Journal of Environmental Health and Sustainable Development

An Adenosine Triphosphate Bioluminescence Method for Evaluating the Microbial Contamination of the Salad-Preparing Tables and Salad-Serving Dishes in Restaurants of Mashhad City, Iran

Monavvar Afzal-Aghaee [1], Zohreh Rahnama Bargard [2, 3], Mohammad Reza Nezakati Olfati [4], Rajab Ali Ahooey [4], Samaneh Gohari [2,3*]

[1] *Department of Statistics and Epidemiology, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*
[2] *Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.*
[3] *Department of Environmental Health Engineering, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*
[4] *Environmental Health Expert of Mashhad Health Center No. 5, Mashhad University of Medical Sciences, Mashhad, Iran.*

ARTICLE INFO

ORIGINAL ARTICLE

Article History:
Received: 25 December 2019
Accepted: 20 February 2020

***Corresponding Author:**
Samaneh Gohari
Email:
Samanehgohari@gmail.com
Tel:
+989159109148

Keywords:
Adenosine Triphosphate,
Food Contamination,
Food Microbiology,
Mashhad City.

ABSTRACT

Introduction: Consumption of vegetable products is increasing commonly in the world because they are recognized as an important source of nutrients, vitamins, and fiber for humans. Salads are among the most widely used foods that are also known as the most contaminated foods in restaurants. This study was conducted to determine the microbial contamination of salad-preparing tables and salad-serving dishes.

Materials and Methods: This study was conducted at 39 restaurants in Mashhad City in Iran during 2017. In this regard, 78 samples were determined from the salad preparing tables and serving dishes. Samples were tested for cleanliness status using Adenosine Triphosphate (ATP)-bioluminescence method as a rapid test.

Results: According to the ATP bioluminescence device, 43.6% of the assessed dishes were clean, 38.5 % were dirty, and 17.9 % were not adequately clean. According to the results of ATP bioluminescence device for tables, 23.1 % of the tested tables passed the test, 15.4 % were classified in the caution status, and 61.5% failed.

Conclusion: The ATP method has been widely used, especially in the food industry, as a rapid method for measuring general levels of cleanliness. However, this method is not a reliable replacement for measuring the microbial contamination. The ATP bioluminescence should not be used as a substitute for quantitation of microbial load.

Citation: Afzal-Aghaee M, Rahnama Bargard Z, Nezakati Olfati MR, et al. ***An Adenosine Triphosphate Bioluminescence Method for Evaluating the Microbial Contamination of the Salad-Preparing Tables and Salad-Serving Dishes in Restaurants of Mashhad City, Iran*.** J Environ Health Sustain Dev. 2020; 5(1): 948-54.

Introduction

To keep a crucial healthy diet, it is essential to consume fresh and worthy vegetables [1]. Previous studies indicated that vegetables may be contaminated by human pathogenic bacteria during the harvest and distribution processes [2]. In recent decades, due to the high costs of effluent treatment and deficiency of the freshwater resources, many

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]

reports showed irrigation of the cultivation lands using infectious effluent, raw sewage, and use of animal and human fertilizers [3]. Considering that no chemical treatments are carried out to reduce the microbial contamination in this field, vegetables can convey pathogenic bacteria that are transmittable through food [2]. Food-borne diseases are considered as the most important health problems in the world [4]. According to the Center for Disease Control and Prevention (CDC), about 41 million cases of disease and 3000 deaths occur a year due to food-borne illnesses in the United States [5]. According to the World Health Organization (WHO), food code commission, ready-to-use cooked foods, and all kinds of salads are classified in the high risk group of foods [6]. Vegetable salads are served raw, which makes them susceptible to microbial contamination during the preparation processes. Time and temperature, personal hygiene, and handling practices are among the most important parameters that affect this process. Hence, to prevent cross-contamination and transmission of food-borne pathogens, these parameters should be strictly considered [7].

In most studies, microbial contamination of foods has been investigated, while the possibility of microbial contamination transmission at other levels, such as contamination of food preparation equipment and consumption should also be investigated [5]. The catering businesses were responsible for more than 59% of the outbreaks caused by food-borne diseases in Europe from 1993 to 1998. Similarly, food-borne diseases were responsible for 55% of different outbreaks in Spain in 2004-2007, which is correlated to the food catering services[7]. In Iran, the prevalence of raw vegetable parasite contaminations was about 1.94% to 68.3% in different areas [8].

During the last decade, Adenosine Triphosphate (ATP)-bioluminescence has been increasingly applied for monitoring the surface cleanliness [9]. The ATP bioluminescence method is an acceptable method for evaluating microbial contamination in the food industry [10]. The ATP reacts with the luciferin-luciferase enzymatic complex and the produced light is expressed in Relative Light Units (RLU) [11, 12]. The ATP is a rapid, user-friendly method of quantifying the surface cleanliness within Hazard Analysis Critical Control Point (HACCP) systems [9, 13, 14]. The aim of this study was to investigate the measure of microbial contamination in salad-contact surfaces such as preparing tables and serving dishes by ATP bioluminescence method in restaurants of Mashhad city as the second metropolis of Iran.

Materials and Methods

This study was conducted at 39 restaurants in Mashhad city in Iran in 2017. The sample size of 78 was determined by observing the 95% confidence interval, 10% relative error, and 0.75 frequency ratio. Samples were taken from salad-preparing tables and salad-serving dishes before the lunch time. To this end, 13 porcelain dishes (33.3%), 10 steel dishes (25.6%), 3 polystyrene dishes (7.7%), 7 melamine dishes (17.9%), 6 polyethylene dishes (15.4%), 3 wood tables (7.7%), 3 steel tables (7.7%), and 33 polyethylene tables (84.6%) were investigated.

The target restaurants were covered by Health Center No. 5 of Mashhad. The restaurants' hygienic and sanitary conditions were checked by the person who took the samples. The geographical location of the study area is displayed in Figure 1.

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]

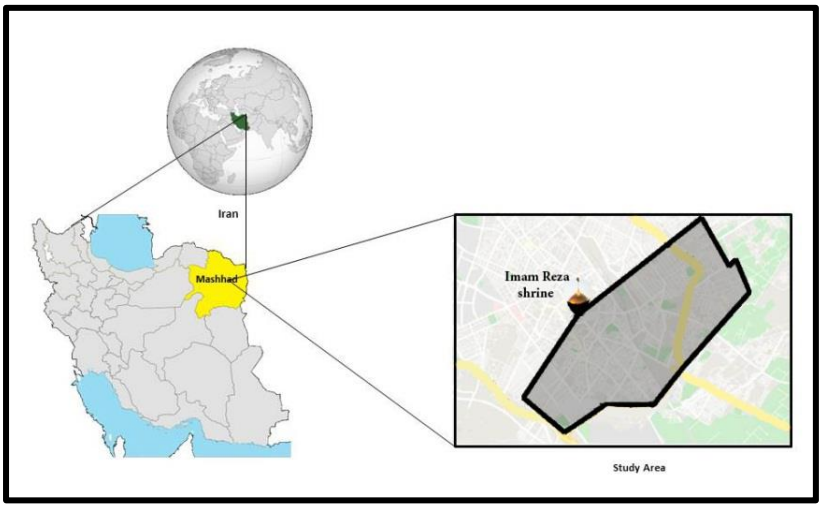

Figure 1: Geographical location of the study area

In each restaurant, two samples (one from salad table and one from salad dish) were collected by swabbing an area of 10 cm × 10 cm using the Ultra Snap Swabs (Hygiena International, Watford, UK) in a day. According to the manufacturer's instructions about using the ATP bioluminescence device, the swabs were placed in the swab tube. Then, the swab tube was broken at the snap valve by bending the bulb forward and backward using the thumb and forefinger. The bulb was squeezed twice and all liquid down the swab shaft was expelled. The swab bud was bathed in liquid by softly shaking for 5-10 seconds. The swab tube was inserted into a portable luminometer "System SURE II" (Hygiena International, Watford, UK) and after closing the lid, results were read. The default setting was employed in the Hygiena luminometer when enzymes in combination with ATP molecules emitted light to the sample. This was measured by the device and its value indicated the degree of contamination or the number of microbes present in the sample. Accordingly, scores less than 10 units showed that the surface was clean (pass), in 11-29 units warned that the surface was not adequately clean (caution), and scores greater than 30 units showed that the surface was dirty (fail).

Statistical analysis

To investigate normality of the data, we used the Kolmogorov–Smirnov test. Considering that the data were abnormal, we restrained the Kruskal-Wallis H test to compare different materials and ATP values. Mann- Whitney U test was also run for comparing ATP values and two groups of dishes and tables. It is worth noting that ATP values were qualitative.

Ethical issues

This study was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences No: IR.MUMS.REC.1396.241

Results

In this study, tables and dishes with different materials were studied. Figures 2 and 3 represent cleanliness status of the salad-serving dishes and salad-preparing tables made from different materials, respectively. According to the ATP bioluminescence device, 43.6% of the assessed dishes (17 samples) were clean, 38.5 % (15 samples) were dirty, 17.9 % (7 samples) were not adequately clean, 23.1 % of the tested tables passed the test (9 samples), 15.4 % were classified in the caution status (6 samples), and 61.5% failed the test (24 samples).

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]

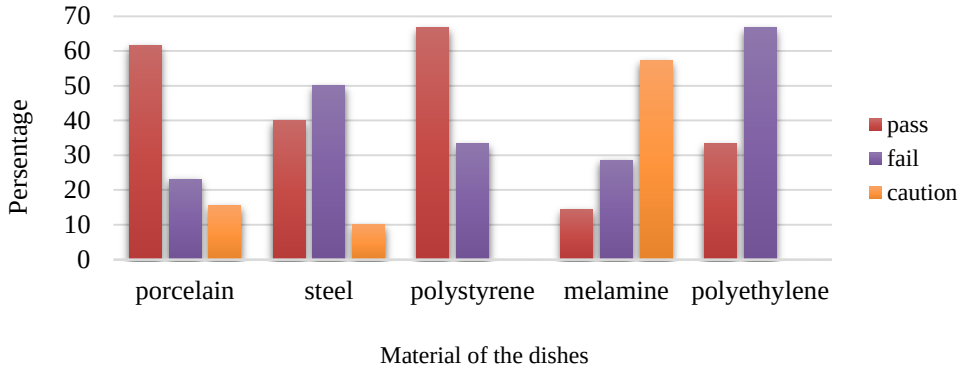

Figure 2: The cleanliness chart of salad-serving dishes of different materials

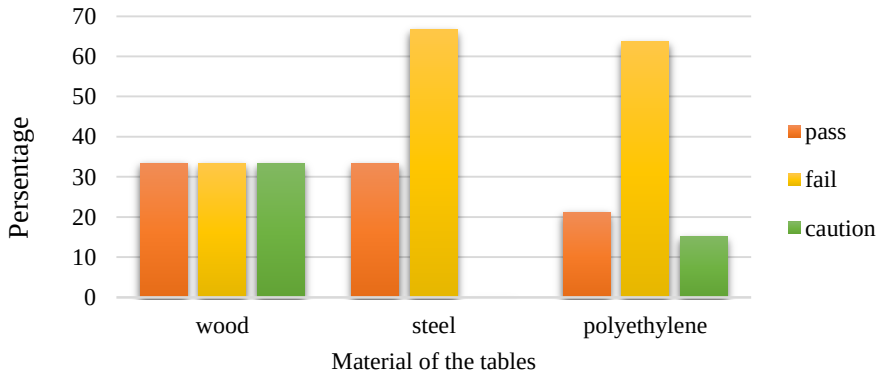

Figure 3: The cleanliness chart of salad-preparing tables of different materials

In this study, the highest median value of ATP was obtained for polyethylene dishes, while the lowest median value of ATP was detected in polystyrene dishes. The ATP bioluminescence results of different materials of dishes are summarized in Table 1.

Table 1: ATP bioluminescence results of dishes made with different materials

Material of Dishes	N	Median	IQR	Minimum (RLU)	Maximum (RLU)
Porcelain	13	6	22	0	1314
Steel	10	29.50	55	0	3069
Polystyrene	3	5	102	0	102
Melamine	7	13	83	1	497
Polyethylene	6	340.50	831	0	876

No significant difference (p = 0.63) was observed between ATP bioluminescence values and materials of salad-serving dishes.

Moreover, the highest and lowest median values of ATP was measured on the steel tables as well as the polyethylene and wood tables, respectively. The ATP bioluminescence results of different materials of tables are shown in Table 2.

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]

Table 2: The ATP bioluminescence results of tables made from different materials

Material of Tables	N	Median	IQR	Minimum (RLU)	Maximum (RLU)
Wood	3	20	151	2	153
Steel	3	128	493	6	499
Polyethylene	33	90	551.5	1	6773

No significant differences (p = 0.62) were seen between ATP bioluminescence values and materials of the salad-preparing tables.

According to the results of ATP, 53.8% of the hygienic restaurants successfully passed the cleaning test, 15.4% were specified at the caution status, and 30.8% were dirty and failed the test. However, 23.1% of the restaurants successfully passed the cleaning test, 17.3% were specified at the caution status, and 59.6% failed the test.

Discussion

The objective of this study was to confirm that application of the quantifiable methods was critical to demonstrate the quality cleanliness levels. The microbiological methods allow identifying the organism and its sensitively, but they take a long time, depend on the organism's viability and suitable transportation, and their costs are high. The microbiological method's limitation limits its daily application[15]. A real time assessment of the total surface cleanliness has been prepared in contrast to the ATP bioluminescence method, including the presence of organic debris and microbial contamination. The capability to provide results into minutes, as contrary to days for microbiological method, enables the ATP bioluminescence to be applied as a monitoring method within HACCP [9].

In this study, sampling the salad-preparing tables and the salad-serving dishes were done before the lunchtime. In other words, they were sampled from the dishes and tables after cleaning, but our results showed that only 43.6% of the dishes and 23.1% of the tables of different materials passed the test successfully. Zambrano et al. showed that 25.37% of the surfaces cleaned by internal personnel and 68.8% of the surfaces cleaned by external personnel were considered clean. These researchers recommended application of the educational and monitoring interventions to improve the quality of cleaning [15]. Lehto et al. studied the fresh-cut vegetable production plants. The ATP bioluminescence on the surface of chopping board, packing board, steel surfaces, and inner surface of plastic box were 1595, 1578, 578, and 793 RLU, respectively. The results of this study and the present study displayed that ATP bioluminescence method was appropriate to test the surface contamination after cleaning. In the case that the samples were taken before cleaning, microbes would have overgrown on the surfaces and the ATP bioluminescence values would also have overpassed the results scale [16]. Furthermore, according to Amodio et al., surfaces exceeding the percent of chosen cut-off limit indicated a significant change in pre-cleaning from 21.2% up to 93.1% and showed a huge rose from 5.3% to 96.5% in the post-cleaning stage [17].

According to our results, ATP bioluminescence detected that the lowest values of contamination were related to the polystyrene in dishes. Rigid PS and PS-related plastics used as food packaging materials have some of the physical characteristics of polystyrene, for instance, they have low impact strength and chemical resistance and are used to develop other food grade plastic [18]. Application of the packaging product is of great importance because they have been properly prepared, washed, and disinfected. Furthermore, the units which are carrying out the packaging process are required to comply with the approved health standards to reach the production permission; so, the contamination load on these products is low.

The results displayed that the tables made of wood and steel passed the test more successfully than the tables made of polyethylene. Stainless steel has often been selected as the most suitable surface material because it has weak adhesion

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]

characteristics and can be cleaned quickly. However, the study by Wilks et al. showed that *E.coli* O_{157} could stay alive for long term when dried onto the surface indicating that the contamination potential factor could occur if a surface was not adequately cleaned [19]. Ak et al. conducted that "wood generally yielded fewer bacteria than plastic after cleaning and the bacteria might be recovered from wood after 12 h at room temperature in high humidity, but numbers were reduced by at least 98% and often more than 99.9%" [20]. However, the other study by Gough et al. found that treatment boards with a proprietary disinfectant was significantly more effective on plastic compared to wooden boards [21]. To compare between 3 surfaces used in our study, the study of Wanyenya et al. was applied mentioning that *Campylobacter jejuni* cells were able to survive longer on wooden and plastic cutting boards than the metal surfaces. This study indicated that wooden and plastic boards could retain *Campylobacter spp.* for a relatively long time [22]. Therefore, the food handlers should be trained over time in this regard to obtain the required knowledge [7].

In the above-mentioned study, surfaces of a raw milk transportation tank, raw milk cooling storage tank, an equalization pasteurizing tank, a pasteurized milk storage tank, a pasteurized milk packaging tank, and a milk centrifuge were evaluated by ATP-bioluminescence method and hygiene monitoring procedures. The results showed that 100% of the studied surfaces were under inadequate hygiene conditions, while the plate count method detected only 50% of contamination based on the American Public Health Association's (APHA) recommendation. Therefore, The ATP bioluminescence method is not a direct replacement for microbiological method, but provides supplementary information and should be used as part of a confederate strategy [12].

Recommendation

As a result of this study, some practical recommendations can be made as following:

- Washing and cleaning practices should be more effective and efficient and suitable detergents should be used for different kinds of surfaces.
- Paying attention to the cleaning equipment in contact with salad-preparing surfaces and disinfecting them properly.
- Training the staffs is very important and attention to hygienic aspects must become a requirement. Hygienic condition, such as cleanness status of surfaces, must be confirmed before and after the working time.
- Staffs must be trained to use gloves during the working time and not leave the boxes and baskets on the tables.
- Surfaces should be monitored regularly and the obtained results should be recorded on a table, so that when the cleaning practices are not performed correctly the staffs would be fined for the wrongdoing.

Conclusion

The ATP bioluminescence method can be used in a few minutes to provide a real time estimate. In addition, this method can not only detect the microorganisms, but also the food residues, which is more beneficial. This is because residues of foods or organic matters indicate the effectiveness of cleaning and hygienic procedures. Furthermore, presence of microorganisms has potential impact on the public health. However, this method is not a reliable replacement for measurement of microbial contamination.

Acknowledgments

The authors would like to thank the Student Research Committee of Mashhad University of Medical Sciences and the Environmental Health Engineers of Mashhad Public Health Department No. 5 for their supports.

Conflict of Interest

The authors confirmed no conflicts of interest regarding the publication of this article.

Funding

This research was funded by the Student Research Committee (Grant: 960233) in Mashhad University of Medical Sciences, Iran.

References

1. Abougrain AK, Nahaisi MH, Madi NS, et al. Parasitological contamination in salad vegetables in Tripoli-Libya. Food control. 2010;21(5):760-2.
2. McMahon M, Wilson I. The occurrence of enteric pathogens and Aeromonas species in organic vegetables. Int J Food Microbiol. 2001; 70(1-2):155-62.
3. Doyle MP. Fruit and vegetable safety-microbiological considerations. HortScience. 1990;25(12):1478-82.
4. Raeisi A. Malaria Elimination in Iran, progress achievements and Challenges. Proceedings of the 6th Metting of National Malaria Programme Managers. 2006.
5. Patel D, Stansell J, Jaimes M, et al. A survey of microbial contamination on restaurant nonfood-contact surfaces. Journal of Food Safety. 2017;37(1):e12287.
6. Basseri H, Moosakazemi S, Yosafi S, et al. Anthropophily of malaria vectors in Kahnouj District, south of Kerman, Iran. Iran J Public Health. 2005;34(2):27-35.
7. Rodríguez-Caturla MY, Valero A, Carrasco E, et al. Evaluation of hygiene practices and microbiological status of ready-to-eat vegetable salads in Spanish school canteens. J Sci Food Agric. 2012;92(11):2332-40.
8. Ezatpour B, Chegeni AS, Abdollahpour F, et al. Prevalence of parasitic contamination of raw vegetables in Khorramabad, Iran. Food control. 2013;34(1):92-5.
9. Davidson C, Griffith C, Peters A, et al. Evaluation of two methods for monitoring surface cleanliness-ATP bioluminescence and traditional hygiene swabbing. Luminescence. 1999;14(1):33-8.
10. Koo O-K, Martin EM, Story R, et al. Comparison of cleaning fabrics for bacterial removal from food-contact surfaces. Food control. 2013;30(1):292-7.
11. Larson EL, Aiello AE, Gomez-Duarte C, et al. Bioluminescence ATP monitoring as a surrogate marker for microbial load on hands and surfaces in the home. Food microbiology. 2003;20(6): 735-9.
12. Costa PD, Andrade NJ, Brandão SCC, et al. ATP-bioluminescence assay as an alternative for hygiene-monitoring procedures of stainless steel milk contact surfaces. Braz J Microbiol. 2006;37(3):345-9.
13. Vilar M, Rodríguez-Otero J, Diéguez F, et al. Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment. Int J Food Microbiol. 2008; 125(3):357-61.
14. Carrascosa C, Saavedra P, Millán R, et al. Monitoring of cleanliness and disinfection in dairies: Comparison of traditional microbiological and ATP bioluminescence methods. Food Control. 2012;28(2):368-73.
15. Zambrano AA, Jones A, Otero P, et al. Assessment of hospital daily cleaning practices using ATP bioluminescence in a developing country. Braz J Infect Dis. 2014;18(6):675-7.
16. Lehto M, Kuisma R, Määttä J, et al. Hygienic level and surface contamination in fresh-cut vegetable production plants. Food control. 2011;22(3-4):469-75.
17. Amodio E, Dino C. Use of ATP bioluminescence for assessing the cleanliness of hospital surfaces: A review of the published literature (1990–2012). Journal of infection and public health. 2014;7(2):92-8.
18. Ahmad M, Bajahlan AS. Leaching of styrene and other aromatic compounds in drinking water from PS bottles. J Environ Sci. 2007;19(4):421-6.
19. Wilks S, Michels H, Keevil C. The survival of Escherichia coli O_{157} on a range of metal surfaces. Int J Food Microbiol. 2005;105(3):445-54.
20. Ak NO, Cliver DO, Kaspar CW. Cutting boards of plastic and wood contaminated experimentally with bacteria. J Food Prot. 1994;57(1):16-22.
21. Gough N, Dodd C. The survival and disinfection of Salmonella Typhimurium on chopping board surfaces of wood and plastic. Food Control. 1998;9(6):363-8.
22. Wanyenya I, Muyanja C, Nasinyama GW. Kitchen practices used in handling broiler chickens and survival of *Campylobacter spp*. on cutting surfaces in Kampala, Uganda. J Food Prot. 2004;67(9):1957-60.

[DOR: 20.1001.1.24766267.2020.5.1.5.0]
[DOI: 10.18502/jehsd.v5i1.2476]