

## SESSION 5 - MEAT PROCESSING: FERMENTED PRODUCTS

Fermentation of meat : an integrated process.

Demeyer<sup>1,2</sup>, D.I., Verplaetse<sup>1</sup>, A. and Monique Gistelincx<sup>1</sup>

<sup>1</sup> Onderzoekscentrum Voeding, Veeteelt en Vleestechnologie, R.U.G. Melle.

<sup>2</sup> Instituut Biotechnologie, V.U.B. Brussels.

### 1. Introduction.

Meat has great nutritional, commercial and social value but a limited shelf life. A comminuted mixture of meat with fat and salt, filled in a casing acidifies and dries, giving a nutritionally safe product with acceptable shelf life : raw, fermented and dry sausage or, for short, dry sausage. This process, based on fermentation and drying forms a major part of the meat industry in Continental Europe, and is gaining interest in the U.S.A., Australia and Great Britain (see e.g. Klettner and Baumgartner, 1980). Comminution distributes the surface flora of the meat throughout the sausage and the presence of salt, the partial exclusion of oxygen by the casing and, possibly, the production of carbon dioxide, prevents growth of pseudomonads but permits growth of the more salt tolerant lactic acid bacteria (Smith and Palumbo, 1973) (Egan, 1983)(Erichsen, 1983). Interaction of the lactic acid formed with the meat proteins solubilized by salt, lowers pH to final values between 4.5 and 5.5 and denatures the protein solution to the desired slicable gel-like texture. The low pH, in combination with the water activity lowered by salt and drying ensures sausage stability and safety. In Europe, regional differences exist : Mediterranean countries rely most on drying to ensure product stability and safety, whereas in North and Central Europe, fermentation is combined with smoking, allowing less extensive drying. Processes used in the U.S.A are based on rapid fermentation at elevated temperatures and drying may be omitted completely (Bacus, 1984). Lücke (1985) distinguishes "natural" and "rapid" processes. The former methods use nitrate, low amounts of sugar and low fermentation temperatures whereas in the latter nitrite, high amounts of sugar and elevated fermentation temperatures are used. In large-scale industrial production sausage ripening (e.g. the whole process between case filling and the final product), can be clearly divided into two periods :

1. A first short fermentation period (1-2 days) in which pH drops to its lowest value between 4.5 and 5.0.
2. A second longer drying period (3-4 wks) in which the sausage loses up to about 30% of its weight and develops full flavour, colour and texture.

Adaptation of traditional empirical methods to industrial production has generated research into the biotechnology of sausage ripening (Lücke, 1985). It has also resulted in energy costs up to 35000 Megajoules per ton of finished product, 90% of which being consumed by air conditioning during drying (Stiebing et al, 1983). Less energy consuming systems using brine immersion have been described (Prändl et al, 1967). I do not have the intention to try and review all aspects of sausage ripening and its technology as recent excellent reviews on this subject are available (Bacus, 1984)(Lücke, 1985). This paper will totally neglect certain aspects (e.g. mould growth and mycotoxins) and focus on work, carried out in our laboratory in which sausage ripening is studied as an integrated process. Such study requires simultaneous evaluation of several

interrelated dynamic aspects of the complex system of sausage ripening, and their effect on sausage quality. The paper will describe simple mathematical and metabolic models used for such evaluation, in an extension of earlier work published locally (Demeyer, 1981)(Demeyer and Verplaetse, 1985) or as abstract (Demeyer, 1982) (Demeyer and Verplaetse, 1985).

### 2. Quality characteristics of dry sausage.

Quality of dry sausage may be defined here as

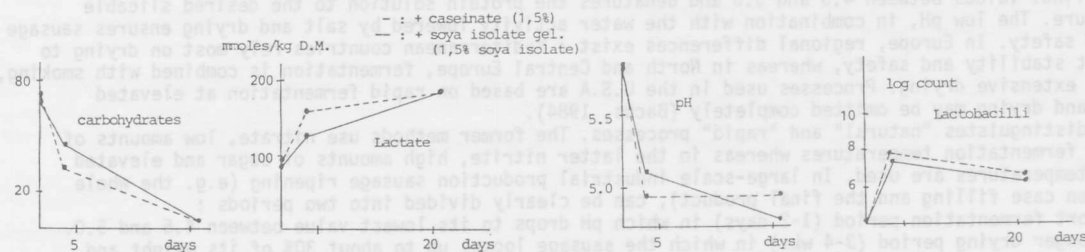
- a. Shelf life and nutritional safety
- b. Sensorial characteristics : texture, colour and flavour.

#### 2.1. Shelf life, nutritional safety and microbiology.

The combination of low pH and low wateractivity ( $a_w$ ) prevents growth and toxin production of e.g. Salmonella and Clostridium botulinum respectively. Staphylococcus aureus enterotoxin production is less inhibited by low  $a_w$  but is sensitive to anaerobiosis (Labots and Stekelenburg, 1979). Lactic acid bacteria furthermore produce anti-bacterial compounds such as hydrogen peroxide, antibiotics and proteins (Egan, 1983)(Talon et al, 1980) (Barefoot and Klaenhammer, 1983). Apart from low pH and  $a_w$ , nitrite provides an additional defense against C. Botulinum toxin and according to Genegeorgis (1978), 50 ppm  $\text{NaNO}_2$  is sufficient to prevent C. Botulinum development, when starter cultures and dextrose are used. Public health considerations are mainly responsible for the emphasis put on a rapid and reproducible pH drop in dry sausage manufacture. Recent information even suggests that nitrite in dry sausage has little or no effect in controlling organisms such as Staphylococcus aureus, Salmonella and Clostridium sporogenes (Collins - Thompson et al, 1984). Improved chilling and sanitation mean however that the average natural inoculum is likely to contain fewer bacteria than in the traditional processes of the past. This may delay fermentation, necessitating a period of pan-curing before stuffing or the use of "starter cultures" or of a backinoculum. It should be mentioned that, depending on temperature pan-curing permits nitrate reduction to nitrite, prior to its inhibition by lactic acid production, whereas even with starter cultures, fermentation may be delayed because of the presence of antibiotic substances, from natural sources (Gill and Penney, 1979) or from antibiotic drug residues (Vandendriessche, Personal communication). Starter cultures were initially developed in the U.S.A. using Pedococcus sp., not normally occurring in meat but resistant to lyophilisation. Later frozen cultures of Lactobacillus plantarum were introduced, showing lower optimal growth temperatures and more akin to the atypical Lactobacilli found in traditional sausage (Lücke, 1985). In Europe, where lower fermentation temperatures are common and nitrate was used more frequently, the rapid lactate production suppressed organisms producing catalase and nitrate reductase, inducing flavour and colour defects. This resulted in the development of starter cultures containing both Lactobacilli and micrococci in Europe. It should be emphasized however that the use of a starter culture is not necessarily associated with a rapid fermentation and vice-versa. Indeed, added starter cultures have to compete with bacteria present in the raw materials and dominance of individual strains determining product characteristics will be affected by many factors. The development of an indigenous meat micro-flora, simultaneous with the

starter culture may even be essential for optimal product quality : Dickson and Maxcy (1985) recently showed that *Pediococcus Cerevisiae* lowered pH of an irradiated sausage mix to 4.38 whereas in a non-irradiated control, final pH was 4.69. The use of starter cultures is reported to deplete nitrite and to lower amine and nitrosamine production as well as proteolysis (Bacus and Brown, 1981)(Smith and Palumbo, 1973). Lipolytic and proteolytic activity of lactic acid bacteria found in meat is a neglected area in research however (Egan, 1983). Lactic acid bacteria used as starter cultures in sour dough manufacture were shown to be proteolytic (Spicher and Nierle, 1984) whereas *Lactobacillus* sp. isolated from meat utilises arginine after glucose depletion (Dainty et al, 1983) with the possible production of putrescine (Vandekerckhove, 1977). Gilliland (1985) discusses intracellular peptidase activity in the utilisation of milk proteins by lactobacilli. It can perhaps be mentioned here that in my opinion total counts of bacteria and their identification may not be very revealing to sausage metabolism. Indeed, environmental changes can cause bacteria to shift their metabolism to different pathways with different end products without any changes in numbers or species (Hobson and Summers, 1967). Also, changes in amounts of end products formed may not be reflected in bacterial counts because of the logarithmic nature of the counts and their errors. Spices e.g. doubled rate of lactate production without any significant effect on lactobacilli counts (Vandendriessche et al, 1980) and a similar effect has been observed with soya isolate (Vandekerckhove and Demeyer, 1978)(fig.1).

Fig.1 : Effects of caseinate and soya isolate on sausage metabolism. (Vandekerckhove and Demeyer, 1978)



Rather than to changes in the bacterial flora, the stimulatory effect of spices was shown to be due to Manganese (Zaika and Kissinger, 1984), an element absent in muscle (Marchello et al, 1984) and essential for *Lactobacilli*, possibly as scavenger of toxic oxygen species (Archibald and Duong, 1984). The presence of unknown growth factors in soya isolate is the most likely hypothesis to explain its stimulatory effect on acidulation (Vandekerckhove and Demeyer, 1978).

## 2.2. Texture.

Salt solubilises sarcoplasmatic and myofibrillar proteins which coagulate and form a gel surrounding lard and

meat particles upon acidification. The pH necessary for coagulation increases with increasing salt concentration and is 5.3 at the often used salt concentration of 3% (Ten Cate, 1960). A rapid drop of sausage pH to values near the isoelectric point of the meat proteins will accelerate drying as observed with the use of PSE meat (Townsend et al, 1980) and spices (Vandendriessche et al, 1980). Texture can be measured using an Instron Universal Testing Machine (Instron Ltd, High Wycombe, G.Britain) to determine e.g. hardness (force necessary to compress a sample to a determined extent) and cohesiveness (the ratio between the work done during a second compression of the same sample to that of the first compression) (Friedman et al, 1963). Such determinations can be done on sausage sections (Vandendriessche et al, 1980) or on whole sausages (Demeyer et al, 1984).

## 2.3. Colour

The red, stable colour of sausage is due to the production of nitroso myo-chromogen, from added potassium nitrate and/or sodium nitrite, the latter added as colouring salt ( $\text{NaCl} \approx 0.6\% \text{NaNO}_2$ ). The formation of the curing red colour in dry sausage is initiated by the initial oxydation of both oxygenated (red) myoglobin and nitrous acid to metmyoglobin and nitrate. These oxidations are followed by reductions (using indigenous or added reductants) forming nitric oxide and myoglobin which combine to form red nitric oxide myoglobin (NoMb) at a rate increasing with falling pH. The protein moiety of NoMb may be denatured and the nitric oxide myochromogen (NoMc) formed improves colour stability, however, at low pH and redox potentials, NoMc may be attacked by peroxides, bleaching the colour to grey, brown or green. This stresses the importance of peroxide and for catalase production by *Lactobacilli*. Peroxidase activity is generally attributed to *Micrococcaceae* (*Staphylococcus*), whose numbers are increased in the presence of oxygen (Lücke, 1985). Use of nitrate requires bacterial reduction to nitrite, which is inhibited by pH values below 5.5. The use of nitrite allows fast acidification as its transformation to nitroso myo-chromogen is not inhibited by low pH. Low amounts of  $\text{NaNO}_2$  ( $\leq 50\text{ppm}$ ) should be sufficient for colour development and inhibition of *Clostridia* and total omission of nitrite in dry sausage manufacture seems a possible alternative (Skjelkvale et al, 1974). Lücke (1985) however states that fermented sausages made without nitrite or nitrate have a poor flavour and spoil rapidly due to oxidative rancidity. Spectrophotometric determination of extracted haem can be used as a measurement for colour, although such measurements do not account for the reflective properties of the sausage surface.

## 2.4. Flavour

The typical flavour of dry sausages is due to lactic acid, salt and a series of end products of bacterial metabolism of carbohydrates, proteins and lipids such as free amino acids, peptides, carbonyl compounds and free fatty acids (Dierick et al, 1974)(Demeyer et al, 1974)(De Ketelaere et al, 1974). The latter substances in combination with flavour compounds produced e.g. by yeast (Milano sausage), surface fungal growth (*Penicillium nalgiovensis*) and spices determine final aroma. Recently acid taste of sausage has been associated with the excessive presence of D(+)-lactate (Burchard et al, 1984). Determination of flavour is best done by trained analytical taste panels using scales although hedonic panels using relative judgements can be applied (Demeyer

et al, 1984).

### 3. Steering of metabolism or processing control : the need for models.

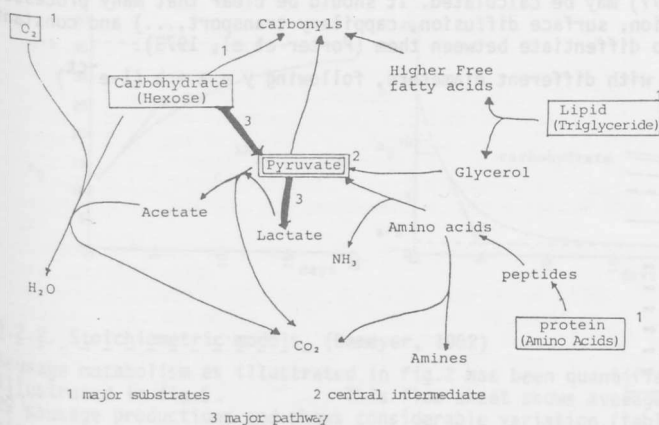
It is clear that the final quality characteristics of dry sausage as discussed above are determined by a complex system of interacting physical (drying, gel formation...), chemical (colour formation...), biochemical and microbiological changes. The final results of these changes as well as their kinetics (e.g. changes in pH,  $a_w$ , texture etc...) are important in the evaluation of quality (Demeyer, 1982).

The manufacturer has the possibility to manipulate or steer this complex system of interacting changes to optimize his product. Such steering can involve numerous actions such as use of spices and non-meat proteins, changes in temperature, relative humidity, time frames, sausage diameter, casing, etc..., use of starter cultures (lyophilized or frozen, activated or not,...) and/or different kinds and amounts of carbohydrate substrates (glucose, lactose, dextrans,...) etc...

Besides economical considerations, main emphasis is placed on rapid and reproducible acidulation of the sausage in the evaluation of a steering action. It is clear however that evaluation has to include as much of the complex system of sausage metabolism as possible. Failure to do so may result e.g. in the loss of traditional flavours associated with the cured meats by the use of starter cultures and nitrite (Bacus, 1984). We have represented

sausage metabolism as a set of overall reactions involving carbohydrate, protein and lipid degradation (fig.2)(Demeyer, 1982) and have characterized kinetics of formation of end products and quality characteristics by simple equations (Vandendriessche et al, 1980) (Demeyer et al, 1984). These representations characterize the dry sausage system, can be used to quantify components of the system and are thus called models (Distefano III et al, 1976). Such models can be used to describe the sausage system in terms of simple kinetic parameters and stoichiometric relations (Demeyer et al, 1984) allowing to evaluate a steering action in terms of the changes it produces in these parameters and relations, eventually subject to statistical analysis. Such descriptive models do not necessarily confirm to established scientific relationships, but adhere closely to experimental data. In contrast, interpretative models evaluate the extent to which sausage metabo-

Fig.2 : Representation of dry sausage metabolism



lism obeys established scientific relationships, and help to better understand the system rather than to evaluate its manipulations. The large variability in metabolism during dry sausage manufacture within the same factory (table 1) indicates the need for better control or steering of sausage production (Demeyer, 1982).

Table 1 : Compounds degraded and produced during dry sausage manufacture (mmoles/kg D.M.)<sup>x</sup>

	Fermentation Period (0-3 days)	Drying period (3-21 days)	Variation Coefficient (%)
Hexose	39	46	50
Lactate	72	56	90
Acetate	11	6	140
NH <sub>3</sub>	7	14	45
α-NH <sub>2</sub> -N	30	55	45

<sup>x</sup> From Demeyer (1982). Only data for industrial productions (n=11) are presented.

The need for better understanding of sausage metabolism is perhaps best illustrated by the variable production of amines (Vandekerckhove, 1977)(Tiecco et al, 1985). Amine production is associated with public health considerations (Vandekerckhove and Demeyer, 1977) and technology (Demeyer and Vandekerckhove, 1977) but may also be associated with the production of desired flavours and with bacterial proteolytic activity. Amines are present in all fermented foods (Pechanek et al, 1983) and it is not clear if and how their production is associated with e.g. the use of starter cultures (Nordal and Slind, 1980)(Bacus and Brown, 1981)(Bacus, 1984). Rate of proteolysis may limit amine production (Lücke, 1985) although free amino acids accumulate during sausage ripening (Dierick et al, 1974). These problems can be overcome by a more fundamental study of the nature and production of flavour compounds and amines and of the properties of starter cultures. Such studies will eventually allow total control of product and process, including e.g. selection of starter based on Arrhenius energies of activation (Racciah, 1984) and on recombinant DNA methods for starter strain improvement as already practised for the dairy industry (Huggins, 1984).

### 4. Models for steering of dry sausage metabolism.

In earlier work, we have developed interpretative models for changes of pH and  $a_w$  in dry sausage production, whereas work on drying losses is in progress. We have also used descriptive models involving kinetics and stoichiometry in the evaluation of spices, starter cultures and sausage diameter as steering actions. Finally, case studies involving the evaluation of steering through starter cultures and sausage diameter using descriptive models will be discussed.

#### 4.1. Interpretative models

#### 4.1.1. Drying losses. (Demeyer, 1981)

Weight losses during drying are assumed to be identical to water evaporation from muscle tissue, neglecting water content and losses from fat tissue and gas production during fermentation. A further assumption fixes water content of fat-free matter as 0.75, allowing development of the formula

$$I = \frac{100}{D} \left( D - 0.75 A - 25 - \frac{1875F}{1000 - 75F} \right)$$

with I = % drying weight loss

D = % dry matter in sausage

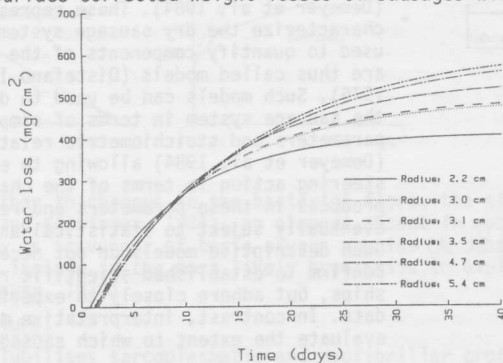
F = % chemical fat in sausage

A = % dry additives in sausage batter (NaCl, sugar, spices, non-meat proteins, etc...).

Evaporation and diffusion controlled drying cannot be differentiated from correlations of evaporation rate with sausage diameter (Verplaetse and Demeyer, unpublished results). It is generally assumed that evaporation controlled drying is very brief during dry sausage ripening (Palumbo et al, 1977).

Fig.3 shows that surface corrected weight loss during dry sausage ripening can be represented by an exponential function following  $y = a + b(1 - e^{-ct})$ . Intersections with the abscissa indicate lag-times, increasing with sausage diameter, reflecting water condensation on the sausage during the fermentation period. From asymptotes, empirical diffusion coefficients (Palumbo et al, 1977) may be calculated. It should be clear that many processes affect drying (e.g. liquid diffusion, vapour diffusion, surface diffusion, capillary transport,...) and constantly changing factory conditions make it impossible to differentiate between them (Porter et al, 1973).

Fig.3 : Surface corrected weight losses of sausages with different diameters, following  $y = a + b(1 - e^{-ct})$



#### 4.1.2. Water activity. (Demeyer, 1979)(De Jaeger et al, 1984)

Water activity in dry sausage is mainly determined by the concentration of salt in the water phase and can be calculated from salt and water content, incorporating a correction for initial  $a_w$  values due the other compounds than NaCl and for drying. Use of these assumptions allows development of the formula :

$$a_w = 1.0014 - 0.6039 \frac{\%NaCl_s}{\%H_2O_s} \left( 1 + \frac{0.0189 \%H_2O_b}{\%NaCl_b} \right)$$

with the index s referring to sausage and b to initial batter.

The validity of the model was experimentally verified in a factory (De Jaeger et al, 1984). Knowledge of drying kinetics (see under descriptive models) allows calculation of the time needed to reach a determined value of  $a_w$  (e.g.  $a_w = 0.95$ ).

#### 4.1.3. pH. (Demeyer et al, 1979)

Acidification of dry sausage from pH 5.8 to 4.8 requires more lactic acid than titration of sausage batter, indicating the production of basic substances during dry sausage production.

Analytical data of 55 different brands of dry sausage were used in multiple regression analysis, showing that 54% of variation in pH was explained following

$$pH = 4.317 - 1.152 x_1 + 0.751 x_2 \quad R^2 = 0.54$$

with  $x_1 = \log \frac{(H_2O)}{(NH_3)}$  and  $(H_2O)$  in g/100g Crude protein

$x_2 = \log \frac{(NH_3)}{(Lactate)} \cdot 100$  with  $(NH_3)$  and (lactate) in mmoles/100g Crude protein.

A similar relation was established for pH changes during fermentation and drying (Vandendriessche et al, 1980). The data indicate the importance of proteolysis followed by desamination in dry sausage metabolism.

### 4.2. Descriptive models

#### 4.2.1. Kinetic models.

Kinetics for the following characteristics are judged to be important in determination of sausage quality : quality characteristics : Dry matter (%DM), pH, colour (luminosity and/or No-Mc) and texture (hardness in Newton) and cohesiveness.

metabolic characteristics : Lactate, total carbohydrates, free amino acids ( $\alpha$ -NH<sub>2</sub>-N), NH<sub>3</sub>, acetate, higher free fatty acids and carbonyl compounds, all in mmoles/kg DM.

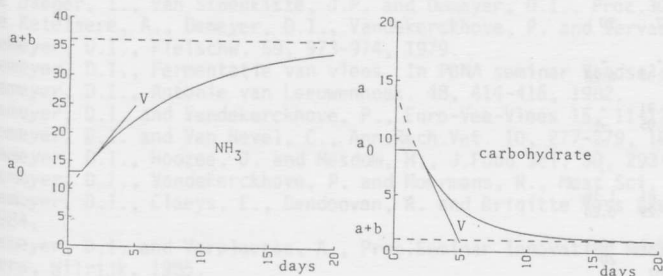
The characteristics listed are incorporated routinely in kinetic models in our laboratory, with the exception of higher free fatty acids, acetate, colour and aldehydes. With the exception of cohesiveness their kinetics in time

t (days) follow a similar pattern : a lag period, followed by rapid change and slow change to a final value. Such kinetics can be fitted to the model  $y = a + b(1 - e^{-ct})$  developed by Orskov and McDonald (1979) analogous to the first-order reaction  $\frac{dy}{dt} = k \cdot y$ , incorporating an initial value a and a final value a + b.

The coefficients a, b and c can be calculated by iterative fitting to experimental data and the model can be corrected for a lag-time  $t_0$  equal to the intercept of  $y = a_0$  and  $y = a + b(1 - e^{-ct})$ ,  $a_0$  being the experimentally determined value of y at  $t = 0$  (McDonald, 1981). So far, mean determination coefficients vary between 0.86 (for  $NH_3$ ) and 0.99 (for DM) in our experiments, involving 6 sampling times at 8 a.m. on days 1, 2, 3, 7, 14 and 21 after stuffing. More accurate determination of the kinetic parameters  $t_0$ , a, b and c is possible through increase of the number of samples, taken at more accurately determined times (hours instead of days). The model does not account for all typical changes during dry sausage production (e.g. slight increase in pH at the end of drying) but allows comprehensive presentation of data and quantification of the effects of a steering action in terms of changes in (fig.4)

- lag-time  $t_0$  (days)
- initial absolute rate of change  $V = \left(\frac{dy}{dt}\right)_{t=t_0} = bc \cdot e^{-ct_0}$
- final value  $y = a + b(1 - e^{-ct})$  with  $t = \text{end of production}$  (e.g.  $t = 21$  days).

Fig.4 : Kinetic model following  $y = a + b(1 - e^{-ct})$



#### 4.2.2. Stoichiometric models. (Demeyer, 1982)

Sausage metabolism as illustrated in fig.2 has been quantified with the exception of lipid metabolism as illustrated in fig.5. This flow sheet shows averages calculated over the whole production period for 29 sausage productions and shows considerable variation (table 1). The model assumes lactate and acetate production from carbohydrate through glycolysis with pyruvate as intermediate. Part of the carbohydrate is assumed to

be completely oxidized to  $CO_2$  and  $H_2O$  with consumption of  $O_2$ . The validity of the latter assumption was tested by comparison of lactate, acetate and  $CO_2$  production with carbohydrate and  $O_2$  consumption during dry sausage metabolism in the laboratory.  $CO_2$  production and  $O_2$  consumption, calculated from stoichiometry were found to be not statistically different from measured values (Demeyer and Vandekerckhove, in preparation). The model further assumes lactate and/or acetate production from amino acid fermentation with pyruvate as central intermediate and in molar amounts equal to ammonia. The validity of such overall stoichiometry for amino acid fermentation has been established for rumen fermentation (Demeyer and Van Nevel, 1979). Finally the model accounts for small but significant amounts of amino acids (mainly tyrosine, arginine and lysine) decarboxylated to amines (Vandekerckhove, 1977). Lipolysis with production of glycerol (Demeyer et al, 1974) contributing to lactate and acetate production (fig.2) has not been incorporated in quantitative stoichiometry. Using the data presented in table 1 and fig.5 for carbohydrate disappearance, lactate, acetate  $NH_3$  and  $\alpha-NH_2-N$  production, sausage metabolism can be characterized following stoichiometric calculations as illustrated in table 2. The model clearly shows that, on average, amino acid fermentation contributes 10% to pyruvate equivalents metabolized, whereas only 60% of these equivalents are used in lactate production and 35% are converted to  $CO_2$  and  $H_2O$ . Such calculations were done for the whole production period, but can be applied to the fermentation period (0-3 days) or drying period (4-21 days) only.

Fig.5 : Flowsheet of sausage metabolism (Demeyer, 1981)\*

\* Data indicate average values for carbohydrate and protein metabolism during fermentation and drying. Numbers are mmoles tricarbon equivalents (MW 100) per kg DM and arrow size relates to the quantities formed.

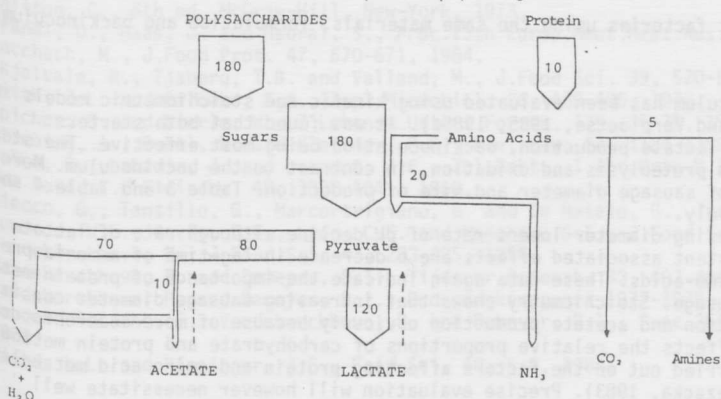


Table 2 : Metabolic pattern for dry sausage production (mmoles pyruvate equivalents per kg DM)

Metabolized		% of total (1)	
Hexose	180	90	
Amino Acids	20	10	
Total (1)	200	100	
Formed			
Lactate	120	60	
Acetate	10	5	
Total (2)	130		
Oxidized : (1)-(2)	70	35	
Free amino acids accumulated	75	100	

Table 3 : Effects of diameter and site of production on kinetics of sausage ripening

	Expt. 1 <sup>1</sup>		Expt. 2a <sup>2</sup>		Expt. 2b <sup>2</sup>	
	47 <sup>3</sup>	90	47	90	47	90
Lag time to (days)						
for % DM	1.84	3.26	0.43	0.01	-	-
pH	1.04	1.37	0.02	-	0.07	0.28
mmoles/kg DM -lactate	4	0.82	0.13	0.15	0.31	-
-NH <sub>3</sub>	1.34	0.95	0.13	-	0.05	-
-α <sup>2</sup> NH <sub>2</sub> -N	-	0.56	0.19	-	-	-
initial rate (day <sup>-1</sup> )						
for % DM	2.17	1.27	1.17	1.19	1.81	1.10
pH	0.49	0.40	0.64	0.44	0.29	0.20
mmoles/kg DM -lactate	19.7	35.9	25.2	27.8	16.2	15.1
-NH <sub>3</sub>	56.0	29	48	43	31	20
-α <sup>2</sup> NH <sub>2</sub> -N	29	76	223	156	107	95
final values						
for % DM	74.1	62.7	70.4	63.8	74.1	67.7
pH	4.93	4.74	4.92	4.95	5.00	4.82
mmoles/kg DM -lactate	194	249	178	201	172	206
-NH <sub>3</sub>	300	343	481	442	354	394
-α <sup>2</sup> NH <sub>2</sub> -N	986	1253	1361	1448	1184	1413

Footnotes 1, 2, 3 and 4, see table 4

Table 4 : Effects of diameter and site of production on stoichiometry of Dry sausage ripening

	Expt. 1 <sup>1</sup>		Expt. 2a <sup>2</sup>		Expt. 2b <sup>2</sup>	
	47 <sup>3</sup>	90	47	90	47	90
Pyruvate equivalents (mmoles/kg DS)						
Metabolized						
Total	154	178	156	162	129	175
% of total						
hexoses	93.2	91.3	89.4	90.1	93.6	92.9
A. Acids	6.8	8.7	10.6	9.9	6.4	7.1
Formed						
% of total						
lactate	71.4	94.7	46.3	67.0	49.2	61.0
acetate	6.5	5.3	15.0	11.7	7.7	6.6
oxidized	22.1	-	38.7	21.3	43.1	32.4
A. Acids accumulated						
per kg DS	434	714	688	894	506	752
per mmoles lactate	3.9	4.2	9.5	8.3	7.8	7.0

1. From Demeyer and Verplaets (1985).

2. Expt 2a and 2b were prepared in different factories using the same materials, formulation and backinoculum.

3. Sausage diameter (mm).

4. - : no lag-time detected.

The effect of a starter culture and backinoculum has been evaluated using kinetic and stoichiometric models in earlier work (Demeyer et al, 1984)(Demeyer and Verplaetse, 1985, 1985a). It was found that both starters accelerate rates of acidulation, drying and lactate production, backinoculation being most effective. The starter culture clearly shifted metabolism away from proteolysis and oxidation, in contrast to the backinoculum. More recent experiments investigated the effect of sausage diameter and site of production. Table 3 and Table 4 show kinetic and stoichiometric results respectively.

Careful evaluation of data shows that increasing diameter lowers rate of pH decline although rate of lactate production is not decreased. The only consistent associated effects are a decrease in lag-time of ammonia production and higher final amounts of free amino-acids. These data again indicate the importance of protein metabolism as it affects change of pH in dry sausage. Stoichiometry shows that increasing sausage diameter consistently shifts metabolism towards less oxidation and acetate production obviously because of more anaerobic conditions. The site of production obviously affects the relative proportions of carbohydrate and protein metabolism. It is clear that more research should be carried out on the factors affecting protein and amino acid metabolism during dry sausage ripening (Perzacki and Pezacka, 1983). Precise evaluation will however necessitate well

controlled laboratory conditions rather than variable factory conditions.

Acknowledgement : the authors acknowledge financial support from the I.W.O.N.L., Brussels and Hansen's laboratory, Copenhagen.

Dendooven R. helped with calculations.

References :

- Archibald, F.S. and Mingh-Ngoc Duong, *J.Bact.* 158, 1-8, 1984.
- Bacus, J., *Food Technol.* 38(6), 59-63, 1984.
- Bacus, J.N. and Brown, W.L., *Food Technol.* 35(1), 74-83, 1981.
- Bacus, J., Utilization of microorganisms in meat Processing Res. Studies Press. Ltd., Letchworth, Hettfordshire, England and John Wiley & Sons Inc., New York, 1984.
- Barefoot, Susan F. and Klaenhammer, T.R., *Appl.Env.Microbiol.* 45, 1808-1815, 1983.
- Burcharies, C., Girard, J.P., Sirami, J. and Pascal, S., *Sci.Aliments*, 4, no hors serie III, 137-143, 1984.
- Collins-Thompson, D.L., Krusky, B., Osborne, W.R. and Hausschild, A.H.W., *Can.Inst.Food Sci.Technol.J.* 17, 102-106, 1984.
- Dainty, R.H., Shaw, B.G. and Roberts, T.A., In *Food Microbiology : Advances and prospects.* eds. Roberts, T.A. and Skinner, F.A. Acad.Press, London, P.151-178, 1983.
- De Jaeger, I., Van Steenkiste, J.P. and Demeyer, D.I., *Proc.30th Europ.Meet.Meat Res.work*, Bristol, 1984.
- De Ketelaere, A., Demeyer, D.I., Vandekerckhove, P. and Vervaeke, I., *J.Food Sci.* 39, 297-300, 1974.
- Demeyer, D.I., *Fleischw.* 59, 973-974, 1979.
- Demeyer, D.I., *Fermentatie van vlees.* In PBNA seminar Voedselconservering. p.1-28, ed.PBNA, Arnhem, 1981.
- Demeyer, D.I., *Antonie van Leeuwenhoek.* 48, 414-416, 1982.
- Demeyer, D.I. and Vandekerckhove, P., *Euro-Vee-Vlees* 15, 11-12, 1977.
- Demeyer, D.I. and Van Nevel, C., *Ann.Rech.Vet.* 10, 277-279, 1979.
- Demeyer, D.I., Hoozee, J. and Mesdom, H., *J.Food Sci.* 30, 293-296, 1974.
- Demeyer, D.I., Vandekerckhove, P. and Moermans, R., *Meat Sci.* 3, 161-167, 1979.
- Demeyer, D.I., Claeys, E., Dendooven, R. and Brigitte Voss Akero, *Proc.30th Europ.Meet.Meat Res.work*, Bristol, 1984.
- Demeyer, D.I. and Verplaetse, A., *Proc.Seminar Innovatibe Meat Technol., Flanders Technol.Int., Ghent*, ed. Dera, Wilrijk, 1985.
- Demeyer, D.I. and Verplaetse, A., *J.Sci.Agric.* 36, 1345-1346, 1985.
- Dickson, J.S. and Maxcy, R.B., *J.Food Sci.* 50, 1007-1013, 1985.
- Dierick, N., Vandekerckhove, P. and Demeyer, D.I., *J.Food Sci.* 39, 301-304, 1974.
- Distefano III, J.J., Stubberud, A.R. and Williams, I., *J.Feed back and Control Systems.* Schoum's outline series. Mc Graw, H.J. Book Co. New-York, 1976.
- Egan, A.F., *Antonie van Leeuwenhoek* 49, 327-336, 1983.
- Erichsen, I., In *Food Microbiology : Advances and Prospects.* eds. Roberts, T.A. and Skinner, F.A. Acad.Press, London, p. 271-286, 1983.
- Friedman, H.H., Whitney, J.E. and Szczesniak, A.S., *J.Food Sci.* 28, 390-394, 1963.
- Genegeorgis, C., *Proc.Meat Proces.Conf.* March 30-31, 1978 p.21, Univ.Calif., Davis, California, U.S.A.
- Gil, C.O. and Penny, N., *Appl.Environment.Microbiol.* 37, 667-669, 1979.
- Gilliland, S.E., In *Bacterial starter for foods.* ed.Gilliland, S.E., p.41-72, CRC Press.Inc., Boca Raton, Florida, 1985.
- Hobson, P. and Summers, R., *J.Gen.Microbiol.* 47, 53-65, 1967.
- Huggins, A.R., *Food Technol.* 38(6), 41-50, 1984.
- Klettner, P.G. and Baumgartner, P.A., *Food Technol.* Australia 32, 380-384, 1980.
- Labots, H. and Stekelenburg, F.K., *Voedingsmiddel.Technol.* 12, 25-28, 1979.
- Lücke, F.K., In *microbiology of fermented Foods* ed. Wood, B.U.B., Vol.2,p. 41-83, Elsevier Applied Science Publ., London, 1985.
- Marchello, M.J., Hilne, D.B. and Slinger, W.D., *J.Food Sci.* 49, 105-106, 1984.
- McDonald, I., *J.Agric.Sci.* 96, 251-252, 1981.
- Modic, P., Trumic, Z., Polic, M. and Turbatovic, L., *Proc.24th Europ.Meet.Meat Res.work*, Kulmbach, 1978.
- Nordal, J. and Slinde, E., *Appl.Environment.Microbiol.* 40, 472-475, 1980.
- Orskov, E.R. and McDonald, I., *J.Agric.Sci.* 92, 499-503, 1979.
- Palumbo, S.A., Komanowsky, M., Metzger, V. and Smith, J.L., *J.Food Sci.* 42, 1029-1031, 1977.
- Pechanek, U., Phannhauser, W. and Woidich, H., *Z.Lebensm.Unters.Forsch.* 176, 335-340, 1983.
- Pezacki, W. and Pazacka, E., *Fleischw.* 63, 625-630, 1983.
- Porter, H.F., McCormick, P.Y., Lucas, R.L. and Wells, D., In *Chemical Engineer's Handbook* eds. Perry, R.M. and Chilton, C., 5th ed. McGraw-Hill, New-York, 1973.
- Prändl, O., Haas, J. and Habral, S., *Proc.13th Europ.Meet.Meat.Res.work*, F2, Rotterdam, 1967.
- Racchach, M., *J.Food Prot.* 47, 670-671, 1984.
- Skjelvale, R., Tjaberg, T.B. and Valland, M., *J.Food Sci.* 39, 520-524, 1974.
- Smith, J.L. and Palumbo, S.A., *Appl.Microbiol.* 26, 489-496, 1973.
- Spicher, G. and Nierle, W., *Z.Lebensm.Unters.Forsch.* 179, 36-39, 1984.
- Stiebing, A., Rodel, W. and Klettner, P.G., *Fleischw.* 63, 1164-1169, 1983.
- Talon, R., Labadie, J. and Laspent, J.P., *Zbl.Bakt., I.Abt.Orig.B* 170, 133-139, 1980.
- Ten Cate, L., *Fleischw.* 40, 1038-1041, 1960.
- Tiecco, G., Tantillo, G., Marcotrigiano, G. and De Natale, G., *Ind.Alim.* 24, 122-126, 1985.
- Towsend, W.E., Davis, C.E., Lyon, C.E. and Mescher, S.E., *J.Food Sci.* 45, 622-626, 1980.
- Vandekerckhove, P., *J.Food Sci.* 42, 283-285, 1977.
- Vandekerckhove, P. and Demeyer, D.I., *Tijdschr.Geneesk.* 33, 407-408, 1977.
- Vandekerckhove, P. and Demeyer, D.I., *Onderzoeksrapport 1977-1978*, Conv. no.25061, I.W.O.N.L., Brussels, 1978.
- Vandendriessche, F., Vandekerckhove, P. and Demeyer, D.I., *Proc.26th Eur.Meet.Meat Res.work*, Colorado-Springs, 1980.
- Zaika, L.L. and Kissinger, J.C., *Food Sci.* 49, 5-9, 1984.

...the authors acknowledge financial support from the U.S. National Science Foundation and the National Institutes of Health.

...the authors acknowledge financial support from the U.S. National Science Foundation and the National Institutes of Health.

...the authors acknowledge financial support from the U.S. National Science Foundation and the National Institutes of Health.