Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(8):661-666 **ISSN 2347-9515 (Print)**
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(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI : 10.36347/sajb.2016.v04i08.011

Original Research Article

Importance of microbial analysis of Cling film in food packaging industry

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Abstract: Plastic packaging which is a critical control point is very often contaminated with microorganisms during production. This aim of present study is to isolate and characterize dominant species found in food quality Cling films. Fifteen Cling film samples from various countries were chosen. Standard plate count for total microorganism was used. Then, Biochemical methods were used to identify microbial community on Cling film. Among tested Cling films, seven samples were devoid of any contamination. No fungus contamination was observed at all, while eight samples had bacterial contamination. The total colony counts for the microorganisms were a follow: 3.2×10^3, 5×10^4, 1.6×10^4 and 3×10^2 CFU. Based on the colony morphology, gram staining and biochemical tests, three different bacterial isolates were identified including *Bacillus spp*. (except *B. anthracis*), *Klebsiella spp*. and *Staphylococcus aureus*. It is necessary to incorporate a Hazard Analysis and Critical Control Points based program to ensure quality through the packaging operation and determination procedures for the presence of microorganisms need to be established which analyze them exactly and acceptable microbial limits set.
Keywords: Cling film, packaging, microbial contamination, foodborne disease.

INTRODUCTION

Cling films are typically made from polyvinyl chloride (PVC) or polyethylene (PE). This product is high-quality food wrap film which makes food available with greater safety assurance from microorganisms, biological and chemical changes, keeps it fresh, and also, decreases the risk of food wastage by enhancing its shelf life [1-3]. From the time; plastics emerged as the favored choice of packaging material for various products including groceries, beverages, chemicals, electronic items and etc, they have become an essential component in the food manufacturing process [4]. Plastic packaging which is a critical control point is very often contaminated with microorganisms during production. As a result, HACCP systems (Hazard Analysis and Critical Control Points) are set to identify microbiological risk factors. Moreover, food producers are progressively demanding compliance with microbial limits for packaging and consequently, packaging manufacturers are obliged to optimize the hygienic conditions of their production [5- 6].

Foodborne diseases (FBD) encompass a wide spectrum of illnesses. It can be caused by a variety of microbial pathogens that have entered the food chain at some point from farm to fork [7-8]. A key aspect here is the microbiological state of the packaging surface at the time of wrapping, namely the level of contamination with bacteria, mold, and yeast [9]. According to the FDA (Food and Drug Administration) declaration, pathogenic bacteria such as *Bacillus spp*. (*B. cereus*) and *Staphylococcus aureus* have been reported with FBD [10]. *Bacillus spp*. (*B. cereus*) is an infectious cause of FBD, accounted for 2% of outbreaks with confirmed etiology that were reported to the CDC (Centers for Disease Control and Prevention), whilst Staphylococcal FBD is one of the most common FBD, a major concern in public health programs worldwide and, it is one of the most common causes of reported FBD in the USA, with attack rates up to 85% [11-12]. In addition, investigations have shown *Klebsiella* spp. has the capability to be a cause of FBD, despite the fact it was not directly introduced by the FDA [13]. The vast majority of FBD cases, although unpleasant, are mild and self-limiting, while a significant number of death do occur. According to the WHO, up to one-third of people in developed countries are suffered by food-borne pathogens each year. Its prevalence is

significantly higher in developing countries. The cost and burden of FBD remain high making it a global concern [14].

Unintended transfer of microbes from one surface to another is one of the means of spreading FBD. The physical interactions between bacteria and a given material will influence the degree to which the microbes can be transmitted from that material. The bacteria's growth potential while attached and the ease of removal are pertinent factors in cross contamination considerations. The environments and processes that the material and microbes are subjected to physical interactions are correspondingly significant [15]. In this case, proper hygienic condition and effective packaging with the right materials are important measures to reduce chances of food contamination, spoilage and its implication in FBD [16]. Although, efforts have carried out on monitoring the microbial purity of packaging, no clear criteria or standards have been released about the microbial community of Cling films. The aim of present study is to isolate and characterize dominant species found in food quality Cling films.

Experimental section

Fifteen Cling film samples from various countries including Canada, Germany, Iran, Korea, Poland and USA with different brand names were chosen. The name of product is not mentioned.

Samples characteristics

All the Cling films used in the present study had household consumption**.** The reels of Cling film were obtained from the local supermarkets of different countries. They were put in aseptic packages and transferred to our laboratory. The average length and width of all samples was 161.85 m and 29.49 cm, respectively. The average thickness of samples, after assessment with thickness measurement gauge, was 11±2 µ. The polymer used for producing Cling films is listed in table 1. Typically, one meter of the samples length was discarded in order to avoid contamination during microbial analysis of Cling films. All the stages were done under sterile condition and in triplicate.

Total count of microorganism per gram

We have used standard plate count for total microorganism. One gr of each sample weighed and then a serial dilution (10^{-1} to 10^{-6}) of samples prepared in nutrient broth (DNB) and incubated for 24 h. The grown colonies was counted and multiplied by the dilution factor of the plate in order to determine the average number of microorganism cell in the original population. If the concentration of microorganism was too great the colonies had grown into each other and the plate reported uncountable. The experiments were carried out in triplicate. Subsequently, 0.1 ml from a liquid culture was spread on agar plate (Blood, MacConkey, Potato Dextrose and Sabouraud Dextrose Agar) and incubated at 37 °C for 48 h. for bacterial identification. All culture medium were purchased from Merck (Germany) company.

Identification of bacteria isolated from samples

Then, Biochemical methods were used in appropriate medium culture to identify microbial community on Cling film. These test were as follow: gram staining, shape, catalase, coagulase, oxidase, capsule formation, spore formation, motility, DNase, gelatinase, lipase, phosphatase, urease, lecithinase, hemolysis, ONPG, nitrate reduction, indole production, methyl red, vogesproskauer, citrate utilization, TSI, H2s, glucose fermentation, lactose fermentation, mannitol fermentation, novobiocin susceptibility, lysostaphin susceptibility, penicillin susceptibility and furazolidone susceptibility.

RESULTS AND DISCUSSION

The results of biochemical tests are as follow:

Bacillus spp.:gram positive Bacilli;catalase, oxidase, spore formation, motility, gelatinase, lipase, phosphatase, lecithinase, hemolysis, nitrate reduction was positive and the test for capsule formation was negative. Also, the isolate was penicillin resistant.

Klebsiella spp.: gram negative Bacilli;the result of catalase, capsule formation, urease, ONPG, nitrate reduction, methyl red, vogesproskauer, citrate utilization, TSI (A/A), glucose, lactose and mannitol fermentation tests were positive, while it was negative for coagulase, oxidase, spore formation, motility, indole production and H2s.

Staphylococcus aureus: gram positive Cocci; catalase, coagulase, capsule formation, DNase, gelatinase, lipase, phosphatase, urease, hemolysis, nitrate reduction, vogesproskauer, glucose and mannitol fermentation tests were positive, whilst oxidase, spore formation, motility, methyl red, citrate utilization and lactose fermentation tests were negative. The isolate was novobiocin and lysostaphin resistant and susceptible to furazolidone.

Our finding showed that from fifteen tested Cling films, seven samples were devoid of any contamination. No fungus contamination was observed in all samples, while eight samples had bacterial contamination. According to dilution series result, four samples had uncountable colonies and bacterial contaminations from other four samples were counted. The total colony counts for the microorganisms were a follow: 3.2×10^{3}, 5×10^{4}, 1.6×10^{4} and 3×10^{2} CFU for second, fifth, eighth and eleventh sample, respectively. The results are shown in table 1.

Table 1: Samples and total colony number

Sample	Polymer type	Colony forming unit	Result
Canada	PVC	uc	Bacillus spp.
Canada	PE	3.2×10^3	Bacillus spp.
Canada	PE	No bacteria	-
Canada	PVC	No bacteria	-
Germany	PE	5×10^4	Staphylococcus aureus
Iran	PVC	No bacteria	-
Iran	PVC	No bacteria	-
Iran	PE	1.6×10^4	Bacillus spp.
Iran	PE	No bacteria	-
Iran	PVC	No bacteria	-
Korea	PVC	3×10^2	Klebsiellaspp
Korea	PVC	uc	Staphylococcus aureus Bacillus spp.
Korea	PVC	No bacteria	-
Poland	PVC	uc	Bacillus spp.
USA	PVC	uc	Bacillus spp.

PVC: Polyvinyl chloride; PE: Polyethylene; uc: uncountable

Based on the colony morphology, gram staining and biochemical tests, three different bacterial strains were identified including positive motility *Bacillus spp*. (except *B. anthracis*), *Klebsiella spp*. and coagulase positive *Staphylococcus aureus*. Six samples from various countries were contaminated with *Bacillus spp*., while contamination of Cling films with *Staphylococcus aureus* and *Klebsiella* was seen in smaller number. Among tested Cling films, Korean Cling film was the only sample contaminated with two different bacteria including *Staphylococcus aureus* and *Bacillus spp.*

Primary packaging material has a dual role in comprising the material and in avoiding contamination with microorganism. If packaging is not appropriately sterilized, it can serves as a starting point of microbial contamination. Practically, the packaging often contributes considerably to the total bio-burden of the product when using non-sterile product. The microflora of packaging material is dependent upon both its composition and storage conditions [17]. Food safety control and management (ISO 22000:2005) systems such as better packaging procedures and improved new pathogen detection techniques have spent considerable amount of time, effort and money for enhancing food safety and quality. Nonetheless there is still little sign within official statistics of significant reductions in the incidence of FBD within EU countries [18]. Particular requirements for the hygienic state of food packaging clarified for the first time by EU Regulation 852/2004 on food hygiene in Section X [9]. Despite the fact that it is expected to have vast degree of legislation owing to control such contamination on cling films, but there is no enacted law by regulatory authorities around the world. In addition, the European Union has not issued any specific demands concerning paper and paper board designed for contact with food [19]. According to FDA declaration, the limit value recommendations for packaging materials vary from 1 fungal cfu dm^{-2} to 250 bacterial cfu g^{-1}. In general, the total accepted counts of yeasts, molds and bacterial must be low and no pathogenic bacteria including *Enterobacteria* and *Escherichia coli* must be detected [20-21]. On the other side, it is believed by Iranian National Standards Organization number 3341 to have 500 colonies of mesophilic aerobic bacteria and 20 colonies of mold which are acceptable, whilst more than this number is not admissible. Accordingly, no coliforms, *Streptococcus*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, coagulase positive *staphylococci* and yeast must be seen in one gram of food contact cardboard sheet [22]. These guideline values will give greater overall assurance when evaluating the hygienic state of food packaging.

Johansson et al. findings on microflora and the content of endotoxin in paper revealed that endotoxins were identified in all the tested samples, while outcome of McCusky Gendron et al. showed that the tested paper contained between 10^2-10^5 CFU/g microorganisms, specifically *Bacillus* genera [23]. Accordance with our

results, Cling films comprised six *Bacillus spp*. which is more than *S. aureus*and *Klebsiella*, but colony counting indicating the range of 3.2×10^3 to 1.6×10^4 for *Bacillus spp*. for two samples, while others had uncountable colonies. This result is comparable with Mohammadzadeh finding which showed the range of 0.2×10^3 to $>1\times10^5$cfu/1g bacterial contamination in paperboard food packaging. Vaisanen et al. found that *B. licheniformis* was the most and *B. brevis*and *B. megaterium*were the least contamination from the family of Bacillaceae, whereas the most prevalent detected bacteria were *Bacillus licheniformis* and *Bacillus subtilis*in the maximum and minimum number according to other studies [24-25]. Also, strains of *Bacillus polymyxa* group (*B. polymyxa*, *B. circulans*, *B. macerans*, *B. pabuli*), *B. cereus* group (*B. cereus*, *B. mycoides*, *B. thuringiensis*), *B. brevis* and *B. licheniformis*were most regularly found as major spore formers in tested samples [26]. Correspondingly, we did not carry out specific tests to differentiate *Bacillus* species, as the *Bacillus* genus comprises more than 268 species in category [27]. Interestingly, one study based on phylogenetic data and phenotypic and chemotaxonomic characteristics on microflora from food-packaging board was carried out. Clearly, that microflora of board was most dominated by *paenibacilli*; it is proposed that the isolates represent a novel species, *Paenibacillus stelliferspp*[28]. Also, *Paenibacillus barengoltzii*, and *Paenibacillus odorifer*was lately reported as a microflorain a carton board sample. It is believed that forming heat-resistant spores which describe its survival during the drying phase of paper board machine operation can be a main reason of finding the *Bacillus* genera. *Bacilli* are known to stick to biofilms as monolayers as well [29].

Despite reporting coliforms and coagulase positive *staphylococci* is unacceptable, but we have observed *Klebsiella spp*. and *Staphylococcus aureus* in tested Cling films which is in agreement with Ibrahim and Sobeih finding who evaluated the effect of packaging containers including plastic and cardboard on the bacteriological profile of Egyptian soft cheese at plant level and bacterial isolates including *Kliebsilla ozaenae*, *Staphylococcus epidermis*, *Enterobacter cloacae*, *Bacillus subtilis*, *Micrococci* and *Enterococcus mutans* was reported [24]. Their result indicated that plastic containers and cardboard packaging enhanced the bacterial contamination in cheese; signifying that packaging materials are substantial source of cheese contamination. The average bacterial counts in the examined soft cheese samples were increased from 2.7×10^2 to 3.1×10^2 for Coliforms, 1.6×10^3 to 7.4×10^3 CFU/g for *Staphylococci*, after packaging in the plastic containers [30], while this figure is equivalent for number of coliform or *Klebsiella spp*. (3×10^2) and it is lower for *S. aureus* (5×10^4) in comparison with our finding. On the other hand, reporting *Klebsiella* as microflora of Cling films is in disagreement with Namjoshi et al. which did not find any coliform bacterium in their investigation [25]. According to Public Health Agency of Canada, Infectious dose for *Bacillus spp*. (*B. cereus*), *Staphylococcus aureus* and *Klebsiella spp*. is 10^4-10^9, 10^5 and 10^8, respectively [31]. In comparison to this authorized organization, high microbial load in our study was 3.2×10^3-1.6×10^4 for *Bacillus spp*. indicating the borderline for induction of FBD, whereas it is lower than infective dose for *S. aureus* and *Klebsiella spp*.

Historically, bacteria are considered for further and extensive researches. Because they have been isolated much more regularly from paperboard packaging than molds and yeasts. This possibly will be ascribed to the belief that chemicals and heat used during paperboard production are more in effect against fungi than bacterial spores [32]. In the majority of studies, no mold, yeast or fungus contamination is reported similar to our study, while Hladikova et al., detected molds in three paper samples in concentrations of 5×10^2 to 1×10^3 CFU/g. Besides, *Penicillium biforme* and *Penicilliumspinulosum* species were also isolated from the paperboard from other study [23, 33].

Interestingly, FBD Outbreak Surveillance System declared that *B. cereus* and *S. aureus* were a main factor of one in ten FBD outbreaks during 1998–2008; indicating the importance of these microorganisms in threatening process safety. Thereupon, presence of these pathogens in food products imposes potential hazard for consumers and causes serious economic loss and loss in human productivity via FBD [11, 34]. Seemingly, our tested samples were contaminated in high degree as it is not comparable to data mentioned above. Tested Cling films in our study were from Canada, Germany, Iran, Korea, Poland and USA. It is noteworthy to know that one of five Iranian samples had bacterial contamination. One of the Korean samples which had uncountable colonies contained two different contaminations and sample from Germany had the highest bacterial contamination. Since international trade can be a factor of spreading diseases, a worldwide limit value is felt. According to Association of Food and Drug Officials (AFDO), simple packaging or repackaging operations can result in an opportunity for the contamination or recontamination with pathogens if strict aseptic conditions are not adhered to. Testing for these organisms at particular control points prepares the best means of quality control. Continuous surveillance and good manufacturing practice are the best techniques for prevention of contamination [35].

CONCLUSION

Taken all together, it is necessary to incorporate a HACCP-based program to ensure quality through the packaging operation and determination procedures for the presence of microorganisms need to be established which analyze them exactly and standard microbial limits set. Since microorganisms present in food industry as a microflora may penetrate into foodstuffs and lead to undesirable effect on public health, not only food packaging, but also storage and distribution practices must also be evaluated as part of

an effective quality control program. Besides, regulation of authorized organization must be accessible to all. This investigation was merely of an introductory character. Further work is needed in that direction to prevent microbial foodborne outbreaks as a public health and provide food grade Cling films in the nearest future.

Acknowledgment

This study was financially supported by Food Security Research Centre, Faculty of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran (Grant No: 293310). The authors would like to thank Parnian Hosseini for her thoughtful editing and constructive comments on this manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Lau OW, Wong SK; Contamination in food from packaging material. Journal of Chromatogrraphy, 2000; A 882: 255-270.
2. Malhotra B, Keshwani A, Kharkwal H; Natural polymer based cling films for food packaging. International Journal of Pharmacy and Pharmaceutical Sciences, 2015; 7(4): 10-18.
3. Castle DA, Gibbins B, Hamer PS; Physical methods for examining and comparing transparent plastic bags and cling films. Journal of Forensic Science Society, 1994; 34(1): 61-68.
4. Allahvaisi S; Polypropylene in the Industry of Food Packaging. In Polypropylene, Fatih Dogan editor, INTECH Open Access Publisher, 2012: 3-22.
5. Tacker M, Hametner C, Wepner B; Determination of microbial contamination of plastic cups for dairy products and utilization of electron beam treatment for sterilization. Food Additives and Contaminants, 2002; 19(S1): 178- 184.
6. Cutter CN; Microbial control by packaging: a review. Critical Reviews in Food Science and Nutrition, 2002;42(2): 151-161.
7. Raj M, Mahendra P; Sanitation in Food Establishments. LAP Lambert Academic Publishing, United States, 2015.
8. Hanson LA, Zahn EA, Wild SR, Döpfer D, Scott J, Stein C; Estimating global mortality from potentially foodborne diseases: an analysis using vital registration data. Population Health Metrics, 2012;10(5).
9. Hennlich W; Guideline values for microbiological contamination provide increased assurance for assessing the hygienic state of food packaging. Pt.I: Packaging materials and packaging systems made of non-absorbent materials. Food Marketing and Techology, 2011;25(2): 54-55.
10. Foodborne Illnesses: What You Need to Know. Available from http://www.fda.gov/.
11. Kadariya J, Smith TC, Thapaliya D; Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Research International, 2014;2014.
12. Epidemiologic Notes and Reports Bacillus cereus Food Poisoning Associated with Fried Rice at Two Child Day Care Centers - Virginia, 1993. Available from http://www.cdc.gov/.
13. Kalantar E, Soltani J, Hatami S, Habibi N, Pormazaheri H, Marashi SMA, et al;Assessment of the Bacteriological Quality of Food Samples May Offer Clues the Antibiotic Resistance Pattern. International Journal of Enteric Pathogens, 2015;3(1).
14. Scott E; Food safety and foodborne disease in 21st century homes. The Canadian Journal of Infectious Disease, 2003;14(5): 277-280.
15. Boersig MR, Cliver DO; The role of pallets in microbial food safety.Food Protection Trends, 2010; 30(10): 576- 579.
16. Ajala L, Ibrahim TA, Adebote VT; Effect of different packaging materials on bacteriological quality of Egidi. AmericanJournal ofFood Nutrition, 2011; 1(2): 79-81.
17. Jeje JO, Oladepo KT; A study of sources of microbial contamination of packaged water. Transsnational Journal of Science and Technology, 2012; 2(9):63-76
18. Roasto M, Reinmüller B, Vokk R, Dinçer Baysal AH, Polanc J, Veskus T, et al; Bacterial foodborne pathogens of concern. Microbial contaminants and contamination routes in food, VTT Symposium, 2007; 116.
19. Guzińska K, Owczarek M, Dymel M; Investigation in the microbiological purity of paper and board packaging intended for contact with food. Fibres and Textiles in Eastern Europe, 2012; 6B(96): 186-190.
20. Pirttijärvi T; Contaminant aerobic sporeforming bacteria in the manufacturing processes of food packaging board and food. University of Helsinki, Finland, 2000: 13.
21. Suihko ML, Skyttä E; A study of the microflora of some recycled fibre pulps, boards and kitchen rolls. Journal of Applied Microbiology, 1997; 83(2): 199-207.
22. Iranian National Standards Organization. Available from http://www.isiri.org.
23. Hladikova Z, Kejlova K, Sosnovcova J, Jirova D, Vavrous A, Janousek S, et al; Microbial Contamination of Paper-based Food Contact Materials with Different Contents of Recycled Fiber. Czech Journal of Food Sciences, 2015; 33(4): 308-312.
24. Mohammadzadeh-Vazifeh MM, Hosseini SM, Khajeh-Nasiri S, Hashemi S, Fakhari J;Isolation and identification of bacteria from paperboard food packaging. Iran Journal of Microbiology, 2015; 7(5): 287-293.

25. Namjoshi K, Johnson S, Montello P, Pullman GS; Survey of bacterial populations present in US-produced linerboard with high recycle content. Journal of Applied Microbiology, 2010; 108(2): 416-427.
26. Välsänen OM, Mentu J, Salkinoja-Salonen MS; Bacteria in food packaging paper and board. Journal of Applied Bacteriology, 1991; 71(2): 130-133.
27. Slepecky RA, Hemphill HE;The genus Bacillus - nonmedical. In The prokaryotes, Springer US, 2006: 530-562.
28. Suominen I, Spröer C, Kämpfer P, Rainey FA, Lounatmaa K, Salkinoja-Salonen M; Paenibacillusstellifer sp. nov., a cyclodextrin-producing species isolated from paperboard. International Journal of Systematic and Evolutionary Microbiology, 2003; 53(5): 1369-1374.
29. Kemppainen H; Mapping of causations for edospore formation and process optimization at pulp-and paper mill. KTH Royal Institute of Technology and Billerud Korsnäs AB, Sweden, 2013: 2-9.
30. Ibrahim EMA, Sobeih AMK; Effect of Packaging Containers on the Bacteriological Profile of Soft Cheese. Journal of Global Veterinaria, 2010;4(4): 349-356.
31. Public Health Agency of Canada.Available from http://www.phac-aspc.gc.ca.
32. Sammons L; Migration of *Penicillium spinulosum* from paperboard packaging to extended shelf life milk. Virginia Polytechnic Institute and State University, United States, 1999: 10.
33. Narciso JA, Parish ME; Endogenous Mycoflora of Gable-top Carton Paperboard Used for Packaging Fruit Juice. Journal ofFood Science, 1997; 62(6): 1223-1239.
34. Bennett SD, Walsh KA, Gould LH; Foodborne disease outbreaks caused by Bacillus cereus, Clostridium perfringens, and Staphylococcus aureus - United States, 1998–2008. Food Safety, 2013; 57(3): 425-433.
35. Nma ON, Ola AA; Microbiological Analysis of some Packaged Fruit Juices sold in Port Hacourt Metropolis, Nigeria. Nature and Science, 2013; 11(4): 30-40.