THE USE OF LACTIC ACID AND OZONE FOR THE REDUCTION OF THE BACTERIAL COUNT IN OVINE CARCASS SURFACES

Antonella Goyeneche,[1*] Gianni Bianchi,[2] and Oscar Bentancur[3]

[1]Departamento de Calidad, Frigorífico Casa Blanca S. A., Casablanca, Paysandú 60000, Uruguay
[2]Departamento de Producción Animal y Pasturas and [3]Departamento de Estadística, Facultad de Agronomía, Universidad de la República, Paysandú 60000, Uruguay
*antonellagoyeneche@fricasa.com.uy

Abstract – The effect of ozone in low concentrations (2.4 mg × m^{-3} × h^{-1}) and/or a warm lactic acid solutions (50mL/carcass, 3% m/m, 45 °C) over the Total Plate Count (TPC) and Enterobacteria count (EB) in 60 ovine carcass surfaces after 24 hours of post sacrifice maturation was studied. The TPC was not affected by any treatment. On the other hand, only the carcasses treated with ozone showed a reduction in the EB count (-1.08 log ufc / cm^2; p≤0.05).

I. INTRODUCTION

A wide variety of antimicrobial treatments have been tested in order to reduce the microbial count in bovine and ovine carcass surfaces (1). Internationally, organic acids, such as lactic acid, and hot water or steam are the most common. In Uruguay, the use of lactic acid has been recently introduced due to EU regulation N° 101/2013 (2). At the end of the slaughter line the carcass is washed with tap water, and even though this is not meant to improve the microbial count, it contributes to wash out the bacteria and to prevent meat superficial color deterioration and dry out (1,3). Another USDA authorized compound is ozone, which is an effective pathogen reducer either in aqueous solution or as a gas (4). It is widely used in the food industry because it does not leave any toxic residues, making it versatile and compatible with HACCP programs. As a result, it has been used in slaughter plants as disinfectant for facilities and equipment and in other industries, such as vegetable and fish, for microbial reduction during processing as well (5). Two of these studies were carried out locally by our group (6, 7). No references were found regarding the use of lactic acid in the slaughter line of bovines or ovines. The purpose of this paper was to evaluate the effect of the use of lactic acid in the slaughter line as well as ozone exposure during post sacrifice maturation.

II. MATERIALS AND METHODS

The study was carried on in a local slaughterhouse (FRICASA). The sources of variation in the initial microbial count in carcass surfaces are several, from operational practices (of the slaughter line) and personal hygiene habits, to original microbial charge of the fleece (depending on the establishment of origin and the transport), being all of them of great incidence. For this paper it was chosen to cancel as much of these variation factors as possible, because any of them have influence in the effectiveness of the treatments, only in the initial microbial counts. In order to this, sixty ovine carcasses from a lot (single producer, single day) were used (Corriedale ewes, 17.26 ± 0.51 kg). Carcasses and treatments: 1-15: AL 3%; 16-30: ozone; 31-45: AL3%+ozone; 46-60: blank. In order to emphasize the effect of the treatments used, no routine washing of the carcasses was performed at the end of the slaughter line. This was a conservative approach, because if the treatments turned out to be effective, they would be much more in normal operational conditions. And besides, it was conducted this way for two purposes: to avoid the possibility of getting final (post-treatment) counts of zero ufc/cm^2 (that cannot be included in statistical analysis), and to protect the confidentiality of the microbiological baseline of the slaughter process of the exporter establishment. For the ozone exposure studies, the ozone-production equipment was installed inside one of the chillers (LER S.A., 2.4 mg × m^{-3} × h^{-1}). For the blank and the carcass treated only with lactic acid a different chiller was used. But for what it counts for this study, the parameters of maturation of both chillers were the same (dimensions, chilling equipment performance, and number of carcasses inside). For lactic acid applications, a backpack fumigation unit with a solution of food grade lactic acid in

warm water was employed (50mL/carcass, 3% m/m, 45 °C). The 60 carcasses were subjected to microbiological analysis before the treatments and after chilling for 24 hours. For each assay, four delimited zones (80 cm^2 total) were sampled in each case, two by side, forequarters and hindquarters. The sample collection was made using a sponge (non-destructive). The cell recovery was in peptone water and the growth in 3M ® petrifilms for TPC (ISO 4833) and for EB (ISO 2158-2) during 24 hours at 37 °C. Two sets of data were obtained for TPC and EB. Each set made of the counts before the treatments and after chilling for 24 hours. For the statistical analysis, viable cell count reduction (R) was selected as a variable, expressed in $\log_{10}$ base (being ufc colony forming units):

$$R = \log_{10}[\text{initial}(\text{ufc} / \text{cm}^2)] - \log_{10}[\text{final}(\text{ufc} / \text{cm}^2)],$$

A variance analysis was performed for the reduction in each case, and the variance homogeneity was tested.

III. RESULTS AND DISCUSSION

The TPC was not affected by any treatment ($p > 0.10$). The figure 1 shows the initial and final counts for the lactic acid treatment. On the opposite of what it was expected, there was an increment in the microbial counts for most cases. Considering that the concentration and temperature used are within the limits of the cited reference (2), this could be due to the application method of the acid, more volume of solution per carcass or an arch with pressurized nozzles should be tested in order to improve the treatment.

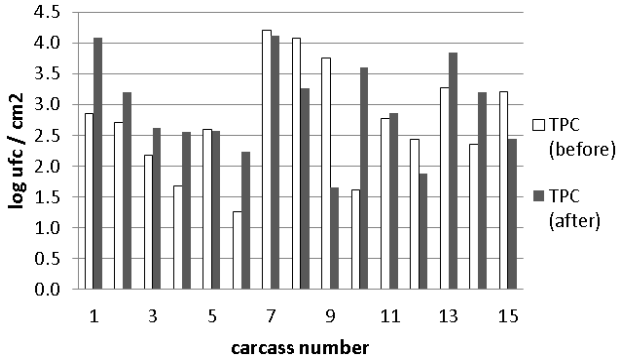

Fig. 1. Variation in the Total Plate Count for the 3% lactic acid treatment, before and after the 24 hour maturation.

The table 1 shows the effect of both treatment, and their interaction, in the TPC. As it can be seen the R turned out to be negative (the counts increased after the treatments and maturation), although the R values of the ozone treatments were less negative than the lactic acid ones. A Tukey (5%) comparison was made, and no significant difference was detected. These results could be due to variability [$\mu = 2.8 \pm 0.74$ $\log(\text{ufc}/\text{cm}^2)$], and high values [up to 4.2 $\log(\text{ufc}/\text{cm}^2)$] of the initial counts of the TPC, which is expectable for a microbiological indicator and for a superficial sampling, in which different (adjacent) places are sponged every time, and also could be due to the shield effect of such high concentration of microorganisms per superficial unit, which interferes with the reaching of the gas and with the contact of the acid solution. Both of these last effects could be eliminated by reducing the initial count by means of the routine washing of the carcasses on the line, as discussed in the methodology.

Table 1. Effect of both treatments in the TPC reduction.

Lactic	Ozone	Reduction log(ufc/cm2)	(± St.dev)	Tukey 5%
0%	No	-1.35	0.49	a
0%	Yes	-0.79	0.49	a
3%	No	-0.21	0.49	a
3%	Yes	-1.17	0.49	a

Different letters indicate significant difference, with $p \leq 0.05$.

The EB was affected only by the ozone treatment. The carcasses treated with ozone showed a reduction of 1.08 units ($p \leq 0.05$). Table 2 presents the reduction comparison for the ozone treated group and the blank.

Table 2. Effect of the ozone treatment in the EB reduction.

Treatment	Reduction ± st.dev. log(ufc/cm2)
Blank	0.20 (± 0.18) a
Ozone	1.08 (± 0.18) b

a,b: $p \leq 0.05$.

The results of Table 2 show that the use of ozone during the post sacrifice chilling of the carcasses reduces the microbial count. This is in agreement with international (8, 9) and national literature (6, 7).

The variations in the EB count for both treatments (ozone and blank) are shown in Figures 2 and 3. Note that the initial counts are variable, but not as variable nor as high as the TPC discussed before. This is expected because EB count is not an indicator, and is normal to find it in lower counts. In this study, this was an advantage.

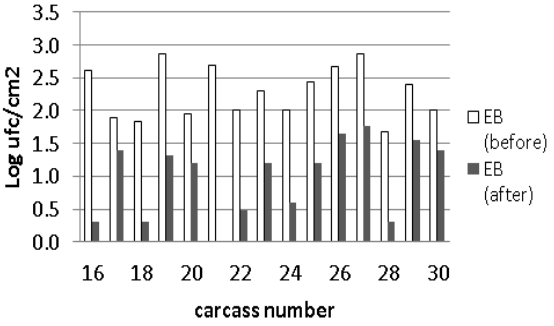

Fig. 2. Variation in the enterobacteria count for the ozone treatment, before and after the 24 hour exposure.

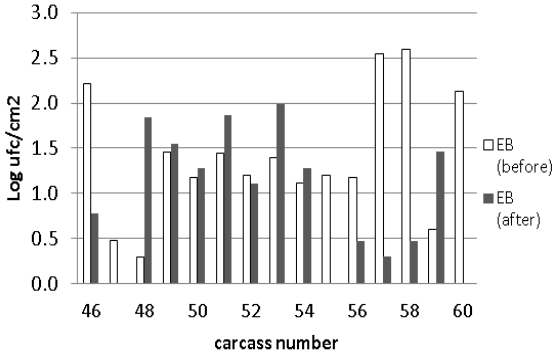

Fig. 3. Variation in the enterobacteria count for the blank, before and after the 24 hour maturation.

Nevertheless, considering that the variable selected for the analysis is a subtraction, the effect of the variation in the initial count is cancelled, and the result of the treatment is evidenced. After the 24 hour exposure period, the reductions reached levels as high as 2.5 log (ufc/cm^2). This is a very promising result, and demands further investigation to improve the ozone treatment parameters inside the chiller, either during the maturation process or afterwards.

On the other hand, more studies should be done regarding the use of lactic acid. Different application methodologies should be tested. And real process conditions, such as washing routines of the carcasses, should be accounted in the experimental designs for both, ozone and lactic acid, treatments.

IV. CONCLUSION

The anti-microbial effect of the ozone treatment is promising. However, the effect on the quality of the meat products has not been studied locally yet as it has been internationally (5). Clearly, it is necessary to explore the use of different alternatives besides ozone, such as ozonized water (10), and to compare with other sanitization products, such as hot water and lactic acid in different temperatures and concentration combinations. The possible effects on different quality parameters and consumer preferences should also be investigated.

This paper shows that ozone could be an option as a disinfectant when it comes to slaughter lines and carcass maturation processes, and it could be a mayor improvement in the meat export industry. The reduction of the superficial microbial count at a previous step to the deboning and packing process, or even before the introduction of the fresh meat to the local market, could mean an extension in the shelf life of meat products.

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