Prevalence and identification of Bacillus species in cellophane, foil, and disposable plastic containers used for food packaging

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Abstract:

Background: Foil, cellophane, and disposable plastic containers are commonly employed in food packaging, namely as coatings on food items. Nevertheless, the existence of microbes on the surface and inside the internal composition of these materials gives rise to concerns, as they have the capacity to multiply under ideal circumstances and spread to food items, thereby jeopardizing the safety and quality of the food.

Objective: This study aimed at examining the number of microorganisms and identifying specific bacterial species within frequently used food packaging materials like foil, cellophane, and disposable plastic containers.

Methods: samples of foil, cellophane, and disposable plastic containers were collected from various popular brands in Iran. The overall bacterial count wضas determined using defibering, flooding, and smear techniques followed by culturing on Tryptone Glucose Extract Agar (TGEA) medium. To identify the bacterial species, various biochemical tests were performed, including fermentation, motility testing, catalase test, oxidase test, and methyl red-Voges Proskauer (MR-VP) test.

Results: Bacterial quantity ranged from 0 CFU/g to 8.3×10^3 CFU/g in analyzed samples. All the samples had primary contaminants mainly Bacillaceae family bacteria that could produce spores. Disposable plastic containers had the lowest bacterial count whereas cellophane showed the highest bacterial contamination level. Among Bacillaceae family members Bacillus licheniformis was dominant.

Conclusion: The study's findings emphasize the possibility of microbial contamination in food packaging materials, including cellophane. These results indicate that the food packaging industry should adopt a rigorous approach to hazard evaluation and Important Control Points (HACCP) in order to guarantee the microbiological safety and efficacy of packaging materials.

Keyword: Foil, Cellophane, Disposable plastic container, Food packaging, Family *Bacillaceae*.

معدل انتشار وتشخيص أنواع وجود العصيات البكتيرية في السيلوفان والرقائق والحاويات البلاستيكية ذات الاستخدام الواحد في تغليف المواد الغذائية

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الخلاصة

الخلفية: تستخدم العبوات البلاستيكية والسيلوفان والرقائق المعدنية على نطاق واسع في تغليف المواد الغذائية. ومع ذلك، فإن وجود الكائنات الحية الدقيقة على الاسطح او داخل البنية الداخلية لهذه المواد يثير القلق، لأنها قد تتكاثر في ظل الظروف المثالية وتنتقل إلى المواد الغذائية، مما قد يعرض سلامة الأغذية وجودتها لخطر التلوث. يهدف هذا البحث إلى دراسة التلوث الميكروبي وتحديد أنواع البكتيريا الموجودة في مواد تغليف الاغذية شائعة الاستخدام، بما في ذلك الرقائق والسيلوفان والحاويات البلاستيكية التي يمكن التخلص منها. تم جمع عينات من رقائق القصدير والسيلوفان والحاويات البلاستيكية التي تستخدم لمرة واحدة من مختلف الشركات المشهورة في إيران. تم تحديد العدد الإجمالي للبكتيريا باستخدام طرق مختلفة، وكذلك الزراعة على الوسط الزرعي(Tryptone Glucose Extract Agar (TGEA , تم إجراء الاختبارات البيوكيميائية (fermentation, mobility, catalase, oxidase, methyl red-Voges Proskauer كذلك النمو اللاهوائي. اظهرت ان اعداد الخلايا البكتيرية تتراوح من 0.0 CFU/g إلى 8.3×10^3 CFU/g للعينات التي تم فحصها. حيث كانت البكتيريا المكونة للأبواغ من عائلة Bacillaceae هي الملوثات السائدة في جميع العينات. ولوحظ أدنى عدد من البكتيريا في الحاويات البلاستيكية التي تستخدم لمرة واحدة، في حين أظهر السيلوفان أعلى مستوى من التلوث البكتيري. كانت Bacillus licheniformis هي الأنواع الأكثر انتشارًا بين عائلة Bacillaceae. من خلال هذه الدراسة نستنتج ان التلوث الميكروبي في مواد تغليف الاغذية، وخاصة السيلوفان يسبب خطر ومشكلة صحية للمجتمع.

الكلمات المفتاحية، رقائق معدنية، سيلوفان، الاواني البلاستيكية التي تستخدم مرة واحدة، تغليف الاغذية، عائلة العصويات.

Introduction

Nowadays, cellophane, foils, and disposable plastic containers are important in packaging specifically for food products. Some of the advantages of foil and cellophane are resistance against heat, impact and its light levity for foil, and transparency and ability of high tension for cellophane. Foil serves as a protective barrier against the movement of moisture, oxygen, and other gases. It also has a stronger resistance to light compared to plastic laminate materials, making it effective in preserving volatile aromas (1). Aluminum foil is an aluminum alloy made from 92 to 99 percent aluminum (2).

Cellophane contains an adhesive material stretched to adhere the material to the surface. Cellophane tape is classified as a type of adhesive called pressure-sensitive tape. This tape is primarily marketed in the labeling industry and includes general-purpose cellophane tape, masking tape, packaging labels, and perhaps the best-known clear tape (3).

Disposable dishes are often made of polystyrene, which is a product of styrene processing. Styrene is a clear, transparent, colorless liquid with a pungent smell, which is found both in nature and is obtained artificially from petroleum materials (4; 5)

These two after-producing come in the needed sizes of the market, which cannot be sterile for food packaging, furthermore, it can be mentioned that the raw materials also are not prepared in a free-bacteria condition. Therefore, there are threats to the health of users due to the existence of pathogenic and non-pathogenic bacteria. The primary objective of packaging is to safeguard the food product against degradation in quality caused by microbes, pests, or chemical or physical alterations (6). The nutritional and

quality attributes of foods can be altered with modern food packaging (7).

If the food packaging contains germs, it is possible for microorganisms to grow and transfer to the food. In general, it can be said that these microorganisms in food packaging can increase and be transferred to food if there are suitable conditions for their growth. Global awareness of the potential hazards associated with microbial contamination in Packaging for food products remains insufficient. Food packaging is a crucial stage in the process of food production. Hence, To improve the overall wellness and effectiveness of the product during the production of food packages, it is necessary to conduct microbiological investigations on the equipment, hands of staff, and air. The best way is the control critical points and hazard analysis (HACCP) during the production line of foil, cellophane, and disposable plastic containers and when food is packaged with them (8; 5).

Despite the seriousness of the issue, there is currently a lack of global focus on the potential dangers posed by microbial contamination in food packaging.

Until now, no test has been conducted regarding the bacterial load of foil, cellophane, and disposable plastic container, nor has a clear criterion been determined for the microbiological conditions of food packaging (9). This research was conducted with the aim of investigating the presence of bacteria in disposable food packaging, including cellophane, foil, and disposable plastic container while identifying the key bacteria in these packaging. If the number of microorganisms in these packages is high, assuming that the microorganisms are not pathogenic, they will increase the protein and nucleic acid load of the food, which can cause diseases such as poisoning, nausea, vomiting, dizziness, heartache, acute or persistent diarrhea, and even abscesses and ulcers, and in the presence of a pathogenic microorganism, there is a possibility of people suffering from a disease related to that bacteria, which is due to neglecting the microbiology effect of these packages.

The objective of this study was to ascertain the level of microbial contamination and identify the specific types of bacteria present in the existing food packaging materials, including foil, cellophane, and disposable plastic containers.

Materials and Methods

Food packaging, including foil, cellophane, and disposable plastic containers, was collected from some famous companies in Iran.

The Tryptone Glucose Extract Agar medium (TGEA) was utilized to isolate live bacteria in the samples. The culture contained 5 grams of Casein enzymatic hydrolysate, 1 gram of Glucose, 3 grams of Meat extract, and 15 grams of Agar per liter (10).

Total count of bacteria per gram of sample using the defibering method

Weigh 1 gram of each sample and then mix thoroughly in a 100 ml sterile Ringer solution. The samples were subjected to serial dilution ranging from 10^{-2} to 10^{-3}. The diluted samples were then poured onto 9 cm Petri dishes using the pour plate method to cover the TGEA medium. Each sample requires three replicates. The cultures were placed in a controlled environment at a temperature of 37 °C for duration of 48 hours. (10; 11).

Total count of bacteria per centimeter of a sample using the Flooding method

One square centimeter of each sample was sliced and placed directly into 9 cm Petri dishes. The Petri dishes were filled with TGEA medium using the pour plating

method. The Petri dishes were incubated at 37 °C for 48 hours (12).

Bacterial contamination of the surface of the sample using the Smear method

The surface of each 20×20 cm^2 sample was wiped using a sterile swab soaked in sterile Ringer solution, then agitated for 30 seconds in 20 ml of the same solution. The pour plating method was used to pour 1 cc of the solution onto a 9-centimeter petri plate. The Petri dishes were placed in an incubator and kept at a temperature of 37 °C for a duration of 48 hours. The sterile distilled water was substituted with normal Ringer solution, and all subsequent steps remained the same. Two replicates were used for each swapped sample (13).

Identification of bacteria isolated from samples:

Biochemical methods were used to identify bacteria (Fermentation test, Mobility, Catalase, Oxidase, MR-VP, Anaerobic growth, and Growth at 37℃) (Table 4) (14, 15).

Results

The presence of microorganisms in a given sample is illustrated in Table 1. The minimum and maximum bacterial counts obtained using the defibering approach are for disposable plastic containers A and B, and Cellophane B, respectively. The range of bacterial counts observed was from 0.0 CFU/1g to 8.346×10^3 CFU/1g.

In the Flooding method, all the cellophane illustrates high contamination, and None of the plates were uncountable. All foils and disposable plastics container were 0.0 CFU/1g (Table 1).

The smear method does not show any noticeable contamination in examined samples. Results are approximately similar and with the use of Distilled water and Ringer solution is not a significant difference.

Table 1–Bioburden of examined samples.

Kind of sample		Procedure for determining the total bacterial count: Sample disintegration method – Bacteria number. CFU/1g	Flooding method – Bacteria number CFU /1 g	Flooding method – Bacteria number CFU /1 cm^2	Smear method – Bacteria number. CFU /1 cm^2 Distilled water	Smear method – Bacteria number. CFU /1 cm^2 Ringer solution
Foil	**A**	3.03×10^3	0.0	0.0	0.0	<0.5
Foil	**B**	1.15×10^3	0.0	0.0	0.0	<0.5
Foil	**C**	0.05×10^3	0.0	0.0	0.0	<1.0
Cellophane	**A**	0.846×10^3	$>10\times10^5$	$>10\times10^5$	0.0	0.0
Cellophane	**B**	8.346×10^3	$>10\times10^5$	$>10\times10^5$	0.0	0.0
Cellophane	**C**	$4/8\times10^3$	$>10\times10^5$	$>10\times10^5$	0.0	0.0
Disposable plastic container	**A**	0.0	0.0	0.0	0.0	0.0
Disposable plastic container	**B**	0.0	0.0	0.0	0.0	0.0
Disposable plastic container	**C**	$0/75\times10^3$	0.0	0.0	<0/5	0.0

Table 2 shows the minimum and maximum number of bacteria by the Defibering method. Disposable plastic containers A and B show 0.0 CFU/1g.

Table 2–Results of bacterial counts in the examined samples

Defibering method			
Kind of sample		**Min CFU/1g**	**Max CFU/1g**
Foil	**A**	1.2×10^3	6×10^3
Foil	**B**	$<10^2$	2×10^3
Foil	**C**	$<10^2$	0.2×10^3
Cellophane	**A**	$<10^2$	1.4×10^3
Cellophane	**B**	4×10^3	$17/0\times10^3$
Cellophane	**C**	$2/0\times10^3$	$\times10^3 10/0$
Disposable plastic container	**A**	0/0	0/0
Disposable plastic container	**B**	0/0	0/0
Disposable plastic container	**C**	$0/1\times10^3$	$2/0\times10^3$

The results of Table 3, according to Bergey's manual bacteria were identified.

Table 3–Biochemical tests

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

NDO*: Not Done

In Table 4 numbers, percentages, and the type of bacteria are demonstrated (The biochemical tests of Table 3 are considered).

Table4–Incidence of different bacteria isolated from the examined samples by defibering method

Defibering method								
Kind of sample			***Bacillus Pantothenticus***		***Bacillus Licheniformis***		***Bacillus Subtilis***	
			No	%	**No**	%	**No**	**%**
Foil	**A**		ND	0/0	ND	0/0	75	100
Foil	**B**		ND	0/0	19	37.25	32	62.75
Foil	**C**		ND	0/0	ND	ND	3	100
Cellophane	**A**		15	45.46	18	54.54	ND	ND
Cellophane	**B**		170	70.83	70	29.17	ND	ND
Cellophane	**C**		78	57/8	50	37	7/0	5/2
Disposable plastic container	**A**		ND	0/0	ND	0/0	ND	0/0
Disposable plastic container	**B**		ND	0/0	ND	0/0	ND	0/0
Disposable plastic container	**C**		6/0	66/67	3/0	33/33	ND	0/0

ND*: Not Detected

Table 5 type of bacteria illustrated (The biochemical tests of table 3 are considered).

In the flooding method, the bacteria were uncountable in the cellophane. *Bacillus Pantothenticus* was observed in all samples.

Foils and Disposable plastic containers showed no bacteria with the method.

Table 5–Incidence of different bacteria isolated from the examined samples by Flooding method

Flooding method					
Kind of sample		**Gram-positive Cocci**	***Bacillus Pantothenticus***	***bacillus Licheniformis***	***Bacillus Subtilis***
Cellophane	**A**	ND	+	ND	ND
Cellophane	**B**	ND	+	+	ND
Cellophane	**C**	ND	+	+	ND

In Table 6 type of bacteria is illustrated (the biochemical tests of Table 3 are considered).

Table 6–Incidence of different bacteria isolated from the examined samples by smear method

Smear method (Ringer solution)					
Kind of sample		**Gram-positive Cocci**	***Bacillus Pantothenticus***	***bacillus Licheniformis***	***Bacillus Subtilis***
Foil	**A**	ND	ND	+	+
Foil	**B**	ND	ND	ND	+
Foil	**C**	+	ND	ND	ND

Discussion

The food packaging that can be used for human consumption should be clean and odorless, economical, suitable, easily filled and sealed, and withstand shock and pressure throughout transportation and storage (16; 17). Food packaging serves multiple purposes, including the prevention of product spoilage, the preservation of the positive effects of processing, the extension of shelf life, and the maintenance or improvement of food quality and safety. Through packaging, protection is provided against three primary categories of external incursions, namely chemical, biological, and physical intrusions. (18; 19).

The study was on the amount of bacterial load and the type of bacteria in three food packaging materials, including foil, cellophane, and disposable plastic container. In all samples containing bacteria, *Bacillaceae* family bacteria were the most common and *Bacillus leishiniformis* was the most numerous among *Bacillaceae*. The lowest number of bacteria in a disposable plastic container and the highest bacterial contamination was from cellophane. Foil as metal packaging was significant in terms of bacterial contamination on its surface. In the case of cellophane, as a coating and packaging that has gained high consumption today, it had high bacterial contamination considering that no standard has been set for this type of packaging regarding its microbial load.

In general, it can be said that these microorganisms in food packaging can increase and be transferred to food if there are suitable conditions for their growth. So far, microbial testing has not been done on these food packages. There is also the possibility of the presence of other microorganisms in food packaging materials, which, related to the type of microorganism and its growing conditions, may cause harm to the consumer of the food. According to this research, the need for a serious approach related to HACCP for food packaging industries can be understood. In this way, it is felt to create measures related to the microbiological purity of packaging materials in food.

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Author contributions

M.M.V. and N.S.H. are responsible for Conceptualization, Investigation, and Resources. E.A. is responsible for Supervision, Writing – Original Drafts, Writing – Review & Editing, and replication of data. B.K.U. was responsible for analyzing, writing, and editing the manuscript. F.G. is responsible for the Investigation and replication of data. M.M.V. is responsible for Supervision, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration, Data Curation, Conceptualization, and Investigation.

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