Evaluation of baseline cleanliness of food contact surfaces in Basrah Governorate restaurants using ATP-bioluminescence to assess the effectiveness of HACCP application in Iraq

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Abstract

The Hazard Analysis and Critical Control Points (HACCP) system prevents and manages physical, chemical and biological risks at places where foods and beverages are processed, packaged, distributed and consumed. The present study (1) assessed the level of microbial contamination of food contact surfaces using adenosine triphosphate (ATP)-bioluminescence in Iraq restaurants; (2) investigated the level of microbial contamination of food contact surfaces; and (3) evaluated the efficiency of sanitizers in removing biological hazards from food contact surfaces. The ATP-bioluminescence discovered the presence of *Escherichia coli* and *Staphylococcus aureus* on surfaces and tools. Results also showed that the HACCP application was very effective in the amelioration of food quality.

Keywords: ATP-bioluminescence; bacteria; contamination; food safety; HACCP

Introduction

A significant number of people worldwide contact various types of diseases each year as a result of consuming food that has been exposed to physical and chemical pollutants and pathogenic microorganisms (Chatterjee and Abraham, 2018; Randhawa *et al*., 2018). According to the World Health Organization (WHO), pathogenic microorganisms transmitted through food or water are among the primary causes of 600 million annual cases of foodborne diseases (Chlebicz and Śliżewska, 2018; Etter *et al*., 2017). In the United States, an estimated 5 million cases of illness caused by foodborne bacteria are recorded annually, while the number of recorded cases due to parasite infections is 2 million, and 30 million cases are recorded due to viruses (Nakao *et al*., 2018; Sankarankutty, 2014). Food contamination because of microorganisms is attributed to numerous reasons, including contact between the food and surfaces, and various contaminants from sources such as air, water, hair, dirt, animal and human waste, exposed wounds etc. (Barbosa *et al*., 2019; Gursoy, 2019), in addition to

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the natural pollution that occurs from raw materials (Forsythe, 2020).

Several tests have been used to detect microorganisms that cause contamination in foods and on surfaces, utensils, and equipment. The most important of these tests are live cell counting (Rajapaksha *et al.*, 2019), staining (Wang *et al.*, 2019), carbohydrate fermentation tests, enzyme-linked immunosorbent assay (Chae *et al.*, 2020), polymerase chain reaction (Liu *et al.*, 2019), UV detection (Etheridge *et al.,* 2019) and spectrophotometric technology (Batani *et al.*, 2019).

Despite the development of numerous analytical techniques using automated devices and multiple monitoring methods for detection of microorganisms in contaminated food, these methods have several drawbacks. For example, current methods require a large number of samples and highly skilled personnel. In addition, these methods are expensive and require extended time frame to obtain results (Duffy and Moore, 2017). As a result, attention has focused on newer and faster alternatives characterized by their high sensitivity for assessing the level of food contamination and the degree of cleaning and sterilization of food contact surfaces (Nemati *et al.*, 2016; Poghossian *et al.*, 2019).

Among the newer methods that have been developed are biosensors, characterized by their accuracy and speed compared to conventional methods (Ali *et al.*, 2020; Mishra *et al.*, 2018). For example, adenosine triphosphate (ATP)-bioluminescence method (RLU/100 cm^2) is one of the most important biosensing methods used for assessing the levels of surface and food contamination in various food preparation and handling situations (Chollet and Ribault, 2012; Patel, 2020). The principle of bioluminescence is based on the production and emission of light by living organisms and is the product of chemical reaction that releases energy in the form of light. It is based on the presence of ATP, the primary energy carrier in all living microorganisms, which is indicative of the presence of live microorganisms that are capable of growth and reproduction, resulting in food contamination (Jayan *et al.*, 2020; Simmons *et al.*, 2014).

The Hazard Analysis and Critical Control Points (HACCP) system is one of the systems related to ensuring food safety. Food safety risks occur anywhere with food contaminant sources, whether natural, chemical or biological, and such risks are to be determined where food is processed, manufactured, prepared and served. Thus, adherence to follow the steps of the HACCP system is a preventive public health measure that reduces potential risks (Gehring and Kirkpatrick, 2020; Tesson *et al.*, 2020).

The objectives of this study were to (1) assess the level of microbial contamination of food contact surfaces in restaurants located in Basrah Governorate, Iraq, using ATP-bioluminescence (RLU/100 cm^2) as a rapid, user-friendly method for quantifying surface cleanliness within HACCP system; (2) investigate the level of microbial contamination in food contact surfaces; and (3) evaluate the efficiency of sanitizers, namely stabilized hydrogen peroxide (H_2O_2), in removing biological hazards from food contact surfaces.

Materials and Methods

Collection of samples

Samples were collected from 10 restaurants located in the Basrah Governorate of Iraq, with two restaurants selected randomly from each of the governorate's five regions (Algeria, Al-Jabila, Al-Ashar, Al-Zubair and Abi Al-Khasib) from January 2020 to February 2021. The samples were collected from the most popular foodstuffs of restaurants, and included beef burgers, salads, kebabs and potato sticks. Each food sample (15 g) was transferred to a sterile polyethylene bag (3M™, Saint Paul, MN, USA), and the bag was closed tightly, transferred to the laboratory, and kept under refrigeration until the testing was conducted.

Samples were also taken from food contact surfaces, which included knives, food cutting boards, utensils, flamenco for the manufacture of falafel and beef burgers, and other tools used in food processing. The samples were obtained from these surfaces by using cotton swabs included with surface cleanliness measuring device (3M™ Clean-Trace™ Surface ATP Test Swab XL100) in order to measure the value of ATP-bioluminescence. A cotton swab with transport medium was also used to withdraw samples in order to count the number of microorganisms on surfaces and tools exposed to food. Samples from surfaces related to food preparation, washing and cooking water were taken using a water cleanliness device scanner (3M™ Clean-Trace™ water ATP Test Swab XL100) to estimate the value of ATP-bioluminescence test. In addition, 1 mL of water sample was taken to calculate the number of microorganisms in samples using the conventional counting method.

Detection of Vibrio cholerae

Freshly prepared thiosulphate citrate bile salt sucrose agar medium (TCBS), 89 g, was dissolved in 1 L of distilled water (pH = 8.9) without sterilization. The suspension was then incubated at 35°C for 18 h to detect *Vibrio*

cholerae and the intestinal pathogen *Vibrio parahaemolyticus*, according to the method reported by American Public Health Association (APHA, 1998) and Andrews (1992).

Detection of Salmonella Typhi

In order to activate *Salmonella* bacteria and ensure its presence in sample, 1 g of sample was added to 9 mL of tetrathionate broth. The sample was then incubated for 24 h at a temperature of 35°C, after which 1 mL of bacterial suspension was cultured in *Salmonella Shigella* agar, which was prepared by dissolving 63 g in 1-mL distilled water without sterilization using a rotary heater. The dishes were incubated at 37°C for 24–48 h, after which the total number of bacteria was calculated (Andrews, 1992; APHA, 1998).

Total aerobic bacterial count

Dilutions were prepared from the withdrawn samples, and 1 mL of prepared dilution was transferred to a Petri membrane and incubated at 37°C for 24–48 h.

Total coliform/E. coli count

Dilutions were prepared from the withdrawn samples; then 1 mL of the prepared dilution was transferred to a Petri membrane and incubated at a temperature of 37°C for 24–48 h. Coliform and *E. coli* were enumerated by counting the developing colonies, prepared according to the instructions and which appeared as blue and red (3M™).

Staphylococcus aureus count

Dilutions were prepared from the drawn samples, and 1 mL of the prepared dilution was transferred to a Petri membrane and incubated at 37°C for 24–48 h. *Staphylococcus aureus* bacteria, which appeared as a reddish-purple color, were counted for colonies according to the instructions (3M™).

Yeast and Mold Count

Dilutions were prepared from the drawn samples, and 1 mL of the prepared dilution was transferred to a Petri membrane and incubated at 25°C for 48–72 h. Yeasts and molds, which had colors ranging from dark pink to greenish blue, were calculated for colonies according to the instructions (3M™).

HACCP Application

Preparation of Samples

The two most consumed meals in restaurants were chosen, which included kebabs and beef burgers. Then the HACCP system was applied to the components of the selected meals, and the types of potential risks and methods of controlling them were identified. The upper limit of the existing risk was documented in a special record and corrective action was taken as shown in Tables 1 and 2, and Figures 1 and 2, for each food item.

ATP-bioluminescence Value

A device (3M™ Clean-Trace™ NGi Luminometer) was used to measure the cleanliness of the following food contact surfaces during the preparation period of kebabs and burgers: meat mincer, knife, plastic cutting board, mixing bowl and workers' hands. Swabs were taken from surfaces before and after applying the HACCP system.

Microorganism Count for Burger and Kebab Samples

Total aerobic bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds were carried out using Petri membranes.

Evaluation of Baseline Cleanliness of Food Contact Surfaces

The cleanliness levels of food contact surfaces of wooden board, plastic board, knife, food preparation table and serving table before and after the daily washing process were determined by estimating the value of ATP-bioluminescence test using the (3M™ Clean device-Trace™ NGi Luminometer) in addition to calculating the total number of bacteria using the Petri membrane.

Effect of exposure of food contact surfaces to different concentrations of hydrogen peroxide

Hydrogen peroxide was found to be a strong oxidizer in detoxification process and had a very little shock regarding the physical and chemical properties of foods when treated. The positive aspect is that H_2O_2 can be removed with common practices (e.g. drying) and is stable at room temperature (Tabata *et al.*, 1994). H_2O_2 at high concentration can produce skin irritation; therefore, its dosage has to be limited to an amount sufficient for the purpose. Heat treatment, such as cooking, quickly decomposes H_2O_2 (Shen and Singh, 2022), and thus has been approved for usage in food industry (US Food and Drug

Table 1. Steps in the burger production process according to the HACCP system.

Critical control point	Hazard type	Control measures	Critical limit	Monitoring frequency/ documentation	Corrective action	Monitoring: responsible	Records and documentation
Receiving	Biological/ chemical	Temperature monitoring	Specified tolerances	Starting with arrival of every lot Record time/date in the work book of HACCP system at each arrival	Reject the product	Purchasing responsible	Thermometer calibration record Temperature control record
Frozen storage	Biological	Temperature	Product temperature ≤ –18°C	Record the results in the work book of HACCP system, twice a day	Discard the product	Storage responsible	Thermometer calibration record Temperature control record
Storage at room temperature	Biological, physical	Standard compliance	Presence of insects or foreign material If the temperature exceeds 20°C, the product has to be cooked rapidly	The worker must check presence of insects and temperature thrice a day	Discard the product	Storage responsible	Thermometer calibration record Temperature control record
Thawing	Biological	Time monitoring	Final temperature 0–7°C	Check temperature/ thawing time	Verify temperature/ time and risk assessment	Thawing responsible	Thermometer calibration record Temperature control record
Cooking	Biological Chemical	Time monitoring	Product temperature ≤80°C	Check temperature/ cooking time	Complete cooking	Cooking responsible	Thermometer calibration record Temperature control record

Administration (FDA), 2022). In addition, small amounts of H_2O_2 given orally produce no toxic effects because of rapid decomposition by catalase of the intestinal cells (Joint FAO/WHO Expert Committee on Food Additives [JECFA], 2004).

In this study, certain surfaces, such as a wooden board, plastic board, kitchen knife, food preparation table and food serving table, exposed to contact with food, were subjected to different concentrations of H_2O_2. A response surface methodology was designed to determine the optimal conditions using different concentrations of H_2O_2 and different periods (Table 3). The level of cleanliness of the surfaces before and after treatment was measured with an ATP-bioluminescence test using the 3M™ Clean-Trace™ NGi Luminometer (3M™), and the process of calculating the total number of bacteria was carried out using the Petri membrane method. The final concentration was suggested based on the ATP-bioluminescence test and the total number of bacteria. The results were recorded, and the experiment results were compared with the predicted results through the Design-Expert software (Stat-Ease Inc., Minneapolis, MN, USA) (Table 3).

Design and Statistical Analysis

The parameters were determined using the Response Surface Methodology based on the design of the Box–Behnken program. Two factors (concentration and timing) were used for five types of surfaces and tools. Different timings used were 15, 97.5 and 180 min, and different concentrations were 600, 400 and 200 parts per million (ppm), respectively. Some symbols were used to determine factors, such as the concentration of H_2O_2 was represented by symbol A (ppm), and the time was symbolized by the symbol B (min). The level of significant differences in the response models was determined using the ANOVA table ($a = 0.05$). The data were statistically analyzed using the statistical program (GenState), and the studied factors were tested using the least significant difference *t*-test at $p = 0.05$.

Results

ATP-bioluminescence method and microbial content of surfaces and tools

Table 4 shows the values of ATP-bioluminescence test and the total bacterial count, coliform, *Escherichia coli*,

Table 2. Steps in the productive process of kebab according to the HACCP system.

Critical control point	Hazard type	Control measures	Critical limit	Monitoring frequency/ documentation	Corrective action	Monitoring: responsible	Records and documentation
Receiving	Chemical	Certified supplier with HACCP program	Specified tolerances	Starting with arrival of every lot Record the results corrective actions, and record time/ date in the HACCP system workbook	Recipient rejects the meal	Purchasing responsible	Thermometer calibration record Temperature control record
Storage	Biological	Monitoring the temperature of the product	Product temperature ≤ –18°C	Record the results in the HACCP system workbook	If proper temperature is not maintained, meal must be rejected	Storage responsible	Thermometer calibration record Temperature control record
Storage at room temperature	Physical	Standard compliance	Unusual material must be disposed of If the temperature exceeds 20°C, the product has to be cooked rapidly	Worker must check the temperature thrice a day	Discard the meal	Storage responsible	Thermometer calibration record Temperature control record
Frozen storage	Biological	Temperature recording	Product temperature ≤ –18°C	Temperature recording for food meal twice a day	If proper temperature is not maintained, meal must be rejected	Storage responsible	Thermometer calibration record Temperature control record
Thawing	Biological	Control of time assumed for the dissolution procedure	Final temperature 0–7°C	Check temperature/thawing time	Verify temperature/ time and risk assessment	Thawing responsible	Thermometer calibration record Temperature control record
Mixing/ mincing	Biological	Standard compliance	Check mixing procedures to prevent surface contamination of components	The worker must check temperature periodically	Discard the meal	Mixing/ mincing responsible	Thermometer calibration record Temperature control record
Cooking	Biological/ chemical	Time control	Product temperature ≤ 80°C for 10 min	Check temperature/ cooking time	Adjust cooking time and temperature	Cooking responsible	Thermometer calibration record Temperature control record

Staphylococcus aureus, and yeasts and molds for the restaurant surfaces and tools that were checked (knife, wooden cutting board, plastic cutting board, kebab preparation utensil, fries preparation utensil, salad utensil, serving dish, food preparation table in kitchen, and the food serving table for customers). The observed values of ATP-bioluminescence test for the food contact surfaces and tools were higher than the recommended values indicated by the manufacturer (3M™). Moreover, ATP-bioluminescence values higher than 150 RLU/100 cm^2 established that the food contact surfaces and tools were not clean. The accurate and quick method of investigating cleanliness of food contact surfaces was used by Zambrano *et al*. (2014) to check the cleanliness and contamination rate of surfaces and tools at a hospital in South America.

Osimani *et al*. (2014) referred to the adequacy of using the ATP-bioluminescence method in evaluating the cleanliness for food serving table, knife, food preparation table, cutting board, meat cutting board and meat mincing board in a cafeteria of applied sciences university in Italy. The authors clarified that this method was superior for detecting microbial content and its results helped to improve cleaning process and mitigate contamination. Our findings demonstrated that the percentage

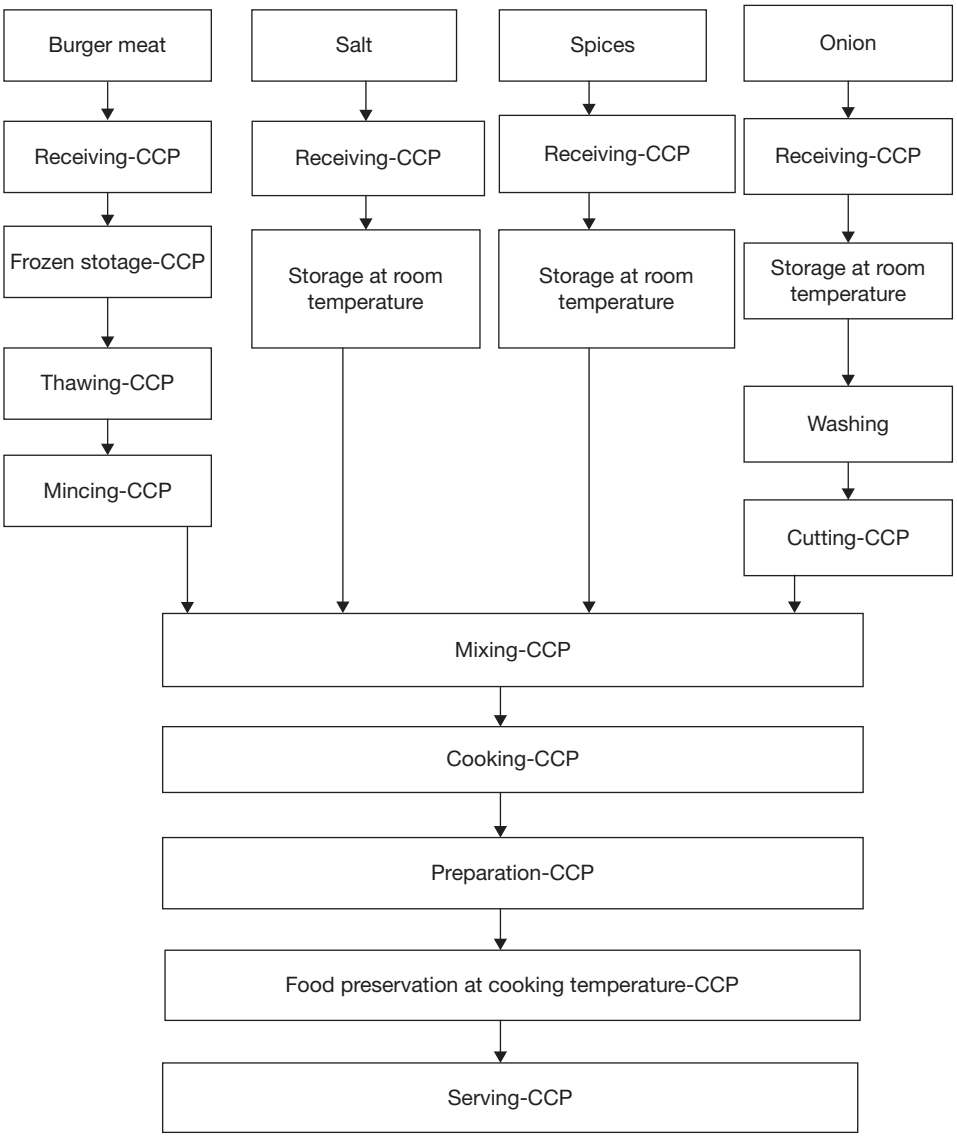

Figure 1. Flow chart and determining of critical control points for burger preparation.

of contamination on food serving tables was minor compared to that on preparation tables.

Correlation between ATP-bioluminescence assay and conventional method

The values of correlation coefficient (R^2) between ATP-bioluminescence and total bacterial count were calculated for tools and surfaces (wooden cutting board, plastic cutting board, knife, food preparation table and serving table). The R^2 values were 0.7284, 0.5377, 0.5923, 0.4457 and 0.8808 for wooden cutting board, plastic cutting board, knife, food preparation table and food serving table, respectively. There was a clear correlation between the value of ATP-bioluminescence and the total bacterial count in case of some tools, but the correlation differed according to the type of surface. This result was in agreement with the results obtained by Shama and Malik (2013), who determined $R^2 = 0.759$ between ATP-bioluminescence and the total bacterial count. The results showed a decrease in R^2 values of cutting board and food preparation table because of the fact that ATP-bioluminescence method can detect live bacteria cells only. In addition, the ATP-bioluminescence method determines the remnants of organic matter compared to the conventional method, which can only measure live microbial cells. This was in agreement with Harper *et al.* (2014), who used ATP-bioluminescence method and total bacterial count method to detect the level of microbial contamination of tools and surfaces in an Italian hospital. The authors confirmed that viable but non-culturable (VBNC) can increase the bioluminescence values of ATP-bioluminescence test, which can affect

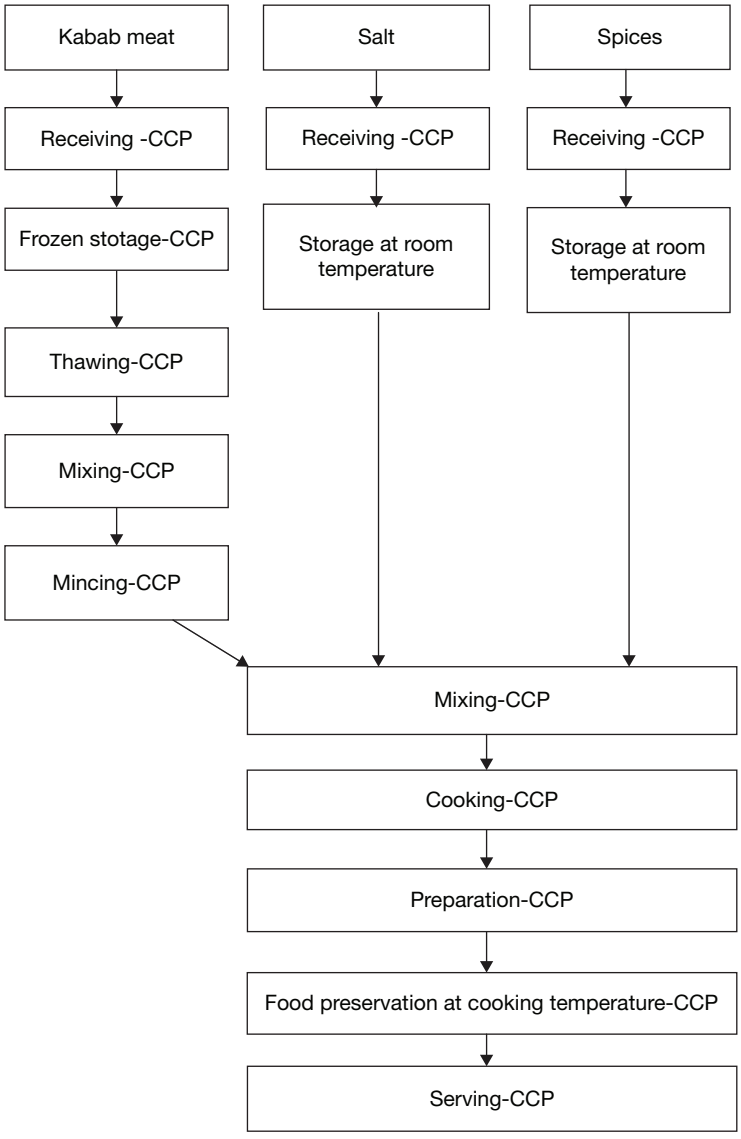

Figure 2. Flow chart and determining of critical control points for all components of kebab.

Table 3. Composite center design matrix for mitigation of microbial load for some surfaces and tools after treatment with different concentrations of H_2O_2 and contact timings.

Run	H2O2 concentration (ppm)	H2O2 contact time (min)
1	600.00	180.00
2	600.00	15.00
3	400.00	97.50
4	400.00	180.00
5	400.00	97.50
6	200.00	97.50
7	400.00	97.50
8	400.00	15.00
9	200.00	180.00
10	600.00	97.50
11	200.00	15.00

R^2 value between ATP-bioluminescence and total bacterial counts. The results also revealed that R^2 values of surfaces varied according to the type of surfaces exposed to food contact. This variation could be attributed to the quality of the material used in the manufacture of surfaces and the type of microorganisms. Van Arkel *et al.* (2021) explained differences in R^2 values between the use of ATP-bioluminescence method and total bacterial count method due to differences in both type of surface and methods used for assessing the level of correlation between surfaces.

Microbial content of different types of restaurant meals

Table 5 shows the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for beef

Table 4. ATP-bioluminescence and microbiological counts for surfaces and tools used at Basrah governorate restaurants, Iraq.

Surfaces and tools	Number of samples	ATP-bioluminescence (RLU/100 cm^2)	Total count (Log CFU/100 cm^2)	Coliform (Log CFU/100 cm^2)	*E. coli* (Log CFU/100 cm^2)	*Staphy lococcus aureus* (Log CFU/100 cm^2)	Yeasts and molds (Log CFU/100 cm^2)
Knife	42	1345.74 ± 130.09	7.62 ± 1.26	2.81 ± 0.46	1.92 ± 0.11	1.91 ± 0.32	2.21 ± 0.13
Wooden cutting board	28	1327.56 ± 142.38	6.96 ± 1.13	2.31 ± 0.31	1.87 ± 0.64	1.61 ± 0.14	2.34 ± 0.43
Plastic cutting board	30	2186.82 ± 189.53	7.07 ± 2.05	2.35 ± 0.18	1.92 ± 0.57	1.93 ± 0.17	2.15 ± 0.18
Kebab preparation utensil	16	552.88 ± 90.82	4.66 ± 1.04	2.32 ± 0.12	1.51 ± 0.13	Not detected	1.68 ± 0.22
Potato fingers preparation	28	415.05 ± 59.10	3.79 ± 0.75	2.22 ± 0.15	1.08 ± 0.12	Not detected	1.25 ± 0.12
Salad dish	26	427.92 ± 39.67	4.41 ± 0.83	1.83 ± 0.12	1.25 ± 0.15	1.33 ± 0.11	1.47 ± 0.11
Serving dish	24	1044.27 ± 110.33	2.33 ± 0.13	1.12 ± 0.12	Not detected	Not detected	1.79 ± 0.41
Food preparation table	20	1044.27 ± 110.33	6.59 ± 0. 46	2.49 ± 0.13	1.81 ± 0.13	1.54 ± 0.15	1.97 ± 0.55
Food serving table	40	547.38 ± 50.87	4.92 ± 0.58	2.2 ± 0.11	1.34 ± 0.16	1.52 ± 0.14	1.48 ± 0.34

Table 5. Total bacterial counts for different types of restaurant meals in Basrah, Iraq.

Types of meals	Number of samples	Total count (Log CFU/g	Coliform (Log CFU/g	*E. coli* (Log CFU/g	*Staphylococcus aureus* (Log CFU/g)	Yeasts and molds (Log CFU/g)
Beef burgers	24	0.52 ± 6.61	0.21 ± 4.37	0.18 ± 1.50	0.06 ± 1.22	0.23 ± 1.98
Potato fingers	28	0.33 ± 3.75	0.21 ± 1.42	0.12 ± 1.10	Not detected	0.35 ± 1.59
Salads	26	0.56 ± 6.18	0.13 ± 1.47	0.24 ± 1.19	0.22 ± 1.49	0.16 ± 1.27
Falafel	18	0.67 ± 4.59	0.15 ± 1.21	Not detected	Not detected	0.32 ± 1.32
Kebabs	16	0.86 ± 7.09	0.34 ± 2.87	0.30 ± 1.69	0.14 ± 1.52	0.43 ± 1.73

Table 6. ATP-bioluminescence and total bactrrial counts in water used for cooking and washing in some restaurants of Basrah, Iraq.

Water	Number of samples	ATP-bioluminescence (RLU/100 cm^2)	Total count (Log CFU/ mL)	Coliform (Log CFU/ mL)	*E. coli* (Log CFU/mL)	*Staphylococcus aureus* (Log CFU/mL)	Yeasts and molds (Log CFU/mL)
Water used in cooking	10	59.03 ± 9.42	Not detected	Not detected	Not detected	Not detected	Not detected
Washing water	10	269.54 ± 5010.70	7.48 ± 0.93	2.68 ± 0.19	1.72 ± 0.20	1.49 ± 0.17	1.07 ± 0.27

burgers, potato fingers, salad, falafel and kebab. The highest microbial contamination levels were found in kebab, beef burgers and salads, which could be due to the quality of raw materials used in the industry. Tesson *et al.* (2020) assessed microbial contamination in meat and found that increase or decrease in the microbial load was due to environmental conditions, storage conditions and temperature, transporting, and cooking methods.

ATP-bioluminescence and total bacterial count for water used in cooking and washing

Table 6 shows the values of ATP-bioluminescence and the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the samples of water used in cooking and washing. The value of ATP-bioluminescence for the water used in cooking was 59.03 RLU/100 cm^2, and there was no growth for coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds. The results showed that the ATP-bioluminescence value for water used for washing was 5010.70 RLU/100 cm^2, while the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds was 7.48, 2.68, 1.72, 1.49, and 1.07 Log CFU/mL, respectively. The washing water used in restaurants was more polluted compared to the water used for cooking, which may be attributed to the inefficiency of water sterilization or the presence of organic pollutants as well as the lack of cleanliness of water conveyance pipes. These results were consistent with those found by Singh and Gupta (2016), who indicated that the high level of pollution in water was due to the high percentage of organic pollutants. The ATP-bioluminescence method for detecting pollution in water was an efficient method. Zhang *et al.* (2019) indicated that this method was effective at detecting pollution in water, as the method was capable of detecting living cells as well as those unable to grow in culture media (VBNC).

Detection of Vibrio cholerae and Salmonella Typhi

The results showed the absence of *Salmonella* bacteria on food contact surfaces and tools (knife, wooden cutting board, plastic cutting board, kebab pot, frying pan, salad bowl, serving bowl, food preparation table in the kitchen and serving table for customers) and in foodstuffs (beef burger, potato sticks, salad, falafel, kebab). In addition, there was absence of *Vibrio cholera* bacteria in the water samples used for cooking and washing purposes.

Bioluminescence of food contact surfaces

The results (Table 7) showed the values of ATP-bioluminescence for food contact surfaces (meat mincer, plastic cutting board, mixing bowl and workers' hands) during food preparation and by applying the HACCP system. The results showed that the use of ATP-bioluminescence method was rapid and efficient for detecting contamination of surfaces exposed to food. Champiat *et al.* (2001) confirmed that application of the HACCP system, combined with ATP-bioluminescence method, was fast and effective for detecting the contamination of food processing tools, and to find out whether the contaminants were microorganisms or remnants of organic material. There was a significant decrease in ATP-bioluminescence values of some tools and food contact surfaces during the preparation of kebabs and beef burgers after applying the HACCP system. The reason for the low values of ATP-bioluminescence could be the use of sterile and clean surfaces during preparation when applying the HACCP system compared to the surfaces and tools used in restaurants without applying the HACCP system. Khairallah (2014) indicated that equipment or machines used for chopping purposes were prone to the accumulation of many microorganisms, particularly the parts of equipment that were exposed to foodstuffs.

Microbial content of kebab and beef burger samples before and after application of HACCP

Figure 3 shows decrease in the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the defrosting step of frozen meat during kebab preparation before and after applying HACCP. The total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds before applying HACCP accounted

Table 7. ATP-bioluminescence of food contact surfaces during food preparation steps after application of the HACCP system.

ATP-bioluminescence (RLU/100 cm²)		Type of contamination surface	Food preparation steps	Type of product
After HACCP	Before HACCP			
109.11 ± 23.09	97.21 ± 745.66	Meat mincer	Meat mincing	Kebab
34.09 ± 4.02	154.23 ± 1233.64	Knife	Onion cutting	
133.67 ± 20.27	172.23 ± 1560.78	Plastic cutting board		
92.81 ± 23.03	72.23 ± 286.45	Mixing bowl	Meat mixing	
25.71 ± 1.91	84.23 ± 567.67	Worker's hand		
166.39 ± 17.89	124.23 ± 934.12	Meat mincer	Meat mincing	Beef burger
102.27 ± 19.22	43.89 ± 322.84	Mixing bowl	Meat mixing	
41.71 ± 2.18	27.12 ± 141.49	Worker's hand		

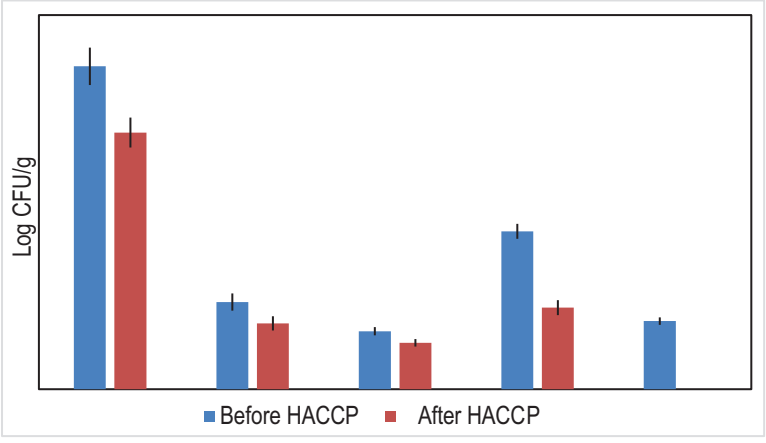

Figure 3. Total count of microorganisms (Log CFU/g) in the defrosting step of frozen meat for making kebab.

for 8.63, 2.33, 1.55, 4.22, and 1.82 Log CFU/g, respectively. All these contaminants decreased significantly ($p < 0.05$) after application of HACCP, lowering to 6.86, 1.76, 1.24 and 2.18 Log CFU/g, respectively, and no yeasts and molds detected.

The results in Figure 4 show that the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the meat mincing step before applying HACCP were 6.85, 2.36, 1.66, 2.33 and 1.15 Log CFU/g, respectively.

Figure 5 shows that total bacterial count, and yeasts and molds for the onion chopping step before applying HACCP were 4.38 and 2.46 Log CFU/g respectively, with no counts recorded for coliform, *E. coli*, and *Staphylococcus aureus*. However, after applying HACCP, total bacterial count, and yeasts and molds decreased significantly ($p < 0.05$) to 2.25 and 1.22 Log CFU/g, respectively, with no counts recorded for coliform, *E. coli*, and *Staphylococcus aureus*.

Figure 6 shows that the values of total bacterial count, and yeasts and molds for kebab spice test before

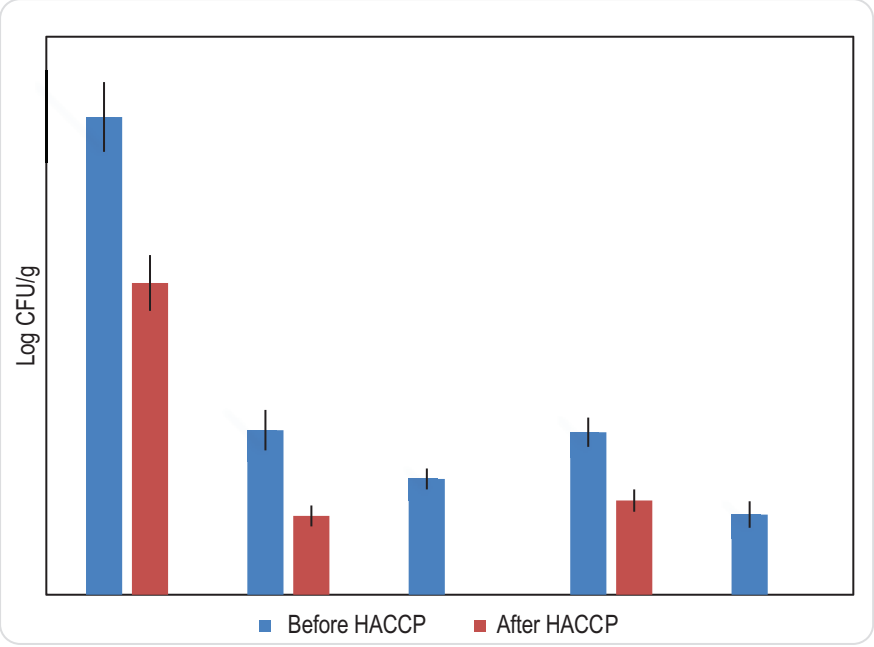

Figure 4. Total count of microorganisms (Log CFU/g) in the meat mincing step during making of kebab.

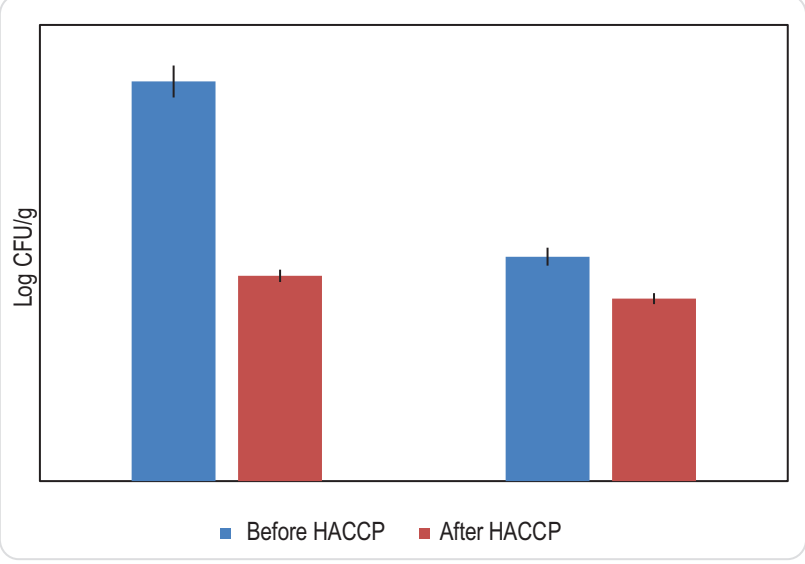

Figure 5. Total count of microorganisms (Log CFU/g) in the onion chopping step during making of kebab.

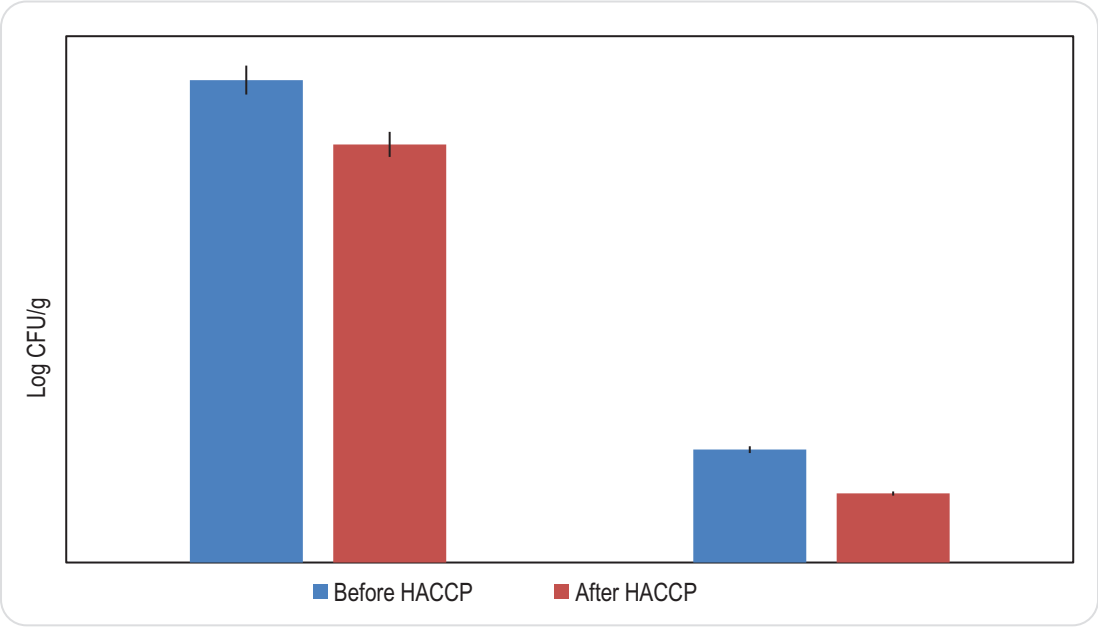

Figure 6. Total count of microorganisms (Log CFU/g) in the kebab spice test step during making of kebab.

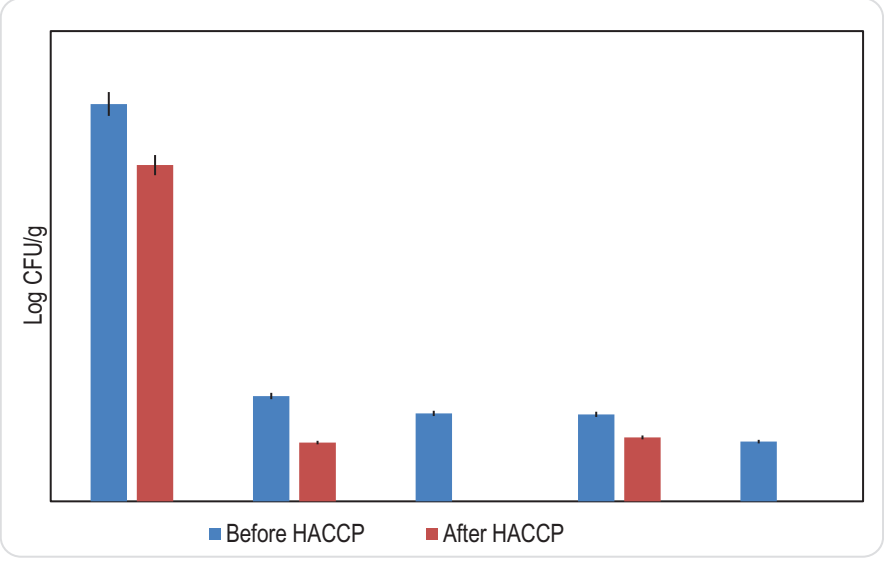

Figure 7. Total count of microorganisms (Log CFU/g) during mixing of meat with ingredients while making kebab prior to serving step.

applying the HACCP system were 8.25 and 1.93 Log CFU/g respectively, with no counts of coliform, *E. coli*, and *Staphylococcus aureus*. After applying the HACCP system, The total bacterial count, and yeasts and molds decreased significantly ($p < 0.05$) to 7.15 and 1.18 Log CFU/g, respectively, with no counts of coliform, *E. coli*, *Staphylococcus aureus*.

Figure 7 shows the values of total bacterial counts, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds during mixing of meat with ingredients prior to serving step without applying the HACCP system at 8.45, 1.85, 2.24, 1.87 and 1.27 Log CFU/g, respectively. However, after applying the HACCP system, the total bacterial count, coliform and *Staphylococcus aureus* decreased significantly ($p < 0.05$) to 7.15, 1.25, 1.36 Log CFU/g, respectively, with no counts of *E. coli*, and yeasts and molds were recorded.

Figure 8 shows that the values of total bacterial count, and yeasts and molds before applying the HACCP system for the meat test step after grilling prior to serving were 6.62

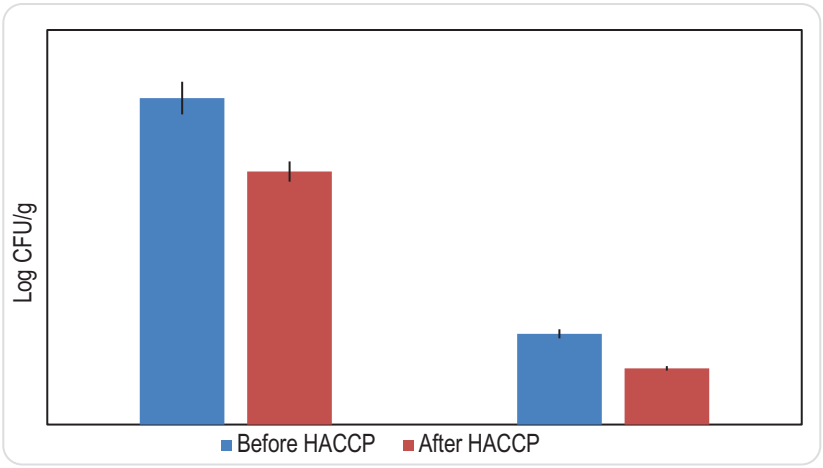

Figure 8. Total count of microorganisms (Log CFU/g) during after-grilling prior to serving step when making kebab.

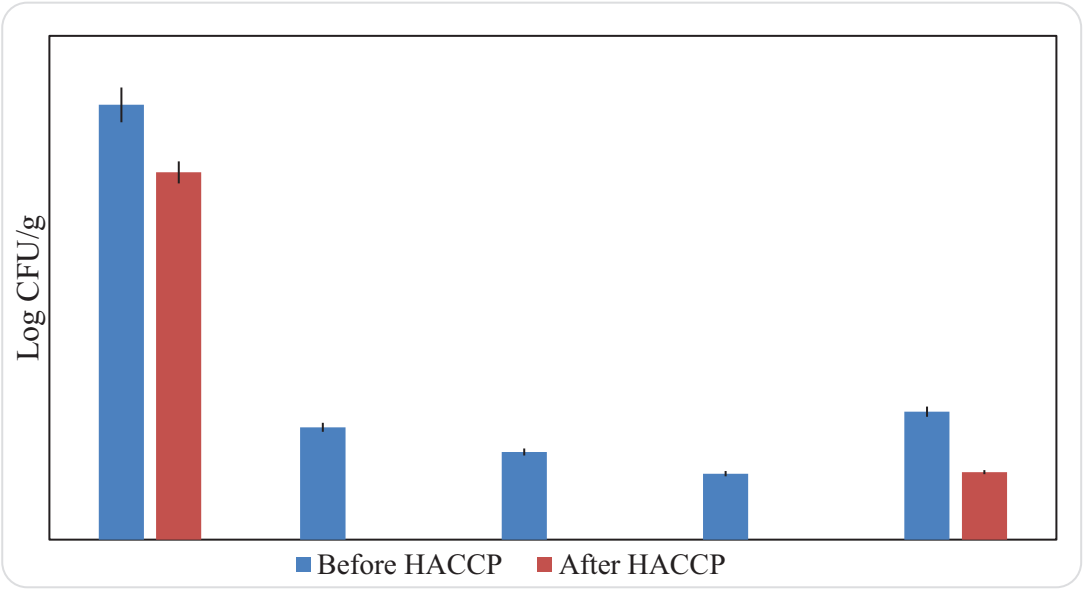

Figure 9. Total count of microorganisms (Log CFU/g) in frozen meat when making beef burger.

and 1.84 Log CFU/g, respectively, with no bacterial counts were recorded for coliform, *E. coli*, and *Staphylococcus aureus*. After applying the HACCP system, the total bacterial count decreased significantly ($p < 0.05$) to 5.13.

The total bacterial count for kebab before applying the HACCP system was 6.23 Log CFU/g and it was 4 Log CFU/g after applying the HACCP system. Results could not be recorded for coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds. Figure 9 shows that the values of the total bacterial counts, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the defrosting step of frozen meat when making beef burger before HACCP were 8.63, 2.23, 1.74, 1.31 and 2.54 Log CFU/g, respectively. After applying HACCP, the total bacterial counts, and yeasts and molds significantly ($p < 0.05$) amounted to 7.29 and 1.34 Log CFU/g, respectively, with no counts recorded for coliform, *E. coli* and *Staphylococcus aureus*.

Figure 10 indicates that the values of the total bacterial count, coliform, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the meat mincing step before applying the HACCP system were 8.53, 2.26, 1.84, 4.18 and 1.38 Log CFU/g, respectively. After applying the HACCP system, total bacterial count and *Staphylococcus aureus* decreased significantly ($p < 0.05$) as these reached 6.37 and 2.15 Log CFU/g, respectively, and no counts recorded for coliform, *E. coli*, and yeasts and molds.

The value of total bacterial count for beef burger spice step before applying the HACCP system was 6.16 Log CFU/g, but after applying HACCP, it decreased significantly ($p < 0.05$) to 5.44 Log CFU/g, and no counts were

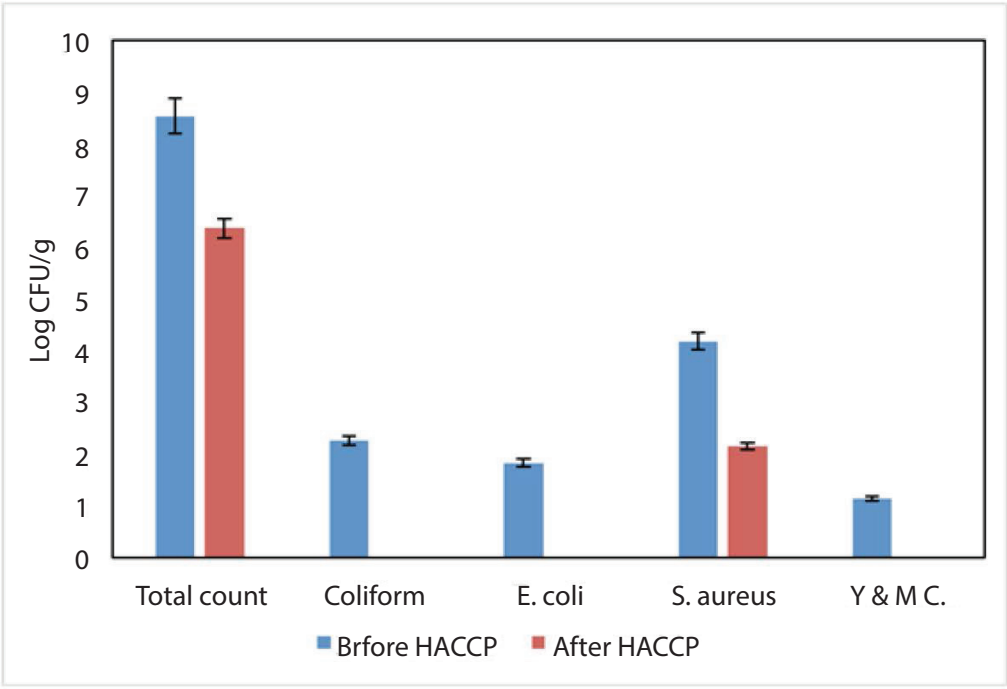

Figure 10. **Total count of microorganisms (Log CFU/g) during meat mincing step when making beef burgers.**

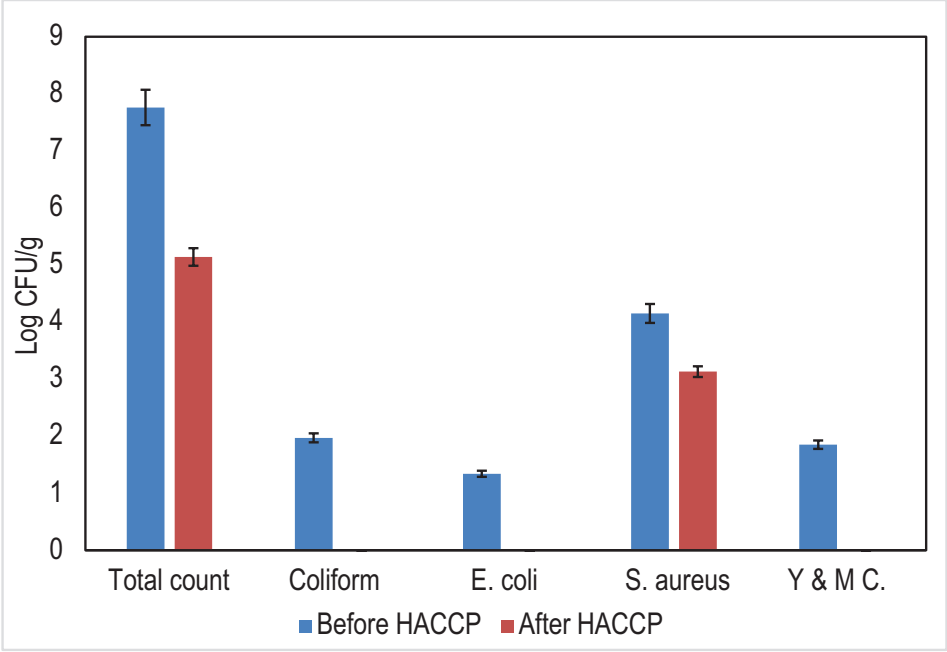

Figure 11. **Total count of microorganisms (Log CFU/g) during the step of mixing beef with ingredients when making beef burgers.**

recorded for coliform, *E. coli*, *Staphylococcus aureus*, and yeasts, and molds.

The values of total bacterial count, coliforms, *E. coli*, *Staphylococcus aureus*, and yeasts and molds for the step of mixing beef burger with ingredients before applying the HACCP system were 7.76, 1.34, 1.97, 4.15 and 1.85 Log CFU/g, respectively (Figure 11). After applying the HACCP system, the total bacterial count and *Staphylococcus aureus* decreased significantly ($p < 0.05$) to 5.14 and 3.13 Log CFU/g, respectively, with no counts recorded for of coliform, *E. coli*, and yeasts and molds.

The values of the total bacterial count, coliform and *Staphylococcus aureus* for the post-grill test step before applying the HACCP system, as shown in Figure 12, were 6.56, 1.25 and 3.54 Log CFU/g, respectively, and no counts recorded for *E. coli*, and yeasts and molds. After applying the HACCP system, the total bacterial count and *Staphylococcus aureus* decreased significantly ($p < 0.05$) to 5.15 and 2.15 Log CFU/g, respectively, with no counts recorded for coliform.

Figure 13 shows that the value of total bacterial count for food preservation step before serving prior to applying the HACCP system was 6.14 Log CFU/g, with no results recorded for coliform, *E. coli*, *Staphylococcus aureus*, and yeasts, and molds. However, after applying the HACCP system, the total bacterial count decreased significantly to 5.25 Log CFU/g ($p < 0.05$).

The above results demonstrated that the application of the HACCP system significantly reduced both microbial content and pollution that could have been transmitted in the product during manufacturing stages from different sources such as workers in the kitchen, utensils or food contact surfaces. Khairallah (2014) indicated that the cleaning of surfaces and equipment prevented the accumulation of microorganisms. The results revealed a decrease

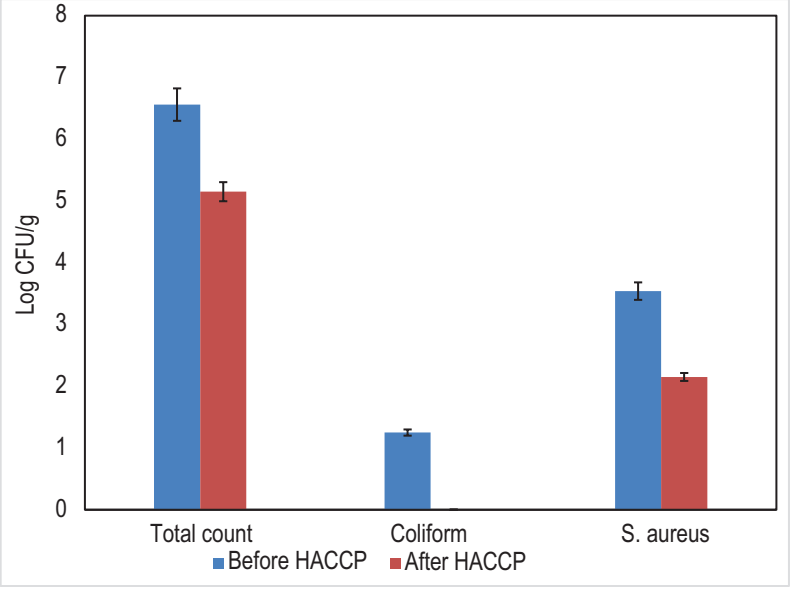

Figure 12. Total count of microorganisms (Log CFU/g) for post-grill test step when making beef burgers.

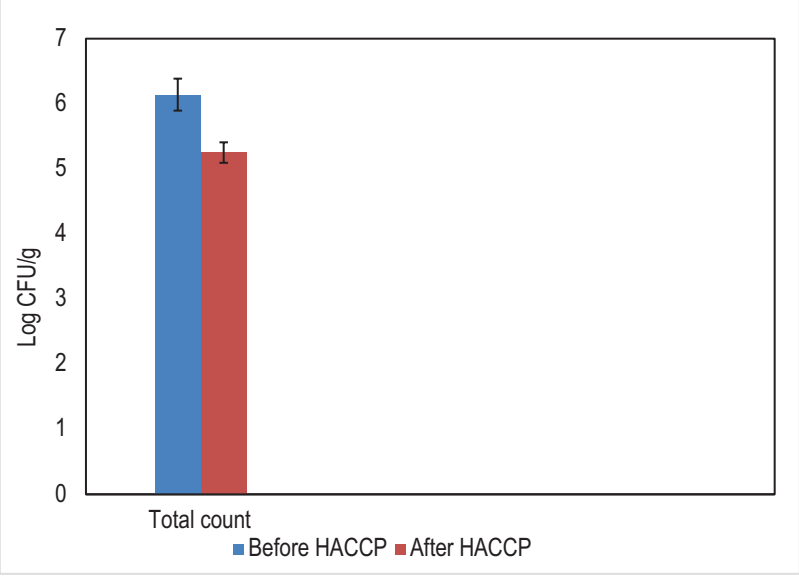

Figure 13. Total count of microorganisms (Log CFU/g) for food preservation step when making beef burgers.

in the number of microorganisms for most of the steps after applying the HACCP system. The results further demonstrated that utilization of this system in restaurant kitchens was important for reducing the microbial load of foodstuffs manufactured there. Another important step in reducing microorganisms was the use of high temperatures that usually reach 76–80°C during grilling process.

Yousif *et al.* (2013) established the importance of applying the HACCP system to reduce microbial load of meat in the kitchen of a nephrology hospital in Egypt. Tesson *et al.* (2020) emphasized the importance of adhering to the HACCP steps to reduce public health risks related to the manufacturing steps of meat and meat products. For example, preservation of meat at low temperatures reduced the counts of *E. coli* and other microorganisms that could be the source of foodborne illnesses.

Quantification of the effect of daily washing on the level of hygiene and microbial content of food-contact surfaces

Table 8 shows decrease in ATP-bioluminescence values and total bacterial counts for surfaces and tools (wood board, plastic board, knife, food preparation table and food serving table) before and after daily washing procedures in the examined restaurants. The results demonstrated a very high reduction (64–69%) in ATP-bioluminescence values and total bacterial count after performing daily washing processes. Results in Table 8 indicate that the use of the ATP-bioluminescence method was effective for detecting the cleanliness level of surfaces after daily washing operations and for predicting the level of contamination on these surfaces.

Harper *et al.* (2014) highlighted the importance of using the ATP-bioluminescence method to detect the level of microbial contamination on clean surfaces exposed to food contact, as this was a fast and highly sensitive method for detecting contamination. The researchers emphasized to use the ATP-bioluminescence method instead of conventional methods. The results also indicated differences in the values of ATP-bioluminescence and total bacterial count for different surfaces following daily washing processes. The difference could be due to the nature of the surface exposed to food contact and the quality of food exhibited to the surface. The wooden cutting board was the least efficient surface studied in the daily washing processes used in restaurants (Osimani *et al.*, 2014). The authors indicated that the efficiency of cleaning processes was affected by the type of surface, because poor surfaces contributed to high microbial content and in reducing the effectiveness of cleaning processes.

Evaluation of stabilized hydrogen peroxide as a sanitizer

Tables 9 and 10 show the effect of treating surfaces and tools exposed to food contact (wooden board, plastic board, knife, food preparation table and serving table) with different concentrations of H_2O_2 and at different times on the values of ATP-bioluminescence and total bacterial count. The results indicated that the lowest value of ATP-bioluminescence and total bacterial count for wood cutting board, plastic cutting board, food preparation table and serving table were 256.33, 262.66, 280.66 and 168.66 RLU/100 cm^2, respectively.

It was noted that the use of H_2O_2 clearly reduced the contamination of surfaces and tools. These results were in agreement with the results of the research conducted by Ukuku *et al.* (2001), who evaluated the effectiveness of H_2O_2 in reducing the number of microorganisms on surfaces and tools. The researchers emphasized the effectiveness and efficiency of H_2O_2 compared to chlorine and other solutions. The chemical composition of H_2O_2 makes it a safe and suitable substance for workers as well as the environment if used in low concentrations on surfaces. H_2O_2 diluted with water is widely used on surfaces and is safe and effective at eliminating microbial loads. McDonnell (2009) indicated the effectiveness of using H_2O_2 in sterilization processes with no adverse effects, and confirmed that it was effective at eliminating bacteria.

Table 8. Change in ATP-bioluminescence values and total bacterial counts for food contact surfaces before and after daily washing operations in some restaurants.

		ATP-bioluminescence (RLU/100 cm^2)			Total bacterial count (Log CFU/100 cm^2)		
Surfaces and tools	Number of samples	Before washing	After washing	Reduction percentage	Before washing	After washing	Reduction percentage
Wood cutting board	12	350.23 ± 7493.328	261.18 ± 2875.662	61.62%	0.96 ± 6.52	0.26 ± 2.33	64.25%
Plastic cutting board	12	176.34 ± 2470.272	108.23 ± 815.885	66.97%	1.34 ± 6.78	0.29 ± 2.21	67.40%
Knife	12	150.11 ± 1218.885	45.67 ± 407.161	66.60%	1.58 ± 6.89	0.31 ± 2.14	68.96%
Food preparation table	12	247.23 ± 2161.55	91.23 ± 742.828	65.63%	1.11 ± 6.15	0.18 ± 1.90	69.11%
Food serving table	12	320.12 ± 1924.05	58.09 ± 714.495	62.87%	1.23 ± 6.9	0.16 ± 2.27	67.28%

Table 9. Composite center design matrix for estimating ATP-bioluminescence values for some surfaces and tools after treatment with H_2O_2 at different concentrations and timings.

Food serving table	Food preparation table	Knife	Plastic cutting board	Wooden cutting board	Time (min)	H2O2 (ppm)	Run
643.33	532.33	343.66	691.66	443.66	180.00	600.00	1
168.66	280.66	271.66	262.66	256.33	15.00	600.00	2
423.66	468.33	364.33	538.33	1468.66	97.50	400.00	3
694.33	689.33	398.33	769.33	1535.66	180.00	400.00	4
425.33	450.33	335.66	540.33	1443.66	97.50	400.00	5
600.33	673.33	290.33	794.66	1813.66	97.50	200.00	6
421.66	474.66	333.66	558.33	1462.66	97.50	400.00	7
299.66	272.33	215.66	393.66	917.66	15.00	400.00	8
702.33	738.33	401.66	806.66	2090.33	180.00	200.00	9
367.66	345.33	295.33	443.66	349.33	97.50	600.00	10
252.66	311.33	353.66	686.66	1156.33	15.00	200.00	11

Table 10. Composite center design matrix for estimating total bacterial counts (Log CFU/10 cm^2) for some surfaces and tools after treatment with H_2O_2 at different concentrations and timings.

Food serving table	Food preparation table	Knife	Plastic cutting board	Wooden cutting board	Time (min)	H2O2 (ppm)	Run
2.12	1.82	2.01	2.04	2.19	180.00	600.00	1
1.64	1.59	1.67	1.51	1.47	15.00	600.00	2
1.72	1.66	1.77	1.84	1.65	97.50	400.00	3
2.19	1.79	1.91	2.09	1.94	180.00	400.00	4
1.70	1.61	1.79	1.85	1.72	97.50	400.00	5
1.95	1.72	1.82	1.91	2.21	97.50	200.00	6
1.75	1.65	1.85	1.87	1.76	97.50	400.00	7
1.67	1.47	1.63	1.65	1.52	15.00	400.00	8
2.17	1.84	2.09	2.14	2.27	180.00	200.00	9
1.81	1.77	1.86	1.74	1.61	97.50	600.00	10
1.88	1.69	1.78	1.96	2.13	15.00	200.00	11

Correlation between ATP-bioluminescence method and total bacterial counts detected on some surfaces and tools

Multiple regression was applied to obtain regression coefficients for independent variables in Tables 11 and 12. The values of R^2 for the proposed model in estimating the microbial load of wooden chopping board using ATP-bioluminescence and total bacterial count were 0.7956 and 0.9660, respectively. In addition, the lack of fit was significant for wooden chopping board. The R^2 values of the proposed model of plastic cutting board for estimating the microbial load using ATP-bioluminescence and total bacterial count were 0.9604 and 0.9585, respectively. The lack of fit was significant for plastic cutting board. The results of the statistical analysis further indicated that R^2 values of the proposed model of knife for estimating the microbial load using ATP-bioluminescence and total bacterial count were 0.7469 and 0.9980, respectively, and the lack of fit was significant. The R^2 values of the proposed model of food preparation table for ATP-bioluminescence and total bacterial count were 0.9982 and 0.9569, respectively, and the lack of fit was significant as well for the food preparation table. The R^2 values for the proposed model of food serving table for ATP-bioluminescence and total bacterial count were 0.8826 and 0.9522, respectively. The value of lack of fit for the food serving table was also significant.

Response Surface Methodology

The results expressed (Figures S1–S10) the three-dimensional (3D) response surface in estimating changes in ATP-bioluminescence values and total bacterial count for each of the tools and surfaces, such as wooden board, plastic board, knife, food preparation table, serving table, after treating them with different concentrations of H_2O_2

Table 11. R^2 value, *p*-value and regression coefficients for some surfaces and tools using the ATP-bioluminescence method (RLU/100 cm^2).

Regression coefficient	Wooden cutting board	Plastic cutting board	Knife	Food preparation table	Food serving table
b_0	587.29970–	7066.26671+	1755.91424–	397.52366+	88.14045+
b_1	0.98023+	35.05399	3.41602+	0.27674-	0.085313+
b_2	57.93806+	33.42995+	54.91717+	5.20207+	7.98992+
b_1b_2	0.087071	0.067288–	0.094985–	7.33833E-003–	9.86364E-003–
$b1^2$	0	0.040882+	0	8.33355E-005+	0
$b2^2$	0	0.032632+	0	0.038763+	0
c.v. *(%)	61.47	36.61	95.15	3.90	26.12
R^2	0.7956	0.9604	0.7469	0.9982	0.8826
p-value of the model	0.0082	0.0016	0.0170	<0.0001	0.0012
p-value of lack of fit	<0.0001	0.0002	<0.0001	0.0039	0.0001

*CV: coefficient of variation

Table 12. R^2 value, *p*-value and regression coefficients for some surfaces and tools using the total bacterial count method (Log CFU/100 cm^2).

Regression coefficient	Wooden cutting board	Plastic cutting board	Knife	Food preparation table	Food serving table
b_0	587.29970–	7066.26671+	1755.91424–	397.52366+	88.14045+
b_1	0.98023+	35.05399	3.41602+	0.27674-	0.085313+
b_2	57.93806+	33.42995+	54.91717+	5.20207+	7.98992+
b_1b_2	0.087071	0.067288–	0.094985-	7.33833E-003–	9.86364E-003–
b_1^2	0	0.040882+	0	8.33355E-005+	0
b_2^2	0	0.032632+	0	0.038763+	0
c.v.* %	61.47	36.61	95.15	3.90	26.12
R^2	0.7956	0.9604	0.7469	0.9982	0.8826
p-value of the model	0.0082	0.0016	0.0170	<0.0001	0.0012
p-value of lack of fit	<0.0001	0.0002	<0.0001	0.0039	0.0001

*CV: coefficient of variation

and for different timings. The results demonstrated that the reduction level of microbial contamination of tools and surfaces studied differed according to H_2O_2 concentration and time of exposure. This difference varied between treated tools, and the effectiveness of H_2O_2 decreased with increase in time.

Hassan *et al.* (2013) used H_2O_2 to clean and sterilize surfaces and tools exposed to food contact in a fish processing plant. After H_2O_2 treatment, a clear decrease in the number of microorganisms was observed on surfaces and tools. Thus, the researchers concluded that H_2O_2 was a rapid and effective cleaning and sterilization agent. The results showed that the high concentration of H_2O_2 over a certain exposure period reduced the microbial load of tools and surfaces. However, the level of decrease in microbial content varied with different timings and concentrations, and effectiveness of some of disinfectants decreased with increase in both time and microbial load.

These results were in agreement with the results obtained by Hassan *et al.* (2013), who studied the effect of using H_2O_2 on the level of cleanliness of surfaces and tools exposed to food contact in food manufacturing plants. The researchers pointed out that H_2O_2 was effective as a fast acting cleaning and sterilization agent in getting rid of microbial content, with resultant decrease in microbial content of tools and surfaces.

Studying optimal conditions

Table 13 shows the optimal conditions for surfaces and tools (wooden board, plastic board, knife, food preparation table and serving table) at different concentrations of H_2O_2 and for different periods after applying a second-order polynomial model. The most effective concentration of H_2O_2 was 566 ppm with timing of 15 min, which achieved the lowest value of ATP-bioluminescence for the studied surfaces and tools.

Table 14 shows that the best concentration of H_2O_2 was 580 ppm with the best time of 17 min, which achieved the lowest value of total bacterial count for the studied

Table 13. Practical and predicted values of ATP-bioluminescence for some tools and surfaces under optimal conditions (H_2O_2 concentration = 566 ppm and contact time = 15 min).

Independent variables			ATP-bioluminescence (RLU/100 cm²)	
H_2O_2 (ppm)	Time (min)	Dependent variables	Practical values	Predicted values
566	15	Wooden cutting board	205.33 ± 10.969[a]	197.26[a]
		Plastic cutting board	268.66 ± 10.504[a]	262.66[a]
		Knife	198.33 ± 13.013[a]	195.32[a]
		Food preparation table	292.66 ± 11.151[a]	291.95[a]
		Food serving table	181.33 ± 13.204[a]	172.51[a]

*Statistically significant difference shown levels a, b compared with same other column ($p \leq 0.05$)

Table 14. Practical and predicted values of total bacterial counts for some tools and surfaces under optimal conditions (H_2O_2 concentration = 580 ppm and contact time = 17 min).

Independent variables			Total bacterial count (Log CFU/100 cm²)	
H_2O_2 (ppm)	Time (min)	Dependent variables	Practical value	Predicted value
580	17	Wooden cutting board	1.48 ± 0.095 [a]	1.42[a]
		Plastic cutting board	1.44 ± 0.074[a]	1.38[a]
		Knife	1.59 ± 0.106 [a]	1.54[a]
		Food preparation table	1.37 ± 0.060 [a]	1.32[a]
		Food serving table	1.44 ± 0.096 [a]	1.36[a]

*Statistically significant difference shown levels a, b compared with same other column ($p \leq 0.05$)

surfaces and tools. Table 14 also shows that there were no significant differences in ATP-bioluminescence values for wooden board, plastic board, food preparation table and food serving table. The ATP-bioluminescence value was 198.33 RLU/10 cm², and the predicted values were 197.26, 262.66, 195.32, 291.95 and 172.51 RLU/100 cm² for wooden board, plastic board, food preparation table, food serving table, respectively.

Table 14 shows that there were no significant differences between total bacterial count and the practical values for wooden board, plastic board, food preparation table and food serving table, which were 1.48, 1.44, 1.37 and 1.44 Log CFU/ 100 cm², respectively.

Conclusions

Implementation of HACCP principles in the restaurant industry varies as much as the products produced. Owing to this diversity, textbook-level HACCP performances are inapplicable in most catering establishments. However, as with many other quality assurance programs, the HACCP principles provide a reasonable approach for identifying and controlling risk factors. Many food safety management systems in the restaurant industry incorporate some, if not all, HACCP principles. Although a complete HACCP system is an ideal solution, different types of food safety management programs could be used to control risk factors. Food safety policy is based on high standards that are implemented to protect and contribute to the health of the consumer. This article provided practical information for nutritionists to follow and develop a working system that could provide quality and safe food to consumers. Our results demonstrated that compared to conventional methods, using ATP-bioluminescence is a rapid alternative to conventional methods for predicting the level of contamination and the degree of cleanliness of food contact surfaces.

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Conflicts of Interest

The authors declared no conflict of interest.

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Supplementary

Supplementary Figures S1–S10 describe the three-dimensional (3D) response surface in estimating changes in ATP-bioluminescence values and total bacterial count for different tools and surfaces (wooden board, plastic board, knife, food preparation table and serving table) after H_2O_2 treatment at different concentrations and for different periods.

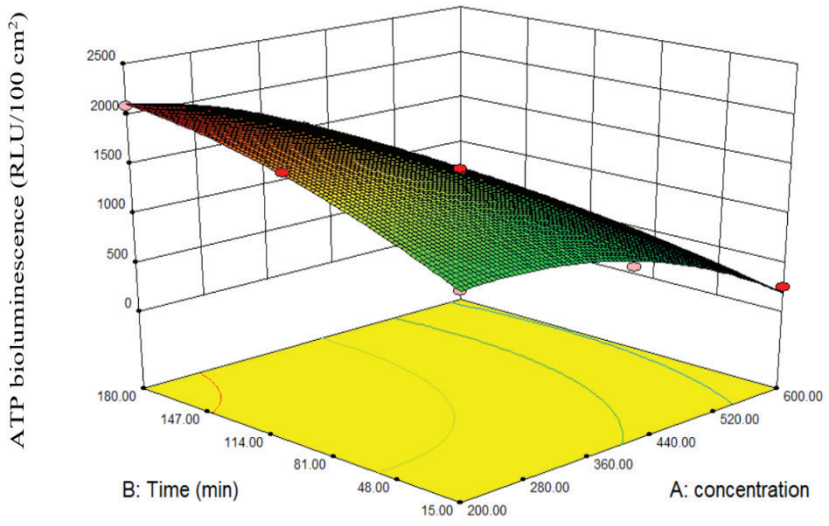

Figure S1. Three-dimensional (3D) response surface for estimating ATP-bioluminescence values (RLU/100 cm²) for wooden cutting boards treated with H_2O_2 at different concentrations and timings.

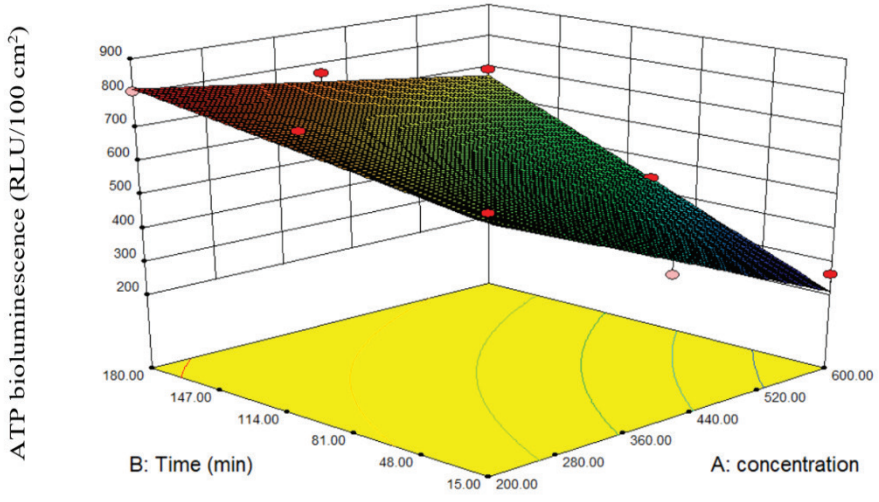

Figure S2. Three-dimensional (3D) response surface for estimating ATP-bioluminescence values (RLU/100 cm²) for plastic cutting boards treated with H_2O_2 at different concentrations and timings.

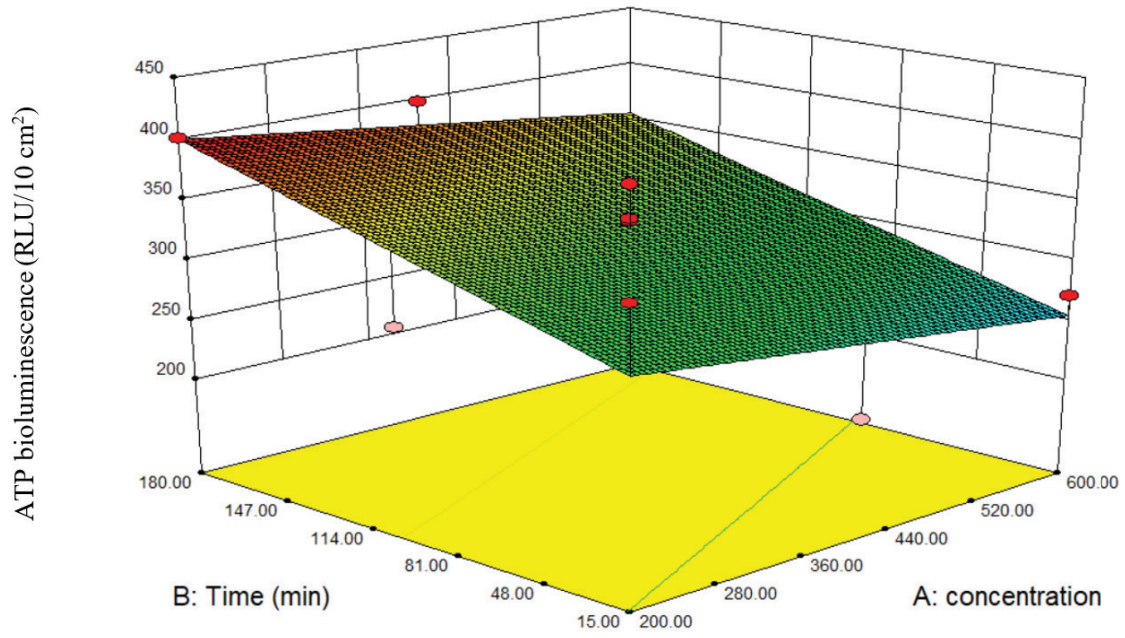

Figure S3. Three-dimensional (3D) response surface for estimating ATP bioluminescence values (RLU/10 cm^2) for knives treated with H_2O_2 at different concentrations and timings.

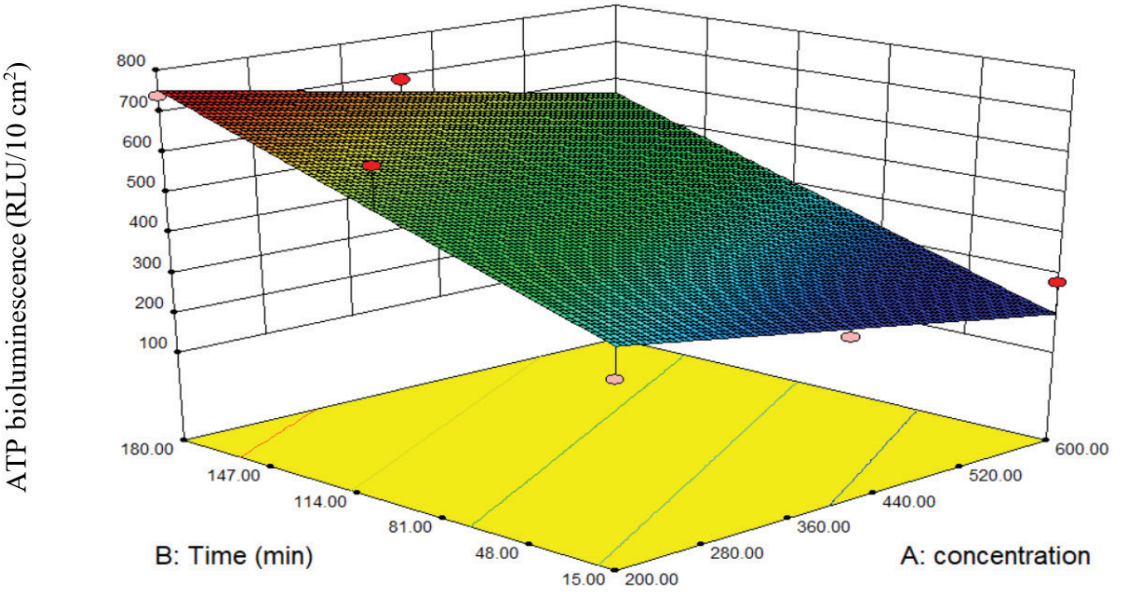

Figure S4. Three-dimensional (3D) response surface for estimating ATP bioluminescence values (RLU/100 cm^2) for food preparation tables treated with H_2O_2 at different concentrations and timings.

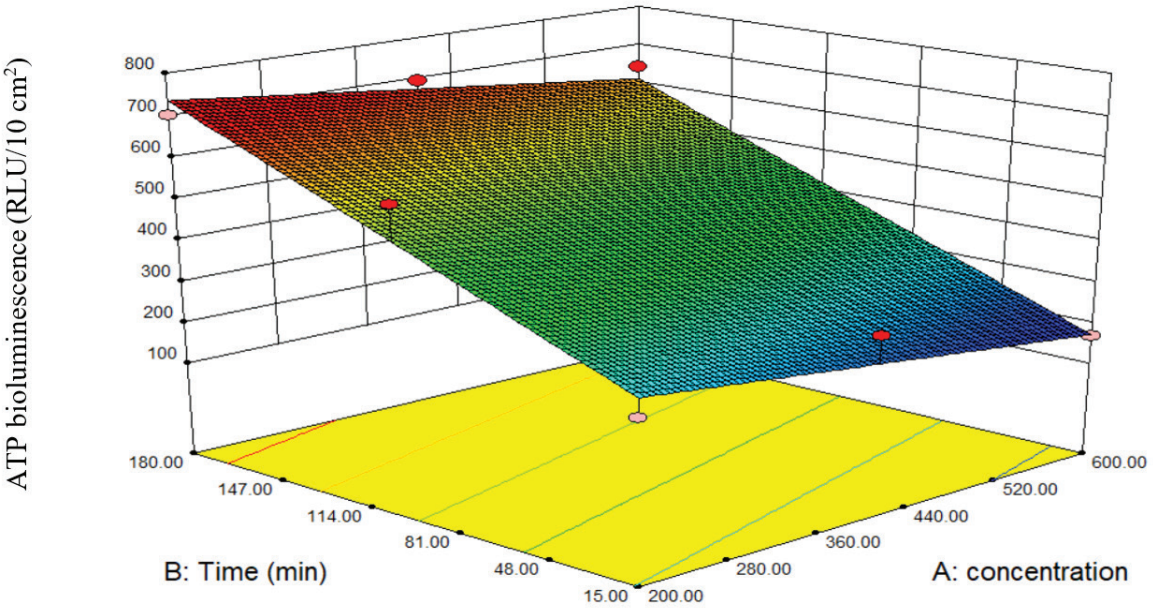

Figure S5. Three-dimensional (3D) response surface for estimating ATP-bioluminescence values (RLU/100 cm^2) for dining tables treated with H_2O_2 at different concentrations and timings.

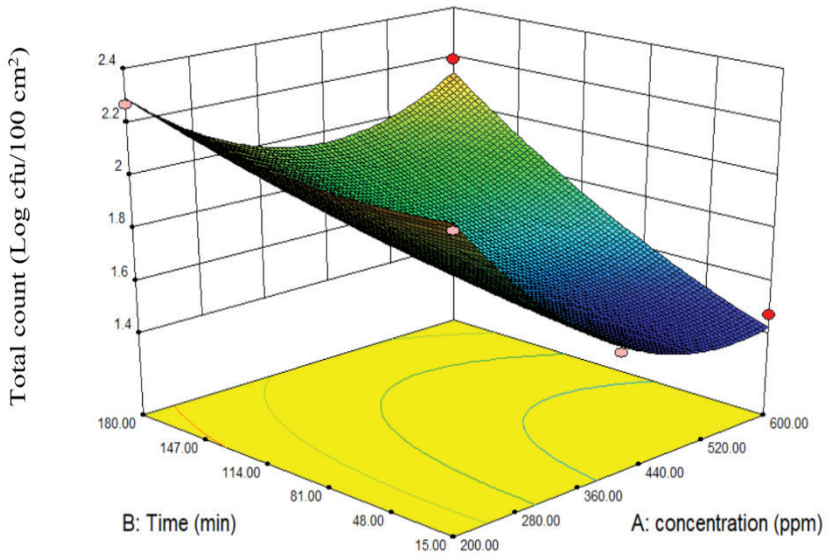

Figure S6. Three-dimensional (3D) response surface for estimating total bacterial count (Log CFU/100 cm^2) for wooden cutting boards treated with H_2O_2 at different concentrations and timings.

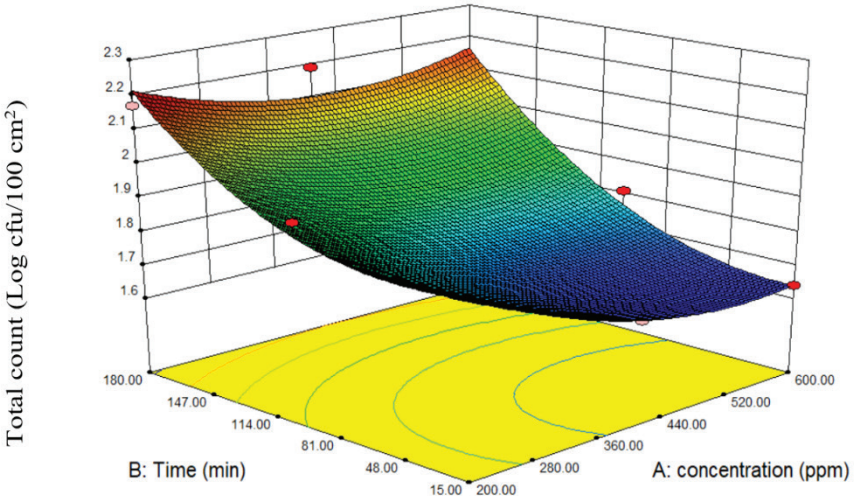

Figure S7. Three-dimensional (3D) response surface for estimating total bacterial count (Log CFU/100 cm^2) for plastic cutting boards treated with H_2O_2 at different concentrations and timings.

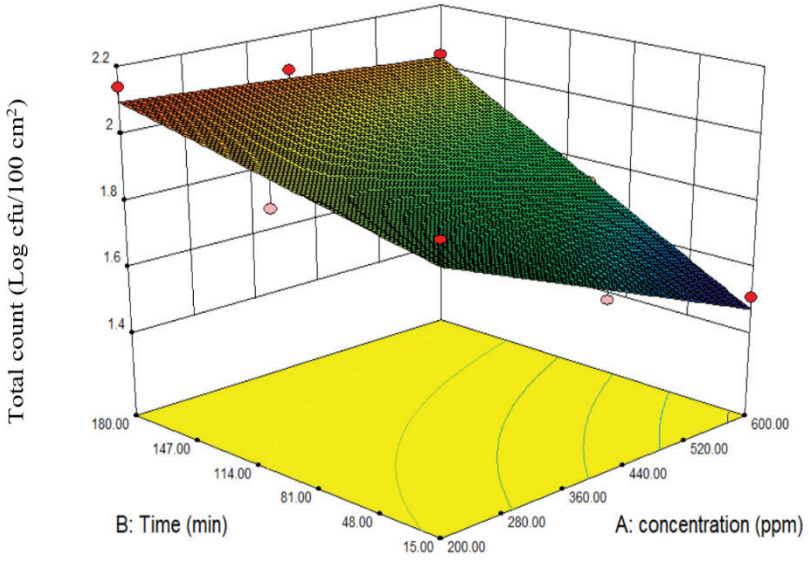

Figure S8. Three-dimensional (3D) response surface for estimating total bacterial count (Log CFU/10 cm^2) for knives treated with H_2O_2 at different concentrations and timings.

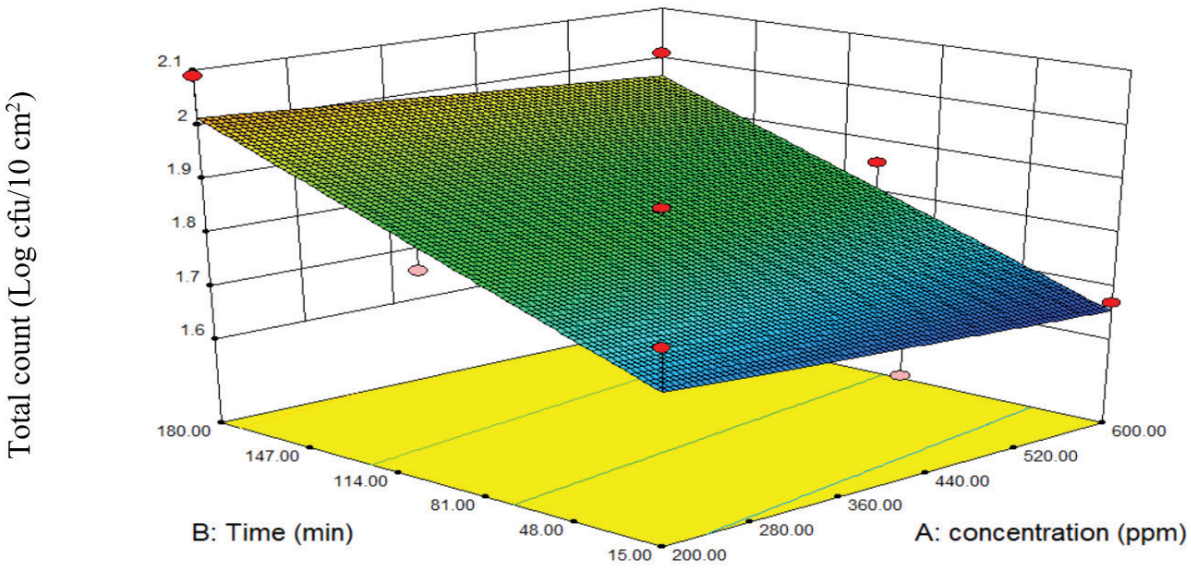

Figure S9. Three-dimensional (3D) response surface for estimating total bacterial count (Log CFU/100 cm^2) for food preparation tables treated with H_2O_2 at different concentrations and timings.

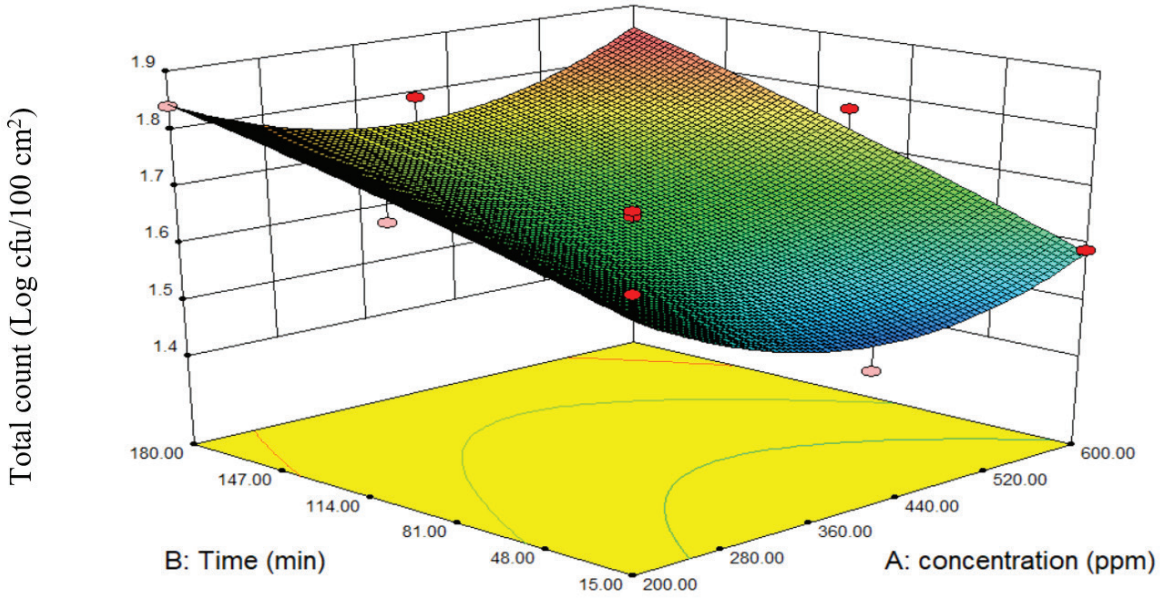

Figure S10. Three-dimensional (3D) response surface for estimating total bacterial count (Log CFU/100 cm^2) for dining tables treated with H_2O_2 at different concentrations and timings.