

**CHARACTERIZATION OF AEROBIC  
SPOREFORMERS FROM SOME ETHIOPIAN SAUCE  
SPICES AND THEIR SPOILAGE POTENTIAL ON  
VARIOUS SAUCES**

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## TABLE OF CONTENTS

page

ACKNOWLEDGMENT	i
TABLE OF CONTENTS	ii
LIST OF TABLES	iii
LIST OF FIGURES	iv
ABSTRACT	v
1. INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1. Spices and condiments	5
2.2. Spices as sources of microorganisms	8
2.3. Control and preventive measures of spices from contamination by spore formers	10
2.4. The genus Bacillus	12
2.5. Industrial, medicinal and veterinary importance of Bacillus species	15
2.6. Role of Bacillus species as spoilers	16
3. MATERIALS AND METHODS	20
3.1. Collection of samples	20
3.2. Preparation of samples	20
3.3. Spore count	20
3.4. Characterization of isolates	20
3.5. Identification of Bacillus species	21
3.6. Determination of proteolytic, lipolytic and amylolytic activities of some Bacillus spp. isolated from spices.	27
3.7. Growth potential of aerobic spore forming bacteria in traditional sauces	29
4. RESULTS	31
5. DISCUSSION	42
6. CONCLUSION AND RECOMMENDATIONS	47
7. REFERENCES	49

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## LIST OF TABLES

	Page
Table 1. Average counts (log cfu/g) of aerobic spore formers in five different spices.	33
Table 2. Frequency distribution of <i>Bacillus</i> isolates from five different spices	35
Table 3. Spoilage pattern of the various sauces by test organisms	40
Table 4. Proteolytic, lipolytic , and amylolytic activities of some <i>Bacillus</i> species isolated from spices.	41

## LIST OF FIGURES

	Page
Figure 1. Growth pattern of <i>B. pumilus</i> (a) and <i>B. subtilis</i> (b) in legume-based, vegetable-based and meat based sauces.	38
Figure 2. Growth pattern of <i>B. cereus</i> in legume- based, vegetable-based and meat based sauces.	39

## ABSTRACT

One hundred twenty five samples from five different spices namely , Fenugreek (*Trigonella foenum-graecum*), 'abish'; Black kumin (*Nigella sativa*), 'tikur azmud'; Ethiopian caraway (*Trachyspermum ammi*), 'nech azmud'; Ginger (*Zingiber officinale*), 'zingibel' and Korarima cardamom (*Aframomum corrorima*), 'korarima' were examined for the incidence and level of contamination by *Bacillus* species.

A total of seven hundred eighty one isolates of *Bacillus* species were isolated and identified. The highest average spore count (log 8.32 cfu/g ) was noted in ginger and this was not significantly different within samples (C.V., <10%). Although black kumin yielded the smallest average count of spores (log 1.63 cfu/g), the counts, in 13 of the 25 samples was below detectable levels (< log 1 cfu/g), resulting quite significant variation within samples (C.V., 117%). The most frequently isolated aerobic sporeformers were *Bacillus pumilus* (43.7%), *B. subtilis* (16.6%), *B. circulans* (11.2%), *B. licheniformis* (8.2%) and *B. cereus* (4.9%). *B. pumilus* was most important in ginger and korarima than the other spices tested.

The proteolytic, lipolytic and amylolytic activities of *B. pumilus*, *B. subtilis* and *B. cereus* were assessed. The *B. pumilus* isolates were active in proteolysis and lipolysis, but amylolysis activity was negative in all isolates. *B. subtilis* isolates were more proteolytic than lipolysis and amylolysis, while *B. cereus* showed lower lipolytic activity than proteolytic and amylolytic activity.

The growth pattern and spoilage potential of *B. pumilus*, *B. subtilis* and *B. cereus* isolated from the above mentioned spices were tested on legume-based, meat-based and vegetable-based sauces. The growth pattern of *B. pumilus* followed almost similar growth pattern in all sauces. *B. subtilis* showed a sharp increment of growth between 6h and 12h in legume-based and meat-based sauces. For the *B. cereus* the highest growth was observed at 18h in legume-based and meat-based sauces. However, growth rate in was low in all cases.

# 1. INTRODUCTION

Food is essential for survival. However, in spite of gaining good nutrition and satisfaction from eating food, occasionally human beings consume undesirable chemical and biological agents and toxins resulting in food borne illnesses (Fung, 1992). Microorganisms are always associated with harvested plant and slaughtered animals, the raw materials of the food industry. Except for foods that are heat processed to a degree of sterilization, some microorganisms may cause spoilage, others may cause food borne disease, and still others may bring about desirable changes as a result of growth in foods with which they are associated. There are two categories of food borne diseases. Food poisoning is caused by either chemicals or the ingestion of a toxin (intoxication) that might be found naturally in certain plants or animals or be a toxic metabolic product excreted by a microorganism. Food infection, on the other hand, is caused by growth of the microorganisms in the human body after the contaminated food has been eaten (Frazier and Westhoff, 1978; Brock and Madigan, 1991).

Spices are natural substances consisting of whole or ground aromatic and pungent part of plants (Parker, 1984). Although spices are considered as minor crops, their significance in Ethiopia can hardly be over-estimated. Spices are needed every day in considerable amounts for the preparation of the main dish of the day (Goettsch, 1991).

They are used as flavoring agents. Spices also have some other properties as antioxidants, preservatives and fermentation aids (William and Brown, 1987).

On the other hand spices may serve as major sources of many food spoilage microorganisms. This is because spice bearing plants are exposed to a wide variety of microorganisms from the environment in which they are grown and harvested. Numerous species of bacteria, yeasts and molds constitute the normal microflora of dried spices (Christensen *et.al*; 1967), although aerobic spore forming bacteria usually predominate (Kavacs-Domjan, 1988).

The spores are biochemically inert, and therefore not themselves responsible for spoilage. Spoilage is actually affected by vegetative cells, and the capacity of spores to spoil food, depends on their ability to germinate, out-grow, and achievement of extensive vegetative multiplication in the food. Interruption of this chain of events at any point will prevent spoilage (Ingram, 1969).

Due to their versatile metabolism, strong saccharolytic and proteolytic activity as well as the high resistance of their spores (Prist 1977), the aerobic spore forming bacilli are among the most important groups of microorganisms occurring in foods. Furthermore, it would be evident that with increasing degrees of heat treatment, spores become increasingly important, because they are likely to survive while competing species are eliminated (Ingram, 1969).

The endospore is distinguished from other bacterial cells by showing refractility when examined by phase-contrast microscope, by not staining with basic dyes and by being resistant to the action of many enzymes. The bacterial spore is also unique in that it contains large quantities of dipicolinic acid (DPA). This compound has been found only rarely in other organisms and has been related to the heat resistance of the spore (Brock & Madigan, 1991; Ross, 1983). The bacterial spore is notorious for its ability to survive, to a much greater extent than vegetative cells, when exposed to cold, drying, chemicals, ultra-violet light and other destructive agents (Walker, 1976).

*Bacillus* species can be the dominant spoilage flora in different foods such as sauces (Mogessie, 1996). Ethiopian sauces are usually hot-spiced made of a variety of ingredients. Different sauces have different flavours depending on the type and amount of spices and other constituents, the extent of cooking and other factors. The sauces are basically legume-based, vegetable based or meat based. Legume-based sauces are frequented in low income families and during fasting periods, whereas meat based sauces are luxury for most families. In most households, sauces are usually prepared early in the day and are supposed to last until dinner and kept over night at ambient temperatures.

Although cooking ( usually  $> 85\text{ }^{\circ}\text{C}$  for 15 - 60 min. ) is supposed to eliminate most of the initial contaminants, the sauces are, in many instances, subjected to

recontamination from equipment, utensil surface, hands of workers, dust and air borne contaminants after cooking and during serving (Mogessie, 1996). In addition, a variety of spices are added during the preparation of Ethiopian sauces. Spices are known to be heavily contaminated with microorganisms including spoilage types to food products (Julseth and Deibel, 1974).

The objectives of this study was to characterize the common aerobic spore formers from spices used in the preparation of Ethiopian sauces. The proteolytic, lipolytic and amylolytic activities of some of the dominant *Bacillus* isolates will be determined to assess their potential as spoilers; time and type of spoilage of some common sauces will be evaluated using the frequently isolated *Bacillus* spp.

## 2. LITERATURE REVIEW

### 2.1. *Spices and Condiments*

The words spices and condiments are used to denote plants or plant products that are used to flavor foods or beverages before, during or after their preparation. The literature generally admits that the distinction between spices, condiments and culinary herbs is not clear. Some authors prefer to restrict the term spices to those culinary plants (or their products) that are of tropical origin. Usually condiment is considered to comprise also flavoring of non-vegetable origin (e.g., salt) (Jansen 1981).

The major use of spices in Ethiopia is in the preparation of the traditional sauces called 'Wot'. 'Wot' is a thick, usually highly spiced sauce. Two groups of 'wot' can be distinguished. In one group *Capsicum* pepper ('Berbere') is the main spice and is referred to as 'Kai-wot' ('red sauce'). It is quite hot sauce. The other group is sauce without *Capsicum* pepper ('alicha wot' or mild sauce). Both kinds are either based on meat ('siga wot' or beef sauce, 'doro wot' or chicken sauce) or on pulses ('misir wot' or Lentil sauce; 'kik wot' or 'shiro wot', split pea sauce or sauce from roasted and powdered bean, respectively). Vegetable-based sauces are usually considered as mild-type sauces and served as side-dishes. As meat is rather expensive, most people are obliged to prepare sauces based on pulses. Onions, garlic and butter or vegetable oil are also components of every 'wot'

Spices and condiments are used extensively in many countries as flavoring agents in food preparation. Their importance lies in the fact that they improve the flavor acceptability of cooked food preparations and make them more appetizing. The value of spices and condiments may be broadly considered from various aspects; improvement of taste and flavor; effects on appetite and digestion; effect on intestinal flora; and harmful effects of excessive amounts of spices. (Swaminathan, 1974).

Although, there are many different spices used for many purposes the following 5 different spices are given attention.

a) Fenugreek (*Trigonella foenum-graecum*), 'abish'. It is grown widely in India, Mediterranean countries, North Africa, and some Latin American countries. It contains about 5% of a bitter fixed oil. The oil has a strong celery- like odor. Fenugreek extract is used in USA in the preparation of imitation - maple syrup. In India, fenugreek seed is used as an ingredient in sour pickles and soup powders (Swaminathan, 1974). Moreover, the seeds are most often used as a food spice (curry) and in traditional medicine. Fenugreek seeds are assumed to have several biological properties such as anti fungal, hypocholesterolemic and appetite stimulant (Saivaire *et al.*, 1996).

b) Black Kumen (*Nigella sativa*), 'tikur azmud'. It is cultivated in Central and

Southern Europe, USSR, Northern Africa, Sudan, Ethiopia, Kenya, Somalia, Djibouti, Iran, Afghanistan and India (Cufalontis, 1953; Tutin, 1964 cited in Jansen 1981). Whole or crushed seeds are used in or on bread in India, Srilanka, Egypt, Turkey and the USSR. In Ethiopia, the seeds are used in the preparation of bread, and hot and mild sauces. *Nigella* seeds in wheat bread are often replaced by other spices, as *Nigella* seeds blacken the bread. *Nigella* seed powder may be added, up to 30% of the weight of the *Capsicum* powder, to reduce the pungency of the pepper and to add to it flavor and color (Janson 1981).

c) Ethiopian caraway (*Trachyspermum ammi*), 'nech azmud.' Although its country of origin is unknown, according to Thelung (1925) (Cited in Janson 1981), it is endemic in Egypt, Ethiopia and in the area from SW-Asia to E. India. In India, the fruits are used as condiment in food. In Ethiopia the fruits are ingredient of the powdered *Capsicum* pepper or are added to various sauces types as a flavoring agents (Jansen 1981).

d) Ginger (*Zingiber officinale*) 'zingebel'. It is the underground stem or rhizome which is commonly used as a spice after drying. It is cultivated extensively in India, Nigeria, Jamaica, Haiti, Taiwan, Japan and China (Swaminathan, 1974). Ginger owes its characteristics aroma to about 1 to 3 % of volatile oils, the principal constituents of which are sequiterpenes, bisabolene, zingiberene, and zingiberol. The pungency of the plant part is attributed to ginger oleoresin from which aromatic

ketones, zingerone and shogaol, have been isolated. (Tyler *et al.*, 1976). The major importance of ginger is serving as flavoring pickles; puddings, meat preparations and syrups (Swaminathan, 1974; Pederson, 1979). Hence, ginger is more largely used as a condiment than as a drug (Trease and Evans, 1978).

e) Korarima cardamom (*Aframomum corrorima*), 'korarima'. It is known only from Ethiopia, where it grows in the forests of Kefa, Sidamo, Illubabor and Wellega. Korarima cardamom seeds are used in Ethiopia to flavor all kinds of sauces, for which they are ground and usually mixed with other spices. Another wide spread use of the seeds in Ethiopia is as a flavoring to coffee (some times also to tea) and some times to flavor a special kind of bread.

## 2.2. Spices as sources of microorganisms

Spices are known to be contaminated with bacteria, yeast and molds, causing spoilage of foods (Krishnaswamy *et.al.*,1971). Some spices harbor great numbers of microorganisms including spore forming bacteria. However, the incidence and number of amyolytic and proteolytic organisms, thermophilic spore formers, yeasts and molds, and total microorganisms vary among the different spices as well as within each type of spices (Julseth and Deibel 1974). This variation may be because of the inhibitory or toxic effect of spices to some microorganisms due to their essential oils.

The microbial profile of different spices can be summarized as follows: among the fungi *Aspergillus flavus* and *A. ochraceus* are frequently isolated from red and black pepper. Among the bacteria, *Escherichia coli*, *E. freundii*, *Serratia* spp., *Klebsiella* spp, *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp. are isolated from the same spices (Christensen *et. al.*, 1967). However, *Salmonellae* and *Shigella* are rarely, if ever, found in spices (Julseth and Deibel, 1974; christensen *et al.*, 1967). Moreover, spices contain considerable numbers of bacteria, the majority of which are aerobic sporeformers which may or may not hydrolyze starch. Molds and yeast are also common in spices, especially in ginger. Although the level of consumption of spices by man kind is low when compared to the consumption of staple cereals like corn, Tjaberg *et. al.* (1972) and Madhyastha and Bahat (1985) reported that among the spices, ginger and red pepper are considered to be a high risk commodities in supporting fungal growth, aflatoxin and heavy loads of bacteria

Most workers have come to the conclusion that cloves, cinnamon and mustard exert a greater preservative action than other spices and herbs. As reported by Corran and Edgar (1933) cited in Hersom and Hulland (1969), it was found that brown mustard flour to be the most potent, followed by cloves and cinnamon. Cardamom, cummin, coriander, caraway, celery had little or no effect. Similarly, volatile oil of mustard was found to have a greater preservative action than the essential oils of other spices and herbs.

The effect of certain spice oils on the thermal resistance of microorganisms has been investigated. For example, chillies and mustard oil were found to be the most effective to lower the heat resistance of *B. subtilis* (Rose and Roy, 1960; cited in Hersom and Hulland, 1969). However, the addition of 10 ppm of allyl isothiocyanate (volatile oil of mustard) to a buffer solutions and to apple and grape juice caused a marked reduction in the thermal resistance of *Aspergillus niger* and *Saccharomyces ellipsoideus*. The effect was less marked against *Bacillus thermoacidurans*. In general certain spice oils may have a considerable effect on the thermal stability of yeast cells than spores of bacteria (Hersom and Hulland, 1969).

### *2.3 Control and preventive measures of spices from contamination by sporeformers*

Microbiological concerns are governed by the fact that end use spices to be used as table condiments should be free of salmonellae. Spices containing high numbers of spore forming bacteria may cause spoilage during the heat processing of certain luncheon meats; those containing thermophilic spore formers may cause spoilage of canned foods. In these instances, spices as ingredients constitute a critical control point and are generally monitored via specifications. Ethylene oxide and irradiation treatments are appropriate control procedures when necessary (Sub-committee on Microbial criteria, 1985).

Ethylene oxide is a gas at ambient temperature and pressure. For an effective sterilization process, the gas concentration, time, temperature and humidity are parameters that must be considered and controlled. The sterilizing action of ethylene oxide requires three hours or more, but is effective against spores as well as vegetative bacteria, yeast, molds and viruses (Banwart 1979; Draughon, *et al.*, 1981).

Ultraviolet radiation is the major bactericidal agent supplied by the sun. Aromatic amino acids of proteins and the purine and pyrimidine bases of nucleic acids absorb UV radiation. The effect of radiation treatment becomes more important when used in combination with hydrogen peroxide. That is, irradiation of hydrogen peroxide with UV light results hydroxyl radicals and the spores are killed by hydroxyl radicals resulted from the decomposition of H<sub>2</sub>O<sub>2</sub> (King and Gould 1969; Waites *et al.*, 1988).

Moreover, decontamination of spices by gamma radiation can result in a good Microbiological quality of spices (Tjaberg, *et al.*, 1972; Soedarman, *et al.*, 1984; Alam and Choudhury, 1992) and irradiation did not give significant variations either in state or odour of spices such as peppers, ginger and nutmeg (Tjaberg, *et al.*, 1972).

In addition to ethylene oxide and gamma radiation treatment of spices, the bacteriological safety can be achieved by other practices such as propylene oxide treatment (Draughon, *et al.*, 1981), treatment with saturated steam, although problems are encountered if this method is used for the reduction of microbes in powdery spices

(Bruehart, *et al.*, 1986).

#### 2.4. *The Genus Bacillus*

The genus *Bacillus* consists of rod-shaped cells, sometimes in chains, capable of producing endospores which are cylindrical, ellipsoidal or spherical, and which are located in the center of the cell, sub-terminally or terminally. It is motile by means of peritrichous flagella or non-motile, Gram-positive and aerobic or facultatively anaerobic. Oxygen is the terminal electron acceptor replaceable in some species by alternatives. Colony morphology and size are variable. The genus exhibits a wide diversity of physiological ability. It is psychrophilic to thermophilic, acidophilic to alkalophilic, some strains are salt tolerant, others have specific requirements for salts. (Claus and Berkeley, 1986). An outstanding characteristic of this genus is the formation of heat resistant endospores with not more than one spore formed in a sporangial cell and sporulation occurs in the presence of oxygen (Walker, 1976).

Members of the genus *Bacillus* have a ubiquitous environmental distribution. Because of the resistance of their spores and the capacity of the vegetative cells to secrete enzymes capable of degrading many organic materials, the genus represents arguably the most commercially important groups of bacteria.

In contrast to other bacterial genera, the genus *Bacillus* encompasses a great diversity

of species and strains. *Bergey's Manual of systemic bacteriology* (Sneath, 1986) lists 34 recognized species. They are easy to isolate and culture, and the persistence of the endospore assists both the isolation and maintenance of strains in the laboratory.

The process of identification is based on the existence of a classification scheme containing taxa to which unknown strains may be compared and related. Of the various schemes proposed, that of Gordon *et al.*, (1973) cited in Kramer and Gilbert (1989) remains preeminent. Following a detailed study of more than a thousand strains, these authors proposed a primary division of the genus into three groups according to their cellular morphology and physiological properties. Group 1 is composed of Gram positive species that form ellipsoidal or cylindrical spores that do not appreciably distend the sporangia. Spores of group 2 and 3 are differentiated on the basis of having swollen sporangia and either ellipsoidal (group 2) or spherical (group 3) spores. Members of these latter two groups could be gram-positive, Gram-negative or variable. Group 1 species are further sub divided according to their vegetative cell dimensions and the presence of lipid globules in the protoplasm. Hence the large celled "species (group 1A) having cell width greater than 0.9  $\mu\text{m}$  and producing lipid globules, include *Bacillus megaterium*, *B. cereus*, and the closely related *B. cereus* var. *mycoides*, *B. thuringiensis*, and *B. anthracis*, where as the "small-celled" species (group 1B) (cell diameter < 0.9 $\mu\text{m}$ , lipid globules not formed) include *B. subtilis*, *B. pumilus*, *B. licheniformis* and *B. coagulans*. The spore may be defined or recognized by its refractility impermeability to stain and by its increase

resistance to heat, chemicals, enzymes, radiation and freezing and thawing (Murrell, 1961).

Endospores occur in several genera of bacteria. such as *Bacillus*, *clostridium*, *Thermoactinomyces*, *Desulfotomaculum*, *Sporolactobacillus*, and *Sporosarcina*. All these genera share the ability to form endospores. Of primary interest to food microbiologists are the spore forming species of the genera *Bacillus* (aerobic) and *Clostridium* (anaerobic) (Frazier and Westhoff, 1978).

The bacterial endospore is a resting or dormant stage which is the end result of many changes in cellular structure during the transformation of a vegetative cell in to a spore. This differentiation of bacterial cell in to a spore appears to begin near the end of exponential growth and proceeds by a complex sequence of biochemical and morphological changes culminating in a dormant spore freed from the mother cell which surrounded it. The spore has a core believed to be related to the cytoplasmic portion of the vegetative cell in that it probably contains most of the spore's enzymes and nucleic acids (Spudich, 1970).

The dormant state of the spore is an inactive phase characterized by minimal metabolic activity. The spore can remain in this inactive but viable condition for years and then under suitable conditions can germinate and produce vegetative cells. The changing of a dormant spore into a vegetative cell can be described as occurring in

three successive stages: activation, germination and out growth (Walker, 1976).

Spores of different species of both bacilli and clostridia exhibit a wide range of heat resistance. Among the more sensitive are *Bacillus megaterium* and *Clostridium botulinum* type E, while among the more resistant are the thermophiles *Bacillus stearothermophilus* and *Cl. thermosaccharolyticum* (Roberts and Hitchins 1969).

### ***2. 5 Industrial, Medical and Veterinary importance of Bacillus species***

Aerobic sporeformers are active and versatile producers of hydrolytic enzymes and consequently can utilize a wide variety of proteins, carbohydrates, lipids, glycosides, alcohols and organic acids. Some of their enzymes are produced in commercial quantities and are used in industrial processes. Therefore, the synthetic capabilities of microorganisms are not only confined to food, drink and pharmaceuticals. Microorganisms also produce industrial chemicals that can either serve as or be employed to make solvents, lubricants, dyes, cosmetics, digestive aids etc.

Although, the greatest variety in structure and number of antibiotics is found in the Actinomycetes, the antibiotics from fungi and bacteria are also important. For example many different peptide antibiotics such as bacitracin, polymyxin etc. are produced by the species of the genus *Bacillus*. Other *Bacillus* spp. (sometimes the same) are both infamous and shunned because they grow in many sorts of valuable

commodities, producing spoilage and cause economic loss to human beings (Frobisher, 1968).

Some species of aerobic spore formers are considered as attractive alternatives to chemical insecticides due to their entomopathogenicity against specific pest species. Therefore, among others, *B. thuringiensis*, *B. popuilliae* and *B. sphaericus* are widely used as effective microbial insecticides.

On the other hand, *B. anthracis* is the only pathogens to members of the animals kingdom. Its pathogenicity is frequently associated with a high degree of mortality resulting from the production of extremely potent toxins in the infected animal (Halvorson and Szulmajster, 1973). Moreover, *B. cereus* is one of the most important member of this genus that causes food poisoning. Although there are various other species of *Bacillus*, which are regarded as saprophytic, a few, notably *B. pumilus* and to a lesser extent *B. subtilis* and *B. licheniformis* have been reported sporadically as pathogens ( Karmar and Gilbert, 1989).

## ***2.6. Role of Bacillus species as spoilers***

Spoilage of food involves any changes which render food unacceptable for human consumption and may result from variety of causes such as insect damage, physical injury, the activity of indigenous enzymes in animals and plant tissues and the activity of microorganisms, particularly bacteria, yeasts and molds. However, spoilage

caused by microorganisms is undoubtedly the most important of the other factors (Hayes, 1992; Jay, 1992).

On the basis of susceptibility to spoilage, foods may be classified as stable or non-perishable, semi-perishable and perishable. For example, flour is intrinsically a stable food because of its low water activities but poor storage conditions which facilitate the absorption of moisture, could convert it into a perishable commodity (Hayes, 1992).

According to Ingram (1971), the amount of chemical changes caused by a single microbial cell is very small, so that large number of bacteria ( $10^8$  cells/gram) may be necessary to induce measurable spoilage in foods over a number of days.

The species of bacteria which form spores are diverse in their biochemical activities. Some ferment carbohydrates with formation of acid and/or gas. Others cause breakdown of proteins, with liberation of ammonia and more complex amino compounds, and may or may not produce gas in the process. Some can reduce nitrates or nitrites, present in cured meats, to nitrogen or oxides of nitrogen. These variable action of the spore formers on different foods will obviously depend on the nature of the food, as well as on the nature of the spore forming organisms (Ingram, 1969).

The importance of spores springs from two main causes: (i) their remarkable powers

of dormancy and resistance which leads to (ii) their almost ubiquitous distribution (Ingram, 1969). Spores of aerobic *Bacillus* species are the most frequent in number and variety (Ingram 1969). Therefore, the presence of spores throughout the environment leads to contamination of any portions of nutritious material wiped or spilled on to utensils or left lying in imperfectly cleaned equipment.

The ubiquitous reservoirs of spores ensure that all foods are themselves likely to carry some spores. This is especially so with foods like vegetables or spices which are apt to suffer contamination with soil, and hence arises the unusual difficulty of processing foods containing vegetables and spices.

Among the parameters significant in determining food spoilage are intrinsic factors, which are the expression of the physical properties, the chemical composition, and some biological attributes of the food itself, and extrinsic factors such as temperature, humidity, oxygen and partial pressure of the environment in which a food is stored (Mossel, 1977).

Spices can be a source of spoilage microorganisms when they are used as seasoning for processed foods. For example, they may introduce heat-resistant spores into canned foods. In any attempt at disinfection, bacterial spores are apt to present a special problem because of their unusual powers of resistance; and the preparation for example of canned foods is governed by rules largely aimed to control the activities of

heat-resistant spores which would otherwise lead to spoilage of the food (Ingram, 1969).

The other important food spoilage by *Bacillus* species is called “ropiness” which is a serious problem in both home made and commercially produced bread because of the out growth of spores of *B. subtilis* in the product. The internal temperature may reach 100°C for a brief time during baking, which is not sufficient to eliminate the spores. Ropiness in bread first becomes evident as brownish spots or patches accompanied by an unpleasant odor in the interior of the loaf. Even though spores are present in the product, ropiness will not develop unless the environment is warm and moist and the acidity of the bread is low (Mossel, 1977; Walker, 1976; Jay, 1992).

were picked from countable plates, purified by repeated plating and transferred to nutrient agar slant for further identification to the species level.

### **3.4. Characterization of isolates**

The isolates from aerobic mesophilic bacteria count plates were characterized to their respective genus using the following tests.

**3.4.1. Colony morphology:** different colonies were characterized based on their colour, elevation, margin and size.

**3.4.2. Microscopic observation:** Wet mounts were prepared from young cultures and examined under a Microscope. The following were the morphological criteria considered in the observation:

Cell shape- rods, cocci

Cell grouping- single, pairs chains

Endospores- present, absent (this was determined by spore stain).

**3.4.3. Oxidation-fermentation test:** The test was conducted as in Aneja (1993).

Ingredients:-

Peptone	2.0g
NaCl	5.0g
K <sub>2</sub> HPO <sub>4</sub>	0.2g
Bromothymol blue(.2%)	0.08g

Agar	2.5g
Distilled water	1000ml
	PH=7.1

The basal medium was distributed to test tubes, 3 to 4ml per tube, and sterilized at 121° C for 15 minutes. After sterilization, glucose (10% solution) was aseptically added to each tubes to give a final 1% concentration. Two tubes of each culture were inoculated by stabbing and a sterile paraffin oil added to one of the tubes and incubated at 32 °C for 3 to 4 days. Acid formation in the open (with out paraffin) tube only indicated oxidative utilization of glucose. Acid formation in both the open and sealed tube was indicative of a fermentative reaction.

#### 3.4.4. *KOH-test (Test on lipopolysaccharide).*

One or two drops of 3% KOH solution were placed on a clean glass slide. A colony was picked from the surface of PC plates with an inoculating loop. The material was stirred in the KOH solution for 5-10 seconds and the inoculating loop was then raised slowly from the mass. When the KOH solution became viscous, the tread of slime followed the loop for 0.5 to 2cm or more. This was observed in Gram-negative bacteria. When there was no slime, a watery suspension that did not follow the loop, the reaction was negative and this was seen in Gram positive bacteria.

### 3.4.5. Catalase test.

The colonies were flooded with 3% solution of H<sub>2</sub>O<sub>2</sub>. Formation of bubbles indicated a positive reaction.

## 3.5 Identification of *Bacillus* Species

### 3.5.1 Colony morphology

Morphological characteristic of colonies was based on their colour elevation, margin and size.

### 3.5.2. Biochemical test

#### 3.5.2.1 Anaerobic growth

This test was conducted by anaerobic agar developed by Dea'k and Timar (1988).

Ingredients:- Glucose	5 g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	1g
KCl	0.2g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2g
Yeast extract	1g
Sodium thioglycollate	2 g
L-cystine	0.5g
Bromocrespiperple (10% solution)	2.8ml
Agar	15g
Distilled water	1000ml
	pH = 7

After distribution in to test tubes it was autoclaved at 121° C for 15 minutes. The agar was allowed to solidify forming a deep base (approx.50mm) and a short upper slope. A 24h young culture was used for inoculation. The culture was inoculated by stabbing with a straight wire to the bottom and incubated at 32° C for 3 days. Aerobic growth was restricted on the surface of the slope while facultative anaerobic strains showed growth also along the stab.

### 3.5.2.2. Production of acetyl methyl carbinol (acetoin)

Determination of acetoin production was made on MR-VP medium (OXOID). Since the phosphate present in MR-VP medium may interfere with the production of a acetoin by *Bacillus* spp., 1% NaCl was added to the medium (Collins and Lyne, 1976).

A 24h pure culture was used to inoculate the broth and the tubes were incubated at 32°C for 5 days. To detect acetoin formation, first 1ml of Reagent A and later 1 ml Reagent B were added to the broth culture. The formation of a pink color within 60 minutes was indicative of acetoin production.

#### Ingredients:

Reagent A:  $\alpha$ -naphthol, 6.0g; ethanol (96%) 100ml

Reagent B: KOH, 40.0g; creatin, 0.3g; distilled water, 100ml.

### 3.5.2.3. Nitrate reduction

Nitrate broth was inoculated with a young pure culture and incubated at 32°C for 3 days.

Ingredients - Nutrient Broth and 5g of NaNO<sub>3</sub> in 100ml of Distilled water pH=7.

Test reagents (Edward's and Ewing 1972)

- A. Dissolve 8g of sulfanilic acid in 1000ml of 5N acetic acid
- B. Dissolve 5g of  $\alpha$ -naphthylamine in 1000ml of 5N acetic acid

Immediately before use, equal parts of the solutions A and B were mixed and 0.1 ml of the mixture was added to each culture. Positive test for reduction of Nitrate to Nitrite was indicated by the development of a red color within a few minutes. A very small amount of zinc dust was added to negative culture tubes. Appearance of red color was confirmative for absence of nitrate reduction.

### 3.5.2.4 Starch hydrolysis

To see the enzymatic hydrolysis of starch by amylases, starch agar was inoculated with a young culture and incubated at 32°C for 3 to 5 days. The growth was then flooded with Lugol's iodine solution. Starch hydrolysis was indicated by a clear zone around the growth. Unchanged starch gave a blue color.

Ingredients. Starch agar - (Claus and Berkeley, 1986) 1 g of soluble starch was

suspended in 10ml of cold distilled water and mixed with 100ml of nutrient agar.

#### Lugol's iodine solution

Iodine	5 gm
Potassium iodide	10 gm
Distilled water	100ml

#### 3.5.2.5. Acid gas production from glucose: as modified by (Aneja, 1993)

##### Ingredients -

Peptone	10.0g,
Glucose	5.0g
sodium chloride	5.0g
phenol red	0.018g
Distilled water	1000ml

pH = 7.3

The medium was sterilized at 115C° for 15 minutes in a test tube containing inverted Durham tubes to detect gas formation. The test organisms were inoculated and incubated at 32°c for 7 days.

#### 3.5.2.6 Growth in 7% NaCl

Nutrient broth (3 ml/tube) containing 7% (W/V) sodium chloride was inoculated with

a loopful of a young culture and incubated at 32°C for 3 days. Growth was observed after 3 days of incubation.

#### **3.5.2.7 Growth at 50° C and 65°C**

The ability of organisms to grow at 50°C and 65°C was determined by inoculating the test isolates into the nutrient broth and immersing tubes containing inoculated culture in water baths at the appropriate temperature. The water levels in the baths were carefully maintained. The temperatures were stable, with a variation not greater than  $\pm 0.5^\circ$ . Growth was observed after 3 to 5 days.

#### **3.5.2.8. Utilization of citrate as a sole source of carbon**

This test was conducted on Simmon's citrate agar (OXOID). The agar was streak plated with a young culture and incubated at 32°C for 24 hours. Utilization of citrate was indicated by appearance of a blue color in the medium.

### **3.6. Determination of proteolytic, lipolytic and amylolytic activities of Some *Bacillus* species isolated from spices**

#### **3.6.1 *Proteolytic activity***

Seventy-one isolates of 3 different species of *Bacillus* were separately inoculated onto Calcium Caseinate Agar to detect their protein degrading activity.

Ingredients:	Lab-lemco broth (meat extract)	8g,
	Sodium chloride	5g,
	Casein	2.5g,
	Calcium hydroxide	0.15g,
	Calcium chloride	0.05g
	Agar	13.0g
	Distilled water	1000ml,
		pH=7.

The medium was filtered and sterilized. Prepared plates were incubated overnight to check for sterility. This was streak-plated with an overnight culture of the test organisms and incubated for 48-72 hours at 30C°. Clear zones around the colonies resulted from the hydrolysis of casein and this indicated a positive test for proteolysis.

### 3.6.2. *Lipolytic activity*

Lipolytic activity of the test strains was determined by using Tributyrin agar.

Ingredients	Peptone	5g
	Yeast extract	3g
	Tributyrin	10ml
	Agar	12g
	Distilled water	1000ml
		pH. 7.5

After sterilization at 115 C° for 15 minutes, prepared plates were incubated overnight

to check for sterility. This was streak-plated with an overnight culture of the test organisms and incubated at 32°C for 24-48h. Colonies of lipolytic organisms degraded Tributyrin and, thus, cleared the surrounding medium.

### **3.6.3 Amylolytic activity**

To prepare Starch Agar (Claus and Berkeley, 1986), 1 g of soluble starch was suspended in 10ml of cold distilled water and mixed with 100ml of nutrient agar. Sterilized medium was streak-plated with an overnight culture of the test organisms and incubated at 37° C for 3 to 5 days. The growth was then flooded with Lugol's iodine solution as in 3.2.2.4. Starch hydrolysis was indicated by a clear zone around the growth. Unchanged starch gave a blue colour.

### **3.7. Growth potential of aerobic spore forming bacteria in traditional sauces**

Legume-based, vegetable-based and Meat-based sauces were prepared following traditional methods and the sauces were filled in test tubes in 20ml amounts and sterilized at 121C° for 15 minutes.

### **3.7.1 Inoculation of sauces with test strains**

For the determination of growth and spoilage potential of *Bacillus* spp. in various sauces the most frequently isolated spp. and the known food poisoning spp. were selected. Ten isolates of each of *B. pumilus*, *B. subtilis* and *B. cereus* were considered for this study. A loopful of the test strains was inoculated into a sterile Brain Heart Infusion broth (OXOID) separately. After incubation for 24h at 37°C, the growth was compared with 0.5 McFarland turbidity. The standard was made by adding 0.5ml of 0.048M BaCl<sub>2</sub> to 99.5ml of 0.35N H<sub>2</sub>SO<sub>4</sub> following Barry (1980) and this corresponded to the turbidity produced in a broth that contains 10<sup>8</sup> cfu/ml. This was further diluted serially to give a final inoculum level of 10<sup>2</sup> - 10<sup>3</sup> cfu/ml in the various sauces. The inoculated sauces were mixed thoroughly and left at ambient temperatures for 72 hours.

### **3.7.2. Determination of growth of test strains in sauces**

About 0.1ml of appropriate dilution from freshly inoculated sauces were surface plated on Nutrient agar in duplicate to determine the initial inoculum level. The various sauces were then sampled at 6 hour intervals. Inoculated plates were incubated at 37°C for 24-48 hours for colony counting. Inoculated sauces were kept at ambient temperature and checked periodically at 6h intervals for signs of food spoilage (foul odour or gas production) (Mogessie 1996,1997). The signs of food spoilage were checked by three different individuals aseptically in a disinfected hood.

## 4. RESULTS

One hundred twenty five samples from five different spices, namely Fenugreek (*Trigenella foenum-graecum*), “abish”; Black cumin (*Nigella sativa*), “tikur azmud”; Ethiopian caraway (*Trachyspermum ammi*), “nech azmud”; Ginger (*Zingiber officinale*), “Zingibel” and Korarima cardamom (*Afirmomum corrorima*) ‘korarima’ were examined for the incidence and level of contamination by *Bacillus* species. The highest average spore count (log 8.32 cfu/g) was noted in ginger and this was not significantly different within samples (Coefficient of variation, C.V., <10%) (Table 1). Although black cumin yielded the smallest average count of spores (log 1.63 cfu/g), the counts in 13 of the 25 samples was below detectable levels (< log 1 cfu/g), resulting in quite significant variation within samples (C.V., 117%). The average spore counts in the other three spices ranged between log 4 and log 5 cfu/g and the coefficient of variation ranged between 15 and 25%.

A total of seven hundred eighty one isolates of *Bacillus* spp. were obtained from the above mentioned spices and, using the simplified identification scheme of Berkeley *et. al.* (1984), were identified to the species level. The most frequently isolated aerobic spore formers were *Bacillus pumilus* (43.7%), *B. subtilis* (16.6%), *B. circulans* (11.2%), *B. licheniformis* (8.2%) and *B. cereus* (4.9%). The other ten *Bacillus* species constituted <10% of the total isolates (Table 2).

Fenugreek yielded a total of 181 isolates were isolated of which the most frequently isolated species were *B. pumilus* (42.5%), *B. subtilis* (18.2%), *B. circulans* (10.4%), *B. cereus* (7.7%) and *B. licheniformis* (6.5%) (Table 2). Of the total of 52 isolates obtained from black cumin, the most common were *B. licheniformis* (23.0%) *B. pumilus* (21.1%), *B. circulans* (19.2%), *B. cereus* (11.5%) and *B. subtilis* (5.7%). A total of 179 isolates were obtained from Ethiopian Caraway and the most frequently observed species were *B. pumilus* (28.4%), *B. subtilis* (22.9%), *B. circulans* (15.6%), *B. licheniformis* (8.3%), and *B. cereus* (5.0%).

Table. 1. Average counts (log cfu/g) of aerobic sporeformers in five different spices.

Spices	No.	Count			standard deviation	% Coefficeint of deviation
		Max	Min	Mean		
Funegreek	25	7.68	3.30	4.55	1.15	25
Black Cumin	25	4.98	0	1.63	1.91	117
Ethiopian Caraway	25	5.90	3.38	4.33	0.67	15
Ginger	25	8.32	6.30	7.53	0.48	6.3
Korarima cardamom	25	7.80	3.68	5.33	1.07	20

Ginger yielded 186 isolates of spore formers out of which the most frequently isolated *Bacillus* species were *B. pumilus* (55.3%), *B. subtilis* (12.9%), *B. circulans* (10.2%), *B. licheniformis* (8.6%). Although the highest count of spores was observed in ginger the number of *B. cereus* in this spice was lower than that encountered in the other spices.

A total of 183 isolates were isolated from Korarima Cardamom and the dominant species were *B. pumilus* (54.3%), *B. subtilis* (15.9%), *B. licheniformis* (7.6%), *B. circulans* (6.5%), and *B. cereus* (4.3%).

The extent of distribution of the test isolates in various spices showed that, although *B. pumilus* was dominant in all spices, the highest number was observed in ginger and the least in black cumin (Table 2). For *B. subtilis* the highest and lowest counts were obtained in Ethiopian Caraway and Black Cumin, respectively. The *B. cereus* count in all spices is lower than that for the above mentioned test strains. However, relatively higher number of isolates were obtained from black cumin and the least from ginger.

Table 2. Frequency distribution of *Bacillus* isolates from five different spices

Isolated spp.	FG	BK	ET	GG	KO	Total	
						No.	%
<i>B. Pumilus</i>	77	11	51	103	99	341	43.7
<i>B. subtilis</i>	33	3	41	24	29	130	16.7
<i>B. circulans</i>	9	10	28	19	12	88	11.3
<i>B. licheniformis</i>	12	12	15	16	14	69	8.8
<i>B. cereus</i>	14	6	9	1	8	38	4.9
<i>B. megaterium</i>	9	2	11	8	4	34	4.4
<i>B. sphaericus</i>	12	3	7	3	8	33	4.2
<i>B. alvei</i>	1	2	7	4	-	14	1.8
<i>B. brevis</i>	2	-	1	2	3	8	1
<i>B. firmus</i>	1	1	4	1	-	7	0.89
<i>B. stearo-</i> <i>thermophilus</i>	-	1	3	1	-	5	0.64
<i>B. coagulans</i>	-	-	1	-	3	4	0.5
<i>B. larvae</i>	-	1	-	1	2	4	0.5
<i>B. lentimorbus</i>	1	-	1	2	-	4	0.5
<i>B. papilliae</i>	-	-	-	1	1	2	0.25
<b>Total</b>	181	52	179	186	183	781	100

FG, Fenugreek; BK, Black Cumin; ET, Ethiopian Caraway;

GG, Ginger; KO, Korarima cardamom

Of the 15 different *Bacillus* species identified in this study, two of the most frequently isolated species, *B. pumilus* and *B. subtilis*, and the potentially food poisoning species, *B. cereus*, were selected and their growth pattern and spoilage potential in legume-based, vegetable-based and meat-based sauces was determined.

*B. pumilus* showed almost a similar growth pattern in all sauce types, but counts in legume-based sauces was higher than in the other sauces (Fig. 1). *B. subtilis* increased by about 3 log units between 6h and 12h in legume- and meat- based sauces and reached counts as high as log 7 cfu/ml at 24h. Its growth was, however, markedly low in vegetable-based sauce and the count at 24 h was slightly higher than log 5 cfu/ml (Fig. 1). *B. cereus* increases steadily in legume- and meat- based sauces and reached maximum counts at 18 h. In vegetable-based sauce, however, it increased by slightly over 1 log unit within 24 h (Fig. 2).

Spoilage of the various traditional sauces by the test strains was observed at 6 h intervals for 72 h. Spoilage was manifested mainly through production of foul odor and no gas production was detected in all cases (Table 3). Twenty six of the thirty test isolates showed in foul odor in legume-based sauces within 24 h. Foul odor in Meat-based sauces was detected between 24 - 48 h and in vegetable-based sauces, between 36-60 h.

Half of the *B. pumilus* strains used in the test showed strong proteolytic activity and almost all showed strong lipolytic activity. None manifested amylolytic activity (Table 4). *B. subtilis* also showed strong proteolytic and lipolytic activity, but most were more proteolytic than lipolytic. Amylolytic activity was noticed in few. Although about half of *B. cereus* test strains exhibited strong proteolytic and lipolytic activity, all were also able to manifest a degree of amylolytic activity (Table 4).

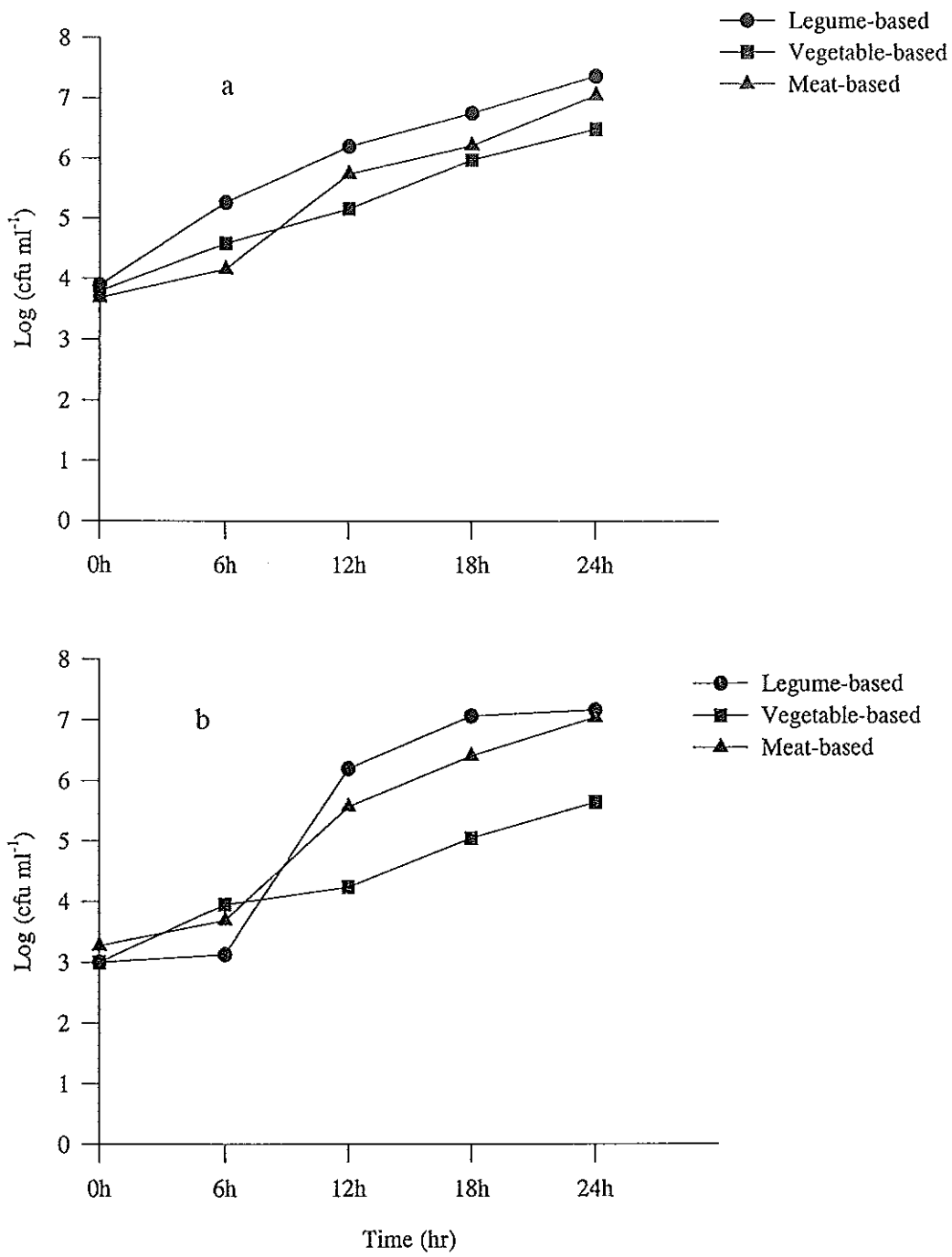


Figure 3 . Growth pattern of *Bacillus pumilus* (a) and *Bacillus subtilis* (b) in legume-based,vegetable-based and meat-based sauces.

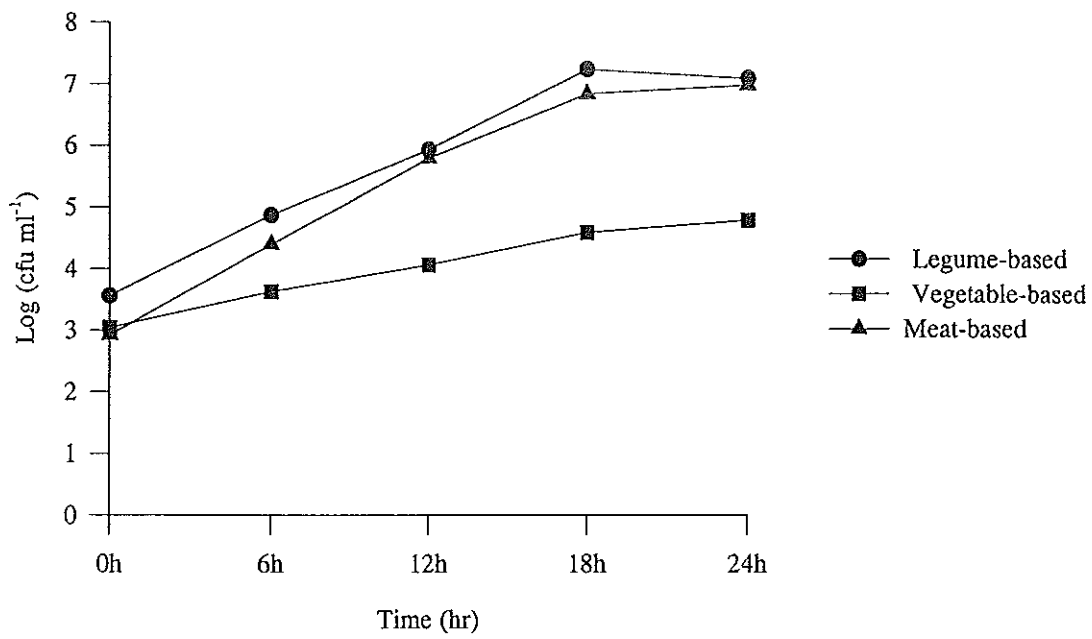


Figure 4. Growth pattern of *Bacillus cereus* in legume-based, vegetable-based, and meat-based sauces.

Table 3 Spoilage pattern of the various sauces by test organisms

			Legume-based					Vegetable-based					Meat-based				
			12h	24h	36h	48h	60h	12h	24h	36h	48h	60h	12h	24h	36h	48h	60h
<i>B. pumilus</i>	10	Foul odor	-	8	2	-	-	-	-	2	5	3	-	2	5	3	
		Gas prod.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. subtilis</i>	10	Foul odor	-	10	-	-	-	-	1	1	5	3	-	7	3	-	-
		Gas prod.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. cereus</i>	10	Foul odor		8	2	-	-	-	-	-	4	6	-	5	2	2	1
		Gas prod.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Proteolytic, Lipolytic and Amylolytic activities of some *Bacillus* species isolated from spices. (++, Strong positive; +, positive, -, No odor)

Test organisms	Number of isolates	Proteolysis	Lipolysis	Amylolysis
<i>Bacillus pumilus</i>	25	13/25 ++	22/25 ++	25/25 -
		12/25 +	3/25 +	
<i>Bacillus subtilis</i>	25	20/25 ++	12/25 ++	20/25 +
		5/25 +	13/25 +	5/25 ++
<i>Bacillus cereus</i>	21	9/21 ++	10/21 ++	21/21 +
		12/21 +	8/21 +	
			3/21 -	

## 5. DISCUSSION

The dominant role in the microbiology of spices is played by aerobic spore formers (Kovacs-Domijan, 1988) and other vegetative forms are usually absent in spices mainly due to their susceptibility to desiccation and toxicity of spices (Christensen *et al.*, 1967; Julseth and Deibel, 1974).

Of the spices considered in this study, ginger had the highest count of spore formers and this was consistently observed in all samples. This is in agreement with the findings of Tjaberg *et al.* (1972). This may be due to the underground nature of the spice part of the plant, thus resulting in close contact with soil. In addition to the fact that ginger contains sufficient moisture that can allow the survival of certain groups of microorganisms and the essential oils of ginger are reported to be less effective against microorganisms (Meena and Sethi, 1994). According to Madhyastha and Baht (1985), ginger and black pepper are better substrates for fungal growth and aflatoxin production. Ginger is commonly added in mild-type sauces of all types and, in certain cases, in some hot meat-based sauces (e.g., chicken sauce) as an important condiment. It may, thus, be an important source of spoilage microorganisms to sauces.

Korarima cardamon, Ethiopian caraway and fenugreek also contained substantial amount of spore formers in all samples and may be considered as possible sources of spoilage microorganisms to the various sauce types. The fact that no spore formers

were detected in half of the black cumin samples makes this spice not a major source of spoilage microorganisms to sauces. On the other hand, the low count may be due to the heat processing of the samples. According to Blank *et al.* (1988), heat treatment at 80°C for 10 minutes in certain spice mixtures resulted in a great reduction of the initial count. In addition, melanthin, a black cumin component, is a very active fish poison and is very toxic for other animals too (Janson, 1981). The higher frequency of isolation of *B. cereus* in black cumin than in the other spices could be due to the nature of the spore which is reported to have great resistance to essential oils of some spices (Meena and Sethi, 1994).

Of the 15 different *Bacillus* spp., *B. pumilus*, *B. subtilis*, *B. licheniformis*, *B. circulans* and *B. cereus* constituted about 85% of the total isolates. These were isolated from all types of spices at varying frequencies and may be considered as important sources of spoilage microorganisms to various sauces. Similar studies from other countries showed that *B. subtilis* and *B. pumilus* were the most frequently isolated species from spices in Malaysia (Mohammed *et al.*, 1986) and these species were also the dominant isolates in spices and other foods in Hungary (Deak and Timar, 1988). In Nigeria, however, *B. polymyxa* and *B. coagulans* were part of the dominant flora in addition to *B. subtilis* and *B. cereus* (Antai, 1988). In Canada, the dominant isolate was *B. cereus* (Seenappa and Kempton, 1981).

Eight other *Bacillus* spp., although encountered in the various spices constituted a

very small proportion of the *Bacillus* flora. This may not, thus, be considered as spoilage species in sauces where these spices are included. The number of thermophilic 'flat sour' groups are limited in all spices as reported by Krishnaswamy *et al.* (1971) and Deak and Timar (1988). In agreement to our observation, however, from among the thermophilic types, *B. coagulans* was dominant in food ingredients including spices.

All the test strains effected spoilage through production of foul odor and no gas production as sign of spoilage was noted. In other studies on spoilage, only a very small proportion of *Bacillus* spp. were able to produce gas (Mogessie, 1997; Al-Diejaili and Anderson, 1991). In Ethiopian sauces, the major gas producing spoilers belonged to the Enterobacteriaceae (Mogessie, 1997).

*B. pumilus* dominated the *Bacillus* flora in almost all spices and may be taken as the most important spoilage organisms of sauces. The spoilage pattern of *B. pumilus* was mainly lipolytic although half of the test strains showed strong proteolytic activity. This may be indicative of the spoilage role of *B. pumilus* in sauces with sufficient fats.

The other *Bacillus* spp. with a higher frequency of isolation was *B. subtilis*. All types of spices were good sources of this species. As most *B. subtilis* test strains showed quite strong proteolytic activity, *B. subtilis* may rather be considered as a very strong spoiler by producing foul odor. This could be noted in the production of foul odor in

the protein-rich sauces ( legume-based and meat-based), where all or most of our *B. subtilis* test strains showed foul odor within 24 hours. Production of foul odor in vegetable-based sauces was mostly detected after 48 hours.

A good proportion of the *B. cereus* test strains could produce foul odor in legume-based sauces within 24 hours. Similar pattern was also observed in meat-based sauces but to a lesser extent.

Production of foul odor in vegetable-based sauces by all test strains was mainly detected after 48 hours. In most households in Ethiopia, sauces are usually consumed the same day they are prepared. Therefore, spore-formers may not play a major role as spoilers in traditional sauces. However in compelling situations where sauces have to be kept longer at ambient temperatures, legume-based and meat-based sauces seem to be susceptible to spoilage by such microorganisms. Meat and legumes contain sufficient proteins to be acted upon by proteolytic organisms and to produce foul odor.

Vegetable-based sauces, on the other hand, were able to retard the rate of production of foul odor mainly due to the absence of sufficient degradable proteins or fats and also due to the presence of certain antimicrobial substances that retard microbial growth (Marchetti *et al.*, 1992).

The growth pattern of the test strains in the various sauces supports this argument. In all cases the test strains did not reach numbers high enough to cause spoilage at 24 h.

Growth was steady in legume-based and meat based sauces in most cases and the number reached at 24 h (log 7 cfu/ml) which was high enough to result in sufficient amounts of proteolytic or lipolytic enzyme activity effect spoilage through foul odor production. The delay of spoilage in vegetable-based sauces could, however, be due to the low rate of growth of the various test strains in the sauces and consequently the low count at 24 h.

Although *B. cereus* was shown to produce spoilage at 24 h in legume-based and meat-based sauces, it is also important from the stand point of toxin production. Thus, sauces stored for longer periods, if contaminated with *B. cereus*, could be sources of food poisoning.

It may also be noted that, although spices may be considered as sources of spoilage microorganisms, they may also exhibit some inhibitory effect on certain microorganisms (Aureli *et al.*, 1992).

## 6. CONCLUSION AND RECOMMENDATIONS

*Bacillus* species are widely distributed in nature because of their versatile metabolism, strong saccharolytic, lipolytic and proteolytic activity as well as higher resistance of their spore. The ability to survive adverse environmental conditions has made the *Bacillus* species of a particular importance in the processing and preservation of foods in which they may cause spoilage or may be a public health hazard.

*Bacillus* species can readily be isolated from raw and processed dairy products, meat, dried foods such as spices. It is well established that most spices have antimicrobial properties. However, they can harbor numerous number of amylolytic, lipolytic and proteolytic bacteria such as spore formers, yeast and molds. Due to many intrinsic and extrinsic factors however, the total microorganisms varied among the different spices as well as within each type of spices.

The result of this study demonstrates that, among the spices ginger appears to be high risk commodities for food spoilage by aerobic spore formers. Although, the total spore count in black cumin is lower compared with the other spices tested the relative distribution of *B. cereus* was higher. Therefore, this can be exploited further containment of toxin contamination of foods.

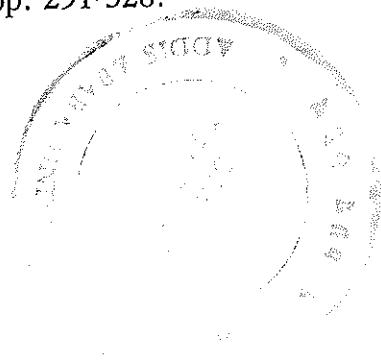
It would appear that spices, like other food ingredients, may be in rare instances

become contaminated with microorganisms of public health significance. However, multiplication in this dried products is low and longevity of vegetative cells in the dry state appears to be limited.

The *Bacillus* species may not be killed by a temperature that most sauces cooked rather this temperature may serve as a heat shocking for activation of spores to germinate and out-grow, therefore, the recognized methods of control include adequate cold storage throughout processing and adequate cooking followed by immediate eating or rapid cooking and continued cold storage. It may be difficult to use cold storage in most households in Ethiopia. However, to control spoilage by spore formers consumption of cooked food immediately is recommended.

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