STUDY OF THE MICROBIAL CONTAMINATION IN FRESH VEAL TONGUES INTENDED FOR HUMAN CONSUMPTION AND ANALYSIS OF ALTERNATIVES TO REDUCE IT

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INTRODUCTION

The motivation for carrying out this study is framed within the project being considered by the company Escorxador Sabadell, S.A., to open new markets for its products in third countries through the export of fresh beef tongues to Japan.

Regulations (EC) 852/2004 and 853/2004 establish the legal frameworks that set the hygienic and sanitary standards for meat industries, ensuring food safety.

Additionally, Regulation (EC) 2073/2005 outlines the microbiological hygiene and safety criteria applicable to food products, including those of animal origin.

Unlike muscle tissue, organs from the digestive tract exhibit high contamination levels when extracted from the animal (Gill et al., 1999). Despite this, Regulation (EC) 853/2004 does not mandate additional processing requirements for fresh tongues intended for human consumption.

This study aims to assess the hygienic conditions of fresh beef tongues, currently processed by thorough washing with cold potable water, and to evaluate alternative methods to reduce contamination and improve their sanitary condition.

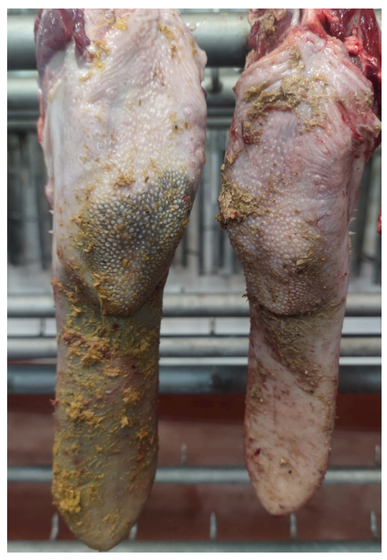

Figure 1. The cleanliness status of the tongues following their extraction from the oral cavity.

OBJECTIVES

- Perform a quantitative assessment of the microbiological contamination levels in the 50 collected samples.
- Compare the efficacy of various treatments in reducing microbiological contamination, identifying the treatment that achieves the highest reduction.
- Provide evidence-based recommendations to the Escorxador Sabadell, S.A slaughterhouse, highlighting the most effective decontamination method to reduce bacteria load on fresh veal tongues.

METHOD

In five trials, ten veal tongues were randomly collected after slaughter. The tongues were selected randomly, starting from a uniform level of ruminal contamination. Sampling was done by swabbing a 25 cm^2 area on the tip of each tongue with an abrasive sponge.

Progressive decimal dilutions (up to 10^-6) were prepared, and samples were mass inoculated in duplicate onto sterile Petri dishes.

Method	Samples	Determination	Medium	Plating Technique	Incubation	Confirmatory Tests
Washed with water	10	Mesophilic aerobic flora	TSA	Pour plate	37ºC for 24-48 hours	---
5% Lactic acid	10	Enterobacteriaceae	VRBG	Pour plate	37ºC for 48 hours	---
Hyperchlorinated water (300 ppm)	10	*Salmonella*	SALMA XLD Agar	Streak plate	37ºC for 24 hours	Latex agglutination test
Scalding <82ºC	10					
Scalding >82ºC	10					

Table 1. Microbiological procedures.

RESULTS

From the plates of aerobic and enterobacterial colonies, a detailed count of the bacterial colonies present was conducted. The averages of the results obtained from the duplicates of each sample were calculated and expressed as Log Colony Forming Units (log UFC) per cm^2.

Table 2. Aerobic colonies counts in the initial samples according to the applied treatment.

Aerobic Count Plates (log UFC/cm^2)												
Method	Sample										$\bar{x}^a$	SD^b
	1	2	3	4	5	6	7	8	9	10		
Washed with water	5.80	5.83	6.04	6.04	6.20	6.07	6.25	6.14	5.80	6.07	**6.02**	**0.16**
5% Lactic acid	4.00	5.04	3.69	5.86	4.84	4.17	5.38	4.17	4.00	4.00	**4.51**	**0.72**
Hyperchlorinated water (300 ppm)	4.00	4.60	4.69	4.95	5.77	4.39	5.44	5.00	4.65	5.25	**4.87**	**0.52**
Scalding <82ºC	4.39	4.47	5.11	4.65	5.49	5.88	5.77	4.76	4.20	5.57	**5.02**	**0.62**
Scalding >82ºC	4.60	4.39	3.69	4.39	4.39	2.00	3.69	2.54	4.60	2.17	**3.64**	**1.03**

a $\bar{x}$, mean of log10 counts
b *SD*, standard deviation of log10 counts

Table 3. Enterobacteria counts in the initial samples according to the applied treatment.

Enterobacteriaceae Count Plates (log UFC/cm^2)												
Method	Sample										$\bar{x}^a$	SD^b
	1	2	3	4	5	6	7	8	9	10		
Washed with water	2.59	2.64	2.27	2.55	2.17	2.53	2.04	3.32	2.59	2.51	**2.52**	**0.34**
5% Lactic acid	<10	<10	<10	2.14	<10	<10	0.69	<10	<10	<10	**0.28**	**0.68**
Hyperchlorinated water (300 ppm)	<10	<10	<10	<10	<10	1.30	<10	<10	<10	<10	**0.13**	**0.41**
Scalding <82ºC	2.04	2.30	2.71	1.81	2.04	1.54	3.27	2.57	2.53	3.11	**2.39**	**0.55**
Scalding >82ºC	<10	<10	0.69	<10	<10	0.69	<10	<10	<10	<10	**0.13**	**0.29**

a $\bar{x}$, mean of log10 counts
b *SD*, standard deviation of log10 counts

The presence of Salmonella was assessed by observing growth on specific culture media. Although some plates showed favorable growth, none of the 40 plates tested positive for Salmonella when subjected to the latex agglutination test.

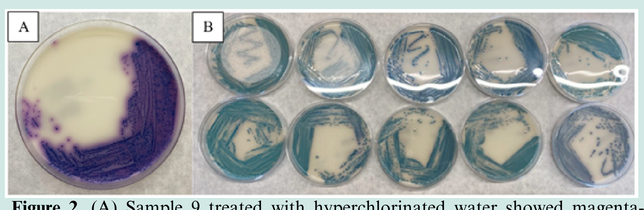

Figure 2. (A) Sample 9 treated with hyperchlorinated water showed magenta-colored bacterial colonies initially suspected to be Salmonella. However, an API 20E test identified the bacteria as Aeromonas hydrophila/caviae/sobria with a 97% probability. Similar esterase activity to Salmonella allowed these bacteria to interact with chromogenic substrates. (B) In contrast, samples treated with the scalding method at temperatures below 82ºC showed no bacterial growth

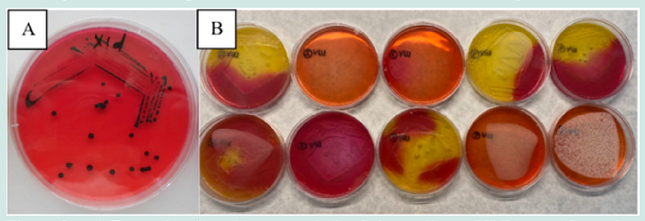

Figure 3. (A) Example of positive growth for Salmonella on XLD agar (Samad et al., 2019). (B) Negative growths were obtained from the 10 samples taken using the scalding method at temperatures above 82ºC.

To determine values exceeding the 5.0 log limit for aerobic colonies as per Regulation (EC) 2073/2005, the hygiene criteria for meat and meat products in Annex I Chapter 2.1 were used.

Treatments with 5% lactic acid, hyperchlorinated water (300 ppm), and scalding above 82ºC met the 5.0 log reduction requirement, whereas scalding at insufficient temperatures did not.

For enterobacteria, the regulation's limit is 2.5 log. All alternative methods, except for scalding at insufficient temperatures, complied with this limit as well.

Regarding Salmonella, all methods effectively ensured its absence in the samples, thus complying with current regulations.

COMPARATIVE

The study concludes that compared to the standard beef tongue washing process, the four evaluated decontamination alternatives significantly reduce contamination levels in fresh beef tongues ($p<0.05$).

However, the group subjected to insufficient scalding (below 82°C) showed a p-value close to the significance level ($p = 0.0005433$) for reducing aerobic colonies, but it did not significantly reduce enterobacteria compared to water-only treatment, making it unsuitable.

Comparations	Aerobic colonies (A) Enterobacteriaceae (E)	*p-value*
Water & 5% Lactic acid	A	0.00007405
	E	0.000000397
Water & Hyperchlorinated water (300 ppm)	A	0.00003992
	E	0.00000000005678
Water & Scalding <82ºC	A	0.0005433
	E	0.5416
Water & Scalding >82ºC	A	0.000009556
	E	0.000000000003657
5% Lactic acid & Scalding >82ºC	A	0.04413
	E	0.5505

Table 4. P-values for the comparisons among the various treatments in relation to the Water group and between the methods 5% Lactic Acid and Scalding >82°C.

CONCLUSIONS

- Veal tongues exhibit high contamination when extracted, and washing with cold potable water alone does not ensure acceptable hygienic conditions for consumption.
- Complementary methods to the current washing process effectively reduce microbial contamination on veal tongues, including aerobic bacteria at 30°C and enterobacteria, and ensure Salmonella absence.
- Decontamination with 5% lactic acid, hyperchlorinated water (300 ppm), and scalding above 82°C for 20 seconds significantly reduces bacterial presence compared to the current method.
- Scalding at >82°C for 20 seconds is the most effective decontamination method without altering the tongues' physical appearance.
- Despite the high level of contamination typically observed in veal tongues after their removal at the end of the slaughtering process, Salmonella does not appear to pose a significant health hazard in these types of meat offals.
- It is recommended to implement a complementary decontamination process alongside the usual washing with cold potable water to improve hygiene.

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