SPOILAGE OF VALUABLE SPICES BY MICROBES

Dr. Kuljinder Kaur

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Preface

The present study was undertaken to assess microbial contamination of commonly used spices viz. Coriander powder, Ginger powder, Turmeric powder, Black pepper powder and Red chilli powder. Samples were examined for total count of aerobic mesophiles, coliforms, aerobic spore formers, yeast and mould, *E coli*, *Faecal Streptococci* and *Salmonella.* Detail of sample collected is given in table-1.General appearance, moisture content and pH of coriander powder is presented in table-6. Sample CP-3 showed least aerobic mesophiles as 58×10^2 cfu/g while coliform were 159 cfu/gm and yeast and mould 90 cfu/gm. The moisture content of the sample was 6.80% sample CP-1 with minimum moisture 5.97% showed maximum no. of aerobic mesophiles (215×10^2) and high number of coliforms (145 cfu/gm) and yeast/mould (136 cfu/gm).

Sample GP-8 of ginger powder showed aerobic mesophiles of 160×10^2 cfu/g and count of GP-4 was 218×10^2 cfu/gm. GP-3 and GP-7 showed coliform count 15 cfu/gm, yeast and mould count were highest in GP-1 i.e. 332 cfu/gm. The total count of aerobic mesophilic bacteria in turmeric powder sample was highest in TP-2(182 x 10^2cfu/gm) and least aerobic count was in TP-3(52 x 10^2cfu/gm). Five samples have less than 100 x 10^2 cfu/gm mesophilic count. However, the total coliforms was more than 600 cfu/gm in two samples (TP-1, TP-3) while TP-4 showed 400 coliforms/gm. Yeast and mould count was maximum as 382 cfu/gm in TP-1 and minimum 160cfu/gm in TP-10. Samples of Black pepper BPP-8, BPP-3, and BPP-1 showed mesophilic count as 155 x 10^2 cfu/gm, 148 x 10^2cfu/gm and 139 x 10^2 cfu/gm which was higher than other samples. Total coliforms count was in range of 80 to136 cfu/gm. While yeast and mould count was less than10 cfu/gm in BPP-1 and 60 cfu/gm in BPP-4. Aerobic mesophilic count in Red chilly powder was more than 200 x 10^3 cfu/gm in three samples RCP-2, RCP-4, RCP-8 (234 x 10^3 cfu/gm, 224 x 10^3 cfu/gm, 220 x 10^3 cfu/gm) and near about 200 x 10^3 cfu/gm in three samples RCP-6, RCP-9, RCP-10(180 x 10^3 cfu/gm, 198 x 10^3 cfu/gm, 188 x 10^3 cfu/gm). The range of coliforms count was 472 in RCP-1, 144 x 10^2 cfu/gm in RCP-2 and RCP-4), Yeast and mold count was highest in RCP-1(312 x 10^2 cfu/gm). All samples were found to be contaminated with *E. coli.* and a few were contaminated with *Feacal Streptococcus* (Table-10). Highest MPN 5.3/gm was in sample RCP-9. MPN count of *E.coli.* 4.4/gm was also found in 2 samples RCP-4 and RCP-7 which was nearest to maximum. MPN

range of *Feacal Streptococcus* was 1.0/gm to 2.7/gm. Sample RCP-9 was highly contaminated with *E. coli.* and *Feacal Streptococcus* having MPN 5.3/gm in *E. coli.* and 2.7/gm in *Feacal Streptococcus.* Similarly *B. cereus* was confirmed in four samples RCP-1, RCP-4, RCP-6 andRCP-9 and counts was 10 cfu/gm. Red chilli powder sample was highly contaminated category of spices sample collected for analysis. *Salmonella* was not detected in any of the red chilli powder sample and it was absent in all samples of this category of sample

Salmonella was absent in all samples of spices. *E. coli* was present in four categories of spices except ginger powder sample. The contamination of spices is highly variable and does not depend upon a single factor.

Dr. Kuljinder Kaur

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This book is based on research conducted on contamination of valuable spices. First, I give thanks to God for giving a chance by encouraging me to start the work, preserve with it, to Complete this work and finally to publish it.

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Dr. Kuljinder Kaur

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CHAPTER-I

INTRODUCTION

Food borne illness is common in India, although it can be prevented with proper care and handling of food products. Bacteria related to food poisoning are fewer than 20 of the many thousands of different species of bacteria found in the environment. More than 90% of the cases of food poisoning each year are caused by *Staphylococcus.aureus, Salmonella, Bacillus cereus* and enteropathogenic *E.coli.* These bacteria are found in many raw foods and also contaminate cooked food. Normally food poisoning bacteria must be present in large numbers to cause illness. The temperature ranges in which most of bacteria grow fall in the category of mesophiles (25^0C- 40^0C) although few psychrophiles and thermophiles are known to cause food poisoning.

The microbiological quality of ten spices or herbs was determined by a national survey at retail level. Aerobic plate count values for ten products ranged from >100 to 3.1 x 10^8 /gm ; mean values of the individual spices or herbs ranged from 1.4x10^3 to 8.2 x 10^5/gm. Coliform count range from > 3 to 1.1 x 10^6 /gm ; however mean values were > 20/ gm for all products. *E.coli* counts ranged from > 3 to 2300 /g., yeast and mould counts were made for five of the ten products.

Major food pathogens are Gram negative bacteria (*Pseudomonas Enterbacteriaceae , Acinetobacter)*, Gram positive bacteria (*Lactobacillus ,Staphylococcus, Enterococcus , Clostridium*) , yeast (*Sacchromyces, Candida , Pichia*) and moulds (*Penicillium, Aspergillus, Rhizopus*) Spices can be contaminated with microorganisms that survive the harvesting , primary processing and drying process. The microorganisms in spices originate from soil, plants and animal source contamination during harvesting, processing, storage or post harvest treatment (MCkee 1995). High total plate counts of bacteria, mould and yeast are common in spices. Contamination in spices is caused by *Salmonella*, *Clostridium* p*erfringens*, *coliforms* and aerobic spore formers such as *Bacillus subtilis, B. cereus, B.licheniformis* and thermophillic anaerobes. *E. coli* is more frequent in case of spices contamination. Contamination level of spices ranges for coliform, plate 0 to 10^6 per gram and yeast and mould 0 to10^7 per gram depending upon the nature and moisture content of the product. Some products such as pepper (Black & White), Chillies and Coriander powder are more susceptible to mould contamination. Food pathogens contaminating spices can be hazardous. When spices are contaminated with 10^4 microorganisms per gram, they would lead to contamination level of 10 to 10^2 microorganisms per gram when added to other product at addition level of 0.1 to 1.0 by weight (McKee, 1955).

Coliforms are indicator microorganisms to serve as a measure of fecal contamination & confirm the presence of enteric pathogens in food. If a sample is contaminated with *Salmenella* or *Shigella* , it also contains *E.coli* because of fecal contamination. Although some spices such as ginger and coriander powder have antimicrobial activity but number

of total plate count and coliform count show increased levels with storage. Contamination of black pepper with species of *Staphylococcus, Bacillus, E.coli , Mucor* and *Candida* has been reported. Aerobic mesophillic count of black pepper results in increase of the spoilage, which promotes fungal growth (Omafuvbe and Kolawole, 2004).

OBJECTIVE AND METHODOLOGY USED

It is therefore important to determine the microbial loads of spices at the time of processing and packaging to prevent the spoilage of spices during storage. With this aim in view, the present study was undertaken to determine:-

a. Total counts of aerobic spore formers in dry spices black pepper, coriander powder, ginger powder, turmeric and red chili powder.

b. Total counts of coliform as indicator of fecal contamination will be taken by pour plate method and MPN method.

c. Total counts of fungal spores particularly *Aspergillus niger*, and *Cleviceps purpura* by using hemocytometer and plating on Yeast extract Dextrose Chloramphenicol Agar medium (YDA).

CHAPTER – II

REVIEW OF LITERATURE

During August – October 1996, 3 different statistical commodities of spices (including 37 samples of black pepper, 41 samples of turmeric, 26 samples of sumac) were taken randomly in west of Tehran, Iran. These spices were examined for fungal and bacterial contamination using EC, VRBL agar, LST and Briliant green media. Result were then compared with the National Iranian spices standards, 81.7% of the evaluated samples showed traces of fungal and bacterial contamination . Microorganisms, isolated from spices included coliforms , *E.coli, Penicillium, Mucor* and *Aspergillus*. Overall 86.5%, 95% and 53.8% of Black pepper, turmeric and sumac samples were contaminated with fungi and bacteria. (Imandel and Adibnia, 2000)

Fecal coliforms are distinguished from main coliform group by their ability to grow in a selective growth medium incubated at an elevated temperature 43-45^{0}C. Fecal coliforms ferment lactose at 44.5^{0}C within 24 hrs with production of gas. Coliforms are historically used as indicator microorganisms to serve as a measure of fecal contamination and thus potentiality of the presence of enteric pathogens in foods. In this study Hilas et.al. (2001), five hundred food samples in 10 different groups, mainly diary products, salads, spices were analyzed for natural contamination by fecal coliform and *E.coli* by MPN method. The difference between means of fecal coliforms and *E.coli* counts were 0.246 $\log_{10}$ MPN/g. It can be said that fecal coliform preferably *E.coli*. Analysis is sufficient for rapid routine determination of fecal contamination. Besides *E.coli* , *Enterococcus* a subgroup of fecal streptococci that includes at least 5 species *: S.faecalis S. faecium, S. durans, S. gallinarum, S.avium,*were present.. Of these *S. faecalis and S. faecium* are most frequent species in spices. *Streptococcus faecalis* is the only species that has been genetically characterized. Its genome is 3 mb in size. The two genetic mechanisms first discovered in enterococi were conjucative transposons and sex pheromone plasmids. Some strains require vitamins B and amino acids for growth. *S. faecalis* resists 60^{0}C for 30 min, forms NH_3 from arginine, tolerates 0.04% Potassium tellurite and form acid from glycerol, mannitol, sorbitol, lactose and sucrose .(Buchanan and .Gibbons 1974)

Enterococci occurs naturally in soil. They have been reported routinely in frozen seafood, cheese, dried whole egg powder, frozen fruits, fruit juice and vegetables. They are capable of producing extracellular proteinases and peptidases to hydrolyse large peptides and transport them into the cell to convert them to amino acids.

A range of ethnic foods was examined for their microbiological contents. In relation to total viable count of aerobic bacteria, count of presumptive coliforms, yeast and mould count, presence of *Salmonella , E. coli*. was also determined (Candlish Smith, Kelly, Irvine 2001). Samples such as nuts, dried fruits, herbs and spices were obtained from local retail outlet and distributed. Peanut showed low counts whereas spices and nuts showed much higher counts. *Cl*ostridium *perfringens*, *Staph. aureus*, *Bacilius*.spp. were common in spices and nuts.

The microbiological quality of some spices consumed in Diyarbakr, Turkey was assessed. The spice samples (90 samples) were examined for presence of total mesophillic aerobic bacteria, *Enterobacteriaceae*, *Coliform* bacteria, *E.coli*, *Staphylococcus* spp., sulfate reducing anaerobic bacteria and yeast /moulds. Total aerobic mesophillic bacteria counts (mean) were $6.6X10^6$,$4.8X10^6$,$5.5X10^6$,$8.5X10^6$,$3.9X10^6$ cfu/g for black pepper, Cumin, all – spice, ground red pepper and flaked red and black pepper samples, respectively. Also 66.67 , 60.1%, 53.34% , 100%, 53.34% & 26.68% of the samples contained microorganisms higher than the acceptable level, respectively. These results showed poor microbiological quality of spices sampled.

Seventy-three samples of spices harvested from meat industry and milk industry were analyzed. Twenty one (21%) was found to be contaminated with coliforms bacteria, *E.coli*, yeast and moulds and sulphite reducing clostridia. It was observed that some of the analyzed spices had a degree of contamination up to 15 times higher than the permitted value. In the case of semi smoked products a thermal treatment at 72^0C for 10 minutes, resulted in reduced contamination, the products not subjected to thermal treatment (Chilli pepper powder, lard, smoked sausage , ground meat paste) a significant effect of microorganisms in the spice was observed, where the microorganisms are more numerous in the final products (Apost et. al.).

The microflora of black & white pepper during processing and storage were enumerated, isolated and identified.

The fresh untreated pepper samples gave appreciable total aerobic mesophillic bacteria (TAMB) counts of 6.65 $\log_{10}$ cfu/gm in white pepper and 7.04 $\log_{10}$cfu/gm in black pepper. Coliforms counts ranged in number from 6.23 – 6.80 $\log_{10}$ cfu/gm while yeast mould counts ranged from 2.00 – 3.74 $\log_{10}$ cfu/gm sample. The microflora associated with untreated fresh pepper samples included species of *Staphylococcus*, *Micrococcus*,*Bacillus*, *Serratia*, *E.coli*, *Aspergillus* , *Fusarium* , *Intersonila* , *Botrydiplodia*, *Penicillium*, *Mucor* , *Candida* and *Brettanomyces*. (Omafuvbe and Kalawole, 2004) Pretreatments involving steeping in boiling water for up to 20 min, surface disinfection with 2% formaldehyde solution and washing reduced the microbial load of pepper samples to zero or less than 1$\log_{10}$cfu/gm. This was accompanied by the disappearance of yeast, coliforms and certain other species of bacteria.

Microbiologists have acknowledged that traditional spices can increase the bacterial levels of some foods which may result in their deterioration and possibly cause food poisoning. The bacterial isolates were identified according to the schemes of Harrigan and Mccance (1976) and Buchanan and Gibbons (1974). Yeast and moulds were identified as described by Collins and Lyne (1970) , Barnett and Hunter (1972) and Lodder (1971).

Untreated sun dried whole black pepper samples however still had appreciable numbers of microorganisms ranging from 4.00 – 4.48 $\log_{10}$ cfu/gm while oven – dried portion gave approximately 3.88 $\log_{10}$ cfu/gm. It is interesting to note however , that the number of Microorganisms isolated during drying and storage falls within the acceptable ICMSF (International Commission on Microbiological specification for foods) level ($>10^6$ cfu/gm) reported by Banerjee and Sarkar(2003).

Studies in 1930, 1940 and 1970s shows that many spices and herbs are heavily contaminated at imports. Christen et. al. (1967) reported the analysis of 11 samples of black pepper obtained from retail stores, interstate carriers or food service establishment. Aerobic plate counts values ranged from 8×10^6 to 7×10^8 /gm with mean of 1.94×10^8 /gm. In 1975 study of 114 samples of 7 spices obtained from military basis (A. H. Schwab et. al. 1982). Power et. al. found that APC values were markedly lower (>100 to 9.1×10^6 /gm) than those found by Christen et. al.(1967) for black pepper. Coliforms counts were generally > 30 /gm and fecal coliforms were not found.

Although microbiological criteria for spices have been recommended, few specific criteria have been suggested. The International commission for microbiological specifications for foods, however has recommended the following microbiological criteria for spices: APC values of n=5, c=2, $m=10^4$ per gm and $M=10^6$ per gm where n is the number of units in a sample, c is the maximum number of marginal quality units, m is the bacterial count that separates goods from marginal and M is the bacterial count that separates marginal from defective quality. The Commission recommended microbiological criteria for *E. coli* of n=5, c=2, m=10 per gm and $M=10^3$ per gm, only 3 samples of the 14,080 tested for *E. coli* exceeded the M value and all were thyme. Criteria for yeast and moulds of n=5, c=2, $m=10^2$ per gm and $M=10^4$/gm were also recommended.

A microbiological study of ready to eat foods with added spices or spice ingredients was undertaken to identify any risk factors in the production, storage and display of this product and to establish their effect on microbiological quality. Examination of 1946 ready to eat foods from sandwich bars, cafes, public houses, restaurants, bakers, market stalls and mobile vendors found that 1291 (56%) were of satisfactory microbiological quality, 609 (32%) were of unsatisfactory quality and 46 (2%) were of unacceptable quality. Unacceptable sample were high for *Bacillus. cereus* and/or other *Bacillus spp.* ($>10^5$cfu/gm). Unsatisfactory results were mostly due to high Aerobic colony counts (up to $>10^7$ cfu/g), Enterobacteriaceae ($>10^4$ cfu gm), *Escherichia coli* ($>10^2$cfu/gm) and *Bacillus spp*. ($>10^4$ cfu/gm).

Examination of 750 spices and spice ingredients revealed that *B. cereus* were present in 142 (19%) samples, other *Bacillus spp*. in 399 (53%) samples and *Salmonella spp.* (*S.enteritidis* PT11) in one (<1%) sample. Approximately, a third (222) of spice and spice

ingredients examined contained high counts (>10^4 cfu/gm) of *B. cereus* and/or other *Bacillus spp*. and appeared to be associated with the corresponding ready to eat food containing similar high counts of these organisms ($p<0.0001$) (Cl Little et.al 2003).

Samples of raw meat prior to roasting, and tsire-suya were analyzed bacteriologically for total viable, coliform, staphylococcal counts and the presence of *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus. aureus* and *Escherichia coli*. The sensitivity of the bacterial isolates to some antibiotics and spices was evaluated. The total viable count varied from $20x10^2$ to $289x10^2$ cfu/gm for the raw meat and $7x10^2$ to $171x10^2$ cfu/gm for the tsire-suya. The coliform count was $4x10^2$ to $71x10^2$ cfu/gm for the raw meat and $1x10^2$ to $42x10^2$ for the tsire-suya while the staphylococcal count ranged from $1x10^2$ to $60x10^2$ cfu/gm for the raw meat and $1x10^2$ to $12x10^2$ cfu/gm for tsire-suya. From results obtained, bacterial count was higher in raw meat than in tsire-suya. *P. aeruginosa*, *B. cereus*, *S. aureus*, and *E.coli* , were isolated from the raw meat and tsire-suya. The isolates were sensitive to some of the antibiotics and spices tested. However, *E.coli* was only sensitive to gentamycin. While *P. aeruginosa* on the other hand was resistant to *Afromomum melegueta*, *Piper quinense* and *Capsicum fructescens*, the three spices tested for this study. (R. E. Uzeh, 2006).

Microbiological investigations of 64 spice samples (ground pepper and paprika) showed that aerobic spore formers including thermophiles play a dominant role in the microbiology of spices. Determination of the numbers of *coliforms* as fecal indicators must be substituted by determination of the numbers of the Enterobacteriaceae family since the former occurs in very small numbers in these products. In the case of the detection of the presence of *Salmonella* the antimicrobial effect of the spices, as well as their micro ecological conditions makes it necessary to use resuscitation of samples in large quantities of medium. With due consideration to the fact that in spices, as much as 50% *B. cereus* contamination may be expected, their direct detection is considered a necessity in those food products where multiplication may occur. (Kovacs - Domjan, H. 1988)

A total of thirteen genera of bacteria and two genera of yeasts were detected in surface sterilized and unsterilized Brazilian commodities and spices such as cashew kernels, Brazil nut kernels, black and white pepper. The genus *Bacillus* with eight species was by far the most common. The yeasts isolated were *Pichia* sp., *P. guillermondii* and *Rhodotorula* sp. Besides *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus* were detected in cashew and Brazil nut kernels (Chagas and Freire, 2001).

Studies conducted in South Africa revealed light contamination of processed and non-processed cashew kernels by *Bacillus* spp. As for Brazil nut kernels, bacteria associated with decaying was first reported in 1921. Mesophile bacterial counts on roasted and salted Brazil nut kernels ranged from 5.3×10^3 to 1.2×10^4 cfu/gm during storage period. Yeasts

occurred in very low counts. The authors, however, did not identify bacteria. Concerning to black pepper, which accounts for some 35% of the world trade in ground spice, counts are by far the highest among the spices most used. Total number of bacteria ranging from 8.3×10^6 to 7.09×10^8 cfu/gm (mean 1.9×10^8) has been confirmed. Other workers have also found high levels of bacterial contamination on black pepper, although yeast populations had always been low. White pepper, to a lesser extent, can also be a source of contamination. Bacteria such as *Bacillus* spp., *Escherichia freundii, E.coli, Klebsiella* sp., *Serratia* sp., *Staphylococcus* spp. and *Streptococcus* spp. have been identified on black and white pepper. A comprehensive review on spices microbiology has been provided. (Freire and Offord Lisa, 2002) .

Spices are known to be heavily contaminated with microorganisms. Ground black pepper may carry as many as 10^8 bacteria/gm. White pepper, to a lesser extent, may also be infected. Most bacteria present in spices are aerobic spore-former. (Baxter and Holzapfel 2002) found that approximately 90% of colonies in viable counts were *Bacillus* spp. In the present study we have obtained similar results, with six species of *Bacillus* being identified. Our counts, however, are far lower compared to the workers above. Among the species we isolated only few of them have been associated with food poisoning. Indeed, *B. cereus* has been recognized as the etiological agent of food poisoning outbreaks in Europe as far back as 1906.

According to Silliker (2002), black pepper contaminated with aerobic spore formers enormously increased the microbial count of sausage. Aerobic spore formers have also been detected in white pepper powder. Coliforms and faecal streptococci were present in significant numbers in black and white pepper. We did not isolate faecal species from our black and white pepper samples but S*almonella* has been found in black pepper produced in the Brazilian Amazonian Region (Dr. Maria L. Duarte, personal communication).

Microbiological quality and nutrient composition of dry tomato sold in Nigerian markets was assessed over four month period. Microbial load in market samples was significantly higher compared to laboratory prepared dried tomato. Staphylococci counts were highest in market sample ranged at $\log_{10}$ 3.56+ or -0.26 to 3.81+ or -0.37 cfu/g followed by coliform counts $\log_{10}$ 3.31+ or -0.7 to 3.56+ or 0.7 cfu/ g and yeast and moulds $\log_{10}$ 3.11+ or -0.21 to 3.47+ or -0.37 cfu/ g (AKpan et. al.2004).

A total of 162 samples were analysed at different stages of the elaboration process. These were grouped in eighteen lots, three for each type of dry sausages. Through out the ripening process a decrease in some microbes groups (enterobacteria, coliforms, *E.coli*) occurred in all cases. Presence of *Salmonella* in some samples of unripened product was confirmed (Lopez et. al. 2000).

Microbiological quality of girelle (Italian ready to eat filled pasta product) was studied. Three industrially produced batches of girelle with ham and cheese were analysed for total aerobic mesophiles, anaerobes, *staphylococci* and *coliforms*. Highest microbial counts were found in finished girelles; individual ingredients contained relatively low microbial counts. The batch with highest microbial load contained aerobic mesophiles at $2 \times 10^5 - 10^8$ cfu/gm, while counts of *staphylococci* in this batch arranged from $5 \times 10^2 - 10^4$ cfu/gm and coliform reached maximum value of 10^5 cfu/g (Paris *et. al*.2005)

CHAPTER -III

METHODOLOGY USED

The present investigation was carried out to test the extent of bacterial contamination in commonly used house hold spices viz. Turmeric powder, Ginger powder, Red Chili powder, Coriander powder and Black Pepper powder. The samples were analyzed for the presence of Total plate count, Coliform count, Fungal spore count, Yeast and Mould count, *E. coli*, *Salmonella, Feacal streptococcus* and aerobic spore former *Bacillus cereus*. Indian standard were followed for analysis of all samples. The methods used in analysis of the samples for mentioned pathogens were:

- Total plate Count(Quantitative)
- Hemocytometer method for fungal spore count
- Most probable number(Quantitative)
- Detection method(Qualitative)
- Confirmatory Tests (Biochemical tests) for Identification

3.1 SAMPLING PROCEDURE: Representative samples of the powdered spices from Agro processing unit were taken after thorough mixing and homogenization as per IS: 5404(Methods for Drawing and Handling of Food Samples for Microbiological Analysis). Each sample weighing about 100g was taken aseptically with sterile scoop in a sterile zipper pouch for microbiological analysis. Ten samples of each category of Coriander Powder, Ginger Powder, Turmeric Powder, Black Pepper Powder and Red Chili Powder were taken for analysis. Total 50 samples were taken. Detail of the samples collected with ID is given in Table-1.

Table-1: Types of samples taken.

Samples of Spices					
Sample No.	**Coriander Powder(CP)**	**Ginger Powder(GP)**	**Turmeric Powder(TP)**	**Black Pepper Powder(BPP)**	**Red Chilli Powder(RCP)**
1	CP-1	GP-1	TP-1	BP-1	RCP-1
2	CP-2	GP-2	TP-2	BP-2	RCP-2
3	CP-3	GP-3	TP-3	BP-3	RCP-3
4	CP-4	GP-4	TP-4	BP-4	RCP-4
5	CP-5	GP-5	TP-5	BP-5	RCP-5
6	CP-6	GP-6	TP-6	BP-6	RCP-6
7	CP-7	GP-7	TP-7	BP-7	RCP-7
8	CP-8	GP-8	TP-8	BP-8	RCP-8
9	CP-9	GP-9	TP-9	BP-9	RCP-9
10	CP-10	GP-10	TP-10	BP-10	RCP-10

3.1.1 TOTAL PLATE COUNT: IS: 5402 standard method is used for determination of total microbial count for quality assessment of sample. For total plate counts, 10g sample of the powdered spice was taken and suspended in 90ml sterile diluents (peptone salt solution). This was the initial tenfold dilution equivalent to 10^{-1}. Most of the time the particulate matter float, the particles were allowed to settle for five minutes. Then 1ml of initial suspension was added to 9 ml of diluent to prepare next dilution that is equivalent to 10^{-2}. Similarly sample was serially diluted up to $10^{-5}/10^{-6}$ dilution. One ml sample of each dilution was poured into sterile petriplate in duplicate. 15 to 20ml of the molten PCA (cooled to 42-45oC) within 15 min from the time of preparation of original dilution was added to the plates carrying dilutions of samples. The culture media and dilutions were mixed by swirling gently clockwise, anti-clockwise, to and fro thrice with taking care that the contents did not touch the lid. Plates were allowed to solidify. The plates inverted were ad incubated at 30^{o}C for 72 hours. Following incubation, colonies on plates containing 30-300 colonies were counted and recorded the results. It was presumed that each colony has arisen from a single cell. Thus, the number of colonies multiplied with dilution factor and it gives the total number of bacteria present in the sample per unit volume of the sample. Colonies were counted as per the formula given below:

$$N = \frac{\sum C}{(N1 + 0.1N2)\, D}$$

Where

$\sum C$ - Sum of colonies counted on all plates

N_1 – No. of plates retained in first dilution

N_2 – No. of plates retained in second dilution

D– Dilution factor corresponding to first dilution

3.1.2 TOTAL COLIFORM COUNT: IS: 5401 standard method was used for total coliforms count in spices. For Coliforms counts crystal violet neutral red bile lactose Agar media was used. The medium was sterilized by autoclaving at 121^{0}C for 15 minutes. Serial dilutions up to 10^{-5} of the sample were prepared as in total plate count method. One ml of each different dilution was poured in sterile Petri plates (in duplicate for each dilution) and then 15-20ml sterile VRBL medium was added. After mixing, the plates were allowed to solidify. After complete solidification added 4ml of VRBL medium at 45^{0}C + 0.5 ^{0}C on to the surface of inoculated medium. Inverted the plate after complete solidification and incubated at 37^{0}C for 24-48h. Following incubation, colonies on plates containing 0-150 colonies were counted and recorded the results. The number of colonies were counted after incubation (Purple color colonies having diameter of 0.5mm) using same formula as in total plate count.

3.1.3 YEAST AND MOULD COUNT: For yeast and mould, **Yeast extract Dextrose Chloramphenicol Agar medium (YDA)** media was used. As following the procedure of total plate count, 1ml of sample from different dilutions was transferred to sterile petriplate (in duplicate for each dilution) and then 15-20ml of YDA was poured into it. The medium was cooled at 45^0C. The plates were mixed well by rotating clockwise and anti clockwise and media was allowed to set. After complete solidification, the plates were incubated at 25^0C for 4-5 days. After incubation, the number of colonies was counted using same mentioned formula.

3.1.4 FUNGAL SPORE COUNT: Hemocytometer was used to count fungal spores in spices. Suspension of sample was prepared in normal saline. 10µl of suspension was transferred to the centre of the hemocytometer and covered it with cover slip.The hemocytometer was placed under microscope with 10X objective to facilitate localization of the grid. Focused on large square consisting of 16 small squares at each corner and number of spores on each large square were counted. Same process was followed on each of the 4 large corner squares. Spores in each of the four 0.1 mm^3 corner squares were counted. The spore count was calculated using the equation:

Spores/ml = (n) x 10^4,

Where: n = the average cell count per square of the four corner squares counted

3.2 Enumeration of *E. coli* in dry spices by Most Probable Number (MPN): The most probable number also called MTD was used for estimating the number of microorganisms in a test sample by assessing the presence or absence of target organism at different dilution level of test sample. It is useful for application to samples, which contain very low no. of microorganisms i.e. < 10 / g. A dilution series is prepared from the test sample and inoculated into MacConky broth media. Three sequential dilutions are inoculated into MacConky broth media in three replicate tubes. After incubation, the presence or absence of test organism in each tube is determined by observing growth in tubes. The number of positive growth tubes was determined. This creates a profile that was translated into MPN of test sample by use of statistical table.

Determination of MPN

1. Added 25 g of sample into 225ml of sterilized diluent, left it on shaker for 10-15 minutes. It was initial suspension. (10^{-1}).
2. Prepared further serial dilution from initial suspension upto 10^{-3} dilution.
3. Transferred 10ml from initial suspension (10^{-1}) into 3 tubes containing sterilized 10ml of double strength Mac Conky broth with Dhuram's tubes in each of 3 tubes.
4. Transferred 1ml from initial suspension (10^{-1}) into 3 tubes containing sterilized 10ml

of single strength Mac Conky broth with Dhuram's tubes in each of 3 tubes.

5. Transferred 1ml from 10^{-2} dilution into 3 tubes containing sterilized 10ml double strength Mac Conky broth with Dhuram's tubes in each of 3 tubes.
6. Transferred 1ml from 10^{-3} dilution into 3 tubes containing 10ml of single strength Mac. Conky Broth with Dhuram's tubes in each of 3 tubes.
7. Incubated all the tubes at 44^{o}C for 48 hours.
8. Examined the tubes after incubation. After incubation if there was gas production in some tubes, assessed by air bubbles in Dhuram's tube and Acid production was confirmed by color change of medium front red pink to yellow.
9. By noting the number of positive tube, MPN of *E.coli* per gm of sample was obtained using MPN table.
10. Further confirmation of *E.coli* was done by Gram Staining, biochemical tests and inoculation of EMB plates.Pre sterilized peptone water tubes were inoculated with positive tubes. Incubated the inoculated EMB plates and peptone water tubes at 37^{o}C for 24 hrs. Metallic sheen colonies on EMB plate confirmed the presence of *E.coli*. Addition of 2 drops of Kovac's reagent to peptone water tubes after incubation resulted in formation of red ring confirming a positive test for *E.coli*.

Fig-1: Metallic sheen colonies of *E.coli*. On EMB

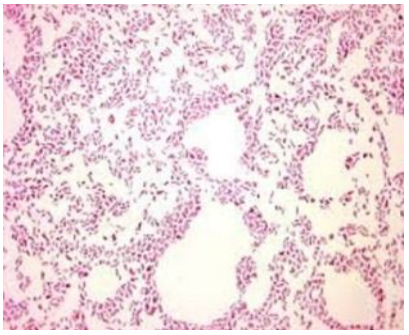


Fig-2: Gram negative rods of *E.coli*.

3.2.1. BIOCHEMICAL TESTS OF *E.coli*: The confirmation of ***E.coli*** was done by Gram staining and following biochemical tests. Suitable positive controls were run to eliminate any false positive results.

Gram Staining: Prepared a thin smear from suspected colony with saline. Heat fixed the smear. Flooded the slide with crystal violet stain for 1min and washed off with tap water. Then added Lugol's Iodine solution on the slide and left it for 1min. After 1min,washed off

it with tap water. The smear was decolourised with 70% Ethyl Alcohol. Washed the smear with tap water and counter stained the smear by flooding the slide for 30 sec with Saffranine solution. It was washed off with tap water. Dried the slide and observed with oil immersion under microscope with 100X objective lens. Bacteria appeared purple were Gram +ve and in appeared pink in color were Gram negative.

1. Indole production: Inoculated a colony into tube containing 5 ml of sterilized Peptone water. Incubated at 37°C for 24 hours. after incubation, added 1.0 ml of Kovac's reagent to it. Formation of red ring indicated a positive test.

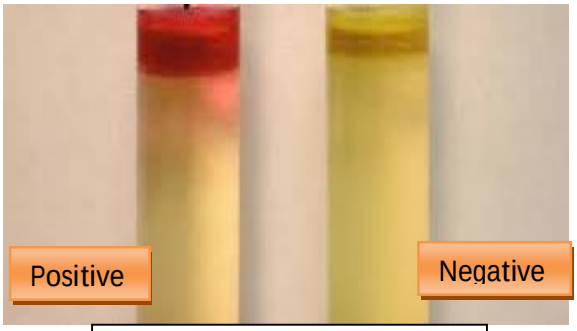


Fig-3: Indole test

2. Methyl Red Test: Inoculated a tube containing test medium with suspected colony and a tube containing test medium with pure culture of *E.coli* incubated both the tube at 37°C for 2 days. After incubation added 2 drops of methyl red solution to each of the tube.Red color formed in tube inoculated with suspected colony, confirmed the presence of *E.coli* as the positive control also shown a positive MR test.

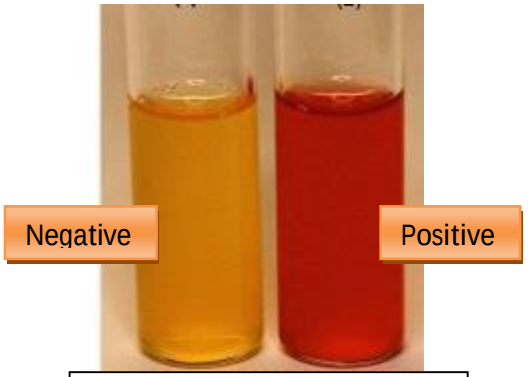


Fig-4: **Methyl Red Test**

3. Voges–Proskauer Test: Suspended a loopful of suspected colony into tube containing 0.2 ml of sterilized VP medium. Incubated at 37°C for 24h. After incubation added a drop of 0.5 % creatine solution, 3 drops of ethanolic solution of napthol and 2 drops of KOH

solution. Appearance of pink red color within 15 minutes confirmed positive test. It was compared with positive control shown same result.

4. Citrate Utilization: Inoculated the Simmon's citrate agar slope with suspected colony by stabbing the butt using a straight wire. Incubated at 37^{o}C for 4 days. There was no change in the color of medium indicating a negative test.Whereas positive test was indicated by blue color.

5. Urease Test: Inoculated one urea agar slope with suspected colony and another urea agar slope with pure culture of *Proteus* (Urease +ve). Incubated both at 37^{o}C for 24 hours. No change in the color of medium in the slope indicated negative test. Brilliant pink color formation due to accumulation of ammonia is shown by positive control. Both slopes were compared for confirmation of the test organism.

6. Test for H_2S Production: Inoculated triple sugar iron agar medium by stabbing the suspected colony into butt and streaking the slope. Incubated at 37^{o}C and observed for 7 days. There was no color change of medium which indicated that there was absence of H_2S production. Compared it with +ve control (*Salmonella*) resulted in blackening of Agar

Table-2: Biochemical confirmation tests for presumptive *E.coli*(IMVIC)

Tests	Positive control *E.coli*.
Gram staining	Pink colored Gram negative rods
Indole	Positive
MR	Positive
VP	Negative
Citrate utilization test	Negative
Urease test	Negative
Test for H_2S production	Negative

3.3 DETECTION AND DETERMINATION OF FEACAL STREPTOCOCCI: Detection of feacal streptococci was done in order to find out the feacal contamination of dry spices. As per methods explained in IS: 5887(11), Plate count method and MPN methods were used for detection of faecal streptococci.

1. Plate Count: Took 25gm. of sample and added 225 ml of diluting fluid to have a dilution of 10^{-1}. Ten-fold serial dilutions with diluting fluid (Peptone salt solution) in duplicate series up to 10^{-6}.were prepared from initial dilution. 0.1ml of each dilution was spread on to Ethyl Violet Azide Dextrose Agar (EVDA) medium plates from each dilution tube. Plates were incubated at 37^{o}C for 48 hours. Dark red colonies were appeared on EVDA after incubation. Colonies were counted and confirmed by Grams staining and by growth at 44^{o}C in MacConky Agar for typical pink colonies. Gram positive cocci were in chains. The number of viable colonies per gram of the sample was determined by

multiplying with dilution factor and dividing by mass of the sample.

Fig-5*: Feacal streptococcus* colonies on EVDA

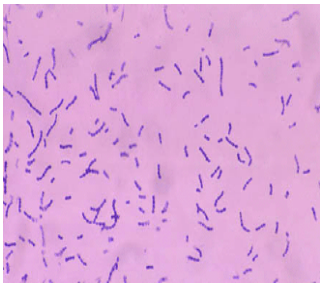


Fig-6*:* Gram positive chains of cocci in *Feacal streptococcus*

2. MPN method for detection: Prepared serial dilutions of sample upto 10^{-5}. Transferred 1ml of the homogenized mixture of the five serial dilutions in triplicate to the tubes of 10ml ethyl violet azide dextrose broth. When number of feacal streptococci was expected to be very small, added 10ml of homogenized mixture in triplicate 10ml of double strength broth. Incubated at 37^{o}C for 48 hours. Recorded the positive tubes which have developed turbidity and growth having been confirmed as faecal streptococci as above in plate count method. Using MPN index result of feacal streptococci have been noted per gram of the sample.

3.4 DETECTION OF AEROBIC SPORE FORMER *Bacillus cereus*

Spice samples were also analyzed for detection of *Bacillus cereus* to find out the reason of food poisoning caused by aerobic spore formers. As per methods of IS:5887(6), Plate count method using Mannitol-egg yolk-polymyxin (MYP) agar culture media was chosen to detect *Bacillus cereus.*

Procedure: 10 g of sample was taken in 90 ml of dilution medium (peptone salt solution). This was 10^{-1} dilution. Further serial dilutions of sample up to 10^{-5} were prepared from it. 0.1ml of each dilution was transferred onto 2 plates of MYP agar plate. The inoculum was spread by sterile spreader using fresh spreader for each plate. The plates were left with lids on for about 15 minutes at ambient temperature for the inoculum to be absorbed into agar. The plates were incubated at 30oC for 48 hrs. After period of incubation, presumptive colonies of Bacillus cereus were counted from each plate containing less than 150 colonies. The presumptive large eosin pink (mannitol fermentation positive) colonies surrounded by zone of precipitation (due to lecithinase activity) were transferred to nutrient agar slants for confirmation tests. Five presumptive colonies were selected from each plate and were confirmed by Gram Staining, spore staining and biochemical reactions as given in table-3.

3.4.1 BIOCHEMICAL TESTS OF *Bacillus cereus*: The confirmation of *Bacillus cereus* was done by Gram staining and following biochemical tests.

1. Gram Staining: Prepared a thin smear from suspected colony with saline. Heat fixed the smear. Flooded the slide with crystal violet stain for 1min and washed off with tap water. Then added Lugol's Iodine solution on the slide and left it for 1min. After 1min, it was washed off with tap water. The smear was decolorized with 70% Ethyl Alcohol. Washed the smear with tap water and counter stained the smear by flooding the slide for 30 sec with Saffranine solution. It was washed off with tap water. Dried the slide and observed with oil immersion under microscope with 100X objective lens. *Bacillus. cereus* appeared as large Gram positive bacilli in short to long chains. spores were ellipsoidal, central to sub-terminal

2. Spore staining: Prepared smear of culture on a clean microscope slide and air dried it after heat fixing. Placed a small piece of blotting paper (absorbent paper) over the smear and place the slide (smear side up) on a wire gauze on a ring stand. Heated the slide gently till it starts to evaporate as the paper begins to dry added a drop or two of malachite green to keep it moist. Remove the heat and reheat the slide as needed to keep the slide steaming for about 3-5 minutes. After 5 minutes, the slide was removed and allowed to cool. The slide was rinsed thoroughly with tap water (to wash the malachite green from both sides of the microscope slide) and smear was stained with safranine for 2 minutes. Rinsed both side of the slide to remove the secondary stain and air dried.

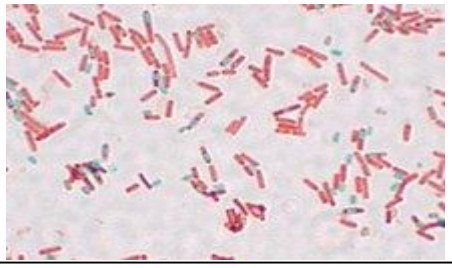


Fig-7: Spore staining of *Bcillus. cereus*

Bacteria under 1000X (oil immersion) total magnification were observed after staining was completed. The vegetative cells appeared pink and the spores appeared green. The spores of *Bacillus. cereus* were ellipsoidal, central to sub-terminal.

3. Glucose fermentation test: Inoculated selected colonies, using a stab inoculation wire into tubes containing glucose Agar medium. Incubated for 24 hours in an incubator at 30^{o}C.Development of yellow colour throughout the stab in the tube indicated the positive reaction. It was compared with the positive control.

4. Voges–Proskauer Test: Suspended a loopful of suspected colony into tube containing

0.2 ml of sterilized VP medium. Incubated at 37°C for 24hours. After incubation, added a drop of 0.5 % creatine solution, 3 drops of ethanolic solution of napthol and 2 drops of KOH solution. Appearance of pink red color within 15 minutes confirmed positive test. It was compared with positive control shown same result.

5. Nitrate reduction test: A loop full of the culture was inoculated into a tube containing (3ml) nitrate broth and incubated for 24 hours in incubator at 30°C. After incubation, 2 drops of sulphanillic acid (solution A) and two drops of alpha naphthol solution (solution B) were added to the tube. The appearance of red color in the medium after 3 minutes indicated a positive test i.e. the reduction of nitrate. Negative tests were confirmed by the addition of about 10 mg of zinc dust. Red coloration on addition of zinc dust was a confirmation of the negative result. Nitrate reduction test is positive in *Bacillus Cereus*.

Fig-8: Nitrate reduction test in *Bacillus Cereus*

Table-3: Biochemical confirmation tests for *Bacillus cereus*

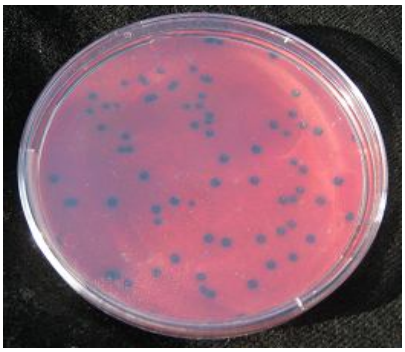
Test	Result	Positive control(*Bacillus cereus)*
Gram staining	Purple colored Gram positive bacilli in short to long chains	Positive
Spore staining	Green colored ellipsoidal spores	Positive
Glucose fermentation test	Acid produced (color changes from red to yellow).	Positive
Nitrate test	Reduces nitrates to Nitrites(red color of the medium)	Positive
VP test	Positive(pink color)	Positive

3.5 DETECTION OF SALMONELLA

Determination of the presence or absence of Salmonella was done in order to find out the contamination accompanied by member of Enterobacteriaceae.

PROCEDURE: Added 25gm of the sample in 225ml of sterilized Buffered Peptone Water. It was incubated at 37°C for 24 hrs. For enrichment culture, 01.ml of enriched culture was transferred to a tube containing 10ml of RV medium. Another 10ml of

enriched culture was transferred to a flask containing 100ml of Selenite Cystine medium. Incubated the inoculated RV medium at 42°C and inoculated Selenite cystine medium at 37°C for 24hrs. After incubation by means of a loop, a loopful of broth was streaked from RV medium on to 2 plates of BGA. Similarly loopful of broth was streaked from fluid Selenite cystine medium on to 2 plates of BGA. RV medium and Selenite cystine medium were also streaked on each of 2 plates of another selective medium XLD. All the inoculated plates at were incubated 37°C for 24 hrs. A pure culture on BGA & XLD plate was also streaked and incubated at 37°C for 24 hrs. After incubation, the colonies on each plate were observed. On XLD (after 24h) - Pink colonies with black centre on XLD and dark red colonies on BGA were appeared. The colonies were compared with the positive control (*Salmonella typhi*). Suspected colonies from each plate were streaked on Nutrient Agar plates and Incubated at 37°C for 24 hrs. After incubation colonies appeared on Nutrient Agar plates were used as pure culture for biochemical and serological confirmation



. Fig-9: Colonies of *Salmonella* on XLD

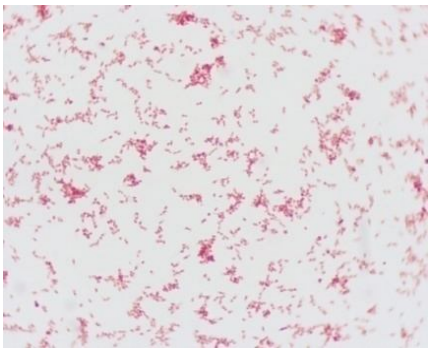


Fig-10: Gram negative rods of *Salmonella*

3.5.1 BIOCHEMICAL CONFIRMATION OF *Salmonella*: The confirmation of *Salmonella* was done by Gram staining and following biochemical tests.

1) Lactose/glucose fermentation and H2S production

Streaked a portion of suspected colony from NA plate on to a slant of TSI Agar slant & stabbed the remaining portion of colony into butt portion. Similarly TSI slant was inoculated with pure culture (*Salmonella* typhi). Both the slants were incubated at 37°C for 24 hrs. Test culture slant showed no appearance of typical *Salmonella* characteristics which were appeared in the positive control i.e. red slant (alkaline) with gas production. Yellow butt with acid production and blackening of Agar (H2S production) confirmed the absence of salmonella.

Fig-11**:** Lactose/glucose fermentation and H2S production on TSI agar in *Salmonella typhi*

2) Urea degradation

A portion of colony from NA plate was streaked onto a tube of urea Agar slant. A tube of urea agar slant was also inoculated with pure culture (*proteus*) which is positive for this test. Both tubes were incubated at 37^{o}C for 24 hrs. After incubation there was change in urea agar slant of positive control culture from phenol red to rose pink and no color change in urea agar slant inoculated with test culture. This reaction confirmed the absence of *salmonella* where urea degradation is absent.

3) L-lysine decarboxylose test

Inoculated tube containing lysine decarboxylase medium with culture just below the surface of medium. It was overlaid with sterile liquid paraffin and tube was incubated at 37^{o}C for 24 hrs. Colour change of the medium was observed after incubation. Yellow color indicated the negative test and purple colour indicated the positive test. It was compared with positive control (*Salmonella*) which developed purple colour.

4. ß-glactosidase test

Suspended a loopful of the suspected colony in a tube containing 0.25 ml of saline solution.Added 1 drop of toluene and shook the tube. Placed the tube in a water both set at 37^{o}C and left for several minutes. Added 0.25 ml of reagent for detection of B-glactosidase and mixed. Incubated the tube in a water bath at 37^{o}C and left for 24 hrs. Yellow colour appeared indicated that reaction is positive which confirmed the absence of salmonella because salmonella is negative for this test.

4) Voges- Praskauer test: (VP)

VP test was performed as described earlier for *E. coli*. There was formation of pink/red colour within 15 minutes after adding 2 drops of creatine sol., 3 drops of ethanolic solution of 1-napthol and 2 drops of KOH. It was compared with the positive control (*E. coli*) which showed the same reaction for this test that indicated the absence of *Salmonella.*

5) Indole Test

This test was performed as described earlier for *E. coli*. There was formation of red ring after additon Kovac's reagent. It was compared with the positive control (*E. coli)* which showed the same reaction for this test that indicated the absence of *Salmonella*.

3.5.2 SEROLOGICAL CONFIRMATION OF *Salmonella*: The presence of *Salmonella* O, H antigens was tested by slide agglutination with appropriate sera. One drop of saline solution was placed on a cleaned glass slide. A portion of suspected colony was mixed on it. The slide was gently rocked for 30-40 seconds. There was no agglutination which indicated that strain was not autoagglutinable.

3.5.2.1 Examination for O-antigens

Using another pure colony isolated from sample, same procedure was followed using one drop of anti-O-serum. One drop of anti-O-serum was rocked with colony of pure culture on another glass slide. Both the slides were observed for agglutination. No agglutination on test slide was observed but agglutination observed on the positive control slide indicated absence of *Salmonella* in test portion.

3.5.2.2 Examination for H antigens

Same procedure was followed with one drop of anti-H serum. No agglutination observed in test portion. Positive control showed agglutination indicated the absence of salmonella in test portion **(Table-5)**.

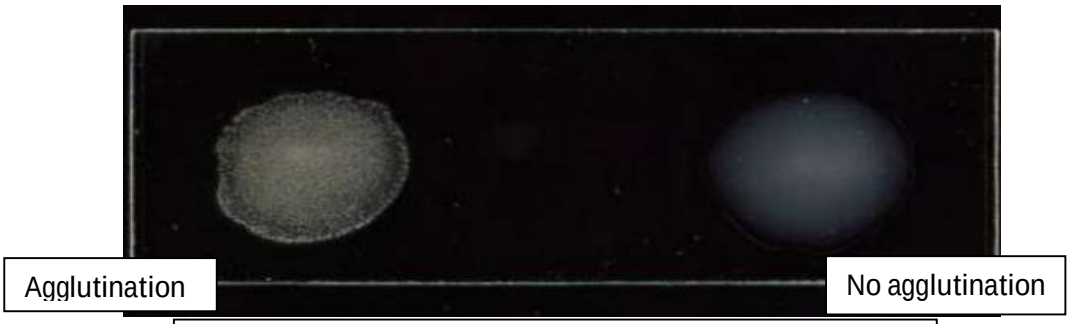


Fig-12**:** Slide agglutination test in *Salmonella* typhi

Table-4: Biochemical confirmation tests in *Salmonella*

Test	Result	Positive control(*Salmonella typhi)*
Lactose/glucose fermentation and H2S production		
TSI slant	Alkaline (red)	**Positive**
TSI Butt	Acid (Yellow)	**Positive**

H_2S production	blackening of agar throughout the tube	**Negative**
Urea degradation	No change in yellow color of medium	**Negative**
L-lysine decarboxylose test	Purple of medium	**Positive**
Voges-praskauer test (VP)	No red color	**Negative**
Indole test	No red ring appeared	**Negative**
Beta-Glactosidase test	yellow color of medium	**Negative**

Table-5: Serological confirmation tests for *Salmonella*

Test	**Result**	**Positive control(*Salmonella typhi)***
Anti-O-serum	Agglutination	**Positive**
Anti-H-serum	Agglutination	**Positive**

CHAPTER – IV

RESULTS

Spices are an integral part of Indian cookery. Some of the spices are added during the process of cooking while others are used for the cooked food generally before serving. Thus the microbiological quality of the spices is important particularly for the later. The contamination of spices is dependent on the production and processing technology used, which is highly variable, and the sanitary conditions of the workers and the processing unit. Conventional production of spices implicates number of hygienic problems and may be exposed to wide range of microbial contamination caused by food pathogens. Presence of spore formers, coliform and related species may pose serious health risk. In view of this, the present study was undertaken to assess microbial contamination of commonly used spices viz. coriander powder, ginger powder, turmeric powder, black pepper powder and red chilli powder. Samples were examined for total counts of aerobic mesophiles, coliform aerobic spore formers and yeast and mould. Samples were further examined for enteropathogenic *E.coli, Faecal Streptococcui* and *Salmonella.*. Ten samples of coriander powder were randomly collected from different sources were free flowing, dry of greenish yellow in color. General appearance, moisture content and pH of coriander powder is presented in table-6. The moisture content was in the range of 5.97% – 10.95% and pH 5.20 to 5.76. Counts of aerobic mesophilic bacteria, coliforms and yeast and mould were highly variable. No relationship was indicated to the moisture content or pH. Sample CP-3 showed least aerobic mesophiles count as 58×10^2 cfu/gm while coliforms count and yeast & mould was 159 cfu/gm and 90cfu/gm. The moisture content of the sample was 6.80% sample CP-1 with minimum moisture 5.97% showed maximum no. of aerobic mesophiles (215×10^2 cfu/g) and high number of coliforms (145cfu/gm) and yeast and mould (136cfu/gm). All results of different bacterial counts were correlated with one another. Raw materials may comprise a very wide range of items from primary agricultural products coming directly from the field to highly processed materials. Within a production environment, air may make contact with the product on many occasions and this will introduce a risk that any microorganisms present in the air may enter the product. The sources of contamination could be different that resulted in variable number of counts. However, the presumptive test based on most probable number (MNP) indicated positive test for *E. coli* and *Fecal Streptococcus* but *Salmonella* and aerobic spore former, *Bacillus cereus* (Table-6) were absent in this selected category of spices. Highest coliform count (172cfu/gm) and yeast and mold count(140cfu/gm) was in sample CP-2. All 10 samples were found to be contaminated with coliforms, *E. coli* and *Fecal Streptococcus*. MPN of *E. coli* was highest in CP-1(3.6/gm). *Fecal Streptococcus* was also positive in CP-1 and CP-10, although less in number (1.0/gm). Details of all results of parameters analyzed in coriander powder samples are presented in table-6.

Ten samples of ginger powder were collected and had uniformly light off white appearance. The moisture content (as determined according to BIS standard) was found to

be in the range of 6.96% in GP-5 to 12.04% in GP-2. This was within limits as per the BIS standards. The pH was in the range of 4.72 (GP-3) to 5.21 (GP-8). Details of all results of parameters analyzed in ginger powder samples are presented in table-7. Total viable counts of mesophilic bacteria were in the range of 160×10^2 cfu/gm for GP-8 and highest of 218×10^2 cfu/gm for GP-4. Coliform count was less than 10 cfu/gm for GP-1 , GP-4 and GP-8 while GP-2, GP-5, GP-6, GP-9, GP-10 showed 10 cfu/gm and GP-3 and GP-7 had 15 cfu/g. Coliform, *E. coli* , *Fecal Streptococcus, Bacillus cereus* and *Salmonella* counts were determined as per the BIS standard. Yeast & mould count were highest in sample GP-1 i.e. 332 cfu/g and lowest of 20 cfu/g in GP-5. Samples GP-6,GP-7 and GP-2 also showed highest Yeast & mould count205,250 and195 cfu/gm respectively. The counts of different samples were highly variable and did not show any particular as samples were from different sources (Table–7). Presumptive test for contamination using MPN showed the presence of pathogenic *E.coli*, Faecal *Streptococcus* but *Salmonella* and aerobic spore former *Bacilus. cereus* were found absent. MPN counts were taken from MPN tables per the BIS standards. Presumptive test indicated that the samples were contaminated with fecal matter (Table – 7).Details of the bacterial counts and the data of presumptive tests have been consolidated in the table-7.The results of ginger powder analysis showed that although samples were contaminated but pathogens of high risk (*Salmonella* and *B. cereus*)were not present.

Table-6: Results of different pathogens in Coriander powder samples taken for analysis

Sample No	Appearance	Moisture (%age)	pH	Aerobic Mesophillic Count (cfu/g)	Coliforms (cfu/g)	Yeast / Mould (cfu/g)	E. coli	Feacal Streptococci	Salmonella	Aerobic Spore former (*Bacillus Cereus*)
CP-1	Greenish Yellow Powder	5.97	5.20	215 x 10^2	145	136	3.6/gm	1.0/gm	Absent/25g	Absent/gm
CP-2	Pale Color Powder	9.90	5.41	86 x 10^2	172	140	2.4/gm	Absent/gm	Absent/25g	Absent/gm
CP-3	Greenish Yellow Powder	6.80	5.62	58 x 10^2	159	90	2.1/gm	Absent/gm	Absent/25g	Absent/gm
CP-4	Greenish Yellow Powder	10.95	5.35	134 x 10^2	145	40	2.0/gm	Absent/gm	Absent/25g	Absent/gm
CP-5	Greenish Yellow Powder	7.92	5.48	176 x 10^2	60	10	1.0/gm	Absent/gm	Absent/25g	Absent/gm
CP-6	Greenish Yellow Powder	7.52	5.76	165 x 10^2	120	80	2.3/gm	Absent/gm	Absent/25g	Absent/gm
CP-7	Greenish Yellow Powder	8.16	5.38	158 x 10^2	110	102	2.0/gm	Absent/gm	Absent/25g	Absent/gm
CP-8	Greenish Yellow Powder	9.10	5.69	140 x 10^2	115	120	2.7/gm	Absent/gm	Absent/25g	Absent/gm
CP-9	Greenish Yellow Powder	7.85	5.64	120 x 10^2	135	81	2.6/gm	Absent/gm	Absent/25g	Absent/gm
CP-10	Greenish Yellow Powder	6.82	5.39	135 x 10^2	122	73	2.8/gm	1.0/gm	Absent/25g	Absent/gm

Table-7: Results of different pathogens in Ginger powder samples taken for analysis

Sample No	Appearance	Moisture (%age)	pH	Aerobic Mesophillic Count (cfu/g)	Coliforms (cfu/g)	Yeast / Mould (cfu/g)	*E. coli*	Feacal Streptococci	*Salmonella*	Aerobic Spore former (*Bacillus Cereus*)
GP-1	Off white Powder	10.63	4.83	204 x 10^2	< 10	332	Absent/gm	Absent/gm	Absent/25g	Absent/gm
GP-2	Off white Powder	12.04	4.80	162 x 10^2	10	195	Absent/gm	Absent/gm	Absent/25g	Absent/gm
GP-3	Off white Powder	9.90	4.72	191 x 10^2	15	145	Absent/gm	Absent/gm	Absent/25g	Absent/gm

GP-4	Off white Powder	7.92	4.79	218 x 10^2	< 10	60	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-5	Off white Powder	6.96	4.84	179 x 10^2	10	20	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-6	Off white Powder	7.53	5.12	180 x 10^2	10	205	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-7	Off white Powder	8.65	4.85	172 x 10^2	15	250	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-8	Off white Powder	9.12	5.21	160 x 10^2	< 10	180	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-9	Off white Powder	7.89	4.89	158 x 10^2	10	160	Absent/gm	Absent/gm	Absent/25 g	Absent/gm
GP-10	Off white Powder	8.12	4.73	162 x 10^2	10	98	Absent/gm	Absent/gm	Absent/25 g	Absent/gm

Table-8: Results of different pathogens in Turmeric powder samples taken for analysis

Sample No	**Appearance**	**Moisture (%age)**	**pH**	**Aerobic Mesophillic Count (cfu/g)**	**Coliforms (cfu/g)**	**Yeast / Mould (cfu/g)**	***E. coli***	**Feacal Streptococci**	***Salmonella***	**Aerobic Spore former (*Bacillus Cereus*)**
TP-1	Yellow Color Powder	6.54	6.23	99 x 10^2	627	382	4.2/gm	2.0/gm	Absent/25g	Absent/gm
TP-2	Yellow Color Powder	5.79	6.31	182 x 10^2	536	227	3.4/gm	Absent/gm	Absent/25g	Absent/gm
TP-3	Yellow Color Powder	4.96	6.48	52 x 10^2	600	240	2.4/gm	Absent/gm	Absent/25g	Absent/gm
TP-4	Yellow Color Powder	11.99	6.35	85 x 10^2	400	172	2.7/gm	Absent/gm	Absent/25g	Absent/gm
TP-5	Yellow Color Powder	7.96	6.42	125 x 10^2	204	259	2.6/gm	Absent/gm	Absent/25g	Absent/gm
TP-6	Yellow Color Powder	5.80	6.45	102 x 10^2	250	170	4.4/gm	2.2/gm	Absent/25g	Absent/gm
TP-7	Yellow Color Powder	4.92	6.55	110 x 10^2	315	185	1.0/gm	Absent/gm	Absent/25g	Absent/gm
TP-8	Yellow Color Powder	6.53	6.72	95 x 10^2	248	202	1.0/gm	Absent/gm	Absent/25g	Absent/gm
TP-9	Yellow Color Powder	5.83	5.89	89 x 10^2	320	215	1.0/gm	Absent/gm	Absent/25g	Absent/gm

TP-10	Yellow Color Powder	5.76	6.48	115 x 10^2	306	160	2.9/gm	1.0/gm	Absent/25g	Absent/gm

Table-9: Results of different pathogens in Black pepper powder samples

Sample No	Appearance	Moisture (%age)	pH	Aerobic Mesophillic Count (cfu/g)	Coliforms (cfu/g)	Yeast / Mould (cfu/g)	*E. coli*	Feacal Streptococci	*Salmonella*	Aerobic Spore former (*Bacillus Cereus*)
BPP-1	Grayish Black Powder	9.76	5.70	139 x 10^2	118	< 10	2.0/gm	Absent/gm	Absent/25g	Absent/gm
BPP-2	Grayish Black Powder	11.93	5.65	100 x 10^2	136	25	2.1/gm	Absent/gm	Absent/25g	Absent/gm
BPP-3	Grayish Black Powder	8.30	5.80	148 x 10^2	90	40	2.0/gm	Absent/gm	Absent/25g	Absent/gm
BPP-4	Grayish Black Powder	7.97	5.75	63 x 10^2	114	60	2.8/gm	1.0/gm	Absent/25g	Absent/gm
BPP-5	Grayish Black Powder	11.99	5.69	116 x 10^2	80	35	1.0/gm	Absent/gm	Absent/25g	Absent/gm
BPP-6	Grayish Black Powder	9.78	5.79	120 x 10^2	115	10	1.0/gm	Absent/gm	Absent/25g	Absent/gm
BPP-7	Grayish Black Powder	10.93	6.21	135 x 10^2	85	20	2.3/gm	Absent/gm	Absent/25g	Absent/gm
BPP-8	Grayish Black Powder	8.50	5.82	155 x 10^2	92	35	2.6/gm	Absent/gm	Absent/25g	Absent/gm
BPP-9	Grayish Black Powder	8.97	5.96	78 x 10^2	112	30	1.0/gm	Absent/gm	Absent/25g	Absent/gm
BPP-10	Grayish Black Powder	10.29	5.75	82 x 10^2	82	15	1.0/gm	Absent/gm	Absent/25g	Absent/gm

Table-10: Results of microbial count in Red Chilli Powder Samples

Sample No	Appearance	Moisture (%age)	pH	Aerobic Mesophillic Count (cfu/g)	Coliforms (cfu/g)	Yeast / Mould (cfu/g)	*E. coli*	Feacal Streptococci	*Salmonella*	Aerobic Spore former (*Bacillus Cereus*)
RCP-1	Dark Red Powder	3.96	4.69	150 x 10^3	472	312 x 10^2	1.0/gm	Absent/gm	Absent/25g	10 cfu/g
RCP-2	Dark Red Powder	11.82	4.70	234 x 10^3	144 x 10^2	46 x 10^2	4.2/gm	2.2/gm	Absent/25g	Absent/gm
RCP-3	Dark Red	9.95	4.62	160 x 10^3	47 x 10^2	128 x 10^2	4.3/gm	2.0/gm	Absent/25g	Absent/gm

	Powder									
RCP-4	Dark Red Powder	4.88	4.72	224 x 10^3	144 x 10^2	87 x 10^2	4.4/gm	1.0/gm	Absent/25g	10 cfu/g
RCP-5	Dark Red Powder	7.97	4.71	103 x 10^3	128 x 10^2	1364	2.8/gm	1.0/gm	Absent/25g	Absent/gm
RCP-6	Dark Red Powder	8.55	5.10	180 x 10^3	112 x 10^2	42 x 10^2	3.5/gm	2.2/gm	Absent/25g	10 cfu/g
RCP-7	Dark Red Powder	7.65	4.86	175 x 10^3	80 x 10^2	54 x 10^2	4.4/gm	1.0/gm	Absent/25g	Absent/gm
RCP-8	Dark Red Powder	8.64	4.92	220 x 10^3	95 x 10^2	63 x 10^2	4.2/gm	2.3/gm	Absent/25g	Absent/gm
RCP-9	Dark Red Powder	8.59	4.97	198 x 10^3	102 x 10^2	83x 10^2	5.3/gm	2.7/gm	Absent/25g	10 cfu/g
RCP-10	Dark Red Powder	9.12	4.79	188 x 10^3	92 x 10^2	79 x 10^2	3.6/gm	2.0/gm	Absent/25g	Absent/g

Ten samples of turmeric powder TP-1 to TP-10 were collected and processed separately. General appearance of turmeric powder was yellow colored and powder was free flowing and dried in nature. The moisture content of the samples was highest in TP-4(11.99%) and lowest (4.92%) in sample TP-7. The pH range varied from 6.23 to 6.72. Results of analysis are presented in table-8.

The total counts of aerobic mesophilic bacteria ranges from 52×10^2cfu/gm to 182×10^2cfu/gm. Highest aerobic count was in sampleTP-2 and lowest in TP-3.However, the total Coliform count was highest in two samples TP-1 and TP-3(627cfu/gm and 600cfu/gm) while TP-4 showed 400 cfu/gm,TP-9 (320 cfu/gm) and TP-7 (315 cfu/gm) Coliform count respectively. and TP-5, 204 as total coliform colonies/g. This could be taken to be contaminated with fecal matter. Total counts for yeast and mould were also found to be higher compared to other samples of spices. Maximum yeast and mould conut 382 cfu/gm was reported in TP-1 and lowest of 160 cfu/gm inTP-10.

However, the presumptive test showed the presence of pathogenic *E.coli* as well as presence of *Faecal Streptococcus* in two samples. All samples were contaminated with *E.coli* with two samples TP-1 and TP-6 showed highest MPN count of 4.2 and 4.4/gm when results compared with MPN table. Two samples were contaminated with *Faecal Streptococcus* showed MPN count of 2.0 and 2.2/gm when results compared with MPN table. *Salmonella* and aerobic spore former, *B. cereus* was also found to be absent (Table - 8). The detailed results of analysis are presented in table- 8. It appeared that the samples are contaminated with animal feacal matter as showed the presence of *E.coli*. and *Faecal Streptococcus*.

Black pepper powder samples showed grayish black colour and afrom outer appearance it was looking that it has been prepared from healthy, black pepper seeds. Moisture content determined as per BIS standards showed nearly 12% in two samples BPP-2 (11.93%) and

BPP-5 (11.99%) and other samples has moisture content of range 7.97% to 10.93% respectively. pH of the samples varied in from 5.65 to 6.21 (Table-9).

Total counts of aerobic mesophilic bacteria, coliforms and yeast/mould are presented in table-9. Aerobic mesophiles which includes the members of enterobacteriaceae were also in fairly low numbers, the minimum being 63×10^2 cfu/gm in (BPP-4) and maximum counts were observed in sample BPP-8 as 155×10^2 cfu/gm. Other samples BPP-1, BPP-3 and BPP-7 showed mesophilic counts as 139×10^2 cfu/gm, 148×10^2 cfu/gm and 135×10^2 cfu/gm respectively. Total coliforms were in the range of 80 to 136 cfu/gm while yeast and mould count were less than 10cfu/gm in one sample (BPP-1) and 60 cfu/gm in another sample (BPP-4) which was the highest. Rest of the samples showed only 25, 40,20,30,15 and 35 cfu/gm in rest of the samples respectively (Table-9).

However, the presumptive MPN test of *E. coli* indicated the presence of pathogenic bacteria. MPN range of *E. coli* in samples was from 1.0-2.8/gm. Highest MPN count was in sample BPP-4(2.8/gm). It showed that all samples were contaminated with *E. coli* and one sample was contaminated with Faecal *Streptococcus* having MPN count 1.0/gm. Tests for other pathogens tested *Salmonella* and aerobic spore former (*B. cereus*) were negative. This may be taken to conclude that the mesophiles and coliforms were of aerial and animal origin. Detailed results of analysis are presented in tabl-9. The absence of pathogens *Salmonella* and aerobic spore former *B. cereus* indicated that samples are not related to dangerous health hazards.

Red chilli powder collected were deep red in appearance with moisture content in limits. One sample (RCP-2) showed highest moisture content of 11.82% followed by RCP-3 with 9.95% and RCP-10 with 9.12 % while RCP-1 had the lowest moisture content of 3.96%. pH of the samples was observed in range of 4.69 and 5.10. pH range showed that samples were acidic in nature. Detailed results of analysis of red chili powder samples are presented in table-10. Total viable counts of aerobic mesophiles, coliforms and yeast and mould count determined by using BIS standard methods were found to be in the higher range as compared to other samples of spices taken for analysis. Counts of mesophiles were higher by a factor of 10 while coliforms and yeast and moulds were high up to by a factor of 100. Results presented in table-10 showed highest counts of mesophiles in RCP-2 (234×10^3cfu/gm). This sample along with sample RCP-4 also had highest coliforms count of 144×10^2 cfu/gm. Mesophilic bacterial counts 224×10^3 cfu/gm in RCP-4, 220×10^3 cfu/gm in RCP-8 were close to RCP-2 sample. Coliforms count ranges from 472- 144×10^2 cfu/gm. Yeast and mould counts range in all RCP samples was 42×10^2 cfu/gm - 312×10^2 cfu/gm respectively RCP-1 was found to have high number of yeast and mould count 312×10^2cfu/gm, however coliforms count was only 472 cfu/gm. Presumptive test for pathogenic *E. coli., Feacal Streptococcus, Salmonella* and *Bacillus cereus* (an aerobic

spore former) was conducted as MPN. All samples were found to be contaminated with *E. coli.* and few were contaminated with *Feacal Streptococcus*(Table-10). MPN count range in *E. coli* was 1.0 to 5.3/gm. Highest MPN 5.3/gm was in sampe RCP-9. 4.4/gm MPN of *E.coli.* was also found in 2 samples RCP-4 and RCP-7 which was nearest to maximum. Three samples RCP-2, RCP-3, RCP_8 were in similar range of MPN count of *E. coli* i.e 4.2/gm, 4.3/gm,4.2/gm. MPN range of *Feacal Streptococcus* was 1.0/gm to 2.7/gm. Sample RCP-9 was highly contaminated with *E. coli.* and *Feacal Streptococcus* having MPN 5.3/gm in *E. coli.*and 2.7/gm in *Feacal Streptococcus.* Similarly *B. cereus* was confirmed in four samples RCP-1, RCP-4,RCP-6 andRCP-9 and counts was 10 cfu/gm. However, other samples were found to be free from pathogenic bacteria *B. cereus*. *Salmonella* was not detected in any of the red chilli powder sample and it was absent in all samples of this category of sample.

The presumptive test for red chilli powder showed positive results for *E. coli* and *Feacal Streptococcus and Bacillus cereus.* Selected colonies from the positive samples were subjected to confirmation on the basis of specific biochemical tests. For *E. coli* besides Gram staining and morphology, following biochemical tests were performed viz. indole production, MR test, VP test, citrate utilization, urease test and H_2S production. A positive control was also run. *E. coli.* appeared as small straight Gram negative rods. Presumptive isolates were positive for indole production and MR test while showed negative results for other four tests. Results tallied with the positive control. Four samples of red chilli powder were detected to be positive for *Bacillus cereus.* Selected colonies of *Bacillus cereus* were subjected to confirmation on the basis of biochemical tests. Besides Gram staining and spore staining was also performed. Growth was confirmed with MYP agar and glucose agar medium,VP test for acetyl methyl carbinol production and nitrate reduction test were performed. A positive control of *B. cereus* was also run along with the test colonies. All the tested colonies showed Gram positive rods and produced endospore as indicated by endospore staining. The spore were eliptical to round and centrally lcoated. *B. cereus* showed pink coloured colonies in Mannitol yeast extract polymyxin medium and was positive in glucose fermentation test as indicated by the change of purple colour to yellow colour. Cultures were also positive for VP test and nitrate reductase test. *Salmonella* in samples was confirmed on the basis of indole test, VP test, TSI, H_2S production, urease test, L-lysine decarboxylase and ß–galactosidase activities. None of the sample was found to be positive for *Salmonella* .Further confirmation was made by serological agglutination tests with O antigen and H antigen. Slide agglutination test was performed by taking standard antiserum for H antigen and O antigen. The absence of agglutination confirmed that there was not any contamination with *Salmonella* .

Production and marketing of spices in small scale industry, before they reach the consumer these are harvested and subjected to drying, cleaning, removal of dirt and grit, grinding and

packing. Quality will depend upon the quality of raw material, processing technology and sanitary conditions.The food borne illness could result from unsatisfactory sanitary conditions and contamination of food including spices.(Sehwab et. al. 1982).Spices can also be contaminated during transport from one place to another. The present study was under taken on the contamination of commonly used spices by aerobic mesophilic bacteria, coliforms, aerobic spore former *Bacillus cereus* and yeast and mould. Among the coliform *E. coli, Feacal streptococcus, Salmonella* were checked. Ten samples of each category of the spices were selected randomly and analyzed for contamination by these microorganism as per the BIS standards IS: 540,**1S**: 5402, **1S** :5403, **1S**:5887(1), **1S** :5887(2), **1S** :5887(3), **1S** :5887(6) and ISO: 6579.All the samples of Coriander powder, Ginger powder, Turmeric powder and Black pepper powder confirmed to the BIS standards and were considered safe from food illness point of view.

However, in case of red chilli powder samples, four samples RCP-1, RCP-4, RCP-6 and RCP-9 were found to be contaminated with *Bacillus cereus,* nine samples were contaminated with *Feacal streptococcus* and all samples were contaminated with *E.coli*. The presence of these bacteria was confirmed on the basis of biochemical tests performed for *E. coli.* & *Bacillus cereus*. Few presumptive colonies of bacteria looking like colonies of *Salmonella* were also tested with biochemical confirmation tests but failed to give test positive for *Salmonella* indicating that none on the sample was contaminated with *Salmonella*. *Salmonella* is considered as potential health hazard besides *S.aureus* (Wagner,Ir 2007). Similar results have been reported by Sehwab et. al. (1982); Omafuvbe and Kolawole (2004).Our analysis ensures that all samples were not carrying *Salmonella.*

CHAPTER-V

DISCUSSION

Spices are an integral part of Indian cookery and can be contaminated with microorganisms that survive harvesting, primary processing and drying process. Contamination in spices is caused by *salmonella, clostridium perfringens, coliforms* and aerobic spore formers such as *Bacillus cereus, B.licheniformis* and thermophillic anaerobes. Thus the microbiological quality of the spices is important. In view of this, the present study was undertaken to assess microbial contamination of commonly used spices viz. Coriander powder, Ginger powder, Turmeric powder, Black pepper powder and Red chilli powder. Samples were examined for total count of aerobic mesophiles, coliforms, aerobic spore formers, yeast and mould, *E coli*, *Faecal Streptococci* and *Salmonella.* Detail of sample collected is given in table-1.General appearance, moisture content and pH of coriander powder is presented in table-6. Sample CP-3 showed least aerobic mesophiles as 58×10^2 cfu/g while coliform were 159 cfu/gm and yeast and mould 90 cfu/gm. The moisture content of the sample was 6.80% sample CP-1 with minimum moisture 5.97% showed maximum no. of aerobic mesophiles (215×10^2) and high number of coliforms (145 cfu/gm) and yeast/mould (136 cfu/gm).

Sample GP-8 of ginger powder showed aerobic mesophiles of 160×10^2 cfu/g and count of GP-4 was 218×10^2 cfu/gm. GP-3 and GP-7 showed coliform count 15 cfu/gm, yeast and mould count were highest in GP-1 i.e. 332 cfu/gm. The total count of aerobic mesophilic bacteria in turmeric powder sample was highest in TP-2(182 x 10^2cfu/gm) and least aerobic count was in TP-3(52 x 10^2cfu/gm). Five samples have less than 100 x 10^2 cfu/gm mesophilic count. However, the total coliforms was more than 600 cfu/gm in two samples (TP-1, TP-3) while TP-4 showed 400 coliforms/gm. Yeast and mould count was maximum as 382 cfu/gm in TP-1 and minimum 160cfu/gm in TP-10. Samples of Black pepper BPP-8, BPP-3, and BPP-1 showed mesophilic count as 155 x 10^2 cfu/gm, 148 x 10^2cfu/gm and 139 x 10^2 cfu/gm which was higher than other samples. Total coliforms was in range of 80 to 136 cfu/gm. While yeast and mould count were less than 10 cfu/gm in BPP-1 and 60 cfu/gm in BPP-4. Aerobic mesophilic count in Red chilli powder was more than 200 x 10^3 cfu/gm in three samples RCP-2, RCP-4, RCP-8 (234 x 10^3 cfu/gm, 224 x 10^3 cfu/gm, 220 x 10^3 cfu/gm) and near about 200 x 10^3 cfu/gm in three samples RCP-6, RCP-9, RCP-10(180 x 10^3 cfu/gm, 198 x 10^3 cfu/gm, 188 x 10^3 cfu/gm). The range of coliforms count was 472 in RCP-1, 144 x 10^2 cfu/gm in RCP-2 and RCP-4), Yeast and mold count was highest in RCP-1(312 x 10^2 cfu/gm). All samples were found to be contaminated with *E. coli.* and a few were contaminated with *Feacal Streptococcus*(Table-10). Highest MPN 5.3/gm was in sample RCP-9. MPN count of *E.coli.* 4.4/gm was also found in 2 samples RCP-4 and RCP-7 which was nearest to maximum. MPN range of *Feacal Streptococcus* was 1.0/gm to 2.7/gm. Sample RCP-9 was highly contaminated with *E. coli.* and *Feacal Streptococcus* having MPN 5.3/gm in *E. coli.* and 2.7/gm in *Feacal Streptococcus.* Similarly *B. cereus* was confirmed in four samples RCP-1, RCP-4,RCP-6 andRCP-9 and

counts was 10 cfu/gm. Red chilli powder sample was highly contaminated category of spices sample collected for analysis. *Salmonella* was not detected in any of the red chilli powder sample and it was absent in all samples of this category of sample

Salmonella was absent in all samples of spices. *E. coli* was present in four categories of spices except ginger powder sample. The contamination of spices is highly variable and does not depend upon a single factor.

Sanitary conditions are the key to prevention of food infection. For spices, the post harvest technology is most important. The produce should be dried to less than 10% moisture content, remain all type of foreign matter like fecal material, stone, dust, twigs etc. besides personal hygiene and sanitization of all equipments utensils and surfaces used during processing are important in control of microbial contamination of spices. The personnel involved in handling and processing should be suitably trained in personal hygiene and basic knowledge of microorganisms involved in food poisoning should be provided. Such a step will go a long way in prevention of food contamination.

CHAPTER -VI

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CHAPTER -VII

CULTURE MEDIA USED

1 CULTURE MEDIA FOR TOTAL PLATE COUNT

a. Dilution Medium (Peptone salt solution)

Enzymatic digest of Casein	–	1.0 g
NaCl	–	8.5 g
Distilled Water	–	1000 ml

Dissolved the components in water and pH was set so that after sterilization it was 7.0 at 25^0C.

b. Plate Count Agar

Pancreatic digest of Casein	–	20.0 g
Yeast Extract	–	20.0 g
Glucose	–	4.0 g
Agar	–	10.0 g
Distilled Water	–	1000 ml
pH	–	7.0 at 25 ^{0}C

The media was sterilized at 121^0C for 15 minutes.

2 .CULTURE MEDIA FOR COLIFORMS

a. Crystal Violet neutral red bile Lactose Agar (VRBL)

Peptone	–	7.0 g
Yeast Extract	–	3.0 g
Lactose	–	10.0 g
NaCl	–	5.0 g
Bile salt	–	1.50 g
Neutral red	–	0.03 g
Crystal Violet	–	0.002 g
Agar	–	15.0 g
D.W.	–	1000 ml

Mixed the contents and it was sterilized by boiling and adjusted pH to 7.40 before sterilization.

3. CULTURE MEDIA FOR YEAST AND MOULD COUNT

a. Dilution medium (Peptone salt solution)

b. Yeast extract Dextrose Chloramphenicol Agar medium (YDA):

Yeast Extract	–	5.0 g
Dextrose	–	20.0 g
Chloramphenicol	–	0.1 g
Agar	–	15.0 g
Distilled Water	–	1000 ml
pH	–	6.6

The components were dissolved in the water by boiling and medium was sterilized by autoclaving at 121^0C for 15 minutes. The final pH of the medium was 6.6.

4. CULTURE MEDIA FOR *E. coli.*

a. Nutrient broth

Peptone	–	10.0g
Meat Extract	–	10.0g
NaCl	–	5.0g
Distilled Water	–	1000ml

It was sterilized by autoclaving at 12^oC for 15 minutes and adjust pH so that after sterilization it was 7.5 - 7.6.

b. Nutrient Agar

Nutrient agar medium was prepared by adding Agar (2%) in nutrient broth and sterilized by autoclaving at 121^oC for 15 minutes. Slants and plates were prepared by pouring of 15-20ml of media in petriplate and 10 ml media in tubes in case of slants. After complete solidification tubes and plates were kept for isolation of pure culture.

c. Mac Conky Broth

Peptone	–	20.0g
Lactose	–	10.0g
Bile salts	–	5.0g
NaCl	–	5.0g

Neutral red	–	0.07g

A Durham tube was transferred into it before autoclaving .Durham tube was adjusted so that no air bubble was present in it. I was Sterilized by autoclaving at 121^{o}C for 15 minutes and pH was adjusted so that after sterilization, it was 7.5+0.2.

d. Mac Conky Agar

Added 2% of Agar in single strength Mac Conky broth and it was sterilized by autoclaving at 121^{o}C for 15 minutes.

Bile salts	–	5g
Peptone	–	20g
NaCl	–	5 g
Agar	–	15 g
Distilled water	–	1000 ml

Steamed it untill the solids were dissolved. Adjust pH 7.6. Autoclaved at 121^{o}C for 15min and filtered while hot. After rejection of filterate adjust pH 7.5 at room temperature. Then added 10 g of lactose and 3.5 ml of 2% solution of neutral red in 50% ethanol. Mixed thoroughly and it was sterilized by autoclaving at 121^{o}C for 15min.

e. Eosin Methylene blue lactose Agar (EMB)

Peptone	–	10.0g
K_2HPO_4	–	2.0g
Agar	–	15.0 gm
Lactose	–	10.0gm
Eosin-y	–	0.40 gm
Methylene blue	–	0.065gm
Distilled Water	–	1000 ml

It was sterilized by autoclaving at 121^{o}C for 15 minutes. Final pH after sterilization was 7.1 ± 0.1.

f. Tergitol-7-Agar

Peptone	–	5.0g
Yeast extract	–	3.0g
Lactose	–	10.0g

Agar	–	15.0g
Tergitol	–	10ml
Bromothymol blue	–	0.025g
Distilled Water	–	1000ml

It was sterilized by autoclaving at 121°C for 15 minute and final pH was 6.9.Plates for were prepared for confirmation of colonies.

g. TSI Medium for H_2S Test

Meat Extract	–	3.0g
Yeast Extract	–	3.0g
Peptone	–	20.0g
Glucose	–	1.0g
Lactose	–	10.0g
$FeSO_4$ $7H_2O$	–	0.2g
NaCl	–	5.0g
Sod. Thiosulphate	–	0.3g
Agar	–	15.0g
0.2% Phenol red sol.	–	12ml
Distilled Water	–	1000ml

It was sterilized by autoclaving at 115°C for 20 minutes and poured into sterilized test tubes and cooled to form a slope with deep butts.

h. Medium for Urease test

Peptone	–	1.5g
NaCl	–	5g
KH_2PO_4	–	2g
1:500 aqueous sol.of Phenol red sol.	–	6 ml
Agar	–	15g
Distilled Water	–	1000ml
pH	–	6.8 –6.9

It was sterilized by autoclaving at 120°C for 15 minutes. When solution was cooled to

about 50°C, added a sterile solution of glucose to a final concentration of 0.1% and added 100ml of 20% solution of urea previously sterilized by seitz filteration.

i. Medium for Indole Production

Peptone	–	20 g
NaCl	–	5g
Distilled water.	–	1000ml
pH	–	7.4

Media was poured in tubes in 5 ml amount and was sterilized at 121°C for 15 minutes.

j. Medium for Methyl Red and Voges-Proskauer Tests

Peptone	–	5 g
KH_2PO_4	–	5 g
Glucose	–	5 g
pH	–	7.5
Distilled water	–	1000ml

Media was Poured in tubes in 5 ml amount and was sterilized at 115°C for 10 minutes

k. Simmon's Citrate Agar

Sod. Citrate	–	2 g
NaCl	–	5 g
Magnesium Sulphate	–	0.2 g
Amm. Dihydrogen Phosphate	–	1 g
Dipotassium Hydrogen Phosphate	–	1 g
0.2 % bromothymol blue	–	40 ml
Agar	–	15g
Distilled Water	–	1000ml
pH	–	6.8 + 0.1

Media was sterilized at 121°C for 15 minutes. After sterilization plates were prepared.

5. CULTURE MEDIA FOR *Feacal streptococci*

a. Ethyl violet Azide Dextrose broth

Tryptose	–	20.0 g
Dextrose	–	5.0 g
Dipotassium phosphate	–	2.7 g
Mono Potassium Phosphate	–	2.7 g
NaCl	–	5.0 g
Sodium Azide	–	0.4 g
Ethyl violet	–	0.00083 g
Distilled water	–	1000 ml
pH	–	7

Media was sterilized at 121^{o}C for 15 min.

b. Ethyl violet Azide dextrose Agar: To the ethyl violet azide dextrose broth, 15 g of agar was added and dissolved by heating. The media was sterilized at 121^{o}C for 15mins.

c. MacConkey Agar : Already mention

6. CULTURE MEDIA FOR AEROBIC SPORE FORMER *Bacillus cereus*

a. Base Medium

Beef Extract	–	1.0g
Peptone	–	10.0g
Mannitol	–	10.0g
Nacl	–	10.0g
Phenol red	–	0.025g
Agar	–	15g
Distilled Water	–	900ml
pH	–	7.2

The components were dissolved and media was sterilized by autoclaving at 121^{o}C for 15 minutes.

b. Polymyxin -B solution (FD003) – Procured from M/s Hi media, Mumbai

c. Egg yolk emulsion (FD045) – Procured from M/s Hi media, Mumbai

d. Complete medium: (MYP Agar)

Base Medium	–	90ml
Polymyxin B Solution	–	1ml

Egg yolk emulsion	–	10.0 ml

The base medium was melted and cooled in a water bath set at 50°C. Egg yolk emulsion and Polymyxin -B were added aseptically. The plates were prepared by pouring 15ml portion of complete medium into sterilized Petriplate and allowed to solidify.

e. Glucose Agar

Tryptone	–	10g
Yeast extract	–	1.5g
Glucose	–	10g
Nacl	–	5.0g
Bromo cresol purple	–	0.015g
Agar	–	15g
Distilled water	–	100ml

The contents were dissolved by heating and media was sterilized in an autoclave at 121°C for 15 minutes. Media was dispensed into test tubes and stabs were prepared.

f. Nitrate Medium

Peptone	–	5 g
Beef extract	–	3 g
Potassium Nitrate	–	1 g
Distilled water	–	1000 ml

Media was sterilized by autoclaving at 121°C for 15 minutes. pH was adjusted so that after sterilization it was 7. The medium was dispensed in quantities of 5.0 ml into sterile test tubes.

g. Nitrate Reagent

(A) 5-Amino-2-Napthalene Sulfonic Acid (5-2 ANSA) Solution:

5-2 ANSA	–	0.1 g
Acetic Acid(2.6 mol/ l)	–	100 ml

Contents were dissolved and filtered through whatman H1 filter paper.

(B) Sulfanilic Acid solution

Sulfanilic Acid	–	0.4 g
Acetic Acid	–	100 ml

Contents were dissolved and filtered through filter paper.

Preparation of Complete reagent: Just prior to use, equal volumes of two acid solutions were mixed (A & B).

h. Zinc Dust:(Ready made from MERK)

7. CULTURE MEDIA FOR SALMONELLA

a. Non selective pre enrichment medium: (Buffered peptone water)

Peptone	–	10.0g
NaCl	–	5.0g
($Na_2HPO_4.12H_2O$) Disodium hydrogen	–	9.0g
Phosphate dodecahydrate		
Potassium dihydrogen phosphate	–	1.5g
Distilled water	–	1000ml

The medium was sterilized by autoclaving at 121^oC for 20 minutes.

b. First selective enrichment medium: Rappaport Vassilliadis Magnesium Chloride Malachite green medium (RV medium)

1. Solution A

Tryptone	–	5.0g
NaCl	–	8.0g
Potassium dihydrogen phosphate	–	1.6g
Distilled water	–	1000ml

The components were dissolved in water by heating to about 70^oC.

2. Solution B

Magnesium Chloride Hexahydrate	–	400.0g
($MgCl_2$ $6H_2O$)		
Distilled water	–	1000ml

3. Solution C

Malachite green oxalate	–	0.4g
Water	–	100ml

Malachite green oxalate was dissolved in the water. The solution was kept in brown bottle at room temperature.

4 Complete Medium

Solution A	–	1000ml
Solution B	–	100ml
Solution C	–	10ml

The three solutions were in ratio indicated above were mixed and sterilized by autoclaving at 115^{o}C for 15 minutes.

c. Second selective enrichment medium: (Selenite cystine medium)

1. Base: Tryptone	–	5.0g
Lactose	–	4.0g
Disodium hydrogen phosphate dodecahydrate	–	10.0g
Sodium hydrogen selenite	–	4.0g
Distilled water	–	1000ml

The first 3 basic components were dissolved in the water by boiling for 5 min. After cooling, added sodium hydrogen selenite.

2. L-Cystine Solution

L- Cystine	–	0.1 g
NaOH Solution (1mol/l)	–	15 ml
Sterile water to a final volume	–	100 ml

Placed the components in a sterile flask and diluted to 100ml of sterile water.

3. Complete Medium

Base	–	1000ml
L-Cystine solution	–	10 ml

Cooled the base and L- Cystine solution added aseptically. Adjusted pH at 7.0.

d. Selective Solid Plating out Media:

1. First selective Medium: (Phenol red / Brilliant Green Agar)

1(A) Base

Meat Extract powder	–	5.0g
Peptone	–	10.0 g

Yeast Extract Powder	–	3.0 g
[Na_2HPO_4] Disodium Hydrogen Phosphate	–	1.0 g
[NaH_2PO_4] Sodium Dihydrogen Phosphate	–	0.6 g
Agar	–	15 g
Distilled Water	–	900ml
pH	–	7.0

Media was by autoclaving at 121^{o}C for 15 mins.

I (B) Sugar/ Phenol red solution

Lactose	–	10.0 g
Sucrose	–	10.0 g
Phenol Red	–	0.09 g
Distilled Water	–	100ml

Contents heated in a water bath at 70^{0}C for 20 minutes. It was cooled to 55^{0}C and used immediately.

1(C) Brilliant Green solution

Brilliant Green	–	0.5 g
Distilled water	–	100ml

The solution for was stored for 1 day in the dark to allow auto-sterilization to occur.

1(D) Complete Medium

Base	–	900 ml
Sugar Phenol red solution	–	100ml
Brilliant green solution	–	1 ml

Under aseptic conditions, base, brilliant green solution and sugar/ phenol red solution were mixed and then maintained at 50^{o}C – 55^{o}C.Plates were prepared by pouring 15 ml of complete medium into sterile petri plates.

2 Second (Selective Medium) Medium:[Xylose Lysine Deoxycholate Agar]

Yeast Extract	–	3.0 g
L-lystine	–	5.0g

Lactose	–	7.5g
Sucrose	–	7.5g
Xylose	–	3.50g
NaCl	–	5.0g
Sodium deoxycholate	–	2.5g
Sodium thiosulphate	–	6.8g
Ferric ammonium citrate	–	0.8g
Phenol red	–	0.08g
Agar	–	15g
Distilled water.	–	1000 ml

It was sterilized by heating. It was not autoclaved.

e. Nutrient Agar

Meat Extract	–	3.0g
Peptone	–	5.0g
Agar	–	15g
Distilled water	–	1000ml
pH	–	7.0g

Medium was sterilized by autoclaving at 121^{o}C for 20 minutes. Plates were Prepared from sterilized medium.

f. Triple sugar/Iron Agar: (TSI Agar)

Meat Extract	–	3.0g
Yeast Extract	–	3.0g
Peptone	–	20.0g
NaCl	–	5.0g
Lactose	–	10.0g
Sucrose	–	10.0g
Glucose	–	1.0g
Iron (III) citrate	–	0.3g

Sodium thiosulphate	–	0.3g
Phenol red	–	0.024g
Agar	–	15g
Distilled Water	–	1000ml
pH	–	7.4

Medium was sterilized by autoclaving at 121°C for 10 minutes. It was allowed to set in a sloping position to give a butt of depth 2.5cm.

g. L-Lysine Decarboxylation Medium

L-Lysine monohydrate	–	5.0 g
Yeast Extract	–	3.0 g
Glucose	–	1.0 g
Bromocresol Purple	–	0.015 g
Distilled water	–	1000 ml
pH	–	6.8

The contents were dissolved by heating. It was sterilized by autoclaving at 121°C for 10 minutes. The medium was transferred in quantities of 5 ml into tubes.

h. Beta. Glactosidase reagent

h.I: Buffer solution

(NaH_2PO_4) Sodium dihydrogen phosphate	–	6.9g
Sodium hydroxide 10ml/l	–	3ml
Water to a final volume of	–	50 ml
pH	–	7.0

h-II: ONPG solution:

O-Nitrophenyl Beta-D- glactopyranoside (ONPG)	–	0.08g
Distilled Water	–	15ml

h-III. Complete medium:

Buffer sol.	–	5ml
ONPG sol.	–	15ml

Buffer solution was added to ONPG solution and complete medium was prepared.

i. Saline solution

NaCl	–	8.5g
Distilled water	–	100ml

It was sterilized by autoclaving at 121^{o}C for 20 minutes.

j. Sera

Poly-o-serum containing antibodies for several O antigens(BD Difco).

Poly-H-serum containing antibodies for several H antigens(BD Difco).

CHAPTER-VIII

EQUIPMENT AND MATERIAL USED

1. Two Autoclaves of sufficient size with calibrated thermometer and pressure gauge. One for sterilization of media and another for discarded plates / used media, etc.
2. Balance ranges from 0.1 g to 200 g load.
3. Colony Counter, Blenders / Stomacher, Vortex – mixer.
4. Dilution and media storage bottles.
5. Rimless glass test tubes of 16 x150 mm with cap.
6. Hot air ovens for sterilization of glass and metal ware with range between 150-185oC.
7. Glass bent rods with fine polished edges, 3-4 mm diameter, 15-20 cm long with angled spreading surface 45-55 mm long.
8. Howard mold count chamber and haemocytometer.
9. Incubators to be operated at 25^{o}C 30^{o}C, 37^{o}C, 44.5^{o}C and 55^{o}C.
10. Inoculating loops and wires. (3-5 mm diameter of nichrome or platinum), Magnetic stirrer, Microscopic slides and cover slips, Non-adsorbent cotton, Thermometers.
11. Microscope binocular with 900 x and higher magnification.
12. Petri plate and containers. (Stainless steel or aluminium, with covers for hot air sterilization of glass Petri plates.
13. Micropipettes with capacity from 1ml to 10 ml with error < ±5 % with autoclavable plastic tips.
14. Electronic pH meter with accuracy of 0.01.
15. Test tube racks and baskets to hold test tubes.
16. Water bath for holding media at 44-46^{o}C.
17. Laminar airflow chamber and Biological safety cabinet level II.

ANNEXURE-I

IS : 5887 (Part I) - 1976

TABLE 1 MOST PROBABLE NUMBER (MPN) OF *ESCHERICHIA COLI*
(*Clause* 7.3)

Number of Positive Tubes per Dilution			MPN	Number of Positive Tubes per Dilution			MPN	Number of Positive Tubes per Dilution			MPN
10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}	
(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
0	0	0	0·30	1	1	1	1·1	2	2	3	4·2
0	0	1	0·30	1	1	2	1·5	2	3	0	2·9
0	0	2	0·60	1	1	3	1·9	2	3	1	3·6
0	0	3	0·90	1	2	0	1·1	2	3	2	4·4
0	1	0	0·30	1	2	1	1·5	2	3	3	5·3
0	1	1	0·61	1	2	2	2·0	3	0	0	2·3
0	1	2	0·92	1	2	3	2·4	3	0	1	3·9
0	1	3	1·2	1	3	0	1·6	3	0	2	6·4
0	2	0	0·62	1	3	1	2·0	3	0	3	9·5
0	2	1	0·93	1	3	2	2·4	3	1	0	4·3
0	2	2	1·2	1	3	3	2·9	3	1	1	7·5
0	2	3	1·6	2	0	0	0·91	3	1	2	12·0
0	3	0	0·94	2	0	1	1·4	3	1	3	16·0
0	3	1	1·3	2	0	2	2·0	3	2	0	9·3
0	3	2	1·6	2	0	3	2·6	3	2	1	15·0
0	3	3	1·9	2	1	0	1·5	3	2	2	21·0
1	0	0	0·36	2	1	1	2·0	3	2	3	29·0
1	0	1	0·72	2	1	2	2·7	3	3	0	24·0
1	0	2	1·1	2	1	3	3·4	3	3	1	46·0
1	0	3	1·5	2	2	0	2·1	3	3	2	110·0
1	1	0	0·75	2	2	1	2·8	3	3	3	110·0
				2	2	2	3·5				

ANNEXURE-II

TABLE 1 MOST PROBABLE NUMBER (MPN) OF FAECAL STREPTOCOCCI
(*Clause* 8.2.2)

Number of Positive Tubes per Dilution			MPN	Number of Positive Tubes per Dilution			MPN	Number of Positive Tubes per Dilution			MPN
10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}	
(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
0	0	0	0·30	1	1	1	1·1	2	2	3	4·2
0	0	1	0·30	1	1	2	1·5	2	3	0	2·9
0	0	2	0·60	1	1	3	1·9	2	3	1	3·6
0	0	3	0·90	1	2	0	1·1	2	3	2	4·4
0	1	0	0·30	1	2	1	1·5	2	3	3	5·3
0	1	1	0·61	1	2	2	2·0	3	0	0	2·3
0	1	2	0·92	1	2	3	2·4	3	0	1	3·9
0	1	3	1·2	1	3	0	1·6	3	0	2	6·4
0	2	0	0·62	1	3	1	2·0	3	0	3	9·5
0	2	1	0·93	1	3	2	2·4	3	1	0	4·3
0	2	2	1·2	1	3	3	2·9	3	1	1	7·5
0	2	3	1·6	2	0	0	0·91	3	1	2	12·0
0	3	0	0·94	2	0	1	1·4	3	1	3	16·0
0	3	1	1·3	2	0	2	2·0	3	2	0	9·3
0	3	2	1·6	2	0	3	2·6	3	2	1	15·0
0	3	3	1·9	2	1	0	1·5	3	2	2	21·0
1	0	0	0·36	2	1	1	2·0	3	2	3	29·0
1	0	1	0·72	2	1	2	2·7	3	3	0	24·0
1	0	2	1·1	2	1	3	3·4	3	3	1	46·0
1	0	3	1·5	2	2	0	2·1	3	3	2	110·0
1	1	0	0·73	2	2	1	2·8	3	3	3	110·0
				2	2	2	3·5				

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She has attended and presented papers in many conferences. She has published 5 Research paper in International journals and 2 papers in National Journals.

ABOUT THE BOOK

Dried spices are important food commodity and are used as an ingredient in variety of foods. However, there is a vast spoilage of spices due to contamination with pathogens which is becoming potential hazard for public health. This book was written with objective to identify the biological and chemical hazard that can lead to major natural contamination in food products associated with use of spices. To provide knowledge on biological hazards of spices through analytical methods for appropriate determination of microbes is also the one of the aim of this book. This book is valuable in getting knowledge that how the contributing factors can be analyzed and how the pathogens responsible for spoilage of spices can be detected. This book also has some review of literature on the presence of pathogens in spices showing the evidence of food borne illness outbreak which is correlated with work done. This book enlighten that how the contamination of valuable spices can be detected and preventive measures can be taken timely to avoid product loss. Thoroughly knowledge of pathogens in spices, following control measures for their prevention and making food containing spices safer is my small effort in the form of this book.

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