Evaluation of Starter Cultures for Fermented Sausages in a Model System.

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Introduction.

The traditional production of fermented sausages has been and is very common throughout the world, but probably based on the experience from the dairy industries, the use of starter cultures for dry sausage manufacture has gained much popularity during the last 40 years or so. When using starter cultures in the production of fermented sausages, many of the uncertainties during manufacture are removed, such as failure in the development of the desired and suitable pH drop, and unintended growth of heterofermentative bacteria, causing gas pockets in the sausages, or colour defects. Some of the available starter cultures also comprise Micrococci, the growth of which are are claimed to improve the flavour.
The traditional Danish dry sausage manufacture is mainly based on an initial heavy cure with a subsequent drying and smoking, but with the changing consumer demand for less heavily salted products, the manufacture of true fermented dry sausages using starter cultures has gained a considerable footing.
In this connection it has been of interest to develop a laboratory method to evaluate the various starter cultures available on the Danish market.
In the system described here the purpose is primarily to evaluate starter cultures for their ability to ferment a carbohydrate, glucose, in a system which is as close to sausage fermentation as possible without having to produce a batch of fermented sausages each time.
It was also considered important that the model system contained meat to avoid too drastic a pH-drop within too short a time.
Paradis and Múngal (1980 a and b) have among others evaluated starter cultures for their ability to ferment selected carbohydrates. The system is based on fermentation in a broth and thus does not take the buffer capacity of meat into consideration such as is done in the present work. Further, the incorporation of meat in a model system makes it possible to get some idea about the length of the lag phase, especially of course if the test is carried out at the temperatures normally used during fermentation of sausages.

Materials and methods.

The model system.

At first it was tried to use a meat/fat slurry, so that the system represented as closely as possible the meat and fat ingredients of a sausage mix to be fermented, but a high fat content - approximately 30 per cent in the slurry - also resulted in difficulties in the pH readings. Therefore it was decided to continue with a meat slurry consisting of lean meat only.
Before the lean meat (pig meat) was chopped, it was scalded for 30 seconds in hot water at above 95°C. It was then minced in a pre-sterilized mincer. In this way it was possible to ensure that no accidental heavy bacterial contamination would interfere with the starter culture to be tested.
The final model system comprised the following ingredients: 50 g pre-scalded minced, lean pig meat, 50 g distilled water, 5 g/sodium chloride, 10 mg sodium nitrite and 1 g glucose. The addition of salts results in a brine concentration which is very close to the normal brine concentration in a sausage mix prior to drying.
Since it is of interest to examine the fermentation ability at temperatures close to those used during sausage drying, the tests were run at 15°, 20° and 25°C.

The starter cultures.

The following starter cultures were evaluated:

Laboratory Number	Organism(s)
1	L. plantarum
2	Micrococci spp & L. plantarum
3	M. aurant. & L. plantarum
4	M. aurant. & L. plantarum
5	Micrococci spp. & L. plantarum
6	P. cereviciae
7	P. cereviciae & Micrococci spp.
8	P. cerevisiae

All starter cultures were enumerated before use. In all cases the recommended amounts of starter cultures to be added were used, i.e. equivalent to initial numbers of approximately 10^6 cells per g sausage mix, except in the runs where this is indicated.
For enumeration of the cultures were used APT for total counts, PCA plus 8 per cent sodium chloride for enumeration of Micrococcus, and Rogosa agar for Lactobacillus and Pediococcus.
In further experiments with actual sausage fermentation, where mixtures of L. plantarum and Pediococcus were used it had been wished to be able to divide these two species on an indicative substrate. Since this posed certain difficulties, a special medium was developed which enables an easy distinction between the two species.

After some preliminary tests the final medium was made up as a modification of a Rogosa agar, where glucose is substituted with mannitol, and to which is added 1 g of thalleus acetate per liter. After sterilization of the substrate, 10 ml of a 1 per cent triphenyl tetrazolium chloride solution are added. When surface plating on this media, L. plantarum forms red-violet colonies, whereas colonies of P. cerevisiae are white. It is essential to incubate the plates aerobically, and on overcrowded plates it is very difficult to distinguish between the two species.

Results and Discussion.

The results of the pH measurements during runs in the model system over a period of up to 10 days are shown in tables 1, 2 and 3 for tests carried out at 15°, 20° and 25° respectively. It will be noted that in practically all cases there is a initial pH increase. This is probably due to the formation of alkaline metabolites from the natural flora of the meat slurry and the meat itself in combination with that the added starter cultures have a lag-phase during which they have to adapt to the sausage mix environment. For this reason they initially produce insufficient lactic acid to compensate for the pH increase. This observation has been reported previously by several other authors, e.g. during a symposium on sausage fermentation in Helsinki (1972).

Starter culture	pH at start	pH max.	pH min.	pH start - pH min.	pH max.- pH min.
1	5.56	5.67	4.88	0.68	0.79
2	5.56	5.61	4.90	0.66	0.71
3	5.56	5.68	4.92	0.64	0.76
4	5.56	5.68	4.80	0.76	0.88
5	5.56	5.69	4.96	0.60	0.73
6	5.56	5.72	5.20	0.36	0.52
7	5.56	5.69	5.24	0.32	0.45
8	5.56	5.65	5.15	0.41	0.50
Control	5.56	5.70	5.47	0.09	0.23

Table 1. Development of pH in the model system at 15°C.

Starter culture	pH at start	pH max.	pH min.	pH start - pH min.	pH max - pH min.
1	5.40	5.56	4.41	0.99	1.15
2	5.40	5.56	4.26	1.14	1.30
3	5.40	5.56	4.25	1.15	1.31
4	5.52	5.53	4.38	1.14	1.15
5	5.46	5.61	4.29	1.17	1.32
6	5.40	5.56	5.00	0.40	0.56
7	5.40	5.53	4.99	0.41	0.54
8	5.40	5.47	4.99	0.41	0.48
Control	5.40	5.59	5.09	0.31	0.50

Table 2. Development of pH in the model system at 20°C.

Starter culture	pH at start	pH max.	pH min.	pH start - pH min.	pH. max. pH min.
1	5.69	5.73	4.37	1.32	1.36
2	5.67	5.70	4.24	1.43	1.46
3	5.48	5.50	4.13	1.35	1.37
4	5.66	5.71	4.28	1.38	1.43
5	5.69	5.70	4.32	1.37	1.38
6	5.71	5.80	5.13	0.58	0.67
7	5.50	5.50	4.95	0.55	0.55
8	5.48	5.54	4.97	0.51	0.57
Control	5.46	5.62	5.12	0.34	0.50

Table 3. Development of pH in the model system at 25°C.

Most starter cultures used have a growth optimum at 30° to 35°C. From the results it will be seen that within the interval from 25° to 15°C, which is the actual temperature interval used for fermentation of sausages there is a decline in acid production.
The tables also illustrate how the different starter cultures influence the total pH decrease in the model system.
As mentioned above, for the tests the amount of starter culture recommended by the manufacturers were used. It was also investigated how higher concentrationsof starter cultures would affect the pH development. Table 4 shows that if the concentration of a starter culture is increased, this appears to remove the initial increase in pH, and thus presumably minimizes the risk of faulty fermentation, but the end-pH is essentially the same.
The repeatability of runs using the model system is illustrated in table 5. The results show that the reproducibility of the system is also acceptable.

Starter culture	pH at start	pH max.	pH min.	pH start - pH min.	pH max. - pH min.
1.	5.69	5.73	4.37	1.32	1.36
1. double concentration	5.65	5.65	4.38	1.27	1.27
6.	5.71	5.80	5.13	0.58	0.67
6. 10 x normal concentration	5.69	5.69	5.10	0.59	0.59

Table 4. Development of pH in the model system at 25°C using increased concentrations of starter cultures.

Starter culture	pH at start	pH max.	pH min.	pH start - pH min.	pH max. - pH min.
1	5.69	5.73	4.37	1.32	1.36
1	5.49	5.52	4.17	1.32	1.35
2	5.67	5.70	4.24	1.43	1.46
2	5.48	5.56	4.19	1.29	1.37
4	5.66	5.71	4.28	1.38	1.43
4	4.48	5.59	4.17	1.31	1.42
5	5.69	5.70	4.32	1.37	1.38
5	5.49	5.52	4.15	1.34	1.38
6	5.71	5.80	5.13	0.58	0.67
6	5.48	5.56	4.96	0.52	0.60

Table 5. Repeatability of tests in the model system at 25°C using various starter cultures.

Concluding remarks.

Since it appears to be very difficult to evaluate properties of starter cultures for their ability for colour formation and flavour of the final product without undertaking actual test productions, which are costly and time consuming, it is recommended to screen the suitability of starter cultures by testing them for purity and for concentration. If a culture is claimed to comprise one strain, this does not present any problem, but in the case where a starter culture consists of e.g. Lactobacillus plantarum and Pediococcus cerevisiae, such a mixed culture may easily be evaluated on the modified MRS-thalleus-acetate tetrazolium agar (MRS-TlT) as described above, supplemented with an enumeration on a non-selective agar to check for purity. The further laboratory evaluation can then be made using the model system described previously. In this way an easy assessment of the principal function of the starter culture can be made showing its ability to develop a suitable pH-decrease during fermentation of dry sausage.

References:

Paradis, D.C. and Múngal, M. (a), Die Fleischwirtschaft (1980), 60, p. 1347.

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