

## Research Article

### Microbial Assessment of Spices Available In Different Markets of Karachi, Pakistan: A Food Quality Index Assessment Approach

Tahira Soomro<sup>1</sup>, Rimsha Soomro<sup>1</sup>, Shaista Urooj<sup>1,2\*</sup>, Izhar Ahmed Shaikh<sup>3</sup>, Yasir Raza<sup>1</sup>, Zulfiqar Ali Mirani<sup>4\*</sup> and Mubashir Aziz<sup>5</sup>

<sup>1</sup>Department of Microbiology, University of Karachi-Pakistan, Pakistan

<sup>2</sup>Department of Life Sciences, Shaheed Zulfiqar Ali Bhutto Institute of Science and Technology Karachi-Pakistan, Pakistan

<sup>3</sup>Pakistan Council of Scientific and Industrial Research-Islamabad-Pakistan, Pakistan

<sup>4</sup>Food and Marine Resources Research Centre (FMRR) (Microbiology section), Pakistan Council of Scientific and Industrial Research Laboratories Complex-Karachi-Pakistan, Pakistan

<sup>5</sup>Department of Microbiology and Molecular Genetics, Bahauddin Zakariya University, Multan-Pakistan, Pakistan

#### Abstract

Spices are used in almost all types of food preparation, and their microbial contamination can lead to spoilage and pose a risk to public health. Therefore, the aim of this study was to investigate the microbiological contamination of spices available in the markets of Karachi, Pakistan. 149 samples of 12 different types of spices were collected from different markets in Karachi. The spices were examined using standard microbiological procedures (U.S. Food and Drug Administration) to analyze Aerobic Plate Count (APC) and Mold and Yeast Count (MYC). The number of colonies in salt was

lower (APC  $4.01 \times 10^4$ ), while in mixed spices sample the highest APC ( $5.72 \times 10^5$ ) was recorded. The Food Quality Index (FQI) for bacterial contamination of spices indicates the highest level of contamination (FQI; 3199.15). The Mold and Yeast Count (MYC) was higher in mixed spices ( $3.93 \times 10^3$ ), the lowest MYC was observed in the pomegranate seed sample ( $11.0 \times 10^1$ ). Mold and yeast contamination of spices also indicated a higher contamination level of FQI (743.40). The evaluation of the spices based on APC, MYC, and FQI indicates that the spice samples are of very poor quality. Consumption of these contaminated spices may endanger human health.

**Keywords:** Aerobic plate count; Food quality index; Microbial assessment; Mold and yeast count; Spices

#### Introduction

Spices have always been used in a variety of foods. They can be extracted from the natural parts of the plant such as roots, rhizomes, leaves, flowers, fruits, seeds, tubers and bark [1]. They can be used as colorants, flavorings, preservatives, and folk medicines throughout the [2,3]. The small needle-shaped leaves of the rosemary plant and the seeds of the anise plant are strongly aromatic and are used in cooking and cosmetics, while the oil extracted from rosemary seeds has medicinal properties, such as a relaxing effect on the smooth muscles of the digestive tract and uterus in digestive disorders and menstrual cramps [4]. Food poisoning caused by contamination with bacteria and fungi such as *Campylobacter*, *Salmonella*, *Shigella*, *E. coli*, *Aspergillus*, *Penicillium*, and *Fusarium* is very common and life-threatening for millions of people around the world. Certain plant-derived matrices have been associated with an increase in foodborne disease outbreaks in recent years. Various types of digestive tract dysfunction have been associated with microbial contamination, which can occur at various points from cultivation to consumption [5,6].

Contamination of spices with various bacterial species such as *Salmonella* spp, *Bacillus cereus*, *Staphylococcus aureus*, *C. perfringens*, and *E. coli* has been observed [7-9]. In the past, much less attention was paid to the myco-flora of foods than to the bacterial flora; fungi are considered as the natural flora of foods, but nowadays fungi are considered to be able to cause spoilage by secreting mycotoxins [10,11]. There are a large number of studies reporting the prevalence of toxigenic fungi in various spices [12,13]. Food safety is one of the most important factors in maintaining public health. Almost half of the world's population has been suffering from foodborne diseases for several years. Since spices are used in very small quantities, but due to their high consumption tend to contaminate a wide range of products, it is very difficult to identify contaminated spices that cause foodborne illness. Pakistan is a developing country with limited control over food quality. The hot climate, inadequate processing methods and improper crop storage conditions favor the spread of food contaminants. To prevent serious microbial contamination and possible food deterioration, microbial counts should be as low as possible. There are few studies on the microbiological quality of spices in Pakistan [14]. Apart from this, there is a lack of comparative studies

\*Corresponding authors: Shaista Urooj, Department of Microbiology, University of Karachi-Pakistan Department of Life Sciences, Shaheed Zulfiqar Ali Bhutto Institute of Science and Technology Karachi-Pakistan, Pakistan, Tell: 0092-303-0257290; E-mail: Shaistaurooj\_map@yahoo.com

Zulfiqar Ali Mirani, Food and Marine Resources Research Centre (FMRR) (Microbiology section), Pakistan Council of Scientific and Industrial Research Laboratories Complex-Karachi-Pakistan, Pakistan, Tell: 0092-334-2113633; E-mail: mirani\_mrsa@yahoo.com

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according to national and international standards regarding aerobic plate count and yeast and mold count of spices [15]. Therefore, the aim of the current study is to determine the microbial quality of spices and compare the microbiological status of spices with the national and international standards. Food quality index is also used to evaluate the level of microbial contamination of spices available in the markets of Karachi, Pakistan.

## Materials and Methods

### Study area and period

The study was conducted on spices from retail (N=112) and production (N=37) sites in different regions of South, Central and West Karachi districts. All samples were analyzed consecutively from June 2020 to March 2021.

### Sample size and sample collection

A total of 149 samples of 12 different types of spices were collected in sterile and sealed plastic bags from street markets (N=63), small stores/shops (N=49) and production sites (N=37).

### Laboratory methods

#### Sample preparation

For enumeration of yeasts and molds, 50 g of each sample was collected. Samples were homogenized in 450 mL of 0.1% peptone water to prepare an initial suspension ( $10^{-1}$ ) in sterile vials. As described in bacteriological manual, after shaking, the vials were not moved for 10-15 min to allow the samples to settle, and then serial dilutions were made up to  $10^{-3}$  [16]. For aerobic plate count determination, 50 g of each sample was collected and homogenized in 450 mL of Butterfield phosphate buffer to prepare a starting suspension ( $10^{-1}$ ). After homogenization, centrifugation was performed at 12000 rpm for 2 min, then serial dilutions were made up to  $10^6$  (USFDA, 2001).

#### Molds and yeast count

For analysis of mold and yeast count (MYC) in each sample, 0.1 mL of sample was collected from a serially diluted sample (ranging from  $10^{-1}$  to  $10^{-3}$ ) and poured onto separate solidified dichloran rose-bengal chloramphenicol (DRBC) agar plates. After pouring, the plates were incubated at 25 °C for 5 days in the dark (USFDA, 2001).

#### Aerobic plate count

Aerobic plate count was analyzed in each sample by pouring 1 mL of the serially diluted samples ( $10^{-1}$ - $10^{-6}$ ) onto the plate count agar. After pouring, all Petri dishes were incubated at 35°C for 48 hours. After incubation, the colony forming unit (CFU) was calculated for each sample (USFDA, 2001).

#### Degree of contamination assessment

The degree of contamination with bacteria, molds, and yeasts was evaluated using the food quality index.

#### Food quality index

Food quality was evaluated using the equation of Horton (1965). It shows the composite effect of individual contaminants on food quality. It is a rating index whose values range from zero to one and are inversely proportional to  $S_n$  (recommended standard). It indicates the level of contamination. The critical value of this index is 100. The FQI can be expressed by the following equations of Horton:

$$FQI = \frac{\sum q_n W_n}{\sum W_n} \quad (1)$$

$$q_n = \frac{V_n - Vid}{S_n - Vid} \times 100 \quad (2)$$

$$W_n = \frac{k}{S_n} \quad (3)$$

$$k = \frac{1}{\sum \frac{1}{S_n} = 1, 2, \dots, n} \quad (4)$$

Where,  $S_n$  is a standard value,  $V_n$  is calculated value,  $Vid$  is ideal value and is zero for all contaminants (microbes),  $k$  is proportionality constant.

## Results

149 spice samples were tested using standard microbiological methods to analyze aerobic plate count (APC) and Mold and Yeast Count (MYC). Table 1 shows the APC values of each sample compared to Pakistan Standard and Quality Control Authority (PSQCA) and U.S. Food and Drug Administration (USFDA) standards. The data in Table 1 show that the Aerobic Plate Count (APC) in the mixed spices sample is very high ( $5.72 \times 10^5$  CFU/g). Compared to PSQCA and USFDA standards, the mixed spices are unfit for human consumption. The data also show that the number of colonies in salt is the lowest compared to the other samples ( $4.01 \times 10^4$  CFU/g). Despite the lower number, salt is still microbiologically unsuitable according to USFDA and PSQCA standards. Comparing the APC values of the salt samples with the PSQCA and USFDA standards (PSQCA, 2009) (*Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods*, 2013), it can be seen that these spices have a higher microbial load than the PSQCA and USFDA standards. The Food Quality Index (FQI) value is greater than 100, indicating that the highest risk of bacterial contamination was found in the spice samples. In Food Quality Index (FQI), the last column ( $W_n \cdot q_n$ ) indicates the contribution of bacterial contamination in each spice sample, while the summation gave the total FQI result (3199.15), which proves “that the spice samples are of very poor quality”.

All spice samples were also analyzed for Mold and Yeast Count (MYC). Table 2 shows the data of all samples analyzed for mold and yeast count. According to the data, the yeast and mold count is higher in mixed spices ( $3.93 \times 10^3$ ), followed by turmeric powder ( $3.75 \times 10^3$ ), curry powder ( $3.75 \times 10^3$ ), coriander powder ( $2.91 \times 10^3$ ), bay leaf ( $2.84 \times 10^3$ ), and red chili powder ( $2.72 \times 10^3$ ) compared to other spice samples. In accordance with USFDA and PSQCA standards, these spice samples were shown to be unfit for human consumption. The lowest MYC value is observed in the pomegranate seed sample ( $11.0 \times 10^1$ ) Except for mixed spices, turmeric powder, curry powder, coriander powder, bay leaf and red chili powder, all other samples are within the satisfactory limits of USFDA and PSQCA. To evaluate the contamination level of mold and yeast in the spice samples, the food

Spices	N	APC (CFU/g)	Food Quality Index (FQI)			PSQCA Standards			USFDA Standards		
			Wn	qn	Wn.qn	s	b	u	s	b	u
Mixed Spices	28	$5.72 \times 105 \pm 403.73$	0.083333	5720	476.6648	104	104-106	$>106$	102	104	106
Cinnamon	9	$4.72 \times 104 \pm 244.95$	0.083333	472	39.33318						
Curry powder	6	$5.02 \times 105 \pm 393.23$	0.083333	5020	418.3317						
Turmeric powder	13	$4.96 \times 104 \pm 230.22$	0.083333	496	41.33317						
Bay leaf	9	$5.13 \times 105 \pm 287.30$	0.083333	5130	427.4983						
Black pepper powder	10	$5.02 \times 105 \pm 264.70$	0.083333	5020	418.3317						
Salt	9	$4.01 \times 104 \pm 223.61$	0.083333	401	33.41653						
Coriander powder	18	$4.97 \times 104 \pm 279.28$	0.083333	497	41.4165						
Pomegranate seeds	7	$5.08 \times 105 \pm 415.93$	0.083333	5080	423.3316						
Red Chili powder	20	$5.03 \times 105 \pm 427.78$	0.083333	5030	419.165						
Cloves	8	$4.84 \times 104 \pm 230.22$	0.083333	484	40.33317						
Cumin powder	12	$5.04 \times 105 \pm 311.45$	0.083333	5040	419.9983						
			$\Sigma Wn = 0.999$		$\Sigma qn.Wn = 3199.15$						
			FQI = 3199.17								

**Table 1:** Aerobic Plate Count (APC) of spices samples compared with Pakistan Standard Quality Control Authority (PSQCA, 2009) and U.S. Food and Drug Administration (USFDA, 2013) standards. Food quality index (FQI) indicating, the degree of contamination of the spices samples by the bacteria.

Key: Data (each sample with means  $\pm$  SD, n = 9). APC= aerobic plate count, N= total number of the spice sample, CFU/g = Colony forming unit in 1 gram, FQI = indicating the degree of bacterial contamination in spices, Wn = bacterial concentration as unit in one gram of the n<sup>th</sup> spice sample, qn = quality rating of bacterial load in n<sup>th</sup> spice sample, s = satisfactory limits, b = borderline limits, u= unsatisfactory limits

Spices	N	MYC (CFU/g)	Food Quality Index (FQI)			PSQCA Standards			USFDA Standards		
			Wn	qn	Wn.qn	s	b	u	s	b	u
Mixed Spices	28	$3.93 \times 103 \pm 50.95$	0.0833	3930	327.369	102	102-104	$>104$	102	102-104	$>104$
Cinnamon	9	$17.4 \times 101 \pm 0.30$	0.0833	17.4	1.44942						
Curry powder	6	$2.92 \times 102 \pm 20.12$	0.0833	292	24.3236						
Turmeric powder	13	$3.75 \times 103 \pm 75.83$	0.0833	3750	312.375						
Bay leaf	9	$2.84 \times 102 \pm 19.63$	0.0833	284	23.6572						
Black pepper powder	10	$15.7 \times 101 \pm 0.24$	0.0833	15.7	1.30781						
Salt	9	$19.0 \times 101 \pm 0.40$	0.0833	19	1.5827						
Coriander powder	18	$2.91 \times 102 \pm 20.88$	0.0833	291	24.2403						
Pomegranate seeds	7	$11.0 \times 101 \pm 0.24$	0.0833	11	0.9163						
Red Chili powder	20	$2.72 \times 102 \pm 20.89$	0.0833	272	22.6576						
Cloves	8	$19.7 \times 101 \pm 0.27$	0.0833	19.7	1.64101						
Cumin powder	12	$19.0 \times 101 \pm 0.40$	0.0833	19	1.5827						
			$\Sigma Wn = 0.999$		$\Sigma qn.Wn = 743.10$						
			FQI = 743.40								

**Table 2:** Mold and Yeast Count (MYC) of spices samples compared with Pakistan Standard Quality Control Authority (PSQCA, 2009) and U.S. Food and Drug Administration (USFDA, 2013) standards. Food quality index (FQI) indicating, the degree of contamination of the spices samples by the mold and yeasts.

Key: Data (each sample with means  $\pm$  SD, n = 9). MYC = mold and yeast count, N= total number of the spice sample, CFU/g = Colony forming unit in 1 gram, FQI = indicating the degree of mold and yeast contamination in spices, Wn = bacterial concentration as unit in one gram of the n<sup>th</sup> spice sample, qn = quality rating of mold and yeast load in n<sup>th</sup> spice sample, s = satisfactory limits, b = borderline limits, u= unsatisfactory limits

quality index (FQI) was used. Wn.qn column indicates the contribution of mold and yeast contamination in each spice sample, while the summation gave the total FQI result (743.40), which in turn proves that “the spice samples are of very poor quality” so that the consumers are not able to use them.

## Discussion

Spices are exposed to a variety of microbial contaminants before, during, and after processing, especially when they are sun-dried on the ground and traded in markets. Spices can contain a significant

amount of microbial contaminants, such as xerophilic molds, mesophilic bacteria, and enterobacteria. Most bacterial species are pathogenic, while fungal species are potential producers of mycotoxins that have toxic and carcinogenic effects in humans and animals, e.g., aflatoxins, ochratoxins and sterigmatocystin [1-20].

The initial level of bacterial contamination depends mainly on the hygienic conditions of handling raw materials and final products, as well as on the processing conditions [21]. Therefore, for food quality, hygiene and safety are important at every stage of processing and

sale. In the present study, spices were collected from different markets in Karachi, Pakistan. Microbial load of spices was determined by Aerobic Plate Count (APC) and Mold and Yeast Count (MYC) method. The microbial load values were higher than the standard limits when compared with USFDA and PSQSA standards. Production of these commodities under poor hygienic conditions and in warm and humid climates can increase the risk of contamination with total microbial loads.

According to the results, the lowest Aerobic Plate Count (APC) was observed in salt samples because salt reduces water availability by removing water from the cell through the process of osmosis [22], inhibiting bacterial growth, but some bacteria are halotolerant and can grow at high salt concentration, some predominant bacterial genera are halotolerant, including; *Halomonas*, *Bacillus*, *Streptomyces*, *Oceanobacillus*, and *Pseudomonas* [23]. Dry mixed spices are produced at various stages such as drying, harvesting, and threshing, so there is a possibility of contamination at each stage [24]. Molds and Yeasts Count (MYC) was also observed high in the spice samples, as most fungi are halotolerant and can survive in high salt concentrations [25]. Well-studied examples of halotolerant yeasts and fungi include *Debaryomyces hansenii* black, *Aureobasidium pullulans* and *Hortaea* [26-28]. They can grow even in the saturated NaCl solution. The lowest MYC is observed in the samples of pomegranate seeds. According to a previous study, pomegranate seeds have antifungal activity [29]. Food Quality Index (FQI) was used to determine the level of contamination of the spices with microbes (bacteria, fungi and yeast). It was found that the FQI values of the spices (APC; 3199.17 and MYC; 743.40) were above the critical value (100), indicating that the spices were highly contaminated with bacteria, fungi and yeast. Even most of the spices were microbiologically unsuitable according to USFDA and PSQCA standards. These contaminated spices can cause serious health problems in consumers with acute and chronic diseases [30]. Consumption of these contaminated spices can endanger people's health [31]. Therefore, spices should be processed under standardized hygienic conditions. To reduce the risk of microbiological contamination, several countries and international organizations have enacted strict food and feed legislation. The best preventive measure is to ensure proper pre- and post-harvest practices. For example, spices must be cleared of physical contaminants shortly after harvest and dried to a safe storage moisture level [32]. Drying is one of the oldest physical methods of food preservation that aims to reduce the moisture content of spices [33-38].

## Conclusion

This study evaluated the microbiological quality of the most commonly consumed types of unpackaged spices sold in Karachi, Pakistan. In the local market, spices sold in bulk (in open containers) are highly susceptible to microbial contamination. Hygiene, environmental, and sanitary conditions during processing, distribution, and storage of unpackaged spices and herbs must be controlled to avoid high microbial counts. In this context, food handlers, suppliers, and consumers should be aware of appropriate handling and storage practices to reduce the risk of contamination. Our results also suggest that a food safety management system, the use of good hygiene practices and manufacturing practices, routine inspections, and testing of the final product are necessary to ensure microbial safety at all stages of safer spice production. In addition, the use of irradiation, heat or steam treatment can help inhibit pathogenic and spoilage microorganisms.

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## Conflict of Interest

There is no conflict of interest among the authors

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