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Safety evaluation and microbiological profile of some spices

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ABSTRACT

Samples of Cumin (*Cuminum cyminum),* Curry (*Murraya koenigii*), Galangal (*Alpinia galanga*) and turmeric (*Curcuma longa)* were evaluated to generate data about their microbiological profile. These samples were procured from different regions of Jeddah province, Saudi Arabia (SA). These samples were evaluated for their microbiological qualities i.e. contamination load of total aerobic mesophilic bacteria (TAMB), mesophilic aerobic sporeformer bacteria (MASB), coliform bacteria (CFB), and lactic acid bacteria (LAB), food borne bacterial pathogens such as *Bacillus cereus, Staphylococcus aureus, Clostridium perfringes,* The samples were also evaluated for the presence of pesticide (dimethoate, malathione, prefenofos, dieldrin, hepatochlor and lindane residues. Samples of all the four spices were found to have some load of TAM and MASB in the range of log 1.71 to log 6.80 cfu/gm. Curry and turmesric samples were containing CFB and LAB in the range of log 2.48 to log 6.70 cfu/gm, where as cumin and turmeric samples showed the presence of yeast and molds and *Bacillus cereus.* Clostridium perfringens was detected only in cumin. Only cumin samples showed the presence of organophosphorous pesticide residue.

KEYWORDS

Cuminum cyminum;
Murraya koenigii;
Alpinia galangal;
Curcuma longa microbiota;
Food borne pathogenic bacteria.

INTRODUCTION

Herbs and spices have been used for centuries by humans as food supplements and to treat ailments worldwide. Spices and herbs can be readily contaminated due to conditions in which they are grown and harvested and subsequent processing. At present, food safety is a main concern to consumers and food industries in the light of increasing cases of food associated poisonings.

Spores of several microbes have been found to be present on spices and herbs and their growth and toxin production documented[1,2]. This may be due to the conditions in which these are grown or subsequent harvesting and processing procedures. It is observed that no or very little data is available about the micro biota and its attributes to the microbiological qualities and safety of plant food materials specially spices in the Kingdom of Saudi Arabia.

The present study was designed to generate data on the microbiological profile of the cumin, curry, galangal and turmeric. In this study these spices were evaluated for microbiological qualities, food borne bacterial pathogens etc. Moisture content, pH value and pesticide residues were also determined.

MATERIALS AND METHODS

Samples

The total of 50 samples of cumin, curry, galangal and turmeric were collected each in the following 3 cat-

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egories, whole non packaged (WN), ground non-packaged (GN), ground packaged (GP). The samples were collected from five different retail outlets in Jeddah province, Saudi Arabia. The samples were authenticated and specimen voucher sample deposited at herbarium of the university for future reference.

Microbiological characteristics

The following criteria were monitored in all samples of spices, total aerobic mesophilic bacteria (TAMB), mesophilic aerobic spore former bacteria (MASB), coliform bacteria (CFB), yeast and mold (Y and M), lactic acid bacteria (LAB), *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus*. TAMB, MASB, CFB, Y and M and LAB were assessed in all samples in triplicate while *Bacillus cereus, Clostridium perfringens* and *Staphylococcus aureus* were monitored in duplicate.

1. Preparation of samples

1-g portions of samples were weighed into screw-capped (18×150 mm) tubes containing 9 ml of sterile saline (0.8% w/v), mixed gently, and kept for 30 minutes at room temperature to enable separation of the microorganisms from the plant matrix before vigorously agitating with a vortex for 1 minute. The mixture was left for 5 minutes to allow the coarse material to settle[3]. Appropriate decimal dilutions of the supernatant were made with sterile peptone water (0.1% w/v).

2. Media and incubation methods

Microbiological media used in this study were as follows: For TAMB and MASB, standard plate count agar (PCA; Difco) and aerobic incubation at 30^0C for 72h. However, in the case of MASB test samples were pretreated by laboratory heat treatment at 80^0C for 5 minutes. Violet red bile agar (VRBA; Merck) and aerobic incubation at 32^0C for 48h were used to assess CFB levels. Y and M were determined using phytone yeast extract glucose agar (4%) (Becton Dickinson) and aerobic incubation at 25^0C for 5 days. LAB were counted using MRS agar (Difco) and aerobic incubation at 37^0C for 48h.

3. Pathogenic bacteria were determined in duplicate as follows

Bacillus cereus, using *Bacillus cereus* selective agar (Oxoid) and aerobic incubation at 37^0C for 48h. Confirmation of typical colonies was based on endospore formation stained with malachite green[3]. *Clostridium perfringens* was counted according to the method outlined by Rodriguez-Romo et al[4].

Staphylococcus aureus was monitored in samples adopting the method of Kneifel and Berger[3] with slight modification. Briefly, Baird-Parker agar medium (Oxoid), aerobic incubation at 37^0C for 48h were used. For identification of presumptive coagualse (+) *Staphylococcus aureus*, representative colonies were loop-streaked on columbia mutton blood agar (bioMerieux) and checked for β-haemolysis, after incubation at 37^0C for 48h. In case of possible haemolytic activity occurred, a clumping test was used with rabbit plasma (Oxoid) in tubes inoculated with a loop of test bacterial colony and incubated at 37^0C for 12h.

Determination of pesticide residues

Pesticide residues in samples of cinnamon during the present investigation were determined according to the method of WHO[5]. Standards of pesticides (Organophosphorus and Organochlorines) were purchased from (Chemical Service Inc. PA, USA). The method briefly as follows: 50 grams of grounded spices and herbs were blended with 350ml acetonitrile/water solution (65:35 v/v) for 5 minutes at a high speed. The blend was filtered and mixed in a separating funnel with 100 ml petroleum ether for up to 2 minutes to permit pesticide residues to dissolve in the petroleum ether layer. The extract was transferred to a column of activated florisil to pass through at a rate of 5 ml/minute. It was eluted with a mixture of diethyl ether/light petroleum ether, and then dried to a certain volume for gas chromatographic analysis. A Hewlett-Packard Model 5890 gas chromatography with ^{63}Ni Electron Capture Detector and a flame Ionization Detector was used.

Determination of moisture content and pH values

Moisture content was determined by drying approximately a 10-g portion of sample at 105^0C to constant weight. For pH values, a 10-g spice and herb portions were mixed in 100 ml de-ionized water and stirred for 10 minutes. The pH of the mixture was measured using a digital pH meter (Hannah, Portugal).

Statistical analysis

To determine significance of the differences, data were analyzed by One Way Analysis of Variance (ANOVA) test after converting the microbial counts to a logarithmic scale.

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RESULTS AND DISCUSSION

Accumulated data showed that there were only few studies concerning microflora and mycobiota associated with food and aflatoxin production in general in Saudi Arabia[6,7]. Different microbiological analyses and aspects of food commodities and especially for spices and herbs in Saudi Arabia sneed to be addressed and their microbiological safety attributes are highlighted.Taking into consideration the above facts, present study was designed.

The different samples of cumin, curry, galangal and turmeric were procured in the form of WN, GN and GP. In all 50 samples were collected and evaluated to generate data about heir microbiological profile, TABLE 1. WN samples of cumin, galangal, turmeric showed presence of high load of TAMB, 5.48, 6.18 and 5.18 respectively. MASB count was higherin GN samples of curry and turmeric, 6.30 and 6.48 respectively. LAB was found in high load in GN samples of turmeric, where as all the samples were free from CFB, TABLE 2. The values are reported as $\log_{10}$cfu/gm of the sample. These results revealed that GN samples had high bacterial load more than 6 $\log_{10}$ cfu/g of the sample and in unacceptable range. Levels between 4 to 6 log10 sfu/g were of marginal quality and samples less than 4 were of acceptable quality as per International Commission on Microbiological Specifications for Foods (ICMSF)[8] specifications.

Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and therefore report more about general microbiological quality than possible health risks posed by the food product[9]. Faecal coliforms were absent in all the samples in our study and these results complied with the Germany standards regarding this analysis item[1]. Also these results agreed with those of others[10,11,12] who reported rare and sporadic faecal coliforms in spices.

TABLE 3 showed the mean $\log_{10}$ counts of yeast and mold of spices samples. Only GN samples of cumin found to have count of 5.0, i.e. unacceptable quality as per ICMSF[8]. Other samples of cumin, curry and turmeric showed the presence of yeast and mold, but in low counts and in the acceptable range. Samples of the spices were free from coagulase staphylococci. *Bacillus cereus* was detected in low range in the samples of the spices where as *Clostridium perfringens* was detected only in cumin. Galangal was found to be free from all these. Molds and yeasts were found in earlier studies[1,3,11] also in considerably similar counts compared to the present findings.

Stastical counts on all the samples revealed a low

TABLE 1: Description of spices samples obtained and analyzed in the study

Spice/herb		Parts used	No. of samples			Total
Common name	Scientific name		WN	GN	GP	
Cumin	*Cuminum cyminum*	Seeds	5	5	5	15
Curry	*Murraya koenigii*	Leaves	NA	5	5	10
Galangal	*Alpinia galanga*	Rhizomes	5	5	NA	10
Turmeric	*Curcuma longa*	Rhizomes	5	5	5	15
	Total		15	20	15	50

WN, whole non-packaged; GN, ground non-packaged; GP, ground polyethylene-packaged; NA, not available

TABLE 2: Mean values of total aerobic mesophilic bacteria (TAMB), mesophilic aerobic sporeformer bacteria (MASB), coliform bacteria (CFB) and lactic acid bacteria (LAB) counts as $\log_{10}$ cfu/g of spices samples

Spice/herb	TAMB			MASB			CFB			LAB		
	WN	GN	GP	WN	GN	GP	WN	GN	GP	WN	GN	GP
Cumin	5.48	4.26	3.30	3.85	2.70	1.71	Nil	Nil	Nil	Nil	Nil	Nil
Curry	NA	6.80	5.78	NA	6.30	5.30	NA	Nil	Nil	NA	4.48	2.48
Galangal	6.18	4.60	NA	4.26	3.30	NA	Nil	Nil	NA	Nil	Nil	NA
Turmeric	5.18	6.30	4.30	4.30	6.48	5.30	Nil	Nil	Nil	4.48	6.70	3.26

WN, whole non-packaged; GN, ground non-packaged; GP, ground polyethylene-packaged; NA, not available

TABLE 3: Mean values of yeast and mold (Y and M), coagulase (+) Staphylococci, *Bacillus cereus* and *Cl. perfringens* counts as $\log_{10}$ cfu/g of spices samples

Spice/herb	Y and M			coagulase (+) Staph.			*Bacillus cereus*			*Cl. perfringens*		
	WN	GN	GP	WN	GN	GP	WN	GN	GP	WN	GN	GP
Cumin	3.30	5.00	3.70	Nil	Nil	Nil	3.20	3.26	2.00	1.60	1.00	1.00
Curry	NA	3.00	2.60	NA	Nil	Nil	NA	2.30	1.48	NA	Nil	Nil
Galangal	Nil	Nil	NA	Nil	Nil	NA	Nil	Nil	NA	Nil	Nil	NA
Turmeric	2.70	3.30	2.30	Nil	Nil	Nil	2.30	1.48	1.70	Nil	Nil	Nil

WN, whole non-packaged; GN, ground non-packaged; GP, ground polyethylene-packaged; NA, not available

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TABLE 3: Mean values of moisture content and pH of spices samples

Spice/herb	Moisture content (%)		pH	
	WN	GN	WN	GN
Cumin	11.5	12.3	6.1	6.3
Curry	NA	14.8	NA	5.7
Galangal	16.2	16.5	5.4	6.1
Turmeric	9.30	13.5	6.5	5.8

WN, whole non-packaged; GN, ground non-packaged; NA, not available

count of bacteria in GP commercial presentations ($P<0.05$) when compared with other commercial presentations (WN and GN). It can be concluded that counts were more in non-packaged samples as compared to packaged ones. This was also found in the study of Rodriguez-Romo et al[4].

The results of moisture content and pH values are summarized in TABLE 4. The moisture content was in the range of 9.30% to 16.5% for the samples. It is apparent that ground non-packaged (GN) samples generally reflected higher figures of moisture content compared to whole non-packaged (WN) spices and herbs samples. (GN) samples due to the fact that they were ground non-packaged might be more able to adsorb moisture especially at high atmospheric relative humidity of Jeddah province which lies on the western coast of Saudi Arabia. Our results generally showed that moisture content values were somewhat high coinciding with other reports elsewhere[1,13]. Any appreciable increase in moisture content of spices stimulates faster mold growth that results in inferior quality and a low marketable value[13].

TABLE 4 also showed the pH values for cardamom and ginger were in the range of 5.4 to 6.5. The pH values were in approximate agreement with other report[13].

Organophosphate and organochlorine pesticides are widely used in the agriculture for control of various insects. The residues of these sometimes are present on the spice or herb at the time of consumption by animal or humans. Beginning even before birth, we are exposed to low levels of pesticide residues through our foods. In our study organophosphates were present in high concentration in cumin, curry and galangal. Organochlorines residues were present in relatively lower concentration in galangal and turmeric. Samples of cumin and curry were free of organochlorines where as turmeric samples were found to be free organophosphates, TABLE 5.

TABLE 5: Mean levels (mg/kg) of some pesticide residues detected in some spices samples

Spice/herb	Types of pesticide					
	Organophosphorus			Organochlorines		
	Dimethoate	Malathion	Profenofos	Dieldrin	Heptachlor	Lindane
Cumin	0.104	0.235	0.116	ND	ND	ND
Curry	0.221	0.077	0.051	ND	ND	ND
Galangal	ND	0.215	0.151	0.018	0.030	0.112
Turmeric	ND	ND	ND	0.15	0.045	ND

ND, not detected

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