Combined effect of packaging and fermentation in increasing meat shelf-life.

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SUMMARY: These experiments were designed to study the effect of lactic acid fermentation and two packaging conditions (vacuum package and wrapping in a semipermeable film) in decreasing the population of two spoilage microorganisms: Pseudomonads and Brochotrix thermosphacta, in pork and beef samples stored at 15 and 27°C. In a preliminary experiment, the depth of penetration of bacterial populations and production of lactic acid were recorded up to 4 mm. Reduction of Pseudomonads population was observed in all vacuum packaged samples, and of B. thermosphacta in all samples; pH decreased more markedly in all pork samples as compared with beef. Tyramine concentration, taken as an indicator of spoilage, decreased in samples inoculated with lactic acid bacteria,but putrescine+cadaverine concentrations did not.

INTRODUCTION: In semitropical conditions such as those found in Mexico, surface contamination of carcasses due to handling, is encouraged by high temperatures and relative humidities. Some decontamination methods have been studied under these environmental conditions, such as spraying of humectants and organic acids, mainly acetic and lactic. The last one is very efficient in reducing pathogens and spoilage microorganisms, but is not feasible to be used, due to the cost of the acid in its chemical form. Two alternative methods have been succesfully tried: production of lactic acid in situ, i.e. via lactic acid fermentation; and reduction of oxygen availability by wrapping meat cuts with semipermeable films such as saran. It is also well known the effect of vacuum packaging on meat shelf-life, mainly regarding the reduction of spoilage microorganisms. On the other hand, microorganisms find their way to the inner part of a meat piece following the structural elements of the muscle. This study was designed to: a.know the penetration of lactic acid bacteria and changes in physico-chemical characteristics of the meat; b. to know the contribution of lactic acid fermentation and packaging on the reduction of spoilage microorganisms, mainly Pseudomonads and B. thermosphacta.

MATERIALS AND METHODS: Bacterial penetration and physicochemical changes. I. Meat from beef and pork carcasses was sampled at random from a commercial abbatoir. No breed,sex or age of the animals were recorded. The samples were taken to the laboratory and cut into 5 cm^3 pieces approximately; inoculation was made by immersing the samples for 10 minutes into a cell suspension of lactic acid bacteria (O.D.=0.5) in a 15% sucrose solution. The $2^3 \times 32 \times 5$ factorial designed applied was:

Factors	Levels
Species	beef/pork
Wrapping	unwrapped/saran
Storage temperature	15/27°C
Inocula	Lactobacillus plantarum+Pediococcus pentosaceus/commercial starter (Lactobacillus bulgaricus+Micrococcus kristinae-varians, Vigusa, Mexico City/control
Depth	0, 2, 4 mm
Study time	0, 24, 48, 72, 96 hours

The samples were analysed by slicing with a sterilized scalpel. The response variables were: colour (by Hunter Lab), lactic acid bacteria counts, Pseudomonads counts, titrable acidity, pH, degree of oxidation (as TBA values).

Effect of packaging and lactic acid fermentation. II. Samples obtained in the same manner as above were allocated at random to a complete 2^3 x 3 x 5 factorial design, as follows:

Factor	Levels
Species	pork/beef
Storage temperature	15/27°C
Wrapping	unwrapped/saran
Lactic acid strain	commercial (L. bulgaricus+M. kristinae-varians)/P. pentosaceus+L. plantarum/cont
Storage time	0, 24, 48, 72, 96 hours

The response variables were: pH, titrable acidity, degree of oxidation (as TBA values), colour (by Hunter La bacterial counts (Pseudomonads, B. thermosphacta, lactic acid bacteria). Diamines concentrations (tyramine, putrescine+cadaverine) were analysed in four pork sample lots stored at 15°C during a total study time of 12 hours. The tratments applied were: i. inoculated with the commercial starter; ii. inoculated with a strain o B. thermosphacta; iii. inoculated with the commercial strain and B. thermosphacta; iv. control.The amines we extracted from the samples as described by Spinelli et al. (1974) and separated in a ion exchange Sepharose CL-6B gel (Sigma) column, using sodium chloride 0.15 M buffer as eluent with a pH gradient from 6 to 7. The concentrations were recorded against a standard in a LKB recorder connected to a LKB spectrometer with a 206 nm filter.

III. A third set of samples were allocated at random to treatments arranged into a complete 2^2 x 6 factorial design as follows:

Constant: commercial strain, 15°C storage temperature

Factors	Levels
wrapping	vacuum/unpacked
species	beef/pork
Storage time	0, 24, 48, 72, 96, 120 hours

The response variables were: titrable acidity, pH, diamine concentrations (tyramine, putrescine+cadaverine), bacterial populations (Pseudomonads, B. thermosphacta, lactic acid bacteria). All data were collected from three replicates of the experiments and analysed for analysis of variance and general linear models using a SAS package adapted to a HP personal computer (SAS Institute, 1982).

RESULTS AND DISCUSSION: According to Table 1, storage temperature was the least important factor in the difussion of lactic acid bacteria, and not significant in Pseudomonads difussion. Differences in all respons variables were signifcant with respect to species and time. Conversely, the degree of oxidation was similar all wrapping conditions and storage temperatures, where TBA values were lower than 0.8 in all cases. Colour values (red component) had no significant differences with respect to wrapping. An unexpected not significa difference was observed in pH among inocula and depth, but with highly significant differences for acidity, which can be a result of high lactic acid production by the two inocula applied, or by the production of aci other than lactic by native heterofermentative lactic acid bacteria in meat. In samples wrapped in saran as

compared with unwrapped samples, pH did not varied during the study time (Tables 2 and 3). Wrapping also did not have a significant influence on lactic acid bacteria populations in both species but storage temperature had, with higher counts in pork than in beef. Wrapping did not have a significant effect on the degree of oxidation, with lower TBA values in pork. The inoculation of a commercial strain also reduced TBA values in pork; however, similar lactic acid concentrations were observed in all samples. Colour faded faster in uninoculated samples and kept similar L and a values when a starter was applied. In all inoculated samples, lactic acid bacteria populations increased with time; Pseudomonads counts tend to increase faster in uninoculated samples. In general, higher lactic acid concentrations were found in pork than in beef with lower Pseudomonads counts.

As shown in Table 4, species had no significant effect on bacterial populations but had significant differences with respect to pH and highly significant with respect to acidity and TBA values. As indicated before, pH values were higher for beef than for pork; the reduction condition in the system could affect the degree of oxidation. Time affected lactic acid bacteria populations as well as pH and L values. As lactic acid bacteria counts increased in vacuum packaged beef samples B. thermosphacta and Pseudomonads counts decreased. In unpacked pork samples Pseudomonads counts increased faster in uninoculated samples as compared with inoculated. It can be assumed that in both sets of samples, B. thermosphacta and Pseudomoands counts decreased in samples added with a strater without affecting notably other characteristics such as colour, degree of oxidation and acidity.

Diamine analysis shown a decrease in tyramine concentration in pork samples inoculated with a commercial starter (Figure 1). However, putrescine+cadaverine concentrations were higher in samples inoculated as compared with those uninoculated (Figure 2). In this case, samples treated with the commercial starter plus B. thermosphacta had the lowest diamines concentrations. These samples had no spoilage odour and kept good red colour after 5 days of study. A possible explanation can be related with the presence of Micrococcus in the commercial strater, which decarboxylated proteins into amines (Nychas, et al. 1988), that in small amounts contribute to the characteristic meat aroma, but after prolonged storage at high temperatures (15°C) amines concentrations become too high and have an important contribution to the spoilage odour. When B. thermosphacta is present, this microorganisms can metabolise the aminocompounds present.

CONCLUSIONS: Penetration of lactic acid bacteria was observed up to 4 mm depth, which was considered enough as to protect meat pieces from surface contamination of pathogens and spoilage microorganisms. Inoculation of pork and beef samples reduced the counts of Pseudomonads when oxygen availability is reduced by wrapping the meat with a semipermeable film. When samples were vacuum packaged, a fast decrease of B. thermosphacta counts was observed. Other characteristics such as colour, acidity and TBA values had only slight alterations, mainly due to acid production and packaging. Pork samples inoculated with a commercial starter had a decrease in tyramine concentrations but an increase in putrescine+cadaverine concentrations. These samples however, did not have spoilage synthoms after 5 days of storage at 15°C.

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ACKNOWLEDGEMENTS: This research was supported by CONACYT (Mexico) and IFS (Sweden).

Figure 1. Tyramine concentration in pork

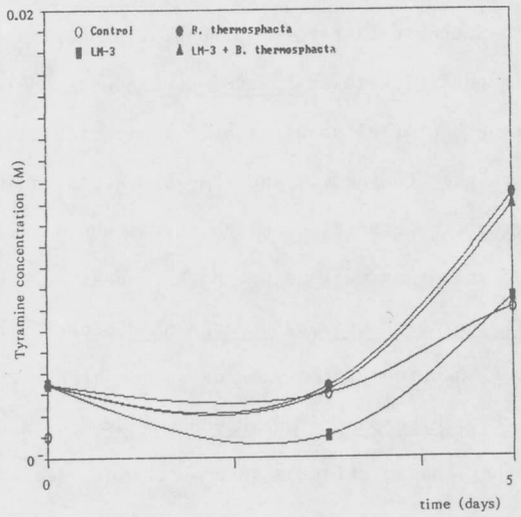

Figure 2. Putrescine+cadaverine concentration in pork.

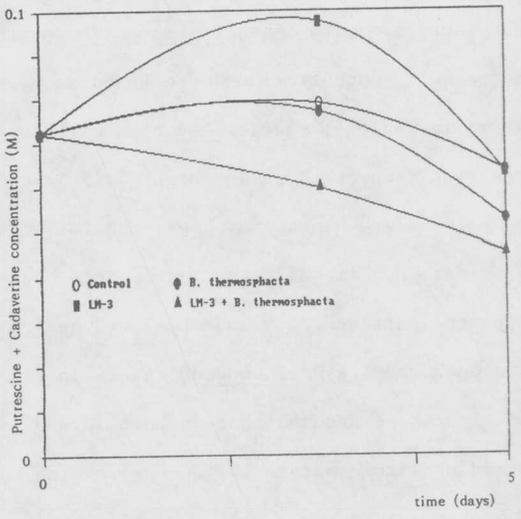

Table 1. Difussion in pork and beef samples (P>)

response variable	model	species	time	wrapping	temp.	inocula	depth
pH	0.0001	0.001	0.01	0.0001	0.001	ns	ns
acidity	0.0001	0.0001	0.0001	0.01	0.0001	0.001	0.0001
TBA values	0.0001	0.01	0.1	ns	ns	0.01	0.0001
L	0.0001	0.0001	0.0001	0.0001	ns	0.0001	0.01
a	0.0001	0.0001	0.0001	ns	0.01	0.0001	0.0001
b	0.0001	0.0001	0.0001	0.0001	ns	0.0001	0.0001
LAB*	0.0001	0.001	0.0001	0.001	0.1	0.0001	0.0001
Pseudomonads	0.001	0.0001	0.0001	0.1	ns	0.0001	0.0001

* Lactic acid bacteria counts
ns - not significant

Table 2. Lactic acid fermentation and packaging: Beef unwrapped/saran wrapped samples (P>)

response variable	inocula	wrapping	temp.	time
pH	0.01	0.001	0.1	ns
acidity	0.0001	0.001	0.0001	0.0001
TBA values	ns	ns	ns	0.0001
L	0.001	0.0001	ns	0.1
a	0.01	0.0001	ns	0.0001
b	ns	0.0001	ns	0.0001
LAB*	0.01	ns	ns	0.0001
Pseudomonads	0.0001	ns	0.1	0.0001

* Lactic acid bacteria counts
ns - not significant

Table 3. Lactic acid fermentation and packaging: Pork unwrapped/saran wrapped samples (P>)

response variable	inocula	wrapping	temp.	time
pH	0.001	0.001	0.01	ns
acidity	0.1	0.1	ns	0.0001
TBA values	0.0001	ns	0.01	0.1
L	0.0001	0.0001	ns	0.0001
a	0.1	0.01	ns	0.001
b	0.0001	0.0001	ns	0.0001
LAB*	0.0001	ns	0.0001	0.0001
Pseudomonads	0.01	0.1	0.001	0.0001

* Lactic acid bacteria counts
ns - not significant

Table 4. Lactic acid fermentation and packaging: vacuum/air (P

response variable	species	time	packaging
pH	0.1	0.0001	0.0001
acidity	0.0001	0.0001	ns
TBA values	0.0001	0.1	0.0001
L	0.0001	0.0001	0.0001
a	0.01	ns	0.01
b	ns	ns	ns
LAB*	ns	0.0001	0.0001
Pseudomonads	ns	0.001	ns
B. thermosphacta	ns	0.001	**

* Lactic acid bacteria counts
ns - not significant
** analysed only in vacuum packaged samples