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Determination of microbiological quality of packed and unpacked bread

Afsana Khanom, Tahmina Shammi and Md. Shahidul Kabir*

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

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Wheat and flour contain essential nutrients such as carbohydrate, minerals, fats and proteins which may aid proliferation of microorganisms in products made from cereal grains and kept under improper storage condition. This study was conducted to determine the microbial load of bread and flour sold in packed and unpacked forms by the local and super markets in Dhaka city. Socioeconomic background, awareness of the microbiological contamination, spoilage of bread and flour and hygiene status of the salespeople were also studied to determine any correlation between the contamination of bread and flour sold by local and super markets. About 20 samples of flour (n=10) and bread (n=10) were included in this study to determine the load of total heterotrophic bacteria (THB), total *Staphylococcus aureus* (TSA), total Enterobacteriaceae (TE), total coliforms (TC), total yeast and mold (TYM). Equal number of unpacked (local market) and packed (supermarket) bread and flour samples were included in this study. TC, TSA and THB counts were found to be ~1.0 $\log_{10}$ cfu/g higher in unpacked (local market) flour samples compared to those of packed (supermarket) samples. TSA counts were significantly higher in unpacked (local market) samples compared to the packed samples ($p<0.05$). Food handlers of both the groups did not have adequate knowledge and training on food contamination and spoilage. However, salespeople in the supermarkets were found to be more educated, experienced than those in the local markets. This study demonstrates that unpacked (local market) bread and flour are more contaminated than the packed (supermarket) samples and may pose risk of foodborne infection to consumers.

Key words: Flour; Bread; Coliform; Enerobacteriaceae; Contamination

Cereals and cereal products constitute large portion of food resources and consumed by a large number of people worldwide. Bakery products and cereals provide adequate nutrients and calories required every day. Cereals have been consumed as basic food since prehistoric times and long before bread making was developed. Bakery products provide the supply of different essential nutrients such as carbohydrates, proteins, lipids, vitamins and minerals (1). Sales of a variety of breads and other bakery products have been increased in the past decades. Flours are made by grinding grains to that of powdery consistency before they are added to bread preparation. Flours are also found to provide primary structure to the final baked bread. Different types of flours are found worldwide which are made from wheat, rye, barley, maize and other types of grains. Wheat flour and bread constitute a large part of daily diet in rural and urban population. Flours contain higher proportion of carbohydrate (starch) and to lower proportion of minerals, fats and proteins (2). In several countries in the world, up to 50% of the total required calories are supplied by bread alone (3, 4).

Flour and breads are generally regarded as safe food from the microbiological point of view as they contain low water activity (5). Although growth of pathogenic bacteria may not be supported under such low water activity they may survive for extended periods of time (6). Cereal grains and bakery products held under improper storage conditions may aid in proliferation of various micro-organisms (7). Food poisoning resulting from contaminated flours has been reported in other countries of the world such as, Australian, European and the USA. Pathogenic microorganisms like *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* and other spoilage micro-organisms were found to be present at low levels in wheat and flour (8-12). In addition to the growth of bacteria, growth of mold in flour is known to significantly deteriorate quality of bread and flour. However, there are only a few studies reported on microbiological status of bread and flour in Bangladesh. Therefore, this study was conducted to determine the bioburden in bread and flours sold in packed (super markets) and unpacked (local market) in Dhaka city. Background information of sales people was also investigated to determine any correlation between their level of knowledge and hygiene practice with the loads of different groups of bacteria in studied samples.

*Corresponding Author: Dr. Md. Shahidul Kabir, Department of Microbiology, and Director, Institutional Quality Assurance Cell (IQAC), Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh. E-mail: kabir@stamforduniversity.edu.bd

MATERIALS AND METHODS

Collection of sample. A total of 20 fresh samples of bread and flour were

collected from different local and supermarkets in Dhaka city. Each sample was collected in a sterile sealed polythene bag, appropriately labeled and immediately brought to the microbiology laboratory of Stamford University Bangladesh and stored in the refrigerator (4-8 °C) before processing.

Sample processing. Each sample (10.0 g) was homogenized with 90.0 ml of sterile normal saline to prepare stock solution. Stocks were serially diluted (1:10) to 10^{-5} by adding 100µl of stock solution to 900µl normal saline in eppendorfs tubes. 100µl of diluted sample was inoculated on Nutrient agar (NA), Mannitol Salt Agar (MSA), membrane Faecal Coliform (mFC), Violet Red Bile Salt Glucose agar (VRBG) and Sabouraud Dextrose agar (SDA) media following spread plate method and incubated at 37 °C for 18-24 hours except for SDA which was incubated at 25 °C for 48-72 hours. All media were procured from Himedia Laboratories Ltd., India.

Determination of total bacterial count. Nutrient agar media was used to determinate the total bacterial count. NA plates were dried and labeled for appropriate dilutions to be used for dilution and spread plate method. Plates were inoculated and incubated at 37 °C for 18-24 hours. Total number of bacteria cfu/g of sample was calculated and recorded for interpretation of the result.

Determination of counts of *Staphylococcus aureus*. MSA was used to determine the counts of *Staphylococcus aureus* in bread and flour samples. The suspected colonies of *S. aureus* showed yellow color for mannitol fermentation and yellow halo for coagulase production around the colony. Suspected colonies were further confirmed by catalase, coagulase tests and Gram staining technique (13). Typical *S. aureus* colonies were counted to calculate cfu per gram of sample.

Determination of total Enterobacteriaceae. VRBG agar was used for propagation of Enterobacteriaceae. After incubation at 37 °C for 24 hours characteristic purple and red colonies were counted as members of Enterobacteriaceae. Suspected colonies were counted to determine cfu per gram of sample (14).

Determination of coliform bacteria. Membrane Faecal Coliform (mFC) media was used to detect the coliform bacteria by using spread plate method. Plates were inoculated and incubated at 37 °C for 24 hours. All blue colored colonies were enumerated to calculate total coliform as cfu/g.

Enumeration of yeast and mold. Diluted samples were inoculated onto SDA medium supplemented with clorampheinicol (40mg/L) by spread plate method as mentioned earlier. The plates were incubated at 25°C for 48-72 hours. Visible colonies were counted and calculated as the total yeast and mold and recorded as cfu/g (15).

RESULTS

Bacterial loads in both packed and unpacked flour samples were tested in this study (Tables 1). Average counts of total heterotrophic bacteria were 1.19×10^7 cfu/g and 3.52×10^6 cfu/g in unpacked and packed flour samples, respectively. Mean concentrations of *Staphylococcus aureus* showed similar pattern having higher concentration in unpacked (3.36×10^4 cfu/g) than packed (1.12×10^3 cfu/g) flour samples. Average counts of total Enterobacteriaceae were 5.68×10^5 cfu/g and 1.24×10^5 cfu/g in unpacked and packed flour samples, respectively. Mean of total coliform counts in unpacked flour (4.0×10^5 cfu/g) was higher than that of packed (1.4×10^4 cfu/g) sample. Mean concentrations of total yeast and mold counts also showed similar pattern and demonstrated higher concentration (2.12×10^5 cfu/g) in unpacked samples than packed (4.08×10^5 cfu/g) flour samples.

Microbial load in both packed and unpacked bread samples tested in this study is shown in Table 2. The average counts of total heterotrophic bacteria were 2.01×10^6 cfu/g and 3.30×10^6 cfu/g in unpacked and packed bread samples, respectively. Averages counts of *Staphylococcus aureus* was found to be higher in unpacked bread (1.93×10^4 cfu/g) than packed bread samples (2.08×10^3 cfu/g). The average counts of total Enterobacteriaceae were 1.22×10^5 cfu/g and 4.84×10^4 cfu/g in unpacked and packed bread samples, respectively. Mean concentrations of total coliform were 1.36×10^4 cfu/g and 1.86×10^4 cfu/g in unpacked and packed bread samples, respectively. The average total yeast and mold counts were 1.05×10^5 cfu/g and 1.01×10^5 cfu/g in unpacked and packed bread samples, respectively.

Table 3 shows the background information of salespeople in both super and local markets engaged in selling flours and breads. All salespeople were found to

TABLE 1. Microbial load (cfu/g) in packed and unpacked flour samples

Sample no.	THB (cfu/g)	SA (cfu/g)	TE (cfu/g)	TC (cfu/g)	TYM (cfu/g)
Unpacked (Local market)					
1	1.2×10^6	8.0×10^4	1.52×10^5	0	0
2	4.2×10^7	4.0×10^3	1.84×10^5	0	6.0×10^3
3	3.20×10^6	1.0×10^3	8.0×10^4	0	3.4×10^3
6	5.20×10^4	3.1×10^3	2.80×10^4	0	5.5×10^4
9	8.0×10^6	8.0×10^4	2.4×10^6	2.0×10^6	1.0×10^6
Average	**1.192×10^7**	**3.36×10^4**	**5.68×10^5**	**4.0×10^5**	**2.12×10^5**
Packed (Super market)					
4	2.80×10^6	0	4.8×10^5	0	6.0×10^5
5	3.20×10^6	0	8.0×10^4	1.60×10^4	5.20×10^5
7	8.0×10^5	4.0×10^2	4.0×10^4	4.0×10^4	4.8×10^5
8	8.8×10^5	1.2×10^3	0	1.2×10^4	4.4×10^5
10	1.0×10^7	4.0×10^3	2.40×10^4	2.0×10^3	0
Average	**3.52×10^6**	**1.12×10^3**	**1.24×10^5**	**1.4×10^4**	**4.08×10^5**

THB= Total heterotrophic bacteria; SA= *Staphylococcus aureus*; TE= Total enterobacteriaceae; TC= Total coliforms; TYM= Total yeast and mold

TABLE 2. Microbial load (cfu/g) in packed and unpacked bread samples

Sample no.	THB (cfu/g)	SA (cfu/g)	TE (cfu/g)	TC (cfu/g)	TYM (cfu/g)
Unpacked (Local market)					
12	5.2×10^4	1.6×10^4	1.32×10^4	0	0
13	1.16×10^4	0	8.0×10^3	0	0
14	3.2×10^6	7.6×10^4	1.6×10^5	5.6×10^4	4.0×10^5
15	2.4×10^6	4.0×10^3	3.2×10^4	0	6.4×10^4
16	4.4×10^6	8.0×10^2	4.0×10^5	1.2×10^4	6.4×10^4
Average	$\mathbf{2.01\times10^6}$	$\mathbf{1.93\times10^4}$	$\mathbf{1.22\times10^5}$	$\mathbf{1.36\times10^4}$	$\mathbf{1.05\times10^5}$
Packed (Super market)					
11	1.32×10^5	7.2×10^3	8.0×10^3	0	0
17	4.8×10^6	0	1.2×10^4	1.2×10^3	4.8×10^4
18	4.0×10^6	0	2.0×10^4	1.2×10^4	1.2×10^4
19	3.6×10^6	0	2.0×10^3	0	7.2×10^3
20	4.0×10^6	3.2×10^3	2.0×10^5	8.0×10^4	4.4×10^5
Average	$\mathbf{3.30\times10^6}$	$\mathbf{2.08\times10^3}$	$\mathbf{4.84\times10^4}$	$\mathbf{1.86\times10^4}$	$\mathbf{1.01\times10^5}$

THB= Total heterotrophic bacteria; SA= *Staphylococcus aureus*; TE= Total enterobacteriaceae; TC= Total coliforms; TYM= Total yeast and mold

be male. Their educational qualifications were up to primary or secondary level. It was found that 40% salespeople had education up to primary level in super market, whereas 80% salespeople had education up to primary level in local market. Secondary level education amongst the salespeople in the supermarkets were higher (60%) than those working in the local markets (20%). Most of the sales people handling bread and flour demonstrated poor knowledge of food spoilage in local (60%) and super (20-60%) markets. However, 20-40% of the workers did not have any previous knowledge of food spoilage. Although they have been working in food shops for a varying length of time, they did neither have any training on food contamination nor attended any such workshop. Consequently they did not appear to be aware of the importance of hand washing and using aprons while working in the food shops. Salespeople did not go through any routine medical checkup and 20% of them were found to have long fingernails. According to the disease history, salespeople in the local market (80%) demonstrated higher rate of dysentery than those working in the supermarket (40-60%).

DISCUSSIONS

Bread and bakery products are most widely consumed food all over the world. Even in Bangladesh bread and flour consumption is increasing due to health consciousness and to some extent for higher price of rice. They are good sources of nutrient and protein and provide high biological value in our diet. Variety of breads and other bakery products have gained popularity among consumers in the last decade and their sales have been increased notably.

This study has revealed presence of microorganisms in different types of bread and flour samples from different outlets. International microbiological standards recommended units of bacterial counts for dry and ready to eat foods are 10^3- 10^2 cfu/g for coliforms and $<10^3$ cfu/g for total heterotrophic bacteria (16). Total heterotrophic counts indicate general microbiological quality and hygienic status of any food sample. It was found that both packed (n=5) and unpacked (n=5) flour samples harbored heterotrophic bacteria above the international acceptable limit. Average concentrations of THB in unpacked flours were slightly higher (1.192×10^7 cfu/g) than packed flours (3.52×10^6 cfu/g). This may be due to the environmental contamination or erroneous handling of unpacked samples by the salespeople. However, no significant difference was found between the counts of THB of packed and unpacked bread samples ($p>0.05$).

Environmental sources like, soil, water and air can act as sources of contamination of *S. aureus*. Lack of aseptic handling and personal hygiene can result in contamination of bread and flour with such bacteria. Their incidence is not always regarded as hazardous to consumers, as only coagulase positive *Staphylococcus aureus* can produce enterotoxin which may cause food borne diseases. However, to produce enterotoxin, it requires a high dose of *Staphylococcus aureus* (10^5-10^6 cfu/ml) in food (17-19). In the present study, *Staphylococcus aureus* was observed in all 5 unpacked and 3 packed flour samples. Average count of *S. aureus* was found to be higher in unpacked (3.36×10^4 cfu/g) than packed (1.12×10^3 cfu/g)

TABLE 3. Background information of salespeople in both super and local markets of flour samples

Criteria		Flour sample		Bread sample	
		Super market	Local market	Super market	Local market
Respondent information					
Gender	Male	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
	Female	0	0	0	0
Educational qualification	Primary	2/5(40%)	4/5(80%)	2/5(40%)	4/5(80%)
	Secondary	3/5(60%)	1/5(20%)	3/5(60%)	1/5(20%)
	Higher secondary	0	0	0	0
	Others	0	0	0	0
Working period (year)	Less than 1 year	1/5(20%)	2/5(40%)	2/5(40%)	4/5(80%)
	1-5 years	4/5(80%)	2/5(40)%	3/5(60%)	1/5(20%)
	6-10 years	0	1/5(20%)	0	0
	More than 10 years	0	1/5(20%)	0	0
Knowledge on food spoilage	**Some**	1/5(20%)	3/5(60%)	3/5(60%)	3/5(60%)
	Poor	3/5(60%)	0	2/5(40%)	1/5(20%)
	Fair	0	0	0	
	Good	0	0	0	
	Very good	1/5(20%)	2/5(40%)	0	1/5(20%)
Regular medical check up	Yes	0	0	0	0
	No	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
Disease history in last 1 year	**Yes** (Cholera/ Typhoid/ Tuberculosis/ Skin infection	0	0	0	0
	Dysentery	3/5(60%)	4/5(80%)	2/5(40%)	4/5(80%)
	No	2/5(40%)	1/5(20%)	3/5(60%)	1/5(20%)
Attended seminar/ training	**Yes**	0	0	0	0
	No	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
Personal hygiene	**Hand washing practice**				
	After go to toilet	5/5(100%)	3/5(60%)	5/5(100%)	3/5(60%)
	Before eating	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
	After sneezing, touching hair and clothes	0	0	0	0
	No	0	2/5(40%)		2/5(40%)
	Hand washing with				
	Only water	0	2/5(40%)	0	3/5(60%)
	Soap	3/5(60%)	3/5(60%)	5/5(100%)	2/5(40%)
	Antibacterial soap/savlon	0	0	0	0
	Hand washing liquid	2/5(40%)	0	0	0
	Glove use				
	Yes	0	0	0	0
	No	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
	Hair restriction /tidy hair				
	Yes	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
	No	0	0	0	0
	Clean nails				
	No long finger nails	5/5(100%)	5/5(100%)	5/5(100%)	4/5(80%)
	Long finger nails	0	0	0	1/5(20%)
	Wearing ring				
	Yes	0	1/5(20%)	0	0
	No	5/5(100%)	4/5(80%)	5/5(100%)	5/5(100%)
	Apron/ uniform use				
	Yes	0	0	0	0
	No	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)

flour samples. Both types of flours showed bacterial loads lower than that required for effective enterotoxin production. For bread samples 4 unpacked and 2 packed samples were found to be contaminated with this organism. Average counts of *S. aureus* were 1.93×10^4 and 2.08×10^3 cfu/g in unpacked and packed bread samples, respectively. In another study Daniyan et al. (16) observed *Staphylococcus aureus* count in different stages of bread

production which included mixing, milling and baking. After baking they found 2.20×10^4 cfu/g *Staphylococcus aureus* in bread samples (16).

Members of coliform group are indicator microorganisms which provide evidence about the hygiene standard maintained during production. Presence of coliforms in food sample suggests the presence of other enteric pathogenic bacteria samples contaminated and may pose risk to public health. In this study coliform was found to be present in 1 unpacked and 4 packed flour samples. The highest count of coliforms in unpacked sample was 2.0×10^6 cfu/g and while average count of packed sample were 1.4×10^4 cfu/g. In previous studies done in Pakistan and Australia showed presence of 10^2-10^5 cfu/g coliform count in flour sample (7, 20). In this study 40% (n=2) unpacked and 60% (n= 3) packed bread samples were found to be contaminated with coliforms. Average counts of coliforms were 1.36×10^4 and 1.86×10^4 cfu/g for packed and unpacked breads, respectively. Higher frequency and higher load of coliform bacteria present in packed and unpacked bread and flour indicate ineffective and inefficient packaging of product and contamination through the migration of substances from the packaging into the food products (21).

Members of family Enterobacteriaceae include a number of important pathogen, such as *Salmonella*, *Yersinia enterocolitica* pathogenic *Escherichia coli* (including *E. coli* O157:H7), *Shigella* spp. and *Cronobacter* spp. According to the NSW food authority, count of Enterobacteriaceae, $<10^2$ cfu/g, is considered as good, 10^2 to $<10^4$ cfu/g is considered acceptable and $\geq10^4$ cfu/g is regarded as unsatisfactory in ready to eat food. In present study, 100% (n=5) unpacked flour sample, Enterobacteriaceae counts were higher than the acceptable limit with an average of 5.68×10^5 cfu/g. Most of the packed flour samples (n=4, 80% of) was positive with an average count of 1.24×10^5 cfu/g of Enterobacteriaceae. For bread sample all packed and unpacked samples were positive for this group of organism. In unpacked bread sample average count was 1.22×10^5 cfu/g with 20% (n=1) sample within the acceptable range 8.0×10^3 cfu/g. In packed bread sample 40% (n=2) fall within the acceptable range and showed average concentration of 4.84×10^4 cfu/g.

The recommended limit for yeast and mold in flour is 10^5 cfu/g (22). Molds can produce mycotoxin and higher concentration of these organisms can cause deterioration of food and food borne illness (23). Yeast and mold counts were found to lie below the recommended limit in all samples except one sample which showed 1.0×10^6 cfu/g of yeast and mold in unpacked flour sample from local market.

Based on the questionnaire survey it was found that education level and hygiene status of bread and flour sellers were linked to the observed count of different microorganisms. In most of the cases the salespeople were not educated and trained properly which was reflected by their poor hygiene state. Only 60% salespeople of local market showed hand washing practice after visiting toilet and 40-60% used just water for hand washing. Salespeople in the supermarket were more careful and found to use soap or hand washing liquid before handling foods than those in the local market. It was also found that 20% of the salespeople found to have unclean long fingernails and put on finger rings. According to the previous history of foodborne diseases, 40%-80% salespeople showed infection in the previous year which may further increase the chance of contamination of the bread and flour samples in the study year with coliform and other intestinal pathogens.

CONCLUSION

Bread and flour are known as safe food commodity due to their low water activity. But microbial isolation and enumeration in this study revealed prevalence of microorganisms including pathogens in higher counts than recommended limits for these food items. As flour and bread are most commonly consumed food products, their microbiological quality is important to consumer's health. A questionnaire survey among the salespeople indicated many underlying reasons of such poor microbiological standard, like disease history, hygiene practice and educational status. Therefore, stringent regulatory actions on the microbiological quality control on bread and flour together with the training of salespeople on food spoilage are necessary for the better management of public health condition in Bangladesh.

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