MICROBIAL DEVELOPMENT IN CURING OF NORMAL AND DFD PORK LOINS

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SUMMARY
In this study normal pH loins and DFD-loins were compared as to their microbial composition during curing. On the other hand, the influence of supplementation of the brine with 1 % saccharose was evaluated. Remarkable differences in microflora could be established between normal and DFD pork loins. Saccharose in the brine stimulated the development of microorganisms in cured meat. In regard to safety against food pathogens, curing of DFD-meat should be avoided. pH-Selection of loins is therefore very usefull for making high quality cured meat.

INTRODUCTION
In contrast to fermented sausage production, where the microbial activity is well known and where starter cultures are commonly used, the presence and the role of microorganisms in cured meat still remain obscure. According to the curing procedure, the microorganisms, which originate from the fresh meat and from the brine, are submitted to a variety of changing conditions (pH, redoxpotential, wateractivity,...). These circumstances should lead to a reduction of pathogenic and spoilage flora and forward microorganisms with technological properties. The aim of this paper is to study the composition of the microbial flora in bacon under different conditions of production. Since the development of microorganisms is influenced by pH and since a high final pH value (DFD-meat) is quite common in pork, normal and DFD pork loins have been compared. On the other hand, the supplementation of 1% saccharose to the brine as a substrate for microbial development and acidifying has been studied.

MATERIALS AND METHODS
For this purpose, 80 pork loins have been selected, among which 40 with a normal pH (< 5.8: control group) and 40 with a DFD character (pH > 6.4), measured in the *Mm. spinalis dorsi et cervicis* (do). Of each group 20 loins were injected for 10 % with a 10°Bé brine supplemented with 1 % saccharose; the other 20 were processed in the same way without saccharose (d1). In this manner 4 groups of 20 loins were obtained:
group 1: control group cured without saccharose
group 2: control group cured with saccharose
group 3: DFD-group cured without saccharose
group 4: DFD-group cured with saccharose

The injected meat portions were transferred into 4 different curing tanks, filled with the same brine as used for the injection. Three days later (d4) the brine was decanted, while the loins remained in the tanks for maturation (4 days at 6-8°C). Then the pork was dried for 4 hours at 30°C, smoked for 16 hours at 41°C and further dried during 5 days (15°C; 78 % RH). On day 14 the meat was cooled to 3°C and vacuum packed. The loins were further stored during 1 month under refrigeration in vacuum packing.

Measurements were performed on fresh meat (do), after curing and maturation (d8), after smoking and drying (d14) and after 1 month of vacuum packing (d43). Each time, 5 samples were taken from each group.

pH-Measurements were carried out on the *Mm. spinalis dorsi et cervicis*, on the *M. longissimus dorsi* and on the *M. rhomboideus thoracis*. For this purpose, a combined glass-calomel electrode was used (Orion 91-63, Swiss made) connected with a digital pH-meter (Orion Research, model 701, Massachusetts, USA).

The wateractivity (a_w) was determined at 25°C in the *M. longissimus dorsi* with a Novasina device (type ER84/3, Zürich, Switzerland).

For microbial examinations, a sample of 10 g meat was taken in a sterile way from the *M. rhomboideus thoracis* and homogenized with a Stomacher (Colworth 400, A.J. Seward, London) in 90 ml Buffered Peptone Water (Oxoid-CM 509) + 4 % NaCl. The media were inoculated by means of a Spiral System device (Spiral Systems Instruments, Inc., Bethesda, Maryland). The following counts were carried out:

- Total aerobic mesophilic germs on Plate Count Agar (Oxoid-CM 325) + 4 % NaCl, incubated at 30°C for 48 hours
- *Micrococcaceae* on Mannitol Salt Agar (Oxoid-CM 85), incubated at 37°C for 36 hours
- Lactobacilli on Rogosa SL Agar (Difco 0480-01-8) (pH 5.4), incubated in microaerophilic conditions at 28°C for 5 days
- *Enterobacteriaceae* on Violet Red Bile Glucose Agar (Oxoid-CM 485), incubated at 37°C for 24 hours
- *Staphylococcus aureus* according to Isigidi *et al.* (1989); incubation at 37°C for 24 hours
- Slimeforming leuconostocs according to Mossel and Tamminga (1973); incubation at 30°C for 5 days
- Vibrios according to Buttiaux (1963); incubation at 30°C for 5 days
- Yeasts on Oxytetracycline Glucose Yeast Extract Agar (Oxoid-CM 545), incubated at 22°C for 72 hours

The counts were converted into $\log_{10}$N/g and the mean value was calculated for every 5 samples.

RESULTS AND DISCUSSION

During curing of the loins a quick decrease of pH was observed in the different muscles (fig. 1). In

DFD-loins the pH dropped also, but still remained at a higher level as compared with normal meat. The pH value of DFD-loins at the end of the production (d43) was already reached on the eighth day in normal loins. This difference has undoubtedly an important influence on the microbial composition of the flora. The pH values showed a high degree of correlation between the *Mm. spinalis dorsi et cervicis* and the *M. rhomboideus thoracis* ($r = 0.921$; $Y = 1.081X-0.389$) and the *M. longissimus dorsi* ($r = 0.865$; $Y = 1.341X - 1.611$) respectively. However, the pH measured in the *Mm. spinalis dorsi et cervicis* (mean pH = 5.524) was always significantly higher than in the *M. rhomboideus thoracis* (mean pH = 5.471; $p = 0.003$) and also significantly higher than in the *M. longissimus dorsi* (mean pH = 5.321; $p = 0.0001$). Even the *M. rhomboideus thoracis* differed significantly in pH value from the *M. longissimus dorsi* ($p = 0.0001$). Hence, pH values measured in the *Mm. spinalis dorsi et cervicis* , which are situated superficially, can be extrapolated for deeper located muscles and are suitable for pH-selection of the loins. The results in tabel 1 indicate that supplementation of saccharose into the brine leads to a supplementary pH-drop in the different muscles.

The wateractivity decreased quickly in both normal and DFD loins, but in normal meat the a_w dropped to lower final values (fig. 2). No significant difference in a_w could be observed in curing procedures with saccharose.

The total aerobic mesophilic counts of the fresh meat were rather high (10^3-10^4/g). Poor hygienic conditions during slaughtering must be incriminated for these values. Especially the scalding process is responsible for deep contamination of meat (Ekstam, 1979). After curing and maturation an important increase (1-3 log) in total counts could be observed. This is due to the injection of regenerated brine and to the relatively high environmental temperature. The total counts in DFD meat were 1 log higher than in normal loins, which indicates better microbial growth conditions in DFD meat. The presence of saccharose in the brine leads to slightly higher counts in comparison with the controls. Out of the figures it appears clearly that total aerobic mesophilic counts are not always higher than the sum of the individually determined genera. This can be explained by the choice of adding 4 % NaCl to the medium; at this concentration the total counts reflect especially the number of microorganisms which are capable of quick growth on cured meat and which can cause deterioration (Gardner, 1982).

Micrococcaceae were inoculated into the loins by means of the brine, since they could not be determined in fresh meat. They increased with 3-4 log within 8 days; this was most evident in DFD meat, where the pH is closer to the pH optimum of *Micrococcaceae* (pH = 7.5) (Schleifer, 1986). In DFD loins *Micrococcaceae* constituted the dominant flora. These results agree with the conclusions of Silla *et al.* (1989) and of Molina *et al.* (1989). In normal loins the supplementation of 1 % saccharose into the brine stimulated the growth of *Micrococcaceae*. Although *Micrococcaceae* are able to break down sugars and are described as acidifying organisms, they should rather be considered as pH-stabilizing bacteria (Coretti, 1975). Among this family, Rheinbaben and Seipp

(1986) observed a ratio of 68 % species belonging to the genus *Staphylococcus* to 32 % belonging to the genus *Micrococcus*.

Stapylococcus aureus could be isolated in 2 samples of the fresh loins (max 330 cfu/g), but also one of the final products was positive on a thermonuclease + *S. aureus* strain (220 cfu/g). At a wateractivity of 0.890 growth and enterotoxin production of *S. aureus* is still possible (Tatini, 1973). It has to be noted that this positive sample belonged to the DFD-group, cured with saccharose.

As for *Micrococcaceae*, the brine injection is the main source of lactobacilli in the loins. These bacteria showed also a quick development, but in contrast to Micrococcaceae, it was especially pronounced in normal pH loins (fig. 3 and 4). Lower pH cured meat is indeed a better medium for growth of lactobacilli (Rheinbaben and Seipp, 1986). The highest counts were observed in normal pH loins, cured with saccharose (fig. 4); the extra sugar being a substrate for growth and acid production by lactobacilli. This is also the reason why in loins cured without saccharose, the number of lactobacilli decreased in the last stage of the experiment (fig. 3). In DFD-meat lactobacilli were of less importance (fig. 5 and 6).

Slimeforming leuconostocs were dominant in normal pH meat (fig. 3 and 4). The high affinity for saccharose of leuconostocs is the explanation for their high counts in control loins cured with sugared brine (fig. 4).

Vibrios were inoculated into the different loin groups by the brine injection. Afterwards, their number decreased in normal pH meat, especially in absence of saccharose (fig. 3). In DFD-meat on the other hand, their counts increased, to a bigger extent when saccharose was used (fig. 6). Since in the literature there has been a lot of confusion in regard to the role of vibrios in cured meat spoilage, these vibrios have been determined according to the classification of Gardner (1980-1981). These vibrios could be assigned to the genus *Vibrio costicola* , which designated them as technological flora (Petäjä *et al.*, 1972 and 1973). In DFD-meat *V. costicola* constitutes an important fraction of the total flora (fig. 5 and 6).

During curing, the loins were also contaminated with yeasts, but their numbers quickly decreased during further processing.

Enterobacteriaceae could be isolated aswell in the fresh meat as after curing and maturation of the loins. In the latter stage 85 % of the positive loins belonged to the DFD-groups. This can be explained by the higher pH- and a_w-values in DFD-meat in this stage, in comparison with normal pH meat. In the later stages all samples were negative, the final pH- and aw-values being less favorable for *Enterobacteriaceae*.

CONCLUSIONS

Between DFD-meat and normal pH meat, remarkable differences could be observed during curing of loins. DFD-loins contained higher total counts, based especially on a predominance of

Micrococcaceae. In normal pH meat, on the other hand, leuconostocs and lactobacilli were more important. In DFD-meat a progressive development of *Vibrio costicola* was obvious. Supplementation of the brine with 1 % saccharose tends to stimulate the development of microorganisms in cured meat.

In regard to safety against food pathogens, DFD-meat is less suitable for curing, since pH- and a_w-values become inhibitive at a later stage of the curing process. For this reason, curing of normal pH loins should be persued for making high quality cured meat. pH-values measured in the *Mm. spinalis dorsi et cervicis* are suitable for pH-selection of the loins.

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Tabel 1: pH-decrease in the different muscles during curing

Groups	*Mm. spinalis*	*M. long. d.*	*M. rhomb. thor.*
control	0,574	0,52	0,652
control + sacchar.	0,80	0,58	0,65
DFD	0,93	0,53	0,78
DFD + sacchar.	1,17	0,56	0,96

FIGURES

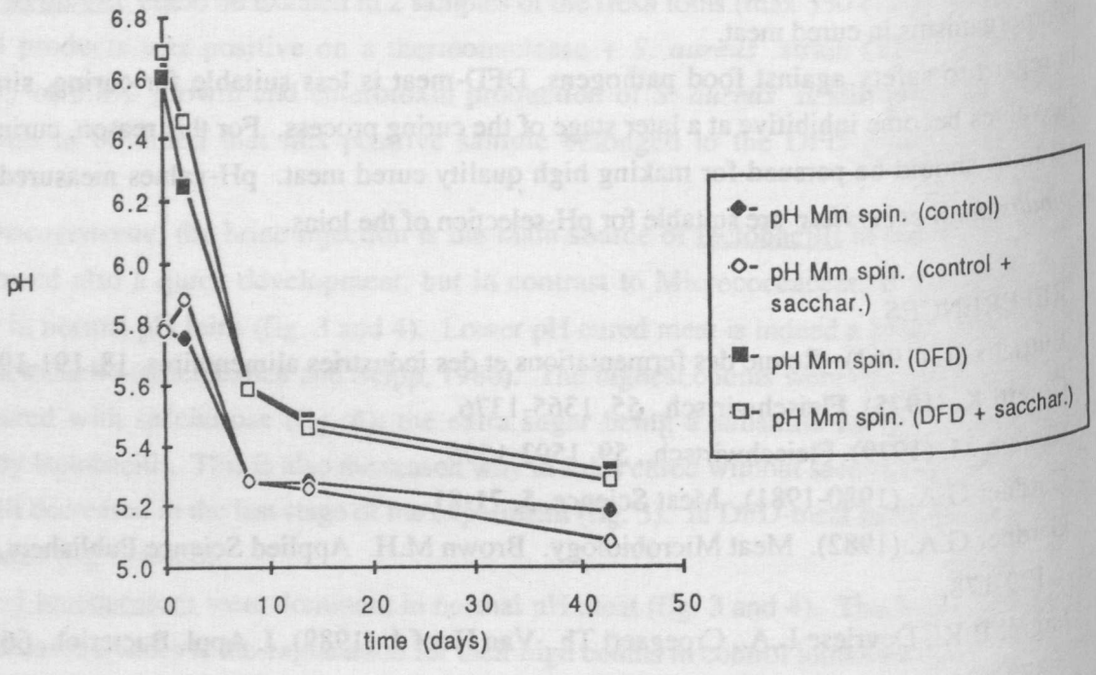

Figure 1: pH values measured in the *Mm. spinalis dorsi et cervicis*

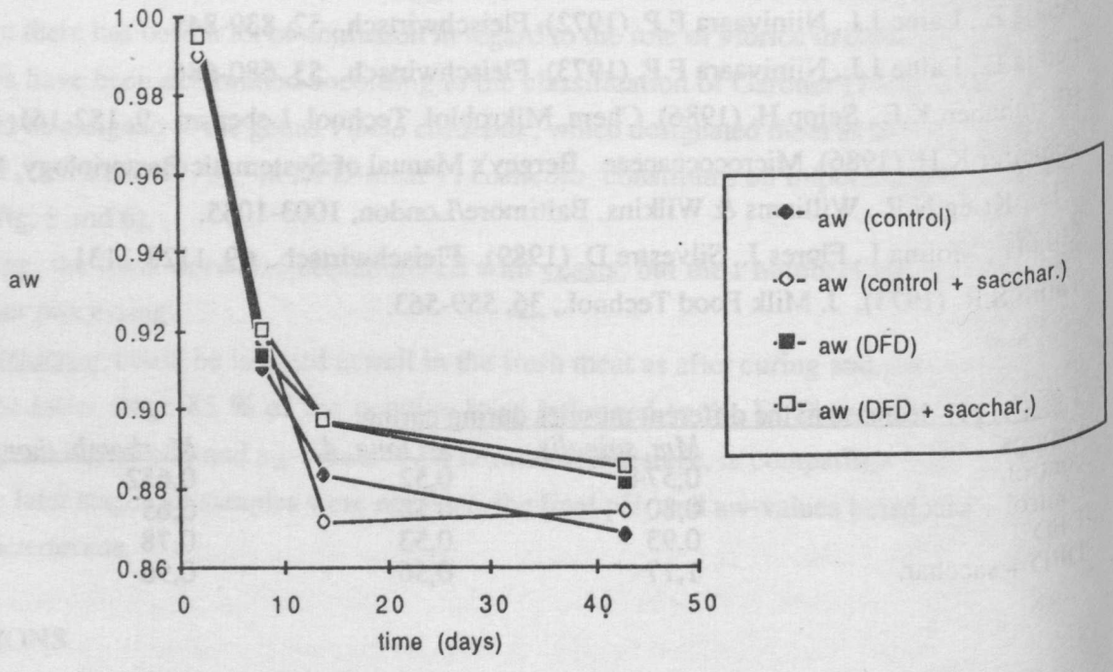

Figure 2: a_W values measured in the *M. longissimus dorsi*

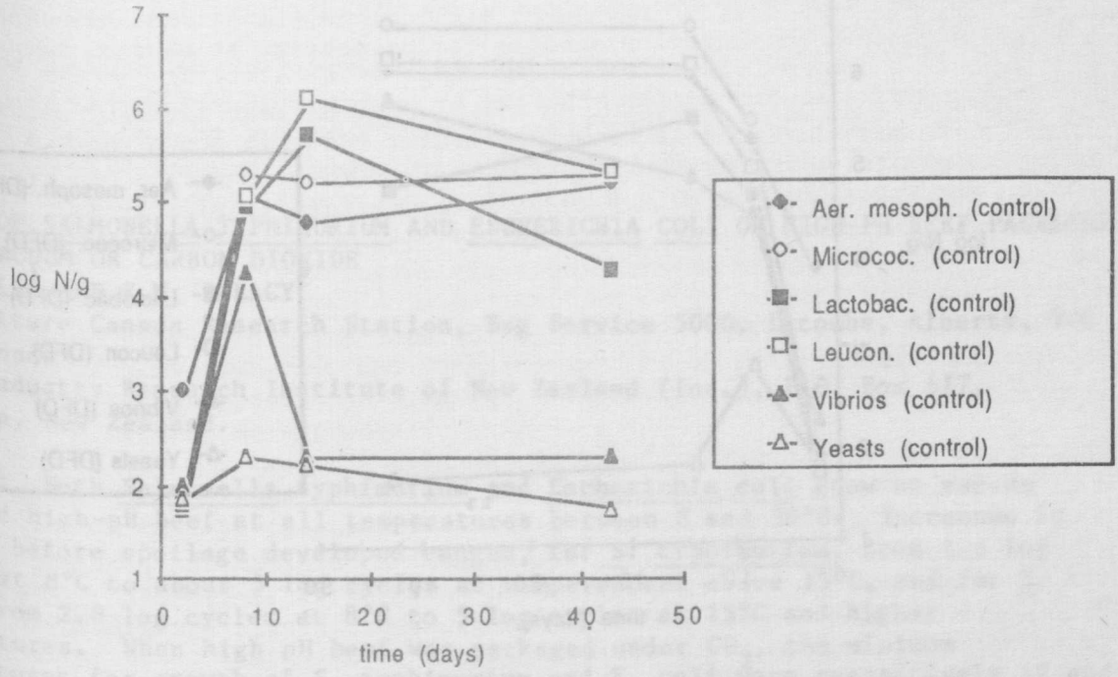

Figure 3: The microbial flora in the control loins

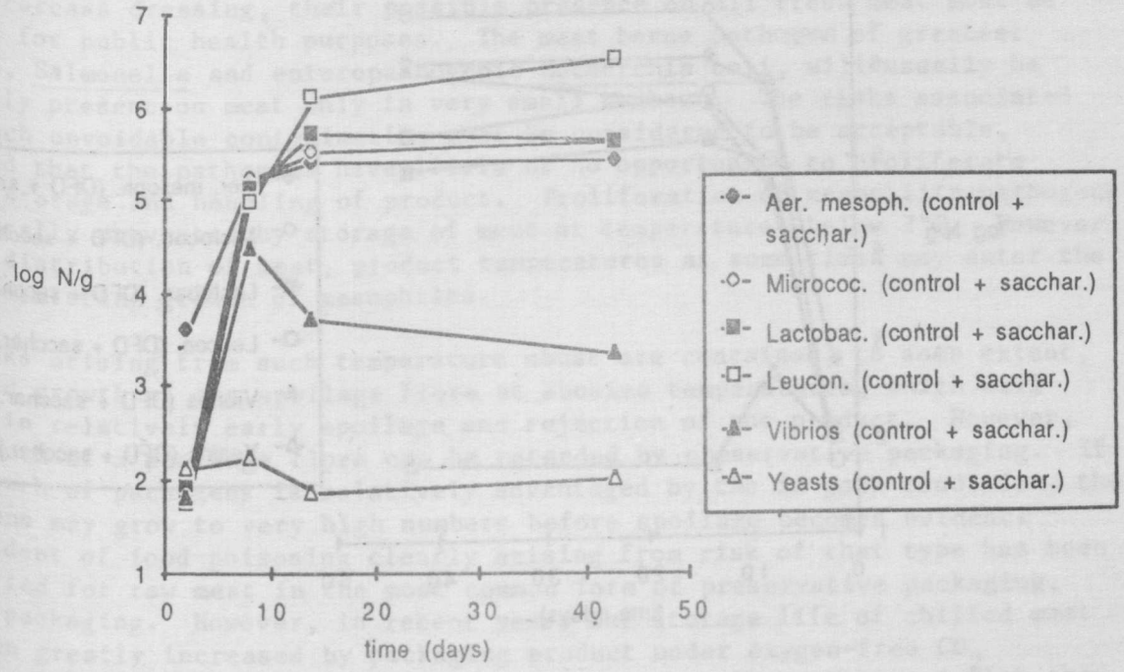

Figure 4: The microbial flora in the control loins cured with saccharose

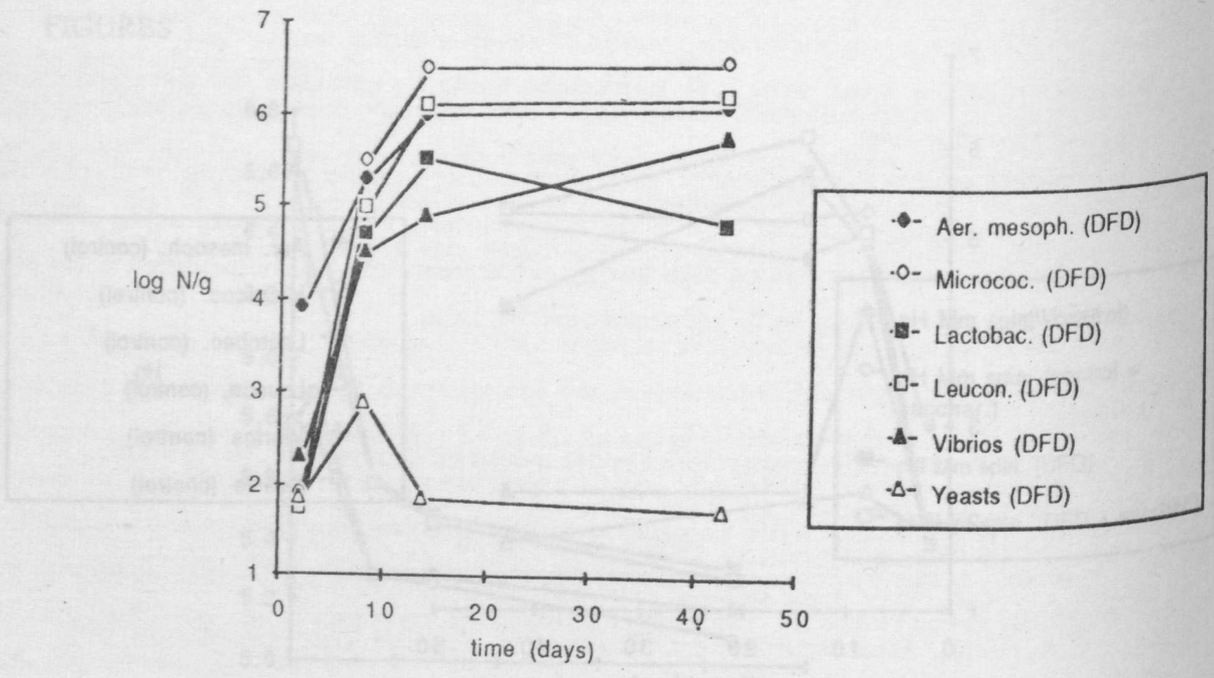

Figure 5: The microbial flora in the DFD loins

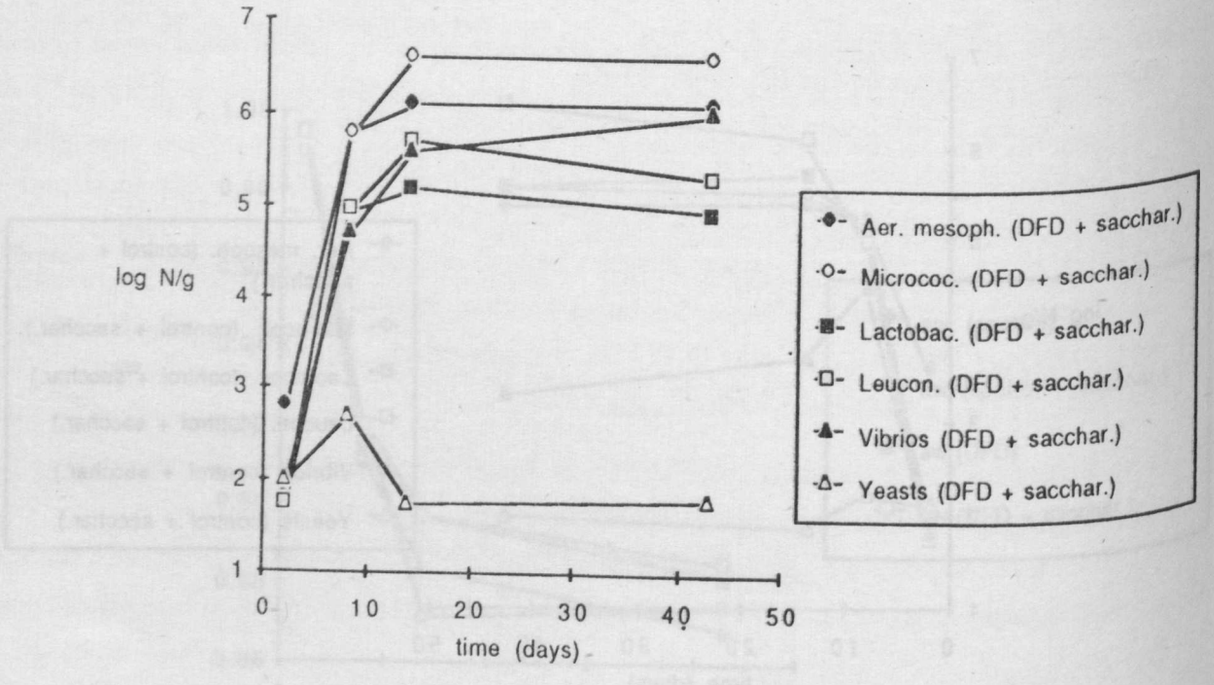

Figure 6: The microbial flora in the DFD loins cured with saccharose