



RESEARCH ARTICLE

Microbial Evaluation of Spices in Ethiopia

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Abstract:

Introduction:

Since spices are taken as ready-to-eat products, they are not subjected to heat treatment. The use of spices contaminated with pathogens can lead to morbidity and mortality.

Materials and Methods:

The study was conducted on 162 samples of 25 spices collected from retail and production sites in different regions of Ethiopia between January 2010 to December 2017 to determine the concentrations of heterotrophic plate count and *Staphylococcus aureus* by pour plate method; for coliforms using NMKL Method No. 44; for mould and yeast enumeration using spread method and for *Salmonella* using ES ISO 6579. The data was analysed using SPSS version 20.0.

Results:

Moulds, yeasts, total coliforms, heterotrophic plate count, total coliforms, thermotolerant coliforms, *E. coli* and *S. aureus* above the acceptable limits were observed in 5 (3%), 7 (4.3%), 2 (1.2%), 20 (12.3%), 10 (6.2%), 9 (5.6%) and 19 (11.7%) samples respectively. *Salmonella* species was not noticed in any of the samples tested. No bacterial and fungal contaminations were observed in 11 of 25 spices.

Conclusions:

Few spices samples had 1.2 to 12.3% of the microbiological indicators, spoilages or pathogens exceeded the ICMFS guidelines. The use of these contaminated spices may pose risk to human health.

Keywords: Spices, Coliforms, Mould, Yeast, *Staphylococcus aureus*, *Salmonella*.

1. INTRODUCTION

Nowadays, consumer's preferences are turned towards healthy, natural products containing herbal and spice substances used mostly in food [1]. Spices are aromatic plants, in whole, broken or ground forms [2] obtained from dried vegetables seeds, roots, fruits or barks. Spices have been used for rituals, cosmetics and perfumery, their colouring, flavouring, preservatives and antimicrobial activity against some microbes that affect the quality of food and their shelf life [3]. Spices in meals also have numerous useful effects such as stimulation of saliva secretion, digestion promotion, cold and influenza prevention, and nausea and vomiting reduction [4, 5] and maintaining the balance of the body humors [6]. In addition, spices also limit salt, sugar and fats consumption [2, 7].

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Spices are considered ready-to-eat products by nature and most people would not take spices to be a food safety hazard and commonly use them without subsequent cooking [8]. Therefore, routine examination of spices for microbes is useful to monitor high rates of contamination entering the products [9].

The level of contamination of spices depends mainly on microbes present naturally on plants, epiphytic microbiota and secondary contamination with water, soil or airborne microbes during harvest, drying, transport and storage [10, 11]. Spices can also be contaminated with sewage, animal or human fecal matters and dust [12].

Recently, the prevalence of pathogenic microbes in spices, such as bacteria and toxigenic fungi have been reported in several research works [13 - 16]. Non-hygienic and improper conditions in production, processing, distribution and storage of spices enhance the risk of microbial contamination [12, 17, 18]. The risk of the growth of the pathogens is also elevated when spices are added in foodstuffs that are not subjected to thermal treatment [16]. The use of these spices which contaminated with pathogens can lead to hospitalization and even death [19].

No information about the microbiological quality and safety of various spices are available in the study sites. Since spices have been associated with various outbreaks, their microbial quality and safety should be examined. The objective of the current study is to find out the presence of heterotrophic bacteria, coliforms, *Salmonella* spp., *S. aureus*, mould and yeast counts in various spices.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted at Ethiopian Public Health Institute on spices samples collected from retail (N=91) and production sites (N=71) in different regions of Ethiopia. Sixty- eight of 72 production sites were devoted to a single spice. The production and retail sites for the spices are located in northern, central, and southern Ethiopia that extend between latitude and longitude 8°00N and 38°00E, and present a diverse topography, ranging from 110 m below the sea level to 4550 m above the sea level.

2.2. Study Design and Period

The study was based on retrospective data obtained from test results of spices, which were kept in Public Health Microbiology Research Team department of Ethiopian Public Health Institute from January 2010 to December 2017 to determine the concentrations of Heterotrophic Plate Count (HPC) and *Staphylococcus aureus* (*S. aureus*) by pour plate method; for coliforms using NMKL Method No. 44; for mould and yeast enumeration using spread method and for *Salmonella* using ES ISO 6579. Laboratory tests were analysed for 26 samples, 19 samples, 17 samples, 24 samples, 20 samples, 23 samples, 14 samples, and 19 samples during consecutive fiscal years from 2010 to 2017 respectively.

2.3. Sample Size and Sampling Technique

Purposive sampling technique was used to select all 162 spices produced in Ethiopia whose routine microbiological examinations were done between January 2010 and December 2017. Of the total spice samples, five samples were physically defective.

2.4. Data Collection

A total of 162 samples of 25 various kinds of spices (46 packaged and 116 unpackaged) were collected from street markets (N= 48 and small shops (N 43) by various regulatory bodies and other health professionals from retail and production sites in various regions of Ethiopia (Addis Ababa N=135, Oromia N=16, Southern Nations, Nationalities, and Peoples' Region (SNNPR) N=10 and Tigray N=1). The data collection method involved the use of available recorded data of microbiological test results of spices.

2.5. Laboratory Methods

All samples were stored at 4°C and examined within 24 hours after collection of Heterotrophic Plate Count (HPC), mould and yeast counts, total coliforms, thermotolerant coliform, *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Salmonella* using accepted methodologies [20 - 24]. For serial dilution, 25-g of spices was weighed and homogenized with 225 ml sterile buffered peptone water (Difco). Serial dilution was made with this diluent and counting plates were prepared up to 1:10⁵ dilutions. One milliliter of each dilution was taken and mixed with molten media. For spreading method, 0.1 ml of the dilution was inoculated on the surface of the plates.

2.5.1. Heterotrophic Plate Count

Spice samples were tested using plate count agar and incubated at 35°C for 48 ± 3 hours. Colonies were counted as colony-forming units per gram [20].

2.5.2. Enumeration of Yeasts and Moulds

Yeast and mould enumerations were done according to ISO 7954 using spread method on Rose Bengal chloramphenicol agar and incubated for seven days at 25°C [21].

2.5.3. Coliform Assay

The coliform enumeration test was based on the Nordic Committee on Food Analysis, NMKL Method No. 44. Approximately, 5 ml of tryptone soya agar was added to 1 ml of 1:10 diluted sample and pre-incubated at 20 - 25 °C for 1-2 hours. Melted violet red bile agar (10-15 ml) was poured on top of the agar and dark red typical colonies surrounded by a red precipitation zone were counted after 24 hours incubation at 37°C and 44.5°C. Selected five colonies from the presumptive coliforms were confirmed by testing for gas production in brilliant green bile salt lactose broth and for thermotolerant coliforms and *E. coli*, EC broth and tryptophan broths were inoculated and incubated at 44°C. The results were reported by calculating the population density from the colony counted and the degree of dilution [22].

2.5.4. Enumeration of *S. aureus*

S. aureus was enumerated according to ES ISO 688-1:2002 using pouring plate method on Baird-Parker agar medium and incubated at 37°C for 24 hours [23].

2.5.5. Detections of *Salmonella* Species

Salmonella was performed by ES ISO 6579: 2002 using a pre-enrichment of buffered peptone water followed by selenite cystine broth selective enrichment and xylose lysine deoxycholate isolation medium incubated at 37° C for 24 hours. Presumptive *Salmonella* spp. were subcultured on an appropriate plate and were biochemically and serologically tested for confirmation [24].

2.6. Data Analysis Procedures

The data was analysed using SPSS version 20.0 (SPSS Inc. Version 20, Chicago, Illinois). Values different from zeros (0.5) were used for negative results to reduce bias in the statistical analyses. Kruskal-Wallis, a non-parametric test was used to observe the differences among the values of variables by regions, spice-type and analysis years. To observe the associations among various organisms; the Spearman Rank Correlation was used. The significance level was fixed at $p \leq 0.05$.

3. RESULT

A total of 162 samples of 25 spices were assessed for heterotrophic bacteria, mould and yeast enumerations, total and thermotolerant coliforms, *E. coli*, *S. aureus*, and *Salmonella* species. Moulds, yeasts, total coliforms and thermotolerant coliforms larger than 10⁴ CFU/g, HPC larger than 10⁶ CFU/g Table (1), *E. coli* larger than 10³ CFU/g Table (2) and *S. aureus* larger than 10² CFU/g were observed in 5 (3%), 7 (4.3%), 20 (12.3%), 10 (6.2%), 2 (1.2%), 9 (5.6%) and 19 (11.7%) samples, respectively. *Salmonella* species was not noticed in any of the samples tested.

Table 1. Enumeration of moulds, yeasts and total heterotrophic bacteria in spices in various regions of Ethiopia between Jan. 2010 and Dec. 2017.

Parameter	Microbial Count (CFU/g)						Total
	<1	1-100	101-1000	1001-10000	10001-1000000	>1000001	
Mould	69	9	50	29	5	0	162
Yeast	134	5	10	6	7	0	162
HPC	29	7	20	25	79	2	162

CFU—colony forming unit, HPC—heterotrophic plate count

Table 2. Enumeration of coliforms in spices in various regions of Ethiopia between Jan. 2010 and Dec. 2017.

Parameter	Microbial Count (CFU/g)						Total
	<1	1-100	101-180	181-1000	1001-10000	10001-1000000	
TC	91	11	1	18	21	20	162
TT	129	5	1	8	9	10	162
<i>E. coli</i>	146	4	0	3	4	5	162

CFU–colony forming unit, TC– total coliforms, TT–thermotolerant coliforms

Moulds, yeasts, total coliforms and thermotolerant coliforms larger than 10⁴ CFU/g, HPC larger than 10⁶ CFU/g, *E. coli* larger than 10³ CFU/g and *S. aureus* larger than 10² CFU/g were found in 3, 3, 8, 5, 2, 3 and 10 spices respectively Table (3). Moulds, yeasts, HPC, total coliforms and thermotolerant coliforms, *E. coli* and *S. aureus* above these ranges were noticed in 4, 5, 1, 10, 3, 2, and 9 spices collected from retail sites, respectively. The maximum levels of mould, total coliform, thermotolerant coliforms and *E. coli* contaminations were observed in turmeric, *berbere* (spice mix mainly red hot chilli), hop and ginger, respectively, while yeast, HPC and *S. aureus* maximum counts were observed in one spice mix.

Table 3. Sites and packaging types of spices samples in different regions of Ethiopia having contaminants above ICMSF limits between Jan. 2010 and Dec. 2017.

S. no	Sample type	No of Contaminants above ICMSF limits							Spices With one or more contamination	Unpackaged	Site	Region
		Mo	Ye	HP	TC	TT	EC	SA				
1	Berberere	2	2	1	4	1	1	5	10/57	9/10	A, O, S,	Sh, P, R
2	Turmeric	1	0	0	1	0	0	1	2/4	2/2	A	R
3	Spice mix	2	4	1	6	3	3	5	11/21	5/11	A, O, T	Sh, P, R
4	Fenugreek	0	0	0	1	0	0	1	2/6	2/2	A	R
5	Rapeseeds	0	0	0	1	0	0	0	1/1	1/1	A	R
6	Moringa p	0	0	0	3	2	0	0	3/7	2/3	A, S	Sh, R
7	Sesame seeds	0	0	0	1	0	0	1	1/6	1/1	A	P
8	Ginger	0	0	0	3	3	1	2	4/7	4/4	A	P, R
9	Garlic	0	0	0	0	0	0	1	1/1	1/1	A	R
10	Black Pepper	0	0	0	0	0	0	1	1/1	1/1	A	R
11	Cinnamon	0	0	0	0	0	0	1	1/1	1/1	A	R
12	tea	0	0	0	0	0	0	1	1/9	0/1	A	R
13	hop	0	0	0	0	1	0	0	1/1	1/1	A	R
14	Basil	0	1	0	0	0	0	0	1/1	1/1	A	R

For the packaged spices, moulds, yeasts, total and thermotolerant coliforms larger than 10⁴ CFU/g, HPC larger than 10⁶ CFU/g, *E. coli* larger than 10³ CFU/g and *S. aureus* larger than 10² CFU/g were observed in 2 (1.2), 4 (2.4%), 4 (2.4%), 2 (1.2%), 0, 2 (1.2%), and 5 (3.1%) samples, respectively.

No bacterial and fungal contaminations were observed in 11 of 25 spices. Some of these spices free of microbes were *Maggi* (spice mix, mainly fenugreek and black pepper), Onion, *Awaze*(spice mix mainly red hot chilli, Nutritional yeast, Ethiopian black cumin, cloves, rue, minced onion and garlic with pepper mix, Ethiopian cardamom, Ethiopian Mustard, and flavour, natural.

Out of 162 spices samples, 139 (85.8%) samples or 11 (44%) various spices had acceptable limits of yeast, mould, HPC, total and thermotolerant coliforms, *E. coli*, *S. aureus* and *Salmonella* spp. However, the rest 40 (24.7%) samples or 14 (56%) of the spices had one or more of the microbes (Table 3).

ICMSF- International Commission on Microbiological Specifications for Foods, Mo–mould, ye- yeast, HP- heterotrophic plate count, TC-total coliforms, TT-thermotolerant coliforms, EC- *E. coli*, Moringa p- Moringa powder, Sesame- Sesame seeds, A-Addis Ababa, O- Oromia, S-Southern Nations, Nationalities, and Peoples’ Region and T– Tigray, Sh–shop, Pr–production, Sm –street market

Spearman’s rank correlation coefficient r results (significant at the 0.05 level, 2-tailed) of HPC, mould, yeast, total and thermotolerant coliforms, *E. coli* and *S.aureus* are indicated in Table 4.

Table 4. Rho values of HPC, mould, yeast, total and thermotolerant coliforms, *E. coli* and *S. aureus* in spices samples between Jan.2010 and Dec. 2017.

Parameters	Mould	Yeast	HPC	TC	TT	<i>E.coli</i>	<i>S. aureus</i>
Mould	1	-	-	-	-	-	-
Yeast	-0.072	1	-	-	-	-	-
HPC	0.180	-0.009	1	-	-	-	-
TC	0.148	0.096	0.295	1	-	-	-
TT	0.054	0.068	.227	0.587	1	-	-
<i>E. coli</i>	0.28	0.130	0.204	0.395	0.635	1	-
<i>S. aureus</i>	0.029	0.086	0.083	0.215	.015	0.139	1

HPC–heterotrophic plate count, TC–total coliforms, TT–thermotolerant coliforms P-values for mould, yeast, HPC, total and thermotolerant coliforms, *E. coli* and *S. aureus*, using Kruskal-Wallis test for spice samples by spice type, region and packed vs unpacked are shown in Table 5.

4. DISCUSSION

In the current study, mycological and bacteriological quality and safety of spices were assessed from different regions in Ethiopia. Some of the samples (3%) contained mould, yeast (4.3%) and total coliforms (12.3%) exceeding acceptable limit of 10^4 CFU/g; HPC (1.2%) exceeding 10^6 , *E. coli* (3%) exceeding International Commission on Microbiological Specifications for Foods (ICMSF) limit of 10^3 [25] and *S. aureus* (11.7%) exceeding 10^2 CFU/g of the total spice samples.

Even though spices are not the most important contributors to foodborne illness, if they are added without further cooking to ready to eat foods, the spices can cause high risk [16]. The contamination of spices by bacteria and fungi might be due to poor harvesting and processing, dust, wastewater, animal and human excreta in production or retail markets [26]. The levels of contamination depend on variations in spices technology [27].

The contamination of spices by yeast was in one of the 154 samples in the study done in India [12], while in the study done in Turkey, Yeasts and moulds were detected in 45.5% of the samples [28]. Similarly, Banejee and Sarkar found the contamination of Indians spices by moulds in 97% of the samples [12]. Fungi are accepted as spoilages in spices. Following cooking, the presence of fungal toxins might cause food poisoning or valuable food products' deterioration. Contamination of mycotoxins is a serious problem if spices are stored for long periods of time, without temperature and moisture controls [29].

The result noticed in the present investigation that the occurrence above the acceptable limit of HPC in the samples being lower than in several researches conducted on different spices samples in Spain (10%) [30] Brazil (25.8%) [31], Saudi Arabia (24.2%), and India (51%) [12]. The presence of heterotrophic plate count above the permissible limit may indicate poor handling and drying conditions or lack of general hygienic conditions [32, 33].

The unacceptable limits of total coliforms, thermotolerant coliforms and *E. coli* being 12.3%, 6.2% and 3%, respectively, in the spices samples were lower than in investigations done on different spice samples in India (total coliforms, 33% and thermotolerant coliforms, 15%) [12] and Iran (*E. coli*, 42%) [34], but higher than in one investigation in Ghana that had acceptable limits of total coliforms and thermotolerant coliforms [35]. The detection of thermotolerant coliforms and *E. coli identified* in the spices was an indication of fresh faecal matter contamination and the presence of pathogens [36]. This could be due to insufficient hand washing by vendors and lack of personal hygiene. This can cause different diseases including cholera [37].

The result observed in the current study above the recommended limits of *S. aureus* in 11.7% of the sample was in line with the research done in India on various spices [12] and lower than the results of the research conducted in Nigeria (24 to 86%) [38]. Although, in one investigation, the presence of this bacteria was higher than that from Turkish research (4%) [32]. The incidence of *S. aureus* may be due to soil or other sources related to unhygienic conditions in the production, primarily handlers, and causes food poisoning [39].

The detection of *Salmonella* spp. in the samples was consistent with the ICMSF requirement for spices that should be free from pathogenic microorganisms [40]. This was consistent with studies conducted by other researchers in Istanbul and Kars [41, 42].

However, in other studies conducted in the United Kingdom [43] and Turkey [28], *Salmonella* spp. has been detected in different kinds of spices. The microbial counts of moulds, yeasts, HPC, total coliforms, thermotolerant coliforms, *E. coli* and *S. aureus* within acceptable limits for 11 of 25 spices are might be due to several compounds

found in spices having an antimicrobial effect against microbes that affect shelf life and quality of the food [3].

The higher microbial loads of non-packaged spices than the packaged products in India (33) were similar to the current study that had 1.5 times, 2.0 times, 4.0 times, 4.0 times, 1.5 times and 4.0 times moulds, HPC total and thermotolerant coliforms, *E. coli* and *S. aureus* higher than the packaged spices, respectively.

The microbial counts of spices collected from street markets had 3.0 times, 4.0 times, 4.0 times, 2.0 times and 8 times mould, yeasts, total coliforms, thermotolerant coliforms and *S. aureus* higher than spices collected from small shops, respectively. The contamination of the spices by fungi that were collected from the retail markets was two times larger than spices collected from production sites.

The correlation test of non-parametric Spearman indicated statistically significant correlations between HPC count and the detection of coliforms (total coliforms, thermotolerant coliforms and *E. coli*), total coliforms and other coliforms, as well as mould and *E. coli*; total coliforms and *S. aureus* were also correlated to each other (Table 4). The Kruskal-Wallis test showed that mould, yeast and HPC statistically differed by Spice type, regions, retail vs production and packaged vs non-packaged. Total coliforms, thermotolerant coliforms, *E. coli* and *S. aureus* differed by the spice type (Table 5).

Table 5. P-values for, mould, yeast, HPC, total and thermotolerant coliforms, *E. coli* and *S. aureus*, in spice samples in Ethiopia by spice type, region packed vs unpacked and retails and production sites between Jan. 2010 and Dec. 2017.

Parameter	Mould	Yeast	HPC	TC	TT	<i>E. coli</i>	<i>S. aureus</i>
Spice type	0.001	0.008	0.001	0.001	0.016	0.028	0.048
Regions	0.002	0.024	0.049	0.396	0.771	0.319	0.177
packaged vs non-pac	0.03	0.006	0.010	0.057	0.136	0.733	0.865
Retails & prodn sites	0.151	0.757	0.001	0.064	0.028	0.028	0.193

HPC–heterotrophic plate count, TC–total coliforms, TT–thermotolerant coliforms, non-pac – non-packaged, prodn–production

CONCLUSION

Majority of the spices samples tested in the present study contained acceptable limits of fungi, bacterial indicators or *S. aureus*, and all the samples did not contain *Salmonella* spp. However, few spices samples had 1.2 to 12.3% of these microbiological indicators, spoilages or pathogens that exceeded the ICMFS guidelines. The use of these contaminated spices may cause high risk to human health. It is suggested that spices should be processed under sanitary conditions and packaging increases the quality of spices.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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