# The Relationship between a failed fermentation process and the properties of a starter culture

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SUMMARY: The fermentation activity of a commercial starter culture derived from *Pediococcus pentosaceus* was compared with the origin strain of the starter culture (*P. pentosaceus* SMRICC 178). The ability to decrease pH was measured during incubation of meat-fat mixtures containing glucose, curing salt and starter culture. The starter culture's ability to decrease pH was not affected by the bacteriological quality of the raw meat used. The ability to decrease pH was affected by the concentration of the starter culture, a concentration of 6 log CFU/g mixture decreased pH by 0.3 units and a concentration of 8 log CFU/g decreased pH by 1.1 units over 6 days at 25°C. The starter culture proved to be less salt tolerant than *P. pentosaceus* SMRICC 178. At a curing salt concentration of 2.5%, the starter culture (7 log CFU/g) over 6 days at 25°C decreased pH from 5.6 to 5.1, while *P. pentosaceus* SMRICC 178 under the same conditions decreased pH to 4.7.

INTRODUCTION: The processing of fermented sausages includes several different steps: formulation, fermentation, smoking and drying (ROCA and INCZE,1990). In Sweden processing is usually based on a rapid fermentation (1-3 days) and the drying period is either short or omitted completely (ERIKSSON, 1961). In order to achieve a rapid and reproducible pH reduction, the use of an active starter culture is necessary. By using frozen bacteria culture concentrates, the pH reduction rate during the fermentation period may be increased, compared to using lyophilized cultures (EVERSON *et al.*, 1970).The efficiency of the starter culture's ability to decrease pH is based on growth rate, ability to form required enzymes and acid forming potential. A rapid decrease in pH prevents the contamination flora from developing (VIGNOLO et al. 1989, SCHILLINGER and LÜCKE, 1990).

In spite of the use of a frozen commercial starter culture (*P. pentosaceus*), process failure occurred repeatedly at a processing plant; the pH did not decrease as expected. The aim of this investigation was to determine:1) how the ability of the starter culture to decrease pH was affected by the bacteriological quality of the raw meat, the initial concentrations of starter culture bacteria and the curing salt concentration; 2) whether the characteristics of the starter bacterium differed from the ones of the starter culture strain origin, maintained in a culture collection.

MATERIALS AND METHODS: The commercial starter culture investigated was produced in 1989. It contained about $10^{10}$ Colony Forming Units (CFU) *Pediococcus pentosaceus*/ml. The starter culture is distributed as a deep-frozen suspension. *Pediococcus pentosaceus* SMRICC 178 (Swedish Meat Research Institute Culture Collection), the origin strain of the starter culture was also studied.

The starter culture's ability to decrease pH was determined in 0.5 to 1.0 kg mixtures of beef meat and pork fat (75:25). Before mixing in a food processor, the meat was minced and the fat was cut into pieces (2 cm x 2 cm). To the meat-fat mixtures, 0.5, 1.0, 1.5, 2.0 or 2.5% (w/w) curing salt (sodiumchloride containing 0.6% sodiumnitrite) and 1% (w/w) glucose were added.The raw meat used was either 1)fresh, 2)stored for 6 days at 8°C or 3) cured (2.5% w/w curing salt) and stored for 6 days at 8°C. Thawed starter culture was added to a concentration of 6, 7, 8 or 9 log CFU/g meat-fat mixture. *P. pentosaceus* SMRICC 178 strain was precultured twice in APT-broth (BBL 10918; Becton Dickinson and Co., Cockeysville, England) for 24 h at 25°C and added to a concentration of 6 or 7 log CFU/g. Both bacteria were diluted in sterile $H_2O$. The meat-fat mixtures were incubated at 25°C for 4-6 days. pH was measured daily (pH electrode, no. 5800 A; Scott-Geräte GmBH, Hofheim, Germany).

From the meat-fat mixtures, a 30 g sample was taken out and homogenized with 270 ml of physiological saline solution (plus 0.1 % pepton). Using the poor plates technique, total bacterial count (Tryptone Yeast Extract agar, Oxoid CM 127; Basingstoke, England; 3 days, 25°C), lactic acid bacteria (MRS, Oxoid CM 361, pH 6.2; 3 days, 25°C) *Brochotrix thermosphacta* (STAA, GARDNER, 1966;

2 days, 22°C) and *Enterobacteriaceae* (Violet Red Bile Glucose agar, Oxoid CM 485; 1 day, 37°C) were estimated. *Pediococcus* spp. were quantified by spreading 0.1 ml homogenised sample on a hydrophobic filter (ISO-GRID 0.45 um, QA Laboratories Limited, Toronto Canada) placed on Modified MRS-agar (HOLLEY and MILLARD,1988). After anaerobic incubation (Anaerobic System, Gaspack; BBL) for 3 days at 25°C the filter was subsequently transferred to a filter paper (Munktell's filter paper no. 3; Stora, Grycksbo, Sweden) which had been premoistened with a 0.4% bromcreosol-solution. After 60 seconds turquoise colonies were counted as pediococci.

The results were statistically evaluated using analysis of variance (SYSTAT software; SYSTAT Inc., Evanston, IL, USA).

RESULTS AND DISCUSSION: Bacterial counts of the raw meat used are shown in Table 1. The total bacterial count varied between 4 and 9 log CFU/g. Lactic acid bacteria were detected in the stored meat and the cured & stored meat, but in substantially lower numbers than the total bacterial count. *Enterobacteriaceae* were only detected in the cured & stored meat. The number of *Brochothrix thermosphacta* was lower than the total bacterial count.

Table 1. The bacteriological status of the raw meat used.

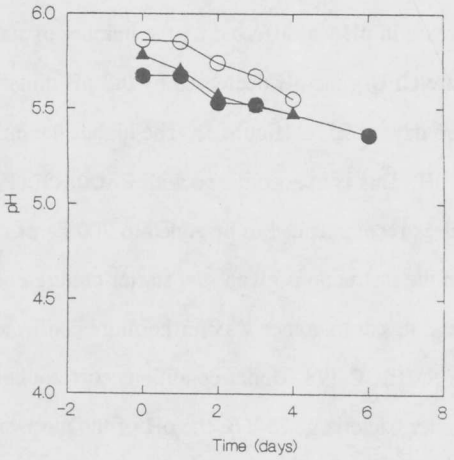
| Meat used | Total bacterial count (log CFU/g) | Lactic acid bacteria (log CFU/g) | *Enterobactericeae* (log CFU/g) | *Brochothrix thermosphacta* (log CFU/g) |
|---|---|---|---|---|
| Fresh | 4.1 | <1.0 | <1.0 | 1.7 |
| Stored1) | 5.4 | 2.2 | <1.0 | 3.5 |
| Cured and stored2) | 8.9 | 4.2 | 3.1 | >3.0 |

1) 6 days at 8°C, 2) 2.5 w/w % curing salt, 6 days at 8°C

Table 2. Analysis of variance showing effect of different bacteria initially present in the meat on the initial 24 hour pH (dpH) decrease during incubation at 25°C. The model used was dpH=constant+total bacterial count+*Enterobacteriaceae* count+lactic acid bacteria count. The concentration of the starter culture was 6 or 8 log CFU/g, ($r^2$=0.01, n=16).

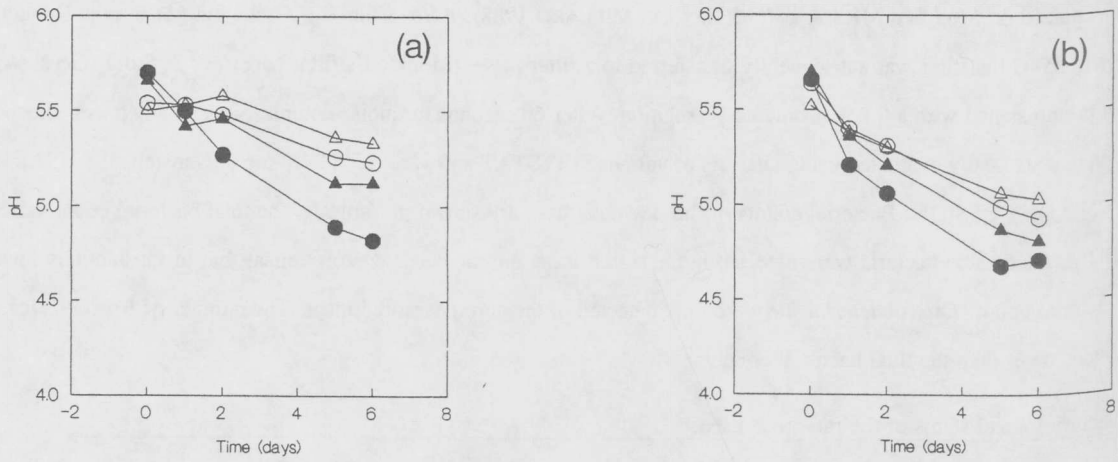
| Bacteria | P-value |
|---|---|
| Total bacterial count | 0.699 |
| *Enterobacteriaceae* | 0.755 |
| Lactic acid bacteria | 0.756 |

Figure 1. pH in meat-fat mixtures prepared from ● fresh meat, ○ stored meat (6 days at 8°C) or ▲ cured (2.5% w/w ) & stored meat (6 days at 8°C). The mixtures contained 1% (w/w) glucose and 2.5% (w/w) curing salt. The starter concentration was 6 log CFU/g and the incubation temperature was 25°C.




The starter culture's ability to decrease pH was not affected by the bacteriological quality of the raw meat used (figure 1). Analysis of variance confirmed that neither total bacterial count, number of lactic acid bacteria nor number of *Enterobacteriaceae* initially present in the raw meat affected the initial 24 hour decrease in pH (Table 2). The statistical model used (dpH=constant+total bacterial count+*Enterobacteriaceae* count+lactic acid bacteria count; dpH=change in 24 hour of incubation) very poorly described what the initial decrease in pH was affected by ($r^2$=0.01). Even though, it was indicated that the bacteriological status of the raw meat was not important for the initial pH-decrease, it is better for the product safety to suppress the contamination microflora. A rapid pH reduction inhibit bacteria such as coliforms and *Staphylococci* from development (VIGNOLO *et al.*,1989, SCHILLINGER and LÜCKE, 1990).

Figure 2. pH in meat-fat mixtures with starter culture concentration of (a) 6 log CFU/g or (b) 7 log CFU/g with different amounts of curing salt, ● 0.5, ▲ 1.0, ○ 1.5, △ 2.0 % (w/w) added. The meat-fat mixtures were made from fresh meat and contained 1% (w/w) glucose. The incubation temperature was 25°C.




The ability of the starter culture to decrease pH in meat-fat mixtures was affected by the curing salt concentration. In the presence of 2% curing salt and 6 log CFU starter culture bacteria/g the pH did not decrease below 5.3. Using the same concentration of bacteria, but at a curing salt level of 0.5%, the pH decreased to about 4.8 (figure 2a). When adding 7 log CFU starter bacteria/g meat-fat mixture, the pH decreased in the presence of 2% and 0.5% curing salt to 5.0 and 4.7, respectively (figure 2b). Thus, even at an initial concentration of 7 log CFU bacteria/g meat-fat mixture, the starter cultures studied were not able to decrease below pH 5 in the presence of 2% curing salt. A concentration of 2-2.5% (w/w) curing salt corresponds to approximately 5% salt in the waterphase. *Pediococcus pentosaceus* is able to grow in the presence of 8% NaCl (TEUBER and GEIS, 1981). The origin strain of the starter culture, *P. pentosaceus* SMRICC 178, is able to grow, at the minimum, in the presence of 5% salt. Thus, it is indicated that the starter culture bacteria had become less salt tolerant.

The decrease in pH was affected by the number of starter culture bacteria added to the meat-fat mixture. With the addition of 6.1 log starter culture CFU/g the pH decreased by 0.3 pH units in 6 days. As opposed to adding 8.0 log CFU/g when the pH decreased by 1.1 pH units in 6 days at 25°C (figure 3). The higher the initial concentration of starter culture in the meat-fat mixture; the faster the decrease in pH. This is in accordance with RACCACH (1986). The starter culture used contained about $10^{12}$ CFU *P. pentosaceus* per package and is recommended to be added to 200 kg of raw sausage mixture, giving $5 \times 10^6$ cells/g sausage mix. Thus, when used according to the instructions given, the starter culture does not reduce pH satisfactorily.

The change in salt tolerance was furthermore confirmed by a direct comparison between the starter culture and the origin strain *P. pentosaceus* SMRICC 178. Under conditions corresponding to the ones used in the processing of fermented sausages (2.5% curing salt, 7 log CFU starter bacteria/g; 25°C), the pH of the meat-fat mixture decreased by only 0.5 pH units in the presence of the starter culture over 5 days of incubation (figure 4). As opposed to, in the presence of *P. pentosaceus* SMRICC 178 when the corresponding decrease in pH was 0.9 units. Furthermore, the initial drop in pH was higher in the presence of *P. pentosaceus* SMRICC 178 than starter culture. The final pH after five days of fermentation was also affected, being pH 5.1 with starter culture and pH 4.7 with *P. pentosaceus* SMRICC 178. This decrease in salt tolerance by the starter culture explained the process failure. If the starter culture had contained the same salt tolerance as *P. pentosaceus* SMRICC 178, the process failure would probably not have occurred. Experience has proven that culture strains can lose some of their desired characteristics with time (NIINIVAARA *et al.*, 1964). It is therefore necessary to expose the starter culture as little as possible, during production, to an environment different to the one it was selected/developed for.

Figure 3. pH in meat-fat mixtures with different concentrations of starter culture , ● 6 log CFU/g, ○ 7 log CFU/g, ▲ 8 log CFU/g or △ 9 log CFU/g. The meat-fat mixtures were made using fresh raw meat, 1% (w/w) glucose and 2.5% (w/w) curing salt. The incubation temperature was 25°C.

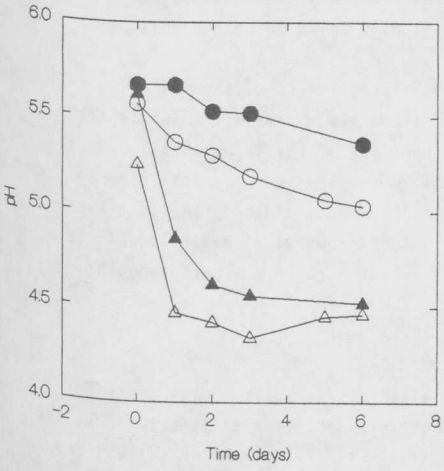
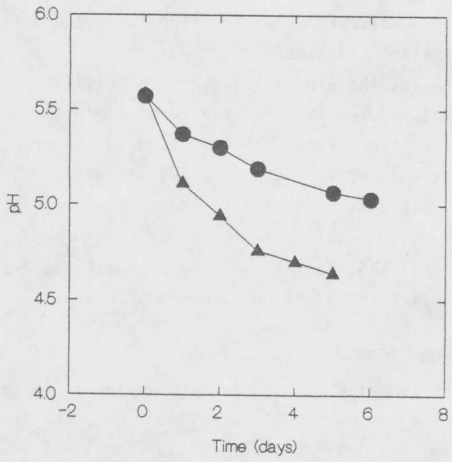



Figure 4. pH in meat-fat mixtures with 7 log CFU/g ● starter culture bacteria or ▲ *Pediococcus pentosaceus* SMRICC 178. Meat-fat mixtures were made from fresh meat and contained 1% (w/w) glucose and 2.5% (w/w) curing salt. The incubation temperature was 25°C.




CONCLUSIONS: The ability to decrease pH was not affected by the bacteriological quality of the raw meat used. The ability to decrease pH was affected by the concentration of starter bacteria added and the concentration of curing salt. Starter culture produced in 1989 was less salt tolerant than the origin strain of starter culture strain (*P. pentosaceus* SMRICC 178) maintained in the Swedish Meat Research Institute Culture Collection. Thus, during the commercial production of starter culture, the *P. pentosaceus* strain had become less salt tolerant.

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