5 : 1

Evaluation of Unconventional Carbohydrates and Enzymes for Fermented Sausages in a Model System

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Introduction

Fermented foods have gained much popularity over the past decennia. In the case of fermented sausages, this has opened not only for an inexpensive way of preservation, but in addition, fermented sausages offer an alternative range of products, as well as cured meat commodities which are less salty, an attribute which is also appreciated by today's consumer. Although it still is common to use "back-slopping" procedures in some regions instead of well defined bacterial starter cultures, it will always be normal practise - and necessary, since the rew material does not contain carbohydrates - to add some kind of carbohydrate to the sausage mix to ensure an efficient fermentation and a decrease in pH to well below 5 during manufacture.
In order to avoid too fast a pH-decrease, which is likely to cause a sharp acid taste instead of a tangy, but more rounded flavour, it has been proposed to use more high-molecular carbohydrates to the mix (Liepe, 1978)
Sometimes this leads to faulty productions though, because the pH-decrease takes place too slowly. If this happens, it is possible for the original contaminating bacterial flora to propagate and cause off-flavours. This is especially seen if heavily contaminated rew materials are used.
Lately, a number of alternative, inexpensive carbohydrates have also been made available and offered for the manufacture of meat products. These include various dextrins, partly hydrolysed starches or by-products from other food productions. An example of this is whey permeate. This compound is guaranteed to contain at least 80 per cent lactose.
Since carbohydrates like this are inexpensive, but may cause delayed pH-decrease when used for fermentation, it was attempted to try these materials in a model system, which besides a meat mix with a starter culture, salt and nitrite, also were added appropriate industrial enzymes.

Materials and Methods

Starter cultures.

The following cultures were used; they are all commercially available:

Culture code	Organism(s)
A	L. plantarum
B	Micrococcus spp., L. plantarum
C	M. auraticus and L. plantarum
D	P. cerevisiae
E	P. pentocace

All starter cultures were used in the concentrations recommended by the manufacturers,i.e. c.10^6 per gram mix.

Carbohydrates.

Whey permeate. According to the manufacturer's specification it has a composition as follows: lactose 82%,protein 4%, ash 9% and fat 0.2%
Maltodextrin, with a DE (dextrose equivalence) of 17 - 19. According to the manufacturer's specification it has a carbohydrate composition as follows: dextrose 4%, maltose 8%, and higher sugars 88%.

Enzymes.

Two commercially available enzymes were used. For whey permeate lactozym (NOVO), with an activity of 3000 LAU/g.
For maltodextrin amyloglucosidase(NOVO), with an activity of 150 AGU/g.

The model system.

This has earlier been described (Zeuthen and Gotfredsen, 1982). Essentially,it comprises a meat slurry which is added salt and nitrite in the concentrations normal for sausage mixes. The slurry is filled in Ehrlenmeyer flasks and added starter culture(s) as required.
In all cases 2% carbohydrate and the amount of the appropriate enzyme, as calculated to yield an optimal substrate/enzyme rate, were added.
Fermentations were carried out at 20^{o} and 25^{o}C, and pH was measured once a day during fermentation.

Sausage manufacture.

The use of a combination of unconventional carbohydrate enzymes and starter cultures for the manufacture of fermented dry sausages was also investigated. The series were essentially identical, except that series 1 was added 2% maltodextrin and amyloglucosidase NOVO, series 2 2% whey permeate and lactozym NOVO, and series 3, the control was added 1% dextrose and 1% glucose syrup. In all cases starter culture B, a combination of micrococci and L. plantarum was used.
The sausages were manufactured according to normal practise, using pork as the only meat ingredient. Drying loss and pH-decrease were followed over a 16 days period, after which the three series were evaluated.

Results and Discussion

In some preliminary tests with the model system the preferable time for adding the enzyme to the sausage mix in relation to adding the starter cultures was first determined. In runs reported here are shown results of fermentations, where the enzyme is added 24 hours before mixing all ingredients,but it wasfound that adding the enzyme and the carbohydrate separately did not offer any specific advantage with regards to the final pH. It was therefore decided to add all ingredients simultaneously, a procedure which also from a working and safety point of view was preferable.
The results of the pH measurements during fermentation runs of the model systems are shown in tables 1 to 4. The experiments show fermentation and pH-decrease in the slurries for 9 to 10 days with whey permeate or maltodextrin without or with enzyme added, at 20^{o} or 25^{o}C.
To enable comparisons between different runs the results show the differences between pH at the beginning and the end of a fermentation, as well as the maximum and minimum pH recordings. Earlier results (Zeuthen and Gotfredsen, 1982) have shown that the less pH increases during a run, the faster is the break-down of the carbohydrate used. The legend of the tables is as follows:

pH-start: pH in the slurry at the beginning of a run. pH-max: the maximum pH recorded during a run. pH-min: the minimum pH recorded during a run. pH(1): the pH-difference between pH-start and pH-min. pH(2): the pH-difference between pH-max and pH-min.

Table 1. pH during fermentation of maltodextrin at 20°C, without and with enzyme added.

Enzyme added / Culture	pH-start		pH-max		pH-min		pH(1)		pH(2)	
	-	+	-	+	-	+	-	+	-	+
A	6.3	6.5	6.5	6.5	5.3	4.8	1.0	1.7	1.2	1.7
B	6.3	6.5	6.5	6.5	4.8	4.4	1.5	2.1	1.7	2.1
C	6.0	6.5	6.1	6.5	4.9	4.8	1.1	1.7	1.2	1.7
D	6.1	6.5	6.1	6.5	4.9	5.1	1.2	1.4	1.2	1.4
E	6.1	6.5	6.2	6.5	5.0	4.9	1.1	1.6	1.2	1.6
Control	6.1	6.5	6.5	6.5	5.4	5.0	0.7	1.5	1.1	1.5

Table 2. pH during fermentation of maltodextrin at 25°C, without and with enzyme added.

Enzyme added / Culture	pH-start		pH-max		pH-min		pH(1)		pH(2)	
	-	+	-	+	-	+	-	+	-	+
A	6.3	6.5	6.5	6.5	4.8	4.9	1.5	1.6	1.7	1.6
B	6.3	6.5	6.5	6.5	4.6	4.5	1.7	2.0	1.9	2.0
C	6.1	6.5	6.1	6.5	5.0	4.5	1.1	2.0	1.1	2.0
D	6.1	6.3	6.1	6.3	5.0	4.9	1.1	1.4	1.1	1.4
E	6.1	6.5	6.1	6.5	5.0	4.8	1.1	1.7	1.1	1.7
Control	6.2	6.5	6.2	6.5	5.2	5.1	1.0	0.9	1.0	0.9

Table 3. pH during fermentation of whey permeate at 20°C, without and with enzyme added

Enzyme added / Culture	pH-start		pH-max		pH-min		pH(1)		pH(2)	
	-	+	-	+	-	+	-	+	-	+
A	6.0	5.9	6.1	6.0	5.5	5.2	0.5	0.7	0.6	0.8
B	6.0	6.0	6.0	6.0	5.4	5.3	0.6	0.7	0.6	0.7
C	5.9	5.9	6.0	5.9	5.4	5.3	0.5	0.6	0.6	0.6
D	5.8	6.0	5.9	6.0	5.6	5.4	0.2	0.6	0.3	0.6
E	5.8	6.0	5.8	6.0	5.5	5.3	0.3	0.7	0.3	0.7
Control	6.0	5.9	6.2	6.0	6.0	5.5	0.0	0.4	0.2	0.5

Table 4. pH during fermentation of whey permeate at 25°C, without or with enzyme added.

Enzyme added / Culture	pH-start		pH-max		pH-min		pH(1)		pH(2)	
	-	+	-	+	-	+	-	+	-	+
A	6.0	5.9	6.1	6.0	5.4	5.1	0.6	0.8	0.7	0.9
B	6.0	6.0	6.0	6.1	5.3	4.8	0.7	1.2	0.7	1.3
C	5.9	6.0	5.9	6.0	5.3	4.9	0.6	1.1	0.6	1.1
D	5.9	6.0	5.9	6.0	5.5	5.5	0.4	0.5	0.4	0.5
E	5.9	6.0	5.9	6.0	5.3	5.0	0.6	1.0	0.6	1.0
Control	5.9	5.9	6.3	6.0	5.8	5.5	0.1	0.4	0.5	0.5

The results show that starter cultures in all cases have an effect on the fermentation, regardless which carbohydrate is used, but that adding the appropriate enzymes in connection with a fermentation improves the pH-decrease. As could be expected, the overall pH-decrease is higher at 25°C than at 20°C. The results also show that, at least in the model system, maltodextrin with a certain initial content of glucose, apparantly is fermented more readily than whey permeate is. This can be observed whether or not enzymes are added.
The price of enzymes is negligble, £0.05 to £0.15 per 100 kg. of sausage mix.

Evaluation of measurements of the fermented sausages.
For all three series the drying loss was 16-18%, and at the end of the drying period the sausages were judged as acceptable, both with regard to texture, colour and flavour.
The development in pH in the treee series during the drying period are shown in figure 1. It will be seen that the pH-decrease is also very similar in all three series.

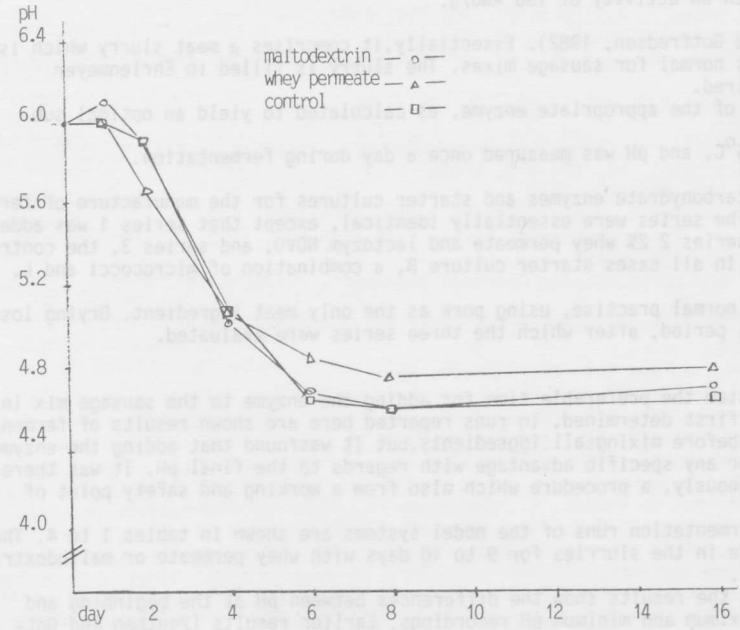

Figure 1. pH-development during drying of fermented sausages.

Concluding remarks.
From the experiments it appears that it is possible to combine the use of inexpensive carbohydrates with commerciably available enzymes for the manufacture of fermented sausages.

References:
Lieps,H.-U.:Zuckerverwendung bei der Rohwuust-Fabrikation, Die Fleischwirtschaft (1978),58, p.1781-82.

Zeuthen, P. and P. Gotfredsen, Evaluation of Starter Cultures for Fermented Sausages in a Model System, Proc. of the 28th EMMRW,Madrid 1982. Paper no. 4.32, p.257-59.

5:2

Selection of micrococci strains for their use as starter cultures for dry fermented sausages.

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The manufacturing of dry fermented sausages occurs in three main steps: mixing of ingredients, fermentation and ripening. During the first step, sugar, salt, nitrate and nitrite, spices and other seasonings are mixed with the meat and lard and filled into casings. During the fermentation two basic microbiological reactions occur simultaneously which influence each other and are directly dependent upon each other: the formation of nitric oxide by nitrate and nitrite reducing bacteria (Micrococcaceae) and the decrease of the pH via glycolysis by lactic acid bacteria. Both processes are two crucial points of sausages production. Several reviews have been published where the mechanisms and interactions of these processes are deeply analyzed (Palumbo and Smith, 1977; Liepe, 1982; Lücke, 1984).

During ripening the sausages lose a 20-40% of their original weigth and lipids and proteins are attacked by enzymes from meat and bacteria. It is in this step when the product adquires the charasteristic texture and flavour. The specific flavour of a given dry sausage is due to many compounds. Some of them are added to the sausages with the ingredients, others are formed without the participation of microorganisms (e.g. by autooxidation reactions) and others are generated as a result of microbial activities on carbohydrates, lipids and proteins. The products of carbohydrates fermentation (lactic acid and small amounts of short-chain fatty acids) seem to give to the semi-dry sausages their typical flavour (Lücke , 1984). Lipids are the source of many flavour compounds (aldehydes, ketones, short-chain fatty acids, etc.). Although some of these substances may be formed from aminoacids (Halvarson, 1973), the micrococci seem to play an important role (Cantoni et al., 1966; Demeyer et al., 1974) through their lipolytic activities (Cantoni et al., 1966; Demeyer et al., 1974). Proteins are also partially hidrolyzed with the consequent increase of non-protein nitrogen mainly as aminoacids (Stanculescu et al., 1970) which may be subsequently decarboxilated or deaminated (Dierick et al., 1974). It has been demonstrated that lactobacilli (Reuter, 1971) and micrococci (Moreno and Kosikowski, 1973) are endowed with proteolytic activities.

It may be concluded that organisms play an important role during the processing of dry fermented sausages. Thus, if it is expected to obtain a normalized final product it is necessary to control the many processes ocurring during dry sausages manufacturing. Despite the very large quantity of pork used in Spain for the production of dry fermented sausages (117.000 Tm), most of these products are manufactured by de "natural" method, i.e., using nitrate and low amounts of sugar but without adding starters. This study is an attempt to select several micrococci strains for their use as starter cultures. Lactobacilli strains have been selected previously (unpublished data).

EXPERIMENTAL METHODS.

Sampling.- Initially, the microbial evolution of two batches of sausages manufactured by the "natural" method was studied. The basic initial sausage mixture contained (% w/w): pork (75-80), lard (5-15), NaCl (3), $NaNO_2$ (0.15), KNO_3 (0.1), glucose (0.22), phosphates (0.17), dextrine (1), lactose (1), paprika (1.7), garlic (0.17), marjoram (0.01), ascorbate (0.04), colouring E-124 (0.008). Samples were taken at different ripening times. A Crison model Digit-501 pHmeter was used to measure the pH.

Microbial analyses.- Total viable counts (TVC) were determinated on Plate Count Agar (PCA); Micrococcaceae on Manitol-salt agar (MSA), both incubated at 30ºC for 3 days; lactobacilli were counted on double layer MRS agar (DIFCO) at pH 5,5 and incubated for 5 days at 30ºC. The separation between micrococci and staphylococci of organisms growing on MSA was made on a 20% of colonies chosen randomly and subjecting them to the O/F test (Evans y Kloos, 1972) and lysostaphin sensibility (Schleifer and Kloos, 1975).

The nitrate reduction test was performed qualitatively by the method of Harrigan y McCance (1976) and quantitatively by adding different amounts of KNO_3 to the medium. The nitrite generated was determined according to the ISO document 2918. The effect of incubation temperature on the growth of isolates was studied by inoculation of 0.1 ml of a 24 hr culture standardized to an absorbance of 0.2 at 600 nm into BHI broth (Difco). Test tubes were inoculated at several temperatures (11ºC, 19ºC, 25ºC and 32ºC) and the growth density measured (600 nm) at different intervals. The effect of a_w on the growth was performed on nutrient broth (Difco) adjusted at defined a_w with NaCl by the graphic interpolation method.

The preparation of exocellular and endocellular enzymatic extracts to study the lipo- and proteolytic activities of micrococci was made by the method described by Ordoñez and Ortiz-Apodaca (1977). The lipolytic activity was studied on agar plates using the basal medium and procedure described by the last authors. Different glycerides (Fluka and Sigma) and fat fraction extracted from pork with chloroform/methanol (2/1) (v/v) were used as substrates at the concentration of 0.06%.

The proteolytic activity was studied by measuring the liberated tyrosine from a mixture of enzymatic extract and substrates (1/3) (v/v). The reaction was stopped after 24 hr at 37ºC adding one volume of a 25% trichloracetic acid solution. A solution of 1% casein (Merck) in phosphate buffer 0.2 M pH 7.5 and other of sarcoplasmic protein extracts in 0.14 M KCl were used as substrates.

RESULTS AND DISCUSSION.

The flora evolution was similar to that observed in other sausages ripened by the "natural" method, consisting of a) a strong increase of lactobacilli during the first days to get stabilized afterwards at an aproximatively count of 10^8 u.f.c. g^{-1} of meat and b) a decrease of micrococci after they have reached the maximun level (about 10^6 u.f.c. g^{-1}) at the 3rd-5th day. Similary, the pH and a_w progresively decrease to achieve a steady state at the values of 4.5 and 0.9, respectively.

At different ripening periods, a 20% of colonies growing on MSA were selected. A total of 629 strains were chosen and submitted to the appropriate tests for their characterization. 27% of strains were characterized as Gram positive, catalasa positive cocci (Micrococcaceae); 71% as Gram positive, catalasa positive bacilli (most of them spore-forming rods) and the remainder 2% were Gram positive, catalase negative cocci, which were considered as belonging to the group D streptococci. The maximum recovery of Micrococcaceae were obtained after 5-7 days of ripening. At the end of the process, all isolates were characterized as bacilli. It is not the first time that a high percentage of bacilli have been detected in dry fermented sausages. They have also been reported by other authors (Palumbo et al., 1976). In fact, Palumbo and Smith (1977) indicated the importance of verifying the colony types growing on micrococci selective agars (salt-containing media) and not rely only on the supposed specificity of a given medium.

All Gram positive, catalase positive isolates were further submitted to O/F and lysostaphin test. Some

strains showed an unclear response to the O/F test, which has been already observed by other authors (Schleifer y Kloos, 1976; Fischer y Schleifer, 1980). Thus, only the 62 strains presenting a negative response to the lysostaphin were considered as micrococci. This test seems to be the most appropriate to separate micrococci from staphylococci (Gutiérrez et al., 1981) and it correlates well with the % G+C of the DNA (Schleifer and Kloos, 1975).

If it is attemped to prepare a starter for dry fermented sausages production, the micrococci used for it have necessarily to be nitrate reducers. Therefore, all micrococci isolated were submitted to the qualitative nitrate reduction test. 42 strains were endowed with this activity.

In order to know the suitability of these strains for their use as starter, the effect of temperature and a_w on growth and the quantitative nitrate reduction capability were studied. Fig. 1 shows the generation time ($\bar{g}$) of each micrococci strain at 11ºC, 19ºC, 25ºC and 32ºC. From this figure it may deduced that a) all strains presented the lowest $\bar{g}$ value at 32ºC, except the Micrococcus-3 which presented a $\bar{g}$ at 25ºC lower than at 32ºC; b) at 25ºC, 35 strains grew faster than at 19ºC but 7 showed a similar $\bar{g}$ value at both temperatures; c) at 11ºC, the growth is deeply delayed. However, the $\bar{g}$ values of three stains were lower than 9 hr, what means an acceptable growth in relation to the remainder strains because 78% of them showed a $\bar{g}$ higher than 40 hr.

The nitrate reduction occurs during the first 24 hr (Palumbo y Smith, 1977) and during this time the sausages are exposed to temperatures of 22-25ºC in order to favour the growth of lactic acid and nitrate reducer bacteria. Most of strains presented a $\bar{g}$ lower than 5 hr at 25ºC. Therefore, any of these can play this important role. Similar considerations may be taken from the results obtained in relation to the a_w studies (Fig. 2). When nitrate reduction is achieved, the a_w of the sausage used in this experience, was higher than 0,94. Thus, most of the strains can grow at this a_w being able, therefore, to reduce the nitrate at an acceptable rate.

Later, the nitrate reduction capability of all strains was quantitatively checked. For that, the basal medium was enriched with KNO_3 at a final concentration between 25 and 500 p/m. Two types of response were observed: a) Those strains (30) yielding a progresive accumulation of nitrite when the concentration of nitrate in the medium was up to 200 p/m aproximatively, but which did not increase the nitrite level when the nitrate concentration in the medium was higher. b) Those strains (12) producing an increase of nitrite proportional to the nitrate concentration in the medium until 500 p/m. The nitrate reduction in sausages is not optimally produced at low pH because it is a process NADH-dependent whose synthesis is inhibited at low pH values (Schiffner et al., 1978). When pH of sausage fall quickly below 5.4 no sufficient nitrate is reduced (Lücke, 1984). To avoid this, it is very important to restrict the rate of lactic acid formation so that the nitrate-reductase from micrococci remains active. Therefore, it is always desirable to use a starter composed by active nitrate reducing strains. Accordingly, in this study only those isolated (6 strains) which accumulated the highest levels of nitrites (more than 60 p/m from 500 p/m nitrate) were selected as potential starter.

Lipolytic and proteolytic activities were studied only in the 6 strains above mentioned. The lipolytic activity was evident by clear zones around the inoculation holes (6 mm diameter) previously made with a corkborer on the agar. The clear zones were visible 4-6 hr after inoculation of the agar plates and increased lineary with incubation time to get stabilized afterwards. The clearness of the areas around the holes reached different degrees. Both, the degree of clearness and the stabilization time of the lipolysis zones were dependent on the strain, substrate, pH and incubation temperature. The clearing zones were therefore subjectively classified on the basis of the transparency degree: (3+) for total transparency, (2+) medium transparency and (1+) representing a still opaque but with a defined area. The reason of the different clearness of lipolytic zones has been related to the solubility of the fatty acids or the partial glycerides liberated from the substrate by the enzymes which, in turn, depends on the positional specificity of lipases.

Experiments to study the lipolytic activity of the 6 micrococci strains finally selected were made at 11ºC, 20ºC and 32ºC and pH values of 5.5, 6, 6.5 and 7, but only the results obtained at 11ºC and at pH values of 5.5 and 6 are shown (Table 1) because these conditions are the nearest to those used during sausages ripening. Three strains (Micrococcus-10A, 10C and 11) did not show exocellular lipolytic activity and two of them presented a very poor endocellular one on monocaprilin and monoolein (Table 1). Two strains (Micrococcus 12 and 30) showed the highest lipolytic activity (Table 1) both exocellular and endocellular. The remainder strain (Micrococcus-3) lacked endocellular lipolytic activity and it presented a poor exocellular one, which only affected to the monoglycerides assayed.

The proteolytic activities of the six strains are shown in table 2. It may be seen that the proteolytic activity was higher against sarcoplasmic extract than against casein, except Micrococcus-11. The endocellular proteolytic activities of all strains were lower than the extracellular one and one strain (Micrococcus-10C) lacked this activity.

Table 3 shows a summary of results. From generation times, four strains (Micrococcus- 10A, 11, 12 and 30) seem to be the most appropriate as potential starter. All strains might be adequate according to the minimum a_w. The six strains displayed in the table were mainly selected according to their nitrate reduction activity. Micrococcus-10C is the less nitrate reducer strain. Micrococci play an important role in fermented dry sausages not only because they can reduce nitrates but also because they can decompose peroxides (Lücke, 1984). Furthemore, it is generally accepted that micrococci are the main organisms responsible for the lipolysis occuring during sausage ripening (Cantoni et al., 1966; Demeyer et al., 1974). Therefore, when it is attempted to prepare a starter, this last property must not be forgotten. According to the data shown in the table 3, only two strains (Micrococcus-12 and 30) presented a great exocellular lipolytic activity. In relation to the proteolytic activity only Micrococcus-3 could be discarded.

In conclusion, from the results described above, we only propose Micrococcus-12 and 30 as potential starters. What is now required is to test this strains through experiments involving the production of dry sausages at a pilot plant scale in order to establish the definitive technological aptitude of the two strains.

REFERENCES

CANTONI, C., MOLNAR, M.R., RENON, P. and GIOLITTI, G. (1966). Proc. 12th Europ. Meet. Meat Res. Work. Oslo, manuscript nº E4.

DEMEYER, D., HOOZEE, J. and MESDOM, H. (1974). J. Food Sci. 39, 293.

DIERICK, N., VANDEKERCKOVE, P. and DEMEYER, D. (1974). J. Food Sci. 39,301.

EVANS, J.B. and KLOOS, W.E. (1972). Appl. Microbiol. 23,326.

FISCHER, V. and SCHLEIFER, K.H. (1980). Fleischwirtschaft 60, 1046.

GUTIERREZ, L., MENES, I., GARCIA, M.L. and MORENO, B.. (1981). J. Appl. Bacteriol. 50, 541.

HALVARSON, H. (1973). J. Food Sci. 38, 310.

HARRIGAN, W.F. and McCANCE, M.E. (1976). "Laboratory Methods in Food and Dairy Microbiology". Academic Press. London.
LIEPE, H.U. (1983). Fleischwirtschaft (Spanish) 1, 34.
LUCKE, F.K. (1984). In "Microbiology of Food Fermentations". (B.J.B. Woos ed.). Applied Science Publishers. London.
MORENO, V. and KOSIKOWSKI, F.V. (1973). J. Dairy Sci. 56, 39.
ORDOÑEZ, J.A. and ORTIZ APODACA, M.J. (1977). Milchwissenschaft 33, 609.
PALUMBO, S.A., ZAIKA, L.L., KISSINGER, J.C. and SMITH, J.L. (1976). J. Food Sci. 41, 12.
PALUMBO, S.A. and SMITH, J.L. (1977). In "Enzymes in Food and Beverage Processing". ACS Symposium Series nº 47. (R.L. Ory and A.J. St. Angelo eds.). Departament of Agriculture. USA, p.279.
REUTER, G. (1971). Fleischwirtschaft 51, 67.
SCHIFFNER, E., HAGEDORN, W. and OPPEL, K. (1978). "Cultivos bacterianos en las industrias carnicas". Ed. Acribia. Zaragoza.
SCHLEIFER, K.H. and KLOOS, W.E. (1975). J. Clin. Microbiol. 1, 337.
SCHLEIFER, K.H. and KLOOS, W.E. (1976). Zbl. Bakt. Hyg. I. Ab. Suppl. 5, 3.
SCHLEIFER, K.H., KUPPER-BALTZ, R., FISCHER, U., FALLER, A. and ENDL, J. (1982). Int. J. Syst. Bacteriol. 32, 15.
STANCULESCU, C., SANDULESCU, C. and SBIRCEA, C. (1970). Proc. 16th Europ. Meet. Meat Res. Work. Varna, p. 1042.

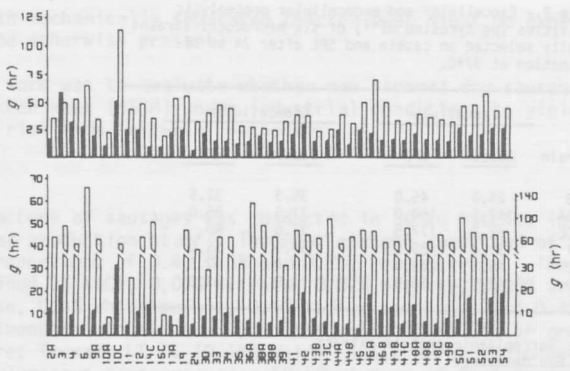

Fig. 1.- Generation times in BHI broth of nitrate reducing micrococci isolated from sausages. In the uppuer figure at 25ºC (white) and 32ºC (black) and in the lower one at 11ºC (white) and 19ºC (black).

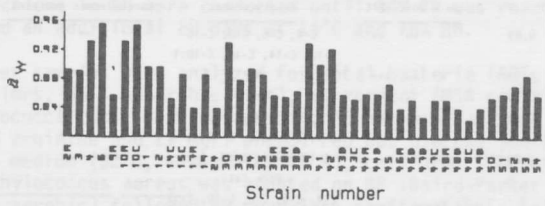

Fig. 2.- Minimum a_w in nutrient broth (adjusted with NaCl) for growth of nitrate reducing micrococci isolated from sausages.

Table 1.- Exocellular and endocellular lipolytic activities (cm^2 of clearness zone) of the six micrococci strains finally selected after 7 days of incubation at 11ºC, pH 5.5 and 6. For meaning of (+) see the text.

EXOCELLULAR

Strain	pH	MC	MO	MS	DP	TCL	TCP	TC	TM	TP	TO	TB	TL	PF
3	5.5	1.4(3+)	-	-	-	-	-	-	-	-	-	-	0.6(1+)	-
	6.0	-	0.5(1+)	-	-	-	-	-	-	-	-	-	-	-
12	5.5	-	1.4(1+)	1.4(1+)	0.9(1+)	2.7(1+)	1.5(3+)	2.0(1+)	0.7(1+)	-	0.7(1+)	3.1(3+)	0.8(1+)	-
	6.0	-	1.1(1+)	-	2.6(1+)	2.4(1+)	1.8(1+)	2.3(1+)	0.9(1+)	-	-	2.9(3+)	1.4(1+)	-
30	5.5	-	1.0(1+)	-	-	2.7(1+)	1.0(3+)	-	1.0(1+)	0.7(1+)	-	3.9(3+)	0.8(1+)	-
	6.0	-	1.2(1+)	1.3(1+)	1.4(1+)	1.4(1+)	2.7(3+)	5.7(3+)	1.4(1+)	0.7(1+)	-	5.0(3+)	1.0(1+)	1.0(1+)

ENDOCELLULAR

Strain	pH	MC	MO	TCL	TCP	TC	TO	TL	PF
10A	5.5	-	0.5(3+)	-	-	-	-	-	-
	6.0	-	0.3(2+)	-	-	-	-	-	-
10C	5.5	0.3(1+)	-	-	-	-	-	-	-
	6.0	0.4(1+)	-	-	-	-	-	-	-
12	5.5	0.5(3+)	0.7(1+)	-	-	-	-	-	-
	6.0	1.3(3+)	0.5(1+)	-	-	-	-	-	-
30	5.5	1.2(1+)	-	0.8(3+)	0.6(2+)	0.4(3+)	0.7(1+)	0.8(1+)	0.2(3+)
	6.0	-	1.6(2+)	1.3(3+)	1.2(3+)	0.5(3+)	1.0(1+)	1.0(1+)	0.4(1+)

Neither exocellular nor endocellular lipolytic activity was detected on tristearin.
Strains Micrococcus-3 and 11 did not show endocellular lipolytic activity.
No endocellular lipolytic activity was detected on MS, DP, TM TP and TB.
MC, Monocaprilin; MO, Monoolein; MS, Monostearin; DP, Dipalmitin; TCL, Tricaprilin; TCP, Tricaproin; TC, Tricaprin; TM, Trimiristirin; TP, Tripalmitin; TO, Triolein; TB, Tributirin; TL, Trilaurin.; PF, Pork fat. (-), Not detected.

Table 2.- Exocellular and endocellular proteolytic activities (µg tyrosine ml^{-1}) of six micrococci strains finally selected on casein and SPE after 24 hr of incubation at 37ºC.

	Exocellular		Endocellular	
Strain	Casein	SPE	Casein	SPE
3	25.0	45.0	35.3	32.5
10A	112.5	180.0	37.5	85.0
10C	92.5	177.5	37.5	ND
11	131.4	80.0	65.0	55.0
12	45.5	260.0	10.0	67.5
30	45.5	55.0	10.0	31.0

SPE: Sarcoplasmic protein extract.
ND: Not detected.

Table 3.- Main characteristics of the six micrococci strains finally selected.

Strain	Generation time		Minimum a_w for growth	Nitrate reduction activity			Exocellular lipolytic activity	Exocellular proteolytic activity
	Hours at			pH 6	20ºC	12ºC	Triglycerides	Sarcoplasmic proteins
	19ºC	11ºC						
3	22.5	49.0	0.89	++	++	++	C-4, C-6, C-8, C-10 C-12, C-14, C-16, C-18:1	++
10A	4.5	8.5	0.93	+	++	+++	ND	+++
10C	31.5	46.0	0.95	+	+	+	ND	+++
11	4.5	25.0	0.89	+++	+++	+	ND	++
12	10.0	44.0	0.89	+++	+++	+	C-4, C-6, C-8, C-10, C-12 C-14, C-16, C-18, C-18:1 Pork fat	+++
30	9.0	30.0	0.93	++	+++	++	C-4, C-6, C-8, C-10, C-12 C-14, C-16, C-18, C-18:1 Pork fat	++

ND: Not detected.
(+) poor, (++) medium and (+++) high activity

Commercial manufacture of raw ripened fermented sausages formulated with mechanically separated chicken meat

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Introduction

Both in the traditional and modern manufacture of raw ripened fermented sausages, successful processing personnel use only the highest quality of meats available. Deviation from this practice leads inevitably to production problems which affect not only the organoleptic quality of the product but also its safety (Acton and Dick, 1976; Terrell et al. 1978; Klettner and Baumgartner, 1980). One key to the production of safe sausages is the effective and rapid initial acidification of the meat mix. This can be brought about by use of the chemical acidulant glucono-delta lactone (GdL) or by the addition of lactic acid bacterial starter cultures. Either or both of these agents allow certain additional latitude in production methods although their use does not completely insure that pathogenic bacteria present in the meat will be eliminated during the curing-ripening period (Goepfert and Chung, 1970; Baran and Stevenson, 1975; Kovacs and Takas, 1979). Other factors also significantly influence both the efficacy of desired acidulation and pathogen survival. The interaction of available carbohydrate, NaCl, curing salts and temperatures of fermentation and drying are the more of important of these (Klettner and Baumgartner, 1980; Liepe, 1983).

In order to improve product selling price - production cost ratios a number of alternatives in manufacturing have been examined. For example, Joseph et al. (1978a,b) added mechanically deboned beef or soy protein fibre to dry sausage formulations. The inclusion of poultry meats in semi-dry and dry sausages has been the subject of a number of studies (Keller and Acton, 1974; Acton and Dick, 1975; Baran and Stevenson, 1975; Dhillon and Maurier, 1975; McMahon and Dawson, 1976; Raccach and Baker, 1979). In some of these products poultry meats were used at levels > 50% of the total (Acton and Dick, 1975). However in most of these studies sausages were cooked at ≥60°C before drying although Baran and Stevenson (1975) heat-treated to 46°C.

Since both Salmonella and Staphylococcus occur regularly in sampled poultry at levels usually higher than in pork and beef (McKinley and Avens 1981; Swaminathan et al. 1978; Bentley and Pettit 1982, Davidson et al. 1985) the formulation of sausages with mechanically separated chicken meat might be expected to contain these organisms in numbers higher than would be otherwise present.

The purpose of the present work was to evaluate whether raw ripened dry sausage could be formulated with mechanically separated chicken meat (MSCM) under industrial conditions to yield an organoleptically acceptable product without additional risk from bacterial pathogens.

Materials and Methods

Sausage preparation Manufacture of sausages was conducted in 50 kg batches in an inspected meat plant by regular production staff. The meat mixture consisted of pork, beef and beef back fat in the proportions of 0.4, 0.35 and 0.25, respectively. The spice-adjunct mixture contained 3% NaCl, 0.02% Nitrate, 0.02% nitrite, 0.05% erythorbate, 0.3% glucose, 0.4% lactose, 0.4% white pepper, 0.4% sodium caseinate and 0.4% modified wheat flour. Experimental batches were formulated with 0.44% GdL or one of two types of starter culture; Trumark LT II (0.1%) which consisted of Pediococcus pentosaceus plus Staphylococcus carnosus or a 50:50 mixture of Duploferment 66 with Duploferment "Spezial" (yielded 70% Staphylococcus carnosus plus 30% Lactobacillus plantarum). The latter starter was added at a rate of 0.05%. After lean meats were chopped, fat was added and this was followed by starter or GdL addition. Sausages were stuffed in 60 mm diameter fibrous casings and weighed about 0.5 kg each. Sausages were held 8 h at 10°C (70% RH) and placed in a smokehouse at 22°C (92% RH) for 17 h. The temperature was reduced to 20°C (90% RH) and after 10 h the RH was reduced to 88% for an additional 14 h. Temperature was then reduced to 18°C for 10.5 h and over the next week gradual reductions in RH were conducted until 84% RH was reached. Sausages were smoked and dried an additional 20 days at 16°C and 76% RH.

Microbial analyses Frozen samples were analyzed for total bacteria (APT, 32°C, 48 h) lactic acid bacteria, LAB (mrs, 32°C anaerobic 48 h), micrococci (MSA containing 3% NaCl, 32°C, 48 h aerobic), pediococci (MRS, 45°C, anaerobic, 48 h) lactobacilli, MRS containing 2% lactose plus 0.1M arginine and 25 mg/l phenol red but glucose and beef extract were not included in this medium (32°C, anaerobic, 48 h), coliforms (VRB with overlay, 35°C, 24 h aerobic), Staphylococcus aureus was counted on BP (Baird-Parker Agar) using spread-plates (35°C, 48 h aerobic) followed by coagulase confirmation. Salmonella

(presence/absence) were examined by the Canadian Health Protection Branch method (MFA-10) using Nutrient Broth, tetrathionate and selenite cysteine enrichments followed by examination of growth on Brilliant Green Sulfa Agar and Bismuth Sulfite Agar. Staphylococci and Salmonella were analyzed using fresh (unfrozen) samples. The identity of Salmonella was confirmed using the micro ID identification kit (Warner-Lambert Co., Morris Plains N.J., USA). Complete cross section slices were taken from sausages for bacterial analyses (11 g treated with a Stomacher 400 and diluted in 0.1% w/v peptone). All media used were obtained from Difo laboratories Ltd., Detroit, USA.

Chemical analyses Nitrate, nitrite, moisture, protein and fat analyses were conducted as described in AOAC (1984). Thiobarbituric acid was measured after Tarladgis et al. (1960) and water activity was assessed using a Novasina model TH2/TH1 (Nova Sina, Zurich) hygrometer. Mesurements of pH were made using electronic metering systems (Fisher Scientific, Ottawa, Canada: Knick Electronische Messgerate, Berlin).

Sensory and instrumental analysis Ten panelists trained in the evaluation of sausage quality evaluated treatments in four separate sessions. Panelists were asked to compare thickness of the outer layer, surface oiliness, greasiness, toughness and smoked flavour using a 15 cm descriptive analysis line scale. A randomized block design was utilized with all six treatments evaluated being present at each session.

A universal food rheometer (interfaced with a personal computer) was used to analyze cohesiveness and firmness of eight replicate cores for each treatment.

An analysis of variance was completed on the sensory and instrumental data and significant differences at the 5% level noted. Correlations between instrumental and sensory data were calculated.

Results and Discussion

Results from preliminary experiments indicated that levels of MSCM ≥20% yielded products of poor organoleptic quality. Subsequent work therefore centered on the use of up to 15% MSCM.

The rate of change in pH from the initial value of sausage meat mixes (pH 5.9 - 6.0) is presented in Table 1. The most rapid and dramatic drop in pH occurred within the first day in GdL treatments, and is similar to that reported in other work (Baumgartner et al. 1980; Gerigk and Gossling, 1981; Metaxopoulos et al. 1981b; Paneras and Bloukas, 1984; Petaja et al 1985). After day 1, the most rapid drop in pH occurred in samples to which Trumark starter had been added. The subsequent acidification of GdL-treated samples proceeded more slowly than in the other treatments. The pH decline in Duploferment samples took place slower than in the other treatments, taking 6 days to reach a value of 5.0, although this might not be considered abnormal in view of the low temperature used for fermentation (22°C). The use of mechanically separated meat did not have a significant influence upon either the initial or final pH, and values obtained were considered acceptable (Diebel et al. 1961; Debevere et al. 1975; Sirvio et al. 1977; Baumgartner et al. 1980) for this type of product.

Total and lactic bacteria in starter-inoculated treatments were close to their maximum numbers at day 4 and within two more days had reached peak levels. Lactic bacteria (MRS, 32°C) dominated the organisms recovered on APT agar in all treatments (Figs. 1, 2 and 3). In Duploferment and GdL treatments lactic bacteria were mainly lactobacilli. Results from other studies of raw ripened (≤24°C) sausages from a number of countries also indicated that lactobacilli were the dominant bacterial component of the microflora within a week of the completion of fermentation (Nurmi, 1966; Debevere et al. 1975; Niskanen and Nurmi, 1976; Hofmann and Scharner, 1980; Gerigk and Gossling, 1981; Metaxopoulos, et al. 1981b; Simonetti et al. 1983; Brankova et al. 1984a,b; Paneras and Bloukas, 1984; Gokalp and Ockerman, 1985) whether or not starter cultures were used.

Starter micrococci were initially dominant in Duploferment treatments (Fig. 1) but they did not grow during sausage maturation in any of the test batches. Nurmi, (1966) pointed out that micrococci do not have to grow in order to contribute to sausage quality. Nonetheless, micrococci have been reported to initially increase in numbers during fermentations at 24°C (Debevere et al. 1975; Sirvio et al. 1977; Gerigk and Gossling, 1981; Holley, 1986) reaching maxima of 10^5-10^7/g although weak growth has also been noted (Metaxopoulos et al. 1981a). In contrast, Simonetti et al. (1981) reported micrococci to reach levels of 10^9/g in Italian salami fermented for two days. It is unlikely that the micrococci of Duploferment and Trumark treatments peaked at levels higher than those noted in Figs. 1 and 2 since their growth on conventional media (Hugh and Leifson's carbohydrate and MSA) was extremely slow at 22°C.

In Trumark treatments, which were rapidly acidulated by the added pediococci, micrococci numbers decreased rapidly. This result was predictable on the basis of previous observations where micrococci were reported to be acid sensitive (Klettner and Baumgartner, 1980). Micrococci remained at a fairly low but stable level through the ripening process in GdL treatments. Pediococci grew initially in all batches but they did not form a significant portion of the microflora in Duploferment treatments (Fig. 1). In contrast, these organisms dominated in Trumark treatments (Fig. 2) where they were the major starter organism added. Pediococci formed the