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Effect of Hydrogen Peroxide in the Scald Tank on the Microbial Count of Pork Skin

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Keywords

Swine – Slaughtering – Hydrogen peroxide – Biological contamination – Meat hygiene – Brazil.

Summary

The objective of this study was to investigate the effect of hydrogen peroxide (H_2O_2) (50% v/v) on pork skin microbial populations. Forty-eight crossbred hogs were analyzed after dehairing and 30 during chilling. Three different concentrations of hydrogen peroxide were added to the scalding water (0.01, 0.05 and 0.1% of the total capacity of the scald tank). In treatment I no addition of H_2O_2 was applied while, in treatment II, H_2O_2 was added at 0 min and, in treatment III, it was added at 0, 30 and 60 min. Both treatments II and III significantly reduced pork skin proliferation of thermophilic bacteria after 30 min of continuous scalding ($P \leq 0.05$). Treatment III decreased the Enterobacteriaceae count after 90 min of scalding ($P \leq 0.05$). During chilling, Enterobacteriaceae and thermophilic bacteria were not detected on pork skin, and hydrogen peroxide lowered values of mesophilic and psychrophilic bacteria ($P \leq 0.05$). This study showed that the addition of hydrogen peroxide to the scalding water decreased the microbial contamination of pork skin.

■ INTRODUCTION

Meat contamination occurs during the conversion of muscle into meat through the slaughter and dressing processes (11, 15). Pork carcasses usually contain higher levels of microorganisms when they are compared to carcasses of other meat animals (4). Pig slaughter offers many opportunities for carcass contamination and thus requires some effort to eliminate or minimize hazards (3). Bacteria counts have been reported to increase on the ham surface after scalding due to the higher contamination of the scalding water after 30 min of operation (5, 10). Hence, the dehairing equipment used right after scalding might be an important source of contamination (4). After dehairing, aerobic mesophilic and coliform bacteria counts were high on the ham, belly, and neck (9).

Intervention strategies for pork harvesting involving mechanical and chemical methods have been used in the US as a measure to eliminate or decrease microbial contamination which occurs during slaughter (6). Additionally, studies involving additives and different methods of scalding gave varying results on pork skin contamination (11, 14). In Brazil, very little research was conducted to study

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similar interventions. However, some research showed that the prevalence of bacteria such as *Salmonella* and *Staphylococcus* in Brazilian pork slaughter plants was lower than that of other countries' slaughter plants (8). Consequently, chemical and mechanical interventions on microbial contamination are not practiced very often. However, although this prevalence was detected, there is no data which correlates food-borne diseases with meat consumption. This experiment was conducted in a commercial abattoir in Brazil with the aim to verify the effects of adding hydrogen peroxide (H_2O_2) (50% v/v) to the scalding water on the counts of aerobic mesophilic, aerobic thermophilic, aerobic psychrophilic, and Enterobacteriaceae bacteria on pork skin. To conduct this study, it was necessary to request authorization from the Brazilian Department of Agriculture for the use of hydrogen peroxide during pig scalding.

■ MATERIALS AND METHODS

Animals

For the microbial analysis of pork skin after dehairing, 48 crossbred hogs were randomly selected from three treatments. Four animals were sampled each day during four days totaling 16 animals per treatment. All the animals used in this experiment were raised and finished in a commercial hog farm located nearby the slaughter plant. Treatments consisted of (I) no addition of H_2O_2 to the scalding water, (II) addition of H_2O_2 at 0 min, and (III) addition of H_2O_2 at 0, 30, and 60 min. For the microbial analysis of pork skin during chilling, 30 crossbred hogs were randomly selected from two treatments (no addition of H_2O_2 to the scalding water and addition of H_2O_2 at 30 min). Before chilling, animals were randomly selected after being scalded for 5 to 6 min, 30 minutes after the scalding procedure had been initiated (five animals were sampled each day during three days totaling 15 animals per treatment). The research was completed in a commercial abattoir under Brazilian federal inspection in Bauru, São Paulo, with a slaughter capacity of 500 hogs per day.

Hydrogen peroxide preliminary treatments

Terplan and Wenzel report that a maximum bacterial load in the scalding water is reached 30 min after the scalding operation begins (13). In the present study, three concentrations (0.01, 0.05 and 0.1% of the total capacity of the tank) of H_2O_2 were thus added to the scalding water collected 30 min after scalding began. The *in vitro* test was carried out to determine which concentration of H_2O_2 added to the scald tank was the most efficient.

Water and carcass sampling

Scalding water samples were taken randomly from the scald tank at 0, 30, 60 and 90 min. The pork skin was sampled immediately after dehairing on the left side of the carcass by swabbing a 10 cm^2 area using a sterilized stainless frame (2 x 5 cm) at 0, 30, 60 and 90 min. The same procedure was performed on the pork skin at 0 and 24 h of chilling. Five different sites with five different swabs were analyzed, totaling 50 cm^2 (ham, belly, neck, shoulder, and loin) (1). Each swab was immersed in a sterilized test tube containing 5 mL of buffered peptone water. Samples were transferred under refrigeration (7 ± 2ºC) to the Agroindustrial Products Technology Laboratory at São Paulo State University (UNESP - Botucatu).

One milliliter of scalding water and 1 mL of fluid containing samples of all five swabs were diluted in 9 mL of peptone water until dilution reached 10^{-7}. Aerobic mesophilic, thermophilic, and psychrophilic bacteria were counted in standard plate count agar after incubation at 32 ± 2°C for 48 h, 55 ± 2°C for 48 h, and 7 ± 2°C for 10 days, respectively. Enterobacteriaceae bacteria were counted in violet red bile dextrose agar after incubation at 37 ± 2°C for 48 h (1).

Statistical analysis

Statistical analysis for microbial count was performed using PROC MIXED of SAS (12). The number of experimental units was calculated by PROC POWER of SAS using means and standard deviations from previous research. Comparisons among treatments at 0.05 required eight experimental units to obtain a power of 99%. When comparing 0.05 vs 0.1, 12 experimental units were required to obtain a power of 86%. In this experiment, 16 experimental units per treatment were used as previously described. Regarding the presented values, all bacteria counts were transformed to log cfu/100 cm^2 for the data on the pork skin and log cfu/mL for the data in the scalding water. Total counts were analyzed as randomized blocks with one site (average of all five analyzed sites) and each individual carcass as a block. Treatment comparisons were analyzed in a 3 x 4 factorial combination considering three treatments (I, II, and III) and four periods (0, 30, 60 and 90 min). A similar analysis was performed for chilled carcasses. However, a 2 x 2 factorial combination was used (two treatments: with or without hydrogen peroxide; and two periods: 0 h and 24 h of chilling). Means were compared with the Tukey test at 5% level of significance.

■ RESULTS AND DISCUSSION

Hydrogen peroxide preliminary treatments

The effects of H_2O_2 in the water 30 min after scalding is shown in Table I. Results indicated that concentrations of 0.05 and 0.1% significantly influenced mesophilic and thermophilic counts when compared with 0 and 0.01%. The mesophilic count decreased from 4 to 2 log cfu/mL, whereas the thermophilic count decreased from 5 to 2 log cfu/mL. Values observed from both concentrations were similar statistically. Therefore, the lowest most effective concentration (0.05%) was chosen to be added to the scalding water during the industrial phase of the experiment.

Table I

Effects of hydrogen peroxide on the total number of aerobic bacteria in samples of water taken 30 min after scalding began

Bacteria	Hydrogen peroxide concentration (%)			
	0*	0.01*	0.05*	0.1*
Mesophilic (log cfu/mL)	4.12^{A}	3.63^{A}	2.95^{B}	2.69^{B}
Thermophilic (log cfu/mL)	5.50^{A}	4.61^{AB}	2.66^{BC}	2.38^{C}
Enterobacteriaceae (log cfu/mL)	< 1.00 est	< 1.00 est	< 1.00 est	< 1.00 est
Psychrophilic (log cfu/mL)	< 1.00 est	< 1.00 est	< 1.00 est	< 1.00 est

* Percentage of the total capacity of the tank

^{A,B,C} Means in the same row with different superscripts are significant at $P \leq 0.05$

est: estimated value

Effect of hydrogen peroxide treatments on the scalding water

Results regarding the effects of hydrogen peroxide treatments on the scalding water are summarized in Table II. For aerobic mesophilic bacteria, treatment I had higher values after 30 minutes of scalding, which remained stable thereafter. These values agree with a previous study which documented that maximum microbial load values are constant and similar after 30 min (14). During treatment II, the addition of H_2O_2 gave lower values of mesophilic bacteria only at 0 min ($P \leq 0.05$). Results after 0 min were statistically similar to results found in treatment I. Treatment III showed lower values of mesophilic bacteria after 90 min and similar values for the period between 0 and 90 min ($P > 0.05$). In addition, the average of all periods was lowest in treatment III (Table II). In the literature, esophilic counts in the scalding water range from 4 to 5 log cfu/mL (4). In the present study, after 90 min, the scalding water from treatment III showed the mesophilic count at approximately 1 log cfu/mL. These results indicated that when H_2O_2 was added periodically at each 30 min of scalding, it prevented an increase of mesophilic bacteria in the scalding water.

Values of thermophilic bacteria gradually increased from period to period during treatment I. When treatment II was performed, lower values were observed in all periods when compared to treatment I ($P \leq 0.05$). Values, however, increased significantly up to 90 min ($P \leq 0.05$). Treatment III values for all periods were also lower than those of treatment I but did not increase across periods as observed in treatment II. Therefore, the lowest mesophilic and thermophilic counts were obtained during treatment III.

Enterobacteriaceae and psychrophilic bacteria were not detected in the scalding water.

Effect of hydrogen peroxide treatments on pork skin

The effects of H_2O_2 on all four bacteria groups of pork skin after dehairing are shown in Table III. Up to 90 min, treatment I showed similar values for all periods ($P > 0.05$). Unlike results obtained with the scalding water, mesophilic counts did not increase on pork skin. When values of all periods were averaged, treatment II significantly influenced the mesophilic count, whereas the average value of treatment III was similar to treatments I and II. Therefore, treatment II was satisfactory regarding the decrease of mesophilic bacteria on pork skin. Values of mesophilic bacteria were previously reported at around 7 log cfu/100 cm^2 (13). In the present study, H_2O_2 lowered these values to 5.39 log cfu/100 cm^2. For thermophilic bacteria, average values of all periods were statistically similar between the treatments ($P > 0.05$). However, a significant increase was observed at 90 min during treatment I ($P \leq 0.05$). Treatments II and III led to lower values compared to treatment I ($P \leq 0.05$) at 90 min. Hence, the authors suggest that thermophilic bacteria on pork skin were sensitive to the H_2O_2 oxidative effect.

Values of Enterobacteriaceae bacteria were constant on pork skin. Average values were significantly lower with the H_2O_2 treatments (II and III) ($P \leq 0.05$). Carcasses of treatment III analyzed at 60 and 90 min showed lower counts than carcasses of the other treatments. In addition, treatment III showed a significant decrease of Enterobacteriaceae bacteria at 60 min. Therefore, the best results were obtained with treatment III. Based on results by other authors, Enterobacteriaceae bacteria counts were found to range between 5 and 5.5 log cfu/100 cm^2 on pork skin after dehairing (2, 14). In the present study, values around 3 log cfu/100 cm^2 were obtained when H_2O_2 was added to the water.

Psychrophilic bacteria counts were constant at 30, 60, and 90 min for treatments II and III. In addition, treatments which contained H_2O_2 had lower average values than treatment I.

Effect of hydrogen peroxide on pork skin during chilling

Carcasses scalded in water treated with H_2O_2 had lower counts of mesophilic bacteria at 0 h of chilling compared to carcasses scalded with no H_2O_2. After 24 h, carcasses from both treatments had similar mesophilic counts ($P > 0.05$). However, when comparing the average for both periods (0 and 24 h), carcasses scalded with H_2O_2 had lower values ($P \leq 0.05$). For psychrophilic bacteria, H_2O_2 treatment

Table II

Effects of different treatments of hydrogen peroxide on the number of aerobic bacteria in scalding water

Bacteria	Treatment	Period (min)				Average
		0	30	60	90	
Mesophilic (log cfu/mL)						
	I*	2.14^{Ba}	3.19^{Aa}	3.21^{Aa}	3.27^{Aa}	2.95^{a}
	II**	1.48^{Cab} est	2.49^{ABab}	2.65^{ABa}	2.90^{Aa}	2.38^{b}
	III***	1.14^{Ab} est	1.58^{Ab} est	1.44^{Ab} est	1.05^{Ab} est	1.30^{c}
Thermophilic (log cfu/mL)						
	I*	2.52^{Ba}	3.01^{ABa}	3.12^{ABa}	3.92^{Aa}	3.14^{a}
	II**	1.25^{Cb} est	1.73^{Bb} est	2.09^{Bab}	2.60^{Ab}	1.92^{b}
	III***	1.16^{Ab} est	1.35^{Ab} est	1.12^{Ab} est	1.11^{Ac} est	1.19^{c}

^{A,B,C} Means in the same row within a bacteria group with different superscripts are significant at $P \leq 0.05$

^{a,b} Means in the same column within a bacteria group with different superscripts are significant at $P \leq 0.05$

est: estimated value

* No addition of H_2O_2

** Addition of H_2O_2 to the scalding water at 0 min

*** Addition of H_2O_2 to the scalding water at 0, 30 and 60 min

Table III

Effects of different treatments of hydrogen peroxide on the number of aerobic bacteria on pork skin after dehairing

Bacteria	Treatment	Period (min)				Average
		0	30	60	90	
Mesophilic (log cfu/mL)						
	I*	6.02	5.63	6.06	5.97	5.92^{a}
	II**	5.24	5.27	5.54	5.53	5.39^{b}
	III***	6.18^{A}	5.57^{AB}	5.52^{AB}	5.12^{B}	5.60^{ab}
Thermophilic (log cfu/mL)						
	I*	2.17^{B} est	$< 2.00^{B}$ est	2.38^{B} est	3.61^{Aa} est	2.54
	II**	2.49 est	< 2.00 est	2.26 est	$< 2.00^{b}$ est	2.19
	III***	< 2.00 est	< 2.00 est	< 2.00 est	$< 2.00^{b}$ est	< 2.00
Enterobacteriaceae (log cfu/mL)						
	I*	4.47^{a}	3.86 est	4.43^{a}	3.59^{a} est	4.09^{a}
	II**	2.63^{Bb} est	3.23^{AB} est	3.44^{Ab} est	3.36^{Aa} est	3.17^{b}
	III***	3.91^{Aa}	3.17^{AB} est	2.26^{Bc} est	2.32^{Bb} est	2.92^{b}
Psychrophilic (log cfu/mL)						
	I*	4.77	3.30 est	3.74 est	3.79	3.90^{a}
	II**	2.77 est	< 2.00 est	< 2.00 est	< 2.00 est	2.19^{b}
	III***	3.56^{A}	$< 2.00^{B}$ est	$< 2.00^{B}$ est	$< 2.00^{B}$ est	2.39^{b}

^{A,B,C} Means in the same row within a bacteria group with different superscripts are significant at $P \leq 0.05$
^{a,b} Means in the same column within a bacteria group with different superscripts are significant at $P \leq 0.05$
est: estimated value
* No addition of H_2O_2
** Addition of H_2O_2 to the scalding water at 0 min
*** Addition of H_2O_2 to the scalding water at 0, 30 and 60 min

showed a significant decrease in counts at both periods and when the values were averaged. Enterobacteriaceae and thermophilic bacteria were not detected during chilling (Table IV).

■ CONCLUSION

Periodic addition of H_2O_2 to the scalding water during each 30 min of continuous scalding decreased water counts of aerobic mesophilic and thermophilic bacteria. Enterobacteriaceae and psychrophilic bacteria groups did not seem to present a health hazard in the scalding water due to their lower values. The addition of H_2O_2 to the scalding water effectively decreased the pork skin microbial counts of all evaluated groups after dehairing. However, addition of H_2O_2 at each 30 min of continuous scalding is recommended for best results. During chilling, when H_2O_2 was added to the scalding water, lower bacteria loads were identified on pork skin. When carcasses are processed further into retail cuts, pork skin contamination can be disseminated to other surfaces (7). Therefore, addition of H_2O_2 to the scalding water is an excellent alternative to improve the quality of pork carcasses and minimize the further contamination which may occur on retail cuts.

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Table IV

Effects of different treatments of hydrogen peroxide on the total number of aerobic bacteria on pork skin at 0 and 24 h of chilling

Bacteria	Treatment	Period (h)		Average
		0	24	
Mesophilic (log cfu/100 cm^2)				
	I*	3.64^{Aa}	2.42^{B}	3.03^{a}
	II**	$< 2.00^{b}$ est	< 2.00 est	$< 2.00^{b}$ est
Psychrophilic (log cfu/100 cm^2)				
	I*	2.25^{Aa}	2.08^{Ba}	2.16^{a}
	II**	$< 2.00^{b}$ est	$< 2.00^{b}$ est	$< 2.00^{b}$ est

^{A,B} Means in the same row with different superscripts are significant at $P \leq 0.05$
^{a,b} Means in the same column with different superscripts are significant at $P \leq 0.05$
est: estimated value
* No addition of H_2O_2
** Addition of H_2O_2 to the scalding water at 30 min

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Résumé

De Mello A.S. Jr., Roça R.O. Effet du peroxyde d'hydrogène dans le bac d'échaudage sur la numération microbienne cutanée des porcs

L'objectif de cette étude a été d'examiner l'effet du peroxyde d'hydrogène (H_2O_2) (50% v/v) sur les populations microbiennes en surface des carcasses de porc. Quarante-huit carcasses de porcs hybrides ont été analysées après épilation et trente pendant le refroidissement. Trois concentrations différentes de H_2O_2 ont été ajoutées à l'eau du bac d'échaudage (0,01, 0,05 et 0,1 p. 100 de la capacité totale du bac). Dans le traitement I, aucun ajout de H_2O_2 n'a été effectué ; dans le traitement II, H_2O_2 a été ajouté à 0 min ; dans le traitement III, H_2O_2 a été incorporé à 0, 30 et 60 min. Les traitements II et III ont réduit significativement la prolifération des bactéries thermophiles au niveau cutané après 30 min d'échaudage continu ($P \leq 0,05$). Le traitement III a diminué le nombre d'entérobactériacées après 90 min d'échaudage ($P \leq 0,05$). Pendant le refroidissement, ni entérobactériacée ni bactérie thermophile n'ont été détectées sur la peau des porcs, et H_2O_2 a abaissé la numération bactérienne mésophile et psychrophile ($P \leq 0,05$). Cette étude a montré que l'addition du peroxyde d'hydrogène dans l'eau d'échaudage des carcasses diminuait la contamination microbienne cutanée des carcasses de porcs.

Mots-clés : Porcin – Abattage d'animaux – Peroxyde d'hydrogène – Contamination biologique – Hygiène de la viande – Brésil.

Resumen

De Mello A.S. Jr., Roça R.O. Efecto del peróxido de hidrógeno en el tanque de escaldado sobre el conteo microbiano en la piel de puerco

El objetivo del presente estudio fue el de investigar el efecto del peróxido de hidrógeno (H_2O_2) (50% v/v) sobre las poblaciones microbianas en la piel de puerco. Se analizaron 48 cerdos cruzados, después de pelados y enfriados durante 30 minutos. Se agregaron tres concentraciones diferentes de peróxido de hidrógeno al agua de escaldado (0,01, 0,05 y 0,1% de la capacidad total del tanque de escaldado). En el tratamiento I no se agregó H_2O_2, en el tratamiento II H_2O_2 se adicionó al minuto 0 y en el tratamiento III H_2O_2 se adicionó al minuto 0, 30 y 60. Ambos tratamientos II y III redujeron significativamente la proliferación de bacterias termofílicas en la piel de puerco después de 30 minutos de escaldado continuo ($P \leq 0,05$). El tratamiento III disminuyó el conteo de enterobacterias después de 90 minutos de escaldado ($P \leq 0,05$). Durante el enfriamiento, las enterobacterias y las bacterias termofílicas no se detectaron en la piel del puerco y el peróxido de hidrógeno bajó los valores de bacterias mesofílicas y psicrofílicas ($P \leq 0,05$). El presente estudio mostró que la adición de peróxido de hidrógeno al agua de escaldado disminuye la contaminación bacteriana de la piel de puerco.

Palabras clave: Cerdo – Sacrificio – Peróxido d'hidrógeno – Contaminación biológica – Higiene de la carne – Brasil.