



## Investigation of Microbial Safety and Shelf-life of Locally Produced Bread and Cake in Tangail City, Bangladesh

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### Abstract

The study was carried out to assess the microbial quality for shelf life of different bread and cake samples, locally produced in Tangail city of Bangladesh. A total 10 samples of different brand bread and cake were collected from different shop kipper for microbiological analysis. The total viable count (TVC) of samples ranged from  $1.0 \times 10^2$  cfu/g to  $1.3 \times 10^6$  cfu/g at first day,  $1.17 \times 10^8$  cfu/g to  $7.3 \times 10^{10}$  cfu/g at third day and  $2.35 \times 10^{14}$  cfu/g to  $1.2 \times 10^{18}$  cfu/g at fifth day, where WHO permitted maximum  $2.0 \times 10^5$  cfu/g for TVC. Total coliform count (TCC) ranged from 2 cfu/g to 244 cfu/g at first day,  $1.07 \times 10^5$  cfu/g to  $3.3 \times 10^8$  cfu/g at third day and  $2.08 \times 10^{10}$  cfu/g to  $1.9 \times 10^{14}$  cfu/g at fifth day (maximum permitted value 200 cfu/g). Again, total fungal count (TFC) ranged from 6 cfu/g to  $1.1 \times 10^6$  cfu/g at first day,  $1.23 \times 10^6$  cfu/g to  $2.2 \times 10^{12}$  cfu/g at third day and  $1.82 \times 10^{13}$  cfu/g to  $9.2 \times 10^{17}$  cfu/g at fifth day (maximum permitted value  $1 \times 10^5$  cfu/g). According to the WHO Standard, the microbiological parameters of all collected bread and cake were out of the permitted standards after third day. It was postulated that lack of good manufacturing practices including poor hygiene and sanitation, nutrient rich bread and cake makes a good medium for microbial growth. Therefore, an immediate step should be taken to aware the bakery owner as well as consumers about the sanitation and hygiene to make safety food products.

**Keywords:** Bread, Cake, Microbial Quality, Sanitation and Shelf-life

### Introduction

Bread and cake is common ready food item in Bangladesh. Thus, a large number of people start their daily life by eating bread or cake at their breakfast. These are valuable sources of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements. The nutrients in bakery products are carbohydrates, proteins, lipids, vitamins and minerals. They are very important for consumers particularly for middle and low income sectors of population who depend on street foods for their main food intake. In developing countries, a large proportion of ready to eat foods (bread, cake) are sold on the street (Mensah *et al.*, 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat street food every day (FAO, 2007). Microorganisms play an important role in the production of bakery products (consistency, formation of flavoring) also cause damage or even spoilage (Frazier and Westhoff, 1978). Although this spoilage and deterioration cannot be stopped completely; however, it is the desire of food processors to slow this rate of deterioration as much as possible through formulation, processing, packaging, storage and handling (Bailey and Holy, 1993).

Food safety and hygiene is very important in any industries. Food factories have to keep basic good house-keeping and hygiene standards (Huq *et al.*, 2013). In developed countries, the quality of food products especially for bread and cake is strictly maintained under several laws and regulations, while unfortunately many developing countries like Bangladesh could not practices appropriate microbiological safety and hygiene for bread and cake. As a result, the transmission of certain human diseases through these products become a serious problem.

Hygienic standards are not usually maintained during production and also in transport system of bread and cake. During the preparation, bare hands were used for handling ingredients and equipments. The equipment were washed just by dipping in one or more buckets and sometimes without soap because running water is not available at bakery sites.

Use of unhygienic surroundings, the products become contaminated with harmful bacteria. The most common food borne pathogenic bacteria is *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Shigella spp.*, *Salmonella spp.*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Streptococcus pyogenes*, *Mycobacterium bovis*, *Listeria monocytogenes* etc. Contaminated with pathogenic bacteria, such as *E. coli* and *Salmonella spp.* can caused numerous illness and even some fatalities. Serious health hazards due to the presence of pathogenic microbes in food can lead to food poisoning outbreaks (Gerez and Torino, 2009).

Considering the importance and benefits of bread and cake in our modern daily life, it is necessary to maintain microbial quality of bread and cake. An attempt has been made to determine the total viable count (TVC), total fungal count (TFC) and total coliforms count (TCC) of bread and cake available in local market of Tangail district, Bangladesh.

### Materials and Methods

Five different brand bread and five different brand cake were purchased from local market of Tangail district (Table 1). The samples were analyzed in the Department of Food Technology and Nutritional Science (Microbiology Lab), Mawlana Bhashani Science and Technology University, Tangail.

**Table 1.** Name of the collected samples and their identification

Brand name of Bread	Sample ID	Brand name of Cake	Sample ID
Fantasy Bread	S1	Bornali Cake	S6
Master Bread	S2	Master Cake	S7
Bondhon Bread	S3	Delite Cake	S8
Jamuna Bread	S4	Bondon Cake	S9
Tangail Bread	S5	Jamuna Cake	S10

**Serial dilution for microbial analysis**

1 g of bread or cake was diluted with 9 ml of sterile buffered peptone water and mixed well ( $10^{-1}$  dilution). Serial dilutions were prepared and spread plate technique was used on solid media. Serial dilutions of samples were made up to  $10^{-16}$  with sterile distilled water. 0.1ml of each dilution was spreaded evenly on the nutrient agar medium and incubated. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per gram (*cfu/g*).

**Methods of microbial analysis**

For total viable count of bread and cake, Nutrient Agar media (Merck Kgaa, 64271 Darmstadt, Germany) was used and estimation of the bacterial load was performed by standard method (ICMSF, 1998). For total coliform count of bread and cake, Macconkey Agar media was used. Estimation of total coliform count was performed by standard methods. Isolates were then identified according to the Bergey's manual of determinative bacteriology (Buchanan and Gibbon, 1984) and manual for the identification of medical bacteria (Cowan, 1975). Potato Dextrose Agar (PDA) media was used for the isolation and enumeration of yeasts and molds. Total fungal count of bread and cake samples were performed by standard method (ICMSF, 1998). All kinds of media, solutions and glass bottles were sterilized at 15 P.S.I (Pounds per square inch) for 20 minutes at  $121^{\circ}\text{C}$  in an autoclave (Model No. Mc-40 w. Made in Tokyo, Japan). In case of TVC, the final  $\text{p}^{\text{H}}$  of the each medium was adjusted to  $7.4 \pm 0.2$ . Then it was cooled down to  $50^{\circ}\text{C}$  and was poured into Petridish. After solidification of agar, the plates were inverted and placed in an incubator operated at  $37^{\circ}\text{C}$  for 24 hours. After incubation, the plates were taken out from the incubator and the plates which contained 10-300 colonies were selected for counting. Colonies were counted with the aid of a Garbar colony counter. The numbers of colonies were multiplied by the dilution and the total viable count per g of sample was recorded. In this study, two petri plates were used for each sample to obtained duplicate data. Total viable count expressed as colony forming units (*Cfu/g*) of the representative samples determined by standard plate count method. In case of TCC, the media was dispensed into several 200 ml screw cap bottles and sterilized at  $121^{\circ}\text{C}$  (6.795 kg pressure/ sq. inch) for 15 minutes in an autoclave. The final reaction was adjusted to  $\text{p}^{\text{H}}$   $7.1 \pm 0.2$ . The media was ready for plating or storing. Before plating, the stored media was melted and cooled at  $45^{\circ}\text{C}$ . Then it was allowed to cool down and solidify the media. After solidification of agar, the plates were inverted and

placed in an incubator operated at  $37^{\circ}\text{C}$  for 24-48 hours. After incubation, the plates were taken out from the incubator and dark red colonies were counted at least of diameter of 0.5 mm. The numbers of colonies were multiplied by the dilution and the count of coliform organisms per g of sample was recorded selected based on color and characteristics.

In case of TFC, each final medium was adjusted to  $\text{p}^{\text{H}}$   $5.6 \pm 0.2$ . The media was ready for plating or storing. Before plating, the stored media was melted and cooled at  $45^{\circ}\text{C}$ . After solidification of agar, the plates were inverted and incubated at  $21-28^{\circ}\text{C}$  for 3-5 days. After incubation, the plates were taken out from the incubator and colonies were counted. Yeast colonies were characterized by their smooth, moist and elevated surface, where mould colonies were identified by the profuse growth of hypae. Finally, the colony number was multiplied by the dilution and the TFC of per g samples were recorded.

**Results and Discussion**

Table 2 shows the total viable count of different bread and cake at first day (Day 1) to 5 day at every 24 hours interval. According to the WHO Standard (1994), the acceptable range of TVC is  $2.0 \times 10^5$  *cfu/g*. The microbiological parameters of all the collected bread and cake samples were higher than the permitted standards after second days.

It was also observed that the present study of 4 bread and 3 cakes samples (S1, S2, S3, S5, S6, S7, S8) showed permitted bacteria level for consumption at first day, but all of bread and cake samples were unacceptable for consumptions from 2<sup>nd</sup> day to 5<sup>th</sup> day. High TVC of the bread and cake may be due to the unhygienic maintenance during preparing the products. According to the WHO Standard (1994), the acceptable range of TCC is  $200\text{cfu/g}$ .

**Table 2.** Total viable count (TVC) of different Breads and Cakes sample at 1<sup>st</sup> day to 5<sup>th</sup> day (before expire date)

Sample No	1 <sup>st</sup> day cfu/g	2 <sup>nd</sup> day cfu/g	3 <sup>rd</sup> day cfu/g	4 <sup>th</sup> day cfu/g	5 <sup>th</sup> day cfu/g
S1	1.1 × 10 <sup>3</sup>	7.6 × 10 <sup>5</sup>	1.17 × 10 <sup>8</sup>	1.95 × 10 <sup>12</sup>	2.78 × 10 <sup>14</sup>
S2	1.8 × 10 <sup>3</sup>	6.9 × 10 <sup>6</sup>	1.15 × 10 <sup>10</sup>	1.77 × 10 <sup>12</sup>	2.46 × 10 <sup>14</sup>
S3	1.0 × 10 <sup>2</sup>	6.5 × 10 <sup>5</sup>	1.29 × 10 <sup>9</sup>	1.94 × 10 <sup>12</sup>	2.35 × 10 <sup>14</sup>
S4	2.4 × 10 <sup>3</sup>	6.5 × 10 <sup>7</sup>	1.32 × 10 <sup>10</sup>	1.74 × 10 <sup>13</sup>	2.43 × 10 <sup>16</sup>
S5	1.7 × 10 <sup>5</sup>	4.5 × 10 <sup>7</sup>	6.3 × 10 <sup>10</sup>	1.5 × 10 <sup>14</sup>	1.2 × 10 <sup>16</sup>
S6	1.2 × 10 <sup>3</sup>	6.3 × 10 <sup>6</sup>	1.25 × 10 <sup>9</sup>	1.90 × 10 <sup>11</sup>	2.91 × 10 <sup>14</sup>
S7	6 × 10 <sup>2</sup>	3.6 × 10 <sup>5</sup>	8.9 × 10 <sup>8</sup>	1.36 × 10 <sup>12</sup>	2.13 × 10 <sup>15</sup>
S8	1.5 × 10 <sup>2</sup>	6.3 × 10 <sup>6</sup>	1.15 × 10 <sup>9</sup>	1.92 × 10 <sup>12</sup>	2.74 × 10 <sup>15</sup>
S9	2.7 × 10 <sup>5</sup>	1.5 × 10 <sup>7</sup>	3.3 × 10 <sup>10</sup>	2.5 × 10 <sup>14</sup>	1.2 × 10 <sup>18</sup>
S10	1.3 × 10 <sup>6</sup>	4.1 × 10 <sup>8</sup>	7.3 × 10 <sup>10</sup>	1.8 × 10 <sup>14</sup>	1.1 × 10 <sup>18</sup>

The TCC of all the collected bread and cake were higher than the permitted standards after second day to fifth day.(Table 3). It was also observed that 4 breads and 4 cakes sample showed permitted coliform level for consumption at first day, but all of breads and cakes samples were unacceptable for consumptions after 2<sup>nd</sup>

day to 5<sup>th</sup> day. Most of the studied breads and cakes higher level were found to be unfavorable for consumption because most of them showed the presence of coliforms.

**Table 3.** Total coliform count (TCC) of different bread and cake samples at 1<sup>st</sup> day to 5<sup>th</sup> day of production (before expire date)

Sample No	1 <sup>st</sup> day cfu/g	2 <sup>nd</sup> day cfu/g	3 <sup>rd</sup> day cfu/g	4 <sup>th</sup> day cfu/g	5 <sup>th</sup> day cfu/g
S1	120	5 × 10 <sup>2</sup>	9.6 × 10 <sup>5</sup>	1.16 × 10 <sup>7</sup>	2.08 × 10 <sup>10</sup>
S2	2	3.7 × 10 <sup>2</sup>	9.8 × 10 <sup>7</sup>	1.47 × 10 <sup>11</sup>	2.11 × 10 <sup>13</sup>
S3	3	6.8 × 10 <sup>2</sup>	1.27 × 10 <sup>5</sup>	2.08 × 10 <sup>9</sup>	2.77 × 10 <sup>11</sup>
S4	20	3.6 × 10 <sup>3</sup>	8.8 × 10 <sup>7</sup>	1.54 × 10 <sup>10</sup>	2.53 × 10 <sup>13</sup>
S5	220	2 × 10 <sup>4</sup>	5.3 × 10 <sup>6</sup>	7.1 × 10 <sup>9</sup>	1.8 × 10 <sup>13</sup>
S6	40	2.88 × 10 <sup>2</sup>	9.6 × 10 <sup>5</sup>	1.62 × 10 <sup>8</sup>	2.10 × 10 <sup>12</sup>
S7	12	6.5 × 10 <sup>2</sup>	1.07 × 10 <sup>5</sup>	1.43 × 10 <sup>9</sup>	1.86 × 10 <sup>14</sup>
S8	20	6.2 × 10 <sup>3</sup>	1.13 × 10 <sup>5</sup>	1.76 × 10 <sup>8</sup>	2.33 × 10 <sup>13</sup>
S9	1.2 × 10 <sup>2</sup>	7.1 × 10 <sup>6</sup>	3.3 × 10 <sup>8</sup>	5.1 × 10 <sup>12</sup>	1.4 × 10 <sup>14</sup>
S10	244	3.8 × 10 <sup>4</sup>	8.3 × 10 <sup>7</sup>	3.1 × 10 <sup>12</sup>	1.9 × 10 <sup>14</sup>

Table 4 showed the TFC of different breads and cakes at different days. According to the WHO Standard (1994), the acceptable range of TFC is 1.0 × 10<sup>5</sup> cfu/g. The fungal count of all the collected bread and cake were higher than the permitted standards from third day to fifth day. From the result, it is found that 4 breads

and 3 cakes samples (S1, S2, S3, S4, S6, S7, S8) showed the permitted fungal count at first day and 3 breads and 2 cake samples (S1, S2, S3, S6, S7, S8) showed the permitted fungal count at 2<sup>nd</sup> day, but all of bread and cake samples were unacceptable for consumptions from 3<sup>rd</sup> days.

**Table 4.** Total fungal count (TFC) of different bread and cakes samples at 1<sup>st</sup> day to 5<sup>th</sup> day of production (before expire date)

Sample No	1 <sup>st</sup> day cfu/g	2 <sup>nd</sup> day cfu/g	3 <sup>rd</sup> day cfu/g	4 <sup>th</sup> day cfu/g	5 <sup>th</sup> day cfu/g
S1	1.3 × 10 <sup>2</sup>	7.5 × 10 <sup>4</sup>	1.05 × 10 <sup>7</sup>	1.35 × 10 <sup>8</sup>	1.66 × 10 <sup>14</sup>
S2	90	3.3 × 10 <sup>4</sup>	7.5 × 10 <sup>7</sup>	1.29 × 10 <sup>10</sup>	1.82 × 10 <sup>13</sup>
S3	6	5.7 × 10 <sup>4</sup>	1.23 × 10 <sup>6</sup>	1.89 × 10 <sup>10</sup>	2.25 × 10 <sup>16</sup>
S4	5 × 10 <sup>2</sup>	3.9 × 10 <sup>5</sup>	1.31 × 10 <sup>8</sup>	1.93 × 10 <sup>12</sup>	2.97 × 10 <sup>14</sup>
S5	1.1 × 10 <sup>6</sup>	1.7 × 10 <sup>8</sup>	1.2 × 10 <sup>12</sup>	8.5 × 10 <sup>14</sup>	9.2 × 10 <sup>17</sup>
S6	20	3.5 × 10 <sup>4</sup>	8.3 × 10 <sup>7</sup>	1.52 × 10 <sup>10</sup>	2.35 × 10 <sup>14</sup>
S7	5 × 10 <sup>2</sup>	7.7 × 10 <sup>6</sup>	1.20 × 10 <sup>10</sup>	1.65 × 10 <sup>12</sup>	2.18 × 10 <sup>14</sup>
S8	11	4.6 × 10 <sup>4</sup>	8.2 × 10 <sup>7</sup>	1.43 × 10 <sup>10</sup>	1.95 × 10 <sup>13</sup>
S9	2.1 × 10 <sup>5</sup>	1.7 × 10 <sup>8</sup>	1.2 × 10 <sup>11</sup>	6.5 × 10 <sup>13</sup>	1.2 × 10 <sup>16</sup>
S10	3.1 × 10 <sup>5</sup>	1.6 × 10 <sup>8</sup>	2.2 × 10 <sup>12</sup>	6.5 × 10 <sup>15</sup>	9.2 × 10 <sup>17</sup>

In general, an effective level of toxin formation require a large number of microorganisms (approximately 10<sup>5</sup>-10<sup>6</sup> cfu/g of food) (IDF, 1994). In this study, most of bread and cake samples contained higher TVC, TCC

and TFC populations than acceptable range which will cause numerous illness and health hazard.

### Conclusions

This study exhibited the microbial load of available local bread and cake to ensure food safety for a certain public health hazard. From the TVC, TFC and TCC data, it is concluded that consumption of these locally made bread and cake is unsafe because almost all branded bread and cake samples collected from different areas of Tangail city were not in satisfactory level. None of the samples were found to meet the WHO standard from 2<sup>nd</sup> days to 5<sup>th</sup> days. Only few samples were met a limited levels of standard up to second day. These contaminated bread and cake samples contribute substantially to serious health hazards of consumers and lead to food poisoning outbreaks if they are not aware of food spoilage. Therefore, Government should take strong initiatives of regular monitoring and corrective actions. Non-government institutions should also plays role by creating public awareness. This study is a little approach toward the microbial quality assessment of local branded bread and cake in Tangail city, however more research is needed to ensure food safety for better public health aspect.

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