STARTER CULTURE CHARACTERIZATION USING A MODEL SYSTEM

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SUMMARY: It has been the aim of this work to develop a model system designed to examine the effect of environmental factors on the activity of meat starter cultures with a view to optimize the fermentation processes.

Preliminary trials have indicated that it will be possible to develop such a model system, but the system still requires further development work.

The bacteriological quality of the raw material, the initial pH and the temperature of the meat slurry are aspects which will have to be controlled better.

INTRODUCTION: Bacterial starter cultures are used for meat processing in order to improve the flavour, appearance and texture of the final products. However, commercially available starter cultures have not always been successfull in producing the desired aroma in cured meat products.

The reason for this is probably that the processing conditions are unsuitable. There is often a lack of knowledge as to how different proccessing conditions affect the aroma formation. Furthermore the bacterial strains used today may not be the most optimal ones.

Overall knowledge about how metabolic activities which are important for the aroma development with specific starter cultures in fermented meat product are affected by factors such as pH, oxygen level, water activity, temperature, growth phase and ingredients is scarce. Such information would contribute to a better understanding of the behaviour of the starter cultures and to the design of optimal product recipes, processing levels and storage conditions for aroma development.

MATERIALS AND METHODS: The activity of the bacteria were studied in a model system using a Setric fermenter, model SET 2 with a max. working capacity of 1.5 l. The system is provided with a gas solenoid valve allowing the gas supply to open or close (CO_2, N_2, O_2, air).

The substrate was a slurry of pork loin with added curing salts. The chemical composition of the meat slurry is shown in table 1. The meat was chopped twice through a 3 mm plate, mixed and weighed out in portions of approx. 1 kg. The meat was packed in Amilon 15/60 and stored at -20°C until use. Before use the meat was thawed for 15-20 hours at +4°C and mixed with 6% bacon curing brine in the ratio 1 to 1. The mixing was carried out in a food processor for two minutes.

Table 1. Chemical composition of the meat slurry.

Water	78.6%
Fat	9.3%
Protein	8.8%
Sodium chloride	3.0%
Dextrose	0.3%
Nitrate	0.03%
Nitrite	0.01%

After calibration of the oxygen electrode, N_2 was passed through the fermentor until the O_2 concentration was approx. 10% (rel.).

Both strains were grown at +20°C for 24 h. Thereafter the temperature was reduced to +10°C.

The starter cultures used in this study were two strains of Streptococcus lactis subspecies diacetilactis - FARGO 761 and FARGO 762. The starters were supplied by Microlife Technics and used in a concentration of approx. 10^7 - 10^8/ml. The starters were stored at -20°C. Before use, one plastic cup containing 34 g was dispersed directly into 1/2 litre 4% NaCl solution. From this suspension 5 ml were added to the meat slurry.

During the tests data were collected with a Setric electronic control module MOD7F. The system was provided with a temperature probe, an oxygen electrode, a conductivity electrode and a pH electrode. Conductivity was measured with a PW 9526 digital conductivity meter.

Samples were taken daily for bacteriological analyses. The bacteriological analyses included plating on:

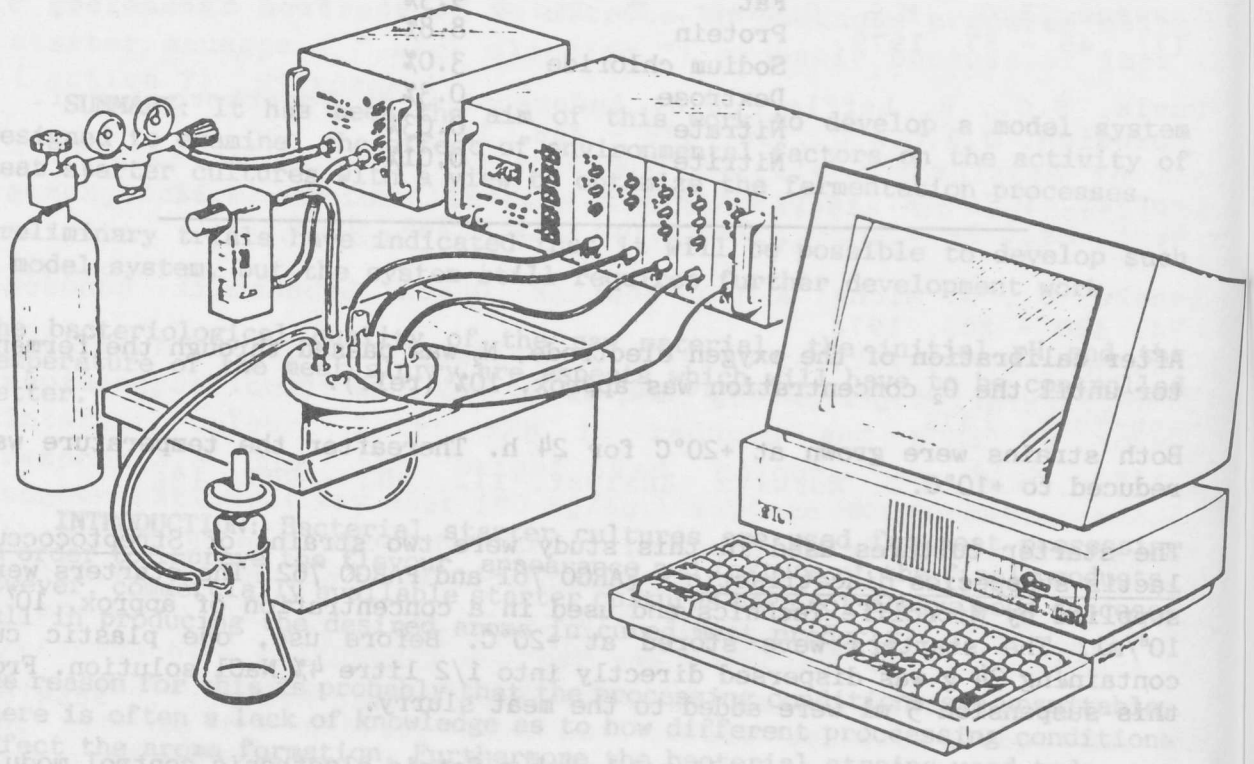
* Plate Count Agar (PCA) with 1% NaCl (5 days, 20°C)
* Agar without carbohydrates and with 1% NaCl (5 days, 30°C)
* Malt Agar (5 days, 20°C)

The model system is shown in figure 1.

RESULTS AND DISCUSSION: Results of the microbiological analyses on PCA and on Agar without carbohydrates are shown in figure 2.

Analyses on PCA of the pure cultures of the two strains before addition to the emulsion in both cases yielded counts of > 10^8 cfu/ml. The bacteriological quality of the meat before adding the starter cultures measured on PCA was 11,800 cfu/g meat when FARGO 761 was used, and 3,400 cfu/g when FARGO 762 was used.

Figure 1. Model system



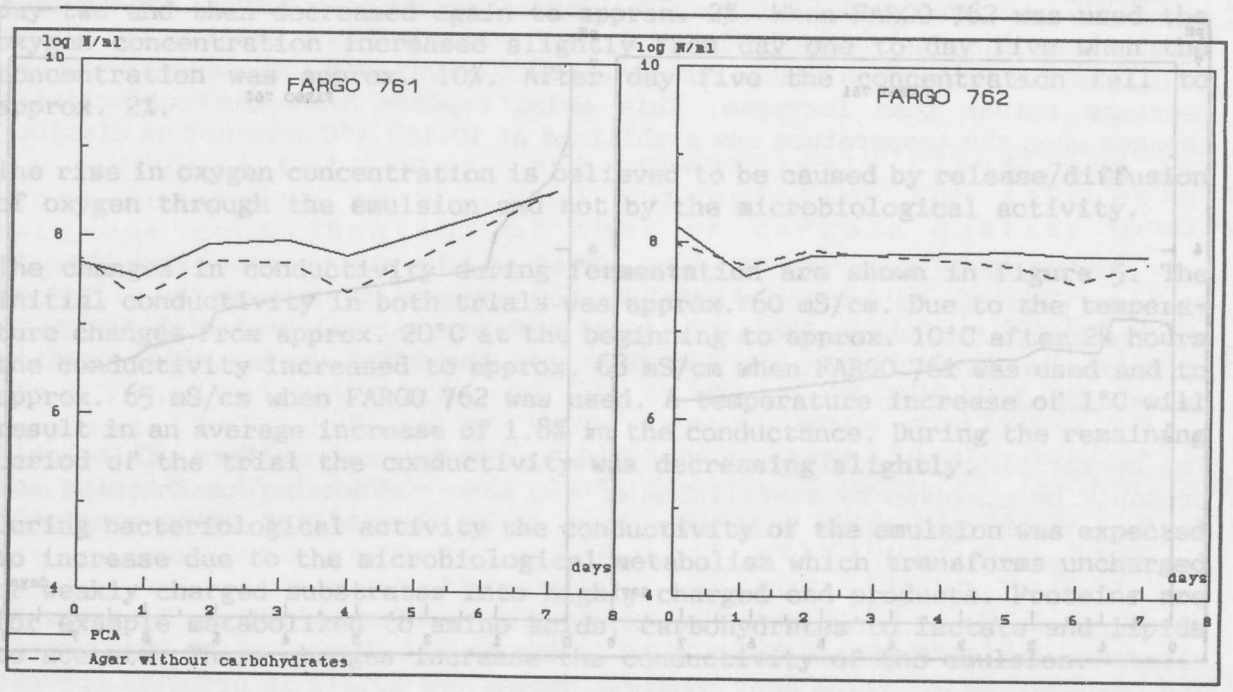
As it can be seen from the graphs the initial counts on both media were $6x10^7$ when FARGO 761 was used. The counts increase during the test period. The initial counts were 10^8 on both media when FARGO 762 was used, but the number of microorganisms decreased slightly during the test period.

As it can be seen from figure 2 the number of bacteria on PCA and on agar without carbohydrates were almost the same. This may be due to the fact that the starter cultures (strains of Streptococcus lactis subspecies diacetilactis) both are able to digest casein, which is one of the ingredients in the agar without carbohydrates.

The results of the microbiological analyses on Malt agar are not shown but in both trials the initial counts were < 100 cfu/ml. In the trial where FARGO 761 was used, the count after seven days was approx. 210,000 cfu/ml. When FARGO 762 was used the count on day two was approx. 6,000 cfu/ml and the number increased to approx. 280,000 cfu/ml on day seven.

The bacteriological counts of the raw meat were at an acceptable level. The high yeast counts in the emulsion will, however, be a disadvantage for the growth and the activity of the starter cultures.

Figure 2. Microbiological analyses on PCA and on Agar without carbohydrates.



The changes in pH during the fermentation are shown in figure 3. The initial pH in the meat slurry was higher when FARGO 762 was used (6.5) while at the end of the period the pH was almost the same independent of which starter culture was used. As a check of the pH electrode, the pH in the sample for bacteriological analyses was measured with a pHM63 digital pH meter immediately after the sample was collected. When measured off-line the pH was 5.5 after one day while it was 6.1 measured on-line. After two days the pH was 5.4 off-line and 5.8 on-line. On the 7th day the pH was almost the same according to both measuring techniques.

The difference in initial pH between the two trials could be caused by a heterogeneous emulsion or by insufficient mixing after chopping. Another problem which could interfere with the pH measurement is that the emulsion after a while separates into two phases - one mainly with fat and one mainly with water.

The changes in oxygen concentration (% rel.) during fermentation are shown in figure 4. As mentioned earlier the oxygen electrode was calibrated, the initial oxygen contentration (% rel.) would always be approx. 100%. After this calibration N_2 was passed through the fermentor until the O_2 concentration was approx. 10% (rel.) or 2% (abs.).

As it can be seen in the graphs the time used to bring down the oxygen concentration to approx. 10% differ between the two trials. In the first trial N_2 was blown through the emulsion for approx. 2 hours, while when FARGO 762 was used N_2 was blown through for approx. 7 hours.

Figure 3. Changes in pH during growth

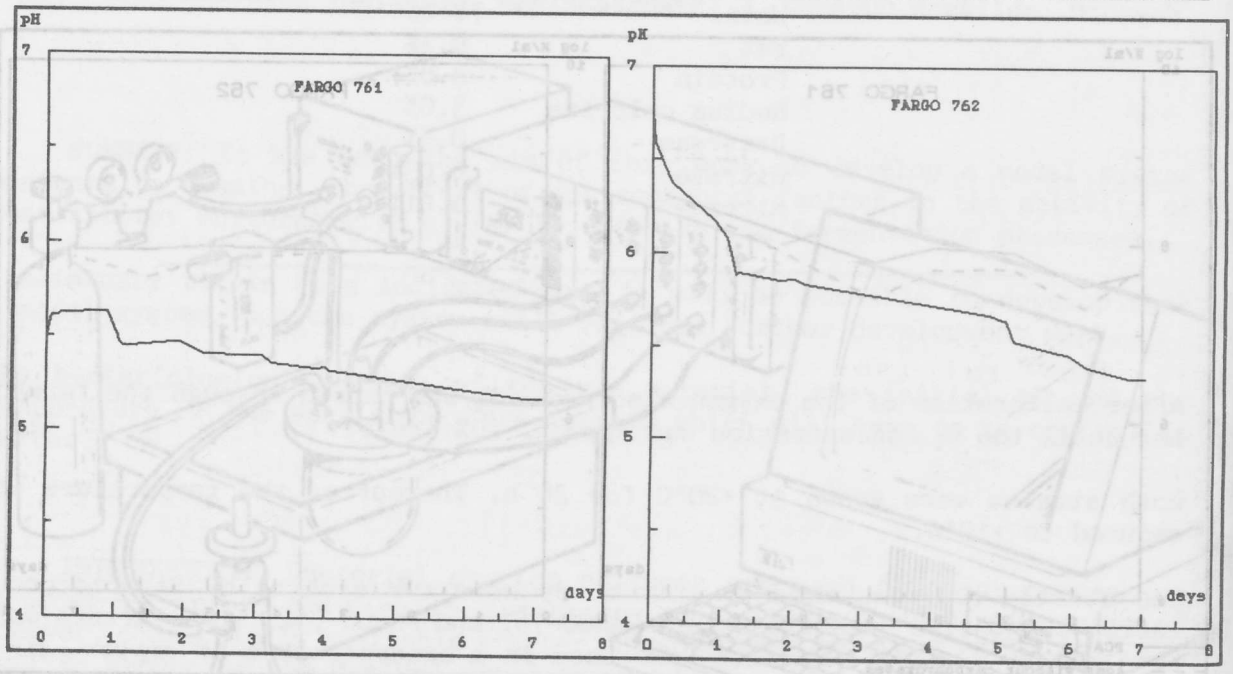
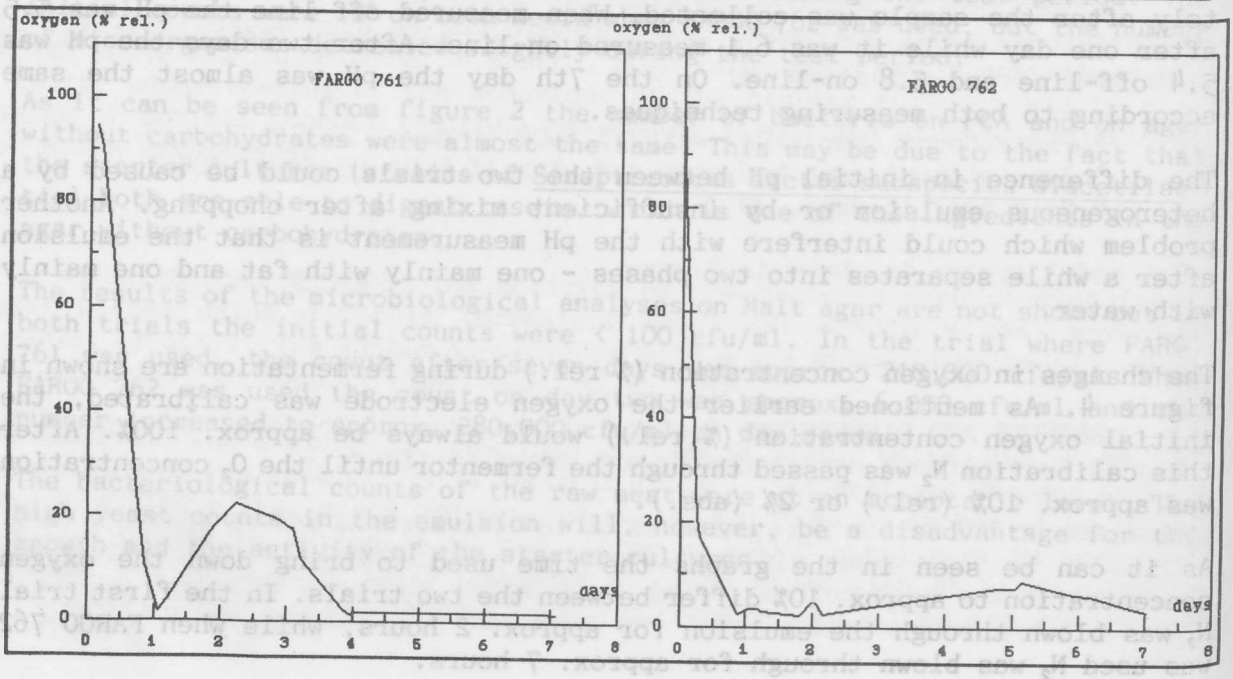


Figure 4. Changes in oxygen concentration during growth



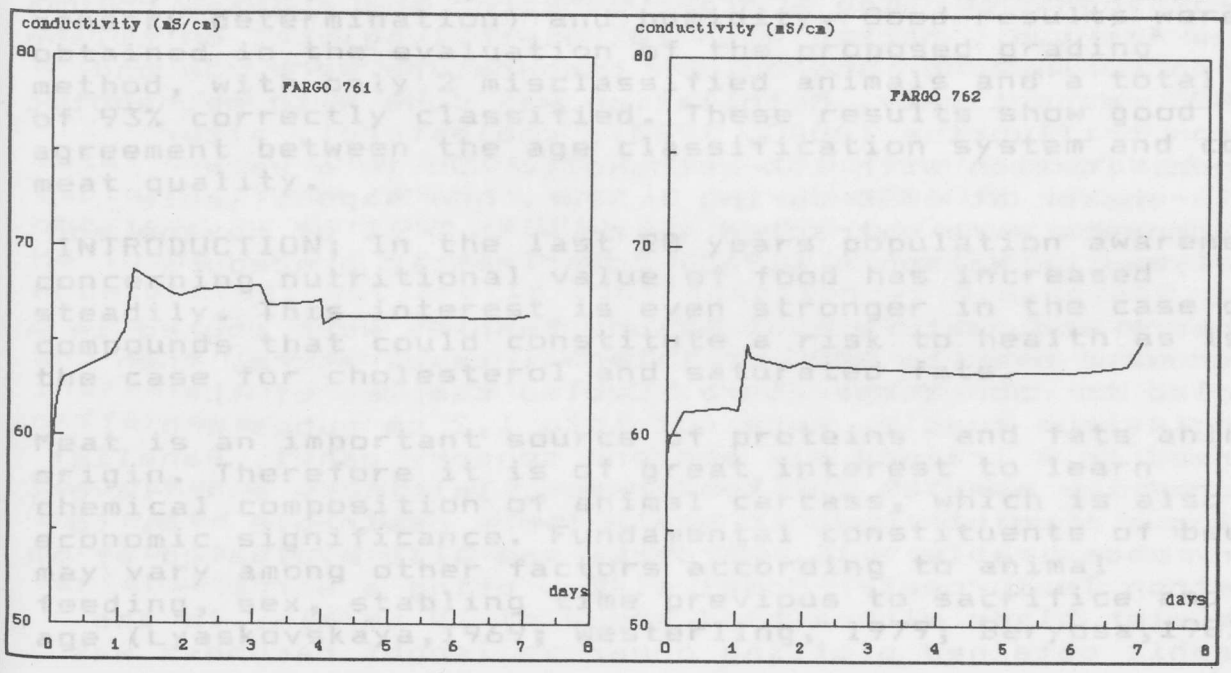
After one day the oxygen concentration was approx. 2% (rel.) in both trials. When FARGO 761 was used the oxygen concentration increased to approx. 20% on day two and then decreased again to approx. 2%. When FARGO 762 was used the oxygen concentration increased slightly from day one to day five when the concentration was approx. 10%. After day five the concentration fell to approx. 2%.

The rise in oxygen concentration is believed to be caused by release/diffusion of oxygen through the emulsion and not by the microbiological activity.

The changes in conductivity during fermentation are shown in figure 5. The initial conductivity in both trials was approx. 60 mS/cm. Due to the temperature changes from approx. 20°C at the beginning to approx. 10°C after 24 hours the conductivity increased to approx. 68 mS/cm when FARGO 761 was used and to approx. 65 mS/cm when FARGO 762 was used. A temperature increase of 1°C will result in an average increase of 1.8% in the conductance. During the remaining period of the trial the conductivity was decreasing slightly.

During bacteriological activity the conductivity of the emulsion was expected to increase due to the microbiological metabolism which transforms uncharged or weakly charged substrates into highly charged end products. Proteins are for example metabolized to amino acids, carbohydrates to lactate and lipids to acetate. These changes increase the conductivity of the emulsion.

Figure 5. Changes in conductivity during growth



Unfortunately the growth of some organisms, such as yeasts, does not result in large changes in the conductance. This may be due to the fact, that these organisms do not produce strongly ionized metabolites, but non-ionized metabolites such as ethanol. In addition, yeast has the ability to absorb ions from the suspension. Hence, under certain conditions the conductivity may decrease rather than increase. This could explain that the conductivity changes when the temperature was stabilized at 10.1±0.1°C were not as distinctive as hoped.

CONCLUSIONS: From all the data obtained, it can be concluded that it would be possible to develop a model system designed to examine the effect of environmental factors on the activity of meat starter cultures with a view to optimize the fermentation process. However, the model system still need to be developed further.

The bacteriological quality of the meat has to be more uniform. This could probably be achieved by sterilizing of the meat surface before chopping and mixing. These operations will then have to be carried out using extremely hygienic precautions.

Furthermore, the initial pH in the slurry has to be standardized in order to eliminate the differences found e.g. in the trials described in this paper. The temperature has to be kept constant during the trials in order to prevent a rise in conductivity caused by a temperature rise.