EFFECTS OF DRY ICE DECONTAMINATION TECHNIQUE ON MICROBIOLOGICAL QUALITY OF POULTRY CARCASSES

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Abstract – The decontamination of poultry carcasses is highly significant because poultry is implicated as a risk factor in human bodies. There is a variety of methods for disinfecting poultry carcasses. In addition to these traditional disinfection methods food processing operations have been developed dry ice blasting technique as a new mechanical decontamination method. Dry ice blasting is a compressed air process, which uses solid carbon dioxide at -78,5 °C as a blast medium. In this study, poultry carcasses were sprayed and immersed with dry ice to determine the effects of dry ice on their microbiological quality. 1 to 2 logaritmic unit reduction was seen in total mesophilic aerobic bacteria as compared to control and it was also observed that dry ice technique was effective on pathogenic microorganisms in some poultry samples. Dry ice blasting was more effective than dry ice immersion and it can also be easily applied to poultry carcasses as compared to other traditional methods. In conclusion, dry ice technique can be used effectively as the industrial technique of the future and dry ice technique significantly reduces microbial contamination in meat industry.

Key Words – Carbon dioxide, Contamination, Pathogenic microorganisms

I. INTRODUCTION

Microbial contamination of meat starts during processing on the slaughter line. First, the microorganisms reach the carcass surface from where they penetrate into deeper layers of the meat. By reducing the primal surface contamination and avoiding or limiting the microbial growth, we can considerably prolong the shelf life of carcasses. Reducing surface contamination would improve food safety and extend shelf life [1].

Poultry has a very complex microflora, which is partly of intestinal origin, due to the production system, flocks of large numbers of fast growing animals and being reared in climatized houses on litter floors [2]. Microorganisms most commonly found in poultry and poultry products are *Campylobacter, Enterobacter, Aeromonas, Alteromonas, Alcaligenes, Esherichia, Bacillus, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Acinetobacter, Moraxella, Corynebacterium, Staphylococcus, Listeria, Clostridium, Yersinia, Shigella* and *Salmonella* [3]. *Esherichia coli, Salmonella spp.* and *Listeria spp.* which are the mains factors of food borne infection and intoxications have an important role in food industry [4].

Various chemicals, including chlorine, trisodium phosphate, ozone and organic acids have been found effective in reducing numbers of surface microorganisms when applied to carcasses at the end of the line. However, disposing of waste chemicals is a significant problem for industry. Residues which remains on carcass surface have a potential risk for human health. Therefore, physical decontamination methods are more likely to gain acceptance [5].

Recently, as a new physical decontamination technique, dry ice blasting technique has been the subject of several research. It has a distinct advantage over conventional cleaning and disinfecting techniques in that there are no residues and no wastes on surface cleaned [4]. Dry ice is the solid form of CO_2, which is a colorless, tasteless, odorless gas found naturally in the atmosphere [6,7]. Though CO_2 is present in relatively small quantities (about 0.03% by

volume), it is one of the most important gases in existence. At atmospheric pressure, dry ice sublimates directly to vapor without going through a liquid phase. This unique property means that the blast media simply disappears, leaving only the original contaminant to be disposed of [6].

The aim of our study was to check the efficacy of dry ice blasting technique on microbiologic quality of poultry in reducing microbial contamination in poultry industry.

II. MATERIALS AND METHODS

Dry ice blasting application was carried out in a private poultry plant working in Manisa, Turkey. Equipment and dry ice required for disinfection was supplied by Yusuf Biricik Temizlik Ürünleri San. Tic. Ltd. Şti. in İzmir, Turkey.Three group of meat were used in three replicates. Two different group were sprayed and immersed with dry ice and the other one group used as a control. After treatment a total of 18 samples immediately transferred to microbiological laboratory under chilled conditions.

All of the samples were analysed for the microbiological characteristics of total mesophile aerobic bacteria (TMAB), yeast and mold, *Esherichia coli* and coliform and also the pathogens, *Salmonella spp.* and *Listeria spp.* Furthermore, it is tried to be validated results by PCR method in parallel with the traditional pathogenic microorganism analysis.

Sample preparation

10 g of the samples was mixed with 90 ml of sterile peptone water, was homogenized in a stomacher under the aseptic conditions. The other decimal solutions were prepared from the same 10^{-1} dilution up to 10^{-7} dilutions and used for analysis. For the search of *Salmonella spp.* and *Listeria spp.* 25 g of the samples were prepared to be analyzed by being put in 225 ml sterile Buffered Pepton Water and Half Fraser Broth, respectively.

Total aerobic mesophilic bacteria count

Plate Count Agar (PCA) medium was used to determine total aerobic mesophilic bacteria count. 1 ml of culture from each dilution spread on sterile duplicate agar plates and mixed with a culture medium. Plates were incubated for 24-48 hours at 30°C. Following incubation, plates containing 30-300 colonies were used to calculate bacterial population [8].

Yeast and mold count

Dichloran Rose Bengal Chloromphenicol (DRBC) agar medium was used to determine yeast and mold count. Agar medium was cooled to 45°C-50°C and approximately 15-20 ml agar was poured on the plates. After pouring, plates were dried at room temperature for 24 hours. 0,1 ml of culture from each dilution was transferred to duplicate sterile plates containing agar medium. Plates were incubated for 3-4-7 days at 25±1°C. Following incubation, plates containing 10-150 colonies were used to calculate yeast and mold population results [9].

Esherichia coli and coliform count

Violed Red Bile Agar (VRBA) and VRBA with 4-methyl-umbelliferyl-β-D-glucuronide (MUG) were used to determine *E.coli*/coliform count. 1 ml of culture from each dilution spread on sterile duplicate agar plates and mixed with VRBA agar. After mixture have became solid, approximately 5-10 ml of VRBA with MUG was transferred to plates. Plates were incubated for

18-24 hours at 35°C. Purple-red colonies surrounded by a reddish zone were evaluated as coliform organism. Red colonies surrounded by a zone and fluoresce blue under long-wave UV light were evaluated *E.coli*. In addition, confirmation for the presence of *E.coli* was done with indol test [10].

Prevalence of Salmonella spp.

25 g of the samples was mixed with 225 ml of sterile buffered peptone water and homogenized in a stomacher under the aseptic conditions. Homogenate was incubated for 18-20 hours at 37° C. Following incubation, 0.1 ml of culture was transferred into 10 ml Rappaport-Vassiliadis Soya Broth (RVS) and was incubated for 24-27 hours at 41,5°C. In addition, 1 ml of culture was transferred into 10 ml Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn) and was incubated for 24-27 hours at 37°C. Following incubation, a loopfull of inoculum from each tube was inoculated duplicate sterile agar plates containing Xylose Lysine Deoxycholate (XLD) agar medium and Xylose-Lysine-Tergitol 4 (XLT4) agar medium. Plates were incubated for 24-27 hours at 37°C. Red colonies with black centers on XLD medium were evaluated as *Salmonella spp*. Also, the colonies appeared black or black centered with a yellow periphery on XLT4 medium were evaluated as *Salmonella spp*. [11] For confirmation and identification of positive cultures Microgen GNA-ID A test strips were used [12].

Quantification analysis of Salmonella spp. with PCR (Polimerase chain reaction)method

Salmonella spp. analysis with PCR method were carried out in a private laboratory with using Light Cycler *Salmonella* Detection Kits (Roche) in PCR. 25 g of the samples were mixed with 225 ml of sterile buffered peptone water, homogenized in a stomacher under the aseptic conditions. Homogenate was incubated for 18-20 hours at 37°C. At the end of the incubation, samples were subjected to DNA extraction. After extraction, reaction mix containing template DNAs of samples were prepared for PCR.

Amplification was performed in LightCycler® 480 Real-Time PCR System. Prepared samples were transferred into PCR plate and then plate was placed on PCR system. The protocol used for amplification was 95°C for 3 min (denaturation), followed by 40 cycles of 95°C for 10 second (annealing) and 55°C for 30 second (extension/elongation), then plate read. At the end of the PCR quantitative results were obtained [13].

Prevalence of Listeria spp.

25 g of the samples was mixed with 225 ml of sterile Half Fraser Broth and homogenized in a stomacher under the aseptic conditions. Homogenate was incubated for 24 hours at 30°C. Following incubation, a loopfull of inoculum from samples was inoculated duplicate sterile agar plates containing Listeria Chromogenic Agar medium. Plates were incubated for 24-48 hours at 37°C. All colonies appearing blue-green or blue-green with an opaque halo on the medium were evaluated as *Listeria spp*. [14] For confirmation and identification of positive cultures Microgen *Listeria*-ID test strips were used [15].

Quantification analysis of Listeria spp. with PCR (Polimerase chain reaction)method

Listeria spp. analysis with PCR method were carried out in a private laboratory with using Light Cycler *Listeria* Detection Kits (Roche) in PCR. 25 g of the samples was mixed with 225 ml of sterile Half Fraser Broth, homogenized in a stomacher under the aseptic conditions. Obteined homogenate was incubated for 24 hours at 30°C. At the end of the incubation, samples were subjected to DNA extraction. After extraction, reaction mix containing template DNAs of samples were prepared for PCR.

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Statistical analysis

The statistical evaluation of the results was performed using the Statistical Package for the Social Sciences (SPSS, 2008). Data collected for all parameters were analyzed by one-way analysis of variance in order to test for significant differences among treatments.

III. RESULTS AND DISCUSSION

Table 1 Bacterial counts found in poultry samples

***** K_1: Control samples$_1$; K_2:Control samples$_2$; O_P:Sprayed samples

Samples	Microbiological Analysis			
	TAMB (logCFU/g)	Yeast/Mold (logCFU/g)	*E.coli* (logCFU/g)	Coliform (logCFU/g)
K_1	7.10^{a}	4.88^{a}	2.33^{a}	3.21^{a}
K_2	6.72^{ab}	4.88^{a}	2.62^{a}	3.37^{a}
O_P	6.24^{b}	2.97^{b}	1.79^{a}	2.32^{a}
O_D	5.36^{c}	0.95^{c}	0.97^{a}	2.27^{a}

O_D: Immersed samples

**Means with different alphabetical superscripts in the same colomn are significantly different ($p<0.05$)

***Each number represents the average value of each parameter for all puoultry samples at the same time.

The evaluation of dry ice technique in the reduction of total aerobic mesophilic bacteria, yeast and mold, *E.coli* and coliform counts of poultry samples are shown in Table I. The results show a significant reduction in total aerobic mesophilic bacteria, yeast and mold counts between control and treated samples and also between sprayed and immersed samples with dry ice ($p <0.05$). No significant differences were found between control groups in total aerobic mesophilic bacteria, yeast and mold counts ($p>0.05$). Similarly, no significant differences were found between all groups in *E.coli*, coliform bacteria counts ($p>0.05$). Statistically higher reduction in total aerobic mesophilic bacteria, yeast and mold counts was found for samples sprayed with dry ice. According to the results, dry ice blasting is more effective than dry ice immersion.

Dinçer *et al.* [17] used 2% lactic acid treatment for poultry carcasses and found 2 logaritmic unit reduction in total aerobic mesophilic bacteria counts of samples. The results described in this study show similar reduction with Dinçer et al.. In another study, Kempt *et al.* [18] found 0,77 logaritmic unit reduction in total aerobic mesophilic bacteria counts of broiler samples which were immersed in acidified sodium chloride. In this study, higher reduction was obtained in total aerobic mesophilic bacteria counts as compared to those found by Kempt *et al.* [18].

Del Rio *et al.* [19] studied antimicrobial effect of trisodium phosphate, acidified sodium chloride, 2% citric acid, peroxy acetic acid and water on poultry samples. They found 1.38; 1.45; 1.38; 1.14; 0.08 logaritmic unit reduction in yeast and mold counts of samples, respectively. These values are less than what were found in our study.

Corry *et al.* [20] investigated effects of hot water treatments at different time and temperatures on *E.coli* count of poultry samples. They have reported 1.2; 1.5; 1.3 logaritmic unit reduction at the end of the study. Compared to this study, higher reduction in *E.coli* counts of samples sprayed with dry ice was obtained.

Kanellos *et al.* [21] reported that lactic acid treatment caused 1.5 logaritmic unit reduction in coliform counts of poultry samples. Similar treatment was conducted in another study which

0.96-1.13 logaritmic unit reduction was found in coliform count [22]. In this study, similar results were obtained with these efforts.

Table 2 *Salmonella* and *Listeria* serotypes identified in poultry samples

Samples	Replications	Pathogen Analysis	
		Salmonella spp.	*Listeria spp.*
K_1	I. Replications	Negative	*L. grayi*
	II.Replications	*S. arizonae*	*L. ivanovii*
	III.Replications	Neg	*L. ivanovii*
K_2	I. Replications	*S. typhi*	
	II.Replications	*S. arizonae*	*L. grayi*
	III.Replications	*S. arizonae*	*L. ivanovii*
O_P	I. Replications	*S. typhi*	*L. grayi*
	II.Replications	Negative	*L. grayi*
	III.Replications	Negative	Negative
O_D	I. Replications	*S. typhi*	*L. grayi*
	II.Replications	Negative	Negative
	III.Replications	Negative	Negative

Table II shows *Salmonella* and *Listeria* serotypes identified in poultry samples. According to the results *Salmonella typhi, Salmonella arizonae, Listeria grayi* and *Listeria ivanovii* are identified in the samples. It is found that two of total six samples (%33) treated with dry ice were contamined with *Salmonella spp.* and also three of them (%50) contamined with *Listeria spp.*

Table 3 *Salmonella spp.* and *Listeria spp.* counts identified by PCR pethods

Samples	Pathogen Analysis	
	Salmonella spp.	*Listeria spp.*
K_1	3.06^a	3.36^a
K_2	2.98^a	3.01^a
O_P	2.56^a	$1,36^a$
O_D	2.43^a	1.18^a

*Means with different alphabetical superscripts in the same colomn are significantly different ($p<0.05$)

Table III shows *Salmonella spp.* and *Listeria spp.* counts identified by PCR methods. According to the results no significant differences were found between all groups in *Salmonella spp.* and *Listeria spp.* counts ($p>0.05$).

Northcutt *et al.* [23] studied effects of chlorine water treatments at different concentration and temperature on microbiologic quality of broiler carcasses and found 0.1 and 0,3 logaritmic unit reduction in *Salmonella spp.* count. Compared to the results, higher reduction was observed in *Salmonella spp.* counts in this study.

Capita *et al.* [24] used trisodium phosphate and sodium hydroxide for disinfecting poultry carcasses and found logarithmic unit reduction in Listeria spp. counts between 1.12-3.34 and 1.80-3.28, respectively. In this study lower reduction values were obtained. Due to the detrimental effect of dry ice on the surface of poultry samples, treatment was conducted in shorter time and so lower reductions could be obtained.

IV. CONCLUSION

This study have showed that microbial loads of poultry samples decreased at the significant rate

with dry ice treatment. But dry ice can't be effective on pathogen microorganisms similarly. This can result from treatment conditions and the negative effects of dry ice in long term contact with the food surface. In the literature, various disinfectans were used for disinfection of poultry carcass. But the results show that dry ice is more effective than most of these disinfectants. Both characteristic features and manner of its application, dry ice provides many advantages in cleaning and disinfection. Even its application is limited in the food sector as a new technique, it is thought that this limit is likely to be increased in the future.

REFERENCES

1. Pipek, P., Houska, M., Jelenikova, J., Kyhos, K., Hoke, K. & Sikulova, M. (2004). Microbial decontamination of beef carcasses by combination of steaming and lactic acid spray. Journal of Food Engineering.
2. Davies, A. & Board, R. (1998). The microbiology of meat and poultry. United Kingdom. St Edmundbury Press.
3. Çetin, B. (2006). Koruyucu kültür ve laktik asit uygulamalarının tavuk etinde raf ömrü ve *Salmonella Typhimurium* gelişimi ve önemli bazı mikroorganizmaların inhibisyonu üzerine etkileri, Doktora Tezi, Atatürk Üniversitesi, Gıda Mühendisliği Anabilim Dalı, Erzurum.
4. Millar, I. (2004). Cold jet- A novel technique for cleaning and decontaminating food processing areas, equipment, carcasses and foods, Final technical report, 114 p.
5. Corry, J. E. L., James, S. J., Purnell, G., Barbedo-Pinto, C. S., Chochois, Y., Howell, M. & James, C. (2007). Surface pasteurization of chicken carcasses using hot water. Journal of Food Engineering 79(3): 913-919.
6. Seibel, B. (2007). Harnessing the cleaning power of dry ice, Finishing Today 83(3): 22-29.
7. Spur, G., Uhlmann, E. & Elbing, F. (1999). Dry-ice blasting for cleaning: process, optimization and application. Wear 233-235/402-411.
8. APHA. (1976). Compendium of methods fort he microbiological examination of foods. American Public Health Association, New York.
9. Anonim, (2008). ISO 21527-1 Microbiology of food and animal feding stuffs- Horizontal method for the enumeration of yeast and moulds- Part I: Colony count technique in products with water activity greater than 0,95.
10. AOAC. (1998). Microbiological methods, chap. 17, p.12c. In Official Methods of analysis, 16th edition, 4th revisio, p.12c.AOAC International, Gaithersburg, Md.
11. Anonim. (2006). TS EN ISO 6579/AC Mikrobiyoloji- Gıda ve Hayvan Yemleri- *Salmonella* Türlerinin Belirlenmesi İçin Yatay Yöntem, Türk Standartları Enstitüsü, Ankara.
12. AOAC. (1999). Biochemical Identification Kit Method : *Salmonella spp, Esherichia coli* and Other Enterobacteriaceae, AOAC Official Method 989.12.
13. Biotecon Diagnostics, (2010). *Salmonella* Detection Kit -5'Nuclease- : PCR system for the quantitative detection of *Salmonella* DNA using real-time PCR instruments, Version 3, October 2010.
14. Anonim. (2006). TS EN ISO 11290-2/A1 Gıda ve hayvan yemlerinin mikrobyolojisi- *Listeria monocytogenes*'in aranması ve sayımı için Yatay Metot Bölüm 2 : Sayım Metodu, Türk Standartları Enstitüsü, Ankara.
15. Microgen bioproducts, (2009). Microgen™ *Listeria*-ID System : An identification system for *Listeria* species, Camberley Surrey, UK.
16. Biotecon Diagnostics, (2010). *Listeria monocytogenes* Detection Kit -5'Nuclease- : PCR system for the quantitative detection of *Listeria monocytogenes* DNA using real-time PCR instruments, Version 3, July 2010.
17. Dinçer, A. H. & Baysal, T. (2004). Decontamination techniques of pathogen bacteria in meat and poultry, Critical Reviews in Microbiology 30: 197.
18. Kemp, G. K., Aldrich, M. L. & Waldroup, A. L. (2000). Acidified sodium chlorite antimicrobial treatment of broiler carcasses, Journal of Food Protection 63:1087–1092.

19. Del Rio, E., Panizo-Moran, M., Prieto, M., Alonso-Calleja, C. & Capita, R., (2007). Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry, International Journal of Food Microbiology, 115: 268-280.
20. Corry, J. E. L., James, S. J., Purnell, G., Barbedo-Pinto, C. S., Chochois, Y., Howell, M. & James, C., (2007). Surface pasteurisation of chicken carcasses using hot water, Journal of Food Engineering

79: 913-919.
21. Kanellos, T. S. & Burriel, A. R. (2005). The in vitro bactericidal effects of the food decontaminants lactic acid and trisodium phosphate, Food Microbiology 22: 591–594.
22. Schneider, K. R., Kemp, G. K. & Aldrich, M. L. (2002). Antimicrobial treatment of air chilled broiler carcasses. Acidified sodium chlorite antimicrobial treatment of air chilled broiler carcasses, Dairy, Food and Environmental Sanitation 22: 102–108.
23. Northcutt, J. K., Smith, D. P., Musgrove, M. T., Ingram, K. D. & A. Hinton, Jr, (2005). Microbiological impact of spray washing broiler carcasses using different chlorine concentrations and water temperatures, Poultry Science 84: 1648-1652.
24. Capita, R., Alonso-Calleja C., Garcia-Arias, M. T., Moreno, B. & Garcia-Fernandez, M. C. (2002). Methods to detect the occurrence of various indicator bacteria on the surface of retail poultry in Spain, Journal of Food Science 67 (2): 765-771.