact with the cell membrane and be lipid soluble. BHA and BHT exhibited these properties since both have a hydroxyl

group and exhibit some degree of lipid solubility (40 - 50 %). BHA and BHT should then be equally effective as antimicrobials; BHA in most cases is, however, more effective than BHT (Kabara, 1980). Phenolic antioxidants were also found to inhibit the synthesis of nucleic acids. BHA and TBHQ inhibited the synthesis of DNA and RNA in Tetrahymena pyriformis (Surak, 1977; Surak et al., 1976a) while TBHQ (50 ppm) slightly decreased (10 - 15 %) the synthesis of staphyloccocal DNase (Raccach and Henningsen, 1982).

3. MATERIALS AND METHODS

3.1. Pastirma Ingredients

3.1.1. Meat and Casings

Fresh beef trimmings (lean and fat) were obtained from a commercial retail store. Natural beef casings (5 cm diameter) were obtained from Canada Packers, Winnipeg. These were pre-soaked for one hour and washed with water before use.

3.1.2. Spices and Sausage Formulas

The spices used in the Pastirma sausage formula are presented in Table 4 . In order to closely simulate the Iraqi Pastirma, these spices, which can vary in pungency depending upon their area of cultivation, were shipped directly from Iraq to the Food Science Department. Other major components of the spice formula included: salt (NaCl) added at levels of 1.5 to 3.0%, sodium nitrite (purity 98.0 %, BDH; added at 100 ppm or 0.1 g/Kg of meat) and garlic added at 1 % w/w. Garlic although classified as a spice did not comprise the dry spice mixture; it was added to the meat as fresh cloves. The standard meat formula presented in Table 5 was used throughout this study. The only components that were altered for special preparation were the spice mixture and salt content.

3.2. Preparation of Pastirma Sausage

The standard procedure used for the preparation of the sausage mixture was as follows: beef ,lean trimmings and fat were mixed in a container by hand for 5 min at room temperature. Garlic, spice mixture (1.5 and 2 % w/w) , salt and sodium nitrite were added and the combined material further mixed by hand for 5-

Table 4. Pastirma spice mixture formula

Ingredient	Rate of addition (%,w/w)
Black pepper	25
Cinnamon	17
Cumin	9
Nutmeg	10.5
Ginger	7
Rose petals	6
Ginger stem	6
Allspice	6
Pepper (hot)	6
Coriander	3
Cardamom	2.5
Cloves	2
Total	100

Table 5. Pastirma formula

Ingredient	Rate of addition
Beef lean	75%
Beef fat	25%
Garlic (fresh)	1%
Sodium nitrite	100 ppm
Spice mixture	1.5%
Salt (NaCl)	3%

10 minutes. This sausage mixture was then ground through a 1.5 cm plate three times using a Hobart Power Meat Grinder. The mixture was then stuffed into beef casings utilizing a F-Dick sausage stuffer.

3.3. Processing of Pastirma Sausage

The Pastirma sausage was pressed into a flat shape (2.5 x 30 cm) by holding it under a 2-5 Kg weight for one day and then hung in a controlled environment cabinet at 40% relative humidity and 15°C . The sausage was considered to be processed and ready for consumption after seven days.

3.4. Quality Tests

3.4.1. Moisture Determinations

Moisture analysis were conducted on the fermenting sausages on a daily basis using a forced draft (air drying) oven method described in A.O.A.C. (24.003). Duplicate analyses were conducted on each sample.

3.4.2. pH and Titratable Acidity

Ten grams of sausage were blended in 100 ml of distilled water for 60 seconds. The pH of the homogenate was then recorded, using a Western Digital pH/mv meter. The titratable acidity (TA) was determined by titrating 10 ml duplicate homogenized sample with 0.1 N NaOH to a pH endpoint of 8.1 (Fields, 1977). The milliliters of NaOH required to achieve this pH endpoint were

converted to % lactic acid using the following equation (Fields, 1977):

$$\% \text{ lactic acid} = \frac{\text{ml of base} \times \text{N of base} \times 0.09 \text{ (equivalent of lactic acid /ml)}}{10 \text{ g sample}}$$

3.4.3. Sensory Analysis

A basic problem with this study was the development of a Pastirma sausage formula that would be similar in sensory quality to sausage made in local Iraqi regions. Initially several formulas were prepared as described in sections 3.2.2., 3.2.3. and 3.2.4. The various sausage products were evaluated by a group of Iraqi families (at the Food Science Department). A preference test procedure was used to compare the sausage products. A simple ballot (Fig. 2) was used to record the data.

The sausage samples were sliced (5 mm thickness) pan fried at 300°F for 5-8 minutes then served. Panelists were asked to compare two coded samples at one sitting. Each sample was presented twice to every panelist.

3.5. Isolation and Identification of Pastirma Lactic Acid Bacteria

Microorganisms obtained from Pastirma sausage (3 % NaCl, 1.5% spice mixture) during the fourth day of storage at 15°C after initial processing, were subcultured on APT, MRS, Rogosa SL and tomato juice agar (Difco) at 30°C for 48-72 h. Approximately twenty isolates were examined microscopically and by Gram staining (Buchanan and Gibbons, 1974). Those isolates chosen for further study were differentiated biochemically using the

Fig. 2

QUESTIONNAIRE FOR

PAIRED COMPARISON TEST

NAME ----------DATE ----------

PRODUCT ----------

Test the two coded samples in the following order

317 225

Which of these two samples do you prefer?

Comments:

following tests:

A) Ammonia from arginine.

A series of tubes containing 5 ml sterile arginine broth (Niven et al., 1942) were subcultured with 2 loopfuls of each isolate previously grown in APT broth for 24 h at 30°C and incubated at 30°C for 48-72 h. Portions of culture broth from each tube were mixed with Nessler's solution (Gerhardt et al.,1981) on a spot plate at 20°C. A positive test for ammonia from arginine was indicated by the development of a yellow or orange color. A series of tubes containing arginine broth without arginine were similarly inoculated and were used as controls for color development with Nesslers'solution.

B) Carbohydrate fermentation.

Purple broth base (BBL) containing 0.5 % w/v yeast extract (Difco) was used as the basal medium. Carbohydrate fermentation was evaluated by the addition of 2 % w/v, of lactose and dextrose into test tubes containing 5.0 ml basal medium with inverted Durham vials. The sterilized tubes were inoculated with two loopfuls of a 24 h culture and incubated at 30°C for 48-72 h. Acid production was noted by a change in broth color from purple to yellow; gas production was evidenced by bubbles in the vial.

C) Growth temperature.

Isolates were streaked on APT and tomato juice agar and incubated at 15 and 45°C for 48-72 h. Growth was recorded for each temperature of incubation.

D) Catalase test.

Isolates were streaked on APT agar and incubated for 24 h at 30°C. The petri plates were then flooded with 0.1 % H_2O_2. Bubble

formation surrounding colonies indicated a positive catalase test.

Upon the completion of these tests two bacteria were chosen for future investigation with spices. The bacteria were designated as lactose fermenting (LF) and non lactose fermenting (NLF).

3.5.1. Culture Maintenance

The LF and NLF cultures were maintained on APT agar slants at 5-7°C after growth at 30°C for 72 h. Cultures were also maintained in APT broth at 5-7°C after similar incubation (stock inoculum).

3.5.2. Initial Inoculum

Inocula used in the study were prepared by the addition of 0.1 ml of the stock inoculum into 5.0 ml APT broth. Incubation at 30°C for 24 h resulted in 10^8- 10^9 CFU/ml.

3.6. Effect of Spices on LF and NLF Cultures

3.6.1. Effect of Spice Mixture

Spice mixture (Table 4) was added to a series of 300 ml flasks containing 150 ml APT broth such that the final concentration was 1, 2, 3, 4 and 5 %, w/v. Following sterilization, all flasks were inoculated with a 24-h LF culture and incubated at 15°C for 168 h. At each 24 h period portions of culture broth were removed and analyzed for colony forming units (CFU), pH and titratable acidity (TA). This procedure was repeated at 20 and 30°C. The NLF culture was similarly evaluated.

CFU were determined by serial dilution using distilled water and APT agar. All plates were incubated at 30°C for 48-72 h. The TA was determined as outlined in 3.4.2.

3.6.2. Effect of Individual Spices on pH Development

In order to evaluate each spice with respect to microbial inhibition, a minimum inhibitory concentration (MIC) for each spice for each culture was determined. The MIC for each spice represents the minimum concentration of spice necessary to inhibit culture pH development by 0.5 units within 24 h. Individual spices (0.0 to 5.0 %, w/v) were added to a series of 300 ml flasks containing 150 ml APT broth. Following sterilization all flasks were inoculated with a 24 h LF culture such that the final concentration was 10^4 - 10^5 CFU/ml. All inoculated flasks were incubated at 30°C without agitation for 24 h. The resultant pH for each culture broth was measured by diluting 10 ml of a culture broth with 50 ml of distilled water, using a digital pH meter. This procedure was also repeated using the NLF culture. Individual spices whose MIC values were below 5 % were then added in concentrations equal to their determined MIC values plus two or three lower concentrations,to a series of 300 ml flasks containing 150 ml APT broth (for the LF culture the following spices and concentrations were used : allspice- 1.0, 0.5, 0.1, 0.0; black pepper- 0.3, 0.2, 0.1, 0.0; clove- 0.5, 0.3, 0.1, 0.0; nutmeg- 0.3, 0.2, 0.1, 0.0; ginger- 1.0, 0.5, 0.0; rose petals- 0.8, 0.4, 0.0; while for the NLF culture the following spices and concentrations were used: allspice- 3.0, 2.0, 1.0, 0.0; black pepper- 0.5, 0.3, 0.1, 0.0; clove -0.3, 0.1, 0.05,

Ø.Ø; ginger- 2.5, 2.Ø, 1.Ø, Ø.Ø; nutmeg- Ø.5, Ø.3, Ø.1, Ø.Ø). The lower concentrations of spices were arbitrarly chosen based on preliminary studies. Following sterilization the flasks were inoculated with a 24h LF culture such that the final concentration was $1Ø^4$ - $1Ø^5$ CFU/ml. All cultures were incubated at 3Ø°C for 24h. Resultant pH development was monitored in all flasks at 12, 14, 16, 18, 2Ø and 24 h of incubation. The same procedure was repeated with the NLF culture.

3.7. Antioxidant Activity of Pastirma Spices

3.7.1. Hemoglobin Peroxidation Test

The antioxidant test system used was a modification of the Hamilton and Tappel (1963) method as outlined by Cort (1974). In this test procedure hemoglobin was used to peroxidize safflower oil in a water emulsion and oxygen removal from the emulsion was measured by an oxygen monitor.

3.7.2. Prparation of Antioxidant-Free Safflower Oil and Emulsion

A 25 %, v/v solution of safflower oil (obtained from a local retail outlet) prepared in petroleum ether (5ØØ ml oil in 15ØØ ml petroleum ether) was stripped of tocopherol (vitamin E) in a method outlined by Chipault et al. (1955). The method first consisted of filtering the safflower oil through an activated alumina column (5 x 22.5 cm) and then through a column containing silicic acid (5 x 1Ø cm). The filtrate was then passed through a Buchner funnel containing a 2.5 cm layer of charcoal placed between 1 cm layers of anhydrous sodium sulfate followed by two

successive filtrations in activated alumina. A 10 %, v/v fresh emulsion of stripped safflower oil in water was prepared daily by homogenization with 1 %, w/v polyoxyethylene stearate (Myrj, 53 ; ICI America Inc, Del) using a high speed mixer for 15 minutes.

3.7.3. Measurement of Spice and Spice Mixture Activity

Dry spice : Dry spice mixture system

The reaction mixture consisted of 3.0 ml of 10 % emulsiontowhichwas added 2 mg spice or spice mixture (0.667 mg/ml). The controls contained 3.0 ml of the 10% emulsion without the spice mixture. Spices or spice mixture added to the reaction mixture were not sterilized.

APT broth spice: APT broth spice mixture system

The reaction mixture consisted of 3.0 ml of a 10% emulsion to which was added 0.5 ml APT broth containing either 2 mg spice or spice mixture (0.57 mg/ml final concentration). The controls only contained 3.0 ml of 10% emulsion to which was added 0.5 ml sterilized APT broth. The APT broth-spice or APT broth-spice mixture was prepared from a stock. These systems were sterilized prior to their use; all sterilization was performed at 121^{o}C for 15 min.

Water spice mixture:

The reaction mixture consisted of 3.0 ml of a 10% emulsion to which was added 0.5 ml of water containing 2 mg spice (0.57 mg/ml final concentration). The water-spice mixture was sterilized prior to its addition to the emulsion. No controls were used.

A YSI (Yellow Springs Instrument) model 53 biological

oxygen monitor was used to measure oxygen dissipation. The reaction vessel consisted of a vial maintained at 30°C by a circulation bath. Mixing of the reaction mixture was achieved by a magnetic stirrer in the vial. The test procedure involved the addition of the emulsion with the spice or spice mixture into the reaction vessel. After insertion of the oxygen probe, into the reaction vial, the instrument was adjusted to read 100 % oxygen after oxygen sparging for 10 min. A hemoglobin solution (50 ul hemoglobin = 68 mg/100 ml, 10^{-5} M) was then injected into the reaction mixture and the time required to remove 50 % of the oxygen from the reaction mixture was determined. The rate of oxygen dissipation (min) was recorded on a strip chart recorder (1 cm/4 min). The antioxidant index for each spice was determined as the ratio of the time required to remove 50 % of the oxygen from the reaction mixture containing the spice to the time required to remove 50 % of the oxygen from the control (Chipault et al., 1952).

4. RESULTS AND DISCUSSION

4.1. Processing of Pastirma Sausage

Fermented meat sausages have been produced by various ethnic people for hundreds of years and many are now well recognized as regional specialities. An inherent characteristic of successful sausage production is the strict control of those microorganisms participating in the fermentation process as well as those spoilage microorganisms which would otherwise deteriorate the product and/ or cause food poisoning. Properly prepared fermented sausage will not support the growth of food poisoning or pathogenic bacteria because of their characteristic high acidity with pH of 4.0-4.6, their quantity of salt and curing agents (sodium nitrite) and their dryness.

The Pastirma sausage produced in Iraq has no set formulation other than certain common ingredients and spices. The processing of this sausage was then undertaken with these constraints in mind.

The effect of salt (1.5 to 3 %, w/w) on the pH development in Pastirma sausage curing at 40 % R. H. containing 2 % spice mixture is shown in Figure 3 . The addition of 2.5 and 3.0 % salt to Pastirma sausage resulted in both a decrease in the rate and extent of acid development during the first four days of curing. At eight days of curing, however, all sausages had similar pH values (4.8 - 4.9). Results of the taste panel (Table 6) indicated that sausages containing 2% spice mixture were unacceptable to the panalists while sausages containing 1.5% spice mixture were prefered. Sausages made with 1.5 % spice

Figure 3. Effect of salt on pH development in Pastirma sausage with 2% spice mixture.

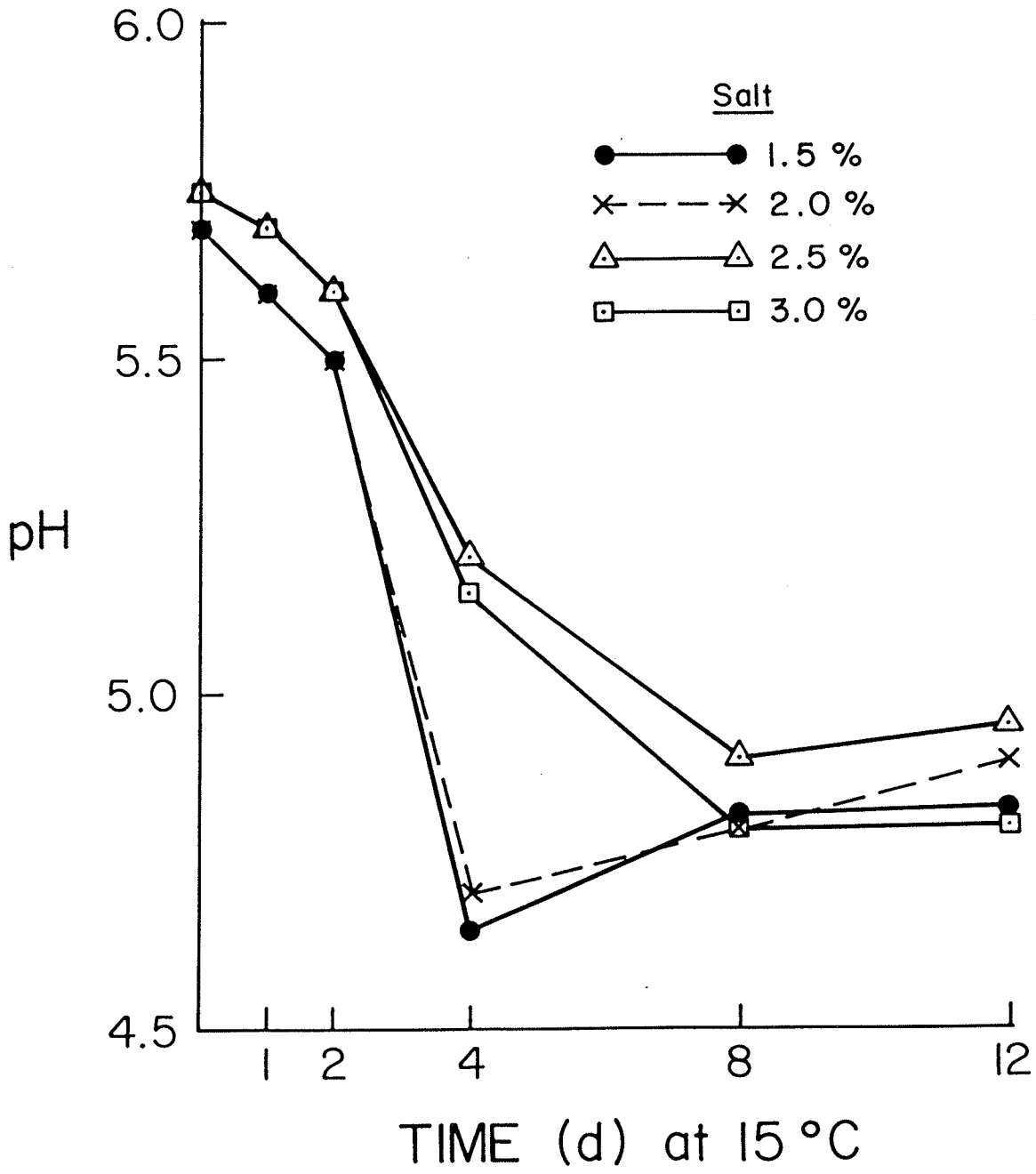


Table 6 . Sensory analysis of Pastirma sausage processed with two levels of spice mixture using the paired comparison procedure (Larmond, 1977).

Treatment Pastirma sausage	Number of acceptance	Probability level for acceptance	
		5%	1%
1.5% spice mixture and 3% salt	18*	17	18
2.0% spice mixture and 3% salt	4*	17	18

* Significant difference in preference between the two levels of spice mixture incorporated into Pastirma sausage.

mixture and 3% salt were then formulated (Table 7). Lactic acid concentration and pH remained constant during the first three days of incubation, then pH decreased to 4.9 after five days and lactic acid increased to 1.18% after seven days. Approximately 3.8 % moisture was lost during the curing process. The texture, color and flavor of this Pastirma sausage (Table 6 and 8) was acceptable to the taste panelists. Although microbiological changes during the fermentation of this product were not performed, it is believed that various lactic acid bacteria are involved and that their presence and or dominance during the fermentation process contributes in part to the taste and texture of the sausage. Most ethnic meat sausages such as salamis or cervelats are known to undergo a lactic acid fermentation. Two important types of organisms are required for the manufacture of fermented meat sausages . One type is nesessary for proper color development (NO_3 reduction); species of the genus Micrococcus are the primary organisms responsible. The other type of microorganisms involved are species of the genera Lactobacillus, Pediococcus and Leuconostoc which are responsible for lactic acid synthesis (Deibel et al.,1960).

The tangy flavor in these types of sausages is largely due to the presence of lactic acid which can represent an increase in total acidity of about 1 % indicated by a reduction in pH from levels of 6.2 - 6.6 to 4.2 - 5.0. The degree of acidity attained depends upon the extent of the fermentation, the particular blend of ingredients and the rate and extent of drying. The addition of salt (1.5 to 3.0 %) in these sausages did

Table 7. Titratable acid, pH and moisture analysis of Pastirma made with 1.5% spice mixture and 3% salt, processed at 15°C and 40% relative humidity.

Time (day)	pH	TA[a]	Lactic acid[b] (%)	Moisture (%)
0	5.60	10.35	0.93	55.1
1	5.60	10.55	0.95	54.3
2	5.60	10.90	0.98	55.3
3	5.55	10.20	0.92	53.6
5	4.90	13.20	1.19	52.4
7	4.90	13.10	1.18	51.3

[a] ml 0.1 N NaOH /10 ml sample.

[b] TA expressed as lactic acid.

Table 8. Sensory analysis of Pastirma sausage processed with two levels of salt using the paired comparison procedure (Larmond, 1977).

Treatment Pastirma	Number of acceptance	Probability level for acceptance	
		5%	1%
1.5% spice and 3.0% salt.	12*	17	18
1.5% spice and 2.5% salt.	10*	17	18

* No signicicant difference in preference between the two levels of salt incorporated inot Pastirma sausage.

not affect the overall taste of the product even though the rate of acid formation was higher with the 1.5 and 2 % salt containing formulations. Salt addition not only adds to flavor but as in many lactic acid fermentations can control the rate and extent of acid production, spoilage organisms and ultimately the final texture of the product (Deibel et al.,1960).

4.2. Isolation and Identification of Pastirma Lactic Acid Bacteria

Two microorganisms originally subcultured and selected from approximately twenty Pastirma sausage isolates were chosen for further investigation with spices. The characteristics of the two isolates designated as lactose fermenting (LF) and non lactose fermenting (NLF) are given in Table 9. Both organisms were shown to be Gram positive, nonmotile and asporogenous. The LF organism (24 h culture grown at 30°C in APT broth) when viewed under phase optics appeared as Gram positive coccobacilli appearing singly or in long chains, while the NLF organisms appeared as Gram positive short rods appearing singly or in short chains. Photomicrographs of these organisms are shown in Figures 4 and 5. These organisms are considered to be homofermentative, since they produce acid but not gas from glucose (Buchanan and Gibbons, 1974). They differ from one another in their ability to ferment lactose. The NLF organism when grown on APT agar at 30°C after 48 h appeared as small, smooth cream colored colonies with entire margins. Microscopic observations of the colony organisms indicated that they were Gram positive rods appearing singly or in long chains. The LF organism when grown on APT agar

Table 9. Some differentiating characteristics in LF and NLF cultures.

Culture	Dextrose		Lactose	Catalase	NH_3 from	Growth at	
	Acid	Gas	Acid		arginine	15°C	45°C
LF	+	-	+	-	-	+	-
NLF	+	-	-	-	-	+	-

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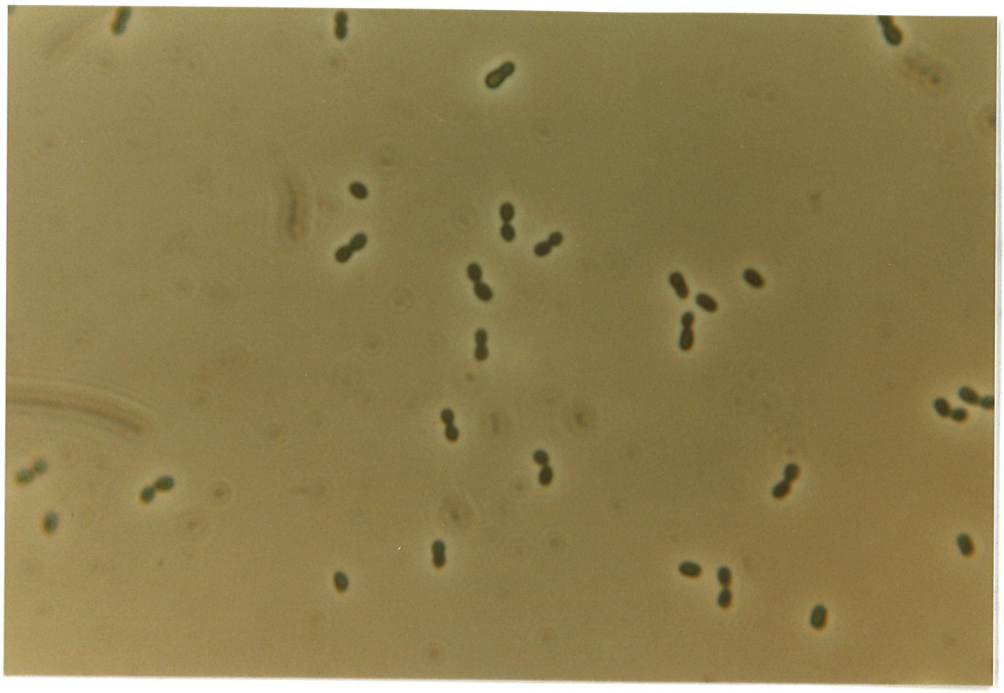


Figure 4. LF cells grown in APT agar, 72 h old.

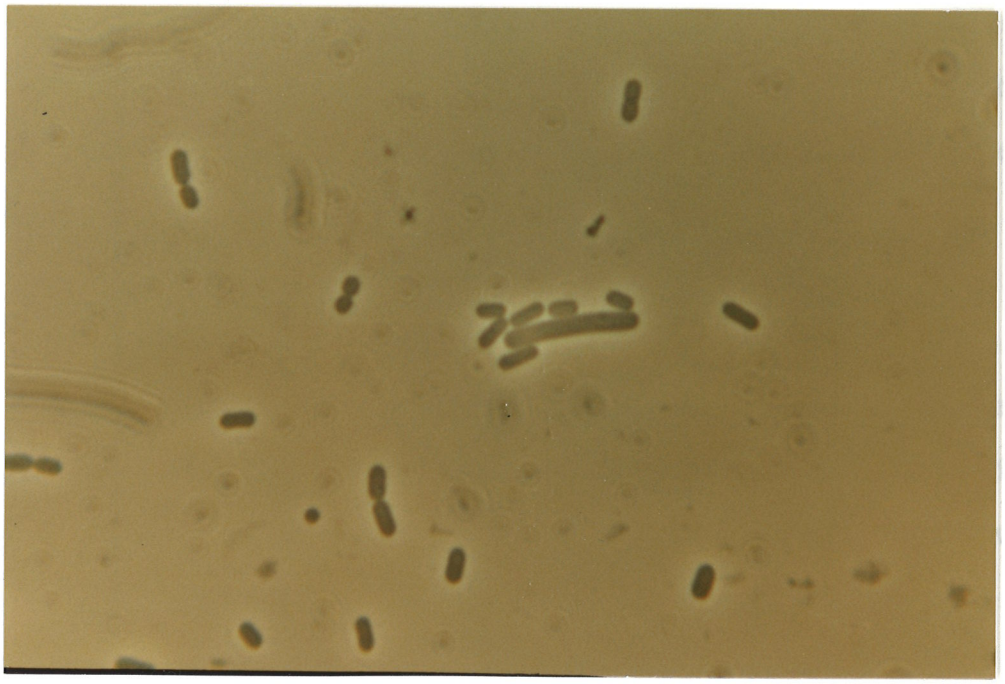


Figure 5. NLF cells grown in APT agar, 72 h old.

at 30°C after 48 h appeared as medium, smooth cream colored colonies. These organisms were Gram positive cocci appearing singly or in long chains. Based on the differentiating characteristics of these organisms it is believed they belong to the family Lactobacillaceae.

4.3. Effect of Spice Mixture

Time course growth studies for the LF and NLF cultures in APT broth containing various concentrations of spice mixture are shown in Figures 6 and 7 . The addition of spice mixture to the NLF culture broth gradually shifted (increased) the time for maximum growth from four days for the Ø, 1 and 2 % spice mixture containing broth to 4-6, 4-6 and 6 days for the 3, 4 and 5% spice mixture containing broth respectively. The latter three spice mixture concentrations severely repressed culture growth during the first two days of incubation with a concomitant suppression in pH and TA development.

All the NLF cultures regardless of spice mixture concentration reached a pH endpoint of 4.2. This value was reached within 4 days for Ø, 1 and 2 % spice mixtures containing broths; broths containing spice mixtures greater than 2 % did not reach this pH end point until 6 days of incubation. The overall effect on pH development by increasing spice mixture concentrations was an alteration in rate, but not in extent.

Increasing the spice mixture concentration also affected the development of TA in the NLF culture. Final values for TA gradually increased with increasing spice mixture concentration. Both the rate and extent of TA development were dependent on

Figure 6. Effect of spice mixture concentration (control, 1 and 2%) on growth, TA and pH development in the LF and NLF cultures at 15°C.

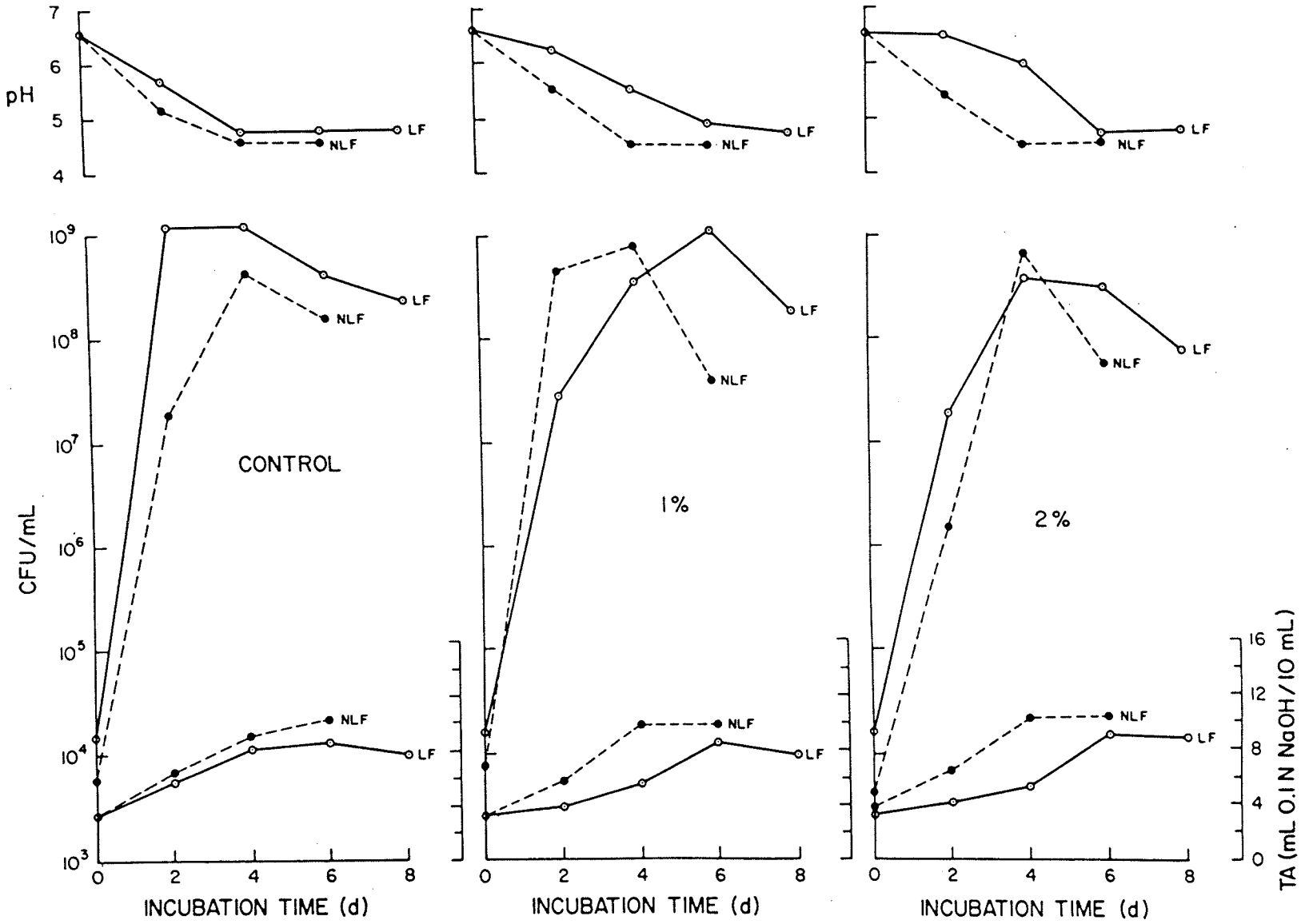
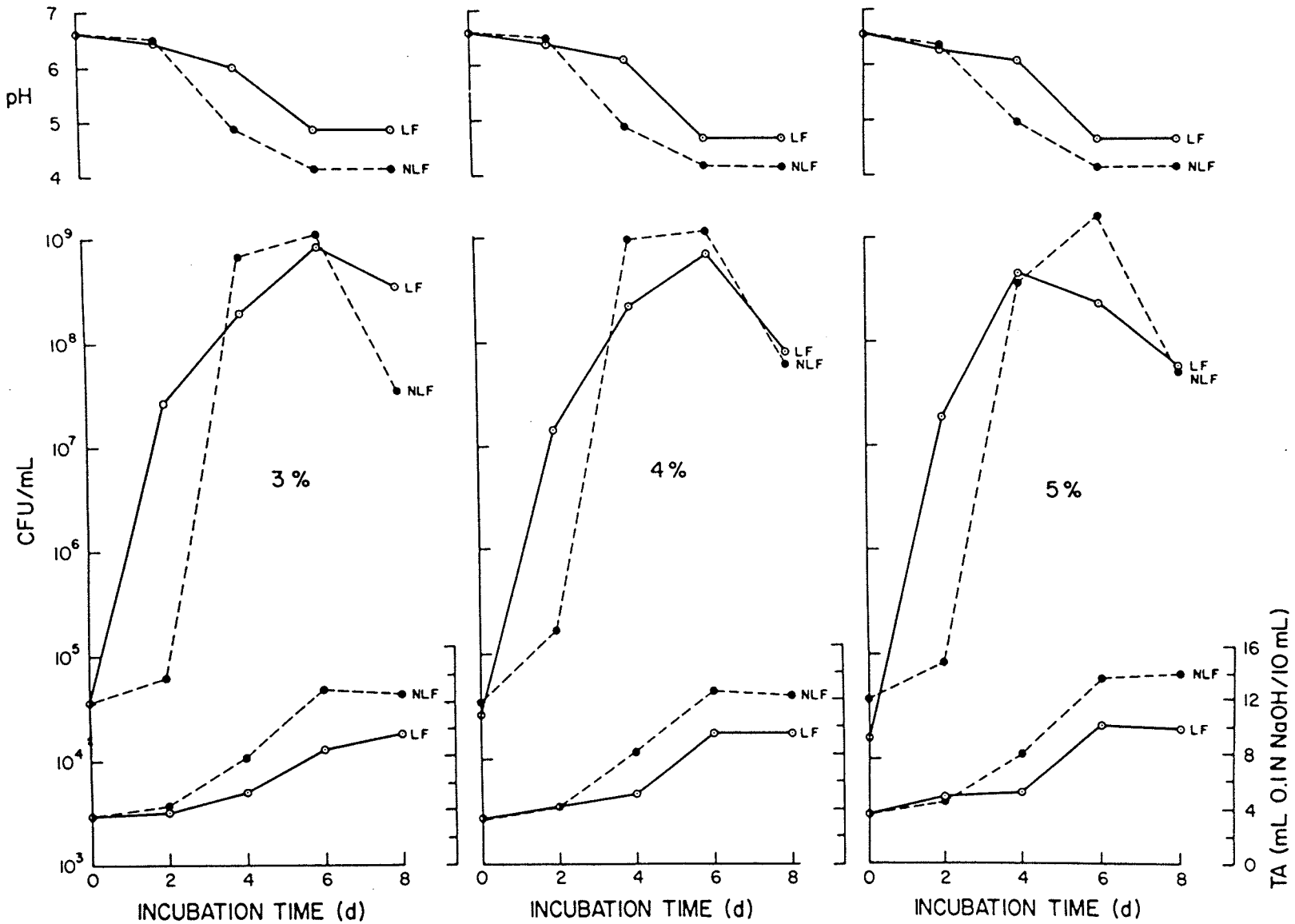

pH
7
6
5
4
LF
NLF
CFU/mL
10^9
10^8
10^7
10^6
10^5
10^4
10^3
CONTROL
1%
2%
0
2
4
6
8
INCUBATION TIME (d)
TA (mL 0.1 N NaOH/10 mL)
16
12
8
4
0

Figure 7. Effect of spice mixture concentration (3, 4 and 5%) on growth, TA and pH development in the LF and NLF cultures at 15°C.


pH
7
6
5
4
LF
NLF
CFU/mL
10^9
10^8
10^7
10^6
10^5
10^4
10^3
3 %
4 %
5 %
0
2
4
6
8
INCUBATION TIME (d)
TA (mL 0.1N NaOH/10 mL)
16
12
8
4
0

spice mixture concentration. The TA in culture broths containing 3, 4, and 5 % spice mixture reached total TA values of 12.6, 12.3 and 13.7 ml in six hours (1.134 , 1.107 and 1.233 % lactic acid) respectively; these values remained constant until the termination of incubation. Final TA values of 9.2, 9.9 and 10.1 ml of 0.1 N NaOH were reached by the 0, 1 and 2 % spice containing broths (0.823, 0.891 and 0.909 % lactic acid) respectively. Maximum TA were normally attained during stationary growth.

The time for maximum growth also increased for the LF culture as the spice mixture concentration was increased (Figures 6 and 7). Growth suppression noted with the NLF culture in 3, 4 and 5 % spice mixture containing broths was not evident with the LF culture. Although a final pH end point of ca. 4.8 was reached by all cultures, the rate of pH development was dependant on spice mixture concentration. The TA development for the LF culture reached a maximum value in 4-6 days with spice mixtures of 0, 1, and 2 %. The final TA values for these cultures was 7.9, 7.7, 8.9 ml of 0.1 N NaOH respectively (0.711, 0.693 and 0.80, % lactic acid). A comparision of the final TA and pH end point for the NLF culture grown in APT broth at 15, 20 and 30°C containing various spice mixture concentrations are given in Table 10 and 11. Increasing the temperature of incubation generally decreased the time for maximum TA development from 6 days at 15°C to 3 days at 30°C. The final TA, particularily at 15 and 30°C, increased with increase in spice mixture concentration. Increasing the temperature of incubation also decreased the time for maximum pH development. Final pH endpoints of 4.15 - 4.17 occurred at 6, 4

Table 10. The effect of temperature and spice mixture concentration on final TA development by the non lactose fermentor.

Spice mixture concentration (w/w,%)	Final TA (ml 0.1 N NaOH /10 ml)		
	15°C[a]	20°C[b]	30°C[c]
0	9.2	10.5	10.8
1	9.9	11.4	11.6
2	10.1	9.9	11.9
3	12.6	12.7	12.3
4	12.3	10.3	12.4
5	13.7	10.3	12.5

[a] after 6 days.

[b] after 4 days.

[c] after 3 days.

Table 11. The effect of temperature and spice mixture concentration on pH development by the non lactose fermentor.

Spice mixture concentration (w/w,%)	Final pH		
	15°C[a]	20°C[b]	30°C[c]
0	4.56	4.31	4.22
1	4.53	4.25	4.19
2	4.50	4.22	4.16
3	4.20	4.21	4.16
4	4.17	4.17	4.16
5	4.16	4.17	4.15

[a] after 6 days.

[b] after 4 days.

[c] after 2 days.

and 2 days at incubation temperatures of 15, 20, and 30°C. Regardless of the temperature of incubation, increasing spice mixture concentrations resulted in lower pH end point values. The effect of incubation temperatures (15, 20 and 30°C) and spice mixture concentration on the production of lactic acid and the final pH in LF culture media are given in Tables 12 and 13. Increasing the temperature of incubation for the LF culture also decreased the time necessary to achieve the final pH end point. This final pH endpoint (4.67 - 4.70) occurred at 4, 2 and 2 days of incubation at 15, 20 and 30°C respectively. Increasing the spice mixture concentration regardless of incubation also resulted in a lower pH end point value. The final TA for the LF cultures increased with increasing spice mixture concentration. Increasing the temperature of incubation, however, did not increase the final TA, although the time required to achieve maximum TA values decreased from 8 days to 3 days at incubation temperatures of 15 and 30°C respectively.

Although many of the spices employed in sausage formulation contain bacteriostatic substances, their relatively low concentrations in these products preclude them as major preservatives. Spices have been reported, however, to affect the preservation of these products indirectly by stimulating the fermentation organisms to produce more acid. Kissinger and Zaika (1978) reported that black pepper, allspice and nutmeg, the major spice components in Lebanon bologna, stimulated acid production by lactic acid bacteria. The authors also reported that the increased acid production was not attributed to increased microbial growth. Acid stimulation of starter cultures and

Table 12 . The effect of temperature and spice mixture concentration on final TA development by the lactose fermentor.

Spice mixture concentration (w/w,%)	Final TA (ml 0.1 N NaOH/10 ml)		
	15°C[a]	20°C[b]	30°C[c]
0	7.9	8.1	7.85
1	7.7	8.2	8.20
2	8.9	7.4	8.60
3	9.6	7.5	8.70
4	9.5	7.7	8.70
5	9.6	9.2	8.80

[a] after 8 days.

[b] after 4 days.

[c] after 3 days.

Table 13. The effect of temperature and spice mixture concentration on pH development by the lactose fermentor.

Spice mixture concentration (w/w, %)	Final pH		
	15°C[a]	20°C[b]	30°C[c]
0	4.81	4.81	4.81
1	4.74	4.77	4.78
2	4.72	4.69	4.72
3	4.66	4.69	4.71
4	4.69	4.68	4.70
5	4.67	4.69	4.70

[a] after 4 days.

[b] after 2 days.

[c] after 1 day.

natural microbial flora by spices has been also reported by Zaika et al. (1978) and Zaika and Kissinger (1984).

In this investigation the addition of increasing levels of spice mixture generally increased the time required to reach maximum growth, TA and pH development. Although the maximum growth obtained in each spice mixture broth was ca. Ø.5 log cycle larger or smaller than the controls, it is evident that growth, TA and pH development was delayed particularly during the first two days of incubation; maximum growth for the LF cultures in spice mixture broths at two days of incubation was ca. 1.5 to 1.75 log cycle lower than the control at this time period. The NLF cultures also showed delayed growth in spice mixture broths, particularly evident in the 3, 4 and 5 % spice mixture broths. The notable exception to this early delayed growth occurredwith the NLF organism in 1 % spice mixture broth where growth appeared to be stimulated. The delay in growth shown by both cultures, particularly during the first two days of incubation may have been due to cell acclimatization. Except for the NLF culture in the 1 % spice mixture, it would appear that the NLF organism was more sensitive to the spice mixtures than the LF culture. The increased TA observed with both cultures, particularly when grown in 3, 4 and 5 % spice mixture containing broths cannot be attributed to population growth. Zaika and Kissinger (1984) identified manganese as a factor in spices responsible for enhanced acid production by starter cultures. The authors reported that fermented sausages without spices but with added manganese and those with spices developed a similar level of acidity. The level of stimulation shown by manganese was media

dependent. Growth media containing relatively high levels of manganese (7.1×10^{-4}M Mn^{++} in APT and 2.2×10^{-4} M in MRS) which normally allowed optimum growth of the starter cultures, did not show significant acid enhancement (Zaika and Kissinger, 1984). Since both cultures were grown in APT broth in this study, acid stimulation due to manganese should not have been observed. It is possible that the cultures used in this study responded to higher levels of manganese or that certain spices and/ or their oil fractions were involved in reducing the activity of manganese. The total available concentration of manganese, contributed by the broth and the addition of spice, may have therefore been reduced. Raccach (1981) also reported that the addition of manganese to sausage accelerated fermentation. The author reported that manganese was effective in reducing or eliminating inhibition of the starter bacteria by BHA and BHT, two common phenolic antioxidants added to fermented meat products. Since many of the essential oils of the spices employed in this study also act as phenolic antioxidants, it is possible that their interaction with manganese may have effectively reduced the total concentration of available manganese. Although enhanced acid production was noted for each culture with increasing spice mixture concentration at a particular temperature, the effect of similar spice mixture concentrations on TA development at different temperatures was less obvious and in some cases decreased with increased temperature of incubation.

4.4. Effect of Individual Spices on pH Development

The minimum inhibitory spice concentrations which retarded pH development of the LF and NLF culture for 24 h are given in Table 14. Clove , nutmeg and black pepper were the most effective spices capable of retarding pH development for 24 h with the LF culture. Cardamom, cinnamon, coriander and cumin showed the poorest effect, requiring concentrations greater than 5 %, w/v to retard pH development. Clove, followed by nutmeg and black pepper were the most effective spices to suppress pH development with the NLF culture. Cardamom, cinnamon, coriander, cumin and rose petals each required concentrations greater than 5 %, w/v to suppress pH development. Based on the minimum concentration of each spice needed to retard pH development, it would appear that the NLF organism was the least sensitive to the individual spices employed. The development of pH in APT broth using the established MIC for each spice (Table 14) and the next two lowest concentrations for each respective spice by the LF and NLF cultures are shown in Figures 8 to 18. For the NLF culture, all controls showed the best rate and extent of pH development; increasing spice concentrations subsequently reduced the rate and extent of pH development. The rate of pH development by the LF culture was also greatest with the controls with some spices, however, the extent of pH development did not diminish with increasing spice concentration, and with some spices- clove, ginger and rose petals, the extent of pH development slightly increased with the first increasment in spice concentration. The inhibitory effects of various spices on bacterial growth is well documented, however, more recent reports have indicated that

Table 14. Minimum inhibitory concentration of spices by the LF and NLF cultures.

Spice	MIC[a]	
	LF[b]	NLF[c]
Allspice	1.0	3.0
Black pepper	0.3	0.5
Cardamom	>5.0	>5.0
Cinnamon	>5.0	>5.0
Clove	0.3	0.3
Coriander	>5.0	>5.0
Cumin	>5.0	>5.0
Ginger	1.0	2.5
Nutmeg	0.3	0.5
Rose petals	0.8	>5.0

[a] minimum inhibitory spice concentration (%,w/v) retarding pH development in growth media by 0.5 pH units for 24 h.

[b] LF: lactose fermentor.

[c] NLF: non lactose fermentor.

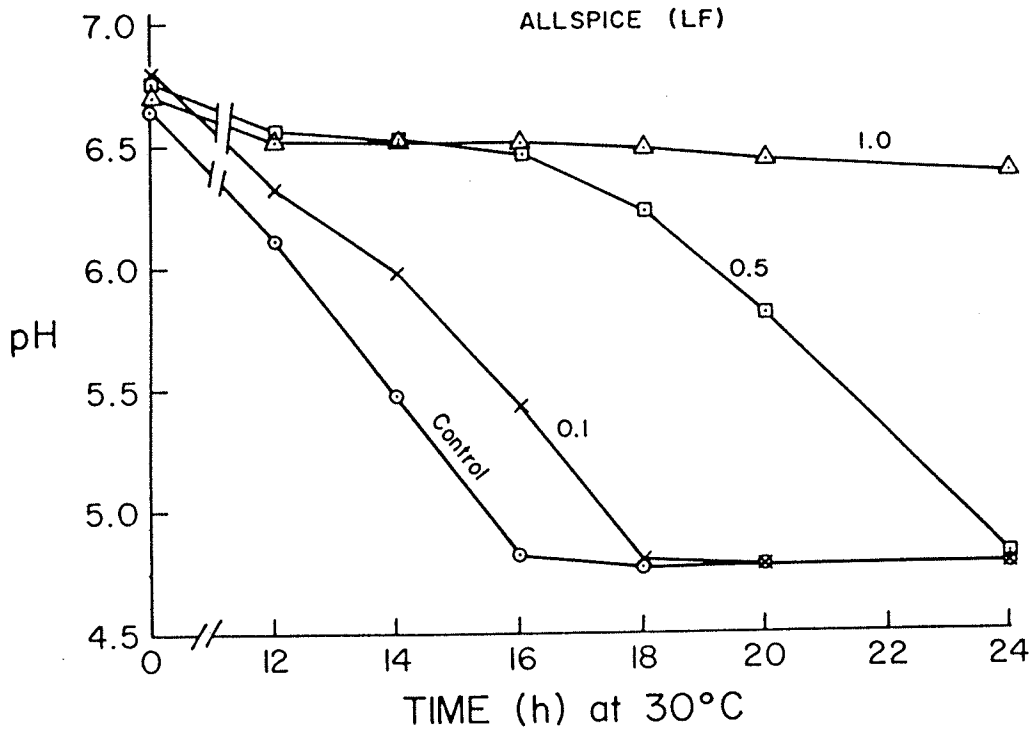


Figure 8. Effect of allspice on pH development by the LF culture.

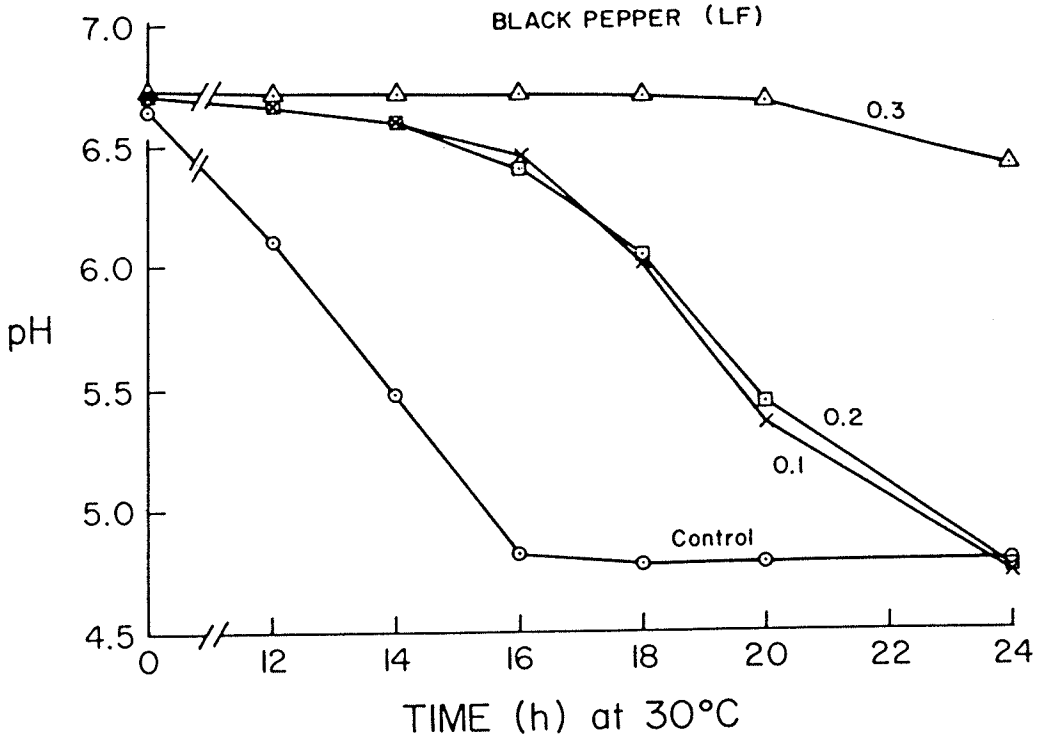


Figure 9. Effect of black pepper on pH development by the LF culture.

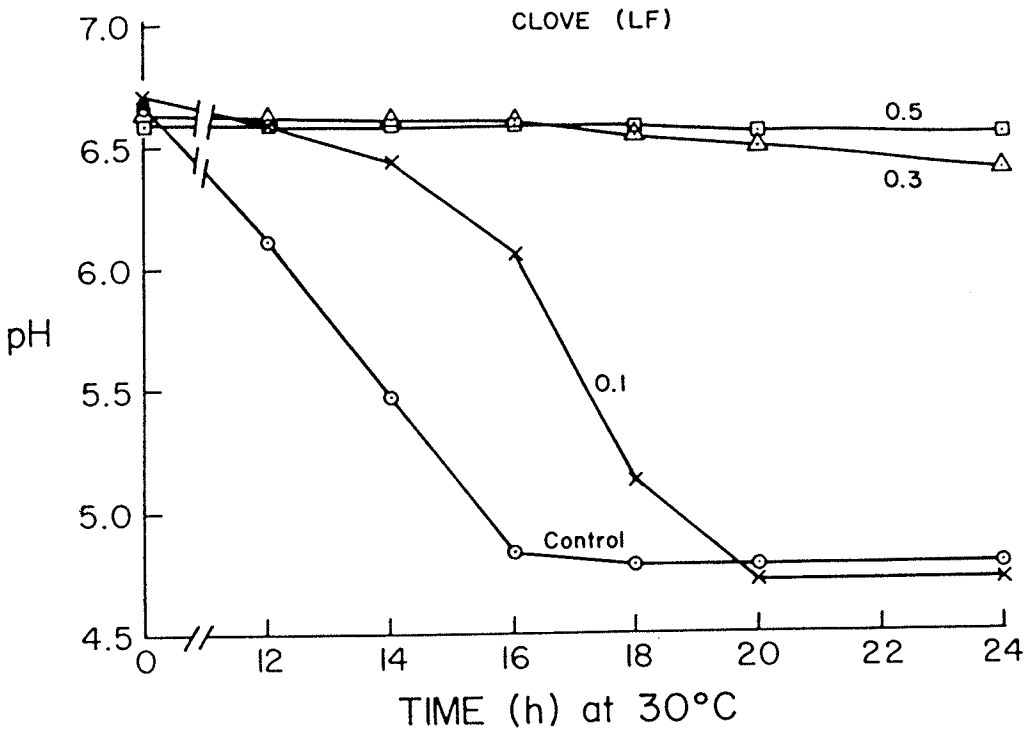


Figure 10. Effect of clove on pH development by the LF culture.

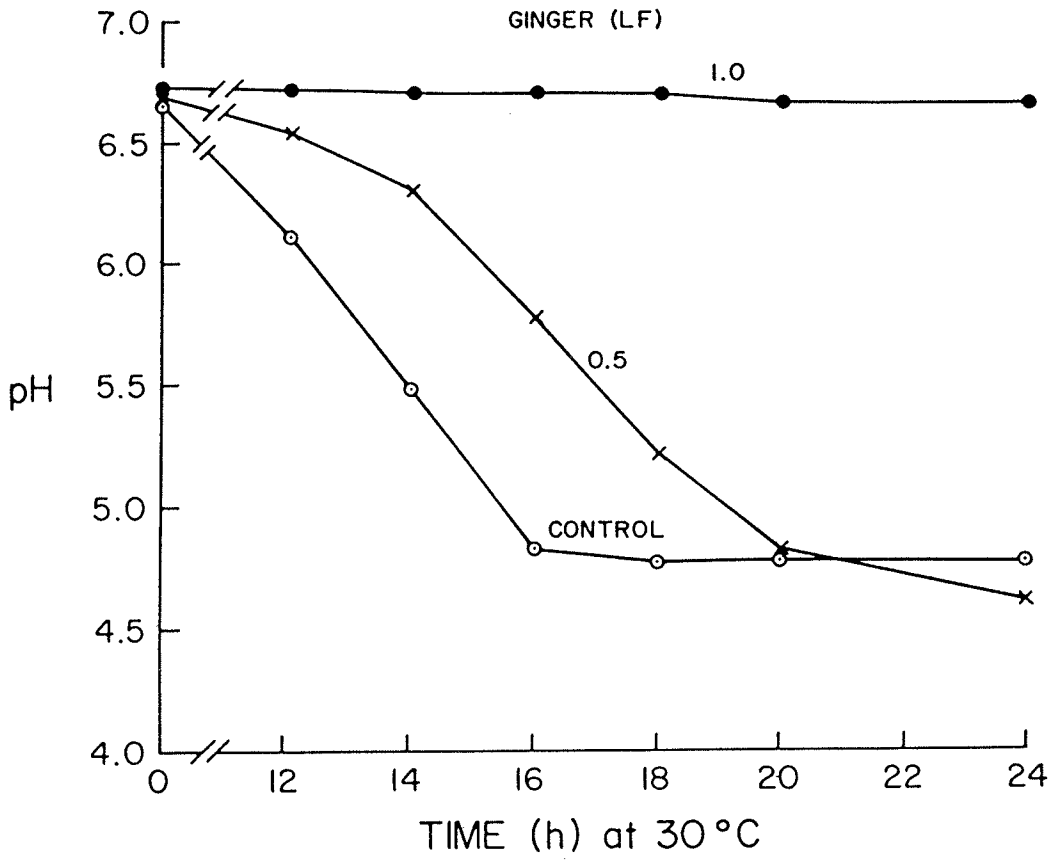


Figure 11. Effect of ginger on pH development by the LF culture.

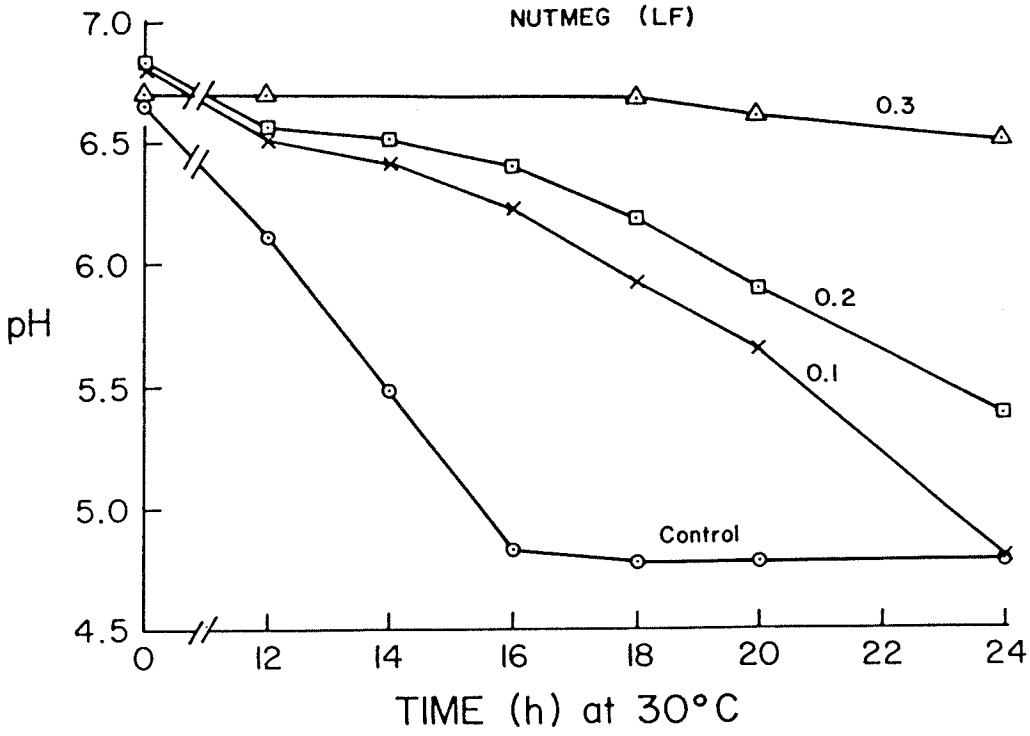


Figure 12. Effect of nutmeg on pH development by the LF culture.

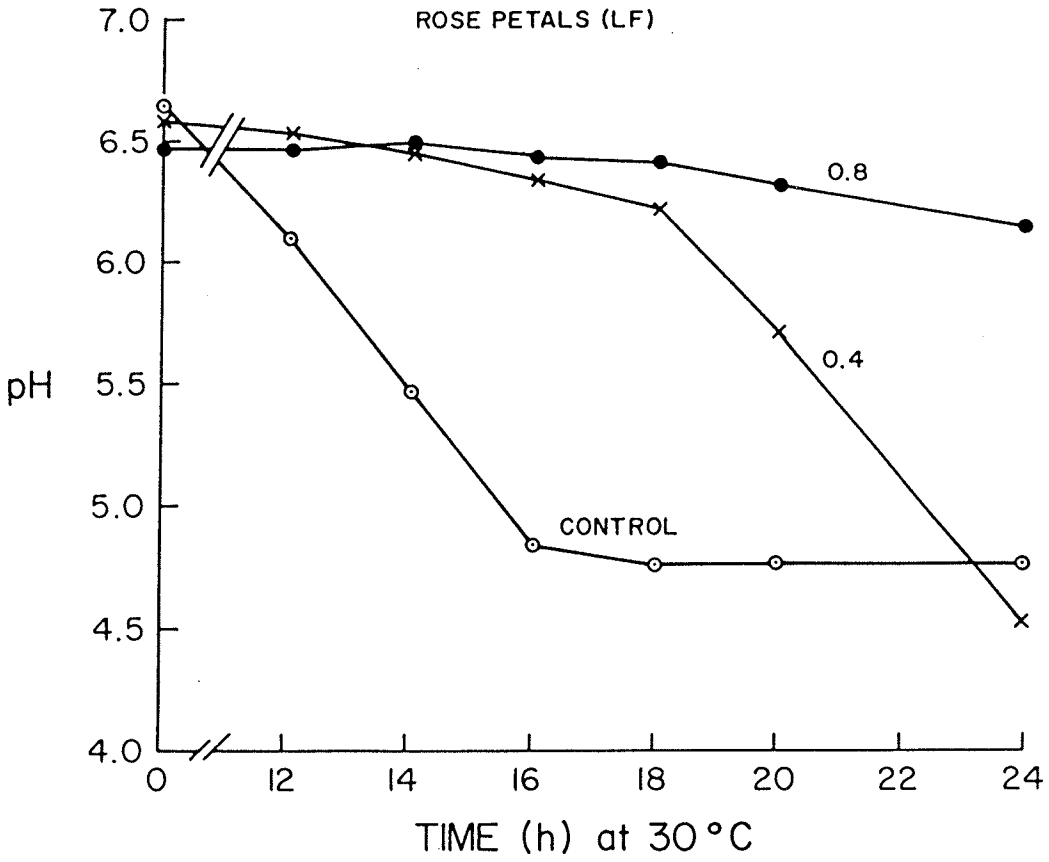


Figure 13. Effect of rose petals on pH development by the LF culture.

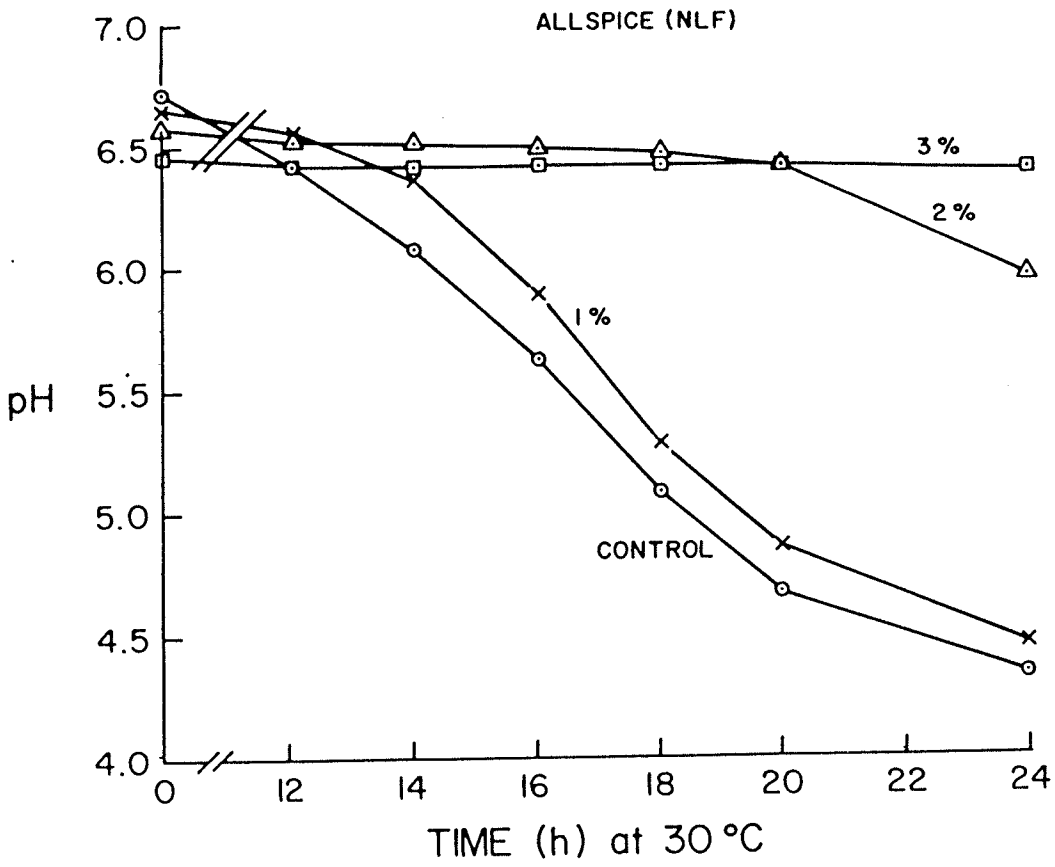


Figure 14. Effect of allspice on pH development by the NLF culture.

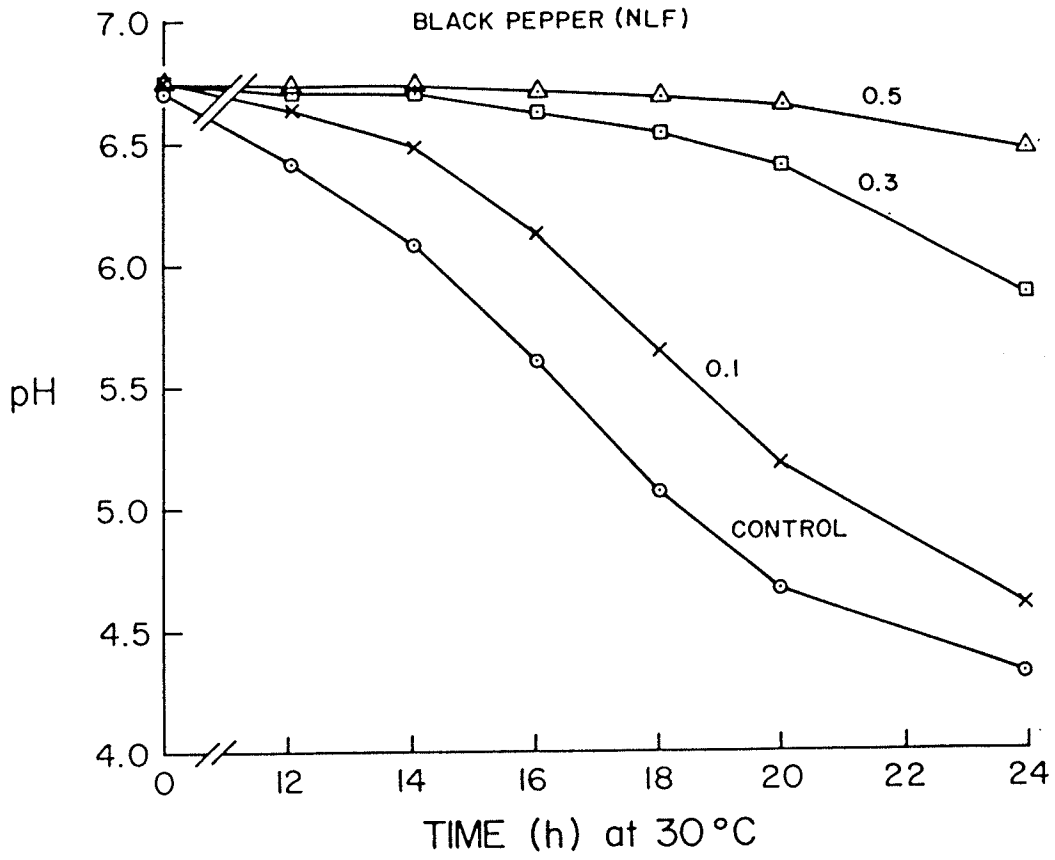


Figure 15. Effect of black pepper on pH development by the NLF culture.

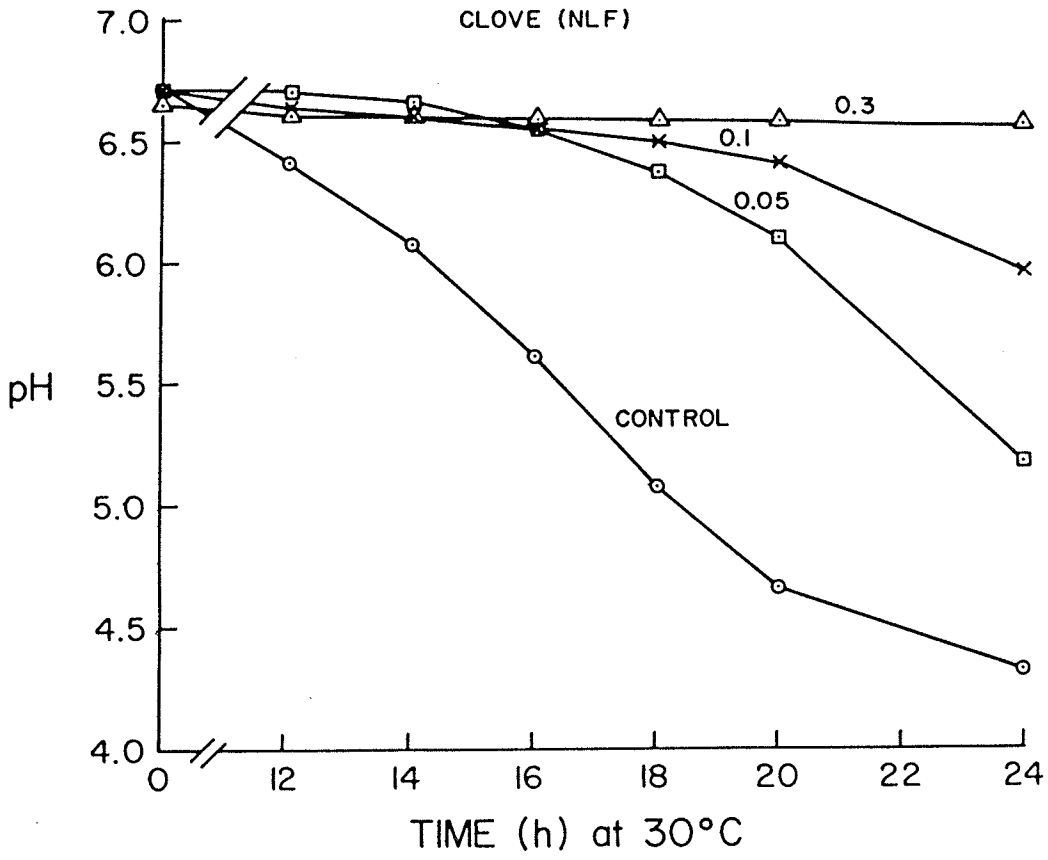


Figure 16. Effect of clove on pH development by the NLF culture.

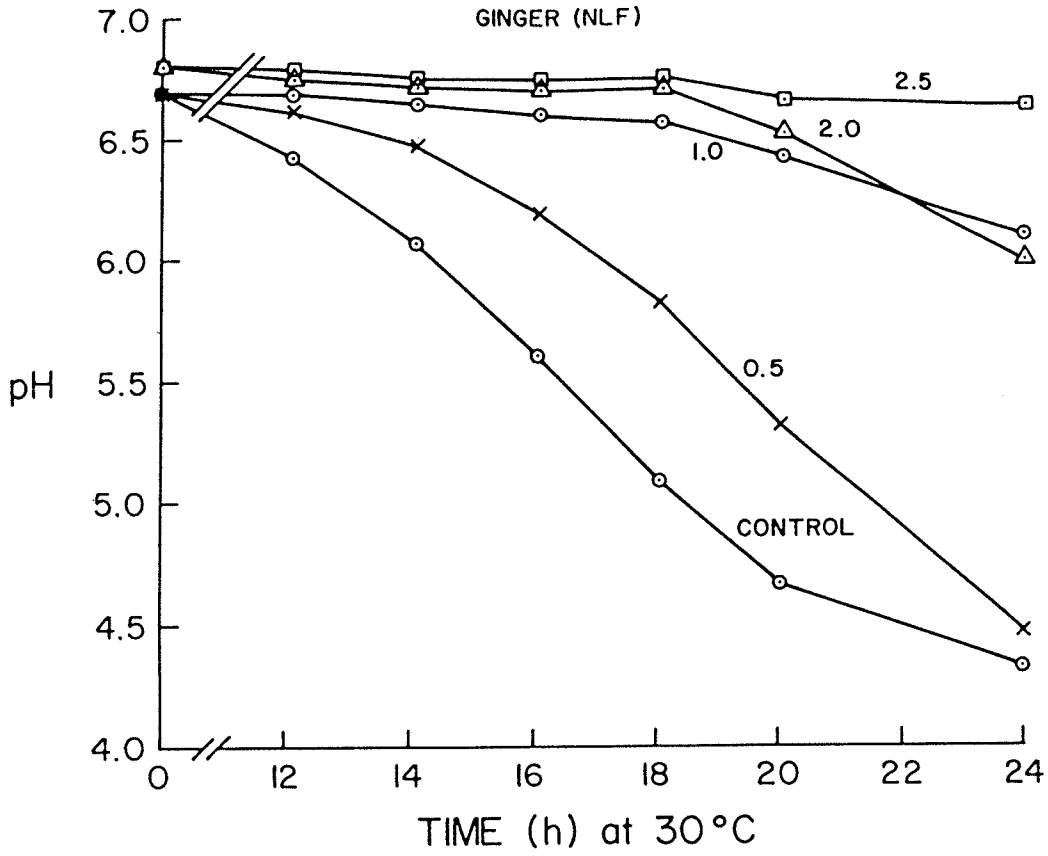


Figure 17. Effect of ginger on pH development by the NLF culture.

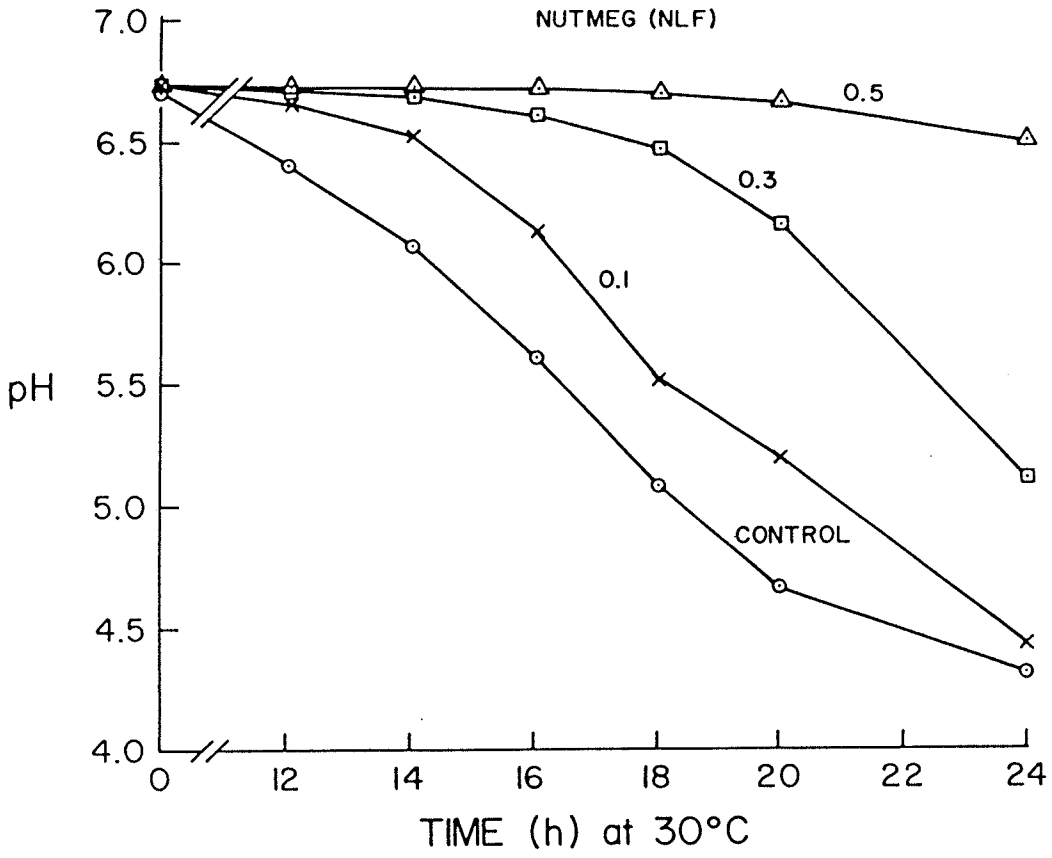


Figure 18. Effect of nutmeg on pH development by the NLF culture.

certain spices may actually stimulate growth and metabolism. Zaika et al. (1983) reported that increasing concentrations of oregano, rosemary, sage and thyme (Ø.5-8 g/L) progressively delayed growth and acid production by Lactobacillus plantarum and Pediococcus acidilacti in a liquid medium. The authors observed that after the bacteriostatic effect was overcome, all four herbs strongly stimulated acid production. Zaika and Kissinger (1984) subsequently reported that manganese was identified as a factor in these spices responsible for enhanced acid production by lactic starter cultures. The authors also reported that acid stimulation was dependant on the starter culture, all other conditions remaining constant. In addition, spice extracts consistently produced slightly higher acidity values when compared to broth cultures supplemented with similar concentrations of manganese, suggesting that spices may contain additional trace minerals or other components that may affect acid production.

4.5. Antioxidant Activity of Pastirma Spices

The antioxidant activities of the various spices and spice mixture used in the formulation of Pastirma sausage are given in Tables 15, 16 and 17. When the individual spices were added to the emulsion in a dry system, clove exhibited the highest antioxidant index (14.Ø) followed by rose petals (11.45) allspice (6.Ø) cinnamon (3.75) and nutmeg (3.Ø5). All remaining spice components exhibited an antioxidant index below 2.Ø. Cardamom showed the lowest index of Ø.7 . When the individual spices were added to the emulsion in APT broth, clove also

Table 15 . Antioxidant index of spices used in Pastirma sausage: APT broth system.

Spice	Time for 50% oxygen removal (min)			
	Trial 1	Trial 2	Mean	Antioxidant[a] index
Control	6.4	6.8	6.6	1.00
Allspice	14.4	14.4	14.4	2.18
Black pepper	17.6	18.4	18.0	2.73
Cardamom	6.0	6.0	6.0	0.91
Cinnamon	8.0	8.0	8.0	1.21
Clove	70.0	69.6	69.8	10.58
Coriander	4.0	4.0	4.0	0.61
Cumin	7.2	6.8	7.0	1.06
Ginger	17.2	17.6	17.4	2.64
Nutmeg	17.2	17.2	17.2	2.60
Rose petals	18.8	17.2	18.0	2.73

[a] Antioxidant index was calculated as the ratio of the stability of each spice to the stability of the control.

Table 16. Antioxidant index of spices used in Pastirma sausage : dry system.

Spice	Time for 50% oxygen removal (min)			
	Trial 1	Trial 2	Mean	Antioxidant[a] index
Control	4.0	4.0	4.0	1.00
Allspice	24.0	24.0	24.0	6.00
Black pepper	6.4	6.4	6.4	1.80
Cardamom	2.8	2.8	2.8	0.70
Cinnamon	14.8	15.2	15.0	3.75
Clove	55.2	56.8	56.0	14.00
Coriander	3.6	4.0	3.8	0.95
Cumin	7.6	8.0	7.8	1.95
Ginger	6.4	6.4	6.4	1.80
Nutmeg	12.4	12.0	12.2	3.05
Rose petals	45.6	46.0	45.8	11.45

[a] Antioxidant index was calculated as the ratio of the stability of each spice to the stability of the control.

Table 17 . Antioxidant index of spice mixture used in Pastirma sausage.

Sample	Time for 50% oxygen removal (min)			
	Trial 1	Trial 2	Mean	Antioxidant[a] index
Control[b]	4.0	4.0	4.0	1.00
Dry	19.2	17.2	18.2	4.55
Control[c]	6.4	6.8	6.6	1.00
APT	5.6	6.0	5.8	0.87
Water[d]	18.4	17.6	18.0	NA[e]

[a] Antioxidant index was calculated as the ratio of the stability of spice mixture to the stability of the control.

[b] Control containing only 3.0 ml emulsion.

[c] Control containing 3.0 emulsion + 0.5 ml APT broth

[d] 3.0 ml emulsion + 0.5 ml water containing 2 mg spice mixture.

[e] NA - not performed

showed the highest antioxidant index (10.58) followed by rose petals and black pepper (2.73) and ginger (2.64). Coriander showed the lowest index of 0.61. Antioxidant indices were generally higher in emulsions containing dry spice than in APT broth, partially due to differences in final spice concentration. In both cases, however, clove followed by rose petals yielded the highest antioxidant index. Cort (1974) also reported that clove showed the highest antioxidant index. A value of 14.0 was reported for clove added either in a dry form or as an ethanol extract. The controls used to calculate the antioxidant index of spices in APT broth showed a mean value of 6.6 minutes as compared to 4.0 minutes for controls without APT broth. The increased time (2.6 min) required to remove 50 % oxygen from the emulsions with APT broth would indicate that some constituents per se exhibited antioxidant or stabilizing properties. APT broth, for example, contains a surfactant, sorbitan monooleate (20 mg/ 100 ml) which could aid the stabilization of the emulsion mixture enhancing its antioxidation. The differences in the antioxidant activities of the same spice in both systems may be the result of several factors including:

1. The difference in the final concentration of the oil emulsion in the reaction mixture (the dry spice system employed 3.0 ml emulsion while the APT broth-spice system employed 3.0 ml emulsion to which was added 0.5 ml APT broth). This diluting effect on the emulsion, per se, may have altered the rate of oxygen removal by altering the oil emulsion/water partition coefficient. Depending on the nature of the antioxidant oil fraction (hydrophobic, hydrophilic or amphiphatic) in each

spice, the antioxidant activity would increase with increased spice oil fraction solubilization in the lipid phase and vice versa (Mattil and Black, 1947).

2. The difference in the final concentration of the spice (Ø.667 mg/ml for the dry spice system; Ø.571 mg/ml for the broth system).
3. Steam sterilization of the APT broth-spice system would be expected to reduce the volatile oil content of some spices (especially those with a relatively high level such as clove) by heat degradation and/ or volatilization (Adejumo, 1985).

Since many of the oil fractions/ oleoresins of spices acting as antioxidants, are phenolic in nature, it is possible that some binding between the spice phenolic components and the APT broth components took place. Several reports have shown that proteins and polypeptides effectively reduced the antioxidant activity of BHA, a phenolic antioxidant. Binding of the antioxidants by these compounds most probably through hydrophobic interaction was reported by Cornell et al. (1971) and Cornell (1979). Rico-Munoz and Davidson (1983) also reported that phenolic antioxidants in the presence of a protein, casein, exhibited less antimicrobial activity.

The spice mixture when added to the emulsion ina dry form yielded an antioxidant index of 4.55. In APT broth, the spice mixture yielded an antioxidant index of Ø.87. Since the majority of the individual spices also exhibited a reduced antioxidant index in the APT broth system, the reduced antioxidant index of the whole spice mixture was expected. The

extent in the reduction of antioxidant index and the time required to remove 50% oxygen was not expected. The reduction in the antioxidant index for the spice system in broth, aside from the possible reasons given earlier may be the result of an additional concentration effect. For example, clove the spice which exhibited the greatest antioxidant index in the APT broth system (Tables 15 and 16) was used in a concentration of 0.571 mg/ml. In the spice mixture-APT broth system , the concentration of clove used was only 2% of 0.571 mg spice mixture/ml or 0.013 mg/ml. Since the addition of the water spice mixture to the emulsion, which was also steam sterilized, gave an average value of 18 min for 50% oxygen removal, it would appear that difference in time taken for 50% oxygen removal between the dry or water spice mixture and the APT broth spice mixture might be attributed to binding of the antioxidant spice oils with APT broth constituents. The addition of spice into APT broth and its subsequent sterilization, however, more aptly reflects the true antioxidant activity of each spice since all treatments in this investigation used this type of preparation.

5. CONCLUSION

Pastirma appears to be a fermented meat sausage involving, at least in part, several homolactic bacteria. The individual spices making up the spice mixture, and the spice mixture per se were shown to influence the growth, pH develpment and titratable acidity of both cultures in vitro. Although the extent of growth and pH development did not differ appreciably from the control treatment, the rate of growth and pH development was shown to be dependant on the spice/spice mixture concentration. The final titratable acidity values increased with increased spice mixture concentration although the rate of acid production particularily during the initial curing time, was slower than observed in the control treatments. Since this product is neither cooked, smoked nor refrigerated, the development of proper acidity is of critical important in preserving this food product. Increasing the temperature of fermentation accelerated acidity development in vitro, however, these sausages are normally cured at average ambient temperatures of 15^{o}C and as such would not normally have this temperature benefit. Slow acid production due to spices/spice mixtures could potentially allow food spoilage-poisioning organisms to grow. In vivo studies are necessary to establish these potential risks since other factors such as salt and moisture also influence the fermentation. The majority the spices used in the sausage formulation exhibited antioxidant activity. Aside from cloves, a correlation between antimicrobial activity and antioxidant activity could not be established. In part, these two activities

are dependant upon the substrate medium and their own chemical behavior and concentration. Compounds normally acting as good antioxidants must be in immediate contact with the lipid food phase. This reduces the effectiveness of these compounds as antioxidants.

6. REFERENCES

Acton, J. C., and R. L. Dick. 1976. Composition of some commercial dry sausages. J. Food Sci. 41: 971.

Acton, J. C., J. G. Williams, and M. G. Johnson. 1972. Effect of fermentation temperature on changes in meat properties and flavor of summer sausage. J. Milk Food Technol. 35: 264.

Adejumo, A. A. 1985. Msc Thesis. Eugenol: A spice-based phenolic antioxidant and its effect on the metabolism of the lactic acid bacteria. Univ. of Manitoba. Unpublished data.

Anonymous. 1972. Improved culture cuts sausage production time. Food Processing 33 :32.

Anonymous. 1984. Irradiation. FDA proposes expanded use for food preservation. Food Proccessing 45 :23.

A.O.A.C. 1975. Official methods of analysis. 12th ed. Association of official analytical chemist, Washington, D. C.

Ayres, J. C., M. J. Orvin and S. E. William. 1980. Microbiology of foods. W.H.Freeman and Company. San Francisco.

Azzouz, M. A. and L. B. Bullerman. 1982. Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J. Food Prot. 45: 1298.

Baxter, R. and W. H. Holzapfel. 1982. A microbial investigation of selected spices, herbs and additives in South Africa. J. Food Sci. 47: 570.

Beuchat, L. R. 1976. Sensitivity of Vibrio haemolyticus to spices and organic acids. J. Food Sci. 41: 899.

Beuchat, L. R., C. F. A. Lin, and J. A. Carpenter. 1980. Growth of Bacillus cereus in media containing plant seed materials and ingredients used in chinese cookery. J. Appl. Bacteriol. 48 :397.

Bikoff, E. M. 1951. Phenolic antioxidants for carotene. J. Am. Oil Chem. Soc. 28: 65.

Boonchird, C. and T. W. Flegel. 1982. In vitro antifungal activity of eugenol and vanillin against Candida albicans and Cryptoccus neoformans. Can. J. Microbiol. 28: 1235.

Bose, S. M., C. N. Bhima Rao, and V. Subrahmanyan. 1949. Influence of organic matter on bacterial efficiency of Indian essential oils. J. Sci. Ind. Res. 8: 157.

Bose, S. M., C. N. Bhima Rao, and V. Subrahmanyan. 1950. Some factors affecting the bacterial property of Lemongrass oil emulsions. J. Sci. Ind. Res., sect. 13 9B 12.

Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. The Williams and Wilkins Company/ Baltimore.

Bullerman, L. B. 1974. Inhibition of aflatoxin production by cinnnamon. J. Food Sci. 39: 1163.

Bullerman. L. B., F. Y. Lieu, and S. A. Seier. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. 42: 1107.

Cavallito, C. J., and J. H. Bailey. 1944. Allicin, the antibacterial principle of Allium sativum. 1. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66: 1950 .

Chang, S. S., B. O. Matijaservic, O. Hsieh, and C. Huang. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42: 1105.

Chipault, J. R., G. R. Mizuna, J. M. Hawkins, and W. O. Lundberg. 1952. The antioxidant properties of natural spices. Food Res. 17: 46.

Chipault, J. R., G. R. Mizuna, and W. O. Lundberg. 1955. Antioxidant properties of spices in oil-in-water emulsions. Food Res. 20: 443.

Chipault, J. R., G. R. Mozuna, J. M. Hawkins, and W. O. Lundberg. 1956. The antioxident properties of spices in food. Food Technol. 10: 209.

Christensen, C. M., H. A. Fanse, G. H. Nelson, F. Bates, and C. J. Mirocha. 1967. Microflora of black and red pepper. Appl. Microbiol. 15: 622.

Christensen, C. M. 1972. Pure spices - how pure? American Society for Microbiology (ASM) News 38 :165.

Cornell, D. G., E.D. DeVilbiss, and M. J. Pallansch. 1971. Binding of antioxidants by milk proteins. J. Dairy Sci. 54: 634-637.

Cornell, D. G. 1979. Distribution of some antioxidants in dairy products. J. Dairy Sci. 62: 861.

Cort, W. M. 1974. Hemoglobin peroxidation test, Screens antioxidants. Food Technol. 28: 60.

Davidson, P. M., and A. L. Branen. 1980. Antimicrobial mechanisms of butylated hydroxyanisole against two Pseudomonas species. J. Food Sci. 45: 1607.

Degre', R., and M. Sylvestre. 1983. Effect of butylated hydroxyanisole on the cytoplasmic membrane of Staphylococcus aureus wood 46. J. Food Prot. 46:206.

Deibel, R. H., and C. F. Niven, Jr. 1957. Pediococcus cerevisiae: A starter culture for summer sausage. Bacteriol. Proc., 5: 14.

Deibel, R. H., G. D. Wilson, and C. F. Jr. Niven. 1960. Microbiology of meat curing. IV- A lyophilized Pediococcus cerevisiae starter culture for fermented sausage. Appl. Microbiol. 9: 239.

Deibel, R. H., C. F. Niven Jr, and G. D. Wilson. 1961. Microbiology of meat curing. III- Some microbiological and related technological aspects in the manufacture of fermented sausages. Appl. Microbiol. 9: 156.

Everson, C. W., W. E. Donner, and P. A. Hammes. 1970. Improved starter culture for semi-dry sausage. Food Technol. 24: 42.

Everson, C. W. 1971. Use of starter cultures in sausage products. In Carpenter J. A. and D. D. Brown (ed.) proceedings 13th annual meat science institute. National independent meat Packers Association and University of Georgia, Athens, Georgia. P. 11-19.

Farbood, M. I., J. H. MacNeil, and K. Ostovar. 1976. Effect of rosemary spice extraction on growth of microorganisms in meat. J. Milk Food Technol. 39 :675.

Fields, M. L. 1977. Laboratory manual in food preservation. The AVI publishing company, INC. Westport, Connecticut.

Flannigan, B., and S. C. Hui. 1976. The occurance of aflatoxin producing strains of Aspergillus flavus in the moulds flora of ground spices. J. Appl. Bacteriol. 41: 411.

Frazier, W. C. 1967. Food microbiology. (2ed.) McGraw-Hill Book Company ,New York.

Frazier, W. C., and D. C. Westhoff. 1978. Food microbiology.(3ed) McGraw Hill, New York.

Furia, T. E. 1980. CRC Handbook of Food Additives. Vol. 11. The CRC press, Inc. Boca Raton, Florida.

Garg, S. K., A. C. Banerjea, J. Verma, and M. J. Abraham. 1980. Effect of various treatments of pulses on in vitro gas production by selected intestinal clostridia. J. Food Sci. 45: 1601.

Gerhardt, P., R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips. 1981. ASM. Manual of methods for general microbiology.

Goepfert, J. M., W. M. Spira, and H. U. Kim. 1972. Bacillus cereus: Food poisoning organism. A review. J. Milk Food Technol. 35: 213.

Guergue. J., and C. Ramirez. 1977. Incidence of aflatoxin-potential contamination in Spanish sausages. Ann. Nutr. Aliment., 31: 485.

Hamilton, J. W., and A. L. Tappel. 1963. Evaluation of antioxidant by a rapid polarographic method. J. Am. Oil Chem. Soc. 40: 52.

Heath, H. B. 1972. Herbs and spices for food manufacture. Proc. Int. Conf. spices, Trop. Prod. Inst. London, 1972, p.39.

Herrmann, K. 1962. Antioxidative action of seasonings and the labiatic acid (Labiat tannin material) contained therein. Z. Lebens.- Unters.- Forsch. 116: 224.

Hitokoto, H., S. Morozumi, T. Wauke, S. Sakei, and H. Kurata. 1977. Mycotoxin production of fungi on commercial foods. In Rodricks J. V. et al.(ed.), Human and animal health, p. 479-487. Pathotox Publisher, Inc., Chicago.

Hitikoto, H., S. Morozumi, T. Wauke, S. Sakei, and H. Kurata. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. Environ. Microbiol. 39:818.

Huhtanen, C. N. 1980. Inhibition of Clostridium botulinum by spice extracts and aliphatic alcohols. J. Food Prot. 43: 195.

International Oranganization for Standardization . 1968. "Spices and Condiments" Nomenclature. ISO Recommendation 1st list. ISO, Budapest.

International Organization for Standardization. 1972. Spices and Condiments, Nomenclature Finalized Draft Proposal, "TC-34/SC-7 (Secritriat-107) 252E, 2nd list. ISO Budapest.

Jay, J. M. 1978. Modern food microbiology. D. Van Nostrand Company , (2ed.) New York.

Jenesen, L. B. 1954. Microbiology of meats. The Garrard Press, (3ed) Champaign, Ill.

Jenesen, L. B., and L. S. Paddock. 1940. Sausage treatment. U.S. patent 2,225,783.

Kabara, J. J. 1980. GRAS antimicrobial agents for cosmetic products. J. Cosmet. Chem. 31: 1.

Karlson, K. E., and M. F. Gunderson. 1965. Microbiology of dehydrated soups. Food Technol. 19: 86.

Katayama, T., and I. Nagai. 1959 (a). Significance of the volatile components of spices from the food preservative viewpoint. II. Antibacterial activity of volatile components of coriander seed. J. Fac. Fish. Anim. Husb., Hiroshima Univ. 2, 349.

Katayama, T., and I. Nagai. 1959 (b). Significance of the volatile components of spices from the food preservative viewpoint. III. Antibacterial activity of volatile components of nutmeg. J. Fac. Fish. Anim. Husb., Hiroshima Univ. 2, 355.

Katayama, T., and I. Nagai. 1960. Chemical significance of the volatile components of spices from the food preservative viewpoint. IV. Structure and antibacterial activity of some terpenes. Nippen Suisan Gakkaishi 26: 29.

Kihara, Y., and T. Inoue. 1962. Antioxidant activity of spice powders in foods. Nippon Shokuhin Kogyo Gakkai-Ski, 9: 290.

Kissinger, J. C., and L. L. Zaika. 1978. Effect of major spices in Lebanon bologna on acid production by starter culture organisms. J. Food Prot. 41: 429.

Klement, J. T., R. G. Cassens, and O. R. Fennema. 1973. The association of protein solubility with physical properties in a fermented sausage. J. Food Sci. 38: 1128.

Kramlich, W. E., A. M. Pearson, and F. W. Tauber. 1973. Process meats. The AVI publishing company, Inc. Westport, Connecticut.

Kramlich, W. E. 1978. Sausage products. P. 484-512. In the science of meat and meat products. Price, J. F. and B. S. Schweigert (ed.). Food and Nutrition Press, Inc. Westport, Connecticut 06880.

Krishnaswamy, M. A., J. D. Patel, and Parthasarthy. 1971. Enumeration of microorganisms in spices and spice mixtures. J. Food Sci. Technol. 8: 191.

Kurita, N., and S. Koike. 1982. Synergistic antimicrobial effect of acetic acid, sodium chloride and essential oil components. Agric. Biol. Chem. 46: 1655.

Larmond, E. 1977. Laboratory methods for sensory evaluation of

food. Publication 1637. Communications Branch (Research Branch), Agriculture Canada, Ottawa.

Lewis, E. J., and B. M. Watts. 1958. Antioxidants and copper binding properties of onions. Food Res. 23: 274.

Maruzzella, J. C., D. Scrandis, J. B. Scrandis, and G. Grabon. 1960. Action of odoriferous organic chemical and essential oils on wood destroying fungi. Plant Dis. Rep. 44: 789.

Masson, A. 1978. The hygenic quality of spices. Lebensmittelunters. Hyg. 69: 544.

Mattil, K. F., and H. C. Black. 1947. A laboratory method for determining the ability of antioxidants to stabilize fats in baked goods. J. Am. Oil Chem. Soc. 24: 325.

Maveety, D. J. 1938. Inhibiting rancidity in oils. U.S. Patent 2,124,706.

Morozumi, S. 1978. Isolation, purification, and antibiotic activity of o-methoxycinnameldehyde from cinnamon. Appl. Environ. Microbiol. 36: 577.

Moubasher, A. H., M. A. Elnaghy, and E. Megala. 1970. Fungi isolated from sclerotia of Sclerotium cepivorum and from soil and their effects upon the pathogen. Plant Soil 33: 305.

Nes, I. F., and R. Skjelkvale. 1982. Effect of natural spices and oleoresins on Lactobacillus plantarum in the fermentation of dry sausage. J. Food Sci. 47: 1618.

Niven, C. F.,Jr., K. L. Smiley, and J. M. Sherman. 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. 43: 651.

Novak, A. F., and M. J. Fisher. 1964. Antimycotic activity of some fatty acid derivatives. J. Am. Oil Chem. Soc. 41: 503.

Parada, J. L., J. Chirife, and R. C. Magrini. 1982. Effect of BHA, BHT and potassium sorbate on growth of Staphylococcus aureus in model system and process cheese. J. Food Prot. 45:1108.

Parker, S. Y., Y. H. Yun, and H. U. Kim. 1980. Studies on the effects of several spices on the growth of Lactobacillus caseiYIT9018. Korean J. Anim. Sci. 22: 301.

Pederson, C. S. 1971. Microbiology of food fermentation. Avi Publishing Company, Inc. Westport, Connecticut.

Powers, E. M., R. Lawyer, and Y. Masuoka. 1975. Microbiology of processed spices. J. Milk Food Technol. 38: 683.

Powers, E. M., T. G. Latt, and T. Brown. 1976. Incidence and levels of Bacillus cereus in procssed spices. J. Milk Food Technol. 39: 668.

Pruthi, J. S. 1980. Spices and Condiments: Chemistry, Microbiology, Technology. Academic Press. New York.

Raccach, M. 1980. Low temperature lactic acid bacteria for meat fermentation. Proc. 26th European Meeting Meat Res. Workers, vol.2: 335.

Raccach, M. 1981. Method and bacterial composition for fermenting meats. U.S. Patent 4,303,679.

Raccach, M., and E. C. Henningsen. 1982. Antibacterial effect of tertiary butylhydroquinone against two genera of Gram positive cocci. J. Food Sci. 47: 106.

Raccach, M. 1984. The antimicrobial activity of phenolic antioxidants in foods: A reveiw. J. Food Safety 6 :141.

Rico-munoz, E., and P. M. Davidson. 1983. Effect of corn oil and casein on the antimicrobial activity of phenolic antioxidants. J. Food Sci. 48: 1284.

Rosengarten, F. Jr. 1969. The book of spices. Livingston Publishing Company. Wynnewood, Pennsylvania.

Saleem, Z. M., and K. S. Al-Delaimy. 1982. Inhibition of Bacillus cereus by garlic extract. J. Food Prot. 45: 1007.

Shelef, L. A., O. A. Naglik, and D. W. Bogen. 1980. Sensitivity of some common food borne bacteria to the spices sage, rosemary, and allspice. J. Food Sci. 45: 1042.

Shelef, L. A., E. K. Jyothi, and M. A. Bulgarelli. 1984. Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. J. Food Sci. 49: 737.

Singer, M., and J. Wan. 1977. Interaction of butylated hydroxytoluene and membrane fluidity. Biochem. Pharmacol. 26: 2259.

Smith, J. L., S. A. Palumbo. 1973. Microbiology of Lebanon bologna. Appl. Microbiol. 26 :489.

Souhair, M. S., and N. M. A. El-Shayeb. 1980. Inhibition of aflatoxin by some spices. Z. Lebensm.-Unter.-Forsch. 7:344.

Subrahmanyan, V., K. Krishnamurthy, V. Sreenivasamurthy, and M. Swaminathan. 1957a. Effect of garlic in the diet on the intestinal microflora of rats. J. Sci. Ind. Res. Sect. C 16: 173.

Subrahmanyan, V., K. Krishnamurthy, V. Sreenivasamurthy, and M. Swaminathan. 1957b. Studies on the antibacterial activity of spices. J. Sci. Ind. Res. Sect. C 16: 240.

Subrahmanyan, V., K. Krishnamurthy, V. Sreenivasamurthy, and M. Swaminathan. 1958. The effect of garlic on certain intestinal bacteria. J. Food Sci. 7 : 223.

Surak, J. G., R. L. Bradley, A. L. Branen, and E. Shrago. 1976. Effect of butylayed hydroxyanisole on Tetrahymena pyriformis. Food Cosmet. Toxicol. 14: 277.

Surak, J. G. 1977. Monotertiary butylhydroquinone effects on Tetrahymena pyriformis. Life Sci. 20: 1735-1740.

Townsend, W. E., and C. E. Davis. 1972. Effect of hanging position on some properties of dry sausage. J. Food Sci. 37:633.

Townsend, W. E., and J. Bard. 1978. Factors influencing quality of cured meats. P. 470-480. In the science of meat and meat products. Price J. F. and B. S. Schweigert (ed.). Food and Nutrition Press, Inc. Westport, Connecticut 06880.

Townsend, W. E., C. E. Davis, and A. J. Mercuri. 1975. Effect of chemically adjusted meat pH and drying air velocity on some properties of dry sausage. J. Milk Food Technol. 38:764.

Wada, Y., M. Kawabata, J. Kiyotani, K. Kobayashi, T. Tada, H. Tsukahira, and S. Matsuda. 1978. Studies on the fungal contamination of spices in Japan. J. Food Hyg. Soc. Jap. 19: 128.

Wardlaw, F. B., G. C. Skelley, M. G. Johnson, and J. C. Acton. 1973. Changes in meat components during fermentation, heat processing and drying of a summer sausage. J. Food Sci. 38:1228.

Webb, A. H., and F. W. Tanner. 1945. Effect of spices and flavoring material on growth of yeasts. Food Res. 10: 273.

Yesair, J., and O. B. Williams. 1942. Spice contamination and its control. Food Res. 7: 118.

Zaika, L. L., T. E. Zell, S. A. Palumbo, and L. J. Smith. 1978. Effect of spices and salt on fermentation of Lebanon bologna-type sausage. J. Food Sci. 43: 186.

Zaika, L. L., and J. C. Kissinger. 1979. Effects of some spices on acid production by starter cultures. J. Food Prot. 42:572.

Zaika, L. L., J. C. Kissinger, and A. E. Wasserman. 1983. Inhibition of lactic acid bacteria by herbs. J. Food Sci. 48:1455.

Zaika, L. L., and J. C. Kissinger. 1984. Fermentation enhancement by spices: identification of active component. J. Food Sci. 49:5.