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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.01.006

The environmental influences on the bacteriological quality of red and chicken meat stored in fridges

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ARTICLE INFO

Article history:
Received 27 Jun 2016
Received in revised form 18 Aug, 2nd revised form 30 Aug 2016
Accepted 19 Nov 2016
Available online 7 Jan 2017

Keywords:
Fridges
Environmental health
Meat
Chicken
Bacteriological
Quality control

ABSTRACT

Objective: To investigate the environmental influences on the bacteriological quality of red and chicken meats on fridges.

Methods: The environmental health status was determined by reliable and valid researcher-made checklist. Then 264 samples were gathered in two phases (at the entrance and three months later) and examined for total bacteria count, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp.

Results: The result revealed that the mean of total bacteria count, *E. coli* and *S. aureus* densities had significant differences in two steps on chicken and the red meat samples ($P < 0.05$). Among the environmental factors, sanitary status, temperature and personal hygiene had significant effects on total bacteria count and *S. aureus* densities in chicken samples ($P < 0.05$), and between wastewater and solid waste disposal with *E. coli* density in red meat samples ($P < 0.05$).

Conclusions: The results implied that the bacteriological quality of red and chicken meat fluctuates with environmental status (especially temperature, sanitary status and personal hygiene). Regular control, improving of sanitary health, and staff training are necessary for elimination of bacterial contamination.

1. Introduction

Regular and technical health inspection of dietary places, including warehouses and fridges and controlling of the effective factors on the bacteriological quality of food and their spoilage is the main duties of sanitation managers and food quality control officials [1]. Exposure with popular consumed food products, including red and chicken meat which had chemical and microbial contaminants can lead to life quality depletion, spoilage and get off the consumption cycle during the storage and preservation in fridges and warehouses [2–4]. Several factors affect the growth and amplification of microorganisms, quality depletion and spoilage of food products among which environmental health and operation and maintenance parameters including temperature, personal hygiene, food storage pattern, product turnover are the most important invest management parameters from point of quality control [5–8]. In addition, health educations of staffs have impacts on the microbial contamination and quality of preserved products, which can lead to bacterial colonization risk, food spoilage and economical loss. Loss of the spoilage food materials is one of the public health concerns which can lead to loss of sustainable food security and invest management.

Kock and Casi reported that fridges operation and maintenance problems were relevant to poor training of operators [9]. Cerveny *et al.* reported that microbial spoilage is one of the most important concerns in worldwide that annually lead to 3.5 million tons of red meat and poultry getting of the consumption cycle by consumers, retailers and restaurants. Animal slaughtering inappropriate packaging and storage conditions are the predominant factors in microbial spoilage [10]. Marsh focused the economic loss of food spoilage [11].

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Foundation Project: Supported by the Health Research Center and Faculty of Health of Baqiyatallah University of Medical Science (Grant No. BG52142).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Several factors have direct and indirect adverse effect of food spoilage, among which psychrophilic bacteria including *Clostridium botulinum* type E, *Yersinia entrotolytica*, entrotoxigenic *Escherichia coli* (*E. coli*), *Listeria monocytogenes*, *Salmonella entritidis*, *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* that are resistant and growth temperature higher than 3–5 °C is very critical in food spoilage. Therefore, inappropriate environmental status in fridges enhance the amplification conditions for their colonization in food material textures and lead to food spoilage.

Due to temperature has a critical role in bacterial colonization, several studies focused on operating and maintenance of fridges in appropriate temperature and keeping records of this parameter for prediction and prevention of bacterial contamination risk and food spoilage [3,5].

According to role of environmental influences and temperature fluctuation on quality of preserved foods in fridges, several researchers [6,11,12] associated the effects of these factors on foods bacteriological quality [6,12]. Boysen *et al.*, clearly associated the effects of temperature fluctuations in warm seasons on red meat contamination with different bacteria [11]. Sumner *et al.* reported the effects of staff's hygiene status on food spoilage [6]. Koro *et al.* demonstrated the effects of inappropriate storage conditions, personal hygiene, building status and non-regulated storage from point of first in/first out pattern on red and chicken meat contamination with pathogens including *Salmonella*, *E. coli*, and *S. aureus* [13]. Based on Cosansu and Ayhan report, more than 30 percent of consumed meats in worldwide are chicken products which have more frequent cross-contamination that originated from devices, equipment and staff [14].

Denny and McLauchlin reported contamination risk of fridge preserved cheese with *L. monocytogenes* that can survive and grow at higher than zero centigrade and inappropriate environmental status and personal hygiene lead to bacterial colonization in dairy products [15]. De Giusti *et al.* reported that staffs hands contamination led to *E. coli* occurrence as a food poisoning and food spoilage agent in dietary markets [16], which revealed the role of personal hygiene and environmental health effects on food poisoning outbreaks and food spoilage.

Soltan Dallal *et al.* reported that 44% of provided meats in Tehran were contaminated by *Yersinia entrocolitica*, in which red and chicken meat had 29% and 71% of contaminations, respectively [17].

Tavakoli *et al.* studied bacterial contamination of red and white meat which is one of the most popular consumed food and reported that 38.9% and 55.6% of the 260 samples were contaminated with *E. coli* and *S. aureus*, respectively. In addition, ground red meat had higher densities of bacterial total count, compared to other consumed meat. They reported that application of contaminated appliances and lack of personal hygiene are the major relevant causes. Similar study was conducted and it reported that 85.4% and 9.57% of raw red meat had contaminated with *S. aureus* and *S. aureus* enterotoxin, respectively [18].

These researches revealed that in Iran only several studies described bacterial food contaminations and analytical research which can declare causalities of these contaminations were not conducted. Therefore, this study was conducted on bacterial contamination of red and chicken meat and identification of the role of environmental status in this phenomenon.

2. Materials and methods

This descriptive-analytical study was performed in 11 sub-zero centigrade fridges in Tehran province. The research was done in 3 separated phases including, evaluation of sanitation status of the fridges, determination of bacteriological quality of red and chicken meat, and association of food bacteriological quality in accordance with sanitation status in fridges.

2.1. Environmental health status

Environmental health status of fridges was evaluated by a researcher-made checklist and its reliability and validity was determined via Cronbach alpha (α = 0.7) expert opinion, respectively. Trained and experienced experts filled the reliable and valid checklist during observation and interview processes with fridges' officials and document analysis. The checklist had 50 questions that were designed in two parts including demographic information and environmental health parameters. Important parameters were relevant to temperature control, goods' storage duration time, building sanitation, appropriate site selection, staffs hygiene, and fridge equipment. The question score and weight coefficient varied between 0–2 and 1–3, respectively, which provided 150 total score for questioner. Three levels were recognized for environmental health status of fridges including excellent (> 120), medium (90–119 points) and poor (< 90).

2.2. Bacteriological quality of meats

2.2.1. Sampling

A total of 264 samples consisting of 132 samples of red meat and the equal number of chicken meat were taken with sterilized bags in accordance with Iranian Standard Industrial Research Institute (ISIRI) procedures (SN: 2836) for food quality control. Sixty-six randomized samples were taken when they were loaded and equal samples after three-month lapse time and preservation in fridges. Five aliquots of 50 g meat specimens were taken from different parts of meats, mixed and transported to laboratory under sterile condition. [19].

2.2.2. Bacterial growth

The samples were defrosted in temperature of 10 °C and 25 g of the samples was mixed and homogenized with 225 mL of diluted solution within the sterilized bacteriological cabinet. Determination of bacteria was performed via culture into brilliant green bile broth and VRBA media, green broth and peptone water, specific environment of Baired Parker and lactose broth, tetrathionate broth, selenite cystine broth culture media for isolation of bacteria and total bacteria count (TBC) of *E. coli*, *S. aureus* and *Salmonella*, respectively and incubation after 24–48 h (37 °C) [19].

Isolation of *E. coli* was done by using green broth media and peptone water for 24 h incubation (37 °C). Kovacs reagent and IMVIC test were used for *E. coli* positive test confirmation.

S. aureus isolation was done by surface culture procedure onto Baired Parker media, positive test confirmation was done by morphological and coagulase tests.

Salmonella positive test was confirmed after culture on selective and non-selective media and culture on *Salmonella*-

Shigella agar selective solid environments and bright green agar in a linear manner. Then, doubtful colonies were transferred to different cultural environments like TSI, lysine iron agar and urea and were tested for the presence or absence of *Salmonella*. Finally, the results of these experiments were compared with the standard tables of the ISIRI and Ministry of Health regarding permissible contamination levels in foods [19].

2.3. Statistical analysis

Descriptive statistics were used for description of data and the pair-*t* tests and Spearman's rank correlations were conducted to analyse the correlation between parameters using statistical package software (SPSS 15) in which statistical results were interpreted at the level of significance $P < 0.05$. All statistics were performed with the culture results of bacteria and environmental health status of fridges which stated in the manuscript.

3. Results

Analysis of checklist showed that 9.1%, 18.2% and 72.2% of fridges have good, medium and weak status, respectively. The results of the measured environmental health parameters are demonstrated in Figure 1. According to Figure 1, 81.9% and 63.7% % of fridges have well to average status with regard to site selection and staff health status. Temperature control and sanitation are important factors which can affect the bacterial quality of preserved foods predominantly have average so, equipment and regulated time storage usually has weak and average status.

Bacteriological quality control in diets shows that several factors affect bacterial amplification and food spoilage, among which environmental factors have predominant roles. The results of this study showed that 9.1% of the studied fridges had good environmental health status. Inappropriate inspection and control of temperature, lack of sanitary condition, application of inappropriate appliance and unregulated preservation duration time were the most unfavorable hygienic status in fridges (Figure 1).

Tables 1 and 2 showed the bacteriological quality of chicken and red meats in entrance time to fridges. According to Tables 1 and 2 all of samples before preservation complies with the ISIRI

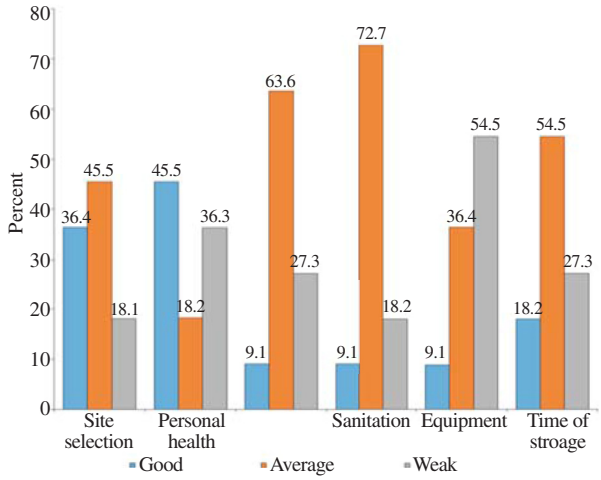

Figure 1. Score of environmental health parameters of fridges (percent).

recommended values. Statistical analysis revealed that the means of bacterial load of meats has significance differences before preservation and after three-month lapse time in fridges ($P < 0.05$).

Analysis of bacteriological quality in chicken meat samples after three months in fridges showed that 6.0%, 4.5% and 4.5% of samples were not complied with the recommended standards with regard to *E. coli*, *S. aureus* and *Salmonella* contamination. All of the contamination was observed in the fridge number 10 which had the lowest score in the environmental health evaluation (Table 1).

Bacteriological quality of red meat after three months preservation in fridges showed that in sixty-six samples, only 3% of samples have no compliancy with goal standard with regard to *E. coli* and *Salmonella* contamination. All of observed contamination was relevant to the fridge number 10 which had the lowest score in the environmental health evaluation (Table 2). According to these researchers and the results of the present study elongated preservation time of meats (red and chicken) can lead to bacterial colonization in meats. Bacteriological quality assessment after three months lapse time preservation showed that the TBC in chicken and red meat samples was permissible (10^7 CFU/g). But 9.1% and 18.2% of samples have no permissible level for *E. coli* and *S. aureus* contamination respectively. Comparison of the statistical results tests on chicken samples showed that the average of TBC, *E. coli* and *S. aureus* counts had significant correlation with unfavorable environmental health status (fridges number 3, 5, 7, 8, and 10) which led to elevated and significant level of contamination ($P < 0.05$) and impermissible levels on contamination (Tables 1 and 2). Also, the results of the statistical test on red meat samples showed that the average of TBC and *E. coli* before and after three months of storage significantly increased in fridges number 3, 5, 7 and 10 ($P < 0.05$). The same results were showed in red meats, which were relevant to unfavorable sanitation status in similar fridges that had lower scores (Table 1). This means that environmental health status and appropriate operation and maintenance have significant impacts ($P < 0.05$) on microbial quality of the preserved foods. Although environmental health factors play a key role in bacterial colonization, unregulated temperature fluctuations and inappropriate status of sanitation are crucial.

According to Table 3, there is a significant statistical relationship between the environment health status of the fridge and bacteriological quality of red and chicken meat (except for *Salmonella*). Table 3, revealed that temperature control, personal hygiene, and sanitary disposal of wastewater and solid waste had significant and non-significant effects on *S. aureus* and TBC, *E. coli* and *Salmonella* spp contamination, respectively, ($P < 0.05$, $P > 0.05$).

As demonstrated in Table 3, in red meat, sanitary status of fridges, temperature control, sanitary disposal of wastewater and solid waste have significant effects on TBC and *E. coli* contamination, respectively ($P < 0.05$). In chicken meat samples, temperature control and staff personal hygiene have significant impacts on TBC and *S. aureus* occurrence ($P < 0.05$). According to ISIRI, *S. aureus* test was not conducted in red meat samples. Sanitation is another environmental parameter which influence the bacterial load of preserved foods in fridges. As demonstrated in Table 3, sanitation has significant effects on TBC of red meat samples.

Table 1
Comparison of TBC, *E. coli* and *S. aureus* (CFU/g) in chicken samples before and after storage in the fridge.

Fridge number	TBC (CFU/g)			*E. coli* (CFU/g)			*S. aeurus* (CFU/g)			*Salmonella* spp (A/P)	
	Before	After	*P*-value	Before	After	*P*-value	Before	After	*P*-value	Before	After
1	2.0×10^5	2.2×10^5	0.09	2.5×10^1	3.0×10^1	0.3	1.4×10^2	1.7×10^2	0.2	A	A
2	2.0×10^5	2.5×10^5	0.06	1.1×10^1	5.3×10^1	0.6	1.9×10^2	3.0×10^2	0.3	A	A
3	2.9×10^5	2.2×10^6	0.03	2.6×10^1	1.7×10^2	0.05	1.4×10^2	1.3×10^3	0.02	A	A
4	1.6×10^5	1.7×10^5	0.6	3.3×10^1	3.7×10^1	0.3	3.5×10^2	3.8×10^1	0.8	A	A
5	5.2×10^5	2.7×10^6	0.03	2.3×10^1	7.7×10^1	0.02	1.7×10^2	6.0×10^2	0.04	A	A
6	4.8×10^5	5.1×10^5	0.1	4.1×10^1	1.7×10^2	0.08	6.6×10	8.0×10^2	0.1	A	A
7	4.6×10^5	6.6×10^6	0.03	6.6×10^1	3.6×10^2	0.02	2.2×10^2	1.7×10^3	0.03	A	A
8	1.4×10^4	5.7×10^6	0.03	1.7×10^2	1.9×10^3	0.03	9.0×10^2	63.2×10^3	0.04	A	A
9	3.4×10^5	4.2×10^5	0.08	5.3×10^1	6.5×10^1	0.9	7.8×10^2	9.0×10^2	0.70	A	A
10	2.4×10^5	4.2×10^5	0.03	6.6×10^1	$43.3^* \times 10^2$	0.05	6.3×10^2	3.3×10^3	0.02	A	P
11	3.1×10^4	3.5×10^4	0.06	1.7×10^1	7.8×10^1	0.08	1.8×10^2	4.9×10^2	0.07	A	A

A: Absent; P: Present; *: Higher than Iranian standard (TBC: 10^7 CFU/g, *E. coli*: 4×10^2 CFU/g, *S. aeurus*: 2×10^3 CFU/g, *Salmonella* A/P).

Table 2
TBC, *E. coli* and *Salmonella* spp contamination in red meat samples before and after preservation in the fridge.

Fridge number	TBC (CFU/g)			*E. coli* (CFU/g)			*Salmonella* spp (A/P)*	
	Before	After	*P*-value	Before	After	*P*-value	Before	After
1	2.1×10^5	2.7×10^5	0.6	3.7×10^1	4.0×10^1	0.3	A	A
2	2.3×10^5	2.4×10^5	0.35	2.2×10^1	2.7×10^1	0.2	A	A
3	4.3×10^5	9.2×10^5	0.03	3.6×10^1	1.1×10^2	0.04	A	A
4	2.1×10^5	2.3×10^5	0.6	3.6×10^1	4.7×10^1	0.08	A	A
5	2.7×10^6	4.0×10^6	0.03	1.0×10^2	2.0×10^2	0.04	A	A
6	2.1×10^6	2.3×10^6	0.3	1.7×10^1	2.1×10	0.06	A	A
7	8.1×10^5	4.6×10^6	0.05	3.3×10^1	2.1×10^2	0.03	A	A
8	4.8×10^6	6.5×10^6	0.06	1.1×10^1	1.7×10	0.07	A	A
9	2.8×10^6	3.3×10^6	0.4	1.7×10^1	1.9×10	0.06	A	A
10	4.2×10^5	3.7×10^6	0.04	7.3×10^1	$4.1^* \times 10^2$	0.02	A	P
11	2.6×10^5	3.2×10^5	0.26	9.7×10^1	1.1×10^2	0.07	A	A

A: Absent; P: present; *: Higher than ISIRI standard (TBC: 10^7 CFU/g, *E. coli*: 5.3×10^2 CFU/g, *Salmonella* A/P).

Table 3
Association of environmental health factors with bacteriological test in red and chicken meat.

Bacteria	Overall status of environmental health		Personnel health		Sanitation status		Temperature control		Solid waste disposal		Wastewater disposal	
	C	RM	C	RM	C	RM	C	RM	C	RM	C	RM
Total count	0.03	0.02	0.10	0.07	0.07	0.02	0.10	0.04	0.10	0.10	0.40	0.20
E. coli	0.03	0.02	0.30	0.09	0.60	0.80	0.20	0.10	0.10	0.10	0.40	0.04
S. aureus	0.04	–	0.04	–	0.20	–	0.02	–	0.02	–	0.03	–
Salmonella spp	0.06	0.10	0.50	0.07	0.07	0.20	0.30	0.40	0.09	0.20	0.04	0.10

C: Chicken, RM: Red meat.

4. Discussion

In the present study, bacteriological assessments showed that all samples in entrance time complied with ISIRI standards, which confirm the role of health inspections/monitoring and purchasing from authorized provider centers which are considered in health regulations of Iranian military organization. These levels of contamination are lower than the contamination level of red and chicken meats that was reported by Soltan Dallal *et al.* and Faramarzi *et al.* on supplied meats in Tehran, which indicates the better status of the fridges in this study. Inappropriate status of personal hygiene and poor staff training may be relevant factors in bacterial contamination level [17,20–22]. The contamination level in the present study is higher than the reported values by Enayat *et al.* in Ahvaz and Sanandaj, Cho *et al.* in South Korea, and Fathi *et al.* in Egypt [23–25].

This phenomenon may be relevant to unfavorable environmental factors including temperature fluctuations, inappropriate sanitation, poor personal hygiene which complies with Meng and Doyle's report [26].

In this research *Salmonella* contamination was 3.0 and 1.5 percent in chicken and red meat samples, respectively. This level of contaminations is lower that than reported by Greeson *et al.* in Saudi Arabia and Donado-Godoy *et al.* in Colombia [27,28]. Lower level of contamination in this study may be related to favorable health status of fridges and environmental condition such as ambient temperature which is another relevant factor that may influence the bacterial colonization in foods during transportation. This environmental factor is very critical in tropical area and warm seasons, which should be considered by health services providers.

Several studies show that personal hygiene is one of the most important factors which can affect the microbial cross-contaminations. As demonstrated in this research poor environmental health status and staffs hygiene had significant effects on *S. aureus* colonization ($P < 0.05$). Similar results were reported by Bhatia and Zahoor, and Dave and Ghaly, who showed the effects of personal hygiene and hand hygiene on cross-contamination of meat [29,30]. Normanno *et al.* associated *S. aureus* contamination in chicken meat samples with personal hygiene and reported that *S. aureus* was 12.8%, which revealed low level of contamination and proper status of personal hygiene [31]. Soares *et al.* reported that in providing and distribution centers despite educational programs for the staff, 53.3% of staffs' hands had positive *Staphylococcus* coagulase test [32]. According to these results it can be concluded that personal hygiene and individual hand hygiene are very crucial in bacterial cross-contamination. So, it is advised that implementation of health education programs with focused hand hygiene has improvement effects on cross-contamination control.

Although in ISIRI standards the permissible storage duration time for frozen red and chicken meat at 18 °C is 9–12 months, preservation lapse time of this study (3 months) and elevated bacterial load in meat samples revealed that operation and maintenance of fridges was not satisfactory which led to higher bacterial colonization in preserved meats. So, it is advised that operation and maintenance, technical inspection of fridges, emergency electrical power supply and environmental factors which had significant effects on bacterial meat contamination should be considered. Also decreasing of preservation duration time can be considered for quality assurance of materials.

As discussed previously, temperature fluctuation and lake of inspection, monitoring and inappropriate operation and maintenance problems led to significant colonization of *S. aureus* and TBC in chicken and red meat samples. Lake of emergency electrical power supply system in studied fridges may lead to fluctuation in fridges temperature which enhance bacterial amplification in preserved foods. Also inappropriate arranges in fridges is another operation parameter which lead to bacterial contamination of meats in fridges. Dave and Ghaly reported that bacterial survival in freezing temperature is dependent to several factors including freezing pattern, microbial flora of meat, and temperature fluctuations [30]. Therefore, it can be concluded that bacterial colonization in meat samples may be relevant to freezing patterns in fridges [30,33,34]. Similar results were reported by Fernandes in which colonization of mesophilic bacteria including *E. coli*, *Salmonella* and *S. aureus* in preserved foods in fridge were related to temperature fluctuation and other hygiene factors during red and chicken meat storage in the fridge [35–38]. Collection and disposal of sewage and garbage are other environmental parameters that lead to significant contamination of red and chicken meat samples by *E. coli* and *S. aureus*, respectively. This phenomenon was observed in fridges which had no appropriate system for sewage and garbage collection and disposal.

Based on this research it can be concluded that environmental sanitation factors are very important in bacterial quality control of preserved meats, among which temperature control, fridges' sanitation, and personal hygiene are the most important factors, which influence bacteriological quality of the preserved meats. Therefore, environmental sanitation and emergency electrical power supply for elimination of temperature fluctuation are crucial factors.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The present study was derived from the thesis "Environmental factors effects on the bacteriological quality of red and chicken meat stored in fridges". We would like to thank the Health Research Center and Faculty of Health of Baqiyatallah University of Medical Science (Grant No. BG52142) for conducted by the financial and technical supports, respectively.

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