Inoculation of steaks with Lactobacillus species and effect on colour and microbial counts

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The shelf-life of refrigerated fresh meat may be extended by using vacuum or modified atmospheres packaging. Using these procedures, typical aerobic spoilage bacteria (Pseudomonas) are often reduced and microbial flora is dominated by anaerobic facultative organisms such as Enterobacteriaceae (Serratia liquefaciens) and lactic acid bacteria. Serratia liquefaciens may contribute to quality deterioration of meat, specially high pH meat, because this bacteria is associated with green discolouration and "off-odor" (DAINTY et al., 1979). Brochothrix thermosphacta also induces spoilage by producing sour and cheesy flavor (EGAN et al., 1980). Lactobacilli are less involved in spoilage than the other organisms : they can produce "off" aroma with some strains, and only few days after the population has reached $10^8/cm^2$ (EGAN and SHAY, 1982). Nevertheless, they are the most desirable organisms because they are able to control the development of spoilage bacteria and therefore lactic cultures could be used to improve the shelf-life of meat. For example Pediococcus cerevisiae and Lactobacillus inoculated on chicken meat inhibit Pseudomonas (RACCACH et al., 1979). Some successful results are also obtained by NTHULI et al., 1977). After addition of lactobacilli to beef meat. Moreover, commercial starter cultures for dry sausage have no inhibitory effect on potential spoilage bacteria in fresh ground pork (BOURGEOIS et al., 1983). On the other way, such inoculation may involve deterioration of meat : after 7 weeks storage NTHULI et al. (1977) report greenish discolouration. SMITH et al. (1980) also observe meat quality deteriorations (flavor, aroma, color).

This present study was undertaken to determine the effects of lactobacilli inoculation, on Brochothrix thermosphacta and Gram negative bacterial growth, on one hand, and on meat color after vacuum packaging and display life of meat at 5°C, on the other hand.

Material and methods

- Bacteria

Lactobacilli strains were choiced among isolates from vacuum packaged beef and pork that had been stored at 5°C. They were identified by determination of various characters according to methods described by SHAW and HARDING (1984).

- Detection of greening

- Greening ability of Lactobacilli were tested in various conditions :

Lactobacilli strains were cultured in M.R.S. broth for 24 hours at 30°C. Then they were streaked on cooked blood agar or inoculated on meat juice prepared as indicated by GILL (1976). Greening of meat surface or juice was examined daily.

- Their inhibitory activity was tested against Pseudomonas species isolated from meat using the procedure of TALON et al. (1980).

- Meat samples

Decontamined steaks were prepared as follows : Semimembranosus muscles trimmed of fat and treated by peracetic acid (0.3 %) were washed with sterile saline solution. The outside layer was removed and then slices of meat (10 g) were cut. These steaks were then placed in steril bags and inoculated with cell suspension adjusted to 10^3 per g. After inoculation, bags were vacuum packed at 9 torrs and stored during 3, 6, 9 days and then were frozen at - 20°C until microbiological analysis. Control steaks without inoculation were treated in the same way.

Steaks were also cut in Longissimus dorsi muscles from carcasses 24 hours post mortem. 50 g were put in polyethylene bags in which 2 ml of lactobacilli suspension containing approximatively 10^3 or 10^5 cells per g were added. Non inoculated steaks were used as control. All steaks were then stored under vacuum.

After appropriate storage period, the bags were opened and steaks were put on fibre board trays, overwrapped with P.V.C. film permeable to gaz and stored in darkness at 5°C from 3 hours to 96 hours (display life). Samples were taken out for bacteriological examination or color analysis.

- Bacteriological analysis

Samples (5 g) were taken from different bags and organisms were enumerated using following procedures :

- Brochothrix on GARDNER's medium (48 hours at 24°C)
- Lactobacilli on M.R.S.'s medium (48 hours at 30°C)
- Gram negative bacteria on V.R.B.G. and D.P.T.S.'s medium (96 hours at 24°C)

- Colour analysis

Colour measurements were done with a Beckman DG-GT spectrophotometer fitted with an integrating sphere and reflectance spectra were recorded in the range 400-700 nm (Source C). Chromaticity (redness "a") and luminosity (L) characteristics were calculated with Hunter Lab system (WYSZECKI and STILES, 1967).

Evolution of myoglobin oxidation on meat surface was determined by reflectance difference R630 - R580 (VAN DEN OORD and WESDORP, 1971).

Results

- Preliminary trial

According to their biochemical characteristics, the selected strains were representative of different sub-groups of bacteria inside of cluster II defined by SHAW and HARDING (1984). None of the strains produced greening on blood agar or in meat juice. All inhibited more or less Pseudomonas.

In a preliminary trial, growth of 8 strains of Lactobacilli were compared on decontamined steaks (this treatment suppress natural population). The evolution of growth shown in figure 1 indicates a lag phase of 3 days for all strains. Between 3 and 6 days there is some slight difference in growth rate between strains and increases at 6 days are the most important on steaks inoculated with cultures 398 and 218. After 10 days, all strains reach a population of 10^7 b/g except strain 401. Moreover, the strain 218 was preferred to the other strains because the meat discolouration, appreciated by the decrease of R630 - R580, was less important after 96 h of display life. Consequently, with these results on bacterial growth and meat colour, strain 218 was selected for further study.

To reduce the 3 days lag phase, different conditions of inoculation were tested. Culture on NWEN's medium rather than on M.R.S. medium did not affect growth. The addition of glucose to the inoculum did not promote development of lactobacilli and the inhibitory effect of strain 218 was not greater when the amount of bacterial inoculum was increased from 10^3 up to 10^5 cells/g

- Effects of strain L 218 on growth of spoilage micro-organisms

The results of the bacteriological examination performed three hours after opening the bags are given in figure 2a. At 5 days total Lactobacillus counts of uninoculated samples are lower than inoculated (at least three log counts difference). Increases in Lactobacillus population over 34 days are greater for the control steaks (2 logs) than for inoculated (1 log). Large numbers of Lactobacilli during the early part of the storage do not exhibit any significant advantage in suppressing the microbial growth. Throughout the storage period, patterns of growth of

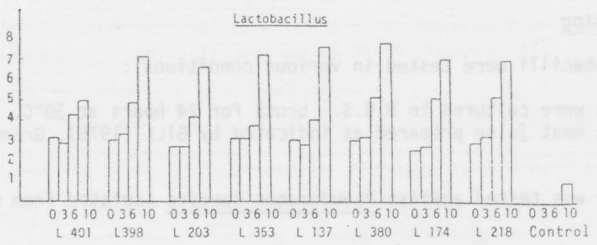

Figure 1 : Evolution of 8 strains Lactobacilli inoculated on decontamined Semimembranosus muscle under vacuum packaging during 3, 6, 10 days at 5°C

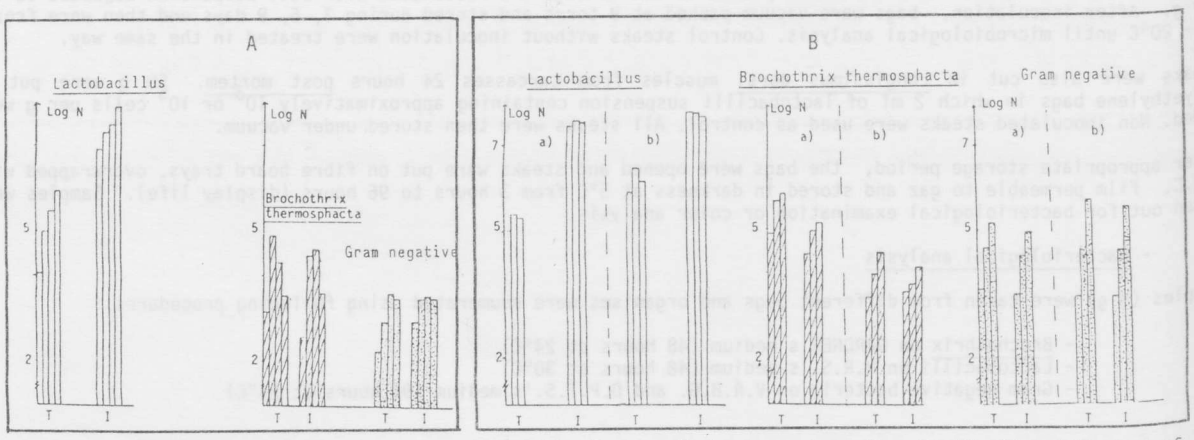

Figure 2 : Growth of bacteria on vacuum packaged beef : A - At bags opening after 5, 14, 20 and 34 days of storage ; B - After 14 (a) and 34 (b) days of vacuum storage and after 3, 48 or 96 hours of display life

T : Control steaks I : Steaks inoculated with strain 218 (10^5/g)

Brochothrix thermosphacta are similar for both inoculated and control steaks : from a count of 5.10^2 (inoculated) or 10^3 (control) at 5 days, the population reachs 10^5/g at 14 days of storage and decreases slightly at 34 days. The Gram negative counts are identical throughout 34 days display period on both inoculated and control samples.

During the display life, Brochothrix thermosphacta grow rapidly, with a more limited growth on inoculated meat, whereas Lactobacilli counts remain constant (figure 2b). It should be also noted that display-life involves a very important development of Gram negative population which has a negative effect on colour (figure 2b) (see below).

- Effect of L 218 strain inoculation on meat colour

When the time of vacuum packaging and display life increase, it can be observed a decrease in redness "a", a value equal to 11 corresponding to 40 % metmyoglobin in meat surface, the redness "a" being highly and positively correlated with panel preference (RENERRE and MAZUEL, 1985). Nevertheless, the differences noted between inoculated and control samples are not significant (figure 3).

If after 5 and 14 days of meat storage in vacuum packaging, the L value of inoculated meat is slightly higher than L value of control samples, after 34 days of vacuum packaging, the L value of inoculated meat becomes lower than those of control samples, these differences being not significant (figure 4). These results are in agreement with those of SMITH and al. (1980) which had shown that use of lactic acid bacteria resulted in darker muscle at the end of the 35 day storage interval. With principal component analysis, RENERRE and MAZUEL (1985) had shown a relation between calculated luminosity and the purple colour impression feeled by the panel.

With increasing times of vacuum packaging and display life, the difference R630 - R580 is decreasing. This is due to a higher percentage of metmyoglobin in the meat surface. Nevertheless, whatever are the storage conditions, the differences in reflectances (R630 - R580) between control and inoculated samples are not significant, even if inoculation seems to have a positively effect on meat colour after 5 days of vacuum packaging and a negatively one after 34 days (figure 5). The limit value of 12.5 corresponding to a 50 % sales ratio (RENERRE and MAZUEL, 1985) is reached only after 34 days of vacuum packaging and 96 hours of display life (figure 5).

Moreover, it is noteworthy to show that, by use of mean values, there is a highly significant and negative correlation between redness "a" and Gram negative bacteria counts (figure 6) : $r = -0.761$ ($P < 1$ %).

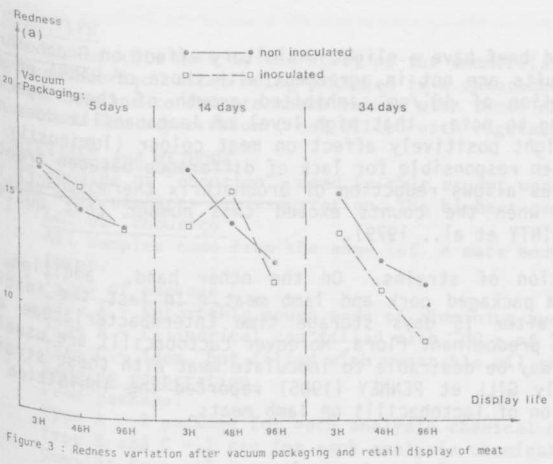

Figure 3 : Redness variation after vacuum packaging and retail display of meat

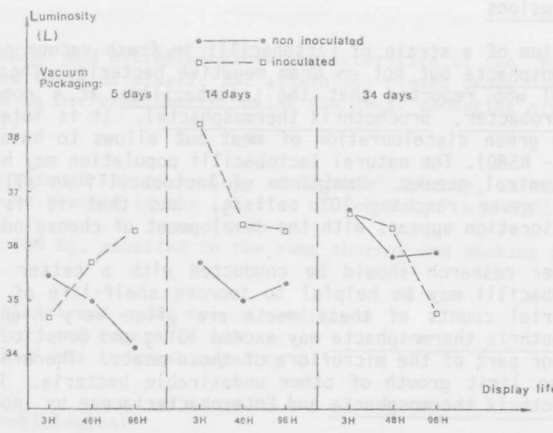

Figure 4 : Luminosity variation after vacuum packaging and retail display of meat

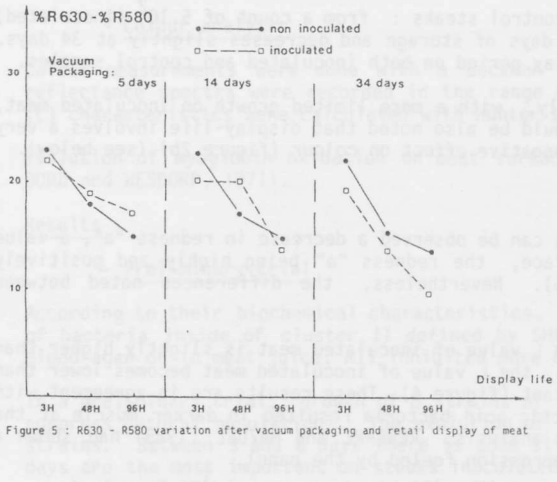

Figure 5 : R630 - R580 variation after vacuum packaging and retail display of meat

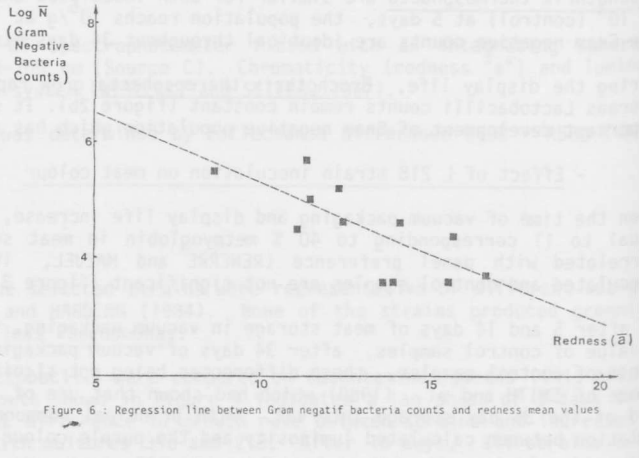

Figure 6 : Regression line between Gram negatif bacteria counts and redness mean values

Conclusions

Addition of a strain of Lactobacilli in fresh vacuum packaged beef have a slight inhibitory effect on Brochothrix thermosphacta but not on Gram negative bacteria. These results are not in agreement with those of HANNA et al. (1980) who reported that the Lactobacillus at a concentration of $10^7/cm^2$ inhibited growth of these species (Enterobacter, Brochothrix thermosphacta). It is interesting to note, that high level of lactobacilli does not cause green discolouration of meat but allows to have a slight positively effect on meat colour (luminosity or R630 - R580). The natural lactobacilli population may have been responsible for lack of difference between treated and control steaks. Dominance of lactobacilli in all samples allows reduction of Brochothrix thermosphacta, its level never reaching 10^8 cells/g, and that it is only when the counts exceed this number that quality deterioration appears with the development of cheese odor (DAINTY et al., 1979).

Further research should be conducted with a better selection of strains. On the other hand, addition of Lactobacilli may be helpful to improve shelf-life of vacuum packaged pork and lamb meat. In fact the initial bacterial counts of these meats are often very high and after 15 days storage time Enterobacteriaceae and Brochothrix thermosphacta may exceed 10^6/g and constitute the predominant flora. Moreover Lactobacilli are usually a minor part of the microflora of these meats. Therefore it may be desirable to inoculate meat with these strains and so limit growth of other undesirable bacteria. Recently GILL et PENNEY (1985) reported the inhibition of Brochothrix thermosphacta and Enterobacteriaceae by inoculation of lactobacilli on lamb meats.

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