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RESEARCH ARTICLE

"TO STUDY THE FOOD SAFETY OF KASURI METHI AND EFFECT OF MICROFLORA ON ITS ORGANOLEPTIC PROPERTIES"

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A B S T R A C T

The present study was aimed to study the food safety of Kasuri Methi and the effect of its microflora on its organoleptic properties. 6 different samples of Kasuri Methi were collected from the nearby areas of Vile Parle and Andheri, Mumbai, Maharashtra. The microbial analysis of all the samples were done which included TPC, YMC, Coliform count, Detection of pathogens like *Salmonella spp., Shigella spp., E. coli, Enterobacter aerogenes, & S. aureus*. Highest TPC was observed in Commercial sample R and lowest was observed in Commercial sample B. Highest YMC was observed in Loose sample collected from Vile Parle and lowest was found in Commercial sample R, Highest count was observed in Commercial sample E and lowest was observed in Commercial sample B. the samples were further confirmed and subjected to identification for the presences of pathogens mentioned above. Al the 6 samples showed presence of Coliforms and *Salmonella* &/or *Shigella spp,* while only 3 samples i.e. Commercial sample E,B, and Loose sample obtained from Vile Parle market showed presence of *S. aureus*. Chemical analysis was done to find out the concentration of compounds responsible for its aroma and flavour. Overall all the 6 samples were found to be of microbiologically unacceptable quality and the results also indicated that the microbial load present in &/or on the sample may affect its organoleptic properties.

INTRODUCTION

Kasuri Methi refers to dried leaves of the fenugreek plant. It is an herb with a bitter taste. Fenugreek, *Trigonella foenum-graecum* L. is an ancient and annual legume crop mainly grown for multiple uses in many parts of the world. Fenugreek contains different alkaloids, flavonoids and saponins, proteins, vitamins, Choline, etc. Fenugreek seed contains volatile oil and fixed oil in small quantities. Fenugreek is widely used in food preparation such as curries, handi recipies, or as garnishing product. Fenugreek (Kasuri Methi) is an amazing magic herb that can cure number of ailments. Like any other object of this planet spices and condiments are also not free from microbial association. The spices may acquire microorganisms during their growth and development or during later stages when these are passed through collection, processing, storage and marketing. Finally at consumers' level these can be found with multitude of organisms. (*Khan M R, 2012*). Products of plant (non-animal) origin are increasingly being recognised as associated with outbreaks of infection of both *Salmonella* and other pathogens (Elviss *et al*, 2009).

MATERIALS AND METHODS

6 Samples of Commercially available Kasuri Methi were collected from retailer: 2 loose and 4 brands (Catch, Everest, Badshah, & Rambandhu) were taken (25g each). Branded samples were obtained from general stores in the nearby areas of Vile Parle and Andheri; one loose sample was obtained from a general store in Andheri market and second loose sample was obtained from a retailer using Kasuri Methi for making snacks to sell.

Samples Were Processed For

(American Herbal Product Association, 2014) Total plate count, Total combined yeast and mold count, Enterobacterial count (Bile tolerant gram negative bacteria), *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Shigella spp.* Samples were mixed with sterile saline and analysed (1g+10ml).

METHODOLOGY FOR MICROBIOLOGICAL ANALYSIS

Total plate count for total aerobic mesophilic bacteria can be carried out in pour plates of plate count agar. Mix 1ml of dilution with 20ml of molten media (45^{o}C) Incubate at room temperature (RT) for 18-24hrs. Count the colonies as cfu/g fresh weight sample.Enumeration of Yeasts and Molds can be carried out in pour plates of potato dextrose agar or Rose Bengal Chloramphenicol agar.Incubate at 28^{o}C for 2-5 days (The representative colonies can be checked for purity before counting). Estimation of Enterobecteriaceae can be carried out in pour plates of Violet Red Bile Glucose Agar without lactose.Incubate at 35^{o}C for 18-24hrs. Confirmation can be done by cytochrome oxidase using oxidase disc and glucose fermentation in stab cultures of purple agar base

supplemented with 1% w/w D (+) glucose.To confirm coliforms, isolate the confirmed enterobacteriaceae isolate isolates onto brilliant green bile broth (BGBB), 2% with inverted derham's tubes.Incubate at 37°C for 24-48hrs. Examine for growth and gas production.For tests of faecal coliforms, incubate the inoculated BGBB tubes at 44+/-5°C for 24hrs. To confirm the presence of *Escherichia coli*; check Indole production by using tryptone water and Kovac's reagent strip. For checking the presence of *Salmonella spp.* And *Shigella spp.* use 25g of sample for pre-enrichment, followed by enrichment and isolation in media: tetrathionate/ selenite F broth for enrichment and brilliant green/ deoxycholate citrate agar. Isolates can be maintained on nutrient agar. The presumptive isolates can be confirmed by appropriate biochemical tests. Selective enumeration of *S. aureus* can be carried out on surface spread plates of Baird-Parker agar. Incubate at 35oC for 24-48hrs. The presumptive colonies can be confirmed by appropriate biochemical tests.

For Chemical Analysis

(Blank I. *et al*; Principle flavour components of Fenugreek)
Ethanol extraction of the samples was carried out. Qualitative and quantitative analysis was then carried out by 2, 4-Dinitrophenylhydrazine (2, 4-DNP) method.

In case of total plate count it was performed using Glucose Yeast Extract Agar medium by pour plate technique. And if we compare the results shows all the 6 samples + 1 sterilized sample were found to be of acceptable quality with the standards (AHPA Standards, 2014). It indicates that there were microbial load present on the surface of the sample but it was not more than the permissible limits provided as per standards (AHPA Standards, 2014). As discussed by scientists Banerjee M., from 154 samples of spices 78 samples were found contaminated with quality unacceptable in case of total plate count. They considered ICMSF specifications according to which total count of less than 10^4 cfu/g is acceptable and 10^4 - 10^6 cfu/g is of marginal quality and 10^6 cfu/g is of unacceptable quality. They used different spices samples; here we took different types of same samples i.e. Kasuri Methi. They found highest count in black pepper i.e. $8x10^7$ cfu/g and lowest count in garlic i.e. $5x10^3$cfu/g. The Total Plate Count was observed to be in between 1.8 $\log_{10}$cfu/g to 3.4 $\log_{10}$cfu/g. Highest count was observed in loose sample collected from Andheri market i.e. $5.2x10^6$ cfu/g and lowest count i.e. $1.0x10^1$ cfu/g in sterilized loose sample collected from a retailer making snacks using Kasuri Methi. (Addo, 2005), tested the microbiological quality of spices and the total aerobic bacterial counts in the spices ranged from 3.6 $\log_{10}$cfu/g to 3.7 $\log_{10}$cfu/g sample.

Table No 1 Consolidated results of Microbial and Chemical analysis of samples

SAMPLE	Total plate count CFU/g	Yeast and Mold count CFU/g	Enterobacterial count CFU/g	*Salmonella spp.* &/or *Shigella spp.*	Coliform	*Staphylococcus aureus*	Concentration of aldehyde and ketone (Sotolone) mg/ml
Commercial Sample C	1.2x105	1.1x103	2.4 x106	Present	Present	Absent	6.85
Commercial Sample E	2.7x104	7.4x102	2.9 x106	Present	Present	Present	6.40
Commercial Sample B	3.1x103	4.7x102	2.6x103	Present	Present	Present	7.80
Commercial Sample R	5.2x106	7.1x101	1.4x105	Present	Present	Absent	7.20
Loose sample 1	1.6x106	3.1x102	7.1x104	Present	Present	Absent	8
Loose sample 2	2.4x105	3.0x103	3.7x105	Present	Present	Present	4
Sterilized loose sample 2	1.0x101	Not Performed	Not Performed	Not Performed	Not Performed	Not Performed	6.60

RESULTS AND DISCUSSION

Products of plant (non-animal) origin are increasingly being recognized as associated with outbreaks of both *Salmonella* and other pathogens as discussed by Willis C, *et al*, 2013. There are multiple opportunities for microbial contamination of these products Pre, during, and post-harvest as well as during processing and in retail, catering and domestic environments. In addition, these products are commonly consumed raw or following minimal processing which is unlikely to eradicate biological foodborne hazards.

Analysis of 6 samples of Kasuri Methi i.e. 4 Commercial samples (C,E,R,B) and 2 Loose samples (one collected from Andheri Market and one collected from retailer making snacks using Kasuri Methi) was performed. Microbial limits for finished botanical products provided by American Herbal Product Association i.e. AHPA (2014) were used for interpretation of our results. The limits provided are total aerobic microbial count-10^7cfu/g, yeast and mould count as 105 cfu per gram, Enterobacterial count (bile tolerant gram negative bacteria) -10^4 (as total coliforms) *E. coli*- absent in 10 grams, *Salmonella spp.* – absent in 25 gms.

The survival of fungal species on dehydrated products is well known (Ahene, *et al*, 2011). Total yeast and mold count was performed for all 6 samples on Rose Bengal Chloramphenicol Agar plates; incubation was done for 48hours at temperature 24-27°C. The results showed all 6 samples were found acceptable with respect to total load of Yeast and Molds on the surface of the sample by comparing to AHPA standards. But this should be confirmed that which species of yeast and mold are present. Identification of species is important as there can be any pathogenic species which may be present in lower amount but it still can be harmful or it may be producing any toxic products which can be harmful to human health and as toxins cannot be denatured even after cooking in most cases (Ahene, *et al*, 2003) it can be proved dangerous. In earlier studies also it was found with spices that though the population of resident micro flora was low, there were other fungal species of pathological importance resident in spice samples (eg. Onion, Aniseed, Rosemary, etc.) They analysed which includes pathogenic fungi like *Aspergillus spp.* (*A. flavus, A. alutaceus, A. niger*) which can produce toxins as well Eg. Aflatoxin produced by *A. flavus* (Ahene, *et al*, 2003). Also scientist Banerjee M. discussed in his studies performed with different samples of spices, he could observe presence of

molds in 97% of the samples and yeasts were detected in 1 out of 154 samples. They used PDA for fungal isolation and incubation of 2-5 days at 28oC. Scientist Toma FM also observed growth of fungi from 16 different samples of spices; which were when identified included *Aspergillus spp.*, *Rhizopus spp., Alternaria spp., Cladosporuim spp., etc.* they used PDA medium for isolation of the same and also DRBC media for mycotoxin detection.

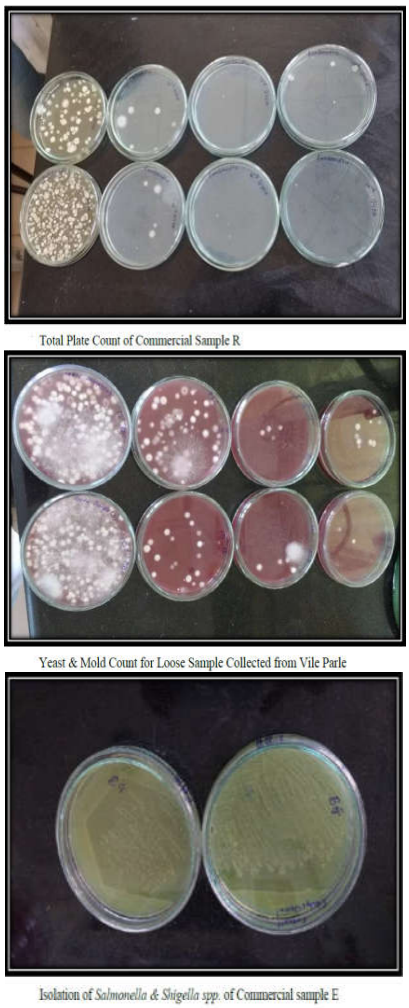

Figure 1

Enterobacterial counts are used more generally as an indicator of hygienic quality of faecal contamination and therefore say more about microbological quality than possible health risks posed by the product (Banerjee M, *et al*, 2003). Enterobacterial counts were performed on Violet Red Bile Agar and incubation was done for 24hours at 37^{o}C. After counting and calculating the results they were compared to AHPA standards. It was found that 5 out of the 6 samples (i.e. commercial sample C,E,R, and both loose samples) were found unacceptable in terms of Enterobacteriaceae counts (bile tolerant Gram negative bacteria).Only one sample i.e. commercial B sample showing count as $2.6x10^3$ cfu/g was of acceptable quality. A highest level of Enterobacteriaceae was found in commercial samples C & E whereas lowest unacceptable count was observed in loose sample obtained from Andheri market.

As discussed by scientist Willic C in his studies for fresh herbs *E. coli* was present at a level of ≥20 cfu/g in 103 samples and counts of $\geq 10^2$ cfu/g (range $1.0*10^2$ – $>1.0*10^7$ cfu/g) were detected in 88 samples and the proportion was higher in leafy spices like curry and paan leaves. In this study, we used dried methi leaves (Kasuri Methi). As it is present in fresh herbs there is a possibility of its persistence in dried ones as well. Presence of *E.coli* was found in 2 out of 6 Kasuri Methi samples i.e. commercial sample E and B and Loose sample obtained from Andheri market showed typical intermediate isolate of *E. coli* and *Enterobacter aerogenes*. Scientist Elvis N (2009) also could detect presence of *E. coli* at ≥102 cfu/g in variety of different herb types such as Basil, Coriander, Mint, Dill, Parsley, Tanagon and other herbs. Also scientist Banerjee *et al* could observe the presence of coliforms in 12 out of 154 samples of spices from which a high level i.e. >104 cfu/g occurred in 14 – 20% of the samples. He also could observe members of Enterobacteriaceae in 23 out of 27 kinds and 32% of the samples of spices. Here Coliforms were observed in all the 6 six samples of spices studied including Commercial samples C, E, R, B, & Loose samples from Andheri market and from a retailer making snacks using Kasuri Methi. Presence of *Enterobacter aerogenes* were observed in 3 samples i.e. Commercial samples E, B and Loose sample collected from the retailer. *Salmonella* spp. have been found in a wide variety of herbs and spices with rates of contamination ranging between 0.6% - 14% (Sagoo *et al*, 2009). In our study, Brilliant Green Agar was used for its isolation and then the identification tests were performed. Presence of *Salmonella* and/or *Shigella* was observed in all the 6 samples under study. Also 3 out of 6 samples showed presence of *Staphylococcus aureus* i.e. commercial sample E, B and Loose sample obtained from the retailer using Kasuri Methi for making his snacks to sell. Other studies also discuss the results showing presence these pathogens in spice/herbs samples; as observed by Sagoo *et al* (2009) *Salmonella* spp. were detected in 1.5% (2/132) of production batches and 1.1% (31/2833) of retail samples.

To determine significance of the difference (p=0.05), Analysis of variance was computed using log cfu values of APC, YMC, and Coliform counts. There was significant difference observed between the APC and YMC and also between YMC and Coliforms indicating that there was correlation between the two results; as APC increased YMC was observed to be decreased; similarly as Coliform counts increased YMC was found to be decreased. But there was no significant difference between Sample no. and APC, YMC & Coliforms and also between APC and Coliforms and between 6 samples the difference was not significant indicating that the results were not significant. Also paired T test was performed significant difference was found only between APC & YMC and YMC & Coliforms. But there was no significant difference between APC and Coliforms, Sample no. and APC, YMC, & Coliforms.

Fenugreek's typical and strong flavour with typical smell is due to the components present in it. Upto date Sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone) has been established as the main impact odour compound from fenugreek seeds responsible for its strong unpleasant odour on the basis of Gas Chromatography Mass Spectrometry (GCMS) and Gas-Olfactometry (GC-O) (Mebazaa R,2009). Sotolone is a sugar lactone having a keto group hence can be considered as Ketone. Therefore its aldehyde and ketone content was estimated using 2,4-Dinitrophenyl hydrazine method using

Benzaldehyde as standard (Concentration range was taken as 2mg – 10mg) and therefore aldehyde and ketone contents were calculated in terms of Benzaldehyde. Highest amount was calculated for loose sample collected from Andheri market i.e.8mg/ml and lowest amount was calculated for Loose sample collected from a retailer using Kasuri Methi for making snacks i.e.4mg/ml. From which both the samples showed presence of coliforms and *Salmonella Shigella* while only the later showed presence of *S. aureus* and the former did not. If we compare TPC the former showed higher counts but for YMC and Coliforms the later showed higher counts. Commercial sample B showed second highest concentration aldehyde and ketone i.e.7.80. Commercial sample B showed lower counts for TPC, YMC & Coliforms when compared with loose sample collected from the retailer showing lowest concentration of aldehyde and ketone. Sample B also showed presence of *Salmonella Shigella* and *S.aureus*. Also Loose sample collected from Vile Parle when sterilized showed higher contents of aldehydes and ketones than that of non-sterilized one. It may indicate that microbial load especially YMC and Coliforms affect the concentration of aldehyde and ketone hence affecting its organoleptic properties. Also GCMS analysis was performed for 3 samples: Sterilized and non-sterilized loose sample obtained from the retailer and one commercial sample i.e. B. We did not get very clear peaks for Sotolone; it may be due to the extraction method used which was methanolic extraction or because of the absence of Sotolone as internal standard. There is a need to check and improve the extraction method of the same. However, there were few peaks obtained near to its molecular weight i.e. 128.13. From those one compound was found from loose sample obtained from Andheri market having molecular weight 120.1485– formula: C_8H_8O named Benzene acetaldehyde. Another compound named 4-hydroxy-2-methyl acetophenone ($C_9H_{10}O_2$) having molecular weight 150.1745 which is a ketone and was found from Sterilized loose sample obtained from the retailer indicating a possibility that Sotolone may be present in the sample. The analysis should be performed again with highly specific facilities including a huge library and also Sotolone should be provided as an internal standard for better identification, quantification, and comparison.

CONCLUSION

Spices and herbs are usually consumed uncooked or following minimal processing which is unlikely to eradicate biological foodborne hazards. As it was found all the samples microbiologically of unacceptable quality in terms of presence of one or more contaminants. All the 6 samples were found of acceptable quality in terms of TPC and YMC the standard limits for which are 10^7 & 10^5 cfu/g respectively. But this does not ensure the safety of the product as bacteria or fungi though present in mall amount can be proved harmful as some species present may be pathogenic for eg. Some fungi can produce mycotoxins. The source of these contaminations should be identified and can be reduced by taking appropriate measures. The study of the spices is important as outbreaks of pathogens eg. *Salmonella spp.* are reported because of the same.

Bibliography

Ahene R.E, Odamtten G.T, Owusu E (2011); Fungal and bacterial contaminants of six spices and spice products in Ghana; *African Journal of Environmental Science and Technology* Vol. 5(9) pp. 633-640

Caroline Willis *et al* (2014); Public Health England Coordinated Food Liaison Group Studies:An Assessment of the Microbiological Safety of Fresh Leaf Herbs From Retail Premises in the United Kingdom with a Focus on *Salmonella* spp.; Public Health England Food Water and Environmental Microbiology Laboratory Porton; pp1-16

Chitra M, *et al* (2014); Antimicrobial and phytochemical analysis of acetone extract of *Trigonela foenum-graecum*; *RSIS International*; 1(2):44-48

Elviss Nicola C. *et al* (2009); Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis*; International Journal of Food Microbiology*; 134:83-88

Erum Shazia, Rashid A. and Shahid M.(2011); Evaluation of Kasuri Methi *Trigonella foenum-graecum*l.var. To establish gi right of Pakistan; *Pakistan j. Agric. Res*.; Vol24:1-4

Khan R M, *et al* (2012); Bacteria associated with common spices and their possible implications; *International Journal of Microbiological Research*; 3(1):53-58

Little C, *et al* (2009); Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom; *food Microbiology* 26: 39-43

Mehrafarin A, *et al* (2011); A review on biology, cultivation and biotechnology of Fenugreek as a valuable medicinal plant and multipurpose; *Journal of Medicinal Plants*; 10(37)

Muman Enas Mehjen, Shifaa J. I, Hedef D. E. (2014); A study of the chemical composition and the biological active components of *Nigella Sativa* and *Trigonellafoenum-graecum L*. seeds; IOSR *Journal of Applied Chemistry* (IOSR-JAC) e-ISSN: 2278 5736.Volume 6, Issue 6; pp 43-45

Toma FM, *et al* (2013); Isolatin and identification of fungi from spices and medicinal plants; Research Journal of Environmental and earth sciences; 5(3): 131-138

Shah Mukhtar Hussain *et al* (2010), Impact of pathogenic fungi and bacteria on fenugreek *(Trigonella foenum-graecum*l) plant stand quality under natural condition; Pak. *J. Phytopathol*., Vol22(2):130-134

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