THE EFFECT OF ELECTRICAL STIMULATION OF MEAT UPON THE SURVIVAL OF BACTERIA DURING SALTING, CURING, FREEZING AND HEATING

H.W. OCKERMAN AND J. SZCZAWINSKI

Department of Animal Science, The Ohio State University, Columbus, Ohio 43210 and The Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA

INTRODUCTION

Available information on the effect of electrical stimulation upon meat microbiology is very inconsistent. Corte et al. (1980) found higher initial superficial contamination of meat from stimulated and hot-boned carcasses than from traditionally chilled carcasses. Jeremiah and Martin (1980) and Kotula (1980) failed to identify any differences in the bacterial counts from electrically stimulated and unstimulated meat initially or after storage. However, a number of research reports have shown a statistically significant reduction in total plate counts as a result of electrical stimulation (Mrigadat at al. ,1980; Ockerman and Szczawinski, 1982; Raccach and Henrickson, 1978, 1980). These inconsistent microbial results would suggest that electrical stimulation causes a slight decrease in the microflora of meat but this decrease is not large enough to be noticed or statistically significant under all experimental conditions.

Several hypotheses on the mechanism by which electrical stimulation can affect the bacteria in meat are possible. They include the hypotheses that a fast reduction in the muscle pH value may retard microorganism growth (Kotula, 1980, 1981; Mrigadat et al. ,1980), that electrical stimulation impairs the metabolism of bacteria cells (Raccach and Henrickson, 1980) or that electrical stimulation has adverse effects on the meat as a growth medium (Riley et al. ,1980). Bacteria may also be destroyed by electrical stimulation initiating the release of some proteolytic enzymes from the meat tissue (Dutson et al. ,1980; Sorinmade et al. ,1978), or by changing meat Eh or by generating free radicals in the stimulated tissue (Mrigadat et al. ,1980). Ockerman and Szczawinski (1982) found that bacteria cells are destroyed directly during the process of electrical stimulation. They also suggested that it is less probable that bacteria cells are retarded only by a drop in pH or by proteolytic enzymes; however, free radicals or other unknown factors acting during electrical stimulation may be responsible for the observed results.

It seems possible that sublethal damage of bacteria may take place during electrical stimulation. This damage can increase the sensivity of microorganisms to other injurious agents such as the presence of salt and nitrite or the effect of low or high temperatures which could be important from a practical processing standpoint. Therefore, the purpose of

this research was to investigate if the synergistic effects of electrical stimulation and other fundamental processes applied in meat technology (salting, curing, freezing and heating) are likely to be encountered.

MATERIAL AND METHODS

M. sterno-cephalicus was collected from both sides of beef carcasses immediately after slaughter, divided into 50 ±10g samples, inoculated by dipping in a suspension containing 5 x 10^7 bacteria/ml (general inoculum prepared from microorganisms removed from cutting table that had previously been used in handling beef carcasses) and subjected to electrical stimulation within 30 min post-mortem. The samples were stimulated with 21 mA (60 Hz) current, 42 V for 4 min, with thirty, 2 sec duration shocks per min. Control samples were not stimulated after inoculation.

In the first experiment, both stimulated and control samples were aseptically ground through a 3.2 mm grinder plate and subdivided into three 50 g portions. The first portion was stored without any additives. The second portion was mixed with 3 % salt (NaCl). To the third portion 3 % salt and 200 mg/kg of nitrite ($NaNO_2$) were added. All samples were stored in Petri dishes at 0-2°C for 14 days. Aerobic plate count (APC) and pH were determined immediately after grinding (before the addition of salt and nitrite) and after 7 and 14 days of storage. The samples were homogenized in distilled water, using a ratio of 1 part meat to 9 parts water, by using a Stomacher Lab-Blender 400. The pH of the slurry was measured using a Beckman pH meter. From another portion of the meat slurry, appropriate dilutions were prepared with a 0.5% solution of Bacto-Peptone and plated using Tryptone Glucose Extract Agar (Difco). Plates were incubated at 25°C for 4 days. This experiment was repeated 6 times.

In the second experiment, inoculated control as well as inoculated and stimulated samples of meat were homogenized in distilled water, using a ratio of 1 part meat to 9 parts water. The meat slurry was poured into 12 test tubes (2.5 ml into each tube) and stored at -21°C for 21 days. The measurement of pH was conducted immediately after homogenization. APC was determined after 0, 7, 14 and 21 days of storage (three repetitions for each time period). This procedure was repeated three times.

In the third experiment, the samples of meat slurry were prepared in the same way as in the second experiment. After determination of pH, the test tubes with meat slurry from control and stimulated meat were heated in the water bath at 60°C for 0, 5, 10 and 15 minutes. Immediately after heating, the samples were cooled in ice water and APC was determined (three repetitions for each heating time). This experiment was repeated three times.

The microbial counts per gram were transformed to logarithms and analyses were conducted on the transformed data. Statistical analyses of data for pH and microbial counts were

carried out using the General Linear Models and Correlation Procedures supplied through the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

As shown in table 1, electrical stimulation caused an initial decrease in APC of meat but this reduction was not large enough to be statistically significant. APC of stimulated meat was also slightly lower in all treatment groups after 7 and 14 days of storage except for meat without salt and nitrite after 14 days of storage. However, significant differences between control and electrically stimulated meats were found only for samples without any additives after 7 days ($P<0.01$) and for meat with salt after 14 days of storage ($P<0.05$).

The overall analysis of variance (table 2) indicates that electrical stimulation as well as treatment and storage time influenced significantly ($P<0.01$) the bacterial counts. However, the interactions of stimulation x treatment, stimulation x storage time and stimulation x treatment x storage time were not statistically significant indicating that NaCl or NaCl and $NaNO_2$ added to the meat had the same retarding influence on bacteria from electrically stimulated or unstimulated meat.

Electrical stimulation also caused a slight decrease in pH for all treatment groups but observed differences were not statistically significant (table 1). The overall analysis of variance (table 2) indicates that pH was significantly ($P<0.01$) influenced only by treatment and storage time.

The obtained results generally confirm previously conducted work (Ockerman and Szczawinski, 1982) in this same laboratory. However, initial differences in the APC and pH between control and stimulated meat had been slightly greater and statistically significant when unground beef cuts had been used for samples in the previous experiments.

In prior studies, the analysis of variance of APC indicated non significant statistical interactions between electrical stimulation and levels of salt and nitrite in the culture media suggesting the same effect for NaCl and $NaNO_2$ on bacteria from control and stimulated beef tissue (Ockerman and Szczawinski, 1982). The results of the present research endorse former observation though the experimental conditions in both works were entirely different.

Although a reduction in the microflora caused by electrical stimulation could be seen initially in this experiment and also after 7 and 14 days of storage of meats subjected to salting and curing, most research workers consider differences in this range as being unimportant in commercial production of meat (Kotula, 1981).

As shown in table 3, the initial difference (0.26 reduction due to stimulation) in APC between samples from control and stimulated meat (significant at $P<0.01$) remained almost

identical for the 21 days of storage at -21°C.

The analysis of variance (table 4) indicates that electrical stimulation as well as the time of frozen storage and individual animals or handling of these animals affected significantly ($P<0.01$) the number of bacteria in the meat slurry.

A non significant interaction for frozen storage time x stimulation indicates that the responses of bacteria, from control and stimulated meat, to low temperature are approximately the same and that a synergistic effect did not occur under these experimental conditions.

Very little information is available on the effect of freezing upon bacteria in stimulated meat. Corte et al. (1980) reported that after 3 months of storage at -20°C thawed cuts from electrically stimulated and hot boned sides showed a tendency to be more contaminated than the control.

As shown in table 5, an initial statistically significant ($P<0.01$) difference in APC between control and stimulated samples increased systematically with time of heating. This decrease in bacterial number at 0 time for stimulated tissue again indicates the effect of stimulation on microorganisms and the increase with heating time suggests a synergistic effect.

A statistically significant difference ($P<0.05$) in the mean D value (table 5) suggests a reduced tolerance for heat by microorganisms in stimulated tissue and the significant ($P<0.01$) interaction for stimulation x heating time found in the analysis of variance (table 6) also shows that electrical stimulation decreases the thermoresistance of bacteria.

This observation could easily be explained by a sublethal damage of bacteria occurring during electrical stimulation. However, it is a well known fact that the pH of the suspending medium in which microorganisms are heated is one of the most important factors that influences thermoresistance (Banwart, 1979). Therefore, it is difficult to determine whether the damage to bacteria is caused by electrical stimulation, or whether a slightly lower pH of the samples from stimulated meat (table 5) is responsible for the synergistic effect observed in this experiment.

In order to examine this question in more detail, additional statistical analyses were conducted. The results obtained from the analysis of variance of APC "adjusted" for pH were very similar to the results shown in table 6 indicating that the small change in pH encountered in this experiment did not affect the thermoresistance of bacteria under these conditions. A statistically non significant correlation between pH and APC, as well as between pH and D values, seems to confirm this conclusion. However, statistical analysis of the D values "adjusted" for pH shows that the difference between control and stimulated samples is not quite large enough to be significant at the 0.05 level (table 5).

Considering all the results, it seems that the damage to bacterial cells occurring

during electrical stimulation was a more important factor than pH in affecting thermoresistance in this experiment.

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Table 1 - Effects of electrical stimulation, salting and curing on pH and APC of inoculated beef stored at 0-2°C

Trait	Time of storage (days)	NaCl 0% $NaNO_2$ 0% Control	NaCl 0% $NaNO_2$ 0% Stimulated	NaCl 3% $NaNO_2$ 0% Control	NaCl 3% $NaNO_2$ 0% Stimulated	NaCl 3% $NaNO_2$ 0.02% Control	NaCl 3% $NaNO_2$ 0.02% Stimulated
pH	0	6.11^{ax}	5.99^{ax}	6.11^{ax}	5.99^{ax}	6.11^{ax}	5.99^{ax}
	7	5.63^{ay}	5.55^{ay}	5.97^{bx}	5.92^{bx}	5.97^{bx}	5.94^{bx}
	14	5.96^{ax}	5.89^{ax}	6.07^{ax}	6.02^{ax}	6.11^{ax}	6.08^{ax}
APC	0	4.69^{ax}	4.45^{ax}	4.67^{ax}	4.45^{ax}	4.67^{ax}	4.45^{ax}
	7	6.11^{ay}	5.76^{by}	4.56^{cx}	4.32^{cdx}	4.30^{cdy}	4.04^{dy}
	14	9.03^{az}	9.06^{az}	6.30^{by}	5.99^{cy}	4.19^{dy}	4.01^{dy}

^{a,b,c,d}Means within the same row bearing different superscripts are different at $P<0.05$

^{x,y,z}Means for the same item (pH or APC) within the same column bearing different superscripts are different at $P<0.05$

Table 2 - Effects of electrical stimulation, salting and curing on pH and APC of inoculated beef stored at 0-2°C (probability of significance of F values in the overall analysis of variance)

Main effect	pH	APC
Stimulation	0.1223	0.0001**
Treatment	0.0067**	0.0001**
Storage time	0.0006**	0.0001**
Stimulation x Treatment	0.0983	0.8159
Stimulation x Storage time	0.8118	0.5366
Treatment x Storage time	0.0891	0.0001**
Stimulation x Treatment x Storage time	1.0000	0.6071

** significance at $P<0.01$

Table 3 - Effect of electrical stimulation on the survival of bacteria in meat slurry during storage at -21°C

Time of storage (days)	Aerobic plate counts Control	Stimulated	Difference
0	5.94	5.68	0.26**
7	5.77	5.51	0.26**
14	5.68	5.45	0.23**
21	5.68	5.42	0.26**

** significant difference at P<0.01

Table 4 - Effect of electrical stimulation on the survival of bacteria during storage at -21°C (probability of significance of the F values in the overall analysis of variance)

Main effect	APC
Stimulation	0.0001**
Frozen storage time	0.0001**
Linear	0.0001**
Quadratic	0.0001**
Cubic	0.4792
Animal	0.0001**
Stimulation x Frozen storage time	0.6978
Stimulation x Animal	0.0001**
Frozen storage time x Animal	0.9500
Stimulation x Frozen storage time x Animal	0.0586

** significance at P<0.01

Table 5 - Effect of electrical stimulation on the thermoresistance of bacteria in meat slurry during heating at 60°C

Trait	Time of heating	Control	Stimulated	Difference
pH		6.28	6.10	0.18
APC	0 nim	5.94	5.68	0.26**
	5 min	3.92	3.59	0.33**
	10 min	2.56	1.99	0.57**
	15 min	2.03	1.37	0.66**
D value[a](min)		3.83	3.48	0.35*
D value adjusted for pH (min)		3.81	3.50	0.31

[a]the time required to reduce the microbial population by 90% at a specified temperature

* significant difference at P<0.05

** significant difference at P<0.01

Table 6 - Effect of electrical stimulation on the thermoresistance of bacteria (probability of significance of the F values in the overall analysis of variance)

Main effect	APC
Stimulation	0.0001**
Heat time	0.0001**
Linear	0.0001**
Quadratic	0.0001**
Cubic	0.0037**
Animal	0.0001**
Stimulation x Heat time	0.0001**
Stimulation x Animal	0.0001**
Heat time x Animal	0.0001**
Stimulation x Heat time x Animal	0.0081**

** significance at P<0.01