INFLUENCE OF TYPE OF MUSCLES ON BACTERIAL GROWTH ON BEEF VACUUM PACKAGED

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INTRODUCTION

As noticed by Egan and Shaw (1984), microbial studies on the conservation of beef under vacuum were numerous. All the articles mentioned the presence of lactic acid bacteria in the total flora after some days of keeping. Some workers imputed them an inhibitory power towards other bacteria, specialy towards *Brochothrix* (Roth and Clark 1972) and *enterobacteria* (Newton and Gill 1978). Lauret (1981) revealed that only *Lactobacillus* capable of growing in presence of a relatively high amount of acetate had this faculty.

On the other hand, some workers (Fournaud and Valin 1978-Taylor and Shaw 1977) found that the bacterial growth might vary according to the nature of the muscle, but without binding this phenomenon to the biochemical characteristics of the muscles.

This study has to object to follow growth of inhibitory *Lactobacillus* among lactic acid bacteria on different muscle. It has also for purpose to confirm or infirm the observations on the differences of bacterial growth according to the muscles;and if the phenomenon exists to try to reveale its relations with some characters of the muscle.

MATERIALS AND METHODS

ORIGIN OF SAMPLES

Four beef (A,B,D,E) of norman race, 36 months old were choosen in the slaughter-house. The mean weight of carcasses was 350 kg. 13 muscles were taken off on every carcass four days after slauthering: *Biceps femoris* (n° 1), *Semi tendinosus* (n°4), *Vastus lateralis* (n°7), *Tensor fascia lata* (n°8), *Psoas major* (n° 12), *Longissimus dorsi* (n° 15), *Tricipitis brachii caput longum* (n° 21), *Semi membranosus* (n° 33), *Rectus femoris* (n° 36), *Supraspinatus* (n° 42), *Adductor femoris* (n° 49), *Infra spinatus* (n°57), *Quadratus lumborum* (82).

Every muscle was cut in three equivalent parts, every piece was then vacuum packaged under retractable film (BB1 Grace Cryovac O_2 permeability 15ml/m2/24 h before retractation).

The pH of all the muscles was taken before packaging. The meat were stored at 0 - 2° C during 28 days maximum. A part of every muscle was taken off, after 7, 14, or 28 days of storage.

MICROBIOLOGICAL SAMPLING

Before packaging two samples of 12.5 cm^2 were done at two different places on the muscles. After 7, 14, or 28 days only one sample of 12.5cm^2 was preleved.

BACTERIOLOGICAL ASSAYS.

They are purchassed according to the techniques described by Fournaud *et al.* (1973). *Lactobacillus* displaying inhibitory power were enumerated on L.B.S. medium (B.B.L.).

STATISTICAL STUDY

Before all calculs, numerical data were converted in logarithmic data. The factorial comparisons of means (test F of analyse of variance), the classification of means according to Newman and Keuls and the correlation were realised according to Snedecor and Cochram (1971). For the factorial comparison of means the animals were seen as forming a bloc.

RESULTS - DISCUSSION

INITIAL CONTAMINATION

Some bacterial populations could not be revelead on all the muscles of all the animals. So, *enterobacteria* were revelead only 3 times for 52,*Lactobacillus* 8 times (4 times on each beef A and D). The lactic acid bacteria was principally met on beef A (11 times for 13) and B (9 times for 13).

One animal, beef A was seen more contaminated that the others for the totality of microorganisms : total count $3.5x10^3$ /cm^2 for A, the average were $3x10^2$ /cm^2 for the other. This difference lies essentially on the presence of *Pseudomonas:* $1.5x10^3$ /cm^2 for A, 20/cm^2 on average for the others. All the muscles of a same carcass were contaminated in the same manner.

BACTERIAL EVOLUTION DURING STORAGE.

1. Influence fo type of muscle.

The bacterial evolution differed according to the muscle with a probability varying from 99% -lactic acid bacteria, total flora- to 99.9 % -*Brochothrix, Pseudomonas*-. One drastic exception, *Lactobacillus* displaying inhibitory power : their growth was independant on the type of muscle and carry on during all the storage.

Brochothrix and *Pseudomonas* together evolued in the same way, on each different muscles (interaction muscle bacteria not significative).Then their evolution differed for each muscle during the conservation. Having regard to the developpement of these two bacteria the muscles could be classed in 3 groups :

Group I:relatively important growth without apparent lag phase: *Supraspinatus* (n°42), *Quadratus lumborum* (n°82); the growth slowed down from the fourteen day (fig 1); For 28 days, on the muscle (n°82) the multiplication of *Brochothrix* was about 7700 times,these of *Pseudomonas* 2500 times.

Group II:growth after an about lag phase of 7 days:*Tensor fasciae latae* (n°8), *Infraspinatus* (n°=57). Like the group I, the growth slowed down from 14 days (fig. 2).

Group III:growth were non existant or negligible on the 9 other muscles, along the storage on 28 days (fig. 3).

For all the muscles tested, lactic acid bacteria multiplied along the storage(P=99.9%). From the second week it formed the dominant flora and the growth rate decreased. After the 28th day the greatest growth was observed on the two muscles of the group I (7.9×10^5 times for muscle n° 82 and 7.2×10^5 for muscle n°42) and the smallest on the two last muscles of the group III(2.1×10^4 times for muscle n°49 and 9.5×10^4 times for muscle n°33)(table 1). The multiplication on the muscle n°57 of group II was among the weakest, it located at the third place from the minimum.

2. Role of the animal.

The bacterial growth differed from one animal to the other with a probability ranging from 99% (*Pseudomonas*) to 99.9% (total flora, lactic acid bacteria, *Lactobacillus*) (table 2). Only *Brochothrix* evolued on the same way on all the animals.

	A	B	D	E
Total flora	2.47a	2.04b	1.92b	1.44c
Lactic acid bacteria	4.04a	3.36b	3.41b	2.43c
Lactobacillus	3.25a	2.16b	3.52a	1.91b
Pseudomonas	-0.36b	0.24a	0.20a	0.29a

Table II. Classement of means of bacterial growth according to the animals. -The means on the same line with the same subscript are equivalent-.

On beef A, *Pseudomonas* displayed the worst growth, inversly lactic acid bacteria got the best (table 2). *Lactobacillus* grew on the same way on A and D animals. We must notice its only on these two animals, that *Lactobacillus* was revealed before packaging. The improved growth of this bacteria could have its origin from a more important initial contamination. It might also result from the substrate meat more suitable, which came from the animal itself, since there was no interference with the muscle. This animal effect -initial contamination, bacterial growth- was also mentioned for the pork (Fournaud *et al.* 1986).

RELATION BACTERIAL EVOLUTION TYPE OF MUSCLE.

The pH value of the different muscles might be classed in about the same order that the *Brochothrix* growth at 28 days (table 1). The highest pH belonged to the muscles of the group I, the lowest to the group III.
The group II included some muscles whose pH connected to the group I (muscle n°57) or group III (muscle n°8). The correlation pH *Brochothrix* growth after 28 days was r=0.86 (P=99%) for all the muscles and 0.90 if muscle n°8 was omitted. These observations on the influence of pH came in the views of those enunciated by Taylor and Shaw (1977), for the total flora of vacuum packaged beef. They confirmed on the whole the results of Grau (1980) about the inhibition of *Brochothrix* in anaerobiosis in presence of lactate, so at relatively low pH. In this hypothesis the muscle n°8 seemed aberrant since the mean of pH (and extreme pH) ranged it in the middle of the second line of the group III (table 1) when the increasing of growth at 28 days classed it higher.It might possible that its lactate amount did not correlate with pH in the same manner that the other muscles.

An another hypothesis might be envisaged for explain the different growths on the muscles. If the inhibitory power of *Lactobacillus* depended on the need of hydrogen peroxide (Fournaud *et al.*1985) the muscle with its own oxidative nature could interfer.
A muscle with an oxidative characteristic, consummating the residual oxygen, release a greater amount of hydrogen peroxide than a glycolytic one ; and then shall restrict the apparent inhibitory power of *Lactobacillus*. The proportion of isoenzyme 1(isoLDH1) of lactate dehydrogenase is directly related with oxidative characteristic of the muscles:the more its rate is high the more the oxidative characteristic is important. Its measurement can be used to classify the muscles. Unfortunately the isoLDH 1 was not investigated on the muscles used in this assays. Nevertheless, if we took up the results got by Bousset (1981) with 10 animals, for the same muscles without the n°82, we could see that the correlation coefficient between *Brochothrix* growth after 28 days and isoLDH 1, was 0.75 and 0.84 if the muscle n°8 was ommited. This hypothesis related to the oxidative characteristic of the muscle is not to be rejected a priori, then more its allows to explain why *Lactobacillus* was the only bacteria whose growth was not influenced by the nature of muscle. In this hypothesis the muscles n°8 seemed one another time aberrant.

CONCLUSIONS

If the bacterial growth varied according to the muscle, excepted for the *Lactobacillus*, it was not possible to find in this present research an definitive explanation. The pH and the more or less oxidative nature of the muscle might be involved. The hypothesis of the pH did not allow to take in consideration the non-influence of nature of the muscle, on the growth of *Lactobacillus*. The theory bounded to oxidative characteristic of the muscle did not be retained, since the amount of isoLDH 1 was not achieve on the same samples. It seemed nevertheless the most probable because it allowed to explain all the phenomenon including the relation with pH, since the content of isoLDH 1 and pH varied in the same direction.

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	Δ Growth			pH		
	Brochothrix	Pseudomonas	Lactic acid bacteria	mean	Minimum	Maximum
Group I						
n° 82	4.89	3.37	5.90	6.03	5.90	6.24
n° 42	2.47	1.82	5.86	5.81	5.69	5.93
Group II						
n° 57	2.30	0.51	5.31	5.82	5.74	5.94
n° 8	1.81	0.85	5.77	5.59	5.50	5.75
GROUP III						
n° 21	1.31	0.14	5.74	5.69	5.59	5.96
n° 36	1.22	0.09	5.44	5.57	5.42	5.65
n° 15	1.02	-0.36	5.64	5.57	5.50	5.64
n° 1	0.89	-0.35	5.77	5.52	5.46	5.60
n° 4	0.60	0.25	5.41	5.46	5.40	5.57
n° 7	0.49	-0.53	5.49	5.51	5.45	5.57
n° 12	0.43	-0.05	5.45	5.46	5.40	5.52
n° 49	0.12	-0.19	4.32	5.53	5.47	5.64
n° 33	-0.07	-0.56	4.98	5.53	5.41	5.72

Table 1 : Increasing (log) of the bacterial count (Δ growth) during 28 days according to the muscles and initial pH of this muscles.

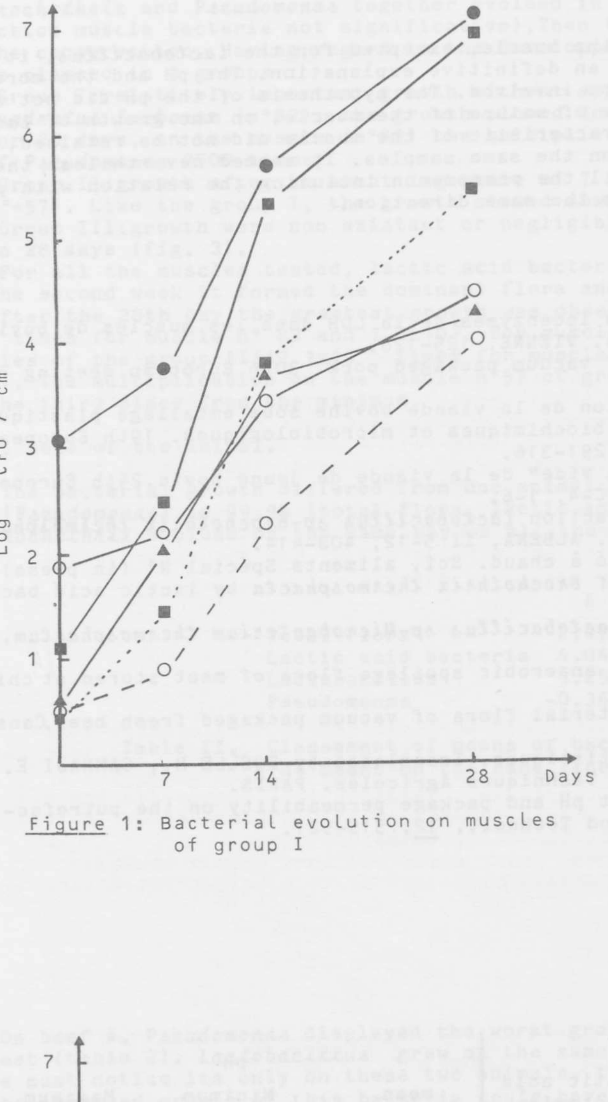

Figure 1: Bacterial evolution on muscles of group I

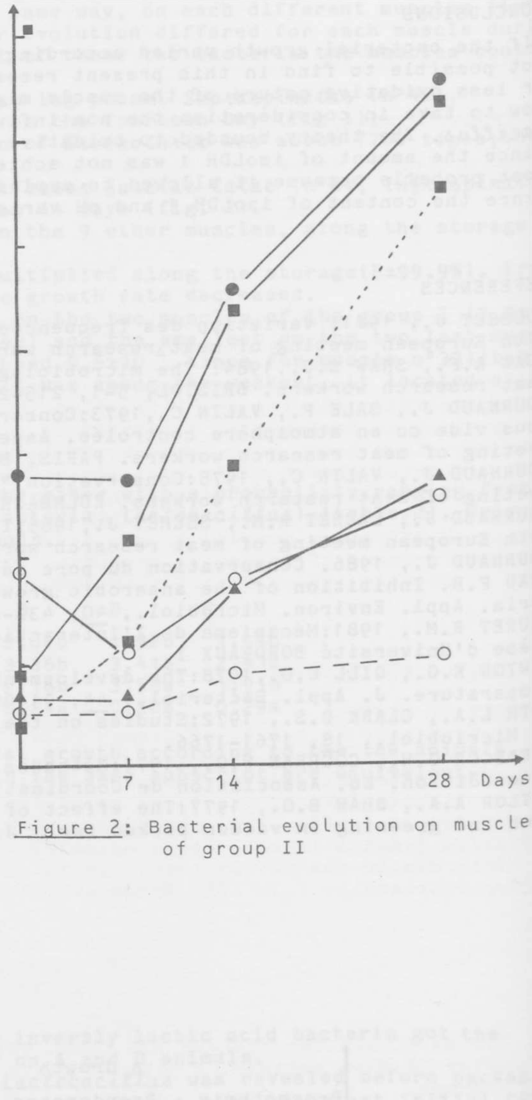

Figure 2: Bacterial evolution on muscles of group II

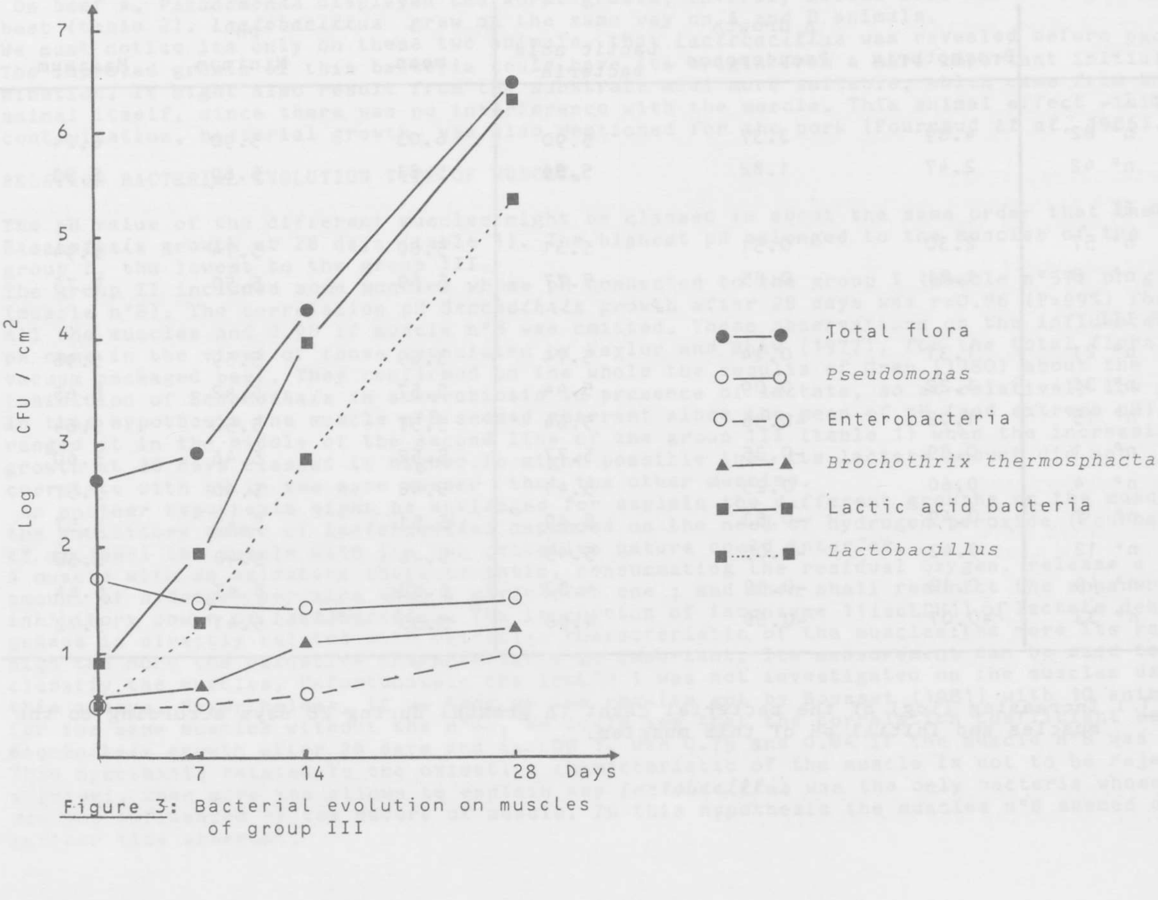

Figure 3: Bacterial evolution on muscles of group III