



Using the Bioluminescent Method in the Food Industry

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Abstract: Foodborne illnesses affect millions of people worldwide each year, resulting from contamination by harmful microorganisms. Preventing these illnesses requires a systematic approach that addresses hazards at every stage of the food supply chain. Understanding how to control microbiological hazards is essential for ensuring food safety and protecting public health. Ensuring hygiene and safe food is the main task of the food industry. The confectionery industry is concerned with the processing of food commodities and the subsequent production of confectionery products. To achieve high-quality and safe confectionery products, a high level of hygiene and sanitation, as well as hygiene control of work surfaces, is essential. Rapid hygiene monitoring tests based on the presence of adenosine triphosphate have been widely used in the food industry to ensure that adequate cleanliness and work surface hygiene is maintained. The use of the bioluminescence method is justified in terms of preventing the occurrence of foodborne diseases. In our study, we evaluated the practical use of the bioluminescence method in evaluated food processing plant, where bioluminescence method is used as a technological advance to facilitate more accurate control of surface hygiene. Based on the results obtained in our study, we can conclude that surfaces hygienic level of evaluated technologies were at an adequate level, except for the package machine surface, where we detected 155 RLU (Relative Light Unit) before production and 301 RLU during production. The hygienic condition of the package machine surface after disinfection improved and ATP counts decreased to zero.

Keywords: Hygiene, ATP, Bioluminescence method, Safety production, Food-borne diseases.

INTRODUCTION

Food contamination leading to foodborne diseases can be caused by air, water, soil, ingredients used in food preparation, equipment, waste, food contact surfaces, and food workers [1]. Food contact surfaces may pose a potential health hazard if they are not properly cleaned and sanitized [2]. Time and product processing temperature, personal hygiene, and handling practices are among the most important parameters that affect food production process. Hence, to prevent cross-contamination and transmission of food-borne pathogens, these parameters should be strictly considered [3]. 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 [4].

The development of new products and food processing technologies, commercial expansion, food safety concerns, and the increase in foodborne illnesses have revealed that the implementation of effective and rigorous cleanliness and disinfection procedures is essential to guarantee quality control. Therefore, the use of fast and sensitive techniques

in food industries for the evaluation of hygiene conditions on living and inert food contact surfaces is necessary [5].

It has been reported that lack of hygiene of food handlers, inadequate hand washing (living surfaces) and disinfection of inert surfaces (countertops and utensils) are some of the factors that can contribute to foodborne disease transmission [6].

During the last decade, Adenosine Triphosphate (ATP)-bioluminescence has been increasingly applied for monitoring the surface cleanliness [7]. The ATP bioluminescence method is an acceptable method for evaluating microbial contamination in the food industry [8]. The ATP reacts with the luciferin-luciferase enzymatic complex, and the produced light is expressed in Relative Light Units (RLU) [9,10]. The ATP is a rapid, user-friendly method of quantifying the surface cleanliness within Hazard Analysis Critical Control Point (HACCP) [11,12].

Traditional methods, such as the swab technique have been the golden standard since the 1900s for the determination of the microbiological quality of surfaces; however, this method requires several days to obtain results, and it is costly. As a result, fast methods based on bioluminescence such as measuring the amount of ATP (adenosine triphosphate) to assess the degree of hygiene, have been implemented. Contaminated equipment and surfaces are among the top risk factors for foodborne outbreaks, emphasizing the importance of proper sanitation protocols. Therefore, it is imperative to establish food safety quality management systems and comply with rigorous hygiene standards in the working environment (surfaces, equipment, and utensils) [13].

The aim of this study was to investigate the level of microbial contamination of surfaces that meet raw materials using the ATP bioluminescence method in a food processing plant.

THE EXISTING RANKING METHODS

In our work, we focused on assessing the hygienic level of technological equipment surfaces (figure 1, 2) in a confectionery operation by using bioluminescence method in three different intervals, before starting the production of puff pastry, during production, after disinfection of technology surfaces.



Figure 1: Conveyor belt.



Figure 2: Laminator.

The monitored processing plant is in central Slovakia, is a large operation, the resulting product is frozen puff pastry and cakes. The evaluated food processing plant produces puff pastry as well as cakes, therefore the sanitation plan must be strictly adhered to and effective. Puff pastry production rotates with cake production at weekly intervals. The disinfection and sanitation program is an integral part of good manufacturing practice. A high level of sanitation helps to maintain high microbiological purity and quality of produced products. The effect of cleaning and decontamination depends on the design of technological equipment. Continuous cleaning is carried out within activity of operation and after completion to such an extent that operational cleanliness is ensured in the production areas. Major disinfection of the entire operation is carried out once a week. As part of the monthly cleaning, a comprehensive inspection of technological equipment is carried out, maintenance, possible repair, and washing of protective flour bags. The disinfectant Aspiral Persteril 15 is used to disinfect surfaces in a 0.4% concentration with a time exposure of 30 min. Aspiral Persteril 15 is a liquid, acidic, non-foaming (without defoamer) disinfectant based on peroxyacetic acid, for cold use. The product is intended for disinfection of tanks, pipes, machines, devices, and equipment. It effectively destroys bacteria, yeasts, molds, viruses and spores. It is effective against listeria. The disinfectant is manufactured by Ronchem, Slovakia. A fast and simple ATP method was chosen to control the hygiene level of the technologies used for processing puff pastry. The HY-LITE NG monitoring system was used. This system consists of a luminometer (figure 3), which evaluates the amount of emitted light generated in the reagent chamber, and a sampling pen.



Figure 3: Luminometer.

After reacting the ATP sample with the luciferase/luciferin reagent in a pen chamber and subsequently measuring it in a luminometer, the light intensity emitted from the sample was measured. The value is determined in relative luminescence units (RLU). The RLU value is directly proportional to the amount of ATP in the test sample. Reference values for the food industry are expressed in RLU. Acceptable values are < 150, limit values (borderline) are 151-299 and unacceptable values are above 300. The ATP value results recorded in Tables 1, 2 are the average values of 5 ATP swabs taken.

EXPERIMENTS AND ANALYSIS

Table 1 shows the results of swabs taken using the ATP method. The highest amount of ATP values was found on the evaluated floors and walls during production. After disinfection, we observed a decrease in the number of ATP values. The hygienic condition of the evaluated technologies surfaces was at a good level, except for the package machine surface, where we detected an increased amount of ATP, specifically 155 RLU before production and 301 RLU during production.

Table 1: Average ATP values of swabs taken from individual surfaces and production technology in the puff pastry production section.

Sampling	Before production Ø (RLU)	During production Ø (RLU)	After disinfection Ø (RLU)
Individual surfaces			
Floor (part of production technologies)	150	300	31
Wall (part of production technologies)	80	151	13
Floor (packing room)	19	160	9
Wall (packing room)	35	157	0
Production technologies			
Sampling	Before production Ø (RLU)	During production Ø (RLU)	After disinfection Ø (RLU)
Worktable	20	40	5
Slicing knife	10	15	0
Retractable conveyor	55	110	10
Package machine	155	301	0
Blending machine	27	47	0
Conveyor belt	15	25	0

The hygienic condition of the package machine surface after disinfection improved so much that the ATP counts decreased to zero. Based on the results obtained, we can conclude that the hygiene level of all monitored surfaces was very good, except for the evaluated package machine surface during production, and 0.4 % Aspiral Persteril 15 with a time exposure of 30 minutes, was sufficiently effective. Five risk factors contribute to

foodborne diseases, namely poor personal hygiene, improper handling, contaminated equipment and cross-contamination, inadequate cooking, and food from unsafe sources. Of these, lack of hygienic measures and cross-contamination (i.e., transfer of harmful microorganisms between food and food contact surfaces) are leading causes of foodborne diseases linked to food establishments [14]. The importance of maintaining and controlling hygiene in the food industry is therefore unquestionable [15]. To ensure surfaces are properly cleaned, cleaning practices should be routinely monitored [16]. With the growing importance of assessing the effectiveness of on-site cleaning, ATP hygiene monitoring tests are being widely used because of their speed, ease of use, quantitative results, and cost-effectiveness [17]. When surface-bound ATP is reduced below a defined value, the surface can be considered clean. The application of the ATP method as a rapid detection method for assessing surface hygiene in the food industry is justified mainly from the perspective of immediate correction in the event of a deficiency during the production process.

CONCLUSION

The globalization of food production, processing and distribution places increasing demands on achieving food safety and health in all parts of the world. New faster and more sensitive detection methods are required for food microbiological contamination quality control throughout the entire handling process. The method of bioluminescence detection of adenosine triphosphate based on the luciferase system is widely used in the food industry due to its practicality, efficiency and speed.

Based on the results obtained, we can conclude that surfaces hygienic level of various evaluated technologies were at a good level, except for the package machine surface, where we detected an increased amount of ATP before production and during production. Based on these findings, corrective measures were taken at the time of production and the condition after disinfection changed to such an extent that we did not record any amounts of ATP on the evaluated surface.

REFERENCES

- [1]. Marriott, N.G., Schilling, M.W. and Gravani R.B., Food Contamination Sources. Principles of Food Sanitation. Springer International Publishing, 2018. p. 83-91.
- [2]. Baghapour, M.A., Mazloomi, S.M., Azizi, K., Microbiological Quality of Food Contact Surfaces in a hospital kitchen in Shiraz. J Health Sci Surveillance Sys., 2015. (4): p.128-132.
- [3]. Rodríguez-Caturla, M.Y. and Valero, A., Evaluation of hygiene practices and microbiological status of ready-to-eat vegetable salads in Spanish school canteens. J Sci Food Agric., 2012. 92(11): p. 2332-40.
- [4]. Patel, D., Stansell, J. and Jaimes M. et al., A survey of microbial contamination on restaurant nonfood-contact surfaces. Journal of Food Safety, 2017. 37(1): p.12287.
- [5]. Lorenzo, F., Sanz-Puig, M., Berto, R. and Orihuel, E., Assessment of performance of two rapid methods for on-site control of microbial and biofilm contamination. Applied Sciences, 2020. 10(3): p. 744.

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- [6]. Suescún-Carrero, S., Avila-Panche, S., Microbiological evaluation in school feeding programs in educational institutions in the Department of Boyacá - Colombia. *Nova*, 2017. 15(28): p. 93-98.
- [7]. Davidson, C., Griffith, C., Peters and A. et al., Evaluation of two methods for monitoring surface cleanliness-ATP bioluminescence and traditional hygiene swabbing. *Luminescence*, 1999. 14(1): p. 33-8.
- [8]. Koo, O.K., Martin E.M. and Story R, et al., Comparison of cleaning fabrics for bacterial removal from food-contact surfaces. *Food control*, 2013. 30(1): p. 292-7.
- [9]. Larson, E.L., Aiello, A.E. and 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): p. 735-9.
- [10]. Costa, P.D., Andrade, N.J. and Brandão S.C.C. et al., ATP-bioluminescence assay as an alternative for hygiene-monitoring procedures of stainless-steel milk contact surfaces. *Braz J Microbiol.*, 2006. 37(3): p. 345-9.
- [11]. Vilar, M., Rodríguez-Otero, J. and Diéguez F. et al., Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment, *Int J Food Microbiol.*, 2008. 125(3): p. 357-61.
- [12]. Carrascosa, C., Saavedra, P. and Millán R. et al., Monitoring of cleanliness and disinfection in dairies: Comparison of traditional microbiological and ATP bioluminescence methods, *Food Control.*, 2012. 28(2): p. 368-73.
- [13]. Amaich, R., E. Ouali Lalami, A., Fadil, M., Bouslamti, R. and Lairini S., Food safety knowledge, attitudes, and practices among food handlers in collective catering in central Morocco. *Heliyon*, 2024. 10(23): p. 40739.
- [14]. Kirchner, R.M., Goulter, B.J., Chapman, J. Clayton, L.-A. and Jaykus, L.A., Cross contamination on atypical surfaces and venues in food service environments. *J. Food Prot*, 2021. 84: p. 1239-1251.
- [15]. Jay, M. J., Loessner, M. J. and Golden, D. A., *Modern Food Microbiology*. Seventh Edition 2005, New York: Springer Science and Business Media. 789.
- [16]. Jones, S.C., Ricke, D.K., Roper, K.E. and Gibson, S.L., Swabbing the surface: critical factors in environmental monitoring and a path towards standardization and improvement. *Crit. Rev. Food Sci. Nutr*, 2020; 60: p. 225-243.
- [17]. Mildenhall, S.A., Rankin, K.B, Implications of adenylate metabolism in hygiene assessment. *J. Food Prot*, 2020. 83: p. 1619-1631.