

Microbiological quality assessment of three selected spices in Bangladesh

¹*Parveen, S., ¹Das, S., ²Begum, A., ²Sultana, N., ²Hoque, M. M. and ²Ahmad, I.

¹Food Microbiology Section, Institute of Food Science & Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh

²Shahjalal University of Science and Technology, Department of Food Engineering and Tea Technology, Sylhet-3114, Bangladesh

Article history

Received: 27 November 2013

Received in revised form:

23 January 2014

Accepted: 24 January 2014

Keywords

Spices

Standard plate count

Coliform count

Yeast and Mould count

Hygienic quality

Colony Forming Unit

Abstract

Spices are used all over the world for culinary purposes. Bangladeshis have strongest preferences for this. Warm and humid condition is favorable for their growth. For this reason spices are easily contaminated by pathogenic microorganisms. 15 packed and 27 unpacked spice sample of three kinds- red pepper, turmeric and coriander were collected from different markets and bazars in Bangladesh. Standard microbiological analysis was carried out for the detection and enumeration of microorganism using standard media. Mean standard plate count values for the 3 unpacked products ranged from 8.53×10^4 to 4.53×10^5 cfu/g and for 3 packed products ranged from 5.26×10^2 to 2.06×10^3 cfu/g. The mean Coliforms counts ranged from 3.65×10^1 to 7.20×10^1 MPN/g and mean yeast and mould count ranged from 3.37×10^3 to 1.90×10^5 cfu/g for unpacked samples. Mean *Escherichia coli* counts ranged from 0.76 to 1.28×10^1 MPN/g in unpacked spice samples. The mean count of *Staphylococcus aureus* in unpacked coriander sample was 3 cfu/g. Results are compared with International Microbiological Standard and found that some microbiological parameters of unpacked sample were above the recommended limits. Therefore, it is suggested to maintain good sanitary practice and hygienic quality during production stages of spices in our country.

© All Rights Reserved

Introduction

Spices constitute an important group of agricultural commodities, which are virtually indispensable in the culinary art. They can be primarily defined as farm products used in various forms, namely fresh, ripe, dried, broken, powdered, etc. which contribute aroma, taste, flavour, color and pungency to food. Spices may be either bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant tops (Takeda *et al.*, 2008).

The present study is based on three type of spices—Red pepper, Coriander and Turmeric. Pepper is grown throughout in Bangladesh. Peppers are of numerous varieties and their degree of pungency varies. It is used as it is or a paste form. Dry peppers are used in various dishes such as curry, meat, fish, etc., give a red color and make the dish attractive. Coriander forms the base of most Bangladeshi dishes. Both its seeds and leaves are used in dishes. Coriander seeds are mostly used in powder form slightly roasted to improve the flavour. Turmeric is the dried rhizome of an herbaceous plant. It is mainly used for making the color of the dishes. It is the most popular spice as a beauty aid. The spice is sometimes called ‘Indian saffron’ (Takeda *et al.*, 2008).

Spices may be contaminated because of the conditions under which they were cultivated and harvested. Contaminated spices have been reported to have been the causes of certain food-borne illnesses and spoilage (Ahene *et al.*, 2011). Indigenous microflora of plants, presence of microorganisms in processing plant, air, dust, using contaminated water and animal/human excreta, pre- and post-harvest procedure including processing, storage, distribution may be the sources of microbial contamination of spices. Therefore, spices pose health problems because they are often added to foods without further processing or are eaten raw (Colak *et al.*, 2006).

During cleaning and processing procedure of spices, there is progressive reduction in the number and types of microorganisms; those remaining are usually aerobic spore-forming bacteria and common moulds. In addition to the contamination of raw food supplies that occurs during growing, shipping and processing, there is the problem of food contamination caused by people who are carriers of pathogens such as *Escherichia coli* and *Staphylococcus aureus* (Ahene *et al.*, 2011). Coliform bacteria occur sporadically and usually in small populations in spices and are associated with fecal contamination. Yeast and mould densities vary considerably with the individual spices, but are usually quite low (Donia, 2008). Spices are

*Corresponding author.

Email: sahana66@gmail.com

mainly used as ingredients to flavour different types of prepared food items or drinks. Many spices have additional commercial uses, e.g., as ingredients of medicine, perfumes, incense and soaps. It is also used as a condiment. Spices are good not only for our taste buds but also for our health (Rathore *et al.*, 2008).

Therefore, this research work was carried out to throw light on the survey of unpacked and packed spices in local market which were used directly by human. This study conducting surveys on microbial status of spices as offered for sale to consumers in retail markets and bazars. The objective of this study was to provide information about microbiological contamination of spices and thus informed any further probable risk consideration. This study represented data on microbial load of 3 different types of locally collected unpacked and packed spice samples (Red pepper, Turmeric and Coriander) in Bangladesh.

Materials and Methods

Collection of spice samples

A total of 15 packed and 27 locally collected unpacked samples representing different type of spices were randomly collected from different market and bazars in Bangladesh. The microbiological analysis was performed to the laboratory immediately after sample collection. All samples were analyzed in duplicate.

Microbiological analysis

All of the samples were carried to the laboratory for microbiological investigations. Enumeration of Total Plate Count, Total Coliform Count, *Escherichia coli* Count, *Staphylococcus aureus* Count and Yeast and Mould Counts were carried out by following Bacteriological Analytical Manual. Total Plate Count was enumerated by pour plate method on plate count agar. Enumeration of Total Coliform Count and *Escherichia coli* Count were carried out by using three-tube most probable number (MPN) method (BAM, 2002). Positive tubes from MPN were streaked onto eosin methylene blue (EMB) agar and incubated for 24 h at 37°C. The typical colonies on EMB agar plate was confirmed by IMViC tests (BAM, 2002). Yeast and Mould Counts were carried out by spread plate technique on potato dextrose agar (PDA) media. Baird-Parker agar medium was used for the detection of *Staphylococcus aureus*. Characteristic black colonies on BPA plate surrounded by a clear zone were selected and subjected to coagulase and mannitol fermentation tests for confirmation of *Staphylococcus aureus* (BAM, 2001).

Table 1. Description of the spice samples collected from different places

| Spice Type | Local name | Scientific name | No. of locally collected unpacked spice samples | No. of collected packed spice samples |
|------------|-------------|---------------------------|---|---------------------------------------|
| Red pepper | Suknamorich | <i>Capsicum annum</i> | 09 | 05 |
| Turmeric | Holud | <i>Curcuma longa</i> | 09 | 05 |
| Coriander | Dhonia | <i>Coriandrum sativum</i> | 09 | 05 |

Table 2. Mean count of microorganisms detected in locally collected unpacked spice samples

| Sample name | Total Plate Count (cfu/g) | Yeast and Mould Count (cfu/g) | Total Coliform Count (MPN/g) | <i>Escherichia coli</i> (MPN/g) | <i>Staphylococcus aureus</i> (cfu/g) |
|-------------|---------------------------|-------------------------------|------------------------------|---------------------------------|--------------------------------------|
| Red Pepper | 3.00×10^5 | 5.56×10^3 | 7.20×10^1 | 3.13 | 0 |
| Turmeric | 8.53×10^4 | 3.37×10^3 | 3.65×10^1 | 1.28×10^1 | 0 |
| Coriander | 4.53×10^5 | 1.90×10^5 | 6.32×10^1 | 0.76 | 3.0 |

Table 3. Mean count of microorganisms detected in packed spice samples

| Sample name | Total Plate Count (cfu/g) | Yeast and Mould Count (cfu/g) | Total Coliform Count (MPN/g) | <i>Escherichia coli</i> (MPN/g) | <i>Staphylococcus aureus</i> (cfu/g) |
|-------------|---------------------------|-------------------------------|------------------------------|---------------------------------|--------------------------------------|
| Red Pepper | 2.06×10^3 | 1×10^2 | 0 | 0 | 0 |
| Turmeric | 8.18×10^2 | 0 | 0.86 | 0 | 0 |
| Coriander | 5.26×10^2 | 0 | 0 | 0 | 0 |

Results and Discussion

The mean microbial load of spice samples collected from different markets and places in Bangladesh was summarized in Table 2 and 3. In the present study, mean microbial load varied from spice to spice. Among the locally collected unpacked spice samples, the highest mean plate count was found in coriander (4.53×10^5 cfu/g). The lowest mean plate count (8.53×10^4 cfu/g) was found in turmeric. Yeast and mould count was also detected. Mean yeast and mould count was higher in coriander (1.90×10^5 cfu/g) and lower in turmeric (3.37×10^3 cfu/g). Coliform bacteria were found in all locally collected unpacked spice samples. The highest Coliform count was found in red pepper (7.20×10^1 MPN/g) and lowest count was in turmeric (3.65×10^1 MPN/g). Highest mean *Escherichia coli* count was detected in turmeric (1.28×10^1 MPN/g). The mean count of *Staphylococcus aureus* in coriander sample was 3 cfu/g. *Staphylococcus aureus* was not found in red pepper and turmeric sample (Table 2).

In the study of packed spice samples, mean plate count was higher (2.06×10^3 cfu/g) in red pepper and lower (5.26×10^2 cfu/g) in coriander. The mean yeast and mould count (1×10^2 cfu/g) was only found in red pepper. Negligible count of Coliform was present in turmeric (0.86 MPN/g). All packed samples were not contaminated with *Escherichia coli* and *Staphylococcus aureus* (Table 3).

In the present study, five microbiological parameters-Total plate count, Yeast and mould count, Total Coliform, *Escherichia coli* and *Staphylococcus aureus* were carried out. This study provided information about microbiological contamination of

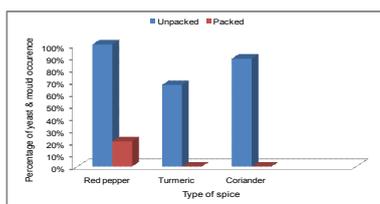


Figure 1. Percentage of occurrence of Yeast and mould count in unpacked and packed spice samples

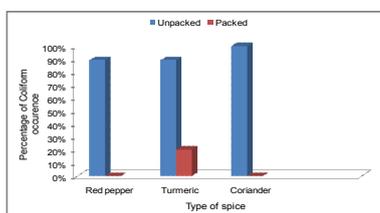


Figure 2. Percentage of occurrence of Coliform count in unpacked and packed spice samples

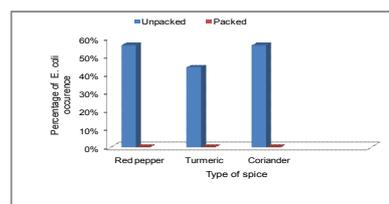


Figure 3. Percentage of occurrence of *E. coli* counts in unpacked and packed spice samples

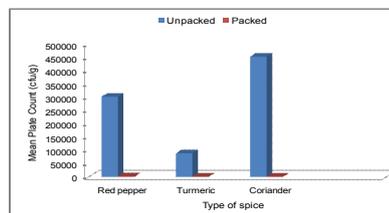


Figure 4. Comparative Mean plate count (cfu/g) between unpacked and packed spice samples

spices and their further risk assessment. While spices have been implicated in large scale outbreaks of food borne illness (Gustavsen *et al.*, 1984), the impact of contaminated spices on the incidence of food borne illness in Bangladesh is not correctly known.

Microbial counts vary according to the region, the year of production and the harvest and storage conditions prior to drying. So, the observed counts were thus a reflection of the original bioload, of growth, as well as of die-off which were probably enhanced by oxidation and the presence of active compounds in herbs and spices (Donia, 2008).

Filiz (2001) counted total mesophilic bacteria at the average numbers of 8.1×10^6 cfu/g in powdered red pepper. Elmali *et al.* (2005) examined 15 powdered red peppers and found 2.7×10^6 cfu/g of mesophilic bacteria. Elmali *et al.* (2005) found at the average number of 1.3×10^4 cfu/g of coliform group microorganisms in powdered red pepper. *Escherichia coli* was identified at the levels of 26.6% in powdered red pepper. Filiz (2001) reported the average number of 1.7×10^2 cfu/g of Coliforms in powdered red pepper. Schwab *et al.* (1982) found very low level of Coliforms and *E. coli* (3-19 cfu/g) in powdered red pepper samples.

Elmali *et al.* (2005) found at the average number of 1.2×10^5 cfu/g of yeast and mould count in powdered red pepper. Filiz (2001) reported the presence of yeast-mould at the average number of 5×10^2 cfu/g. Powers *et al.* (1975) reported 1.0×10^2 cfu/g of yeast and mould in powdered red pepper which is similar to our study for packed samples.

Abu Donia (2008) investigated 15 coriander samples and reported mean count of 4×10^5 cfu/g aerobic bacteria. This count is similar to our study for unpacked coriander sample (4.53×10^5 cfu/g). Abu Donia (2008) also reported 2.3×10^1 cfu/g Coliform

bacteria, 3 cfu/g *E. coli* and 1.2×10^3 cfu/g yeast. The percentage of yeast and mould, Coliform and *E. coli* of both packed and unpacked samples was presented in Figure 1, 2 and 3, respectively. In Figure 1, 100% unpacked red pepper samples were contaminated with yeast and mould. The presence of 100% Coliform bacteria was found in unpacked coriander sample in Figure 2. In Figure 3, highest percentage (56%) of *E. coli* was observed in both unpacked red pepper and coriander sample.

Staphylococcus aureus is considered the third most important cause of disease in the world amongst the reported food-borne illness (Zhang *et al.*, 1998; Ananou *et al.*, 2007). Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in developed countries (Vora *et al.*, 2003). But in the present study, the evidence of *Staphylococcus aureus* was not found. None of the samples except unpacked coriander (3 cfu/g) in our study gave a positive result.

In our study, mean plate count of unpacked spice samples was higher than the packed one (Figure 4). The variation in the microbial count is depend on the different origin of spices, conditions of processing and storage, type of spices and being packed and unpacked. The highest count of bacteria, Coliform and yeast and mould may be due to poor hygienic standard of preparation and or handling. The International Microbiological Standard recommended limits for bacteria contaminants in spices are in the range of 10^1 to 10^3 cfu/g for Coliform, 10^1 to 10^5 cfu/g total microbial plate count, 10^1 to 10^3 cfu/g for yeast and mould, 0/20 g for *S. aureus* and 0/20 g for *E. coli* (Awe *et al.*, 2009). We compared our results to International Microbiological Standard and found that some microbiological parameters count of unpacked sample were above the recommended

limits.

Gallo *et al.* (1992) reported that faulty food handling techniques especially storage of food at improper temperature for long periods of time has been identified as one of the microbial proliferation in contaminated food. The production of these spices by some manufacturer may be under unhygienic environment. The spices may also not be sufficiently dried or contaminated raw materials may be added or final products may be manually packaged. These factors will contribute to the high microbial counts obtained from these products. There is thus a need to ensure that production is done under good sanitary condition. It may also be necessary to reassess production to involve technique that will further reduce the microbial load. It is desirable that aseptic technique should be used at all stages of production, this will go a long way in checking the risk associated with the presence of microbial contaminant. Care must be taken to maintain good hygiene practice, select good leaves while infected one should be discarded, also seeds must be properly dried prior to production (Awe *et al.*, 2009).

Conclusion

It was concluded that spices may be high risk products as it contained many pathogenic bacteria, Coliform, *Escherichia coli* and yeast and mould. Among packed and unpacked spice samples, unpacked spices were highly contaminated with microorganisms. Packed spices were less contaminated than the unpacked samples. Therefore, more studies are necessary to find out the ways of contamination. Aseptic techniques at all stages of production and processing must be ensured to prevent contamination of spices from pathogenic microorganisms.

References

- Ahene, R. E., Odamtten, G. T. and Owusu, E. September 2011. Fungal and bacterial contaminants of six spices and spice products in Ghana. *African Journal of Environmental Science and Technology* 5(9): 633-640.
- Ananou, S., Maqueda, M., Martinez-Bueno, M., Galvez, A. and Valdivia, E. 2007. Bactericidal synergism through enterocin AS-48 and chemical preservatives against *Staphylococcus aureus*. *Letters in Applied Microbiology* 45(1):19-23.
- Awe, S., Sani, A. and Ojo, F. T. 2009. Microbiological quality of some selected spices (*Thymus vulgaris*, *Murraya koenigi* and *Piper nigrum*). *Nigerian Journal of Microbiology* 23(1): 1876 – 1881.
- Internet: BAM (Bacteriological Analytical Manual), January 2001. *Staphylococcus aureus*, Chapter 12. Downloaded from <http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm071429.htm>
- Internet: BAM (Bacteriological Analytical Manual), September, 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria, Chapter 4. Downloaded from <http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm064948.htm>
- Colak, H., Bingol, E. B., Hampikyan, H. and Nazli, B. 2006. Determination of aflatoxin contamination in red-scaled, red and black pepper by ELISA and HPLC. *Journal of Food and Drug Analysis* 14(3): 292-296.
- Donia, A. M. A. 2008. Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. *Global Veterinaria* 2(4): 175-181.
- Elmali, M. and Yaman, H. 2005. Microbiological quality of some spices sold in the markets of Bitlis district. *Journal of Faculty of Veterinary Medicine, University of Erziyes* 2(1): 9-14.
- Filiz, N. 2001. Microbial flora of some ground spices consumed in Bursa. *Journal of the Faculty of Veterinary Medicine of Uludag University* 20: 103-107.
- Gallo, G., Berzero, R., Caltai, N., Recchia, S. and Orefici, G. 1992. An outbreak of group a food-borne Streptococcal pharyngitis. *European Journal of Epidemiology* 8(2): 292-297.
- Gustavsen, S. and Breen, O. 1984. Investigation of an outbreak of *Salmonella oranienburg* infections in Norway, caused by contaminated black pepper. *American journal of Epidemiology* 119 (5): 806- 812.
- Powers, E. M., Lawyer, R. and Y, Masouka. 1975. Microbiology of processed spices. *Journal of Milk and Food Technology* 38: 683-687.
- Rathore, M.S. and Shekhawat, N.S. 2008. Incredible Spices of India: from Traditions to Cuisine. *American-Eurasian Journal of Botany* 1(3): 85-89.
- Schwab, A. H., Harpedsted, S. A., Lanier, J. M., Wentz, B., Duran, A. P., Barnard, R. J. and Read, R. B. 1982. Microbiological quality of some spices and herbs in retail markets. *Applied and Environmental Microbiology* 44(3): 627-630.
- Takeda, J., Silva, S.D., Muthuraman, P., Rahman, S. M. and Lotje, K. 2008. Spices in Sri Lanka, India and Bangladesh with Special Reference to the Usages and Consumptions. *Bulletin of Faculty of Agriculture, Saga University*. 93: 1-25.
- Vajdi, M. and Pereira R. R. 1973. Comparative effects of ethylene oxide, gamma irradiation and microwave treatments on selected spices. *Journal of Food Science* 38(5): 893-895.
- Vora, P., Senecal, A. and Schaffner, D. W. 2003. Survival of *Staphylococcus aureus* ATCC 13565 in intermediate moisture foods is highly variable. *Risk Analysis* 23(1): 229-236.
- Zhang, S., Iandolo, J. J. and Stewart, G. C. 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). *FEMS Microbiology letters* 168(2): 227-233.