# STARTERCULTURE CHARACTERIZATION USING IMPEDIMETRIC PROCEDURES.

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## INTRODUCTION

Startercultures are widely used in the meat industry, especially in the production of sausages.
A problem concerning the use of starters in meat products is the lack of information about their metabolic activity, e.g. their ability to metabolize different sugars and their lipolytic and proteolytic activity, especially in the natural products. Investigating these factors in the products is very complicated and is also very timeconsuming.
Therefore it has been tried to make a model system, in which the startercultures can be characterized in a fast and easy way.
The observation of impedance variations due to microbial metabolism has been reported more than 80 years ago, but when performed with modern instrumentation, it requires relatively new procedures. Impedance instrumentation has been applied in different fields, for example in the enumeration of microorganisms in milk (Gnan and Luedecke 1982, Firstenberg-Eden and Tricarico 1983), in raw meat (Firstenberg-Eden 1983, Martins and Selby 1980, Bultke and Reuter 1984), in fish (Gibson and Ogden 1980), in vegetables (Hadley et al 1977) and for estimation of coliforms and Salmonella (Firstenberg-Eden and Klein 1983, Firstenberg-Eden et al 1983, Martins and Selby 1980).
Growth of microorganisms will cause an increase in both conductance and capacitance.
The changes in conductance are associated with the end products, which are the result of the microbial metabolism of the media.
Uncharged or weakly charged media are transformed into endproducts, with higher charges.
Carbohydrate are for example metabolized to lactate, lipids to acetate and proteins to aminoacids, which all increase the conductivity of the substrate. The metabolism also affects the capacitance, due to the polarization of the electrode. Firstenberg-Eden and Zindulis (1984) have found, that pH plays an important role in this polarization. Okibo et al (1985) have described an activity test for lactic acid bacteria, using pH and impedance instrumentation.
The changes recorded during the growth of microorganisms can be used in the characterization of their metabolism.
The present work specially focus the selection of appropriate media for impedance testing, since the selected media are very important for the quality of the impedance curves.

## MATERIALS AND METHODS

### Cultures:

The startercultures used in this study were *Lactobacillus plantarum* (L115), *Pediococcus acidilactici* (P120) and *Staphylococcus simulans* (M72). The starters were supplied by Lacto-Labo.

### Media used in impedimetric analysis:

1) A modified APT broth was prepared, consisting of Bacto yeast extract (7,5g), Bacto trypton (12,5 g), Tripotassium citrat (1,0g), $K_2HPO_4$ (5,0 g), $MnCl_2$ (0,14 g), $MgSO_4$ (0,8 g), $FeSO_4$ (0,04 g) and $H_2O$ (1000 ml). pH= 6,7
With the modified APT broth as basis, different series were made.

I) To 1000 ml of base medium, 5,0 g NaCl and 5,0 g of sugar were added. Each of the following sugars were used alone: glucose, sucrose, lactose and fructose.
II) As no I, except that the final concentration of sugar was 0,1 mol/l.
III) As no. II, but without NaCl.
IV) As no. I, but added 0,1 g yeast extract /100 ml broth.

2) BHI broth (Difco)
BHI broth with 0,1 g yeast extract/100 ml broth.

3) MRS broth (Difco)
MRS broth with 0,1 g yeast extract/100ml broth.

4) PCA (Difco) without agar.
PCA (Difco) without agar, with 0,1g yeast extract/100 ml broth.

5) M$_2$ broth : Meat extrakt (30,0 g), pepton (Difco) (20,0 g), $Na_2HPO_4$, $2H_2O$ (3,0 g), $MgSO_4,7H_2O$ (0,1g), $H_2O$ (1000 ml).pH=6,9
I) M$_2$ broth with 0,1 mol sugar/l. As sugars were used glucose and lactose.
II) As no. I, but added 0,1g yeast extract/ 100 ml broth.

6) Tributyrin broth : Beef extract (5,0 g), Bacto pepton (10,0 g), NaCl (5,0 g), Yeast extract (3,0g), Tributyrin ( 10 ml), $H_2O$ (1000 ml). pH=7,0
I) Tributyrin broth, with more yeast extract (0,1g/ 100 ml)

7) Gelatine broth: Gelatine (4,0 g), Beef extract (3,0g), pepton (5,0g), $H_2O$ (1000 ml).pH=7,0
I) Gelatine broth with a total of 120,0 g gelatine/l.
II) Gelatine broth with 0,1 g yeastextract /100 ml.

8) Milk powder media: 50 g of skim milk powder (Difco) was added to 450 ml of water.

I) Milk powder medium with 0,1 g yeast extract/100 ml.

Media used for traditional tests:

9) Tributyrin agar : As 6), but with 15 g agar.

10) Bromthymolblue agar : Meat extrakt (10,0 g), Bactopepton (10,0 g), Indicator suspension (12,0 g), agar (15,0 g), $H_2O$ (1000 ml).
The indikator suspension was made of 1,0 g of bromthymolblue, suspended in 25 ml 0,1N NaOH and 475 ml $H_2O$. pH (media)=7,4- 7,6.
Different sugars were added to the bromthymol-blue agar, eg. glucose, lactose, fructose or sucrose (20g sugar /l).
The substrates were sterilized at 115 °C for not more than 15 minutes.

11) Fehlhabers agar : Nutrient agar (Difco) (28,0 g), Skim milk powder (2,5 g), $CaCl_2$ (0,2 g), $H_2O$ (1000 ml). pH=7,4
Tributyrin (10,0 g).

Analysis:

All cultures were grown in APT broth (Difco) for 24 hours at 30°C. 1 ml of broth was then diluted at least a thousand times, to a final concentration not more than $10^{-6}$ CFU/ ml. This suspension was used as inoculum. For dilution, saline with 1% pepton was used.
1 ml of media was added into wells in the modules of the bactometer. The wells were inoculated with 0,1 ml of the above mentioned inocolum. The modules were incubated in the bactometer processing unit at 30°C. 2 different concentrations of inocolum was used. As control, uninoculated wells were used, plus wells inoculated with 0,1 ml of saline with 1% pepton.
For all trials, changes in conductance and capacitance were measured.

The cultures were spread on the surface of bromthymolblue agar with different sugars in order to investigate their ability to metabolize sugars, resulting in a pH decrease.
Also tests were made with round holes in the agar, containing the bacterial suspension. Prior to testing, the cultures were grown in APT (Difco) and in the modified APT broth without sugar.
All cultures were tested against tributyrin, by spreading the cultures on the surface of tributyrin agar. Again tests were made, using agar plates with wells, containing the bacterial suspension.
Fehlhabers agar was used, both to determine lipolytic and proteolytic colonies. The agarplates were developed with a saturated solution of $CuSO_4$ and afterwards with 0,08% acid fuchsin.
Clear bluegreen zones indicated lipolytic activity (Cu-salts of free fatty acids) and clear dark red zones indicate proteolysis. (This dye does not stain amino acids and smaller peptides).
All plates were incubated at 30°C. Acid production from the metabolism of sugar, changes the indicator, bromthymolblue to yellow, while negative colonies are blue. The tributyrin plates were examined for clearing zones.
pH in the wells were measured prior to testing, and the pH was measured in 50 ml inoculated APT broth, incubated at 30°C in the laboratory and tested simultaneusly with runs in the bactometer. pH was selected instead of the titration of acidity, because it was easier to measure during the testperiode.
Preliminary trials were made with MRS broth and BHI in different concentrations, inoculated with a mixture of Staphylococcus simulans (M72) and Lactobacillus plantarum (L115).The same trials were made with extracts of meat, in order to reveal, whether the impedimetric procedure can be used for the quantitative estimation of lactic acid bacteria in meat products.

The mixture of the two cultures was also incubated in a bottle in the laboratory, and each hour 0,1 ml suspension was spread on MRS agar (Difco) and PCA (Difco) in order to determine the concentration of the microorganisms.

## RESULTS AND DISCUSSION

Results on bromthymolblue agar and tributyrin agar are shown in table 1. Results were similar regardless whether spreading or wells were used, although the wells in most cases gave the most visible result. Based on a pH decrease in the media, milk powder media with yeast extract gave the best results when analysing sugar metabolism in the bactometer. Here a good correlation between pH and the change in impedance was found. In fig. 1. the change in pH is plotted versus conductance
A high correlation was found for the change in conductance and the pH change during the first 2 hours of incubation.
This result has also been reported in the work of Waes and Bossuyt (1984) and Okibo et al (1985).
Milkpowder is of course different to meat as a substrate for starter cultures developed for meat products. More suitable for the characterization of meat starters is MS broth with yeast extract. The capacity curves (See fig 2, 3 and 4) give a good picture of the sugar metabolism, although it was not possible directly to correlate the changes in capacitance to the changes in pH. Figures 2, 3 and 4 show the changes in capacity for each of the cultures : Pediococcus acidilactici (P120), Lactobacillus plantarum (L115) and Staphylococcus simulans (M72).

With regard to Pediococcus acidilactici (P120), the curves : M17-broth without sugar and M17-broth with lactose are almost parallel, which suggests, that lactose is not metabolized. If this is so, it is corresponding with the fact, that the bacteria are negative on bromthymol-blue agar plates.
Lactobacillus plantarum (L115) can metabolize other components than sugars, as the curve: M17 broth without sugar is quite steep. The metabolism of M17 broth with lactose gives a characteristic curve. The bromthymol-blue agar, containing lactose turned yellow very fast. The shape of the curve is possibly due to fast lactose metabolism.
Looking at Staphylococcus simulans, the curve showing the metabolism of M17 broth without sugar is almost horizontal, whereas the broth containing lactose, has 2 peaks, and again runs parallel with the M17 broth without sugar. Also here it is close to suggest, that lactose is the component first metabolised. It is worth mentioning, that the media showed horizontal baselines, when they were not inoculated or only inoculated with 0,1 ml saline with 0,1% pepton.

Lipolysis has been investigated using tributyrin as a substrate. Fig 5 shows the results for the tested cultures. It can be seen, that Staphylococcus simulans (M72) starts to metabolize a new component after about 38 hours incubation, while the other two cultures change to a stationary fase. The cultures were spread on quantitative tributyrin agar in the same concentration and after 40 hours incubation clearence zones were visible for in the M72 culture. Both Pediococcus acidilactici (P120) and Lactobacillus plantarum (L115) did not show any lipolytic activity against tributyrin. The use of tributyrin broth as medium for the bactometer shows the importance of using stable emulsions. If the tributyrin is not properly emulsified, very irregular curves can be observed. The tributyrin can be stablized using polyvinylalcohol, but more work is needed in this field. The use of natural fats in a stable emulsion will be the next steep in characterization of the startercultures and will undoubtedly give a more realistic picture of the lipolytic activity of the cultures, taking in account the work of Papon and Talon (1988) and Sugiura (1975).
With regard to the analysis for proteolytic activity, no media was found, which gave distinct results. More investigations are therefore needed. Especially it was a problem to get impedimetric curves of a good quality and the differences between the cultures were not distinct.

The impedimetric procedure may possibly also be used for differentating between Lactobaccilli and Staphylococcus in meat products, but this is still unconfirmed. Preliminary trials showed some favorization of Lactobacilli in the used media, but testing of more selective media is needed, before any conclusions can be made.
Thus the CFU calculated from the impedance curves, compared with the CFU, counted from the agarplates, show, that 10-20% of the Staphylococcus are detected with the bactometer.

## CONCLUSION

Impedance instrumentation may be a very useful tool in the analysis and characterization of meat starter cultures. The bactometer curves can be used to describe and visualize different characteristics and they may especially be suitable for comparing different cultures.
The method is fast and easy to perform. Much research is still needed in order to relate the results obtained with the bactometer, with results from the traditional tests.

Also it is necessary to develop more suitable media, to describe the startercultures and at the same time give impedance curves of a good quality.

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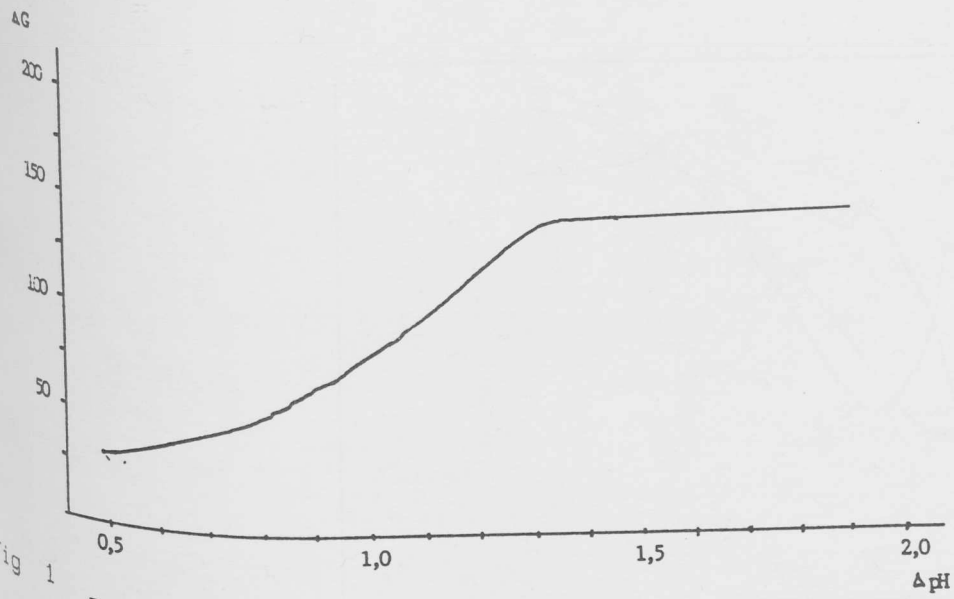


Fig 1

Relationship between pH change in milkpowder medium with yeastextract and change of conductance.

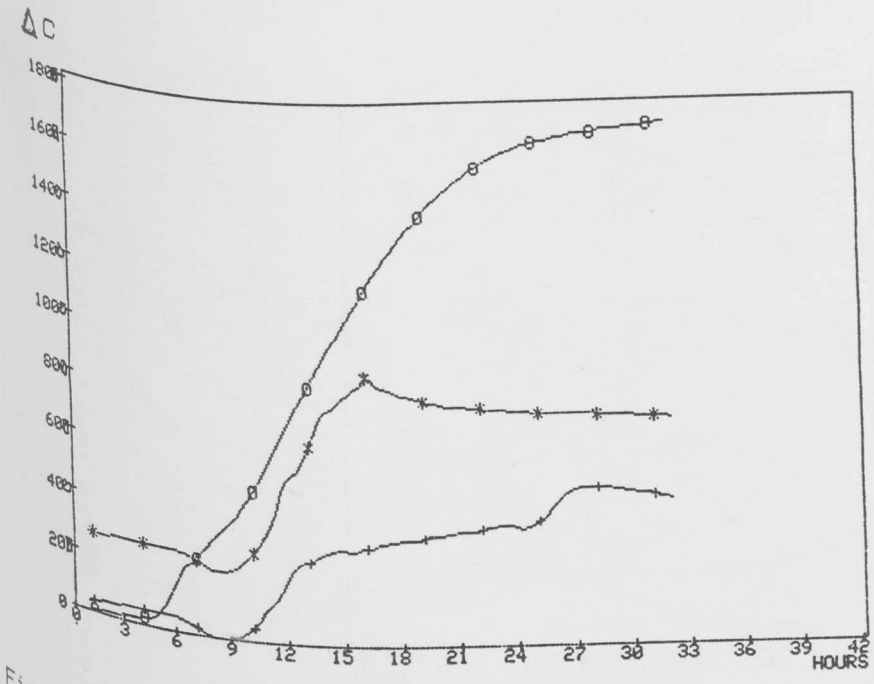


Fig 2

Capacitance changes caused by growth of Pediococcus acidilactici (P120) in $M_{ac}$-broth with yeastextract.

+ – $M_{ac}$-broth with yeastextract without sugar
* – – with lactose
0 – – with glucose

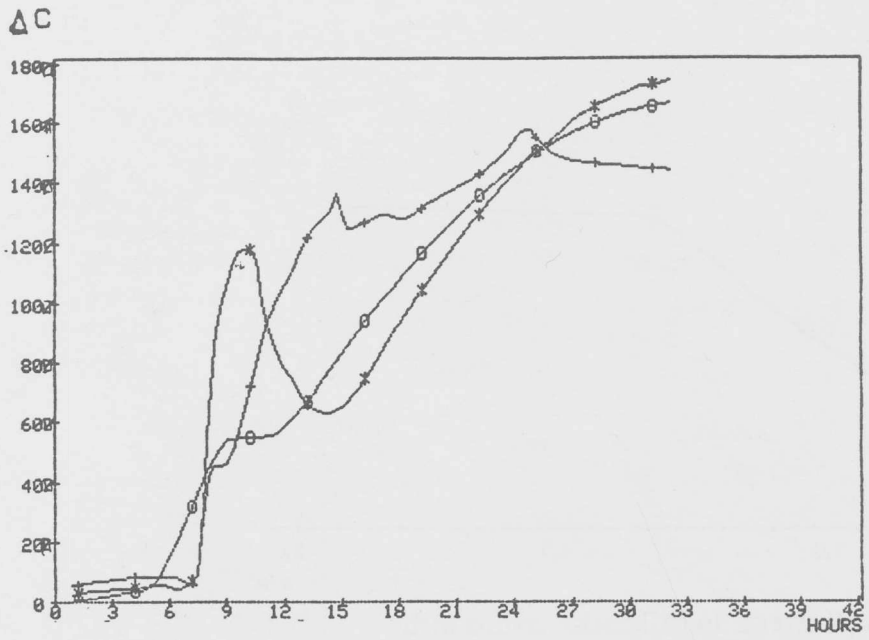


Fig 3 Capacitance changes caused by growth of Lactobacillus plantarum (L115) in M$_{17}$-broth with yeastextract.

\+ – M$_{17}$-broth with yeastextract without sugar
* – – with lactose
0 – – with glucose

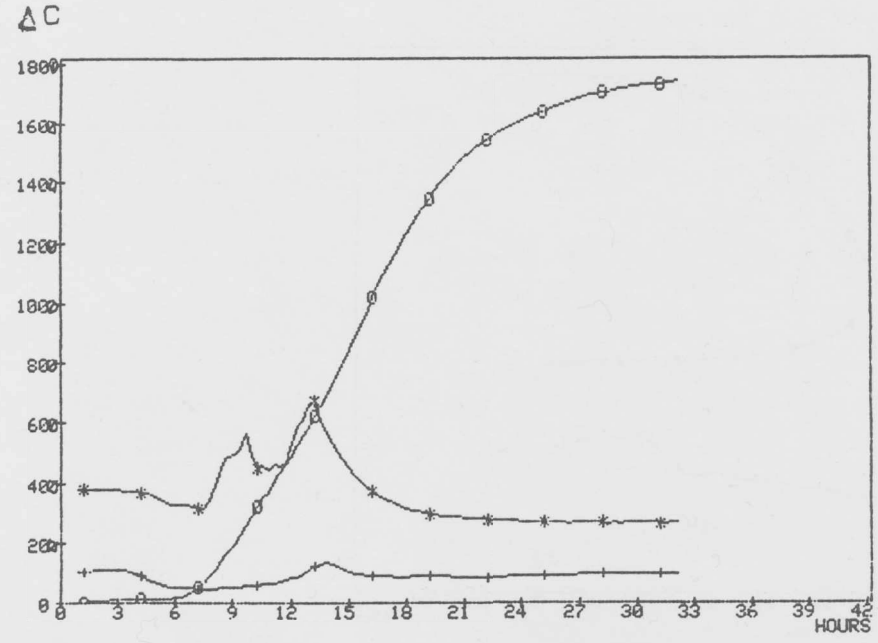


Fig 4 Capacitance changes caused by growth of Staphylococcus simulans (M72) in M$_{17}$-broth with yeastextract.

\+ – M$_{17}$-broth with yeastextract without sugar
* – – with lactose
0 – – with glucose

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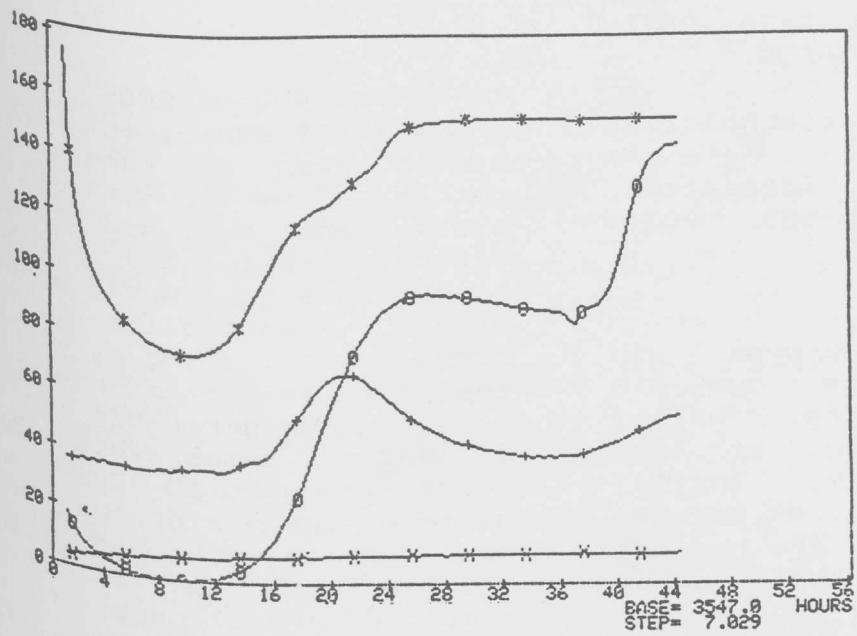


Fig 5

Capacitance curves caused by growth of Pediococcus acidilactici (P120), Lactobacillus plantarum (L115) and Staphylococcus simulans (M72) in tributyrin.

\+ – Pediococcus acidilactici (P120)

* – Lactobacillus plantarum (L115)

0 – Staphylococcus simulans (M72)

X – Control, tributyrin inoculated with saline with 0,1 % pepton

| Culture | glucose | lactose | sucrose | fructose | lipo-lysis | proteo-lysis |
|---|---|---|---|---|---|---|
| L.plantarum (L115) | + | + | + | + | – | – |
| P.acidilactici (P120) | + | – | + | + | – | – |
| S.simulans (M72) | + | + | – | + | + | + |

Table 1. Results on bromthymolagar, tributyrinagar and quantitative tributyrinagar.