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MICROBIOLOGICAL STUDIES ON SOME COMMONLY USED SPICES IN PAKISTAN

Seema Ismat Shamshad, Rabia Zuberi and R.B. Qadri

PCSIR Laboratories, Karachi-39

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Microbiological investigations were carried out on commonly used spices such as turmeric, coriander, red chilli and black pepper, available locally both packed and un-packed. The total bacterial count in packaged samples ranged from 1.3×10^5 CFU/g (coriander) to 2.8×10^7 CFU/g (black pepper). The corresponding total bacterial count in unpacked samples ranged from 1.4×10^5 CFU/g (red chilli) to 4.7×10^7 CFU/g (turmeric). No significant difference was observed in total bacterial count in unpacked and packaged samples. Coliforms were present in most of the samples tested, ranging from 0 to> 1100/g. Chilli samples were found to be contaminated with faecal coliforms.

No *Salmonella* and enterococci were isolated from any sample. *Bacillus licheniformis* was the dominant species found in spices, followed by *B. pumilus, B. subtilis, B. cereus, B. sphaericus, B. megaterium, B. laterosporous, Micrococcus* sp., and *B. coagulans.*

Aspergillus niger and *A. flavus* were the dominating fungi isolated from both types of the samples. No yeast was isolated from any sample.

INTRODUCTION

Spices, as in all tropical countries, are essential ingredients of the Pakistani diet. Various kinds of spices are added to different dishes in varying amounts. Recently certain spices, both in bulk and small fancy packets are exported from Pakistan to many countries. Spices are essential part of flavouring in many daily food products of western countries as well. The bacteriostatic and bactericidal effects of spices were known to the Egyptians, who preserved dead bodies (mummies) by plastering them with spices. Recent investigations [1-7] have, however, shown that spices may harbour large number of microorganisms including bacteria, yeast and fungi. Uncleaned spices have been found to be grossly contaminated and contamination of food by spice carriers has been reported to result in food spoilage and may even lead to food poisoning (3, 4, 8]. Spore formers may cause food spoilage after surviving the cooking process and multiplying under favourable conditions [4, 9].

No information is available on the microbial flora of spices from this region. Locally different spices are available in the market in bulk, both whole and powdered, from wholesalers and retailers. Packaged spices are also available under different commercial brands and are also being exported. In order to compete in the world market, our products must be of good quality. It is, therefore, imperative to maintain rigid microbiological quality control of these valuable commodities meant for external and internal trade.

In the present paper an attempt has been made to determine the microflora in certain spices consumed locally and meant for export. Another purpose was to find out the difference in microbiological quality of unpacked and packed spices available in the market. The data may also serve as a basis for microbiological quality requirements and standards of spices.

MATERIALS AND METHODS

Samples: Most commonly used spices such as turmeric, coriander, red chilli and black pepper were selected for the study. Unpacked samples and packaged samples of six different popular brands were obtained from six different localities at Karachi and designated as A, B, C, D, E, and F.

Preparation of samples: All samples were obtained in powdered form except for the unpacked black pepper which was powdered in a grinder before analysis. 10 g. of each sample weighed into sterile polythene bag, was added to 90 ml sterile diluent (0.1% peptone and 0.85% NaCl), placed in a flask and shaken vigorously for 2-3 min. in order to suspend the organisms, and 10-fold dilutions were made. A 0.1 ml portion of appropriate dilution was spread over various media as detailed below. Unless otherwise stated, all media used in this investigation was from E. Merck, Darmstadt, Germany.

Aerobic bacterial count: Total aerobic bacterial counts were determined on Iso plate count agar after incubation at 30° for 48 hr.

Yeast, and Fungi: Yeast and fungi were enumerated on Sabouraud's dextrose agar and Czepack's Dox agar respectively.

Coliforms and faecal coliforms: Total and faecal coliforms most probable number (MPN) determinations were performed in accordance with the methods decribed by Thatcher and Clark [10].

Staphylococci and Enterococci: Staphylococcus medium 110 (Difco) was used for the enumeration of staphylococci and Azide broth was used for streptococci.

Salmonella: Twenty-five g. sample each was added aseptically to a flask containing 225 ml of lactose broth and incubated at 37° for 24 hr. After incubation, 50 ml of lactose broth culture was added to 50 ml double strength selenite enrichment broth and incubated at 43° for 18 hr. After thorough shaking, the enriched broth was streaked on to the surface of brilliant green agar and bismuth sulphite agar. Brilliant green plates were incubated at 37° for 24 hr. and the bismuth sulphite plates at 37° for 48 hr. Suspected colonies (3-5) from each plate were isolated on nutrient agar slants. All the suspected isolates were examined for Gram staining and oxidase reaction and inoculated into TSI, Urea agar and SIM medium.

Identification of organisms: To determine microbial types a certain number of colonies (20-30) were isolated at random from countable plates. The isolates were identified according to Bergey's "Manual of Determinative Bacteriology" [11, 12] and with the use of the Knight & Proom method 13].

The following tests were performed for the identification of isolates.

Gram reaction, morphology, colony characteristics, motility, nitrate reduction, catalase production, acetoin production, acid and gas from glucose, acid from xylose, and mannitol, starch hydrolysis, growth in anaerobic agar, growth in NH_3-basal medium [13], egg yolk reaction, lecithinase production, haemolysis, oxidation and fermentation reaction in Hugh's Leifson medium. All media were autoclaved at 15 lb. for 15 min. while sugar containing media were sterilized at 10 lb for 15 min.

RESULTS AND DISCUSSION

A total of 46 samples were examined microbiologically (Table 1). In general, the results are in agreement with those found by other workers for similar spices [2, 3, 4, 7]. Total bacterial counts of packed samples ranged from 1.3×10^5 CFU (colony forming unit)g (coriander) to 2.8×10^7 CFU/g (black pepper). The total counts of unpacked samples ranged from 1.4×10^5 CFU/g (red chilli) to 4.7×10^7 CFU/g (turmeric). Similar counts on these commodities have been reported by many workers [1, 2, 7]. Fabian *et al* [1] found that samples of whole and ground spices purchased on the open market had a bacterial count of 0 to 6.7×10^7 CFU/g. Yesair and William [2] reported that cloves, cinnamon, turmeric, onion, red chilli powder, and black pepper were most contaminated, the total count ranging from 12×10^5 to 16.3×10^5 CFU/g. Recently Baxter and Holzapfel [7] reported an exceptionally high count (ie> 10^6 CFU/g) in black pepper, coriander, paprika, pumento and white pepper samples obtained from wholesalers.

Total bacterial counts have their value in indicating sanitary conditions during processing and to a limited extent future keeping quality of the product at the time of examination. Grossly contaminated spices may result in the spoilage of food in which these have been added.

Coliforms were present in most of the samples examined. Faecal coliforms were absent in black pepper and turmeric but present in red chilli (93/g) and coriander (43/g) indicating possible contamination during processing and/or storage. No signficiant difference was found among the various brand samples. However, on the basis of low bacterial counts and the absence of fecal coliforms, the samples of brand 'A' were found to be relatively better in quality than the other brands and unpacked samples.Krishnaswamy *et al* [3] found colifroms to be present in black pepper, coriander, mustard, fenugreek, cumin, fennel and curry powder samples collected from wholesalers in India. Coriander was found to contain maximum load (2.4×10^3/g) and fenugreek minimum (1.3×10^2/g).

No *Salmonella* and enterococci were isolated from any sample. In this investigation the *Staphylococcus* medium 110 (Difco) was used for the isolation of staphylococci. All samples gave counts on this medium but no isolate proved to be *Staphylococcus* on further testing. Gram + ve spore formers of the genus *Bacillus* dominated the bacterial flora. Micrococci were isolated from only two unpacked samples. Organisms of the genus *Bacillus* were identified upto the species level. The dominant species was *B. licheniformis* (Fig. 1 and 2). No signficant difference was observed in the distribution of different types/species in packed and unpacked samples. The order of predominance was *B. licheniformis, B. pumilus, B. subtilus, B. cereus, B. sphaericus, B. megaterium, B. laterosporous, Micrococcus* sp. and *B. coagulans.*

Table 1. Microbiology of different processed packed and unpacked spices

Brand source	Samples	Packed samples				Unpacked samples			
		TPC/g	Coli-forms MPN/g	Faecal colifo-rms MPN/g	Fungi	TPC/g	Coli-forms MPN/g	Faecal Colifo-rms MPN/g	Fungi
A	1. Black pepper	5.8×10^5	11	Nil	*A. flavus & A. niger*	5.2×10^5	15	Nil	*A. flavus & A. niger*
	2. Red chilli	4.7×10^5	150	Nil	*A. flavus & A. niger*	6.2×10^5	150	20	*A. flavus & A. niger*
	3. Coriander	1.3×10^5	Nil	Nil	*Mucor*	*–	–	–	–
	4. Turmeric	5.1×10^5	Nil	Nil	Nil	1.6×10^5	Nil	Nil	Nil
B	1. Black pepper	–	–	–	–	2.5×10^6	Nil	Nil	*A. niger*
	2. Red chilli	6.3×10^5	210	43	*A. flavus & A. niger*	1.4×10^5	> 1100	93	*A. flavus & A. niger*
	3. Coriander	1.5×10^6	Nil	Nil	*A. niger*	3.5×10^5	75	Nil	*A. flavus & A. niger*
	4. Turmeric	2.6×10^6	Nil	Nil	*A. niger*	1.2×10^6	Nil	Nil	*A. niger*
C	1. Black pepper	3.2×10^6	Nil	Nil	*A. niger*	7.1×10^6	21	Nil	*A. niger*
	2. Red chilli	2.6×10^6	> 1100	93	*A. flavus & A. niger*	2.6×10^6	> 1100	210	*A. niger & Mucor*
	3. Coriander	2.3×10^6	460	43	Nil	1.4×10^7	460	43	Nil
	4. Turmeric	1.2×10^6	Nil	Nil	*A. niger*	2.2×10^6	Nil	Nil	*A. niger*
D	1. Black pepper	7.2×10^6	15	Nil	Nil	5.6×10^6	Nil	Nil	Nil
	2. Red chilli	1.3×10^6	23	Nil	Nil	1.2×10^6	23	Nil	Nil
	3. Coriander	2.1×10^5	Nil	Nil	Nil	3.1×10^5	> 1100	Nil	Nil
	4. Turmeric	6.7×10^5	1100	Nil	Nil	2.5×10^6	Nil	Nil	*A. flavus*
E	1. Black pepper	2.8×10^7	Nil	Nil	*Mucor*	3.4×10^7	Nil	Nil	*A. flavus*
	2. Red chilli	1.5×10^7	> 1100	Nil	*A. Flavus & A. niger*	3.5×10^7	> 1100	4	*A. flavus & A. niger*
	3. Coriander	1.5×10^6	150	Nil	Nil	4.9×10^5	93	Nil	*A. flavus & A. niger*
	4. Turmeric	1.9×10^7	23	Nil	*A. flavus*	4.7×10^7	23	Nil	Nil
F	1. Black pepper	–	–	–	–	7.2×10^6	11	Nil	Nil
	2. Red chilli	2.2×10^6	23	Nil	*Mucor*	7.2×10^6	23	Nil	*A. flavus & A. niger*
	3. Coriander	6.8×10^5	Nil	Nil	*A. niger*	7.3×10^5	460	Nil	*A. flavus & A. niger*
	4. Turmeric	4.3×10^6	Nil	Nil	Nil	8.4×10^6	15	Nil	*Mucor*

*– Not determined.

The presence of *B. cereus* in the samples is of particular concern. This organism has been recognized as the etiological agent in food poisoning outbreaks in Europe and was ranked third most common cause of food poisonings during the period 1950-1960. The incidence of this organism on different foods in our country is unknown. More emphasis was, therefore, given to confirm the suspected isolates of *B. cereus* by lecithinase production and haemolysis test. Totally, 6% of the isolates were confirmed as *B. creus* by random isolation from total count plates. If special selective methods were adopted for its isolation the percentage would have been much higher than presently realized. In general, *B. cereus* were isolated less frequently in packed samples as compared to unpacked ones. It was more frequently isolated in unpacked samples from source C & E. Powers *et al* [4] reported large number of *B. cereus* in 53% of spice samples they analysed.

No yeast was isolated from any sample examined. *Aspergillus niger* and *Aspergillus flavus* were the dominant species of fungi in the samples. Again, no singificant difference was noticeable in their occurrence in packed and unpacked samples. Red chillies in particular were found to

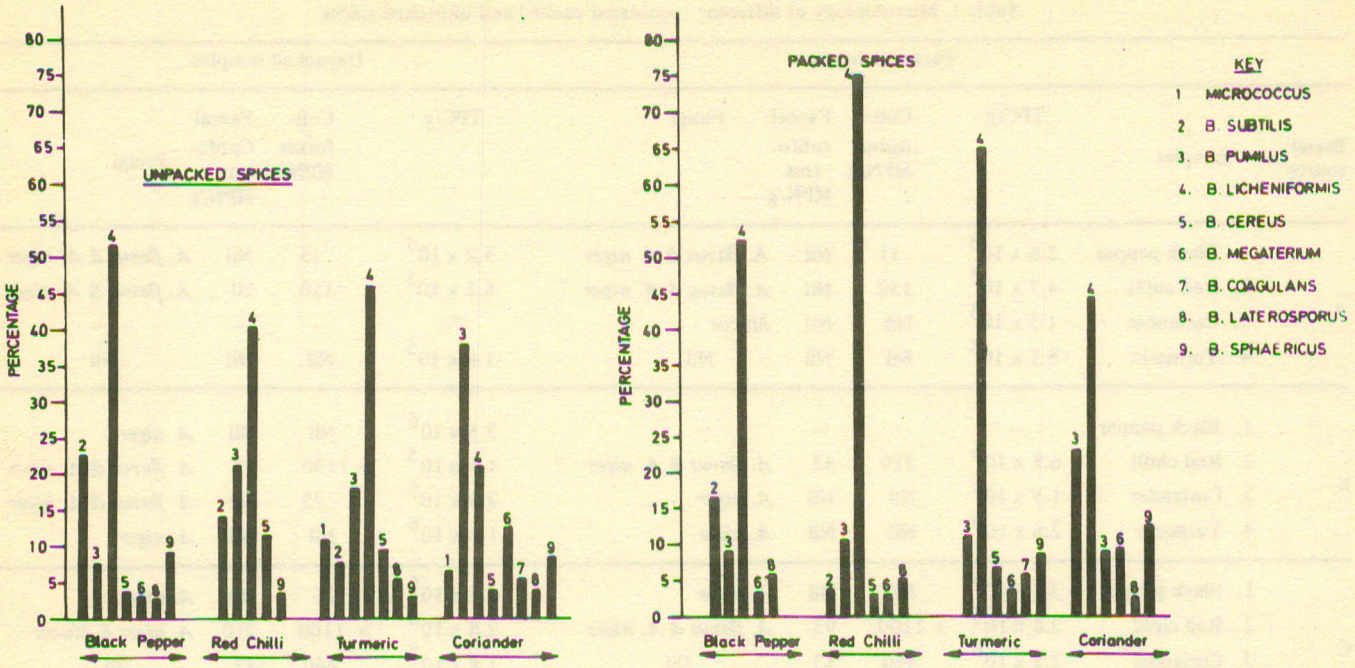

Fig. 1. Percentage distribution of different bacterial species samples.

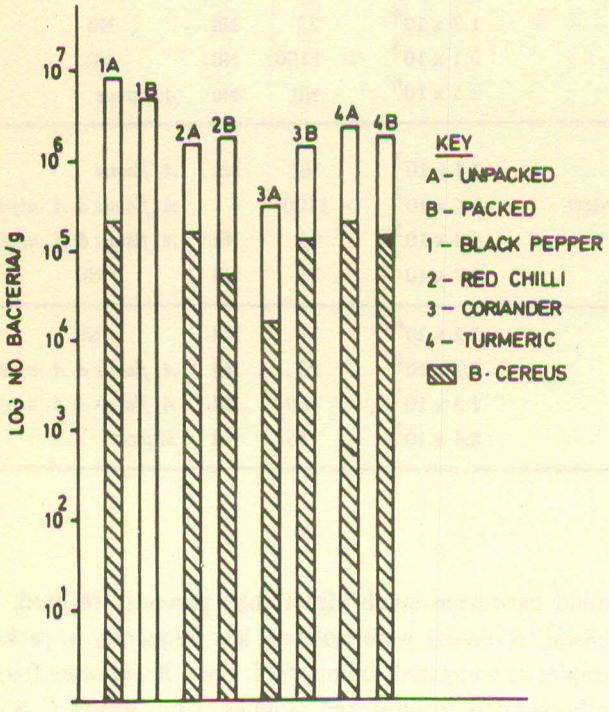

Fig. 2. Relationship between TPC/g and *B. cereus* in spices samples.

be highly contaminated with *A. flavus*. There was no signficant correlation between total bacterial counts and the presence of fungi.

The study of fungi in spices is of significance because of the potential production of mycotoxins [14]. Many workers [15, 16] have reported the presence of *A. flavus* in different spices such as red, white and black pepper. Similar to the findings of this study, *A. flavus* was found to be the prominent component of mycolfora. Christensen [15] Pal and Kundu [16] isolated *A. flavus* strains and confirmed aflatoxin production.

It has been confirmed by most microbiological studies that spices may be grossly contaminated with bacteria and moulds. They are grown and harvested in areas where sanitary conditions are poor and moreover, are grown in warm humid areas where the growth of wide variety of fungi and bacteria is readily supported [19]. Unless necessary steps, such as cleaning, dehydration and fumigation are not performed in a scientific manner, it is not likely that spices with low microbial quality could be prepared readily. Aluminium phosphide is being used for the fumigation of various food grains and spices in Pakistan. In general, the microbial quality of the spices tested was fair, indicating that some attempts are made to prevent contamination in Pakistan. However, the need for more clean and preferably commercially sterile spices is emphasised by these results, especially in view of the presence of high number of viable endospore forming bacteria in particular *B. cereus* and the contamination of 50% chilli samples with *E. coli* and *A. flavus*.

The quality of spices examined compares well with the quality of spices reported in the literature. Considering the results these products can be classed as safe for human con-

sumption. No significant difference was obtained between the quality of packed and unpacked samples. Brand 'A' samples were relatively better in terms of quality than other samples examined.

The bacteriological safety of spices can effectively be achieved by several fumigation practices by ethylene oxide, propylene oxide, or by microwave and gamma irradiation. These methods are, however, limited by cost, the time required to completely destroy microorganisms and their effect on flavour and colour of some spices [20]. According to Weber *et al* [21] ethylene oxide treatment appears to be the best method available. The by-products of this process are said to be consumed in negligible amounts by the general public. Investigations of the effectivity of gamma irradiation for sterilization of spices are in progress in USA [7].

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