

The Effects of Ozone and Chlorine Applications on Microbiological Quality of Chickens During Processing

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ABSTRACT

In this study, effects of two antimicrobial applications (ozone and chlorine) on broiler carcasses after evisceration were investigated. The ozone and chlorine (sodium hypochlorite, NaHClO) were applied to broiler carcasses as 1.5 ppm and 30 ppm for 7 minutes, respectively. During the broiler processing, the samples were taken from 14 different points in the production line, 17 surface points and 5 workers' hands for the microbiological analyses as ten replicates. At the beginning, *Escherichia coli* growth was not observed after ozone treatment. But, *E. coli* growth increased after portioning and grading of broiler carcasses. It is assumed that workers' hands and equipment can be a source of secondary contamination. Ozone can also be used in lower concentration and more safely than the chlorine.

Key words: Broiler carcass, ozone, Na-hypochloride, antimicrobials.

INTRODUCTION

Chicken meat have been widely consumed especially in the recent years due to its low fat ingredient, fast preparation, and being more economical than red meat. Chicken meat is being sold as whole or as pieced depending on the demands of the consumers (Cevger et al 2002). There is an increase on the pieced chicken demand specially in large cities (Şengör 2002).

Protection of quality particularly for safety during processing, storage and marketing of food has gained importance in Turkey, as it has in the whole world (FAO 1998).

Pathogenic and harmful bacteria that are present in chicken's interior organs, skin surface and feather, can easily contaminate the meat during process steps. Contamination is mostly seen at steps like scalding, plucking, and evisceration. In addition to this, cross contamination in the carcasses, dirtiness of the process water and equipment increase the contamination level in the process steps (Anonymous 2002, Tosun and Tamer 2000).

The emphasis has been given to HACCP based programs for the identification and prevention of the possible microbiological risks that can originate from raw material, processing stages, the product and from the food plants (Giaccone et al 2002; Mantouanelli et al 2001).

In order to prevent the microorganisms in the chicken meat, methods like cooling, vapor-vacuum system, vapor pasteurization are being used (Allen et al 2000). Along with this, chemicals like chlorine and chlorine compounds (Erickson 1999), ozone (Chang and Sheldon 1989; Whistler and Sheldon 1989), organic acids (Anonymous 2002), trisodium phosphate (Rio et al 2006) are being widely used for decontamination purposes.

Chlorine inhibits glucose oxidization in the bacteria and shows bactericidal effect. It also decreases the activations of some enzymes that carry sulfide group. However, excess usage of chlorine forms toxic and carcinogenic compounds called tri-halo methane by reacting with the meat (Oguz and Guler 2004).

In 1982, ozone has been generally recognized as safe (GRAS) by the Food and Drug Association (FDA), and in 2001 it was recognized as legal to use ozone directly in food products involving fish, red meat and chicken meat and its usage in the food industry (Mielcke and Ried 2004). Ozone, which is a strong oxidant, is effective against Gram positive and Gram negative bacteria, yeasts, moulds and viruses. Since ozone does not leave any material in the food products, it does not make a change in the taste and the color of the product (Okoyama et al 2002).

This study was made for the purpose of examining the possible effects of ozone and chlorine applications on possible micro contamination sources during chicken processing, preventing economical losses that could be due to the damages caused by micro organisms, and to supply quality and safe products to the consumer.

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MATERIALS AND METHODS

Sampling procedure

The study was made in a private slaughterhouse. The ozoning process of the broiler carcasses was made during the evisceration, by using the 1.5 ppm ozonated water that was obtained by Pacific brand, ORC 60 model ozone generator. The chlorination process was made by chlorinated water containing 30 ppm sodium hypo chloride (NaHClO) by Tekna brand, AXS 602 model chlorine pump. The both treatments were applied for 7 minutes as spraying of broiler carcasses.

As illustrated in figure 1, in the process line, the samples were taken ten times from 14 different pre-determined points from the chicken samples (before evisceration and after evisceration whole chicken, after spraying, after pre-cooling, packaging, grading and also fillet, wing, whole leg, half leg, oven, frying, cutlet, chicken steak, breast). Also samples were taken ten times by 5 different personnel and 17 surface points.

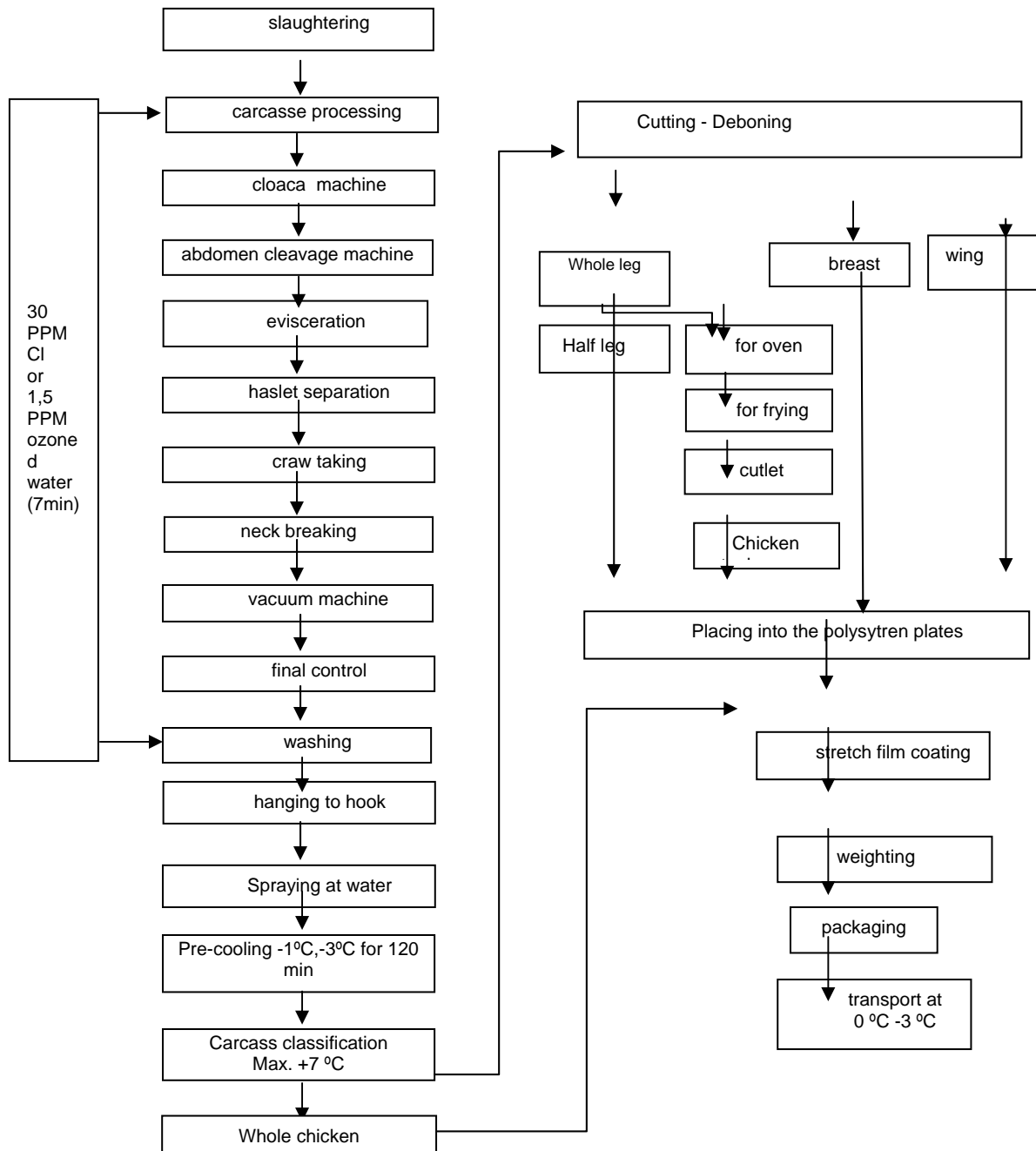


Figure 1. Pieced Chicken Production Flow Chart

Twenty five grams of chicken meat was sampled for the determination microorganisms as indicated in microbiological analyses section (ICMSF 1982). Samples from the personnel hands were taken as follows: workers were let to wear sterile latex gloves and 20 ml 0.1% sterile peptone water was carefully pipetted into the gloves. Hands in gloves were massaged completely and the gloves were carefully taken off, tied at the top and were transferred to the laboratory in pre-chilled insulated containers with chiller packs (De Wit and Kampelmacher 1988). Other samples were removed from the surface by swab method (Diliellol 1982). The samples were diluted up to 10^{-8} with 0.1% sterile peptone water and were plated using appropriate methods for the bacteria indicated in microbiological analyses section (Dore et al 2003). For sampling from environmental air, specific agar plates without lids were kept in a place, where there was no air circulation and were incubated under appropriate temperatures for 15 min. (ISO 1986). Water samples were also collected at different intervals from drinking water used at the plant.

Microbiological analyses

Enumeration of Total Aerobic Mesophilic Bacteria

Plating was performed into Plate Count Agar (PCA, OXOID CM325) from the prepared dilutions by spread plate method. Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted (Swanson et al 1992).

Enumeration of Staphylococcus/Micrococcus

For *Staphylococcus/Micrococcus* counting, spreading was done by surface diffusing method to the Baird Parker Agar (BPA, OXOID CM 275) which was prepared by the addition of Sterile Egg Yolk Tellurite Emulsion (OXOID SR 54) and colonies produced by incubation at 37 °C for 24-48 hours were counted (Bridson 1980).

Presence-absence test of Salmonella spp.

After a non-selective pre-enrichment at 37°C for 16 h in buffered peptone water, samples were transferred to Rappaport-Vassiliadis enrichment broth (RV, OXOID CM 669) for selective enrichment and plates were incubated at 42 °C for 24 h. A loopful of sample was streaked onto bismuth sulphite agar (BSA, OXOID CM 201) for selective growth, and was incubated at 37 °C for 48 h. Brown-grey-black colonies surrounded by a brown-black zone and yielding metallic sheen were regarded as typical suspect *Salmonella* colonies (FDA 1988).

Enumeration of E. coli

For *E. coli* counting, spreading was done by surface diffusing method to Tryptone Bile X-Glucuronide Medium (TBX, OXOID CM945, and the petri dishes were incubated at 44 °C for 24 h , IMVIC test was applied to the bluish colonies produced after the incubation process and the results were determined for *E.coli* (FAO 1992).

Analysis of Water

For the enumeration of aerobic mesophilic bacteria, 0.1 ml from each water sample was pipetted and spread onto Plate Count Agar (PCA, OXOID CM325), and incubated for 48 h at 30 °C. The MPN method was used for the enumeration of coliforms and *E.coli* (Andrews 1992).

Statistical analysis

Statistical software SPSS for Windows was used to determine the differences between the groups in the microorganism numbers that were obtained from the surface samples. Variance analysis method was used in the repeating groups. Kruskal-Wallis test was applied to determine the difference in the microorganism numbers between the personnel (SPSS Inc. 2004).

RESULTS

Minimum, average and maximum (cfu/g) values of the total aerobic mezophilic bacteria, *E. coli* and *Staphylococcus/Micrococcus* numbers of the ozone and chlorine applied chicken samples examined during the chicken processing are given in Table 1.

Table 1 . Microbiological analysis results (cfu/g) of ozone and chlorine applied samples (n=10) during chicken processing:

Samples	Total aerobic mezophilic bacteria						<i>E.coli</i>						<i>Staphylococcus/Micrococcus</i>					
	Ozoned			Chlorined			Ozoned			Chlorined			Ozoned			Chlorined		
	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.
Before evisceration, whole chicken *	3.2x10 ⁴	7.5x10 ⁴	1.1x10 ⁵	3.1x10 ⁴	7x10 ⁴	1.2x10 ⁵	2 x10 ²	3.6x10 ³	5 x10 ²	9x10 ²	3.8x10 ³	7 x10 ³	7 x10 ²	1.5x10 ⁴	1.7x10 ⁴	6 x10 ³	1.6x10 ⁴	2.1 x10 ⁴
After evisceration, whole chicken	3 x10 ³	1.5x10 ⁴	3.4x10 ⁴	1.3x10 ⁴	3x10 ⁴	4.5x10 ⁴	3 x10	< 10	5 x10	2x10 ²	1.6x10 ³	1 x10 ³	6 x10 ²	2.8x10 ²	8 x10 ³	5 x10 ³	8 x10 ³	1.7 x10 ⁴
Spraying whole chicken.	2.8x10 ³	1 x10 ⁴	3 x10 ⁴	1.2x10 ⁴	2x10 ⁴	3.5x10 ⁴	ND	ND	ND	1x10 ²	1.1x10 ³	8 x10 ²	4 x10 ²	2 x10 ³	1.7x10 ⁴	6 x10 ³	6x10 ³	1.8x10 ⁴
Pre-cooling exit	2 x10 ³	9.5x10 ³	3 x10 ⁴	1.2x10 ⁴	1.8x10 ⁴	3.5x10 ⁴	ND	ND	ND	1x10 ²	1x10 ³	9 x10 ²	5 x10 ²	1.7x10 ³	4.4x10 ³	6 x10 ³	5x10 ³	1 x10 ⁴
Packeted whole chicken	1 x10 ⁴	1.3x10 ⁴	3.4x10 ⁴	1.5x10 ⁴	2.2x10 ⁴	3.5x10 ⁴	ND	ND	ND	9x10 ²	1.6x10 ³	4 x10 ³	3.8x10 ³	3x10 ³	7 x10 ³	5.6x10 ³	9.5x10 ³	2.6 x10 ⁴
Grading whole chicken	1.2x10 ⁴	1.2x10 ⁴	3.5x10 ⁴	1.5x10 ⁴	2.1x10 ⁴	3.6x10 ⁴	ND	ND	3 x10	ND	1x10 ³	8 x10 ²	3 x10 ²	3.5x10 ³	6.4x10 ³	1 x10 ³	9x10 ³	1.2 x10 ⁴
Whole leg	8.4x10 ³	1.5x10 ⁴	2.4x10 ³	8.8x10 ³	1.9x10 ⁴	2.4x10 ³	ND	4x10 ¹	1x10 ²	9x10 ²	1.1x10 ³	1.7x10 ³	5 x10 ²	4.7x10 ³	7.6x10 ³	6.4x10 ³	9.5x10 ³	1.2 x10 ⁴
Half leg	3.6x10 ³	1.3x10 ⁴	1.7x10 ⁴	5 x10 ³	2.1x10 ⁴	4.4x10 ⁴	ND	ND	5 x10	1x10 ²	1.3x10 ³	7 x10 ²	8 x10 ²	3x10 ³	6 x10 ³	4.9x10 ³	8.5x10 ³	7.5 x10 ³
For Oven	6.8x10 ³	1.1x10 ⁴	1.2x10 ⁴	1 x10 ⁴	1.6x10 ⁴	2.1x10 ⁵	ND	< 10	1 x10 ²	9x10 ²	1.6x10 ³	2.6x10 ³	3.6x10 ²	4.9x10 ³	6.9x10 ³	9.6x10 ³	9x10 ³	1.2 x10 ⁴
For Frying	1.5x10 ³	9x10 ³	5 x10 ³	1.8x10 ³	1.3x10 ⁴	7.5x10 ³	ND	ND	ND	ND	1.7x10 ³	2.7x10 ³	1.2x10 ²	2.7x10 ³	1.1x10 ³	6 x10 ²	7.8x10 ³	5.2 x10 ³
Cutlet	3 x10 ³	1.2x10 ⁴	3.1x10 ⁴	4 x10 ³	1.7x10 ⁴	3.2x10 ⁴	ND	5x10 ¹	1 x10 ²	8x10 ²	1.7x10 ³	2.8x10 ³	6.6x10 ²	4.3x10 ³	9.6x10 ³	1.6x10 ³	8.6x10 ³	2.2 x10 ⁴
Chicken steak	1 x10 ³	1x10 ⁴	1.2x10 ⁴	1.1x10 ⁴	1.8x10 ⁴	2.2x10 ⁴	ND	ND	ND	5x10 ²	1.9x10 ³	4.2x10 ³	5.6x10 ²	4x10 ³	3.7x10 ³	1.2x10 ³	9x10 ³	2 x10 ⁴
Breast	1.5x10 ³	4.5x10 ³	3.3x10 ³	9.6x10 ³	3 x10 ³	4 x10 ³	ND	ND	ND	ND	1x10 ³	1.2x10 ³	8.4x10 ²	1 x10 ³	1.7x10 ³	1.9x10 ³	3x10 ³	3.9 x10 ³
Wing	2.7x10 ³	2x10 ⁴	3.1x10 ⁴	6 x10 ³	2.3x10 ⁴	4 x10 ⁵	ND	8x10 ¹	1.7x10 ²	1x10 ²	2.5x10 ³	3.5x10 ³	1.8x10 ³	7x10 ³	1.5x10 ⁴	5 x10 ³	1x10 ⁴	1.9x10 ⁴

ND: Not Detected * Not operated with ozone and chlorine

When looked at the before and after taking interior parts of out whole chicken values it was seen that ozone application makes a 80% decrease in the total number of aerobic mezophilic bacteria number, and 57.2% decrease in the chlorine application. Again at the same points, it was found that the average effect of ozone on *E. coli* is 97.77% percent and the effect of chlorine is 57.9%. On the number of *Staphylococcus/Micrococcus* number, ozone has an average effect of 81.33% and chlorine has an average effect of 50%.

After the spraying process in both ozone and chlorine applied samples, the decrease in the number of aerobic mezophilic bacteria number was 33.33%. At this stage, *E. coli* was not observed in ozone applied samples where as there was an average of 25% decrease in the *Staphylococcus/Micrococcus* number, and in the chlorine applied samples there was an average of 31.25% decrease in *E. coli* number and an averages of 28.57% decrease in the *Staphylococcus/Micrococcus* number.

At the exit of pre-cooling, there was a decrease of 5% in the total number of aerobic mezophilic bacteria number in the ozone-applied samples, and a 10% decrease in the chlorine applies samples. No *E.coli* was observed in the ozone applied samples and it was decreased by 9.09% in the chlorine applied samples. As far as *Staphylococcus/Micrococcus* number is concerned, there was a decrease of 15% in the ozone applied samples and 16.6% decrease in the chlorine applied samples in average.

As seen in Table 1, in the samples taken after this stage, there was an increase in the microbiological loads of the chickens. Although no *E. coli* was determined after ozone application, at the grading line, there were *E. coli* in all products such as whole leg, half leg, oven, and wing. Among these samples, wings contain the higher amount of microbiological load.

In none of the groups, *Salmonella* spp. was isolated.

The microbiological analysis results of the samples taken from the surfaces are given in Table 2. When the results in the table are examined, it is seen that microbiological load increases especially in knives of the disintegrating line and surface.

Microbiological analysis results of the samples taken from the personnel's hands are given in Table 3. In personnel's hands, total aerobic bacteria was found as 10^2 cfu/ cm², *Staphylococcus/Micrococcus* number was found as 10 cfu/cm². No *E.coli* was detected in 3 of the personnel and it was in the order of 10 cfu/cm² in two of the personnel.

No growth was observed neither water nor air samples.

Table 2. Analysis (cfu/cm²) results of samples (n=10) taken from surfaces during chicken processing

	<i>Staphylococcus/ Micrococcus</i>		<i>E.coli</i>		Total aerobic mezophilic bacteria	
	Mean	±Std. error	Mean	±Std. error	Mean	±Std. error
Chicken transfer region	16 ^{fg}	3,3	ND	-	27,1 ^{fg}	2,5
Pre cooling hooks	21 ^{fg}	3,7	ND	-	141,5 ^{bcd}	18,6
Pre cooling wall	25 ^{efg}	4,8	ND	-	110,9 ^{bcdef}	11,6
Grading line	34 ^{def}	2,2	ND	-	44,5 ^{defg}	8,2
Calibration division	17 ^{fg}	1,8	ND	-	73,5 ^{cdefg}	7,2
Whole chicken table	56,5 ^{cde}	4,2	13 ^{bcd}	5,3	250 ^a	56,2
Wing knife	100 ^{ab}	3,2	8 ^{cd}	5,5	125,3 ^{bcdef}	32,3
Wing band	61,5 ^{cd}	7,2	13 ^{bcd}	8,1	73,5 ^{cdefg}	5,3
Breast knife	109,5 ^{ab}	4,5	9,5 ^{bcd}	5	93,5 ^{bcdefg}	6,7
Breast band	114 ^a	7,4	19 ^{bcd}	9,7	132,5 ^{bcd}	17,3
Whole leg knife	58 ^{cde}	9,2	29,5 ^{bcd}	10,6	122 ^{bcdef}	17,3
Whole leg band	104,5 ^{ab}	13	15,5 ^{bcd}	5,7	145 ^{bcd}	14,8
Half leg knife	78 ^{bc}	12,8	45 ^{bc}	16	189 ^{ab}	21,3
Halh leg band	86 ^{abc}	8,2	50 ^{ab}	14,2	178 ^{abc}	27,7
Chicken steak knife	79,5 ^{bc}	6,5	41,5 ^{bcd}	11,8	136 ^{bcd}	26,9
Chicken steak table	88,5 ^{abc}	7,8	87,5 ^a	16,3	142 ^{bcd}	19,8
Coffer	15,5 ^{fg}	3,6	6,5 ^{cd}	3,5	37,5 ^{efg}	7,5
Car	12,5 ^{ef}	4,2	9 ^{bcd}	4,8	47,6 ^{defg}	4,5
Packaging material	ND	ND	ND	ND	ND	ND

a-g: Differences between groups containing different letters in the same column are significant (P<0,05).

ND: Not Detected

Table 3. Analysis (cfu/cm²) results of samples (n=10) taken from personnel's hands during chicken processing

Personnel	<i>Staphylococcus/Micrococcus</i>		<i>E. coli</i>		Total aerobic mezophilic bacteria	
	Mean	±Std. error	Mean	±Std. error	Mean	±Std. error
1. Personnel	3.6x10 ¹	25,4	ND	-	1.77x10 ²	134,7
2. Personnel	8.4x10 ¹	67,5	1.5x10 ¹	4,7	1.54x10 ²	215,7
3. Personnel	1.8x10 ¹	5,3	3.5x10 ¹	7,4	1.2x10 ²	88,4
4. Personnel	1.8x10 ¹	6,3	ND	-	1.19x10 ²	204,1
5. Personnel	1.6x10 ¹	3,3	ND	-	2x10 ²	230,7

ND: Not Detected

DISCUSSION

Chang and Sheldon (1989) had indicated an average of 2.7 logarithmic decreases in the number of total organisms by ozoning the water that is used before cooling. In another study, it was indicated that the microbiological load of the chicken carcasses bathed in ozoned water is logarithmically two times lower as compared to those that are not processed (Sheldon and Brown 1986). Yhang and Chen (1979) as observed 1 logarithmic decrease in the microbiological load when the chicken pieces are bathed in ozone containing water. When Table 1 is examined, it is seen that the total decrease in the number of aerobic mezophilic bacteria is parallel to other research results.

Waldroup et al (1993) has announced that the usage of ozone in the cooling water of chicken carcass inhibits *E. coli*. In the final report announced by California energy commission which is about ozone usage in chicken companies indicates a 73-78% decrease in the *E. coli* number by adding 25 ppm chlorine to the washing water, a 87-98% percent in the *E. coli* number by adding 4-8 ppm chlorine addition. The volume of ozoned water used is 30% less than chlorinated water volume (Anonymous 2002).

Güzel-Seydim et al (2004) have studied the effects of ozone on the decrease of bacterial population by using different food components. In the study, after a 10 minute of ozone application *Bacillus cereus* sports, *Staphylococcus aureus* and *E. coli* were inoculated to different food components. Depending on the difference in the food components, a 1.98-6.11 logarithmic decrease in the *E. coli* population, and 1.02-6.48 logarithmic decrease in the *Staphylococcus aureus* number has been detected. A 0.24-4.93 logarithmic decrease in the spor population was observed.

The effects of ozone on different microorganisms were compared with chlorine, and it was found that ozone can kill *E. coli* 125 times faster as compared to chlorine and chlorine products. It was found that ozone is 51 times more effective on bacteria cell membranes as compared to chlorine. It was also reported that ozone has a wide bactericidal effect including Gram negative and Gram positive bacteria (Restaino et al 1995).

The decrease in the number of *E.coli* and *Staphylococcus/Micrococcus* numbers in our study is parallel with other researchers' results.

However, as seen in Table 1, in the samples taken after the pre-cooling stage, there is an increase in the microbiological load especially in pieced chicken samples.

Cleaning and disinfestations are the critical processing steps to prevent secondary contamination which is caused by equipment (Ünlütürk and Turantaş 1999). As seen from the Table 2 the differences between the total aerobic mezophilic bacteria, *E. coli* and *Staphylococcus/Micrococcus* numbers were found meaningful (p<0.05). Contamination was seen mostly in used knives, tapes, tables used in the pieced chicken section.

As seen in Table 3, the differences between the personnel groups were found insignificant. However, when the general microbiological loads of personnel hands were examined, it is seen that it may be a source of aerobic mezophilic bacteria contamination. Also, *E. coli* was detected in the hands of two of the personnel.

After the analysis of the samples taken from the packaging section, it was determined that this part does not have a microbiological risk.

The medium air, which is seen as an important quality criterion for foods that are in contact with air during processes like cooling, freezing due to its technology, is important for microbiological contamination (Tükel and Doğan 2000). It is indicated that the total number of bacteria should not exceed 10³ cfu/m³ in air (Ünlütürk and Turantaş 1999). In the microbiological analysis of water and air samples used in the study, it was determined that no microbiological risk is present.

As a result of the study, it was found that ozone can be used in disinfection of chicken carcasses in lower levels, more safely and more effectively as compared to chlorine, however among the controls examined during the pieced chicken production stages it was seen that equipments that are in contact with personnel hand is a source for secondary contamination and specially for processes that require more manual operations like buttocks, baget, oven and wing production, microbiological quality can be effected negatively. The microbiological increase in the samples taken from the greying line and whole chickens can be related to this secondary effect and also to the facts that the company works over its capacity and general hygiene rules are not obeyed.

For food safety, in order to decrease microbiological threats in chicken companies, the initial microbiological contaminations risks should be decreased and no matter how effective disinfection is applied, necessary hygiene rules should be followed during operation, storing, transporting and sales, and HACCP based systems should be applied.

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