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Microbial Safety of some Selected Spices Sold in Jimma Town, South Western of Ethiopia

Shelema Tulu, Shiferaw Demissie and Anbessa Dabassa
College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia

Corresponding Author: Anbessa Dabassa, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia Tel: 251 911070477

ABSTRACT
Spices have been get Great attention among the world population because of its great value in food floor and aroma. Moreover it is benefiting for sellers by creating job opportunities. However it perceived to be major public health problem due to microbial contamination. The purpose of this study was to evaluate microbial safety of spices in Jimma town. The study involves laboratory analysis for microbiological safety of spice. The standard method were used for the enumeration of Aerobic mesophilic, Entrobacteriaceae, coli forms, *Staphylococci,* yeasts and molds. A total of 40 spice samples Red chilies (*Capsicum* spp.), Turmeric (*Curcuma longa*) were collected from Mercato, Kochi and Agip selling sites. This result show that the overall mean microbial counts (CFU g^{-1}) were dominated by Aerobic mesophilic bacteria (7±o.4), yeasts (6±o.4), coliform (6±0.0), molds (6±0.0), *Staphylococci* (4±0.8) and entrobacteriaceae (5.5±0.5). Out of total 208 isolates characterized, the most dominant were *Bacilli* spp. (83.33%) followed by *Staphylococci* spp. (14.3%). Out of 20 mycopopulation isolates characterized, molds were dominant (75 %) and yeast were 25%. Among the molds isolates, *Mucor* spp. were predominant (41.6%) followed by *Aspergillus* spp. (16.6%) whereas *Pencillium* and *Geotrichum* spp. (12.5%) while *Eurotium, Rhizopus* and *Fusarium* spp. were least recorded fungi (8.33%). Spices contamination problem in the present study could be due to poor preparation, selling site, storage area and materials and poor personal hygiene. Generally, the microbial safety of spices sold in Jimma town was poor and call for special attention.

Key words: Aroma, flavor, enumeration, isolates, Jimma town, microbial safety

INTRODUCTION
Spices are non-timber forest product which extract or obtained from plant vigor such as seeds, fruits, flowers, rhizomes, bulbs, bark, leaves and stems that used as food additive in order to provide odor, smell, taste flavor (Leek *et al.*, 2004). It encompasses of different chemical nutrient and minerals including water, protein, fat, carbohydrates, ash, calcium, potassium, sodium, phosphorus, iron and various essential vitamins (Singh *et al.*, 2002). Spices are essential components of cuisines since ancient and are used in minute amount impart aroma in food preparation to improve their palatability (Hampikyan *et al.*, 2009). They are also used for stabilizing several food items from deterioration (Mandeel, 2005). It has also been prescribed for aiding digestion, raising sexual potency, decreasing blood pressure, controlling metabolism and delaying the onset of degenerative disease (Jayaprakasha *et al.*, 2001). On the other hands, Spices used as refreshing food, drink and daily meal components (Anderson *et al.,* 2000). Nowadays, Spices are known to have some ethno-botanical medicine and antimicrobial properties (Singh *et al.*, 2002; Knefiel and

Berger, 1994). Plants traditionally used for medicinal purpose in different parts of the world have been screened for possible antimicrobial action by severaworkers (Bonjar, 2004). This value of spices also reported by many scientists (Leite de Souza *et al.*, 2006; Karaman *et al.*, 2011). The combined value of spices also generally show synergistic antimicrobial effect specially on fungus (Das, 2002). This is due to the presence of different phyto-chemicals or compounds like alkaloids and terpenoids (Jackson *et al.*, 1995).

Even if, spices are multipurpose non-timber frost products it could be spoiled and contaminated by pathogenic microbes during and post-harvesting in poor hygiene condition (Gurbuz *et al.*, 2000). Hence, they are considered as significant carriers of microbial contamination primarily molds and some bacteria (Dimic *et al.*, 2000; Romagnoli *et al.*, 2007). Therefore, Spices are a primary alimentary intoxication when added to foods in which the pathogen is favorable to grow (Hampikyan *et al.*, 2009). According to Vij *et al.* (2006), the possibility of pathogen growth in higher rate in spices in case of poor hygienic condition when it used in food without subsequent preservation and that may lead to serious disease. Similarly, several studies show that spices contain various types of microorganisms such as pathogenic and toxigenic molds (Banerjee and Sarkar, 2003). Moreover, Martins *et al.* (2009) explained that molds from spices may reduce the quality of food and creates potential risk for human health with the production of toxic metabolites called mycotoxins. To this effect, the present study was designed to assess the microbial safety of some selected spices in Jimma town with the following objectives.

MATERIALS AND METHODS

Study area: This study was conducted in Jimma town located at 353 km south west of Addis Ababa the capital city of Ethiopia. The geographical location of the town is 7°41`N latitude, 36°50` E longitudes and an average altitude of 1780 m above sea level. The average minimum and maximum Temperature of the town is 14 and 30°C, respectively with annual rainfall ranges from 1138-1690 mm (Alemu *et al.*, 2011). This study was covered from September-may 2013.

Sample collection: A Total of 40 samples were collected from spices sellers of Jimma town, from September-May 2013.The sample was purchased from spices sellers at 2-5 AM. The sample was transported to Research and post-graduate laboratory Department of Biology, College of Natural Sciences and Jimma University main campus by investigator. The spices were kept in the Refrigerator at 4°C until analysis was conducted.

Sample preparation and microbial enumeration

Sample preparation: A 25 g of spices sample was suspended in 225 mL of saline solution and homogenized in erylenmer flask for 5 min using shaker at 160 rpm. A 1 mL of homogenized sample was transferred in 9 mL of saline solution and mixed thoroughly by using vortex mixer. The homogenized spices sample was diluted from 10^{-1}-10^{-8} and 0.1 mL of aliquot dilution was plated on pre-solidified plate and incubated at appropriate temperature (32°C) for 18-48 h. The colonies were counted from plate containing microbial colonies between 30-300 and expressed in colony forming unit per gram (CFU g^{-1}).

Microbiological analysis

Determination microbial spectrum: For Spice sample content Total Aerobic Mesophilic Counts (TAMC), Counts of *Staphylococci*, Count of Enterobacteriacea, *Bacillus*, Counts of yeast and molds and Counts of Coliforms were determined according tothe criteria specified in (Dabassa and Bacha,

2012) and by standard plate count methods on PCA (Plat Count Agar) (Oxoid) for TAMC at 32°C during 72 h, Mannitol Salt agar (Oxoid) for staphylococci count,Chloramphenicol Bromophenol Blue Agar for yeast and mold and VRBGA (Violet Red Bile Glucose Agar) (Oxoid) for Enterobacteriacea and VRBA (Violet Red Bile Agar) (Oxoid) for Total Coliform Count (TCC) during 24 h at 32°C for TCC and 44°C for FCC.

RESULTS AND DISCUSSION

Microbial counts: Spices may be in corporate to processed food or may be added as an ingredient or condiment to food prepared in home or food businesses. It may have a high incidence of microbial contamination. The International Commission on microbiological specification in foods recommended that spices can be treated as a raw agricultural commodity with ultimate use of the product determining the acceptable microbial load (Omafuvbe and Kolawole, 2004). In addition, spices have implicated in large scale outbreaks of food borne illness (Frisvad *et al.*, 2005). Additionally, utensil incase container of spices used by sellers has also its own impact in facilitating spice contamination and may have originated from sellers (vendor) hand when touch items uncover (dishcloths) (Cardinale *et al.*, 2005). So that, the mean count of Aerobic Mesophilic Bacteria (AMB) was highest (8.23 log CFU g^{-1}) in red chillies and lower (6.6 log CFU g^{-1}) in Turmeric (hird). The heat resistant spores of spore former bacteria may survive cooking while vegetative bacteria were eliminated (Freire and Offord, 2003). Similarly Fufa and Urga (1996) stated the food items that sold at stalls have spore forming bacteria which are the main concern due to temperature time abuse. The mean count of coliforms was high (6.2 log CFU g^{-1}) in Turmeric and low (5.8 log CFU g^{-1}) in Berber whereas mean count of Entrobacteriaceae low in red chillies (5.3 log CFU g^{-1}) while high (5.8 log CFU g^{-1}) in Turmeric. However, the mean count of *Staphylococcus aurous* was as high as red chillies (4.8 log CFU g^{-1}) in Turmeric and low in red chillies (Berbere). On the other hand, the mean count of yeast was high (6.9 log CFU g^{-1}) in the turmeric whereas low (5.8 log CFU g^{-1}) in the red chillies. But the mean count of molds was high (6.2 log CFU g^{-1}) in red chillies and low (5.8 log CFU g^{-1}) in turmeric. The reason why a wide range of spice contaminates with the moulds is that its spore can be carried out by wind and hence can easily enter into good sample (El-kady *et al.*, 1995).

The highest mean counts of AMB were recorded in spice sample red chillies from Mercato (10.3 log CFU g^{-1}) and turmeric from Kochi (7.75 log CFU g^{-1}). The mean count of total Aerobic mesophilic bacterial observed in the present study is line with Craig (1999) reported the total plate count of AMB in Red chilies' greater than 1×10^7 CFU g^{-1} from Australian spices. Similarly, the highest mean counts of Entrobacteriaceae in spices sample from Mercato (5.5 log CFU g^{-1}) and turmeric from Kochi (5.1 log CFU g^{-1}) and highest mean counts of coliforms were recorded from sample Turmeric of (Mercato)(6.9 log CFU g^{-1}) and Red chillies from Kochi (5.8 log CFU g^{-1}) whereas highest mean of yeast and molds were recorded in spices sample of Turmeric from Kochi (7.88 log CFU g^{-1}) and Red chillies from Kochi (6.8 log CFU g^{-1}) and Turmeric from Kochi (7.47 log CFU g^{-1}) and Red chillies from Kochi (6.5 log CFU g^{-1}), respectively. Furthermore, the highest mean count of *Staphylococcus aereus* was recorded in samples (turmeric) from Kochi (5.1 log CFU g^{-1}) and red chillies (Berbere) from Kochi area (4.9 logCFU g^{-1}).

The maximum and minimum mean counts of both spices samples were shown in (Fig.1). Accordingly the highest mean counts (log CFU g^{-1}) of AMB, coliforms, yeast, molds, *Staphylococcus aereus* and Entrobacteriaceae were recorded in spices samples. The mean count of Entrobacteriaceae in the present study was 5.5 CFU g^{-1} while the coli form count was 6 CFU g^{-1}

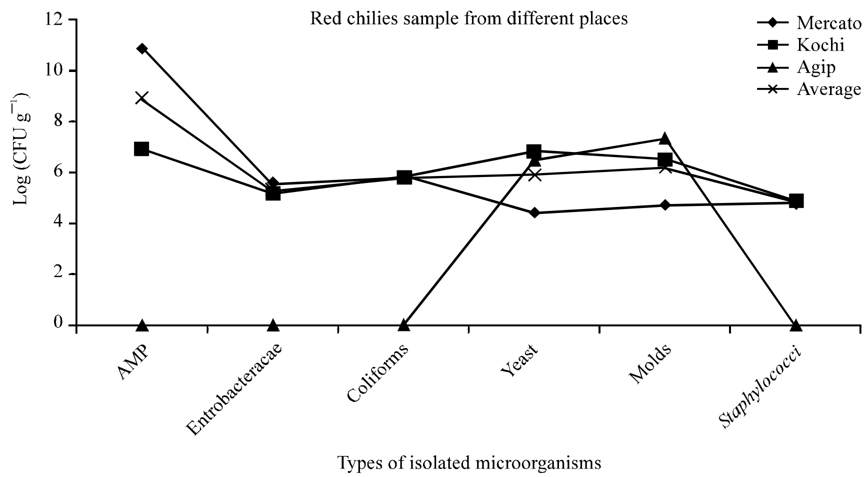

Fig. 1: Mean maximum and minimum microbial count of red chilies spice sample collected from different site, Jimma town

irrespective to (Colak *et al.,* 2006) who reported as 3.79 CFU g^{-1} from turmeric. The mean count of AMB was highest (10.3 log CFU g^{-1}) in red chillies sample collected from Mercato site and the mean counts of coliform was 5.8 log CFU g^{-1}. The mean counts of Entrobacteriaceae in the same sample was 5.5 log CFU g^{-1} followed by *Staphylococcus aereus* 4.8 log CFU g^{-1} but the highest mean count of yeast and molds was 4.4 and 4.7 log CFU g^{-1}, respectively. On the other hand the highest mean count of AMB from Mercato turmeric was 6.2 log CFU g^{-1} but the Entrobacteriaceae mean count was highest (6.5 log CFU g^{-1}) than AMB count. However, the mean count of coliform, yeast, molds and *Staphylococcus aereus* were 6.0, 5.5, 4.45 and 4.55 log CFU g^{-1}, respectively as in Fig. 1.

The results also show that the maximum mean microbial count (log CFU g^{-1}) of yeast, molds, AMB, Entro, coliform and *Staphy* in red chillies collected from Kochi site were 9, 8.7, 6.9, 6.2, 6.07 and 5.04, respectively, meaning they were above detectable level as shown in (Fig. 1). But it is line with (Chandarana *et al.*, 2005) who reported the high count of AMB Turmeric to be greater than 2.5×10^5 CFU g^{-1} from Japan spices. Similarly, the maximum mean microbial count (log CFU g^{-1}) of AMB, yeast, molds, coliforms, *Staphylococcus aereus* and Entrobacteriaceae in turmeric collected from Kochi site were 9.5, 9.43, 8.47, 6.64, 5.3 and 6.1, respectively. They were also above detectable level in samples as it expressed in (Fig. 2.) In contrast to this to this, the recent study indicate that the total count of AMB was greater than the limit.

Only the mean count of yeast and molds were detected from Agip site. Accordingly, the highest mean of yeast count in red chillies and turmeric was 6.5 and 7.3 log CFU g^{-1}, respectively. The mean count of yeast and molds in the current study is in line with Nair and Chanda (2006) who reported counts between 2-6 CFU g^{-1} from prepacked of Indian spices. However, the highest mean count of mold in red chillies and turmeric was 7.3 and 5.4 log CFU g^{-1}, respectively as it shown in (Table 1). In contrast to this study Buckenhuskes and Rendlen (2004) reported that count of yeast and molds to be 3.08 CFU g^{-1} from species in chain stores. INSC also reported that the maximum count number of molds has to be 5×10^3 CFU g^{-1} in contrasts to present study.

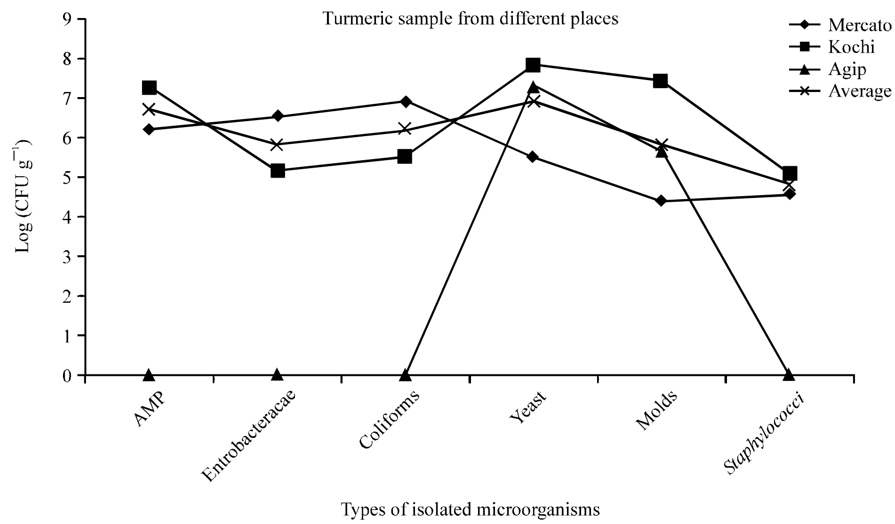

Fig. 2: Mean maximum and minimum microbial count of turmeric spice sample collected from different site, Jimma town

Table 1: Mean microbial count minimum, maximum (log CFU g^{-1})

Sample types	Sampling site	AMP Min	AMP Max	AMP Avg	Entrobacteracae Min	Entrobacteracae Max	Entrobacteracae Avg	Coliforms Min	Coliforms Max	Coliforms Avg	Yeast Min	Yeast Max	Yeast Avg	Molds Min	Molds Max	Molds Avg	*Staphylococci* Min	*Staphylococci* Max	*Staphylococci* Avg
Red chilies	Mercato	9.82	10.83	10.32	4.9	6.1	5.5	5.6	6	5.8	4.07	4.77	4.4	4.34	5.07	4.7	4.7	4.9	4.8
	Kochi	5.4	6.9	6.15	4.1	6.2	5.15	56	6.07	5.8	4.77	9	6.8	5.07	8.07	6.5	4.84	5.04	4.9
	Agip	-	-	-	-	-	-	-	-	-	6.44	6.55	6.5	7.33	7.36	7.3	-	-	-
	Average	7.61	8.86	8.23	4.5	6.12	5.3	5.6	6	5.8	5.09	6.7	5.9	5.6	6.8	6.2	4.8	4.9	4.85
Turmeric	Mercato	5.47	6.9	6.2	5.6	7.4	6.5	6.47	7.34	6.9	5	6.04	5.5	4.1	4.8	4.4	4.1	5	4.55
	Kochi	5	5.9	7.25	4.2	6.1	5.15	4.46	6.64	5.5	6.33	9.43	7.8	6.47	8.47	7.4	4.9	5.3	5.1
	Agip	-	-	-	-	-	-	-	-	-	7.33	7.36	7.3	5.4	5.9	5.65	-	-	-
	Average	5.23	8.05	6.7	4.9	6.75	5.8	5.44	6.99	6.2	6.22	7.61	6.9	5.49	6.24	5.8	4.5	5.15	4.82

Microbial counts minimum, maximum (log CFU g^{-1})

Mycopopulation analysis and characterization: From total of 20 fungal populations isolated *Mucor* spp. Were the predominant fungus in both Turmeric (50%) and Red chillies (33.33%) followed by yeast (25%) in red chilies and 25% in turmeric (hird). On the contrary, both *Pencillium* and *Goetrichum* spp. were the less dominant fungal strain isolated from turmeric (12.5%) and *Aspergillus* species were 16.6% while *Rhizopus*, *Eurotium*, *Fusarium* spp. were the least dominant fungal species isolated from red chilies (8.33%) (Table 2). *Aspergillus*, *Rhizopus* and *Fusarium* were the least dominant fungal isolates. In similar to the present study of Avato *et al.* (2000) who reported *Aspergilus* spp. isolated from Red chillies.

The *Fusarium*, *Rhizopus* and *Aspergillus* spp. were isolated from both turmeric and *Redchillies* that a agreed with Shadanaika (2005) from India. Similarly, Joe *et al.* (2009) reported that *Rhizopus* spp. was found in Turmeric powder.

Microscopically observed and characterized of these fungi indicated in (Table 3). Generally among the isolates fungal strain *Mucor* spp. were predominant (41.66%) followed by yeasts (25%)

Table 2: Frequency distribution of dominant fungi (molds and yeasts) from the selected spices, in Jimma town, south western of Ethiopia

Types of sample	Genus isolates	Frequency (%)
Turmeric	Mucor	4 (50)
	Yeast	2 (25)
	Pencillium	1 (12.5)
	Geotrichum	1 (12.5)
Red chillies	*Mucor*	4 (33.33)
	Yeast	3 (25)
	Aspergillus	2 (16.66)
	Fusarium	1 (8.33)
	Eurotium	1 (8.33)
	Rhizopus	1 (8.33)

Table 3: Mycopopulation distribution of dominant fungi in the selected spices, Jimma town, south western Ethiopia

Isolates fungi	General character (microscopically)
Yeast	Multi-lateral budding, white/cream colonies, no hyphae, smooth appearance
Rhizopus	Non-septate hyphae, consist rhizoids, dark sporangia large sporangiospore with striated cell
Mucor	Non-septate hyphae, has stolon than rhisoids, zygospores formation
Aspergillus	Black conidiophore, green considia, non-septate hyphae
Eurotium	Green conidia, possess ascospores, separate hyphae
Fusarium	Cotony brown colony, sickle shaped conidia, extensive mycelium
Geotrichum	Colorless conidia, antroconidia formation, separate hyphae
Pencillium	Grey conidia and striated conidia, extensively branched mycelium

while *Aspergillus* spp. were (16.6%) and *Goetrichum* and *Pencillium* spp. were (12.55%). *Fusarium*, *Rhizopus* and *Eurotium* spp. were (8.33%).

CONCLUSION

Spices are sourced from the roots, stems and seeds of the aromatic plants and sold at the market place. It can be become contaminated with pathogenic microorganisms during growth, harvesting, post harvest processing and during selling in the market place. The overall microbial quality of species assessed in this study was very poor, as sellers put spices utensil on the floor which easy to soil borne contaminate to it. Furthermore, the most predominant microbial group was *bacillus* spp. *Staphylococci*, *Penicillium* and *Aspergilus* spp. Thus the presence of this microbial group could be a possible predication for the presence of pathogen. Thus, the presence of these microbial groups could be a possible means to produce toxic substances that cause disease or illness to human being upon consumption.

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