

Isolation and identification of bacteria from paperboard food packaging

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ABSTRACT

Background and Objectives: Paper and paperboard packaging play an important role in safety and quality of food products. Common bacteria of paper and paperboard food packaging could grow due to specific conditions included humidity, temperature and major nutrition to contaminate the food. The purpose of this research was to investigate numbers and the types of bacteria in the food packaging paperboard.

Materials and Methods: The surface and the depth of the each paperboard sample were examined by the dimension of one cm² and one gram. The paperboard samples were randomly collected from popular confectionaries and fast food restaurants in Tehran, Iran.

Results: The results indicated the range of 0.2×10^3 to $>1.0 \times 10^5$ cfu/1g bacterial contamination in paperboard food packaging. Also, most detected bacteria were from spore forming and family *Bacillaceae*.

Conclusion: The bioburden paperboard used for food packaging showed high contamination rate more than standard acceptance level.

Keywords: Paperboard, Food packaging, Bioburden, Family *Bacillaceae*, Bacterial contamination.

INTRODUCTION

Paperboard and paper are the most important products in packaging, beside glass, metal and plastic (1). The importance of paper can be seen in corrugated boxes, milk cartons, folding cartons, bags and sacks and wrapping paper (2).

Paperboard and paper are pulpy materials made from an interweave network of cellulose fibers originated from wood using sulfate and sulfite (2). These raw material are biodegradable, therefore microbial growth can occur anywhere in the paper production process. Industrial environments in which papers are produced are exposed to microbial pollution, also the tank and the slurry in it have desired pH, temperature and the water content that is suitable for microbial growth (3). The slurry itself may have microorganism before starting the process. The microorganism may still be diffused in the final product. Microbial content of the paper and paperboard in food packaging have been established by health organizations of several countries. These organizations also determined the number of specific microbes in one gram,

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but with all of this determination still there is no serious global attention to the bio hazardous perils that may arise from microbial pollution in food packaging. Food packaging is an important step in food production. Therefore, to enhance the health and product quality during manufacturing food packages, the equipment, hands of employees and air should go under microbial examination. The best way is, to establish Hazard Analysis Critical Control Point (HACCP) in the paperboard production line process and timing of food packaging. Few studies have been conducted regarding the microbial contamination of paper and paperboard, and there is no clear criteria are specified for microbiological purity and packaging conditions. Most works reported *Bacillus* spore-bearing Gram-positive bacteria as the most prominent families for paper and paperboard contaminant (3). The aim of this study was to determine the bioburden and type of contaminated bacteria in the current food packaging paperboard.

MATERIALS AND METHODS

Paperboard packaging for food products, including pizza, fried chicken and cookie, french fries boxes and parchment paper were collected from famous fast food restaurant and confectionary in Tehran city, Iran.

In Table 1, materials of tested paperboards are shown.

The medium is Tryptone Glucose Extract Agar culture (TGEA) (containing 5 grams of Casein enzymatic hydrolysate, 1 g Glucose, 3 g Meat extract, 15 g Agar per liter of culture) used for isolation of viable bacteria in the samples (5).

Total count of bacteria per gram of sample us-

ing Defibering method. 1 g of each sample weighed and then homogenized in a 100 ml sterile Ringer solution. Serial dilution (10^{-2} to 10^{-3}) of samples prepared and then poured on 9 cm Petri dishes followed by pour plate method to flood medium TGEA. Three replicates use for each sample. The cultures were incubated at 37 °C for 48 hours (5-6-7).

Total count of bacteria per centimeter of sample using Flooding method. 1×1 cm² of each sample cut and transferred in to 9 cm Petri dishes and TGEA medium flooded by pour plating. All Petri dishes were incubated at 37 °C for 48 hours (8).

Bacterial contamination of surface of sample using Smear method. Surface of each 20×20 cm² sample swapped using sterile swap soaked in sterile Ringer solution, Then shaken for 30 seconds in 20 ml of the same solution. 1 ml of contained solution was poured onto 9 cm petri dish with pour plating method. The Petri dishes were incubated at 37 °C for 48 hours. Sterile distilled water was replaced by normal Ringer. Two replicates used for each samples (4-9).

Identification of bacteria isolated from samples. Biochemical methods were used to identify bacteria (Table 4).

RESULTS

Bioburden of tested samples are demonstrated in Table 2. The minimum and maximum of bacteria number with the method of Defibering found to be for parchment paper C, cookie and fried chicken box A, respectively. The minimum and maximum number of bacteria was 0.2×10^3 cfu/lg and $>1.0 \times 10^5$ cfu/lg.

Table 1. Materials of tested samples (2-4).

| Sample | Type of paperboard |
|-------------------|-----------------------------|
| Pizza box | kraft+corrugated paperboard |
| Parchment paper | cellulose fibers |
| Cookie box | White paperboard |
| French fries box | kraft+corrugated paperboard |
| Fried chicken box | kraft+corrugated paperboard |

Table 2. Bioburden of examined samples.

| Kind of sample | | Method to estimate the total bacteria number | | | | |
|-------------------|---|--|----------------------|-------------------------|--|-----------------|
| | | Defibering method | Flooding method | | Smear method | |
| | | Bacteria number. cfu/1g | Bacteria number | | Bacteria number. cfu / 1 cm ² | |
| | | | cfu / 1 g | cfu / 1 cm ² | Distilled water | Ringer solution |
| Pizza box | A | 4.08×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | 0.0 | <0.5 |
| | B | 0.96×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | C | 17.0×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| Parchment paper | A | 0.33×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | 0.0 | 0 |
| | B | 0.397×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | C | 0.2×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | 0.0 | 0 |
| Cookie box | A | >1.0×10 ⁵ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | B | 6.15×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | C | 2.115×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | 0.0 | 0 |
| French fries box | A | 21.74×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <1.0 |
| | B | 8.35×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | C | 1.6×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| Fried chicken box | A | >1.0×10 ⁵ | >1.0×10 ⁵ | >1.0×10 ⁵ | 0.0 | 0 |
| | B | 15.3×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |
| | C | 7.55×10 ³ | >1.0×10 ⁵ | >1.0×10 ⁵ | <0.5 | <0.5 |

A: Sample No 1

B: Sample No 2

C: Sample No 3

In the Flooding method, all the samples showed high contamination (Table 2).

Smear method did not show any noticeable contamination in examined samples. Results are approximately similar using distilled water and Ringer solution as it is not significantly different.

Table 3 shows minimum and maximum number of bacteria using Defibering method. All samples of parchment paper has range between 1.0×10^2 to 1.0×10^3 cfu/ 1g. Samples of fried chicken box have the highest range (between 3.2×10^3 to 1.0×10^5 cfu/ 1g).

In Table 5 numbers, percentages and the type of bacteria were demonstrated (The biochemical tests of Table 4 are considered).

The most common detected bacteria were found to be the family *Bacillaceae* that *Bacillus licheniformis* and the *Bacillus subtilis* were showed the maximum and minimum number of bacteria, respectively.

In the Table 6 type of bacteria is illustrated (the biochemical tests of Table 4 are considered).

In the Flooding method, the bacteria were uncountable. *Bacillus licheniformis* observed in all

samples except the fries box A.

The photos of Figure 1 show the growth of bacteria on 1×1 cm² of samples. The number of bacteria were >1.0×10⁵ cfu/1g using Flooding method.

In Fig. 2, the number of bacteria in each sample are shown using Defibering method.

DISCUSSION

The usable food packages for human use should be clean and inert, economical, suitably packaged, easily filled and sealed, tolerate rough handling throughout shipping and storage.

In study done by Krystyna Guzińska, Monika Owczarek and Marzena Dymel, the number of bacteria by the Defibering method was in the range of 10²– 10³ cfu/1g, highest bacteria number of 1.2×10³ cfu/1g was to fast growth of the bacteria making the computing of the single colonies impossible. They were marked as uncountable (Uc.). In the Flooding method in the

Table 3. Results of bacterial counts in the examined samples

| Defibering method | | | |
|-------------------|---|--------------------|--------------------|
| Max cfu/1g | | Min cfu/1g | Kind of sample |
| Pizza box | A | 1.5×10^3 | 8.0×10^3 |
| | B | $<1.0 \times 10^2$ | 3.0×10^3 |
| | C | 1.12×10^3 | 22.0×10^3 |
| Parchment paper | A | $<1.0 \times 10^2$ | 1.0×10^3 |
| | B | $<1.0 \times 10^2$ | 1.0×10^3 |
| | C | $<1.0 \times 10^2$ | 1.0×10^3 |
| Cookie box | A | $>1.0 \times 10^5$ | $>1.0 \times 10^5$ |
| | B | 3.0×10^3 | 14.0×10^3 |
| | C | 1.0×10^3 | 2.5×10^3 |
| French fries box | A | 6.9×10^3 | 41.0×10^3 |
| | B | 3.7×10^3 | 14.0×10^3 |
| | C | 0.7×10^3 | 4.0×10^3 |
| Fried chicken box | A | $>1.0 \times 10^5$ | $>1.0 \times 10^5$ |
| | B | 8.7×10^3 | 24.0×10^3 |
| | C | 3.2×10^3 | 14.0×10^3 |

Table 4. The results of biochemical tests on isolated bacteria.

| | Gram-positive Cocci | Bacillus Pantothenticus | Bacillus Licheniformis | Bacillus Stearothermophilus | Bacillus Subtilis |
|------------------|------------------------|----------------------------|---------------------------|--------------------------------|----------------------|
| Glucose | Pos. | Pos. | Pos. | Pos. | Pos. |
| Arabinose | Pos. | ±↑ | Pos. | NDO | NDO |
| Galactose | Pos. | ±↓ | ±↑ | Neg. | Neg. |
| Sucrose | Pos. | Pos. | Pos. | NDO | NDO |
| Trehalose | Neg. | Pos. | Pos. | Pos. | Pos. |
| Maltose | Pos. | ±↑ | Pos. | ±↑ | ±↑ |
| Mannitol | Pos. | ±↑ | Pos. | Pos. | ±↑ |
| ONPG | ±↓ | Pos. | Pos. | Pos. | ±↑ |
| Mobility | Neg. | Pos. | Pos. | Pos. | Pos. |
| Catalase | Neg. | NDO | NDO | NDO | NDO |
| Oxidase | Neg. | Pos. | Pos. | Neg. | Neg. |
| Xylose | Neg. | Neg. | ±↑ | Neg. | ±↑ |
| Inositol | Neg. | Neg. | ±↓ | Neg. | Neg. |
| Dulcitol | Neg. | Neg. | ±↓ | Neg. | Neg. |
| Raffinose | Neg. | Neg. | Neg. | Neg. | ±↑ |
| Adonitol | Neg. | ±↓ | ±↓ | Neg. | Neg. |
| Rhamnose | Neg. | Neg. | Neg. | Neg. | Neg. |
| VP | Neg. | Pos. | Pos. | NDO | NDO |
| Salicin | Pos. | ±↑ | Pos. | NDO | Pos. |
| Melezitose | Neg. | Neg. | Neg. | NDO | NDO |
| Sorbitol | Neg. | ±↓ | ± | NDO | ±↑ |
| Anaerobic growth | NDO | NDO | Pos. | Pos. | Neg. |
| Growth at 50 °C | NDO | NDO | Pos. | Pos. | Pos. |

Pos.: Positive Neg.: Negative

NDO: Not Done

Table 5. Percentage of bacteria isolated from the examined samples by Defibering method

| Defibering method | | | | | | | | | | | | | |
|-------------------|---|-------------------|------|---------------------|-------|-------------------------|-------|------------------------|-------|-----------------------------|-------|-----------------|-------|
| Kind of sample | | Bacillus Subtilis | | Gram-positive Cocci | | Bacillus Pantothenticus | | Bacillus Licheniformis | | Bacillus Stearothermophilus | | Other bacteria* | |
| | | No | % | No | % | No | % | No | % | No | % | No | % |
| Pizza box | A | NDE | NDE | 9 | 7.03 | 8 | 6.25 | 100 | 78.13 | NDE | NDE | 11 | 8.59 |
| | B | NDE | NDE | NDE | NDE | NDE | NDE | 22 | 100 | NDE | NDE | NDE | NDE |
| | C | 1 | 0.23 | 14 | 3.24 | NDE | NDE | 263 | 60.88 | 82 | 18.98 | 72 | 16.67 |
| Parchment paper | A | NDE | NDE | 4 | 28.57 | NDE | NDE | 10 | 71.43 | NDE | NDE | NDE | NDE |
| | B | NDE | NDE | 5 | 83.33 | NDE | NDE | 1 | 16.64 | NDE | NDE | NDE | NDE |
| | C | NDE | NDE | 3 | 100 | NDE | NDE | NDE | NDE | NDE | NDE | NDE | NDE |
| Cookie box | C | NDE | NDE | Uc. | 50 | Uc. | 50 | NDE | NDE | NDE | NDE | NDE | NDE |
| | B | 2 | 1.31 | NDE | NDE | 8 | 5.23 | 127 | 83 | NDE | NDE | 16 | 10.46 |
| | A | NDE | NDE | NDE | NDE | NDE | NDE | 64 | 100 | NDE | NDE | NDE | NDE |
| French fries box | A | NDE | NDE | 85 | 22.48 | NDE | NDE | 221 | 58.47 | 55 | 14.55 | 17 | 4.5 |
| | B | NDE | NDE | 25 | 14.13 | NDE | NDE | 142 | 80.23 | NDE | NDE | 10 | 5.64 |
| | C | NDE | NDE | NDE | NDE | NDE | NDE | 24 | 72.72 | 7 | 21.21 | 2 | 6.07 |
| Fried chicken box | A | NDE | NDE | NDE | NDE | Uc. | 33.3 | Uc. | 33.3 | Uc. | 33.3 | NDE | NDE |
| | B | NDE | NDE | NDE | NDE | 129 | 35.84 | 213 | 59.16 | NDE | NDE | 18 | 5 |
| | C | 2 | 1.36 | 23 | 15.65 | 25 | 17.01 | 91 | 61.9 | NDE | NDE | 6 | 4.08 |

NDE: Not Detected Other bacteria: Bacteria that were not identified. Uc.: Uncountable.

Table 6. Type of bacteria isolated from the examined samples by Flooding method

| Flooding method | | | | | | | |
|-------------------|---|-------------------|---------------------|-------------------------|------------------------|-----------------------------|-----------------|
| Kind of sample | | Bacillus Subtilis | Gram-positive Cocci | Bacillus Pantothenticus | Bacillus Licheniformis | Bacillus Stearothermophilus | Other bacteria* |
| Pizza box | A | NDE | NDE | Pos. | Pos. | Pos. | Pos. |
| | B | Pos. | NDE | NDE | Pos. | NDE | NDE |
| | C | Pos. | NDE | NDE | Pos. | Pos. | NDE |
| Parchment paper | A | NDE | Pos. | NDE | Pos. | NDE | NDE |
| | B | NDE | NDE | Pos. | Pos. | NDE | NDE |
| | C | Pos. | NDE | Pos. | Pos. | NDE | NDE |
| Cookie box | A | NDE | NDE | Pos. | Pos. | NDE | NDE |
| | B | Pos. | NDE | Pos. | Pos. | NDE | NDE |
| | C | NDE | NDE | NDE | Pos. | Pos. | Pos. |
| French fries box | A | ND | Pos. | ND | Pos. | Pos. | ND |
| | B | Pos. | NDE | NDE | NDE | Pos. | NDE |
| | C | NDE | NDE | NDE | Pos. | Pos. | NDE |
| Fried chicken box | A | NDE | NDE | Pos. | Pos. | Pos. | NDE |
| | B | NDE | NDE | Pos. | Pos. | NDE | NDE |
| | C | NDE | NDE | Pos. | Pos. | NDE | NDE |

NDE: Not Detected Pos.: Positive



Fig. 1. Agar Flooding method. Growth of: a) bacteria on fries box B, b) parchment paper B, c) pizza box C.
 French fries box B Parchment paper B Pizza box C

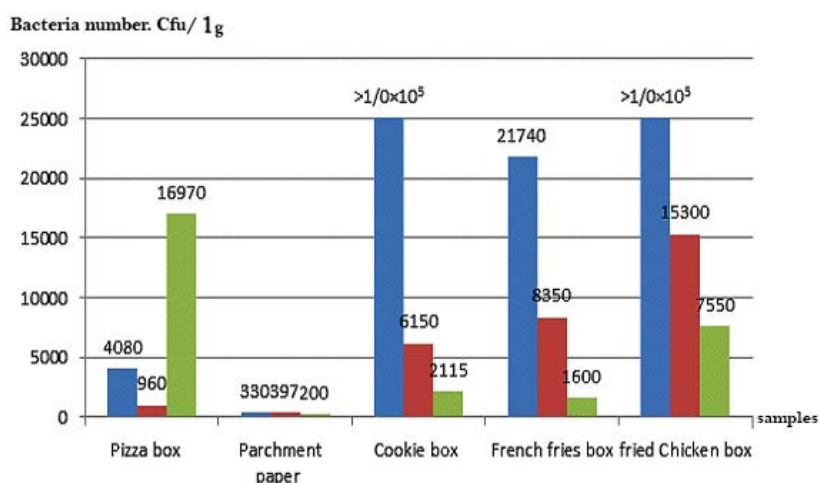


Fig. 2. Bacteria number (cfu/1g) of tested samples using Defibering method.

case of high contamination, the results were unreadable (10).

In our study the most common detected bacteria were belong to the family of *Bacillaceae*. In comparison with Krystina's study (10), in both Smear and Flooding methods, number of bacteria were similar, however, in Defibering method in our samples, bacteria range was between 0.2×10^3 to $>1.0 \times 10^5$ cfu/1g which has a wider range and more contamination in the packaging.

In other study by Ibrahim and Sobeih (2010), the effect of packaging containers (plastic and cardboard) on the bacteriological profile of Egyptian soft cheese

was studied at plant level. *Enterobacter cloacae* (6.67%), *Kliebsilla ozaenae* (13.33%), *Bacillus subtilis* (13.33%), *Staphylococcus epidermis* (6.67%), *Micrococci* (6.67%) and *Enterococcus mutans* (6.67%) were the isolated bacterial strains from cardboard laminated sheets. They concluded that controlling bacterial cross-contamination of cheese during packaging is an important safety issue (11).

Ibrahim and Sobeih were working on *Sporeformers*, *Coliform*, *Staphylococci* and *Enterococci* counts, while we were working on the family *Bacillaceae* that has the highest number among all bacteria families. In compare, the numbers of extracted bacteria in our

samples are much more than Ibrahim's samples.

CONCLUSION

The study was designed to find the type and number of bacteria on the paperboard packaging for food products, including pizza, fried chicken and cookie, french fries boxes and parchment paper, produced in Iran. All samples found to be contaminated with bacteria. *Bacillaceae* family were most common, particularly *Bacillus licheniformis* as isolated in all samples. The lowest number of bacteria was found on parchment paper and the highest belong to fried chicken and cookie boxes. It is recommended to take a serious action leading to establishment of HACCP for food packaging industries to reduce the contamination in food packaging material. In this way, establishing the measures regarding bioburden of packaging materials made out of paper and paperboard is urged.

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