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Comparison of Air and Water Chilling Effects on the Microbiological Quality of Broiler Carcasses*

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Summary: The objective of this study is to compare the effects of air and water chilling on the microbiological quality of carcasses in a commercial broiler slaughterhouse in Bolu Province. For this purpose, a total of 320 carcasses were sampled for 20 weeks (16 broiler carcasses/week). Swab samples were obtained from wing, breast, and dorsal subsections of broiler carcasses before and after air and water chilling procedures, and also water samples were taken from the chilling tank. Samples were analyzed for total aerobic mesophilic bacteria, and numbers of *Enterobacteriaceae,* coliform bacteria, staphylococci and micrococci, coagulase-positive staphylococci, psychrophilic bacteria, yeast and mold. Statistically significant differences in the number of total aerobic mesophilic bacteria, coliform bacteria, staphylococci and micrococci and psychrophilic bacteria were observed between the two chilling methods investigated. In conclusion, chilling methods were found to be effective on the microbial count of broiler carcasses. Total aerobic mesophilic bacteria and psychrophilic bacteria counts were found to be lower in air-chilled carcasses.

Key Words: Air chilling, broiler carcass, microbiological quality, water chilling

Hava ve Su ile Soğutmanın Broiler Karkaslarında Mikrobiyolojik Kalite Üzerine Etkilerinin Karşılaştırılması

Özet: Bu çalışma, Bolu'daki özel bir broiler kesimhanesinde hava ve su ile soğutmanın karkasların mikrobiyolojik kalitesi üzerine olan etkisinin karşılaştırılması amacıyla yapıldı. Bu amaçla çalışmada her hafta aynı kesim gününde 16 karkas örneği alındı. Bu şekilde uygulamaya 20 hafta süresince devam edildi. Çalışmada toplam 320 broiler karkası kullanıldı. Örnekler karkasın kanat altı, göğüs ve sırt bölgelerinden svab tekniği kullanılarak yapıldı. Hava ve su soğutma tekniklerini karşılaştırmak amacıyla karkas örnekleri soğutma öncesi ve sonrası alındı. Ayrıca, su ile soğutma tekniği uygulanan karkasların soğutma tankında bulunan su numuneleri de incelendi. Her iki soğutma tekniğinde ve soğutma tankındaki su numuneleri de aerobik mezofilik genel canlı, *Enterobacteriaceae*, koliform bakteri, stafilokok ve mikrokok, koagulaz pozitif stafilokok, psikrofilik bakteriler, maya ve küf sayıları yönünden incelendi. Aerob mezofil genel canlı, koliform bakteri, stafilokok ve mikrokok, psikrofilik bakteri sayıları bakımından her iki soğutma tekniği arasındaki farklılıklar istatistikî olarak önemli bulundu. Sonuç olarak, kullanılan soğutma tekniğinin broiler karkaslarının mikroorganizma sayısını etkilediği, hava ile soğutulmuş broiler karkaslarında aerob mezofil genel canlı ve psikrofil mikroorganizma sayısının daha düşük olduğu görüldü.

Anahtar Kelimeler: Broiler karkası, hava soğutma, mikrobiyolojik kalite, su soğutma

Introduction

Chilling is a critical step in poultry slaughtering for prevention or inhibition of microbial growth (16, 23). According to USDA, the carcass temperature should be reduced to 4°C within 4 h after evisceration to reduce both internal and external microbial growth (13, 20). Chilling extends the storage period and shelf-life of the final product (26, 27).

In various countries, consumers favor fresh chilled poultry meat instead of frozen poultry meat. Similarly, chilling techniques have changed; three chilling methods are currently prevalent in slaughterhouses: water, air and evaporative air chilling (1, 2, 28). For water chilling, carcasses are submerged in cold or ice water for a specified time following evisceration. Chilling is applied in two stages: pre-cooling and main cooling. With cold water flowing in the counter direction, carcasses move in the direction of declining temperature. Given the low cost of the procedure and the rapid decrease in carcass temperature compared with other chilling methods, this

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method is used extensively in the USA and Brazil (6, 13). However, water chilling may cause cross contamination between carcasses. The factors which cause an increase in the microbial load of chilled poultry carcasses with water were defined as: bacterial contamination of carcasses before chilling, the amount of water required for each carcasses and the rate of carcasses in water chilling (21). Since the European Union has restricted water chilling to minimize such cross contamination, air chilling is used more commonly for carcasses that are to be consumed as fresh meat. In air chilling, carcasses are hung in chilling rooms at temperatures of −4°C to −1°C with an air flow of 3–5 m/s until the carcass breast temperature falls below 4°C. Because carcasses are individually hung on shackles during chilling, the possibility of contact between carcasses is minimized. The absence of the mass washing procedure decreases the risk of cross contamination (13, 29).

Chilling of broiler carcasses have produced varying results on microbiological quality. Sanchez et al (23) reported that with respect to *Salmonella* spp. and *Campylobacter* spp. and psychrotrophic bacteria, the microbiological quality of air-chilled carcasses was better than that of water-chilled carcasses. Fluckey et al (8) reported that after air chilling, the number of total aerobic mesophilic bacteria, coliform bacteria and *Escherichia coli* were considerably reduced.

Poultry slaughterhouse owners either partially or completely turn into air chilling in order to meet customer demands. Because of it is not yet known how much carcasses may affect with respect to microbiological quality. Therefore, the objective of this study was to compare the effects of air and water chilling on the critical control points for microbiological quality of carcasses in commercial broiler slaughterhouses.

Materials and Methods

This study was performed in a commercial broiler slaughterhouse in Bolu Province of Turkey using broiler carcasses (42 to 47 d of age). The slaughterhouse had an average slaughtering capacity of 10,000 broilers/h water and air chilling systems were used in the slaughterhouse. Water chilling included pre-cooling and main cooling. In this system, broiler carcasses are chilled in a pre-cooling section at 13°C–15°C for 15 min with continuously renewed and counter-flowing water. In pre-cooling section 2.5 liters of water is used per carcass weighing 2.5 kg or less. In the main cooling section with ice water at −1 to 1°C for 30 min, 1.0 liter of water is used per carcass weighing 2.5 kg or less. In the air chilling system, carcasses hung on a line are chilled in an air chilling room at −2 and 0°C with a cold air flow rate of 2 m/s for 90 min. Information for water temperature during water chilling and temperature in air chilling were obtained mainly from slaughterhouse instruments.

Sampling

Before slaughtering, wings of randomly selected samples were marked with plastic tags. Each week, 8 carcasses (four air-chilled and four water-chilled) were sampled from the wing, breast and dorsal parts before chilling. Swabbing samples were carried out on 10 cm^2 area using sterile metal templates. Samples from the three parts were considered as a single sample (total 30 cm^2). Each operation was repeated after air and water chilling of the carcasses. The swabs were stored in sterile test tubes containing 10 ml of 0.1% sterile physiological saline and taken to the laboratory. The study continued for 20 weeks. Thus, a total of 320 carcasses were subjected to microbiological analysis (3, 22, 24).

In our study, the broiler carcass mean temperatures were measured by inserting digital thermometer (Testo 915, Ireland) into the center of the breast before chilling and also after 45 min of water chilling and 90 min of air chilling, respectively.

In parallel with the carcass sampling, water samples were taken from pre and main chilling parts of water chilling tank. A total of 40 water samples were taken that 20 of them taken from pre-chilling part, other 20 samples taken from main chilling part during the 20 weeks (21).

Microbiological analysis

The samples were analyzed microbiologically by decimal dilution to 10^{-6} with water containing 0.1% peptone (3, 21, 22, 24). Samples were analyzed for total aerobic mesophilic bacteria, and numbers of Enterobacteriaceae, coliform bacteria, staphylococci and micrococci, coagulase-positive staphylococci, psychrophilic bacteria, yeast and mold (7, 10, 14, 18, 25) (Table 1).

Table 1. Media and incubation conditions for the enumeration of microorganisms in the broiler carcasses and water samples.

		Incubation		
Microorganism	**Growth medium**	**Temperature, °C**	**Time, h**	**Conditions**
Total aerobic mesophilic bacteria	Plate Count Agar (Oxoid CM 325)	30	48-72	Aerobic
Enterobacteriaceae	Violet Red Bile Glucose Agar (Oxoid CM 485)	37	24-48	Aerobic
Coliform bacteria	Violet Red Bile Lactose Agar (Oxoid CM 107)	30	24-48	Anaerobic
Staphylococci and micrococci	Baird Parker Agar (BB-DM 905) Egg Yolk Telluride Emulsion(Oxoid R054)	37	24-48	Aerobic
Coagulase positive staphylococci	Brain Heart Infusion Broth (Oxoid CM 375) Coagulase Rabbit Plasma With EDTA (Oxoid R 21060)	37	24	Aerobic
Psychrophilic bacteria	Plate Count Agar (Oxoid CM 325)	4	168-240	Aerobic
Yeast/Mold	Rose Bengal Chloramphenicol Selective Agar (Oxoid CM 0549), Chloarmphenicol Selective Supplement (Oxoid SRO 78)	25	120	Aerobic

Statistical analysis

In this study, the data obtained from microbiological analysis were transformed to the $\log_{10}$ values. The differences of microbial counts between air and water chilling (before and after chilling stages) were analyzed by using *Student' s t-test*, while differences of microbial counts between pre-cooling and cooling sections in water samples were evaluated by *paired samples t-test*. The analyses were performed in SPSS 18.0 software.

Results

The mean temperatures of the broiler carcasses were determined as 38.2°C and 37.6°C before air and water chilling, respectively. After chilling, carcasses temperatures were measured as 2.6°C and 3.6ºC, respectively. The mean water temperature was 12.7°C in the pre-cooling section of the water chilling tank and 1°C in the main cooling section, and the mean temperature of the air chilling room was 0.5°C.

The effects of air and water chilling on the numbers of microorganisms located on broiler carcasses are shown in Table 2. The microbiological results obtained from pre-cooling and main cooling sections are shown in Table 3.

Table 2. The effects of air and water chilling on the broiler carcasses microorganism counts (log10 cfu/cm2)

	Before Chilling			After Chilling		
	Air n=80	Water n=80		Air n=80	Water n=80	
Microorganism	$\bar{x} \pm s_{\bar{x}}$		**Statistical Significant (Student T test)**	$\bar{x} \pm s_{\bar{x}}$		**Statistical Significant (Student T test)**
Total aerobic mesophlic bacteria	5.13±0.036	5.12±0.040	P>0.05	4.54±0.045	4.93±0.043	P<0.001
Enterobacteriaceae	3.21±0.071	3.10±0.075	P>0.05	2.57±0.098	2.64±0.090	P>0.05
Coliform bacteria	3.15±0.067	2.95±0.081	P<0.05	2.24±0.098	2.53±0.101	P<0.05
Staphylococci and micrococci	3.61±0.077	3.73±0.064	P>0.05	3.12±0.074	3.52±0.044	P<0.001
Coagulase positive staphylococci	2.62±0.063	2.94±0.080	P>0.05	2.43±0.088	2.80±0.074	P>0.05
Psychrophilic bacteria	4.83±0.063	4.61± 0.067	P<0.05	3.89±0.074	4.31±0.083	P<0.001
Yeast	2.65±0.090	2.30±0.106	P<0.05	2.45±0.112	2.21±0.136	P>0.05
Mold	nd	1.44±0.143	-	nd	1.30±0.001	-

nd: Not detected. $\bar{x}$: Mean $s_{\bar{x}}$: Standard error

Table 3. Microbiological analysis results of the water samples from the chilling tank (log10 cfu/ml)

	Pre-cooling n=20	Cooling n=20	
Microorganism	$\bar{x} \pm s_{\bar{x}}$		**Statistical Significant (Paired sample T test)**
Total aerobic mesophlic bacteria	3.81±0.050	3.10±0.041	P<0.001
Enterobacteriaceae	3.04±0.088	2.47±0.025	P<0.001
Coliform bacteria	2.76±0.047	2.15±0.031	P<0.001
Staphylococci and micrococci	2.69±0.083	2.12±0.040	P<0.001
Coagulase positive staphylococci	nd	nd	-
Psychrophilic bacteria	3.61±0.065	2.94±0.056	P<0.001
Yeast	nd	nd	-
Mold	nd	nd	-

nd: Not detected. $\bar{x}$: Mean $s_{\bar{x}}$: Standard error.

The differences between air and water chilling with respect to total aerobic mesophilic bacteria, coliform bacteria, staphylococci and micrococci and psychrophilic bacteria were significant (P < 0.001) (p < 0.05).

Discussion

This study has shown that air-chilled broiler carcasses contain lower total aerobic mesophilic bacteria and psychrophilic bacteria than water-chilled carcasses.

In this study, the mean total aerobic mesophilic bacteria count was determined as 5.12 $\log_{10}$ cfu/cm^2 before water chilling and 4.93 $\log_{10}$ cfu/cm^2 after chilling; in air chilling stage, it was defined as 5.13 $\log_{10}$ cfu/cm^2 before air-chilling and 4.54 $\log_{10}$ cfu/cm^2 after chilling. The number of total aerobic mesophilic bacteria in broiler carcasses sampled

in this study after air chilling was lower than that of after water chilling. Berrang et al (5), who utilized a rinse technique, determined the total aerobic mesophilic bacteria counts in broiler carcasses between 3.83 $\log_{10}$ cfu/ml and 3.40 $\log_{10}$ cfu/ml after air and water chilling. Zhang et al (30) reported that carcass rinse samples analyzed at each of the four processes after evisceration, after disinfectant spraying, after air and water chilling had mean total aerobic mesophilic bacteria counts of 2.98, 1.64, 2.16 and 1.79 $\log_{10}$ cfu/ml, respectively. These data are lower in level than our findings. The researchers used cetylpyridinium chloride as an antimicrobial agent in their studies. Göksoy et al (9) found that in two different slaughterhouses, after air chilling, the numbers of total viable count in the neck skin were 5.18 $\log_{10}$ cfu/g and 5.13 $\log_{10}$ cfu/g. Their results were found to be lower than those we observed. This was caused by sampling difference, that neck skin of broiler carcasses were used by researchers. We propose that the differences are due to general conditions, such as the initial microbial load of carcasses transported from the farm to the slaughterhouse, the number of animals slaughtered per hour, the sampling method, the hygienic conditions of the equipment and working practices, the number of chilled carcasses, the chilling time, the implementation of disinfection after evisceration and the hygienic conditions in the chilling room.

Lillard (15), in two different commercial slaughterhouses obtained that the numbers of *Enterobacteriaceae* decreased after water chilling. Lillard (15) determined that *Enterobacteriaceae* counts in broiler carcasses between 6.01-6.09 $\log_{10}$ cfu/carcass before water chilling and 4.97-4.97 $\log_{10}$ cfu/carcass after water chilling. In contrast, James et al (12) found that the *Enterobacteriaceae* counts did not decrease after water chilling (before water chilling $\log_{10}$ 2.29 cfu/ml – after water chilling $\log_{10}$ 2.32 cfu/ml post chill), but increased after postautomatic cut. The researchers found that adding 25 ppm chlorine in the chilling water decreased *Enterobacteriaceae* counts of from $\log_{10}$ 2.57 cfu/ml (before water chilling) to $\log_{10}$ 1.75 cfu/ml (after water chilling) (11). In our study, *Enterobacteriaceae* counts were determined as 2.64- 2.57 $\log_{10}$ cfu/cm^2 after water chilling and air chilling, respectively. The study results were higher than James et al (11) and James et al (12) but lower than those reported by Lillard (15) findings. We propose that the differences are due to worker hygiene, contamination by intestinal contents during evisceration and the quality of water used in chilling.

The coliform count is an indicator of the hygienic quality of a product. Berrang et al (5), who utilized a rinse technique, determined that the coliform count in broiler carcasses after air and water chilling were between 2.53 $\log_{10}$ cfu/ml and 2.05 $\log_{10}$ cfu/ml. The researcher investigated after cooling stages and approximately 0,5 log cfu/ml reduction was determined half-carcass rinse. In this study, the difference in coliform counts to the two chill method were approximately 0,91 - 0,42 $\log_{10}$ cfu/cm^2 of air and water chilling. The difference could be caused by sampling method. Fluckey et al (8) determined that the coliform count was 2.59 $\log_{10}$ cfu/ml after air chilling. Zhang et al (30) reported that, while disinfectant spraying decreased the numbers of coliforms in carcass rinse samples by 1.29 $\log_{10}$ cfu/ml and 1.37 $\log_{10}$ cfu/ml, air and water chilled carcasses, respectively. Our results were similar to those reported by Berrang et al (5) and Fluckey et al (8), but higher than those reported by Zhang et al (30). We suggest that the difference was due to the application of antimicrobials after evisceration.

Göksoy et al (9) studied in two commercial broiler slaughterhouses after air chilling, the numbers of staphylococci and micrococci in the neck skin were found as 4.11 $\log_{10}$ cfu/g and 3.94 $\log_{10}$ cfu/g. They reported that the numbers of staphylococci and micrococci were extremely high before slaughtering (6.90–6.85 $\log_{10}$ cfu/g). In the broiler carcasses analyzed in our study, the numbers of staphylococci and micrococci counts were found as 3.61 $\log_{10}$ cfu/cm^2 and 3.12 $\log_{10}$ cfu/cm^2 before and after air chilling. We suggest the difference was due to the sampling techniques and type of samples.

In recent years it has revealed that poultry meat was contaminated with staphylococci from the feathers and skins of the birds and that cross contamination was caused by defeathering machines (19). High counts of staphylococci in foods have potentially capable of causing food poisoning (4). In this study, staphylococci has been isolated thought to be important because of it causes food poisoning, accelerate spoilage and also contaminate the final product. But it was not found a study about chilling effects regarding with microorganisms such as staphylococci and micrococci when comparing the effects of air and water chilling on carcass microbiological quality has been performed except Göksoy et al (9).

In our study, psychrophilic bacteria counts were determined as 4.31 $\log_{10}$ cfu/cm^2 after water chilling; it was defined as 3.89 $\log_{10}$ cfu/cm^2 after air-chill-

ing. Sanchez et al (23) found the mean psychrotroph count as 3.2 $\log_{10}$ cfu/ml after water chilling and 1.91 $\log_{10}$ cfu/ml after air chilling. Mead et al (17) reported that *Pseudomonas* counts in the neck skin samples after air chilling were between 2.6 $\log_{10}$ cfu/g and 3.9 $\log_{10}$ cfu/g. The number of psychrophilic bacteria obtained after air chilling in our study is similar to that reported by Mead et al (17) and higher than that reported by Sanchez et al (23). We propose that this difference was due to initial contamination level, the equipment and general processing facility conditions. Considering these findings, the number of psychrophilic bacteria in air-chilled carcasses was lower than that in water-chilled carcasses. We propose that in air chilling, the chilled air circulation removes water from the surfaces of carcasses, thereby reducing microbial growth because of drying effect of air chilling.

In a study by Tuncer and Sireli (26) in which the shelf-life of broiler carcasses was studied, the microbial qualities (for total bacterial counts, for *Pseudomonas* spp., *Enterobacteriaceae,* yeasts and molds) of broiler carcasses stored at various temperatures (0, 4 and 7 °C) after various time periods (0, 4, 8, 10 and 14 days) were evaluated, and it was found that air-chilled carcasses were of higher quality than water-chilled carcasses for all analyzed bacterial counts. These findings were in agreement with those of our study.

In air-chilled carcasses, particularly, the number of total aerobic mesophilic bacteria and psychrophilic bacteria was lower, thereby delaying spoilage and conferring longer shelf-life.

Our findings also have implications in preventing environmental pollution. Because, the chilling water used in poultry slaughterhouses should be returned back to the environment after decontamination. McKee (16) stated that extremely large amounts of water are used in slaughterhouses and that wastewater management is difficult and costly. We also found air chilling to be better than water chilling; the reduction in the amount of wastewater could contribute protection of environment.

Conclusion

In conclusion, although both air and water chilling reduce the microbial count in broiler carcasses, the count in air-chilled carcasses were found to be lower than that in water-chilled carcasses, especially for the number of total aerobic mesophilic bacteria and psychrophilic bacteria. The results show that the air chilling technique is safer than the water-chilling technique with respect to some microbiological count.

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