STUDIES ON MEAT INOCULATED WITH TWO LACTIC ACID STRAINS AS A MEANS OF DECONTAMINATION.

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SUMMARY: THE STUDY CONSISTED IN TWO PARTS, THE FIRST ONE DEALT WITH STUDIES ON THE SUITABLE AMOUNT OF SUCROSE ADDED TO THE MEAT IN ORDER TO START A LACTIC FERMENTATION IN THE SURFACE, AS WELL AS WITH THE DEPTH OF PENETRATION SUCROSE. IN THE SECOND PART THE FOLLOWING FACTORS WERE STUDIED: INOCULA, MEAT SPECIES, PACKAGING CONDITION, STORAGE TEMPERATURE AND STORAGE TIME. THE RESULTS SHOWED A GOOD ADHERENCE OF SUCROSE WHEN THE MEAT WAS IMMERSED IN 15% SUCROSE DURING 15 MINUTES, WITH A FINAL CONCENTRATION OF 2.3% SUCROSE AT 4 MM DEPTH. PSEUDOMONADS POPULATION DECREASED WHEN LACTIC ACID BACTERIA WERE INOCULATED, ALTHOUGH THIS DECREASE WAS LESS MARKED IN BEEF THAN IN PORK. IN GENERAL, SELECTED STRAINS CAN COMPETE SUCCESFULLY WITH PSEUDOMONADS IN MEAT SUBSTRATES IF CONDITIONS OF REDUCED OXYGEN CONCENTRATION ARE FAVOURED AS WELL AS HAVING A CARBON SOURCE.

INTRODUCTION: BACTERIA IN MEAT SURFACE, MOSTLY OF THE GENERA PSEUDOMONADS AND ACHROMOBACTER, ARE THE MAIN RESPONSIBLE FOR ITS DETERIORATION. THESE MICROORGANISMS FIND THEIR WAY TO THE MEAT VIA CONTACT OF THE CARCASSES WITH WALLS AND FLOORS OF THE SLAUGHTERFLOOR, AS WELL AS BY HANDLING. PSEUDOMONADS AND ACHROMOBACTER ARE PSICHROPHILES AND AEROBES, BUT NOT TOLERANT TO ACID. FOR THIS REASON, LACTIC FERMENTATION ON THE MEAT SURFACE CAN BE A WAY TO DECONTAMINATE CUTS, REDUCING THE POPULATION OF PATHOGENS AND SPOILAGE MICROORGANISMS, AND INCREASING MEAT SHELF-LIFE. THE BACTERIOSTATIC PROPERTIES OF LACTIC ACID DO NOT ALTER THE PHYSICOCHEMICAL CHARACTERISTICS OF THE MEAT WHEN ACID IS PRESENT UP TO 1% AT pH=2.4, AND NO OFF-FLAVOURS ARE DETECTED UP TO 2% LACTIC ACID. THE AMOUNT OF LACTIC ACID PRODUCED DURING FERMENATATION, AS WELL AS THE DECREASE IN pH AND INHIBITION OF SPOILAGE MICROORGANISMOS DEPEND ON A LARGE EXTENT TO THE STORAGE TEMPERATURE AND THE CARBOHYDRATE SOURCE. GLUCOGEN IS THE ONLY NATURAL CARBOHYDRATE SOURCE IN MEAT, BUT IT IS DEPLEATED DURING THE ONSET AND RESOLUTION OF RIGOR MORTIS. THEREFORE IT IS NECESSARY TO ADD AN EXTRA SOURCE OF CARBON. ON THE OTHER HAND, ANAEROBIC OR MICROAROPHILIC CONDITIONS PROMOTE THE GROWTH OF LACTIC ACID BACTERIA, BECOMING THESE MICROORGANISMS THE PREDOMINANTE FLORA, AND DECREASING THE POPULATION OF UNDESIRED GENERA. THE OBJECTIVES OF THIS WORK WERE: 1. TO DEFINE THE AMOUNT OF SUCROSE NECESSARY TO START A LACTIC FERMENTATION ON THE MEAT; 2. TO KNOW THE DEPTH OF PENETRATION OF SUCROSE IN THE MEAT; 3. TO KNOW THE EFFECT OF TWO STARTERS, PACKAGING AND STORAGE TEMPERATURE IN THE SHELF-LIFE OF MEAT.

MATERIALS AND METHODS: PORK AND BEEF SAMPLES WERE TAKEN AT RANDOM FROM CARACASSES SLAUGHTERED THE SAME DAY IN A MUNICIPAL ABATTOIR. THE MEAT WAS CUT INTO 5 CM3 SAMPLES AND THESE WERE IMMERSED IN 10, 15 OR 20% SUCROSE SOLUTIONSDURING 15 MINUTES.

Total sugars were analysed at 2 and 4 mm depth. The inocula used were: a commercial starter (LM-3 Biocarna, Vigusa, Mexico City, which consisted in a mixture of Micrococcus kristinae-varians and Lactobacillus bulgaricus) and a mixture of L. bulgaricus and Pediococcus pentosaceus, isolated in our laboratory from fermented corn-based beverages. The strains were inoculated in Rogosa liquid media and incubated until an O.D.=1. The inocula were then diluted with sterile distilled water 1:1; this cell suspensions were used to inoculate the meat samples by immersion. Half of the meat samples already inoculated were wrapped in a saran semipermeable film (polyvinyl chloride, Dow Chemical Co., Mexico City) and stored at 15 and 27 C. The other half were stored without wrapping at the same temperatures. The experimental design consisted in a split-block design as follows:

TABLE 1. Experimental Design

Main block	Split-block 1	Split-block 2	Split-block 3
Inoculum	Temperature	Packaging	Storage time
LM-3	15 C	wrapped	0,2,4,7 days
		unwrapped	id.
	27 C	wrapped	id.
		unwrapped	id.
L. bulgaricus + P. pentosaceus	15 C	wrapped	id.
		unwrapped	id.
	27 C	wrapped	id.
		unwrapped	id.
Blank	15 C	wrapped	id.
		unwrapped	id.
	27 C	wrapped	id.
		unwrapped	id.

Analysis were performed at days 0, 2, 4 and 7 of the study time for the following response variables: pH; degree of oxidation (by a TBA method); colour (by the use of a Hunter Lab colorimeter model D-25, recording L, a nd b values); titrable acidity (as % of lactic acid); Pseudomonads and lactic acid bacteria populations (by standard methods). The data were analysed by an analysis of variance and an analysis of correlation using a SAS package adapted to a PC. All statistical analysis were performed at 5% level of significance.

RESULTS AND DISCUSSION: A 15% SUCROSE CONCENTRATION WAS ENOUGH TO HAVE 2% AT 4 MM DEPTH, AS SHOWN IN THE FOLLOWING TABLE.

TABLE 2. SUCROSE PENETRATION

% Sucrose in solution	depth	% sucrose in meat
Pork:		
10	2 mm	1.98
10	4 mm	0.89
15	2 mm	3.64
15	4 mm	2.36
20	2 mm	7.24
20	4 mm	3.96
Beef:		
10	2 mm	1.57
10	4 mm	0.55
15	2 mm	3.08
15	4 mm	1.28
20	2 mm	3.88
20	4 mm	1.56

FROM THE RESULTS ABOVE IT WAS CONCLUDED THAT A 15% SUCROSE SOLUTION WAS ENOUGH TO HAVE A CONCENTRATION OF MORE THAN 2% AT 4 MM DEPTH, NECESSARY TO START A LACTIC FERMENTATION.

TABLE 3. ANALYSIS OF VARIANCE

Response variable	Source of variation	Error MS	Error df	P>
pH		0.281	38	
	inoculum			0.1
	packaging			0.001
	temperature			0.1
	time			0.1
lactic acid		0.224	38	
	inoculum			0.1
	packaging			0.1
	temperature			0.1
	time			0.001

TABLE 3 (cont.)

TBA values		0.217	38	
	inoculum			0.1
	packaging			ns
	temperature			ns
	time			ns
L (colour)		5.593	38	
	inoculum			0.1
	packaging			0.0001
	temperature			0.1
	time			0.001
a (colour)		1.515	38	
	inoculum			0.1
	packaging			0.001
	temperature			0.1
	time			0.0001
b (colour)		1.359	38	
	inoculum			0.001
	packaging			0.0001
	temperature			ns
	time			0.001
Lactic acid bacteria	population	1.486	38	
	inoculum			0.1
	packaging			0.1
	temperature			0.001
	time			0.0001
Pseudomonads	population	1.285	38	
	inoculum			ns
	packaging			ns
	temperature			0.1
	time			0.0001

* NOTE: THE EFFECT OF ANIMAL SPECIES WAS CONFOUNDED.

A LOW CORRELATION WAS FOUND BETWEEN THE THREE COMPONENTS OF COLOUR (L, A AND B) AND STORAGE TEMPERATURE AND LACTIC ACID CONCENTRATION, THEREFORE IT WAS CONCLUDED THAT COLOUR WAS NOT AFFECTED BY THE AMOUNT OF LACTIC ACID PRODUCED DURING THE FERMENTATION.A HIGH AND NEGATIVE CORRELATION WAS FOUND BETWEEN POPULATIONS OF LACTIC ACID BACTERIA AND PSEUDOMONADS, MEANING A DECREASE IN PSEUDOMONADS POPULATION WHEN LACTIC ACID BACTERIA INCREASED. THIS EFFECT IS MORE MARKED WHEN A COMMERCIAL STARTER IS USED (LM-3). LIPID OXIDATION (TBA VALUES) SHOWN ONLY A SIGNIFICANT CORRELATION WITH RESPECT TO LACTIC ACID CONCENTRATION, DUE THAT THE REDUCING CONDITIONS PROMOTED BY THE ACID DECREASE THE RATE OF OXIDATION IN FATS. THIS AFFECTS AT THE SAME TIME THE OXIDATION STATE OF MYOGLOBINE PRODUCING A MORE INTENSE RED COLOUR IN THE MEAT, DUE TO THE REDUCED FORM OF THE PIGMENT.

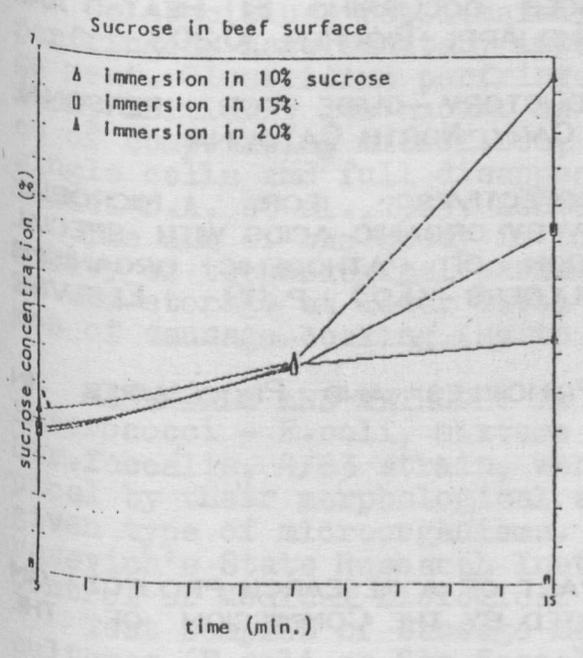
THE MICROAEROPHILIC ENVIRONMENT PRODUCED BY THE SEMIPERMEABLE WRAPPING FILM PROMOTE THE GROWTH OF LACTIC ACID BACTERIA, WHICH METABOLISM AT THE SAME TIME INCREASED THE CONCENTRATION OF LACTIC ACID ESTABLISHING A REDUCING ENVIRONMENT WHICH AFFECTS THE OXIDATION STATE OF THE PIGMENTS. AS THE STUDY TIME PROCEEDED, ACUMULATION OF OXIDATED LIPIDS AS WELL AS THE PRESENCE OF OTHER OXIDATION FACTORS SUCH AS LIGHT AND AIR SHIFT THE OXIDATION STATE TOWARDS OXIDATIVE REACTIONS PROMOTING DEVELOPMENT OF BROWNISH METMYOGLOBINE.

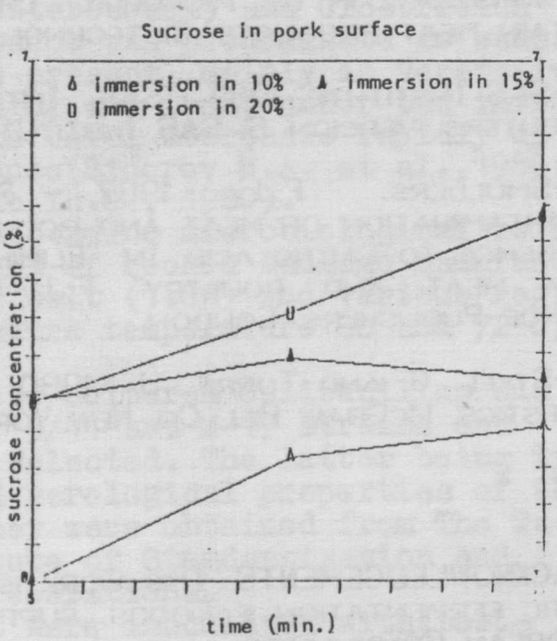
TABLE 4. CORRELATION COEFFICIENTS (PORK)

	TEMPERATURE	TIME	pH	LACTIC ACID	TBA VALUES	L	A	B	LACTIC ACID BACTERIA
pH	-0.201	-0.358							
LACTIC ACID	-0.002	0.466	-0.370						
TBA VALUES	0.225	0.040	-0.121	0.463					
L	0.007	-0.258	-0.324	0.120	0.099				
A	-0.014	0.102	0.300	-0.143	-0.181	-0.614			
B	0.019	-0.481	-0.125	-0.029	0.096	0.773	-0.313		
LACTIC ACID BACTERIA	0.180	0.781	-0.0378	0.325	0.116	-0.179	0.114	-0.216	
PSEUDOMONADS	0.126	0.810	-0.434	0.270	0.031	-0.218	0.164	-0.357	0.919

TABLE 5. CORRELATION COEFFICIENTS (BEEF)

	TEMPERATURE	TIME	pH	LACTIC ACID	TBA VALUES	L	A	B	LACTIC ACID BACTERIA
pH	-0.114	0.192							
LACTIC ACID	0.472	0.382	-0.744						
TBA VALUES	0.121	-0.579	-0.106	-0.135					
L	-0.096	-0.240	-0.357	0.115	0.342				
A	-0.120	-0.668	-0.212	-0.266	0.224	0.369			
B	0.038	-0.328	-0.423	0.190	-0.068	0.743	0.546		
LACTIC ACID BACTERIA	0.139	0.809	0.229	0.397	-0.540	-0.106	-0.609	-0.221	
PSEUDOMONADS	0.101	0.904	0.214	0.445	-0.610	0.215	-0.667	-0.302	0.893





CONCLUSIONS: IMMERSION OF SAMPLES IN 15% SUCROSE SOLUTION WAS ENOUGH TO HAVE A CONCENTRATION OF MORE THAN 2% AT 4 MM DEPTH NECESSARY TO START A LACTIC FERMENTATION. PSEUDOMONADS POPULATION WAS DECREASED WHEN LACTIC ACID BACTERIA WERE INOCULATED, HAVING BETTER RESULTS WITH THE COMMERCIAL STARTER THAN WITH THE STRAINS ISOLATED IN OUR LABORATORY. COLOUR WAS NOT ALTERED WITH THE PRESENCE OF INOCULATED LACTIC ACID BACTERIA. THIS IS PROBABLY DUE TO THE REDUCING CONDITIONS FAVOURED BY THE PRODUCTION OF LACTIC ACID, SHIFTING THE OXIDATION STATE OF MYOGLOBINE TO THE REDUCED (BRIGHT RED) FORM.

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