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Bacterial and Fungal Counts of Dried and Semi-Dried Foods Collected from Dhaka, Bangladesh, and Their Reduction Methods

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Food is a basic necessity for human survival, but it is still the vehicle for the transmission of food borne disease. Various studies have examined the roles of spices, herbs, nuts, and semi-dried fruits, making the need for safe and convenient methods of decontamination a necessity. The current study determined the bacterial and fungal loads of 26 spices and herbs, 5 nuts, 10 semi-dried fruits and 5 other foods. Spices, herbs and semi-dried foods demonstrated the highest bacterial and fungal loads with the majority showing over 10^4 CFU/mL. Nuts and other foods showed growths ranging from 10^2 to 10^6 CFU/mL. The current study also attempted to determine the effects of heat and plasma treatment. The log reduction of bacterial growth after heat treatment (maximum: 120 min for 60℃) was between 0.08 to 4.47, and the log reduction after plasma treatment (maximum: 40 min) ranged from 2.37 to 5.75. Spices showed the lowest rates of reduction, whereas the semi-dried and other foods showed moderate to high levels of decrease after heat treatment. The log reduction of fungal growth after heat treatment ranged from 0.27 to 4.40, and log reduction after plasma treatment ranged from 2.15 to 5.91. Furthermore, we validated the sterilization effect of plasma treatment against *Bacillus* spp. and *Staphylococcus* spp. by using scanning electron microscopy. Both treatment methods could prove to be advantageous in the agriculture related fields, enhancing the quality of the foods.

Key words : Bacterial counts of food / Fungal counts of food / Heat treatment on food / Low-pressure plasma treatment / Scanning electron microscopic study.

INTRODUCTION

Food has always been a human necessity, but that has not ruled it out as a viable source of bacterial and fungal infections (Yoon and Kim, 2012). It still remains a vehicle for food-borne illnesses. Spices and herbs are an integral part of food in the Indian subcontinent, including Bangladesh. They serve as the base for food preparation and are commonly used for flavoring in every dish in the local cuisine. Additionally, various spices possess antimicrobial properties, preserving the foods in which they are used (Elshafie et al., 2002). If contaminated, however, they can serve as a major threat to public health and safety. Various cereals and cereal products have also been contaminated with bacteria and fungi (Zinedine et al., 2006). As they usually originate from dried plant material, the presence of microorganisms or fungi in spices is not unlikely (Kovic-Tanackov et al., 2007). Commensal organisms, which are originally found in plants, may survive the drying process and contribute towards the rise of food-borne illnesses (Hashem and Alamri, 2010). The warm and humid conditions in which they are grown favor the

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growth of microorganisms (Banarjee and Sarkar, 2003). Furthermore, spices are exposed to various amounts of environmental contaminants during processing, such as wastewater, dirt and dust, and human and animal excreta (Banarjee and Sarkar, 2003).

Contamination may occur as a result of mishandling during storage, distribution and processing. These factors contribute to microbial contamination, regardless of the minimal presence of contaminants in cooked foods (Hashem and Alamri, 2010). Further complications may arise due to the consumption of foods which undergo no processing after the addition of spices and herbs (Banarjee and Sarkar, 2003). The situation is made even more complicated with the increase in spice trading, resulting in the wide geographic occurrence of disease (Zweifel and Stephan, 2012). The high bioburden of processed foods also contribute to an increased rate of putrefaction of food products, negatively affecting quality and leading to the production of mycotoxins (Juri et al., 1986). Consuming contaminated foods may contribute towards developing liver carcinoma or other serious diseases (Hashem and Alamri, 2010). Various studies have established the presence of microorganisms and fungi in spices (Banarjee and Sarkar., 2003; Elshafie et al., 2002; Hashem and Alamri, 2010; Zweifel and Stephan, 2012).

As the negative effects are numerous and continue to grow, the need for developing methods for minimizing or eradicating pathogens also grows. Studies by Juri et al. (1986) have used gamma irradiation as a possible means of decontamination, and as mentioned in the same paper, others have attempted the use of fumigation with ethylene oxide. All these methods are associated with several disadvantages consequently, the search for alternative methods still continues. Maintaining Hazard Analysis Critical Control Point (HACCP) principles and establishing good manufacturing and hygiene practices are important in developing safe and healthy foods (Zweifel and Stephan, 2012). However, underdeveloped countries may find it difficult to adhere strictly to these principles and practices, often compromising quality to maintain quantity and profit margins. Therefore, simple and easily replicable methods of decontamination are now more important than ever for the resource-poor settings such as Bangladesh.

Heat has always been used in household and laboratory settings in the form of boiling, cooking, pasteurization, autoclave and heat sterilization (sterilizer and fire). This is a simple method, which can be reproduced in any setting. Heat treatment has always been used in milk decontamination but it is difficult to determine the exact level of decontamination (Raikos, 2010). Another method is low-pressure plasma treatment for inactivating microorganisms, which has been gaining attention as an alternative method in medical instrument sterilization, and in recent times its application has spread to agriculture (Nishioka et al., 2014). The method involves the discharge of plasma, distributing gas in combination with ions, electrons and radicals, which inactivate the organisms upon interaction (von Keudell et al., 2010). This method may be used at low temperatures and without chemicals, removing any possibility of damage to the food and minimizing the presence of harmful residues (Selcuka et al., 2008). Its effect on microorganism growth on artificial surfaces has been widely studied, but its application in the food industry has not been explored in depth (Basaran et al., 2008).

For this reason, the current study would like to explore the effects of applying heat and low-pressure plasma treatment on the presence and survival of microorganisms in spices, herbs and other commonly consumed dried and semi-dried food, and food products available in Dhaka, Bangladesh. The current study also hopes to strengthen the practice of using heat for decontamination of foods, positively affecting public health and the food safety of disease-ridden Bangladesh.

MATERIALS AND METHODS

Sample collection

As seen in Table 1, 46 dried and semi-dried samples were collected from supermarkets and street markets of Dhaka, Bangladesh, and transported by air to Kindai University, Nara, Japan. They were tested as soon as possible to avoid spoilage.

Measurement of airborne and falling microorganisms

Dried samples were bought from a supermarket located in Dhanmondi, Dhaka. Semi-dried samples were bought from an open vegetable market in Rayer Bazar, Dhanmondi, Dhaka.

Microbial Air Sampler (RSC)

The bacteria and fungi present in the air were measured using a Hycon® RSC air sampler (Merck). The machine was held in the air, with the respective agar strip (Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA)), at 3 different points of each sampling area. Air was collected for 2 minutes in the collection area and incubated (at 37℃ for 2 days or 25℃ for 5 days). The resulting colonies were counted and CFU/L was identified by the following formula:

Colony Count ÷ (40 x time in minutes).

Falling Microorganisms

The amount of microorganisms present in the air, with the ability to fall and land on the samples were also measured. NA and SDA (Hi-media Laboratories Pvt.

TABLE 1. Dried and semi-dried food samples

Sample type	Number of samples	Foods
Spices	23	Chili Powder, Turmeric Powder, Cumin Powder, Cloves, Saffron Powder, Nutmeg Powder, Garam Masala Powder, Garlic Powder, Ginger Powder, Coriander Powder, Paprika Powder, Poppy Seeds, Cut Red Pepper, White Pepper, Bay Leaves, Fenugreek Powder, Celery Powder, Nigella Seeds, Sesame Seeds, Mustard Seeds, Cardamom, Cinnamon, Mace
Herbs	3	Thyme, Parsely, Basil
Nuts	5	Cashew, Pine nuts, Pistachio, Peanuts, Almonds
Semi Dried Fruits	10	Raisins, Dates, Plum, Honey Melon, Cherry Tomatoes, Pineapple, Rock Melon, Sweet Mango, Tamarind, Kiwi
Whole Foods	5	Chick Peas, Lentils, Rice, Dabli Boot, Kidney Beans

Ltd) plates were placed in 3 different points of each sampling area for 30 min each. Bacteria falling from the air in the area was allowed to collect in the agar plates after which they were incubated for 2 days at 37℃ or 25℃ for 5 days, and resulting colonies were measured.

Measurement of microbial and fungal counts in dry and semi-dry foods

In the case of powdered dried samples, 10 grams were measured and placed into a stomacher bag. Subsequently 90 mL of sterilized physiological saline was added. For solid dried foods, a tube mill crusher (IKA®-Werke) was used to convert the samples into powder before adding saline. Semi-dried foods were also placed in the crusher for softening, then added to the bag where they were pressed to be mixed with the saline. The filtered saline was then diluted and plated onto NA and SDA containing 50 μg/mL of chloramphenicol. NA plated were incubated at 37℃ for 2 days, while those of SDA were incubated at 25℃ for 5 days.

Detection of pathogenic bacteria in dry and semi-dry foods

Samples tested were plated on MacConkey Agar (MAC) for the detection of *Escherichia coli*. Samples exhibiting growth characteristics of *E. coli* on MAC were further tested for pathogenicity by using Pourmedia Vi EHEC agar (Eiken Chemical Co., Ltd.).

Samples tested were also plated on Mannitol Salt Agar (MSA) for the detection of *Staphylococci*. Samples (43 samples) exhibiting growth characteristics of *Staphylococci* on were further tested for resistance by using oxacillin disc.

Methods to decrease microorganisms in dry and semi-dry foods

Heat Treatment

Ten grams of each sample were placed in a sterilizer set for 60℃ for 30 min, 1 h and 2 h, after which they were diluted and their bacterial and fungal loads were detected.

Low-Pressure Plasma Treatment.

Seven samples were subjected to low-pressure plasma treatment as described by Nishioka et al. (2014). About 2 g of each sample were placed on the mesh sheet between electrodes in the plasma discharge chamber (Fig.1). The chamber was connected to a dry vacuum pump and an argon gas inflow system. The flow rate of argon gas was 0.5 L/min and the running pressure was sustained at 10.7 kPa. The plasma was generated by AC high voltage and the frequency and amplitude of voltage of power supply were 10 kHz and 5 kV, respectively. Plasma treatment time ranged from 5 min to 40 min, coupled with a 5 min pre-treatment prior to every time interval. Serial dilution and determination of microbial and fungal loads were subsequently carried out.

Morphological change in test bacteria treated with low-pressure plasma sterilization as observed by scanning electron microscopy

Scanning electron microscopy was used to observe the test bacteria. Samples were inoculated on MSA and SDA, upon identifying the bacteria through their characteristic appearance and growth colonies along with the application of detection kits (api staph and api 50 CHB/E, Biomerieux).

Each test bacteria strain was pre-incubated by using SCD (Nihon Pharm. Co., Ltd.) broth at 37℃ for 20 h. The bacterial suspension was placed into sterilized plastic tubes, and centrifuged at 3000 rpm for 15 min at 4℃. After discarding the supernatant solution, the cells were washed with 10 mL of the sterilized physiological saline twice. Bacterial cells were re-suspended with sterilized physiological saline, and when adjusted to the

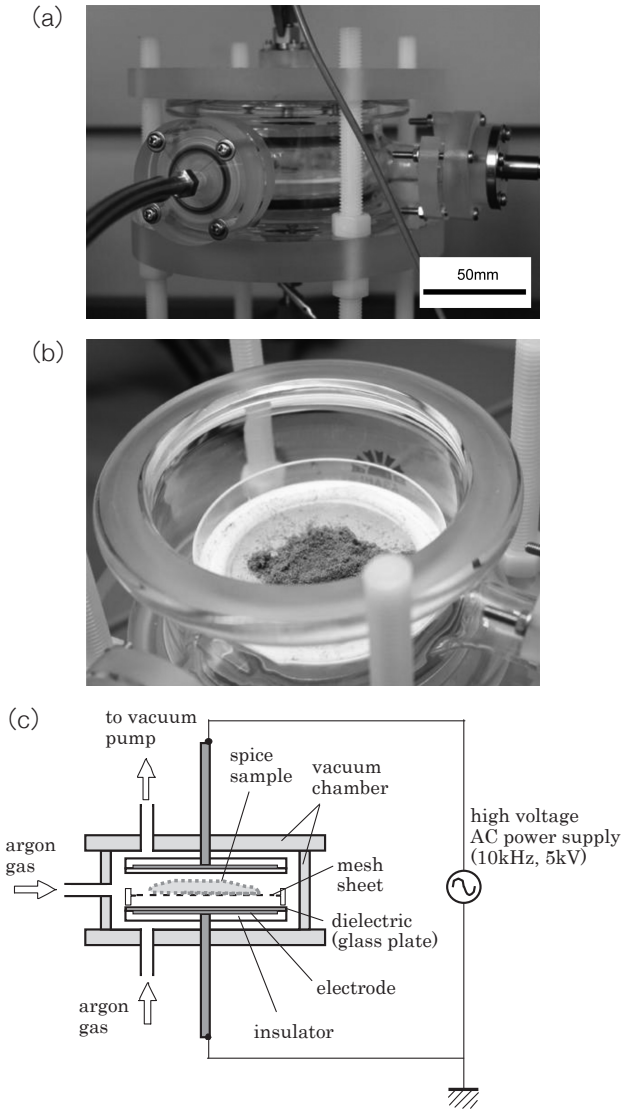

FIG. 1. The photo (a and b) and the schematic diagram (c) of low-pressure plasma sterilization machine.

absorbance at 630 nm the reading was about 0.12. The cell suspension (0.1 mL) was placed onto each slide glass. The slide glass was placed in the center position of the low-pressure plasma machine, and the plasma was irradiated for 5, 10, 20 and 40 min, respectively.

After irradiation of plasma, the sterilized physiological saline (10 mL) was added into each petri dish containing the test bacteria treated by plasma, and the treated test bacteria were recovered. This treatment was repeated again, and total bacterial suspension were transferred into a plastic tube. After centrifugation at 3000 rpm for 15 min at 4℃, 5 mL of modified Karnovsky solution (Karnovsky, 1965) (a mixture of 1% glutaraldehyde and 1% paraformaldehyde) was added into each tube, and the tubes were incubated for 1 h at about 8℃. After centrifugation at 3000 rpm for 15 min at 4℃, the supernatant solution was discarded. Karnovsky solution (5 mL) was added, and incubated for 1 h at about 8℃. The supernatant solution was discarded, and rest of the cells were washed by the sterilized phosphate buffer (pH 7.2) twice. After centrifugation at 3000 rpm for 15 min at 4℃, the cells were treated by 50%, 60%, 70%, 80%, 90% ethanol and absolute ethanol, respectively. After freeze-dry treatment, treated samples were transferred to each tape for measuring by scanning electron microscopy, and vapor deposition of gold was performed. Accelerating voltage was adjusted to 5.0 kV and the scanning electron microscopic study was performed by Hitachi High Technology Series SU 3500 with each treated sample under high vacuum conditions. The morphological change in the test bacteria by low-pressure plasma treatment was observed.

RESULTS

Measurement of airborne and falling microorganisms

The results of measuring airborne and falling microorganisms are shown in Table 2.

The counts of microorganisms at three measuring points in the vegetable market were larger than those in the Supermarket.

Bacterial and fungal counts observed in dried and semi-dried foods tested

Bacterial and fungal counts observed in semi-dried and dried foods tested are shown in Table 3. Of the 23 samples of spices, a majority showed an initial load of 10^4. Similarly, all 3 herbs tested also revealed the same initial load. The microbial and fungal loads of nuts and

TABLE 2. Measurement of airborne and falling microorganisms.

Location	Airborne bacteria by RCS machine (CFU/L)	Falling micro-organisms (CFU/30 min/ plate)
Supermarket, Point 1	NA= 0.24 SDA= 0.31	NA= 15 SDA= 6
Supermarket, Point 2	NA= 0.18 SDA= 0.28	NA= 22 SDA= 2
Supermarket, Point 3	NA= 0.15 SDA= 0.36	NA= 18 SDA= 5
Vegetable market, Point 1	NA= 0.79 SDA= 0.64	NA= 172 SDA= 29
Vegetable market, Point 2	NA= 0.85 SDA= 0.66	NA= 154 SDA= 38
Vegetable market, Point 3	NA= 0.88 SDA= 0.63	NA= 169 SDA= 33

NA: Nutrient Agar, SDA: Sabouraud Dextrose Agar

TABLE 3. Incidence and contamination level of viable bacteria counts and fungal counts in dried and semi-dried foods.

Sample type	Number of samples	Level of contamination, Number of samples										
		Viable bacteria counts (CFU/mL)					Fungal counts (CFU/mL)					
		10^2	10^3	10^4	10^5	10^6	10^2	10^3	10^4	10^5	10^6	$>10^7$
Spices	23		5	10	4	4	1	2	13	3	3	1
Herbs	3			3					2		1	
Nuts	5	1	1	1	1	1	1	1	1		2	
Semi Dried Foods	10			8		2	1		9			
Whole Foods	5		1	1		3	1	1			3	
Total	46	1	7	23	5	10	4	4	25	3	9	1

whole foods were scattered between 10^2 to 10^6 CFU/mL. Semi-dried foods had initial loads of 10^4 to 10^6 CFU/mL for both bacteria and fungus. Among the total of 46 samples, the microbial and fungal loads remained mainly within the range of 10^4 CFU/mL (23 and 25, respectively).

Identification of specific bacteria

E. coli isolated from 26 samples isolated were tested in this experiment. By using poa-media® Vi EHEC agar, O111 was detected in the sample of No.26 (poppy seeds), and O157 was detected in the sample of No.28 (celery) and the sample of No.42 (cardamon). EHEC was detected in 3 samples (11.5%) in 26 samples.

Forty three samples (43/46: 93.5%) also demonstrated resistance to oxacillin.

Effects of heat treatment of the test samples on microbial counts

Effects of heat treatment from 0 min to 2 h on microbial counts in test samples are were shown in Table 4. As shown in Table 4, the log reduction of bacterial growth was between 0.08 to 4.47. Spices showed the lowest rates of reduction, whereas the semi-dried and whole foods showed moderate to high levels of decrease. The most significant rate reduction of 4.47 was observed in dates (semi-dried foods), and the least significant reduction rates of 0.08, 0.10 and 0.12 were observed in Sesame seeds, peanuts and cumin (spices), respectively. In case of tamarind and chick peas, no growth was observed at 2 h.

Effects of heat treatment from 0 min to 2 h on fungal counts are shown in Table 5. The log reduction of fungal growth ranged from 0.27 to 4.40. The highest reduction was observed in almond (4.40), kidney beans (4.32) and rice (4.32). The lowest reduction rates were observed in coriander powder (0.27), chili powder (0.35) and saffron (0.30). In case of dates, no growth was observed after 1 h.

Effects of low-pressure plasma treatment on bacterial and fungal growth

Reduction effects of low-pressure plasma treatment on microbial and fungal counts of dried foods are shown in Table 6. Seven samples were subjected to plasma treatment. In case of bacterial growth reductions, chilli (5.75), turmeric (5.28) and coriander powder (5.40) exhibited the best results, whereas cumin (2.37) showed the smallest rate of reduction. In fungal growth, the highest reduction rate was observed in ginger (5.91) and the lowest in cumin (2.15). No microbial growth was observed in poppy seeds after 20 min and pine nuts after 40 min. No fungal growth was observed in pine nuts after 20 min.

There was no significant change in color when samples tested were treated with plasma up to 40 min.

Morphological change in test bacteria treated with low-pressure plasma as observed by scanning electron microscopy

Fig.2 and Fig.3 show morphological changes in bacteria after treatment with a low-pressure plasma machine. An increase in treatment time resulted in the breakdown of *Bacillus* spp. (Fig. 2). Similarly, low-pressure plasma treatments have also resulted in the breakdown of *Staphylococcus* spp. (Fig 3). According to these test results low-pressure plasma treatment would be a good and suitable method to kill bacteria contaminating food.

DISCUSSION

The current study attempted to reduce microbial and fungal loads of dried and semi-dried foods by, using heat sterilization and low-pressure plasma treatment. This study hopes to positively impact the food industries of Bangladesh, giving them methods which are cheap but still produce safe and healthy food.

Contamination of food may occur due to mishandling

TABLE 4. Effects of heat treatment from 0 min to 2 h on bacterial growth (log values)

Samples	0 min	30 min	1 h	2 h	Log reduction
Chili	5.04	5.20	5.11	3.46	1.58
Turmeric	5.23	5.14	4.30	3.88	1.35
Cumin	4.42	4.18	3.98	4.30	0.12
Cashew	3.78	3.30	2.00	2.70	1.08
Pine Nuts	4.04	3.40	3.08	2.00	2.04
Raisins	4.08	2.95	2.00	3.00	1.08
Dates	4.47	2.30	2.00	0.00	4.47
Saffron	4.38	4.32	4.08	4.00	0.38
Nutmeg	6.36	5.32	3.30	4.11	2.25
Garam Masala	4.65	4.29	4.18	2.00	2.65
Garlic Powder	4.57	4.04	4.18	3.48	1.09
Ginger Powder	4.23	3.72	3.59	4.08	0.15
Coriander Powder	4.18	4.08	4.00	3.65	0.53
Paprika Powder	6.08	5.29	4.97	4.36	1.72
Poppy Seeds	3.92	3.76	3.32	3.30	0.62
Thyme	4.83	3.00	3.70	3.85	0.98
Cut Red Peppers	3.60	3.70	3.36	2.30	1.33
White Pepper	3.23	4.26	4.11	2.95	0.28
Parsley	4.49	5.34	5.32	2.00	2.49
Bay Leaves	4.04	4.00	3.72	3.08	0.96
Fenugreek	4.32	4.20	3.88	3.08	1.24
Celery	5.85	6.41	5.32	5.70	0.15
Pistachio	6.08	5.20	4.40	3.70	2.38
Cloves	3.00	2.59	2.39	2.00	1.00
Peanuts	2.60	3.00	2.48	2.70	0.10
Chick Peas	3.00	2.62	2.00	ND	3.00≤
Lentil	4.79	4.23	4.20	3.93	0.86
Rice	6.28	2.70	2.00	2.00	4.28
Mace	6.00	4.18	3.75	3.93	2.07
Dabli	6.11	2.00	2.00	2.00	4.11
Basil	4.85	6.32	5.32	3.00	1.85
Nigella Seeds	4.93	4.11	4.30	3.95	0.98
Sesame Seeds	3.97	5.41	2.39	3.85	0.08
Mustard Seeds	5.40	3.93	4.14	3.32	2.08
Almond	5.23	2.70	2.00	3.00	3.23
Cardamom	6.18	4.48	3.86	2.00	4.18
Kidney Beans	6.28	3.60	3.00	2.00	4.28
Plum	6.18	3.00	3.00	2.00	4.18
Honey Melon	4.28	3.00	3.73	3.00	1.28
Cherry Tomato	6.11	5.18	4.99	4.11	2.00
Pineapple	4.23	3.30	2.30	2.00	2.23
Rock Melon	4.70	4.00	3.79	2.90	1.80
Sweet Mango	4.53	2.70	2.00	2.00	2.53
Cinnamon	4.65	4.04	3.70	4.30	0.35
Kiwi	4.25	3.78	3.30	3.00	1.25
Tamarind	4.00	3.70	2.00	ND	4.00≤

*ND: Not Detected.

TABLE 5. Effects of heat treatment from 0 min to 2 h on fungal growth (log values)

Samples	0 min	30 min	1 h	2 h	Log reduction
Chili	4.46	4.20	4.34	4.11	0.35
Turmeric	4.59	4.40	4.30	1.04	3.55
Cumin	3.08	2.60	2.40	2.04	1.04
Cashew	4.58	3.30	2.60	2.00	2.58
Pine Nuts	2.60	2.30	2.00	2.00	0.60
Raisins	4.00	3.30	2.00	2.00	2.00
Dates	2.00	1.50	ND	ND	2.00≤
Saffron	2.30	3.30	3.30	2.00	0.30
Nutmeg	7.20	6.36	5.34	5.11	2.09
Garam Masala	5.11	4.08	4.15	3.89	1.22
Garlic Powder	6.08	5.00	4.15	3.72	2.36
Ginger Powder	3.04	4.08	3.99	2.48	0.56
Coriander Powder	4.04	4.26	4.11	3.77	0.27
Paprika Powder	6.46	6.18	6.00	5.89	0.57
Poppy Seeds	4.60	4.30	4.30	3.65	0.95
Thyme	4.52	2.70	2.30	2.00	2.52
Cut Red Peppers	4.66	3.81	2.00	2.30	2.66
White Pepper	4.62	2.69	4.11	3.80	0.80
Parsley	4.57	4.30	4.15	3.48	1.09
Bay Leaves	4.75	3.30	2.00	2.69	2.06
Fenugreek	4.47	4.12	3.86	3.62	0.85
Celery	4.48	5.00	4.30	4.00	0.48
Pistachio	6.04	5.32	5.04	4.70	1.34
Cloves	4.47	4.00	3.40	3.27	1.20
Peanuts	3.78	3.76	2.30	2.00	1.78
Chick Peas	3.00	2.75	2.25	2.00	1.00
Lentil	2.79	2.00	2.00	2.00	0.79
Rice	6.32	2.00	2.00	2.00	4.32
Mace	5.32	3.11	2.90	2.00	3.32
Dabli Boot	6.28	3.51	3.00	2.00	4.28
Basil	6.18	6.36	6.18	4.04	2.14
Nigella Seeds	6.34	4.40	4.30	3.88	2.46
Sesame Seeds	4.57	4.08	3.99	3.30	1.27
Mustard Seeds	4.89	4.11	4.14	3.83	1.06
Almond	6.40	3.99	3.00	2.00	4.40
Cardamom	5.20	3.60	3.30	3.00	2.20
Kidney Beans	6.32	2.70	2.30	2.00	4.32
Plum	4.54	3.18	2.00	2.00	2.54
Honey Melon	4.26	3.48	3.86	2.47	1.79
Cherry Tomato	4.34	3.68	4.36	3.70	0.64
Pineapple	4.20	2.00	3.60	2.00	2.20
Rock Melon	4.32	3.70	2.00	3.00	1.32
Sweet Mango	4.48	3.30	3.70	2.30	2.18
Cinnamon	4.51	2.30	2.70	2.48	2.03
Kiwi	4.15	3.00	3.70	2.60	1.55
Tamarind	4.61	3.00	2.00	2.00	2.61

*ND: Not Detected.

TABLE 6. Effects of plasma treatment on bacterial and fungal growth (log values).

Bacterial growth						
Sample	0 min	5 min	10 min	20 min	40 min	Log reduction
Coriander Powder	6.36	1.30	1.30	1.30	0.96	5.40
Chili Powder	6.05	2.40	2.30	1.30	0.30	5.75
Poppy Seeds	4.11	0.70	0.30	ND	ND	4.11
Ginger Powder	4.70	2.30	2.20	1.30	1.14	3.56
Cumin Powder	3.48	2.10	2.40	1.30	1.11	2.37
Turmeric Powder	6.48	3.30	2.40	2.30	1.30	5.18
Pine Nuts	3.34	1.18	1.00	0.18	ND	3.34 ≤
Fungal growth						
Sample	0 min	5 min	10 min	20 min	40 min	Log reduction
Coriander Powder	4.56	1.20	2.10	1.00	0.93	3.63
Chili Powder	4.08	2.30	1.30	1.20	0.11	3.97
Poppy Seeds	4.30	1.50	0.30	0.30	0.74	3.56
Ginger Powder	6.54	1.50	2.00	0.90	0.63	5.91
Cumin Powder	3.08	2.00	1.40	0.70	0.93	2.15
Turmeric Powder	5.04	2.00	1.00	1.00	0.28	4.76
Pine Nuts	2.60	1.00	0.40	ND	ND	2.60 ≤

*ND: Not Detected.

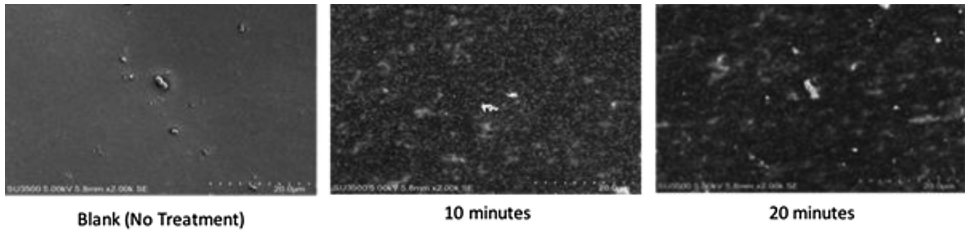

FIG. 2. Scanning Electron Microscopy Image of *Bacillus* spp. after low-pressure plasma treatment. Scale bar: —— 10 µm

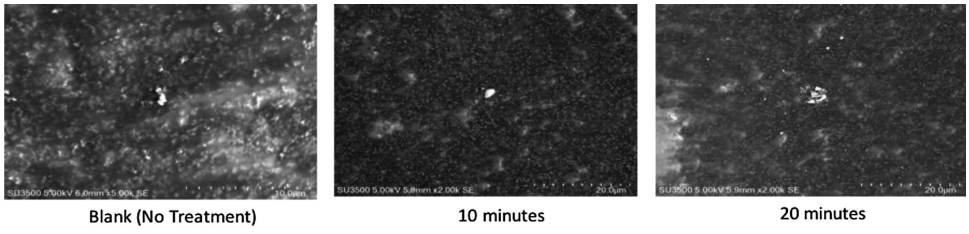

FIG. 3. Scanning Electron Microscopy Image of *Staphylococcus* spp. after low-pressure plasma treatment. Scale bar: —— 10 µm

during storage, distribution and processing (Hashem and Alamri, 2010). Samples in this study included spices and herbs, nuts, whole foods and dried foods. Various studies have reported the presence of microorganisms, similar to what was observed in this study (Banarjee and Sarker, 2003; Donia, 2008; Friere, 2002). According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1974) spices are deemed unfit for consumption if the total bacterial count exceeds that of 6 log CFU/g and yeast and moulds exceed 4 log CFU/g, similar to those levels stated in the European Spice Associations (European

Spice Association, 2004) and the Codex Code of Hygiene Practice (Codex Alimentarius Commision, 2004). Although 15% of the spices and herbs of this study exceeded the limits stated, most samples showed microbial growth between 3 to 6 log CFU/g. In the case of fungus, 81% of samples exhibited growths higher than 4 log CFU/g, exceeding the safety limits set by the International Commission on Microbiological Specification for Foods, and posing a serious threat to consumers. As the rate of food borne illnesses in Dhaka, Bangladesh increases, so does the need for developing more effective methods of decontamination.

Adding to the adverse quality of the foods is the deteriorating state of the air quality in Dhaka, Bangladesh. The current study has found a large concentration of bacteria in open markets, at which the majority of food purchases are made by the masses of Bangladesh. Supermarkets had significantly lower concentrations of bacteria and fungus, hence having little contribution to the spread of organisms via food or air. However, it must also be considered that supermarkets of Dhaka target the upper middle class, selling food products at higher prices. Hence the majority of the population relies on open markets when purchasing produce and meat.

Whole foods such as rice, lentil, dabli boot, chick peas, and kidney beans are dried foods, which are generally consumed after boiling, possibly accompanied by a few more minutes of cooking for flavoring. In most cases of whole foods, heat proved an adequate method to decrease microbial counts by values ranging from 3.00 to 4.28. However, in the case of lentils only a decrease of 0.86 was observed, which could be potentially harmful to the consumer. Other alarming results were seen for peanuts (0.10 log reduction), as they are mostly consumed raw. Other than the results mentioned above, spices and herbs had the lowest reduction rates and the highest original microbial and fungal counts. The effect of heat on spices depends on interpretation, as the log reductions ranged from 0.10 to 2.50 for both microbial and fungal strains. Spices also gave the highest bacterial and fungal counts, proving to be a likely vehicle of food borne illnesses. Aside from cherry tomatoes and plums, the semi-dried foods gave mid-level bacterial and fungal counts, interestingly, they also demonstrated minimal log reductions after heat treatment.

Heat treatment is a conventional method often used in household and commercial settings but it has presented sensory, nutritional and functional changes in food properties (Cruz-Romero et al., 2007). Heat treatment has long been used with foods such as milk to increase shelf life and reduce the presence of microorganisms, but whether it can fully render milk free of organisms is still in question (Raikos, 2010). Studies on the effect of heat treatment on the ecology of forests have also reported a decrease in soil biota (Banning and Murphy, 2008). Although heat treatment has been applied to raw foods, milk and juices; its use in the dried and semi-dried foods is limited. The knowledge of the effects of simple heat treatment on the samples will positively impact the health and safety of the consumers.

Low-pressure plasma treatment uses plasma, ions, electrons and radicals to sterilize objects (von Keudell et al., 2010). It is normally applied in the sterilization of medical equipment but it is being explored for use in the agricultural fields (Nishioka et al., 2014). In the current study, log reductions of bacterial and fungal counts were higher after low-pressure plasma treatment than after heat treatment, often double for the same samples. In many cases, after 20 min bacterial and fungal growth was almost eliminated. Research on using this method om seeds, has been conducted successfully before (Nishioka et al., 2014), further asserting the findings of the current study. Other studies, also in agreement with our findings, using grains and legumes (Selcuka et al., 2008) have likewise seen a significant decrease in surface fungi. This implies low-pressure plasma treatment is a viable method for the reduction of bacteria and fungi. It is made even more interesting by the lack of negative effects due to the absence of harsh chemicals (Selcuka et al., 2008).

The current study only tested seven samples to assess the effectiveness of low-pressure plasma treatment. Although heat reduced microbial loads, higher reductions would be desirable. The study has observed significant reduction of bacterial and fungal growth, therefore, it can be assumed that plasma treatment possess the ability to penetrate deep within food samples and breakdown cells. On this basis, further testing with a larger group of samples would help establish low-pressure plasma treatment as a viable method of decontamination. This could add in-depth insight into its use in the industry, especially as the research on its possible application in packaged foods is gaining interest (Roth et al., 2010). Research has also indicated its effectiveness against spores and prions, further aiding the food industry (Roth et al., 2009). Perhaps the only question may arise on whether this method could be effectively applied on the products of various shapes and sizes, when testing semi-dried or raw samples.

Both examined methods in this study can prove to be advantageous in the agricultural fields. Their application may greatly enhance the quality of the foods. Heat can be applied to the spices prior to distribution to ensure safety. This would be inexpensive as most production areas already have a source of heat additionally, it is a non-chemical method of disinfection. We would like to acknowledge that although lack of chemical use does

render low-pressure plasma treatment as cost effective, perhaps purchasing the machine itself, in Bangladesh, may prove to be difficult and expensive. That said, the subsequent use and increased quality of the food may make up for the financial loss. The use of both these methods would ensure the quality of the food is maintained without compromising nutritional benefits or affecting the profit margins of the food producers and/ or agricultural companies.

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