THE EFFECT OF HEAT TREATMENT AT 60°C ON THE SURVIVAL OF LACTOBACILLUS PLANTARUM, PSEUDOMONAS PUTREFACIENS AND STREPTOCOCCUS FAECALIS FROM ELECTRICALLY STIMULATED AND UNSTIMULATED PORK TISSUE

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INTRODUCTION

In a previous study (Ockerman and Szczawinski, 1982a) in the same laboratory, it was reported that electrical stimulation caused an initial reduction in pH and in aerobic plate count (APC) of a mixed microflora inoculated beef tissue. It was also stated (Ockerman and Szczawinski, 1982b) that electrical stimulation slightly decreased the thermoresistance of bacteria. However, due to the mixed microflora used in these experiments no information was obtained on the influence of electrical stimulation on the survival, growth and thermoresistance of individual strains of microorganisms.

Little information on the influence of electrical stimulation on specific strains of microorganisms can be found in the literature because most research workers have investigated the effect of electrical stimulation on natural, mixed microflora of meat (Berry and Kotula, 1982; Jeremiah and Martin, 1980; Kotula, 1980, 1981; Kotula and Emswiler-rose, 1981; Raccach and Henrickson, 1980; Taylor et al. ,1980; West et al. ,1980).

The objectives of this study were: (a) to determine if selected strains of bacteria are influenced by electrical stimulation; (b) to determine the effect of electrical stimulation on the thermoresistance of selected microorganisms; (c) to compare the thermoresistance of bacteria from stimulated and unstimulated meats heated in water and heated in a phosphate saline buffer solution; and, (d) to compare the thermoresistance of control and stimulated bacteria heated immediately after stimulation (45 min) and heated after 3 days of aerobic storage at 0-2°C.

The microorganisms used in this study represent some of the bacteria most often involved in spoilage of meat and meat products. Pseudomonas putrefaciens has been designated as being important in spoilage of refrigerated meat and poultry (Banwart, 1979). Streptococcus and Lactobacillus may survive an insufficient heat treatment and cause spoilage of such products as cured and cooked meat, sausages and canned meat (Nickerson and Sinskey, 1972; Banwart, 1979). According to Lee et al. (1982), Streptococcus and Lactobacillus were predominant microflora of both hot-boned and conventionally processed beef after 14 days of

vacuum storage at 2°C.

MATERIAL AND METHODS

Collection of samples

M. sterno-cephalicus was collected from both sides of the pork carcasses as aseptically as possible in order to obtain tissue low in microbial numbers and the level of contamination did not exceed 100 organisms per gram of meat tissue. *M. sterno-cephalicus* was used in these experiments because of accessibility immediately after bleeding. The collected muscles were divided into 40±10g samples.

Preparation of inoculum and inoculation of meat

The microorganisms used in this study were obtained from the Department of Microbiology at The Ohio State University. The culture media used for inocula preparation were APT broth (Difco) for *L. plantarum*, nutrient broth (Difco) for *P. putrefaciens* and Trypticase Soy Broth (BBL) for *S. faecalis*. *L. plantarum* and *S. faecalis* were allowed to multiply for 24 hours at 37°C and *P. putrefaciens* was incubated for 48 hours at 25°C. Samples of meat were inoculated by dipping three times for 1 second each time into a suspension containing approximately $5x10^{8}$ viable cells per ml.

Electrical stimulation

Immediately after inoculation (within 30 min post-mortem) one of each pair of samples was stimulated with 21 mA (60 Hz) current, 42 V for 4 min, with thirty, 2 sec duration shocks per min. The probes were attached by alligator clips to opposite ends of the long axis of the muscle. Before and during electrical stimulation the samples were held at ambient temperature (ca. 20°C). After electrical stimulation one-half of the samples were immediately prepared for heating, whereas the other one-half were aerobically stored in Petri dishes for 3 days at 0-2°C prior to heating.

Heat treatment

Every sample of meat was divided into 2 parts (approximately 20 g each) and one part was homogenized in distilled water, using a ratio of 1 part meat to 9 parts water, by using a Stomacher Lab-Blender 400. The second portion of this same sample was homogenized in 0.05 M phosphate saline buffer using a ratio of 1 part meat to 9 parts buffer solution. Meat slurries that were obtained were poured into 13x100 mm test tubes and heated in a water bath for 0, 5, 10 and 15 minutes at 60°C. Immediately after heating, samples were cooled in ice water.

Bacteria enumeration and pH measurement

From every meat slurry, appropriate dilutions were prepared with 0.5% solution of Bacto-Peptone and plated using Tryptone Glucose Extract Agar (Difco) for *P. putrefaciens* and *S. faecalis* or APT agar (Difco) for *L. plantarum*. Plates with *P. putrefaciens* were

incubated for 5 days at 25°C and plates with *S. faecalis* and *L. plantarum* for 3 days at 37°C.

The pH of the meat slurry was measured by using Fisher Accumet pH meter model 610A immediately after homogenization.

Statistical analyses

For each strain of bacteria, 3 samples of meat tissue from 3 animals per treatment group were used and from every meat slurry 2 test tubes for each heating time were prepared resulting in 6 bacteria enumerations per treatment group.

The microbial counts per gram were transformed to logarithms and calculations and analyses were conducted on the transformed data.

Statistical ANOVA analyses of data for pH and microbial counts were conducted 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 initially caused a significant reduction in the pH of the meat samples, but did not decrease the bacterial counts for the non-heated microorganisms tested (table 1, heating time 0 min, storage time 0 days). Statistically insignificant differences between bacterial counts from stimulated and unstimulated meat after 3 days of storage at 0-2°C (table 1, heating time 0 min, storage time 3 days) suggest that electrical stimulation also did not affect the growth of tested microorganisms under these experimental conditions.

It seems, that these results confirm to some extent the observations by Gill (1980) who found no difference in the lag phase, the growth rate or maximum cell density between electrically stimulated and control mutton legs inoculated with *P. fluorescens* . Butler *et al.* (1981) also reported that there were no significant differences in growth of *Lactobacillus sp.* , *Pseudomonas sp.* , *Acinetobacter sp.* or a mixture of bacteria flora in ground meat made from electrically stimulated and nonstimulated muscles. Mrigadat *et al.* (1980), however, observed that electrical stimulation of rabbit muscles caused a reduction of *P. putrefaciens* and *Lactobacillus sp.* when inoculated muscles were held for 45 min after electrical stimulation.

The results shown in tables 1 and 2 also suggest that electrical stimulation slightly decreased the thermoresistance of *L. plantarum* and *P. putrefaciens* but did not exert any influence on the thermoresistance of *S. faecalis* .

Analysis of variance for *L. plantarum* and *P. putrefaciens* (table 2) showed that the effect of electrical stimulation on bacterial counts as well as the interactions for Stimulation x Heating time were statistically significant ($P<0.01$) for bacteria heated in

distilled water and insignificant for microorganisms heated in phosphate saline buffer solutions. This could suggest, that the slight differences in pH between meat slurries from control and electrically stimulated samples prepared with distilled water (table 1) might be responsible for the decrease of the thermoresistance of L. plantarum and P. putrefaciens .

However, statistically significant differences in mean bacterial counts between control and stimulated samples (table 1) can also frequently be found for L. plantarum and P. putrefaciens heated in distilled water after 3 days of storage or heated in the buffer solution immediately after stimulation when pH values of the meat slurries were very similar or even identical.

Therefore, damages to bacterial cells taking place during electrical stimulation seem to be the main reason for the decrease of thermoresistance of L. plantarum and P. putrefaciens . This observation confirms the former investigation conducted in this same laboratory (Ockerman and Szczawinski, 1982b).

Electrical stimulation seems to impair bacteria very slightly because its effect on thermoresistance of L. plantarum and P. putrefaciens could not be found for heating in the buffer solution after 3 days of storage (table 1), when the bacteria were better adapted to their environment (compared to microorganisms heated immediately after stimulation) and had relatively better conditions during heat treatment (compared to bacteria heated in distilled water).

Electrical stimulation had no significant effect on S. faecalis .

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Table 1 - Effect of electrical stimulation, time of storage and heating medium on the survival of bacteria in meat slurries during heating at 60°C (Least square means)

Microorganism	Trait measured	Time of heating (min)	Distilled water				Buffer			
			0 days		3 days		0 days		3 days	
			C [a/]	S [b/]	C	S	C	S	C	S
	pH	-	6.11	5.92**	5.67	5.69	7.04	7.04	7.03	7.04
L. plantarum	Log/ml	0	6.29	6.28	6.48	6.29	6.25	6.32	6.53	6.35
		5	1.53	1.30	1.92	1.20**	3.10	2.95	3.97	3.62
		10	0.47	0.52	0.64	0.57	1.58	1.19**	2.09	2.23
		15	0.39	0.26	0.49	0.29	1.35	0.97*	0.72	0.99
	pH	-	6.24	6.09*	5.91	5.85	7.04	7.04	7.03	7.03
P. putrefaciens	Log/ml	0	5.69	5.75	5.81	5.71	5.96	5.90	6.13	6.06
		5	2.50	1.91**	2.86	2.22**	2.81	2.42**	3.02	3.17
		10	1.76	1.36*	2.24	1.56**	2.16	1.88*	2.58	2.68
		15	1.14	0.66**	1.43	0.68**	1.87	1.66	2.22	2.28
	pH	-	6.25	6.15**	6.02	6.05	7.07	7.07	7.06	7.06
S. faecalis	Log/ml	0	5.84	5.84	6.02	6.05	5.98	5.96	6.07	6.02
		5	5.41	5.41	5.64	5.71	5.31	5.30	5.66	5.65
		10	4.13	4.14	4.83	4.87	4.20	4.15	5.11	5.12
		15	2.37	2.29	2.90	3.09	3.41	3.41	3.74	3.78

* significant difference compared to unstimulated control at $P<0.05$
** significant difference compared to unstimulated control at $P<0.01$
[a/] C = control (unstimulated)
[b/] S = electrically stimulated

Table 2 - Probability of significance of the F values in the analysis of variance for microbial counts

Main effect	L. plantarum		P. putrefaciens		S.faecalis	
	Dist.water	Buffer	Dist.water	Buffer	Dist.water	Buffer
Electrical stimulation	0.0004**	0.0610	0.0001**	0.0524	0.3476	0.6935
Heating time	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**
Storage time	0.0384*	0.0001**	0.0002**	0.0001**	0.0001**	0.0001**
Stimulation x Heat time	0.0096**	0.8540	0.0004**	0.9692	0.9767	0.9326
Stimulation x Storage time	0.0362*	0.0324*	0.0894	0.0013**	0.1647	0.7535
Heat time x Storage time	0.9471	0.0001**	0.3932	0.0053**	0.0001**	0.0001**
Stimulation x Heat time x Storage time	0.4598	0.0060**	0.8577	0.1755	0.5642	0.9685

* significance at P<0.05
** significance at P<0.01