RESEARCH ARTICLE

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Comparative studies on two types of chilling methods in poultry slaughterhouses in Khartoum State, Sudan

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KEYWORDS

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Process steps
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ABSTRACT

The study aimed to evaluate the effect of chilling methods and their related process steps (scalding, de-feathering, evisceration, and chilling) on chicken bacterial load in poultry processing plants in Khartoum State during the period from November 2019 to May 2020. Immersion and air chilling methods were both investigated in eight poultry slaughterhouses, four slaughterhouses for each method. Both checklist and microbiological tests were used in the evaluation. A total of 160 samples were collected from 32 carcasses, four carcasses were taken after each process step, from five parts of broiler carcasses (wings, legs, thigh, breast, and backbone) and all these samples were examined bacteriologically. The samples were subjected to the total bacterial count and isolation of *Enterobacter*iacea and *E. coli*. The result revealed that the mean bacterial load count of legs samples was highly significantly greater in emersion method (2.1±.05) compared to air chilling method (.7±.1), p=0.00 Also, the result indicates highly significant differences between the two chilling methods with regard to *E. coli+Salomnella* and *E. coli+Enterobacter* (0.00), *E. coli+Shigella* (0.001), *E. coli+Citrobacter* (0.00). When comparing the chilling methods with their respective process steps, scalding process showed no significant difference between the two chilling methods. Concerning scalding water change during each shift, all slaughterhouses (100%) were not changing scalding water. Washing process step revealed no significant differences between the two chilling methods in terms of in and out washing of carcasses, P.214 and .500, respectively. For chiller temperature all plants using air chilling practiced optimal temperature (4°C or less), while three quarters (75%) of plants using immersion chilling exceeded the acceptable limit but there were no significant differences between the two chilling methods, with p>0.071. In conclusion the microbial contamination of poultry meat in slaughterhouses in Khartoum State was higher in emersion chilling method compared to air chilling method.

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INTRODUCTION

The legal frame of food safety in Sudan started with the Public Health Act (1939) which deals with food hygiene issues. Poultry industry is growing very fast since the last ten years in Sudan. This rapid growth involved diversified stakeholders in poultry production without having proper training and knowledge in hygienic practices, and food safety system include hazard analysis and critical control point (HACCP) (Mustafa *et al*., 2016).

Mustafa *et al*. (2016) reviewed research studies conducted in Khartoum State. These reflected that the situation of microbial and chemical contamination in poultry and red meat in the slaughterhouses and factories of Khartoum State was higher than the level recommended by international regulatory bodies. This was attributed to the absence of implementation of Good Manufacturing Practices and Good Hygiene Practices. This was also evidenced by Munir *et al*. (2014) who investigated the bacterial contaminants of poultry meat and poultry products in Khartoum State and revealed that *E. coli* represented the highest contaminant with prevalence of 34.6% followed by Proteus spp. 32.2%, then Citrobacter spp. 13.5% and Salmonella spp. 10.4%.

Contamination of broiler meat with Salmonella spp., *Escherichia coli* and Staphylococcus spp. was evident in many studies. Ahmed (2014) assessed the measures of poultry meat safety in one of the Khartoum State slaughterhouses with the aim to detect the bacterial load and types of bacteria on the carcasses of broiler by applying the principles of HACCP. The contaminating bacteria isolated in the study were *E. coli*, *Staphylococcus spp.* and Salmonella spp. organisms. Contamination of broiler meat with Salmonella spp. and *Escherichia coli* were predominant in slaughterhouse processing as reported by Omer *et al*. (2015) who studied poultry meat carcasses at an automatic slaughterhouse in Khartoum State.

Enterobacteriaceae is a useful indicator of hygiene and post processing contamination of heat-processed foods. This family has been used as indicators of food quality and also for food safety. Enterobacteriaceae counts are an effective method to assess environments, such as post process food contact surfaces and help to quickly determine potential sources of contamination (Halkman *et al*., 2014). To this end, quality assurance programs in slaughterhouses are applied to ensure the safety of meat for human consumption (Govender, 2014). The objective of this study was to evaluate the effect of chilling method and its related process steps on chicken bacterial load in poultry processing plants in Khartoum State.

MATERIALS AND METHODS

Study Area

This study was conducted in the three localities of Khartoum State; Khartoum, Omdurman and Bahri.

Study design

A cross sectional and analytical study that lasted for 28 weeks from November 2019 to May 2020 was carried out in eight poultry slaughterhouses. Of these, seven were commercial poultry companies and one was a commercial slaughterhouse serving small poultry farms.

Chilling methods and related process steps

Air chilling and immersion chilling were the two investigated chilling methods, whereas the related process steps were a) scalding (scald temp and turbulence used; scald time per seconds); b) De-feathering (time sprayed with water -seconds); c) Evisceration (viscera mechanically opened; chickens hanged on legs till the end of evisceration; the technique for viscera suction); d) inside and outside washing (water temp.-washing duration).

Two slaughterhouses in Khartoum locality used air chilling method, four slaughterhouses in Omdurman locality used both air chilling method (two slaughterhouses) and immersion chilling method (two slaughterhouses) and two slaughterhouses in Bahri locality used immersion chilling method (one of which was commercial serving small poultry farms). Both checklist and microbiological tests were used in the evaluation.

Collection of Samples and sample Size

Simple random swab sampling of 160 swab samples were collected from the investigated poultry slaughterhouses.

Type of Samples

All samples were taken from 32 carcasses. Twenty swab samples were collected from 4 carcasses in each slaughterhouse from 5 parts: leg, thigh, breast, wing and backbone in 4 different process steps: after scalding, after de-feathering, after evisceration and after chilling.

Sample collection and transport

The samples were taken directly during the processing in the investigated slaughterhouses which operated in the morning shift except one (the commercial slaughterhouse) operated at night.

In the slaughterhouse, an average of 11,000 birds were killed per day during one shift either in early morning or in the evening. Each day, five parts of carcasses were randomly collected before they entered the chilling system and after entering in four different process steps: scalding, defeathering, and in and out carcass washing and chilling.

Samples were packed in sterile polyethylene bags and transported to the laboratory of the College of Veterinary Medicine at University of Bahri using an insulated ice box contained an ice pack at 4°C.

Bacterial Count

The total bacterial count is one of the key indicators in the field of hygiene management. Standard plate or viable count a sample is diluted in a serious of dilution blanks, then the dilutions plated from 5th serial dilution onto MacConkey media for Enterobacteriaceae.

Microbiological analysis

Aerobic colony count (ACC), Enterobacteriaceae and coliforms using ISO 4833 and *E. coli* using ISO 16649-1, ISO 16649- 2 (2008) method were performed.

Statistical Analysis

The collected data were coded and analyzed using Statistical Packaging for the Social Sciences (SPSS/PC version 21 for windows). Data were analyzed for descriptive statistical analysis to test for significant differences ($P < 0.05$) between the different chilling methods and the related process steps.

RESULTS

The mean bacterial load count of legs samples was highly significantly greater in emersion method (2.1±.05) compared to air chilling method (.7±.1), p=.000. Also, the mean bacterial count of breast (1.8±.08 vs. 1.6±.2), thigh (1.9±.1vs. 1.8±.1), backbone (1.8±.08 vs. 1.5±.2) and wings (1.6±.1 vs. 1.5±.1) was not significantly greater in emersion method compared to air chilling method, respectively, p> 0.05 (Table 1).

Table 1: Mean of bacterial load count Log 10 (CFU/ml) X 10^5 in MacConkey Agar (MCA) media for enterobacteriaceae from different carcass sites with different chilling methods

Chilling method		Sample location				
	Statistic	legs	Breast	Thigh	Backbone	Wings
Emersion	Mean± SE	2.1±.05	1.8±.08	1.9±.1	1.8±.08	1.6±.1
	Minimum	1.7	1.3	1.2	1.2	1.0
	Maximum	2.4	2.5	2.8	2.3	2.4
Air chilling	Mean± SE	0.7±.1	1.6±.2	1.8±.1	1.5±.2	1.5±.1
	Minimum	0.3	0.00	1.00	0.00	0.7
	Maximum	2.4	2.5	2.5	2.5	2.5
Total	Mean± SE	1.4± .1	1.7±.1	1.8±.1	1.7±.1	1.6±.1
	Minimum	0.3	0.00	1.00	0.00	0.7
	Maximum	2.4	2.5	2.8	2.5	2.5
ANOVA (P-value)		0.00*	0.445	0.814	0.084	0.681

SE = Standard error of means

*P-value considered significant at less than 0.05 levels

Table (2) represents the incidences and microbial counts for enterobacteriaceae and *E. coli* in emersion and air chilling methods using Ethylene Methylene Blue (EMB). The result indicates highly significant differences between the two chilling methods with regard to *Enterobacter and Citrobacter* (.017), *Shigella* (.004), *Klebseilla* (0.001), *E.coli+Salomnella and E.coli+Enterobacter* (0.00), *E.coli+Shigella* (0.001), *E.coli+Citrobacter* (0.00), *and Enterobacter+ Citrobacter* (.041).

This study also investigated the two chilling methods with their related process steps using a checklist. Concerning scalding water change during each shift, all slaughterhouses (100%) were not changing scalding water during the shift. Also, no significant difference was found between scalding water change during the shift and all chickens immersed together (Table 3).

Table (4) shows the efficacy of defeathering machines to keep chickens not to fall down, proper cleaning of defeathering fingers and chicken pass with feather return to machine for more trimming in the two methods. Nonetheless, no significant differences were found between the two methods, with p> 0.214.

Table (5) shows that no significant difference was found between the two chilling methods in terms of opening viscera mechanically, chicken hanged on legs until evisceration process step finished, technique for viscera suction and in and out washing machine for carcasses. No significant differences were found between the two methods, with p> 0 .214.

Table (6) revealed no significant difference between the two chilling methods in terms of in and out washing of carcass and availability of potable water free from salt with p .214 and .500, respectively.

The study revealed that sodium chloride was used in three quarters 3 (75%) of the slaughterhouses using immersion chilling method and one quarter used citric acid, while half (2) (50%) of the slaughterhouses using air chilling method used sodium chloride. There was no significant difference between the two chilling methods, with p>.500.

The study also revealed that only half of the slaughterhouses using immersion chilling didn't change water of chillers during shifts. There was significant difference between the two chilling methods with p> .018.

Table 2: Incidences and microbial counts for enterobacteriaceae and *E. coli* in emersion and air chilling methods using EMB

Type of bacteria	Chilling method	Mean	SE	Sig.
E.coli	Emmersion	0.163	0.042	0.75
	Air chilling	0.147	0.029	
	Total	0.152	0.024	
Enterobacter	Emersion	0.088	0.032	0.017*
	Air chilling	0.020	0.012	
	Total	0.043	0.012	
Citrobacter	Emersion	0.100	0.034	0.017*
	Air chilling	0.027	0.013	
	Total	0.052	0.015	
Shigella	Emersion	0.075	0.030	0.004*
	Air chilling	0.007	0.007	
	Total	0.030	0.011	
Klebseilla	Emersion	0.075	0.030	0.001*
	Air chilling	0.000	0.000	
	Total	0.026	0.011	
Salmonella	Emersion	0.150	.0402	0.522
	Air chilling	0.120	.0266	
	Total	0.130	0.022	
E.coli+Salomnella	Emersion	0.188	0.044	0.000*
	Air chilling	0.000	0.000	
	Total	0.065	0.016	
E.coli+Enterobacter	Emersion	0.213	0.046	0.000*
	Air chilling	0.013	0.009	
	Total	0.083	0.018	
E.coli+Shigella	Emersion	0.076	0.030	0.001*
	Air chilling	0.000	0.000	
	Total	0.026	0.011	
E.coli+Citrobacter	Emersion	0.088	0.032	0.00*
	Air chilling	0.000	0.000	
	Total	0.030	0.011	
Enterobacter +Citrobacter	Emersion	0.075	0.030	0.04*
	Air chilling	0.020	.0115	
	Total	0.039	.0128	
Enterobacter +Citrobacter+shigella	Emersion	0.038	0.021	0.09
E.coli+Salomnella +Enterobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
Enterobacter +salmonella	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+Enterobacter +Citrobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+klebsiella +Citrobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+kebseilla	Emersion	0.025	0.018	0.052
	Air chilling	0.000	0.000	
	Total	0.009	.0061	
Salmonella+Citrobacter	Emersion	0.000	0.000	0.47
	Air chilling	0.007	0.007	
	Total	0.004	0.004	

*P-value considered significant at less than 0.05 level

EMB= Ethylene methylene blue, SE= Standard error of means

Table 3: Scalding processes with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Scalding water change during shift	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	Not computed
	No	4 (100%)	4 (100%)	8 (100%)	
Total		4 (100%)	4 (100%)	8 (100%)	
All chickens are immersed together in hard scalding	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.214
	No	2 (50%)	4 (100%)	6 (75%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 4: Defeathering procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-value
		Immersion chilling	Air chilling		
The efficacy of defeathering machines to keep chickens not to fall down; Proper cleaning of de-feathering machine rubber fingers	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Are chickens pass with feather return to machine for more trimming?	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.214
	No	2 (50%)	4 (100%)	6 (75%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 5: Evisceration procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Is viscera mechanically opened? Are chickens hanged on legs till the end of evisceration? Is there any technique for viscera suction?	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 6: Washing procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Is there in and out washing machine for washing carcasses?	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Is potable water free from salt available?	Yes	1 (25%)	2 (50%)	3 (37.5%)	0.500
	No	3 (75%)	2 (50%)	5 (62.5%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 7: Chilling procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Chicken internal breast temperature before entering chiller 40°C	Yes	4(100%)	4(100%)	8(100%)	0.214
	No	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Chiller temperature more than 4°C reach 11°C	Yes	3 (75%)	0 (0.0%)	3 (37.5%)	0.071
	No	1 (25%)	4(100%)	5 (62.5%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Water chiller is changed during shift	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.018*
	No	2 (50%)	0 (0.0%)	2 (25%)	
	NA	0 (0.0%)	4 (100%)	4 (50%)	
Total		4 (100%)	4 (100%)	8 (100%)	

*P-value considered significant at less than 0.05 level

DISCUSSION

This study aimed to evaluate the effect of chilling method and its related process steps on chicken bacterial load in poultry processing plants in Khartoum State.

In this study the mean total values of the standard plate count were lower than values obtained by Abdalla *et al*. (2013) who recorded 8.16±0.11 log10 CFU/ml for legs, 8.68±0.25 log10 CFU/ml for back, 9.18±0.13 log10 CFU/ml and for the breast. The reason for these lower bacterial findings was that this study expressed values of bacterial contamination after emersion or air chilling which logically preceded by lower microbial values.

Similarly, higher results also were obtained by Abdalla *et al*. (2013) who reported that the TVC revealed the highest contamination level of the backs recorded after de-feathering was 9.99±0.01 log10 CFU/ml, while the highest contamination level of the breasts after chilling and packing was 1.86±0.01 log10 CFU/ml and the highest contamination level of the legs after scalding was 9.96 ±0.01log10 CFU/ml.

Also, higher results were revealed by Kabour (2011) who reported mean TVCs 7.69±2.6 in legs, 7.49±1.6 in backs and 8.38±2.1 in breasts after defeathering. But the mean TVCs obtained from chicken carcasses after spray wash and after chilling and packing were lower than those reported in this study.

In this study the results of the effect of chilling methods on microbiological quality of meat revealed that the mean bacterial load count of legs samples was significantly greater in emersion method compared to air chilling method (p-value =.000). This might be due to poor cleaning and disinfection of rails and equipment, poor handling and poor health status of workers as observed by the researcher.

The study conducted by Geornaras *et al*. (1997) reported that contamination may occur due to bacterial contamination associated with water from the scald tank and from rubber fingers at the exit of defeathering machine.

This study revealed that scalding water was not changed during working shift in the two chilling methods. The effect of not changing scalding water during working shift in this study may pose hazards on the safety of broiler meat. This finding is in line with Anand *et al*. (1989) who stated that spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin.

Defeathering process step is considered vital as birds arriving to the poultry slaughterhouse for processing are generally highly contaminated with bacteria, especially with potential human pathogenic bacteria, such as Coliform and Salmonella (Göksoy *et al.,* 2004).

In this study all plants used air chilling had defeathering machines efficient enough to keep chickens not to fall down, while only half of the plants used immersion chilling were efficient. These findings were found true in the bacteriological

analysis in this study where bacterial load count was significantly greater in emersion method compared to air chilling method specifically in legs samples and not significantly greater in emersion method in breast, thigh, backbone and wings samples compared to air chilling method.

This study showed that mechanical opening of the viscera was practiced in all plants that used air chilling, while only half of the plants used immersion chilling practiced it. This was evident in that bacterial load count was significantly greater in emersion method compared to air chilling method in terms of dealing with viscera. This finding is in line with that recorded by Hinton *et al*. (2000) who reported that broiler carcasses can be contaminated by bacteria when contact with ingesta and feces during evisceration.

This study revealed that all operations using air chilling method had in and out washing machines while only 50% of those using immersion method had such a system. Mead (2004) reported the importance of in and out washing machines of broiler carcasses in reducing TVCs and coliform bacteria counts.

Department of Agriculture, Food Safety and Inspection Service, demands the chilling of carcasses below 4.4 °C until 4 hours postmortem (Savell *et al*., 2005).

This study displays the effect of chilling process step on reducing carcass temperature measured at internal breast from 40°C to 4°C. All slaughterhouses using air chilling method practiced optimal temperature. There was no significant difference between the two chilling methods, with $p>0.071$.

These findings were in line to that reported by James *et al*. (2006) who stated that in industrial processing of poultry, immediately after hot water scalding and the further steps poultry carcasses have to be chilled to reduce their temperature from approximately 40 to 4 °C, which contributes to ensure safe products.

The efficacy of air chilling and drying in this study is supported by the microbial load of chicken which was significantly lower (p-value =.000) than the immersion method.

On the other hand, several authors have postulated that surface drying during air chilling reduces water activity, retards bacterial growth, and causes enough injury to pathogenic bacteria to reduce recovery (Huezo *et al*., 2007).

This study revealed that only half of the slaughterhouses using immersion chilling didn't change water of chillers during shifts and thereby practice cleaning and disinfection. This might be one of the reasons why in this study bacterial load in immersion method was higher than air chilling method as birds are usually soaked in a large pool of water with tons of other chickens and their bacteria. This is also evidenced by that stated by Mead *et al*. (2010) who reported that during slaughter, the chilling of poultry carcasses is one of the main points for potential contamination, requiring constant monitoring.

The addition of chemicals in immersion method during chilling resulted in the low microbial load in this study. This is also in line to that recorded by Ismail *et al*. (2001) who stated that the attempts to apply chemicals to reduce the microbial contamination of poultry carcasses such as acetic acid (2.5%), Trisodium phosphate (8%) and/or sodium hypochlorite (800 pm) had resulted in significant reduction in the number of microorganisms.

CONCLUSION AND RECOMMENDATIONS

The study concluded that the situation of microbial contamination of poultry meat in all investigated slaughterhouses in Khartoum State was in the level recommended by International regulatory bodies. It is recommended that HACCP prerequisites programs should be well maintained in all poultry slaughterhouses.

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