EFFECT OF INOCULATING LACTIC ACID BACTERIA IN DECREASING POPULATIONS OF SPOILAGE MICROORGANISMS IN PORK.

ANA MARIA CHAVEZ G., ARELY PRADO B., ISABEL GUERRERO L.
DEPARTMENT OF BIOTECHNOLOGY, UNIVERSIDAD AUTONOMA METROPOLITANA, UNIDAD IZTAPALAPA. APARTADO POSTAL 55-535, 09340 MEXICO D.F., MEXICO.

SUMMARY: BROCHOTRIX THERMOSPHACTA IS ONE OF THE MAIN MICROORGANISMS INVOLVED IN MEAT SPOILAGE. AMONG THE COMPOUNDS PRODUCED BY THIS MICROORGANISM, AS WELL AS BY PSEUDOMONADS, ARE DIAMINES, RESPONSIBLE FOR THE PUTRID ODOURS. THIS STUDY WAS FOCUSED IN DEFINING THE CHANGES IN DIAMINES CONCENTRATION IN MEAT WHEN LACTIC ACID BACTERIA WERE INOCULATED AS ANTAGONISTS OF B. THERMOSPHACTA AND PSEUDOMONADS. WRAPPED AND UNWRAPPED PORK SAMPLES WERE INOCULATED WITH LACTIC ACID BACTERIA; AN IMPORTANT REDUCTION IN DIAMINES CONCENTRATION WAS OBSERVED IN INOCULATED AND WRAPPED SAMPLES. A POSITIVE CORRELATION WAS OBSERVED BETWEEN LACTIC ACID BACTERIA AND B. THERMOSPHACTA POPULATIONS. IT IS ASSUMED THAT DIAMINES ARE DEPLETED (PROBABLY METABOLISED) BUT THE POPULATION OF B. THERMOSPHACTA DID NOT DECREASE WHEN LACTIC ACID BACTERIA WERE INOCULATED.

INTRODUCTION: B. THERMOSPHACTA IS ONE OF THE MAIN MICROORGANISMS RESPONSIBLE FOR MEAT SPOLAGE, BOTH IN AEROBIC AS WELL AS IN ANAEROBIC CONDITIONS. THIS IS THE ONLY GRAM-POSITIVE MICROORGANISM FOUND IN CONSIDERABLE AMOUNTS IN MEAT STORED IN AN AEROBIC ENVIRONMENT. BY AN INCOMPLETE OXIDATION, ITS METABOLISM PRODUCED ACETOIN (3-HIDROXI-2-BUTANONE), DIACETYL (2,3-BUTANONE) AND ACETIC ACID. THE MAIN NON-VOLATILE COMPOUNDS PRODUCED ARE CADAVERINE AND PUTRESCINE, RESPONSIBLE FOR PUTRID ODOURS IN SPOILED MEAT. HOWEVER, THESE COMPOUNDS CAN BE ALSO PRODUCED BY PSEUDOMONADS AND ENTEROBACTERIACEAE METABOLISMS, RESPECTIVELY OR EVEN BY PURELY CHEMICAL REACTIONS DURING THE FIRST DAYS OF STORAGE. WHEN THE MEAT IN WRAPPED WITH A IMPERMEABLE FILM, CARBON DIOXIDE ACCUMULATES IN THE MICROENVIRONMENT, DECREASING THE PSEUDOMONADS POPULATION AND INCREASING THE NUMBERS OF B. THERMOSPHACTA, BECOMING THIS ONE THE PREDOMINANT FLORA. IN THIS SITUATION, GLUCOSE IN CONVERTED TO ACETOIN AND TO SHORT-CHAIN FATTY ACIDS, PRODUCING A "SWEET" ODOUR, CHARACTERISTIC OF PUTREFACTION. SOME STRAINS OF LACTIC ACID BACTERIA CAN ALSO PRODUCE COMPOUNDS RELATED WITH PUTREFACTION, MAINLY IN VACUUM PACKAGED MEAT. HOWEVER, SOME STRAINS USED COMMERCIALLY AS STARTERS IN FERMENTED SAUSAGES CAN BE USED TO COMPETE SUCCESFULLY WITH SPOILAGE MICROORGANISMS, PRODUCING IN SITU LACTIC ACID AND OTHER BACTERIOSTATIC COMPOUNDS. THE OBJECTIVE OF THIS WORK WAS TO STUDY THE SURVIVAL OF PSEUDOMONADS AND B. THERMOSPHACTA WHEN A MEAT SUBSTRATE IS INOCULATED WITH A COMMERCIAL LACTIC ACID BACTERIA STRAIN.

MATERIALS AND METHODS: PORK CARCASSES WERE SAMPLED AT RANDOM FROM DIFFERENT REGIONS IN A LOCAL COMMERCIAL ABATTOIR. THE ANIMALS WERE SLAUGHTERED FROM 3 TO 4 HOURS BEFORE SAMPLING THE CARCASSES NO SEX, BREED, AGE OR NUTRITIONAL BACKGROUND WAS KNOWN. THE

SAMPLES WERE CUT INTO 5 CM3 PIECES AND ALLOCATED AT RANDOM TO THE TREATMENTS IN A COMPLETE RANDOMIZED DESIGN, AS FOLLOWS:

TABLE 1. EXPERIMENTAL DESIGN

TREATMENT	CONDITION
1	VACUUM PACKAGED, UNINOCULATED
2	VACUUM PACKAGED, INOCULATED
3	UNWRAPPED, UNINOCULATED
4	UNWRAPPED, INOCULATED

INOCULATION OF LACTIC ACID BACTERIA IN TREATMENTS 2 AND 4 WAS PERFORMED BY IMMERSION OF THE SAMPLES IN A LACTIC ACID BACTERIA CELL SUSPENSION. A COMMERCIAL STARTER (LM-3 BIOCARNA, VIGUSA, MEXICO CITY, CONSISTING IN A MIXTURE OF LACTOBACILLUS BULGARICUS AND MICROCOCCUS KRISTINAE-VARIANS) WAS FIRST GROWN IN ROGOSA LIQUID MEDIUM AND INCUBATED AT 37 C UNTIL O.D.=1. IT WAS THEN DILUTED WITH STERILE DISTILLED WATER, AND THE SAMPLES SUBMERGED IN THIS SUSPENSION FOR 3 MINUTES.

THE SAMPLES WERE THEN STORED AT 27 C, AVERAGE ROOM TEMPERATURE IN MEXICO, AND ANALYSED AT DAYS 0, 2 AND 4 FOR THE FOLLOWING RESPONSE VARIABLES: PUTRESCINE AND CADAVERINE CONCENTRATIONS; DEGREE OF OXIDATION; PH; LACTIC ACID BACTERIA, PSEUDOMONADS AND B. THERMOSPHACTA POPULATIONS.

THE EXTRACTION OF DIAMINES WAS CARRIED OUT FOLLOWING A MODIFICATION OF THE METHOD REPORTED BY SPINELLI (1974), EXTRACTING DIAMINES FROM THE MEAT WITH PERCLORIC ACID,CHLOROFORM/BUTANOL AND HEXANE. THE EXTRACTS WERE THEN APPLIED TO A SEPHAROSA ION-EXCHANGE COLUMN, USING A NACL BUFFER WITH A ION STREGTH GRADIENT (0.1 TO 0.2 M). THE PEAKS WERE COMPARED WITH PUTRESCINE AND CADAVERINE STANDARDS.

THE DEGREE OF OXIDATION WAS ANALYSED BY THE TBA METHOD.

PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS WERE ANALYSED BY STANDARD METHODS. POPULATIONS OF B. THERMOSPHACTA WERE ANALYSED BY THE MEDIUM REPORTED BY GARDNER (1966); MACRO AND MICRO-MORPHOLOGY AS WELL AS BIOCHEMICAL REACTIONS OF THE MICROORGANISM WERE COMPARED WITH A STANDARD KINDLY PROVIDED BY PROF. W. WAITES, UNIVERSITY OF NOTTINGHAM.

ALL TESTS WERE PERFORMED IN TRIPLICATE, AND THE RESULTS ANALYSED USING A SAS PACKAGE ADAPTED TO A PC.

RESULTS AND DISCUSSION: TBA VALUES HAD A HIGH POSITIVE CORRELATION WITH PSEUDOMONADS POPULATIONS, INDICATION A PROMOTION OF OXIDATION DUE TO THE GROWTH OF THIS MICROORGANISM. THE SAME EFFECT WAS OBSERVED BETWEEN COUNTS OF LACTIC ACID BACTERIA AND B. THERMOSPHACTA. IT SHOULD BE POINTED OUT THAT NO DIFFERENCIATION WAS TRIED BETWEEN NATIVE LACTIC ACID BACTERIA (PROBABLY SPOILAGE PROMOTORS THEMSELVES) AND THE INOCULATED STRAIN.

HOWEVER, IT WAS EVIDENT THE DECREASE IN THE CONCENTRATION OF CADAVERINE AND PUTRESCINE WITH AN INCREASE IN LACTIC ACID BACTERIA POPULATIONS. CONVERSELY, THERE WAS A POSITIVE CORRELATION BETWEEN PUTRESCINE CONCENTRATION AND PSEUDOMONADS POPULATIONS. DUE THAT CADAVERINE IS MAINLY PRODUCED BY ENTEROBACTERIACEAE, THE CORRELATION COEFFICIENT BETWEEN CADAVERINE CONCENTRATION AND POPULATIONS OF B. THERMOSPHACTA AND PSEUDOMONADS IS LOW.

TABLE 2. CORRELATION COEFFICIENTS.

	TIME	TBA VALUES	pH	PUTRESCINE CONC.	CADAVERINE CONC.	LACTIC ACID BACTERIA POPULATION	PSEUDOMONADS POPULATION
TBA VALUES	-0.008						
pH	0.039	-0.479					
PUTRESCINE CONCENTRATION	0.163	0.295	0.321	0.320			
CADAVERINE CONCENTRATION	0.119	0.411	-0.236	-0.170			
LACTIC ACID BACTERIA POPULATION	0.488	0.255	0.402	-0.060	-0.496		
PSEUDOMONADS POPULATION	0.753	0.329	0.542	0.552	0.133	0.318	
B. THERMOSPHACTA POPULATION	0.743	0.253	-0.336	0.723	-0.227	0.641	0.813

There were not significant differences regarding TBA values in vacuum packaged or unwrapped samples. In the same way, with respect to time of storage no significant differences were detected for TBA values in vacuum packaged samples. This was probably due to the reducing condition which prevents oxidation to take place. Conversely, unwrapped samples had a fast oxidation rate. Inoculated samples also had higher TBA values probably due to oxidation caused by the metabolism of lactic acid bacteria.

None of the sources of variation (inculum, packaging condition or time) had a significant difference with regard to putrescine concentration. Conversely, regarding cadaverine concentration the three sources of variation had a significant difference.

As expected, the packaging condition had significant difference with respect to lactic acid bacteria populations; vacuum packaged samples had higher counts..

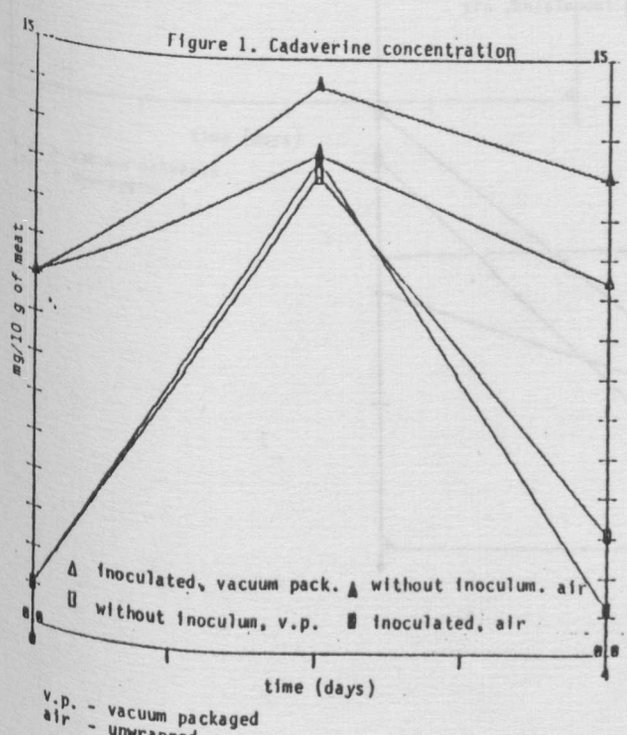
Pseudomonads and B. thermosphacta populations had also significant differences regarding packaging; higher populations were foundin unwrapped samples. However, when the samples were inoculated with lactic acid bacteria, Pseudomonads populations decreased but B. thermosphacta populations did not.

TABLE 3. Analysis of Variance

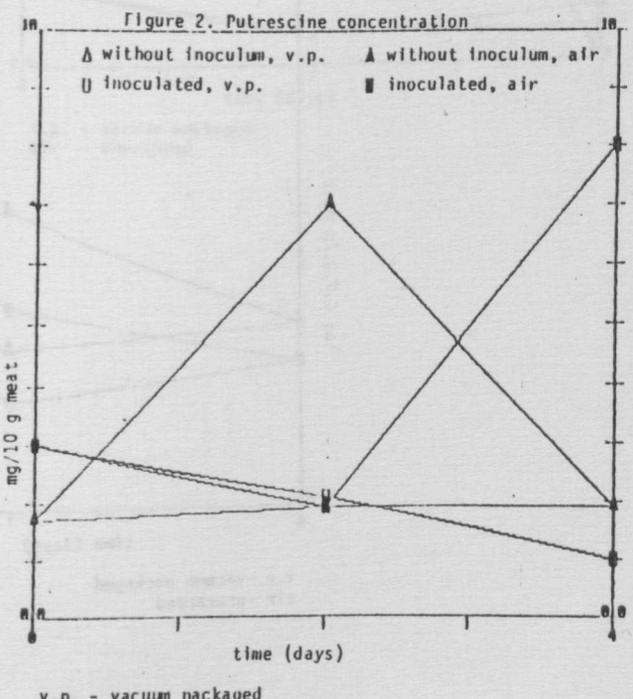
Response variable	Source of variation	Error MS	Error DF	P>
TBA values		0.1644	4	
	inoculum			0.1
	packaging			NS
	time			NS
pH		0.0238	4	
	inoculum			0.1
	packaging			0.1
	time			0.1
Putrescine concentration		0.1827	4	
	inoculum			NS
	packaging			NS
	time			NS
Cadaverine concentration		1.5207	4	
	inoculum			0.01
	packaging			0.1
	time			0.001

LACTIC ACID BACTERIA POPULATION		0.8090	4	
	INOCULUM			0.1
	PACKAGING			0.1
	TIME			NS
PSEUDOMONADS POPULATION		0.0608	4	
	INOCULUM			0.1
	PACKAGING			0.1
	TIME			0.1
B. THERMOSPHACTA POPULATION		0.0406	4	
	INOCULUM			0.1
	PACKAGING			0.1
	TIME			NS

NS - NOT SIGNIFICANT.

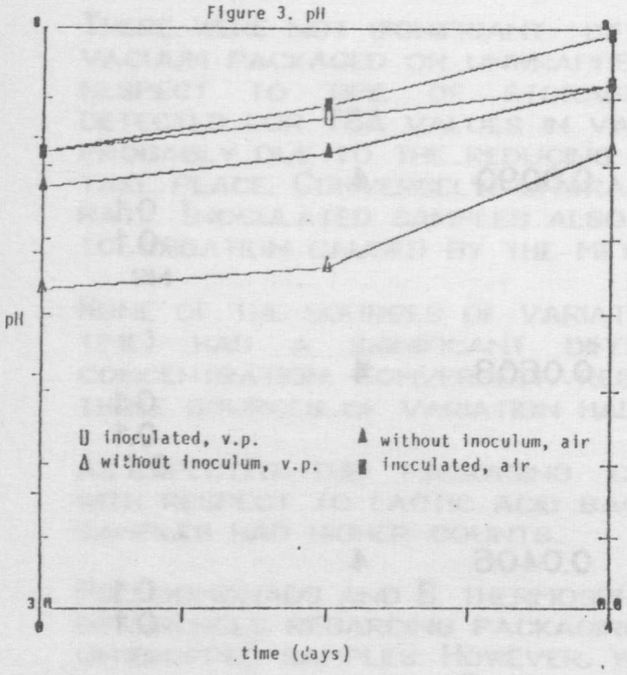


v.p. - vacuum packaged
air - unwrapped



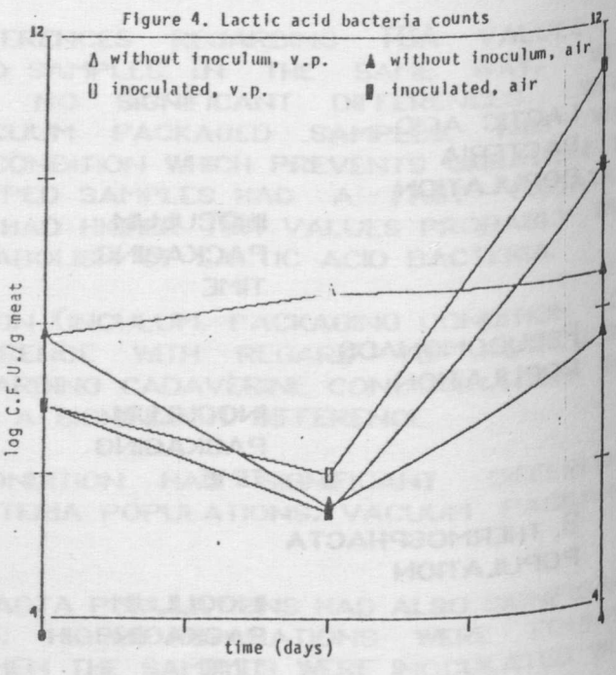
v.p. - vacuum packaged
air - unwrapped

Figure 3. pH



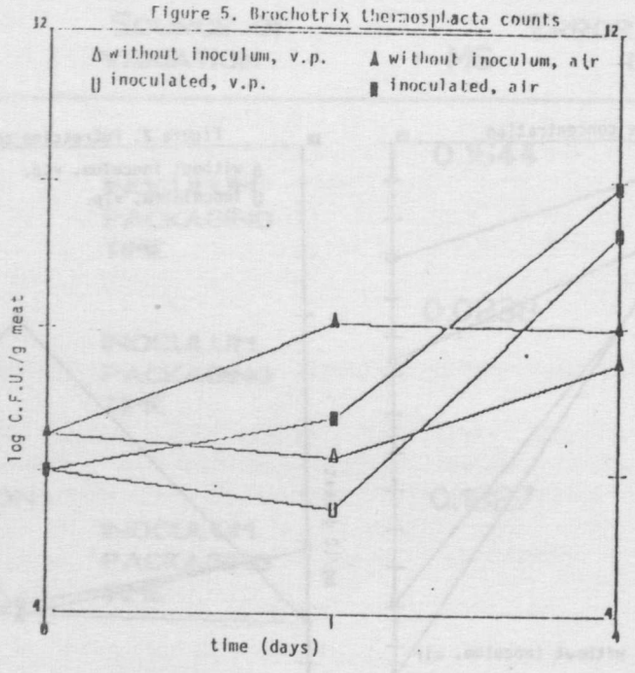
v.p. - vacuum packaged
air - unwrapped

Figure 4. Lactic acid bacteria counts



v.p. - vacuum packaged
air - unwrapped

Figure 5. Brochotrix thermosphacta counts



v.p.-vacuum packaged
air -unwrapped

Figure 6. Pseudomonads counts

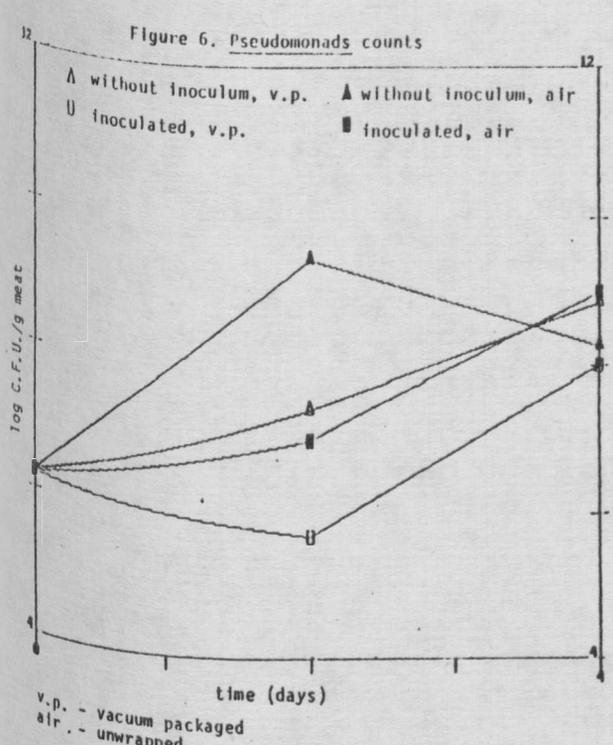
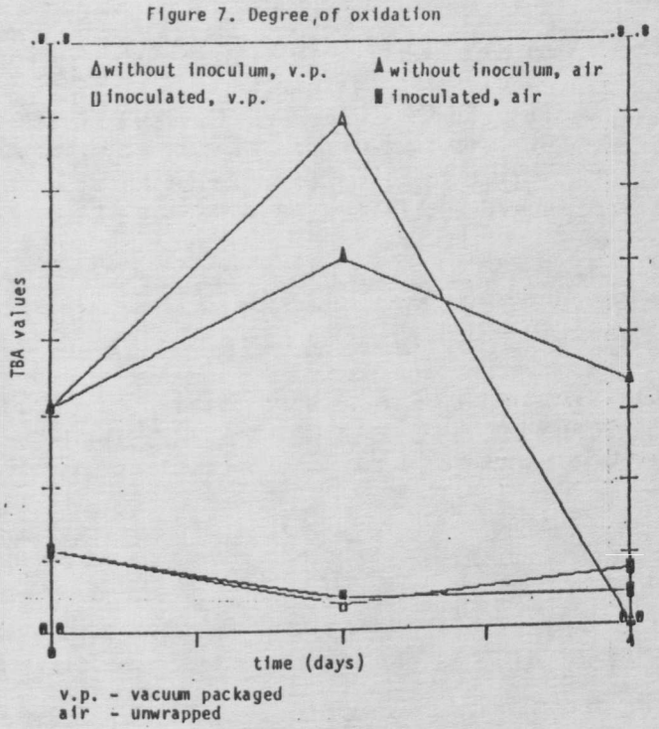


Figure 7. Degree of oxidation



CONCLUSIONS: Inoculation of pork samples with a commercial starter decreased the concentration of cadaverine and putrescine. Vaccum packaging favoured the growth of lactic acid bacteria, whereas in unwrapped samples numbers of Pseudomonads and B. thermosphacta were higher. A high positive correlation between B. thermosphacta and lactic acid bacteria numbers could be due to counting inoculated lactic acid bacteria as well as native ones. The native lactic acid bacteria also contribute to meat spoilage. In general, inoculating strains of homofermentative lactic acid bacteria, together with a microaerophilic environment can reduce the concentration of diamines, but not the population of B. thermosphacta.

REFERENCES:

- APHA. 1976. Compendium of methods for microbial examination of foods. M.L. Speck (Ed.). American Public Health Association. Intersociety Agency Commitee on Microbial Methods of Foods. Washington, D.C.

- Dainty, R.H. 1986. Chemical changes associated with microbial growth on meat stored at chill. 36th Meeting of Meat Research Workers Proceedings. Vol. 1. p. 179. Ghent, Belgium.

- Gardner, G.A. 1966. A selective medium for the ennumeration of Microbacterium thermosphactum on meat and meat products. J. Appl. Bacteriol. 29:445.

- Gardner, G.A. 1988. Brochotrix thermosphacta in the spoilage of meat. J. Appl. Bacteriol. 44:139.

- SAS Institute. 1985. SAS Introductory Guide for personal computers. Version 6. SAS Institute, Cary, North Carolina.

- Spinelli, A.M., Lakritz, L., Wasserman, A. 1974. Effects of processing on the amine content of pork bellies. J. Agric. Food Chem. 22(6):1026.

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