Iranian Journal of Microbiology
Volume 7 Number 5 (Octobr 2015) 287-293

ORIGINAL ARTICLE

Isolation and identification of bacteria from paperboard food packaging

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Received: February 2015**, Accepted:** June 2015

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 cm^2 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 $>1.0\times10^5$ 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).

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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,

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 using 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 cm^2 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 cm^2 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/1g and $>1.0\times10^5$ cfu/1g.

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	$>1.0\times10^5$	$>1.0\times10^5$	0.0	<0.5
	B	0.96×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	C	17.0×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
Parchment paper	A	0.33×10^3	$>1.0\times10^5$	$>1.0\times10^5$	0.0	0
	B	0.397×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	C	0.2×10^3	$>1.0\times10^5$	$>1.0\times10^5$	0.0	0
Cookie box	A	$>1.0\times10^5$	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	B	6.15×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	C	2.115×10^3	$>1.0\times10^5$	$>1.0\times10^5$	0.0	0
French fries box	A	21.74×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<1.0
	B	8.35×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	C	1.6×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
Fried chicken box	A	$>1.0\times10^5$	$>1.0\times10^5$	$>1.0\times10^5$	0.0	0
	B	15.3×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<0.5	<0.5
	C	7.55×10^3	$>1.0\times10^5$	$>1.0\times10^5$	<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 $<1.0\times10^2$ to 1.0×10^3 cfu/ 1g. Samples of fried chicken box have the highest range (between 3.2×10^3 to $>1.0\times10^5$ 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 cm^2 of samples. The number of bacteria were $>1.0\times10^5$ 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 DymelThe, number of bacteria by the Defibering method was in the range of $10^2 – 10^3$ cfu/1g, highest bacteria number of 1.2×10^3 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	$<1.0\times10^2$	3.0×10^3
	C	1.12×10^3	22.0×10^3
Parchment paper	**A**	$<1.0\times10^2$	1.0×10^3
	B	$<1.0\times10^2$	1.0×10^3
	C	$<1.0\times10^2$	1.0×10^3
Cookie box	**A**	$>1.0\times10^5$	$>1.0\times10^5$
	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**	$>1.0\times10^5$	$>1.0\times10^5$
	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 pepar B, c) pizza box C.
French fries box B Parchment paper B Pizza box C

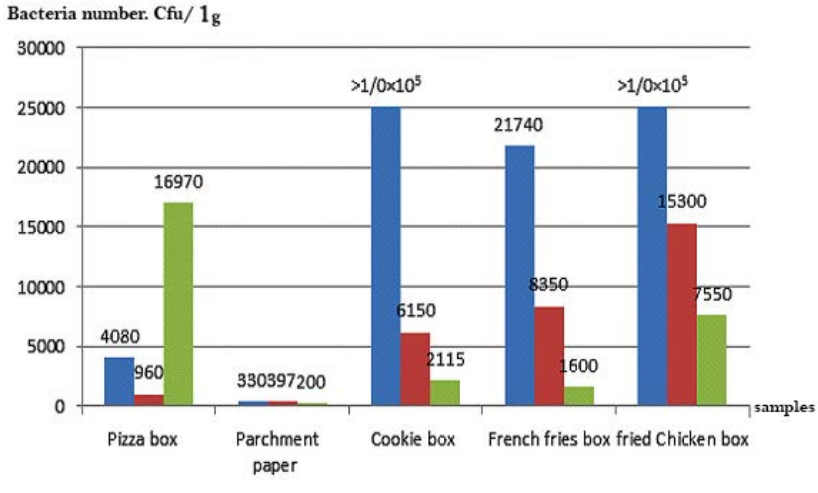

Fig. 2. Bacteria number (cfu/1g) of tested samples using Defibering method.

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 $>1.0\times10^5$ 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 pa¬perboard is urged.

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