EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF PHOSPHATES IN REFRIGERATED CHICKEN CARCASSES

Carlos Eduardo Ruberti Perizzotto[1]; Luciana Miyagusku[2];

[1]Specialization course student at the Centro de Tecnologia de Alimentos – ITAL – Campinas / SP
[2]Research Scientist at the Centro de Tecnologia de Alimentos – ITAL – Campinas / SP

Introduction

In the last years, the number of research on the influence of phosphate in microbial growth is increasing. However, so far, the basic mechanism of how the phosphates interfere in the microbial growth is still not well-known. The most common theory is that the phosphates interfere with the minerals uptake of the cell. The research, agrees that the Gram-positive microorganisms are primarily affected. However, there are some Gram-negative microorganisms that can be inhibited by the phosphates (BOOK, 2000). There are some research on the effect of the phosphates in microbial growth in refrigerated chicken carcasses, however all in pilot-scale. In 1964, ELLIOT *et al.* observed that the shelf life of refrigerated chicken carcasses increased 17 and 25% with the addition of 3 and 8%, respectively, of a mix of polyphosphates in the cooling solution. In 1995, RATHGEBER and WALDROUP observed a significant reduction for coliforms and *E.coli*, by the immersion of chicken carcasses for an hour in a solution with 1.5% of a mix of Sodium Acid Pyrophosphate and Orthophosphoric Acid. In 1999, KIM and MARSHALL verified that chicken legs dipped in a solution of 5% of Trisodium Phosphate for 10 minutes presented an Aerobic Plate Counts 3 logs lower than the control.

Materials and Methods

During normal processing in a slaughterhouse facility, chicken carcasses was collected randomly before and after two chillers, one containing just water and ice (control) and the other containing water, ice and phosphate (treatment), both with the same dimensions and capacity. All the samples collected were packed in polyethylene bags and sent immediately to the freezing tunnel, with residence time of approximately one hour, and right after were sent to the cooling chamber at -3°C. The following phosphates were evaluated: MSP – Monosodium Phosphate (NaH_2PO_4), SAPP – Sodium Acid Pyrophosphate ($Na_2H_2P_2O_7$), TSPP – Tetrasodium Pyrophosphate ($Na_4P_2O_7$), STPP – Sodium Tripolyphosphate ($Na_5P_3O_{10}$), and SHMP - Sodium Hexametaphosphate ($Na_{n+1}P_nO_{(3n+1)}$ n =13–18). The initial concentration of each phosphate was 1.5%. The microorganisms monitored in this experiment were the number of total coliforms and fecal coliforms (MPN - Most Probable Number) and the research of *Salmonella ssp* (VANDERZANT, SPLITTSTOESSER, 1992). To determine if the phosphates influenced the normal processing of chicken carcasses, the pH of the skin surface and the interior of the muscle was measured (pHmetro Digimed - model DM-20). Besides this, the water absorption of the carcasses during the cooling (Absorption Index - Portaria n°210 de 10 de novembro de 1998 / MA) and the loss of exuded liquid during the thawing - Drip Test (recommended by the FAO - *Added Water in Frozen Chicken*) were determined. Temperature of the carcasses before and after the cooling was measured. A sample of the cooling solution from each chiller was taken at the beginning and at the end of the experiment. The analysis of the level of phosphate in the cooling solution was done by liquid chromatography HPLC, using the method of analysis PHO-PF-008 of Astaris Brasil Ltda.

Results and Discussion

Cooling Solution Concentration: The phosphates content in the cooling solution (Table 1) drastically decreased from the moment of its preparation until the end of the experiments. Part of the phosphate initially added was hydrolyzed for being in an aqueous solution. However, it is an almost insignificant fraction, because in low temperatures the half-life time of phosphates is very long. The most significant part of the hydrolysis is probably due to the action of phosphatases, because of the large quantity of carcasses present in the chiller, and of the high content of blood dissolved in the solution. The hydrolysis rate in the presence of enzymes is 10^6 times higher than the absence of enzymes (MONSANTO CO, 1995). Other significant factor was the frequent addition of ice to the chiller for the maintenance of low temperature of the solution. This suspicion can be confirmed by the reduction of MSP content, since this phosphate can not hydrolyze.

Table 1 – Concentration of Phosphates in the Cooling Solution

Phosphate	STPP	SAPP	TSPP	SHMP	MSP
Treatment – Beginning (%)	0.76	0.42	0.34	0.57	0.32
Treatment – End (%)	0.23	0.15	0.29	0.41	0.10

Other relevant suspicion is the hydrolysis that occurred after the collection of samples. As the time spent between the collection and the analysis of the sample was in average 14 days, even though the samples have been frozen right after the collection and maintained freezed during all the stocking time, the presence of phosphatase in the solution could have caused the hydrolysis of the phosphate, mainly during the periods of thawing and of preparing the solution for the realization of the analysis.

Analysis of pH: It can be concluded that a significant alteration of pH occurred ($P \leq 0.05$), only in the surface of the carcasses treated with SHMP and in the muscle of carcasses treated with SHMP and MSP (Table 2). Larger alterations occurred for the SAPP and the STPP. However, the measures of pH by these phosphates were realized in carcasses treated in laboratory. In this case, as we did not have a constant addition of ice, like it occurred in the normal processing, these carcasses were exposed to a more concentrated solution.

Table 2 – Results of the analysis of pH

	Surface – Control			Surface – Treatment			Muscle – Control			Muscle - Treatment		
Phosphate	Avg.	Error	ESE *	Avg.	Error	ESE *	Avg.	Error	ESE *	Avg.	Error	ESE *
MSP	6.25	0.07	1.12 %	6.22	0.14	2.17 %	6.12	0.07	1.15 %	5.79	0.08	1.30 %
SAPP **	-	-	-	4.74	0.09	-	-	-	-	5.99	0.10	1.70 %
TSPP	6.11	0.15	2.52 %	6.31	0.06	0.87 %	5.93	0.14	2.29 %	5.89	0.28	4.69 %
STPP **	-	-	-	8.79	0.21	-	-	-	-	6.03	0.24	4.01 %
SHMP	7.15	0.10	1.38 %	6.06	0.18	2.97 %	6.01	0.10	1.59 %	5.71	0.04	0.76 %

* ESE – Estimate Standard Error. ** Analysis realized in pilot test.

Temperature of Carcasses: Carcass temperature shows that the chiller used for the treatment presented a shorter residence time, because of their higher final temperatures compared to the control chiller (Table 3). With this, potentially two interferences in the experiment can have

occurred: The higher temperature of the carcass can lead higher absorption of water. The second refers to the microbial growth, since elevated temperatures are better for the growth of microorganisms.

Table 3 – Average temperature for chicken carcasses before and after the chiller

Phosphate	MSP	SAPP	TSPP	STPP	SHMP
Initial Temperature (°C)	40.80	41.90	42.30	41.50	43.30
Final Temperature – Control (°C)	9.17	8.23	11.03	8.88	11.17
Final Temperature – Treatment (°C)	7.93	12.20	13.18	10.36	11.22

Absorption Index: The quantity of phosphates used in the cooling solution of the chiller is not sufficient to result in a significant increase in the absorption of water by the carcasses (Table 4). Only the data of the absorption referent to MSP, SAPP and TSPP presented statistically significant differences ($P \leq 0.05$). However, for the TSPP, in average, the absorption was lower for the carcasses treated with phosphates. For the SAPP, a higher absorption observed could have been caused by a higher temperature of the cooling solution.

Table 4 – Results of the test of absorption (%)

Phosphate	MSP		SAPP		TSPP		STPP		SHMP	
Chiller	Control	Treatment	Control	Treatment	Control	Treatment	Treatment	Control	Treatment	Control
Average	14.85	18.72	12.96	17.02	17.29	13.44	13.90	14.45	12.95	13.13
Error	1.11	1.68	2.36	0.93	2.04	1.68	2.80	0.60	2.90	2.54

Dripping Analysis: There was not a statistically significant difference in the results of the analysis of Drip Test the carcasses of control and the treated with phosphates.

Microbiological Analysis: In Tables 5 and 6, are the average values obtained from the microbiological analysis for total and fecal coliforms.

Table 5 - Total Coliforms (log MPN/g)

Phosphate	STPP		SAPP		TSPP		SHMP		MSP	
	Avg.	Error	Avg.	Error	Avg.	Error	Avg.	Error	Avg.	Error
Before the Pre-Chiller	3.38	0.00	3.63	0.32	3.75	0.42	1.24	0.40	3.82	0.16
After Chiller – Control	3.21	0.24	1.52	0.60	1.72	0.61	1.32	0.47	1.76	0.61
After Chiller – Treatment	3.14	0.41	1.58	0.39	1.47	0.63	1.29	0.43	1.56	0.68

Table 6 – Fecal Coliforms (log MPN/g)

Phosphate	STPP		SAPP		TSPP		SHMP		MSP	
	Avg.	Error	Avg.	Error	Avg.	Error	Avg.	Error	Avg.	Error
Before the Pre-Chiller	3.27	0.20	3.58	0.43	3.61	0.74	1.24	0.40	3.75	0.21
After Chiller – Control	2.34	1.00	1.52	0.60	1.52	0.57	1.24	0.39	1.56	0.53
After Chiller – Treatment	2.78	1.30	1.58	0.39	1.41	0.62	0.98	0.68	1.18	0.21

The STPP data will not be considered in the evaluation of the results of this experiment due to the small number of samples available, since many samples were rejected. There was little difference between the control and the phosphate treatment realized with the SAPP. This result can have been influenced by the high temperature of the carcasses treated in the end of the cooling, what did not occur for the carcasses of the control. For the treatment with SHMP, it was observed a small reduction only for the counting of fecal coliforms. For MSP and TSPP, it in such a way had a reduction of the counting of total coliforms, as of the counting of fecal coliforms. As the real concentration of the cooling solution was way inferior than the originally foreseen concentration, it is believed that the reduction of the counting of total and fecal coliforms could have been more extensive if the concentration of the solution was really in 1.5%, as observed by RATHGEBER LDROUP 1995. About the analysis of *Salmonella ssp*, this work was not conclusive because it was not detected the presence of Salmonella in a number of samples that was significant.

Conclusion

The presence of the enzyme phosphatase in the cooling solution causes a fast hydrolysis of the present phosphate in the solution, being necessary a constant replacement of phosphate during the processing, so that it gets the desired effect. In the concentration used in this study (1.5%), the alteration of pH of the surface and of the muscle of chicken carcasses is practically insignificant, and does not affect the final product. The use of phosphates in the cooling solution does not result in a significant increase of the water absorption for the chicken carcasses, being that this variable is more susceptible to the variation of the temperature of the solution than for the presence of the phosphates. The presence of the phosphates also does not affect the loss of liquid during the thawing. The cooling of carcasses by immersion has a very important role in the reduction of the initial microbial level, independent of the presence of the phosphates. Reduction of microbial count for the treatment realized with SAPP was not observed. For the SHMP, it was observed a reduction only of the counting of fecal coliforms. However, the TSPP and the MSP had been the phosphates that had presented the best results of reduction of the counting of total and fecal coliforms. It is believed that in bigger concentrations this reduction can still be more significant. It was not possible to conclude the effectiveness of the phosphates in the reduction of *Salmonella* due to a low incidence of this microorganism in the analyzed samples.

References

BOOK Sharon. *Literature Review of Phosphates & Microorganisms*. Astaris LLC, St. Louis, p. 1 – 11, Nov. 2000.

ELLIOTT, R. P., STRAKA, R. P., GARIBALDI, J. A. *Polyphosphate Inhibition of Growth of Pseudomonads From Poultry Meat*. Applied Microbiology, v. 12, n. 6, p. 517 – 522, 1964.

KIM, C. R., MARSHALL, D. L. *Microbiological, colour and sensory changes of refrigerated chicken legs treated with selected phosphates*. Food Research International, v. 32, p. 209 – 215, 1999.

MONSANTO CO. *Hydrolysis of Phosphates – Technical Update from Monsanto*, St. Louis, p. 1 – 16, Jan. 1995.

RATHGEBER, B. M., WALDROUP, A. L. *Antibacterial Activity of a Sodium Acid Pyrophosphate Product in Chiller Water Against Selected Bacteria on Broiler Carcasses*. Journal of Food Protection, v. 58, n. 5, p. 530 – 534, 1995.

VANDERZANT, C., SPLITTSTOESSER, D. L. Compendium of Methods for the Microbiological Examination of Foods, 3ed. Washington: American Public Heath Association (ALPHA), 1992.