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Original Article *Food Hygiene*

Application of HACCP system to food preparation and services kitchens in Mansoura University hostels

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ABSTRACT

Objective: to assess to what extent the food safety principles of the Hazard Analysis Critical Control Points (HACCP) were applied at all Mansoura university hostels.

Design: Descriptive study.

Procedures: This was carried out through using a food safety checklist (49 attributes covering receiving, storing, preparation, cooking, holding and serving processes) and also through determining the microbiol quality of 100 samples from sterile milk, yoghurt, tabled egg and processed cheese (25 each) that were collected haphazardly from breakfast and dinner meals in Mansoura University hostels, with special reference to total bacterial and coliform count and isolation of Escherichia coli, Salmonella and Staphylococcus aureus which were identified by conventional bacteriological techniques and further confirmed microscopically and serologically.

Results: The finding of the observation checklist revealed that El Gomhoria city achieved the highest score (75.51%) in all investigated dimensions. On the contrary Al Amal was (55.1%), Gehan was (55.1%) and Al Zahraa was (54.42%) obtained the lowest score. The highest total bacterial count was in plain yogurt samples, followed by processed cheese samples then table egg samples, while the lowest total bacterial count was in sterile milk samples. On the other hand, the highest total coliform count was found in processed cheese samples, followed by table egg samples then plain yogurt samples, and sterile milk samples. These results were not compatible with the Egyptian standard (2005). Also, E. coli was found in 40% of the table egg samples which represent the highest percentage, Salmonella was found in 32% of processed cheese samples, and S. aureus was found in 40% of table egg samples. While sterile milk samples were free from E. coli, Salmonella species, and S. aureus.

Conclusion and clinical relevance: The general sanitary conditions concerning the production and handling of milk, dairy products and table eggs frequently introduced to the resident in Mansoura University hostels should be monitored.

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Keywords: Food safety, HACCP, E. coli, Salmonella, Staphylococcus aureus.

1. INTRODUCTION

HACCP has long been globally documented and recognized as an effective food safety organization system [1, 2]. It provides a preventive framework for recognizing the probable contamination and subsequent assessing that the procedure is in control of these steps of the food chain essential for food safety [3].

Food safety is a progressively important public health issue [2] and food safety concerns have become important factors for consumers in defining food from collection till ingestion. Food borne diseases (FBD), a result of deprived hygienic performs [4] are informed globally in which tend to overthrow the purposes of quality food donation [5].

So, assessment of bacteriological standards in dairy products is essential stage to confirm competence of HACCP plan. The total bacterial count is commonly utilized to assess the microbiological quality of foods. On the other hand, coliforms were primarily attributed to insufficient handling or processing techniques [2].

Salmonella species are ubiquitous bacteria in food industry and has been commonly reported to cause food-borne outbreaks. In particular, Salmonella enterica is one of the main causes of enteric diseases globally [6]. Also E. coli is frequently utilized as alternate indicator as food containing E. coli often indicates its fecal contamination [7]. Additionally, SFD (Staph. Food-linked disease) is a public FBD and is a chief concern for public health programs all over the world [8].

So, this study aimed to apply HACCP via a food safety checklist (forty-nine attributes covering receiving, storing, preparation, cooking, holding and serving processes) on all Mansoura university hostels to identify to what extent food safety performs were applied and determine the microbiol quality of 100 samples from sterile milk, yoghurt, tabled egg and processed cheese (25 each) that were collected randomly from breakfast and dinner meals in Mansoura University hostels, with special reference to total bacterial and coliform count and isolation of E. coli, Salmonella and S. aureus that were identified by conventional bacteriological techniques and further confirmed microscopically and serologically.

2. MATERIALS AND METHODS

2.1. Sample collection

100 samples from breakfast and dinner meals of sterile milk, yoghurt, table egg and processed cheese (25 of each type) underwent collection and were examined within 6 months from breakfast and dinner meals in Mansoura University hostels. The samples will be preserved in a protected ice box (4±1oC) to be transported to food hygiene laboratory at Faculty of Veterinary Medicine, Mansoura University and then analyzed either immediately or kept in the refrigerator for future analysis with a minimum of delay.

The checklist for assessment of food management system in university hostels were included 49 attributes covering receiving, storing, preparation, cooking, holding and serving processes are summarized in Table 1 [9].

2.2. Bacteriological examination:

Total aerobic bacterial count: It was carried out using the pour plate method according to [10]. While total coliform count: As shown by [10]

Isolation and identification of E. coli This was performed according to [11]. and serologically identified using the slide agglutination method as explained by Forbes et al. (2007) utilizing rapid commercial analytical polyvalent and monovalent E. coli agglutinating antisera groups (DENKA SEIKEN Co., Japan).

Isolation and identification of Salmonella was performed according to the method explained by ISO 6579 (ISO 2002) for further morphological, biochemical and serologic identification of Somatic (O) and flagellar (H) antigens utilizing DENKA agglutinating antiserum sets (DENKA SEIKEN Co., Japan) of Salmonella.

Isolation and identification of S. aureus. Biochemical identification by catalase and tube coagulase tests was applied as described by Robert et al. [12].

2.3. Ethics statement:

The collecting of samples that were used in this research monitored the rules of Mansoura University also the practice of this research was accepted by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (code No M/54).

2.4. Statistical analysis:

All statistical results were evaluated using Microsoft Excel® 2013 and the SPSS software, and the data was tabulated and assessed. For quantitative findings, the statistical markers of central tendency are the mean, median, standard error and mode.

3. RESULTS

The findings of the observation checklist are illustrated in table (1) summarize the level of food safety practices' achievements in the investigated Mansoura university hostels. As shown in the mentioned table revealed that, El gomhoria achieved the highest score (75.51%) in all investigated dimensions. On the contrary Al Amal (55.1%), Gehan (55.1%) and Al-zahraa (54.42%) hostles obtained the lowest score.

The study revealed that the highest total bacterial count was in plain yogurt samples with a mean count of (8.26 $x10^3$ CFU/ml), followed by processed cheese samples with a mean count of (7.45 $x10^3$ CFU/ml), then table egg samples with a mean count of (4.83 $x10^3$ CFU/ml), and sterile milk samples with a lowest mean count of (12.38 $x10^2$ CFU/ml) as shown in table (2).

The highest total coliform count was found in processed cheese samples with a mean count of ($2.61x10^2$ CFU/ml), followed by table egg samples with a mean count of ($2.35x10^2$ CFU/ml), then plain yogurt samples with a mean value of ($1.77x10^2$ CFU/ml), and sterile milk samples with a lowest mean count of ($0.35x10^2$ CFU/ml) as shown in table (3). These results were not compatible with the Egyptian standard (2005).

In table (4) all investigated samples of sterilized UHT milk were negative for E. coli, Salmonella and S. aureus, while in case of yogurt samples, our results showed that (36%) of samples were tainted with E. coli, (24%) of yoghurt samples were tainted with Salmonella and (20%) of samples were contaminated with S. aureus, also in processed cheese samples, the present study declared that (36%) of the tested samples harbored E. coli, (32%) of tested samples harbored Salmonella and (28%) of tested samples harbored S. aureus, finally in table egg samples, the present study declared that (40%) of the tested samples harbored E. coli, (24%) of samples harbored Salmonella and (40%) of tested samples harbored S. aureus.

The serotypes and pathotypes of the isolated E. coli from the investigated samples were O114:H21 (yoghurt, table egg), O146:H21 (processed cheese, table egg), O119:H6 (yoghurt, processed cheese, table egg), O44:H18 (yoghurt, table egg), O1:H7 (yoghurt, processed cheese), O8:H21 (yoghurt, processed cheese, table egg), and unclassified E. coli (yoghurt, processed cheese, table egg) as shown in table (5).

Serologically identified serotypes, O serogroups and pathotypes of the Salmonella isolates that gathered from the investigated samples included, S. Entertidis (O1.9.12 :Hg, m), S. Typhimurium (O1.4.5.12 :H:1,2), S. infantis (O6.7.14 :H:r:1,5), S. Virchow (O6.7. :H:r:1,2) as well as unclassified salmonellae as shown in table (6).

4. DISCUSSION

The findings of the observation checklist are illustrated in table (1) reflect fair level of food safety practices regarding preparing, cooking, receiving, storing, holding and serving of food in Mansoura university hostels. These findings were lower than that obtained by Ali et al. 2018 who found that El Gomhoria university hostel achieved 83.40%, Al Amal 80.6%, Gehan 79.04% and Al Zahraa 74.9%.

In sterilized milk samples, the results of TBC as shown in table 2 indicate low hygiene during processing, handling and production since they lacked the compatibility with the Egyptian Standard (2005) which states that TBC of UHT milk must not exceed 10 CFU/ ml. This finding was found against

the results by [13] who found that TBC were <10 CFU/ml in investigated UHT milk brands. Also, TCC was not compatible with the Egyptian Standard (2005) stating that UHT milk should not contain Coliforms (table 3) and this was inconsistent with the findings of [13], and this is a feedback of their poor hygienic quality and/or faecal contamination during production that often cause quick deterioration of the products and if consumed could result in significant health hazards [14].

Table 1: Assessment of food safety practices in the investigated Mansoura university hostels.

Items	MANSOURA UNIVERSITY HOSTLES			
	EL GOMHORIA	EL ZAHRAA	GEHAN	EL AMAL
Receiving Section				
1. The receiving area is cleaned and free of trash, insects and rodents.	**3**	**1**	**2**	**1**
2. The receiving equipment is available in a good condition.	**3**	**1**	**1**	**1**
3. Food is rejected if received at unsafe temperatures.	**2**	**1**	**1**	**1**
4. Frozen / refrigerated food is stored immediately.	**3**	**2**	**2**	**2**
5. Food is not accepted in badly, damaged, soiled, and infested condition.	**3**	**3**	**3**	**3**
6. Food labels & dates are checked and controlled in receiving.	**3**	**3**	**3**	**3**
7. Chilled and frozen foods are received in proper temperature.	**3**	**2**	**2**	**2**
8. The delivery vehicles are cleaned, and in good condition.	**2**	**2**	**2**	**2**
Storing area				
a-Dry store				
9. Dry goods are stored in healthy airtight containers / sealed packets / no cartons	**2**	**2**	**2**	**2**
10. Dried food items are stored at least 6 inches away from walls and above the floors.	**3**	**2**	**2**	**2**
11. Dry storage is clean, organized and shelves not rusty.	**1**	**1**	**1**	**1**
12. Temperature / ventilation of dry storage is adequate.	**1**	**1**	**1**	**1**
13. The dry store is clean, well-lighted and protected from insects and rodents.	**2**	**2**	**2**	**2**
14. All products are labeled with name and date (expiry/delivery)	**3**	**2**	**2**	**2**
15. Raw material arranged and utilized on FIFO (first in first out) basis.	**3**	**2**	**2**	**2**
16. Raw materials are stacked properly (heavy cartons, glass jars stored on lower shelves)	**3**	**2**	**2**	**2**
17. Chemicals are stored in separate room	**3**	**3**	**3**	**3**
b-Cold store (refrigerators and freezers)				
18. Proper temperatures are maintained (≤4ºC for chiller and -18ºC for freezers).	**3**	**3**	**3**	**3**
19. General cleaning (walls / floors / doors / shelves / light fitting)	**2**	**1**	**2**	**2**
20. Cooked foods are stored above or separately from raw foods.	**3**	**2**	**2**	**2**
21. Food is stored away from floor and placed in clean containers.	**2**	**2**	**2**	**2**
22. Cold storage room is not over-loaded with food products.	**2**	**1**	**1**	**1**
23. Calibrated thermometer is used for checking temperature.	**0**	**0**	**0**	**0**
24. Food from opened cans is decanted into healthy containers and labeled	**1**	**1**	**1**	**1**
25. Products with strong odors are kept covered.	**3**	**2**	**1**	**1**
26. Frozen foods are kept tightly wrapped or packaged to avoid freezing burns.	**3**	**2**	**2**	**2**

Food preparation and cooking area					
27. The preparation areas are always clean, well-lighted, well ventilated and absence of insects and rodents.	**3**	**1**	**1**	**2**	
28. Preparation and cooking equipment and tools are available in good condition.	**3**	**2**	**2**	**2**	
29. Available colour code of cutting boards and knives for raw & cooked items	**1**	**0**	**0**	**0**	
30. Cutting boards, meat blocks and surfaces cleaned, free from splits and sanitized.	**1**	**1**	**1**	**1**	
31. Food is defrosted under temperature-controlled condition	**1**	**1**	**1**	**1**	
32. Thawed products are immediately utilized and not refrozen	**3**	**2**	**2**	**2**	
33. Vegetables are being properly washed in separate sink	**2**	**2**	**2**	**2**	
34. Prepared foods are always covered.	**1**	**1**	**1**	**1**	
35. Documented cooking methods available	**0**	**0**	**0**	**0**	
36. Minimum cooking core temperatures are checked (75˚C – 167˚F)	**0**	**0**	**0**	**0**	
37. Frying oil/fat is changed immediately when there is color alteration or scum formation.	**2**	**2**	**2**	**2**	
38. Cooked food is not left at room temperature for > 2 hrs.	**3**	**1**	**2**	**2**	
39. All uncooked salads, fresh fruits & vegetables etc. are freshly prepared to the extent possible.	**3**	**2**	**2**	**2**	
Food holding and serving					
40. Hot holding units are being pre-heated before placing of food inside them.	**3**	**0**	**0**	**0**	
41. All units are satisfactorily being pre-chilled before food is decanted for holding/display.	**2**	**2**	**2**	**2**	
42. Food display is presentable, well arranged and attractive.	**3**	**2**	**2**	**2**	
43. Food in hot/cold holding units is sufficiently protected from contamination	**2**	**1**	**1**	**1**	
44. Suitable serving utensils are provided.	**3**	**3**	**2**	**2**	
45. Hot foods are served hot and cold foods are served cold.	**3**	**2**	**2**	**2**	
46. Products displayed are labeled and product's name are written clearly on the label	**3**	**3**	**3**	**3**	
47. Staff of service wear clean uniform and follows good personal hygiene habits.	**1**	**1**	**1**	**1**	
48. Personal hygiene messages displayed on prominent places.	**3**	**2**	**2**	**2**	
49. Table wares are available and in good condition.		**3**	**3**	**3**	**3**
Total		**111**	**80**	**81**	**81**
Max Score		**147**	**147**	**147**	**147**
%		**75.51**	**54.42**	**55.1**	**55.1**

The score (+++ good 3, ++ moderate 2, + fair 1, - Absent 0)
Overall score (≥85% good, ≥65% moderate, ≥50% fair, ≤50% unsatisfactory)

Table 2: Descriptive statistics of total bacterial count (TBC) in sterile milk, plain yoghurt, processed cheese and table egg samples.

Type of samples	Number of examined samples	Minimum	Maximum	Mean ± SE
Sterile milk	25	0.4 $x10^2$	80$x10^2$	12.38 $x10^2$ ± 358.54
Plain yoghurt	25	0.1 $x10^3$	82 $x10^3$	8.26 $x10^3$ ± 3464.80
Processed cheese	25	10 $x10^2$	81 $x10^3$	7.45 $x10^3$±3165.7
Table egg	25	10 $x10^2$	20 $x10^3$	4.83 $x10^3$±835.12

Table 3. Descriptive statistics of total coliform count (TCC) in sterile milk, plain yoghurt, processed cheese and table egg samples.

Type of samples	Number of examined samples	Minimum	Maximum	Mean ± SE
Sterile milk	25	0.00	1.02 $x10^2$	0.35 $x10^2$ ±6.02
Plain yoghurt	25	0.11 $x10^2$	5.11 $x10^2$	1.77 $x10^2$±32.72
Processed cheese	25	0.17 $x10^2$	5.40 $x10^2$	2.61 $x10^2$±32.85
Table egg	25	0.11 $x10^2$	8.90 $x10^2$	2.35 $x10^2$±40.20

In yoghurt samples, the finding of TBC according to table 2 does not agree with the reported range of TBC in examined yoghurt samples in other previous studies of [15]. Higher results were obtained by [16] due to the improper hygienic measures and heat treatment during yoghurt processing and manufacturing. The results of TCC in table 3 failed to comply with Egyptian Standards (2005) which stated that TCC must be nil in yoghurt. Furthermore, higher TCC in yoghurt was revealed by [17],and lower TCC was recorded by [18]. The presence of Coliforms in yoghurt samples is indicative of post-processing yoghurt contamination because Coliforms to survive heating during the manufacturing process.

In processed cheese, the results of table 2 for TBC do not agree with the previous finding of [19], while nearly similar to those observed by [20].Table 3 was shown the results TCC in processed cheese that don't agree with Egyptian standards (2005) as the acceptable critical limit for TCC was 10 CFU/g. The obtained results of processed cheese are higher than the previous results by [21] study that reported a maximum value of TCC in spread processed cheese that reached 2.1x102 CFU/ml. Dairy foods contamination with Coliform could be linked to the poor quality of added ingredients which is considered a plant problem. Moreover, the prevalence of any member of Enterobacteriaceae family is undesired in heat-treated dairy products, which suggests post – pasteurization contamination [22].

Regarding to table egg, results of TBC and TCC were shown in table 2 and 3. [23] stated the mean log counts for table egg contents of 7.26, 6.54, 7.18 and 6.9 for Kukuo, Lamashegu, Aboabo and Tamale city, correspondingly. In addition, [24] stated the mean viable counts of > 7.0 log10 CFU/mL in retail egg contents. In contrast, [25] found lower CFU/mL (3.02 log10) of average TBC in table egg contents obtained from Taif city (Saudi Arabia). Aerobic plate count is a major consideration for food examination. It reveals the sanitary procedures during processing, handling as well as storage. TCC are usually used as a sign of heat treatment failure and post-heat treatment contamination [26].

The absence of sanitation and individual's hygienic measures during production of dairy products makes these products reservoir for E.coli and thus denotes a serious health concern to humans and food safety [27]. Pathogenic strains of E. coli and Salmonella are commonly associated with consumption of raw or insufficiently heat-treated dairy products from in rural areas and local groceries of Mansoura city [28].

In our study, 36% of yoghurt samples showed contamination with E. coli (O114:H21, O119:H6, O44:H18, O1:H7, O8:H21 and unclassified E. coli) serotypes (table 4 and 5) that were also isolated in another previous Iranian research by [29]. Our results not agree with [30] who recorded that the yoghurt samples were free from E.coli.

In our study, 36% of the tested processed cheese samples harbored E. coli (table 4 and 5) which reflects a certain degree of hygienic quality, storage conditions, and heat treatment applied during the manufacturing of tested processed cheese. Greater incidence 96% in cheese was revealed by [31]. On the other hand, lower E. coli prevalence (12.9%) in cottage cheese was revealed by [32].

Table 4. Prevalence of some food poisoning pathogens in examined samples.

Type of samples	Number of examined samples	*E. coli*		*Salmonella* spp..		*Staph. aureus*	
		+ ve	%	+ ve	%	+ ve	%
Sterile milk	25	0	0%	0	0%	0	0%
Plain yogurt	25	9	36%	6	24%	5	20%
Processed cheese	25	9	36%	8	32%	7	28%
Table eggs	25	10	40%	6	24%	10	40%

Table 5: Serotypes of *E. coli* isolates isolated from the examined samples.

Serotypes	Number of isolates	Distribution of E. coli isolates			
		Sterile milk	yoghurt	Processed cheese	Table eggs
O114:H21	2	-	1	-	1
O146:H21	2	-	-	1	1
O119:H6	5	-	1	1	3
O44:H18	3	-	1	-	2
O1:H7	2	-	1	1	-
O8:H21	5	-	1	2	2
Unclassified E. coli	9	-	4	4	1
Total	28	-	9	9	10

In addition, 40% of the tested table egg samples harbored E. coli (table 4 and 5). Such finding was consistent with [33] in Trinidad. They found that 71/184 (38.6%) of table eggs positive for enteric pathogens such as E. coli, Salmonella, etc. Also, another work revealed a 40.30% contamination of table eggs with E. coli as most prevailing organism. Such differences may be because of differences in management, handling and hygienic measures at farm and/or sale outlets. The poultry eggs could become contaminated either horizontally (through their shells) or vertically (trans-ovarial), and can be a potential source of microbes causing FBD [34].

Non-typhoidal Salmonella spp. is an important microbial hazard linked to consuming dairy products that are produced either from raw milk or from milk that underwent contamination post-pasteurization by food handlers predominantly in developing countries due to inadequate hygiene. Although, Salmonella usually undergo destruction or inactivation during the fermentation of high-acid products like yoghurt when the pH is less than 4.55, and this is the main cause of Salmonella absence in investigated yoghurt samples in previous study performed by [35] and Salmonella cannot rule out yoghurt as a source for Salmonella infection [36], but in our study, (24%) of the yoghurt samples showed contamination with salmonella (table 4 and 6).

In spite of the absence of Salmonella in all Egyptian studies examining the processed cheese, the culture method in our work showed that Salmonella existed in 32% of processed cheese samples and they was serologically serotyped as (S.Entertidis, S.Typhimurium, S.infantis, S. Virchow and unclassified salmonellae) which were universally reported as the commonest etiology of salmonellosis and food-borne gastroenteritis outbreaks in humans [37].

The existence of Salmonella spp. in our study may be because of the fact that processed cheeses encounter many materials with high potential of carrying microbes from animal or human feces particularly if processing occurs under unhygienic measures at a lower temperature than that essential for pasteurization [38].

In our study, 24% of tested table egg samples harbored salmonella (S. entertidis, S. typhimurium and S.infantis) (table 4 and 6). Three serotypes were detected including S. enteritidis, S. ohio and S. infantis [39]. In a previous study of the UK Food Standards Agency in 2003, none of the 4753 pooled egg contents of retail samples contained Salmonella [40]. Our results were greater compared with the prevalence in other countries as reviewed by [41]: Italy (3.1%), Austria (1.1%), Spain (8.1%) and Greece (3.8%).

The detection of S. aureus in yoghurt often indicates contamination by food handlers due to hand or arm lesions associated with S. aureus infection or due to cough and sneezing related to respiratory infections or in symptomatic carriers who handle the food. In our study (table 4), 20% of examined yoghurt samples were tainted with S. aureus. Greater incidence was reported by [42].

Our results revealed that 28% of processed cheese samples were tainted with S. aureus. According to [43], in three hundred milk and cheese samples, S. aureus was the predominant species. S. aureus could grow during production depending upon the rapidity of acid production. The significance of detecting S. aureus in food suspected to cause staphylococcal poisoning has to be interpreted cautiously. Though food should contain at least 106 enterotoxigenic S. aureus cfu/g to cause disease, small numbers of S. aureus in thermally-processed food might represent the survivors of very large populations.

Table 6. Serotypes of *Salmonella* isolates isolated from the examined samples.

Serotypes	Antigenic structure	No. of isolates	No. of *Salmonella* isolates from examined samples			
			Sterile Milk	Plain yoghurt	processed Cheese	Table egg
S. entertidis	O1.9.12 :Hg, m	5	-	1	1	3
S. typhimurium	O1.4.5.12 :H:1,2	4	-	1	1	2
S. infantis	O6.7.14 :H:r:1,5	3	-	1	1	1
S. virchow	O6.7. :H:r:1,2	2	-	1	1	-
Unclassified *salmonellae*		6	-	2	4	-
Total		20	-	6	8	6

Regarding to the (Egyptian Organization for Standardization and Quality Control, 2007), which stated that S. aureus must not be found in egg content (Nil), it was noticed that there were samples that failed to achieve the Egyptian Standard levels with incidences of 40% of table egg samples were tainted with S. aureus in our study. Comparable

with this result, Stępień et al. (2009) reported 19.8% S. aureus in table eggs. There are many important points contributing to eggs' contamination with microbes in the pathways of reaching the public. These include environment, storage, transportation as well as handling [34].

Conferring to the definition of UHT procedure, the existence of pathogens in UHT milk must be decreased or not at all [44]. In our study, all investigated samples of sterilized UHT milk were negative for E. coli, Salmonella and S. aureus (table 4). Comparable results of E.coli and S. aureus in UHT milk were revealed by [45].

Conclusions and future prospective

In the present study, extremely pathogenic bacteria were isolated in dairy products and table eggs daily consumed by the resident in Mansoura student hostels. This establishes a potential hazard to human health. So, food safety management system in the investigated properties should focus particular emphasis on following and adopting food safety practices in all food handling procedures receiving, storing, cooking, holding and serving of food in Mansoura university hostels. In addition, the application of well hygienic measures along with hazard examination and risk-based defensive control measures are significantly necessary in the procedure of the HACCP strategy to reduce contamination risk during manufacturing instead of dependence on final-product examination.

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Conflict of interest

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Authors' contributions

All authors contributed equally to this manuscript.

Consent to participate

Not applicable

Consent for publication

All authors agree to publish the findings of the current research.

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