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Food safety: Cleanliness and disinfection of food contact surfaces in gastronomy laboratories at a university in Colombia

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Abstract

The implementation of effective cleaning and disinfection procedures is crucial to ensure quality control and food safety in food services. This study aimed to evaluate the hygienic conditions in the gastronomy laboratories of a university in Colombia. The study conducted ATP bioluminescence detection and microbiological analysis on various surfaces and food handler's hands. The results indicated the presence of aerobic mesophilic bacteria and total coliforms on the surfaces, but no *Escherichia coli, Staphylococcus aureus*, *Listeria monocytogenes,* or *Salmonella* spp. The cutting-board had the highest microbial counts, while the countertop and serving dish had the lowest. Total coliform counts exceeded the acceptable limits on most surfaces. Correlations between ATP levels and microbial counts were not significant. ATP measurements indicated organic contamination, but not necessarily high microbial loads. The findings emphasize the importance of proper cleaning, disinfection protocols, and personal hygiene practices to prevent cross-contamination and ensure food safety.

Keywords: Microbial contamination; Food handler; Hygiene; Cross-contamination; Swabbing; Bioluminescence.

Highlights

- Monitoring cleanliness and disinfection was assessed by culture methods and ATP detection
- The cutting board was the surface with the highest level of microorganisms
- Food contact surfaces were not contaminated but contained a high load of organic matter
- No significant positive correlation between culture methods and ATP detection was found

1 Introduction

The development of new products and food processing technologies, the commercial expansion, the concern about food safety, and the growth of foodborne diseases, have revealed that the implementation of

effective and rigorous procedures of cleanness and disinfection are essential to guarantee quality control in food services. 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 (Lorenzo et al., 2020). 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 (Suescún-Carrero & Avila-Panche, 2017). 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 (Altemimi et al., 2022). This method allows the detection of ATP from microbial contamination as well as from organic contamination like skin flakes, bodily fluids, and food scraps (van Arkel et al., 2021).

Gastronomy laboratories, which resemble industrial kitchens in design and operational models, play a crucial role in providing food services to students, teachers, and administrative staff. These laboratories should prioritize cleaning and disinfection procedures to ensure adherence to good manufacturing practices (Food and Agriculture Organization, 2011; Colombia, 2013), thereby following established and approved protocols. Inadequate procedures pose a high risk of cross-contamination, not only reducing the shelf life of finished products but also increasing the likelihood of foodborne diseases caused by pathogenic microorganisms such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp., which can survive in the presence of food residues remaining on food processing or handling equipment after use (Lim et al., 2019) by forming biofilms (Ripolles-Avila et al., 2019). Cross-contamination between food and contact surfaces is a major contributing factor to outbreaks, leading to both illness and food spoilage according to the Centers for Disease Control and Prevention (İpek & Demirel Zorba, 2018; Possas & Pérez-Rodríguez, 2023). Therefore, stringent measures must be taken to control cleanliness and disinfection in gastronomy laboratories to prevent this problem from worsening over time (Sharma et al., 2022). The verification of surface cleaning and disinfection processes in gastronomy laboratories is crucial for controlling the risk of cross-contamination and implementing measures to ensure the safety of food for consumers. Additionally, it aims to educate food handlers about the importance of cleaning and disinfection processes and the implementation of microbiological controls that comply with regulatory requirements for establishments involved in food production and sale. This study aimed to evaluate the hygienic conditions and the presence of foodborne pathogenic microorganisms, on food contact surfaces in the gastronomy laboratories of a university in Colombia.

2 Material and methods

2.1 Study location

The analysis was performed in the gastronomy laboratories of a university in Medellín, Colombia. Sampling was performed on inert food contact surfaces including countertops, blenders, cutting boards, and serving dishes, as well as the operator's hands (living surface), after the preparation of food and their cleansing, according to the recommendation of the technical director of the laboratories, which indicated that the selected surfaces had a high risk of contamination by food residues and microorganisms, or were exposed to the environment. These surfaces have also been selected by other authors who reported them as critical surfaces for the risk of food diseases (Ríos-Castillo et al., 2021). The sampling was performed from October to November of 2021 and March to May of 2022, periods in which the laboratories are used by students and teachers. The cleansing protocol included initial cleaning using a neutral detergent (Berhlan) and a second step consisting of disinfection using sodium hypochlorite (195 ppm) for 5 minutes followed by rinsing with water. The food handler's hands were washed with neutral hand soap (Berhlan).

2.2 Sampling methods

Surface hygiene was assessed using two methods: bioluminescence detection of ATP and microbiological analysis. 32 samples were made for each type of surface and food handler, each one was collected in triplicate, according to the procedures established by NTC 5230 2017 (ICONTEC, 2017).

2.3 Bioluminescence detection of ATP

Hygiena EnSURE Luminometer ATP was used for the bioluminescent detection of ATP. The method measures light to quantify the amount of biological energy in a sample, specifically ATP. This molecule can be converted to light by a chemical reaction with luciferin and the enzyme luciferase. The amount of light emitted in the reaction is directly proportional to the amount of ATP in the sample, and the amount of ATP, is related to the number of ATP containing cells in the sample (AIDIAN, 2022). Samples were collected with Ultrasnap surface swabs (Hygiena®), according to the manufacturer's protocol by swabbing 100 cm^2 of each surface. The swab stick was mixed in a container to activate the enzyme present on the top. The swab was put in the EnSURE Luminometer equipment (Hygiena®) and the measurement was carried out within 15 seconds. The result of the ATP-bioluminescence assay was expressed in relative light units (RLU/100 cm^2). According to the manufacturer of <10 RLU/100 cm^2 the surface could be considered clean, ≥10 to ≤29 RLU/100 cm^2, indicated that the surface was not adequately clean, and >30 RLU/100 cm^2 the surface required cleaning (Whitehead et al., 2019).

2.4 Microbiological analysis

Microbiological samples were taken with a moistened swab that was constantly rotated while contacting 100 cm^2 of each inert surface. For the food handler's hands the swabbing included the palm, the back of the hand, the interdigital spaces, and nails. Each swab was stored in sample tubes with 9 mL of 0.1% w/v peptone water (Microkit) and was transported at 4 ± 2 °C to the Laboratorio de Control Calidad (LACMA), where they were processed in the next 24 hours (h) after the sampling. According to NTC 5230 (ICONTEC, 2017), serial dilutions up to 1×10^{-3} were prepared for the count of indicator Aerobic mesophilic microorganisms according to ISO 4833-2:2013 (International Organization for Standardization, 2013) on Plate Count Agar (PCA) (Merck®). Total coliform and *E. coli* were performed according to (Noma Tecnica Colombiana, 2007) on Chromocult® agar (Merck®). For *S. aureus,* Baird Parker agar (Merck®) was used as indicated in ISO 6888-1:2021 (International Organization for Standardization, 2021). For detection of *L. monocytogenes* Palcam agar (Oxoid®) and *Listeria* spp. agar according to OTTAVIANI and AGOSTI (Merck®) were used according to ISO 11290-1 (International Organization for Standardization, 2017b). *Salmonella* sp. was detected according to ISO 6579-1 (International Organization for Standardization, 2017a) on *Salmonella-Shigella* agar (Merck®) and Xylose Lysine Deoxycholate agar (Merck®). After incubation plates with typical colonies for each pathogenic microorganism evaluated, according to the technical regulation, the results were confirmed by the automated identification method VITEK®2 (BioMérieux). Results were expressed as Colony Forming Units/cm^2 (CFU/cm^2).

2.5 Statistical analysis

Data analyzes were carried out using IBM SPSS program version 29.0.0 with a significance level of 0.05. Shapiro-Wilk's test was used to test the normality of data distribution and Levene's test was used to test homoscedasticity. Differences between the surfaces were compared using Kruskal Wallis nonparametric test where $p < 0.05$ was considered significant. The correlation between RLU values and CFU was calculated using the Spearman's rank correlation coefficient.

3 Results and discussion

In this study, a microbiological analysis was performed on some food contact surfaces in the gastronomy laboratories of a university in Colombia. Aerobic mesophilic bacteria and total coliform counts on inert surfaces are presented in Figures 1 and Table 1, respectively. On none of the surfaces and food handler's hands, the presence of *E. coli*, *S. aureus*, *L. monocytogenes,* and *Salmonella* spp. were detected. These bacteria are indicators of a dirty environment, unhygienic production procedures, and poor water quality (Wiatrowski et al., 2023). Work areas, cutting boards, sinks, and kitchen faucets are recognized as important surfaces in food service facilities that might result in cross-contamination of food, especially if these surfaces are contaminated by mesophilic aerobic bacteria (Rodríguez et al., 2011). Aerobic mesophilic bacteria count ranged from 0 to 21.8 CFU/cm^2 on all the inert surfaces. The highest counts were presented by the cutting board and blender, without statistically significant differences between them ($p > 0.05$), while the countertop and serving dish had the lowest counts ($p < 0.05$) among all the inert surfaces. For food handler's hands the aerobic mesophilic count was 6.98 CFU/cm^2 (Table 2). Aerobic mesophilic counts of food processing environments are used to estimate the hygiene of the entire food production process (Touimi et al., 2019). Colombian regulation for the microbiological sampling of surfaces and operator's hands classifies the efficacy of a cleaning and disinfection procedure according to the aerobic mesophilic counts, in which the areas are clean (2-10 CFU/cm^2), acceptable (11-100 CFU/cm^2), dirty (> 100 CFU/cm^2) and out of control (101 -1000 CFU/cm^2) (ICONTEC, 2017). The countertop and the serving dish presented less than 10 CFU/cm^2, being classified as clean, while the cutting board and the blender were acceptable. Food handler's hands were classified as clean, as they presented aerobic count lower than 10 CFU/hand. Additionally, Legnani et al. (2004) determined that food contact surfaces are considered satisfactory hygienically when aerobic mesophilic counts are lower than 50 CFU/cm^2. For the surfaces evaluated in this study, the mesophilic counts were lower than this reference value, indicating that the cleansing procedure used was satisfactory and the surfaces were uncontaminated. Similar results were obtained by Oliveira et al. (2014), who evaluated the hygienic condition of food contact surfaces including mixers, cutting boards, dishes, and countertops, and found aerobic mesophilic counts similar to this study and they were lower than the reference value. Similarly, Janjić et al. (2015) found mesophilic counts higher than 10 CFU/cm^2 on working surfaces for food preparation surfaces, wooden and plastic cutting boards. The results obtained in this work are lower than those reported in other studies that evaluated the hygienic conditions on some food contact surfaces including utensils in kitchens (Al-Aejroosh et al., 2021; Touimi et al., 2019).

Table 1. Total coliform counts on surfaces of the gastronomy laboratories and percentage of unsatisfactory surfaces (n = 32).

Surfaces	CFU/cm^2	Unsatisfactory surfaces
Cutting board	0 - 401.6	16 (50%)
Blender	0 – 263.3	10 (31.25%)
Countertop	0 – 7.7	5 (15.6%)
Serving dish	0 – 1.0	3 (9.37%)

Table 2. Microbial counts and ATP bioluminescence results for food handler's hands.

Aerobic mesophilic counts (CFU/hands)	Total coliform counts (CFU/ hands)	ATP bioluminescence (RLU/ hands)
0 – 15633	0 – 600	38 - 4587

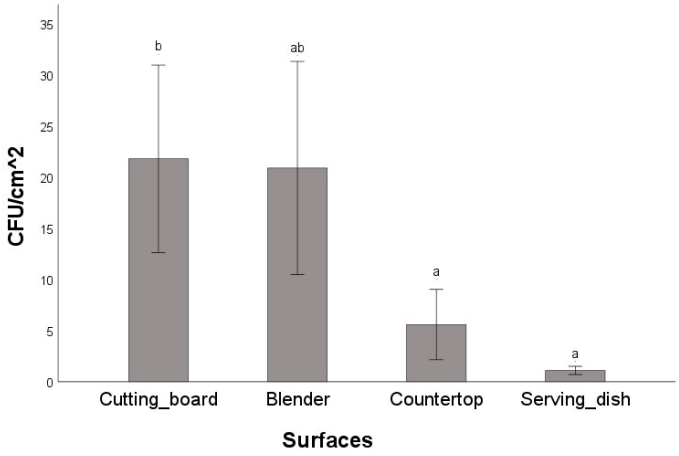

Figure 1. Aerobic mesophilic bacteria count on surfaces of the gastronomy laboratories.

Total coliform counts ranged from 0 to 401.6 CFU/cm^2 within all the inert surfaces; the cutting board and blender showed the highest counts of total coliforms, while the countertop and serving dish presented the lowest count ($p < 0.05$). Because the sampling was conducted immediately after cleansing and disinfection protocol and in accordance with Resolution 2674 of 2013 (Good Manufacturing Practices) (Colombia, 2013) that governs in Colombia, food handler's hands are considered sanitized when they do not show total coliforms, and furthermore, *E. coli* should not be detected on any of the surfaces. The total coliform counts on some food handlers' hands (9.37%) and inert surfaces were unsatisfactory based on this microbiology criteria (Table 1 and 2), so their personal hygiene was not adequate and could contribute to increase the risk of transmission of foodborne diseases. Contradictory results were found in the study conducted by Bumyut et al. (2022), who investigated the food safety conditions at food service premises and the hand hygiene of food handlers in Thailand, finding satisfactory results in food handler´s hands after handwashing. It is of great importance train all employees in the use of effective hand washing procedures, and that the safety of the food chain supply can easily be broken proper enforcement of these procedures (Lambrechts et al., 2014).

Most of the inert surfaces presented coliform counts that exceeded the limit established by this regulation being classified as unsatisfactory. These results may be due to flaws in the cleaning protocol including the use of inadequate concentration, contact time, mechanic action, and temperature. Additionally, other disinfectants could be more efficient, with lower toxicity, fast acting, and not be adversely affected by organic load, for the food industry in the removal of possible pathogenic microorganisms and improve hygienic conditions (Kim et al., 2023). The total coliform counts found in this study are lower than those found in the study of Tenna et al. (2023), who evaluated the microbiological quality of food contact surfaces (utensils) from hotels and restaurants, and their counts ranged from 4.85 to 5.93 Log CFU/100 cm^2. Total coliforms are considered indicators of failures in cleansing and disinfection procedures, which can represent cross contamination and biofilm formation on the surfaces. *E. coli* is considered a contamination indicator submitting evidence for the food to be contaminated with fecal residues and likely to retain pathogenic organisms. Mesophilic bacteria are commonly used for food and raw materials rather than surfaces, as they are considered indicators of temperature fluctuations, humidity, and water content, while total coliforms are directly associated with the efficacy of a cleansing protocol.

Several reports have found that the main sources of cross contamination during food processing come from the surfaces, utensils, and employees (Mohammad & Al-Taee, 2018) and they can harbor a high microbial load if they are not properly cleaned or are excessively used (Trindade et al., 2014). Based on the microbial counts, the cutting board was the surface with the highest level of microorganisms, which could be due to the fact that this surface is made from polyethylene and can present pores or cuts from usage. This surface could be a reservoir for microorganisms that are not destroyed instantly after cleansing and could

become a potential source of cross contamination where microorganisms can be further transferred to food products or other food contact surfaces (Carrasco et al., 2012). Cutting boards should be replaced periodically due to inevitable surface wear or, as soon as they become too worn or develop hard-to-clean grooves. Additionally, color cutting boards should be used to separate different types of foods, such as dairy and bread (white), raw red meat (red), vegetables and fruits (green), poultry (yellow), raw seafood (blue), and cooked meat (brown), helping to reduce the risk of cross contamination (National Environmental Agency, 2016). Microbiological cleanliness for cutting boards depended on the length of time the boards had been in use; only new boards had high cleanliness levels (Wiatrowski et al., 2023). Most often food contact materials found in food preparation settings are stainless steel, plastics, and ceramics (Djekic et al., 2016), while rougher, more hydrophobic, and crusted surface materials have a favoring effect on the attachment of bacteria and the biofilm formation (Lee et al., 2022). In this study, the countertop was made of stainless steel, a material that has high durability and resistance to corrosion, allowing its easy cleaning, the serving dish was made of ceramic and the blender was made from plastic. In a study made by Sahai et al. (2015) who assessed the microbiological quality of utensils after a complete cycle in a dishwasher, finding that when the mean bacterial colony count by material type was compared for individual swabs, it was observed that plastic items had significantly higher counts than metal and ceramic items, same behavior found in this study.

The detection of microorganisms by traditional methods on food contact surfaces and equipment is not the most appropriate option in this case, as it does not provide an immediate evaluation of the hygiene state of the surfaces. Ready to use methods that detect microbial or organic loads are an alternative to rapidly assessing the hygienic status of food contact surfaces. The indirect method of hygiene evaluation using the measurement of ATP levels is one of the fastest measurement methods compared to culture methods, with results obtained in just a few seconds (Wiatrowski et al., 2023). To correlate the results from the culture methods, ATP measurement was carried out. The ATP bioluminescence investigation revealed the presence of ATP on all the surfaces in all the areas (Table 3). The median RLU/100cm^2 value for the total ATP measurements was 1345.89, with a range from 4.67 up to 7954.33. There were no statistically significant differences ($p > 0.05$) among the countertop, blender, and serving dish. This method detected that all the surfaces were unsatisfactory in terms of cleanliness; nevertheless, the bioluminescence assay can be highly variable in determining bacteria present on surfaces due to the presence of organic material that cannot be distinguished from the different types of microorganisms. Values obtained in this study are similar to those reported by Wiatrowski et al. (2023), on five different surfaces on a mobile gastronomy truck (shelf of a refrigerator, cutting board, small utensil such as a knife, serving surface, and worktop surface). Additionally, the amount of ATP that a cell contains depends on its size and vital state so this can underestimate the number of cells viable but not cultivable (Ríos-Castillo et al., 2021). When comparing this result with the microbial counts it could be demonstrated that the food contact surfaces were not contaminated but contained organic matter. These results indicated that ATP measurement cannot be used to quantify the microbial populations due to the presence of non-microbial ATP in the surface, but it is an indicative of cleanliness in food industries (Carrascosa et al., 2012).

Table 3. ATP bioluminescence measurements on the surfaces of the gastronomy laboratories.

Surface	RLU/100 cm^2	
	Range	Mean value
Cutting board	24 – 4552.33	621.72 ± 109.91 [b]
Blender	11 – 7954.33	1922.96 ± 339.93 [a]
Countertop	135.77 – 7145.33	1924.57 ± 340.22 [a]
Serving dish	4.67 – 4328.67	914.32 ± 161.63 [a]

Results were expressed as mean value ± standard error. Means in the same column without a common letter differ significantly ($p < 0.05$).

Spearman's correlation between the measures of ATP (RLU/cm^2) and Aerobic mesophilic counts (CFU/cm^2) showed a weak correlation between both variables (r = 0.044; p = 0.578). Similarly, there was a weak correlation between ATP (RLU/cm^2) and Total Coliform counts (CFU/cm^2) (r = -0.053; p = 0.512) (Figure 2). An explanation could be that ATP measures other biological materials (food scraps, skin flakes, etc.), so the amount of bacterial/fungal contamination could be low while biological contamination (organic materials) is higher (Oliveira et al., 2014). It was not possible to assume that a low CFU would correlate with decreased ATP. Furlan et al. (2019) evaluated the correlation between microbiological culture and ATP bioluminescence assay on five surfaces in a Brazilian clinic and found that only two of them presented a significant correlation: the reception desk (p = 0.002) and the stretcher (p = 0.040). Similar results could be identified, indeed, where a significant weak correlation between the values observed with the luminometer and the bacterial counts was presented by Oliveira et al. (2014), in public schools in Brazil; as well as Hammons et al. (2015), in retail delis and Raia et al. (2018), in hospital environments. The data obtained in this study differs from those found in other studies (Shama & Malik, 2013; Shirai et al., 2016). The differences found in those studies can be influenced by several factors including the load of organic matter present in the surfaces that not necessarily mean that it has a high microbial load, given that ATP is an energy source not only for microorganisms but also for plants, animal cells (Somatic cells) and other organisms such as parasites (Bernardes et al., 2023). Additionally, there are differences in specific ATP content between microbial genera and species (Shama & Malik, 2013).

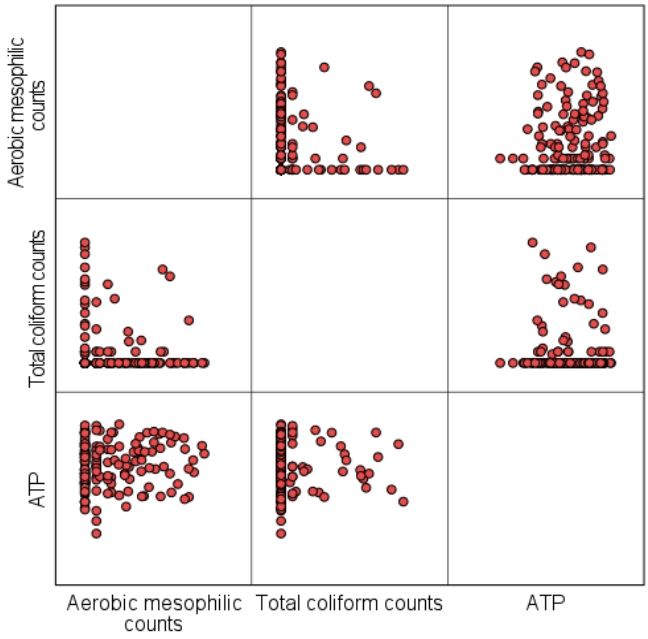

Figure 2. Spearman's correlation between the measures of ATP (RLU/cm^2) and Aerobic mesophilic counts (CFU/cm^2) and ATP (RLU/cm^2) and Total Coliform counts (CFU/cm^2).

The goal of ongoing research on hygiene validation approaches is to develop faster, simpler, and more accurate technologies for the identification of microbial contamination in food processing environments including surfaces, with the aim to be a tool for the management of the environment in food production systems to define possible critical control points. This includes the use of ATP based methods with specific and more sensible swabs that are designed and validated for the detection of specific microorganisms (Micro Snap™). For food safety to be achieved, commercial operators, scientists, and consumers must work continuously. Combining these techniques can give quality managers more knowledge about the characteristics of the contamination discovered, allowing them to continuously enhance their control over hygiene processes. Some factors that can contribute to limiting the contamination of food contact surfaces

include personal hygiene, proper kitchen design, sanitation, and cleaning techniques that follow scientific guidelines. It is necessary to design a disinfection protocol based on the specific needs of the gastronomy laboratories and use cleaning and disinfection products that are specific to the food industry, following Resolution 2674 (Colombia, 2013), and according to the technical data sheets of detergents and disinfectants. Additionally, a microbiological sampling plan must be implemented with trained personnel.

4 Conclusions

This study highlights the importance of effective cleaning and disinfection procedures to ensure quality control and food safety in gastronomy laboratories. The study showed that although the quick method is cheaper, it also gives an overestimated result, as it indicated the presence of ATP on all surfaces analyzed, but due to traditional analysis, it was possible to conclude that they were organic residues. The study found that traditional methods for assessing surface cleanliness are time-consuming and costly, leading to the adoption of fast techniques such as bioluminescence-based ATP measurement. The results showed the presence of organic contamination on all surfaces, indicating the need for improved cleaning practices. While microbial counts were generally low and did not exceed acceptable limits in most cases, total coliform counts on most surfaces exceeded regulatory criteria. This suggests that personal hygiene practices and cleaning protocols need to be enhanced to minimize the risk of cross-contamination and foodborne illnesses. The findings emphasize the significance of proper training and education for food handlers regarding the importance of hygiene procedures and microbiological controls. Furthermore, the study underscores the need for ongoing research and development of advanced technologies to improve hygiene validation approaches and enhance food safety management in food processing environments. Implementing stringent cleanliness and disinfection measures, along with regular monitoring and evaluation, is crucial to ensure the safety and quality of food services in gastronomy laboratories and other food production settings.

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de alimentos y los requisitos para la notificación, permiso o registro sanitario de los alimentos, según el riesgo en salud pública, con el fin de proteger la vida y la salud de las personas. *Diario Oficial*, Colombia.

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