12 The effect of starter cultures on stoichiometry and kinetics of dry sausage metabolism.

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1. Introduction.

An immediate, rapid and reproducible drop in pH is essential to obtain dry sausage of acceptable shelf life, nutritional safety and sensorial quality. Decline in pH is the result of lactic acid production by bacteria, a major reaction in a complex network of biochemical changes involving both fermentation and oxidation of carbohydrate, protein and lipid substrates (Demeyer, 1982). To ensure rapid and predominant lactic acid production, large numbers desirable bacteria are often added to inhibit growth of undesirable species. Such addition may be done as meat preserved from a previous successful fermentation (back slopping or starter sausage) or as commercial starter culture, a technique well known in the dairy industry. (Smith and Palumbo, 1983).
In the present work, the effect of an added starter sausage mix and a starter culture on sausage metabolism and quality was investigated.

2. Materials and Methods.

2.1. Preparation of sausages.

The basic sausage batter (15 kg) contained (% w/w) frozen S. American beef (37.9), frozen pork (31.6) frozen lard (25.3), salt (2.8) sodium caseinate (1.4), lactose (N.V. Roland - Brussel) (0.37) white pepper (0.28) glucose (0.33) and sodium ascorbate (0.02). The salt used was NaCl containing $NaNO_2$ (0.4 %) and KNO_3 (0.2 %). Caseinates (Protevit) were obtained from Dena (Eupen). Materials were mixed in the cutter in the sequence beef, starter culture (suspended in 25-50 ml of tap water 30 min. before cuttering) pepper and additives, pork, lard and finally, salt.
A control series without starter (series C), a series with starter sausage mix obtained from sausages prepared using Micrococci starter cultures (0.06 %) (series S1) and a series with a lyophilyzed starter culture on lactose (Hansen's CS 123 containing 2.10^{12} Micrococci and 10^{12} Lactobacilli) (0.05 %) (series S2) was prepared. Temperature after cuttering decreased from ± 0° C to -3.5 à -4.5°C. The cutter was rinsed with hot water and detergent (Absonal - Arnold - K.G. Hamburg) between batches S1 and S2. Mixtures were vacuum packed, packages opened and filled into casings, (Naturin R2, Naturin, Germany, diameter 7 cm) giving sausages of 800 - 1000 g each. Sausages were transported to a conditioning room (average air velocity: 5m/sec) and subjected to the sequence shown in table 1.

Table 1.

	Temp.	Relative Humidity	Smoking
Start	21° C[3]	67	-
Day 1	24°C	92	1 u
2	22.5°C	92	3 x 1 u
3	22.5 °C	92	3 x 1 u[2]
4[1]	16.0°C	81	-
until end			

[1]: Transferred to drying room after pH 5.0 is reached (required 5 days for series C)

[2]: Sausages sprayed with a Pimaricine suspension (0.1 % active substance in water)

[3]: Internal sausage temp. 14°C after 1 day and 24° C after 2 days.

Samples (sausages) were prepared at Ter Beke N.V. (Waarschoot) and transfered to the "Laboratorium Voeding & Hygiëne" of the University of Ghent immediately after preparation and each day at 8 a.m. on days 1,2,3,7, 14 and 21 after starting for immediate determination of pH, and texture. The rest of the samples was vacuum packed and kept at - 20°C for further analyses.

2.2. Analyses.

After arrival of the sausages at the laboratory, texture (hardness) was immediately determined on the whole sausage using an Instron Universal Testing machine, as described earlier for sausage sections (Vandendriessche et al, 1980). The sausage was then cut transversely and pH measured on transverse sections (Vandendriessche et al., 1980).
The sausage was ground after removal of the casing and 5 g were extracted in 100 ml of 0.6 N $HClO_4$ in duplicate (De Ketelaere et al., 1974) for determination of total carbohydrates expressed as glucose (phenol-sulfuric acid method), lactic acid, ammonia and α-amino N as described earlier (De Ketelaere et al., 1974) (Dierick et al, 1974). Acetate was determined after steam distillation by GLC (De Ketelaere et al., 1974) and carbonyl compounds using benzidine reagent (Demeyer et al., 1974).
Proximate analyses were carried out for dry matter (ISO method 1442-1973) and other compounds as described earlier (Vandekerckhove & Demeyer, 1975).

2.3. Sensorial evaluation.

At the end of the experiment, sausages with starter cultures (series S2) were evaluated by a 10 member non trained taste panel, in preference tests involving the batch without added cultures (series C) and the batch with "starter sausage" (series S1). Each test was repeated once, giving a total of 10 sessions. Panel members were asked to indicate preference for colour, firmness, acid taste and general acceptability. Rank totals (20 replicates) were tested for significance as described by Kahan et al. (1973). Sausages (series S2) or sections of sausages (series C en S2) were vacuum packed and placed at 2°C. One to two hours before a session, sausages were brought out of the vacuum package at room temperature and slices of 8 mm thickness were cut. All slices were cut into 4 segments, segments were pooled and two segments per series and panel member used.

2.4. Calculations.

Data for formation of fermentation end products and carbohydrate disappearance, drying, change in pH and development of texture measured as hardness could be fitted to the model $y = a + b\ (1 - e^{-ct})$ developed by Ørskov & Mc Donald (1979). The model was corrected for a lag-time t_o (McDonald, 1981) and the coefficients a,b,c and t_o were calculated using an iterative procedure and a desk computer HP 85 (Hewlett-Packard, California). We are grateful to Mr. McDonald (Rowett Research Institute, Aberdeen) for making the computer program available.
With y = parameter studied and x = days after stuffing, the values t_o, c and a + b give an estimate respectively of the lag time (days) fractional rate of change (day^{-1}) and the theoretical final value of y respectively. Mean determination coefficients (R^2) were 0.997, 0.992, 0.966, 0.964, 0.943, 0.9220 and 0.914 for % DM, hardness, total hexose, α-NH_2-N, lactate, pH and NH_3-N respectively. Although the model used is an improvement over earlier attemps (Vandendriessche et al., 1980), it still does not account for all typical changes in dry sausage during ripening (Vandendriescche et al., 1980). Nevertheless, the model allows comprehensive presentation of data to evaluate the effect of starter cultures in terms of lag time (t_o in h), fractional rates of change (c as fraction per hour of total value or h^{-1}) and theoretical final values (a + b in the same units as the component measured).

3. Results and Discussion.

Proximate analyses (% of DM) at the end of the ripening period did not show differences between the three series and gave mean values ± SD of 30.54 ± 0.97, 60.93 ± 2.44 and 5.90 ± 0.65 for crude protein, crude fat and NaCl respectively. Individual data for changes in all components measured are presented in a detailed report elsewhere (Demeyer et al, 1985). Data are presented here in terms of initial analyses, lag times (t_o), rates of change (c), theoretical final values (a + b) and final analyses.

3.1 Drying, decline of pH and texture development.

Table 2 shows that the addition of starter sausage increased rates of drying, texture development and pH drop two-, six- and fivefold respectively. Lag time was decreased for texture development only. Measured final values were not different, but theoretical final values were decreased for DM and texture, which may indicate that both parameters are stabilized sooner in the presence of starter sausage.

Table 2. Effect of starter sausage or culture on drying, texture and pH[1]

	Control	S1	S2
Drying (%DM)			
Initial value	47.18	47.68	47.42
t_o	-	-	-
c	0.026	0.055	0.035
a + b	80.1	69.4	78.2
final value	61.17	62.36	63.68
Hardness (kg)			
Initial value	2.57	2.23	2.27
t_o	3.9	1.1	1.6
c	0.009	0.062	0.020
a + b	37.6	10.0	26.0
final value	7.24	7.68	9.87
pH			
Initial value	5.87	5.76	5.83
t_o	1.08	0.97	0.98
c	0.23	1.06	0.64
a + b	5.02	4.99	4.83
final value	5.04	4.92	4.70

[1]Calculations explained in text

The lyophilyzed starter culture had similar but less outsproken effects on rates of change but measured final value of pH was lower whereas hardness and dry matter showed higher measured final values. Rates of hardness development seem to be determined by rates of drying which seem positively correlated by rates of pH drop (see also Vandendriessche et al, 1980). Final measured values of DM and hardness are negatively correlated to final pH values.

3.2. Characteristics of metabolism.

Table 3 shows that starter sausage at least doubles rates of carbohydrate disappearance, lactate, ammonia and α-amino N production. Again, the starter culture is somewhat intermediate in this respect. Starter sausage however does not change very much the total amount of carbohydrate metabolized or lactate formed (similar theoretical final values) whereas these amounts are increased by the starter culture. These increases are accompanied by lower theoretical final values for NH_3 and α-NH_2-N. These rate considerations indicate that starter sausage increases rate of fermentation but does not change very much the total amount fermented or the pattern of fermentation. On the other hand starter culture does not increase rates of fermentation as much, but increases total amounts fermented and shifts fermentation pattern away from proteolysis. The same conclusions are apparent from a study of overall fermentation stoichiometry (Demeyer, 1982).

Table 3. Effect of starter sausage or culture on rates of metabolism

	Control	S1	S2
Carbohydrates[1]			
Initial value	10.41	10.06	9.90
t_o	1.49	0.83	1.16
c	0.19	0.53	0.35
a + b	2.59	5.41	2.01
final value	3.19	1.73	1.61
Lactate[1]			
Initial value	10.68	10.44	10.29
t_o	1.78	1.35	0.72
c	0.16	0.72	0.26
a + b	19.38	20.93	24.72
final value	20.10	24.08	25.76
NH_3-N[2]			
Initial value	22.8	23.2	21.6
t_o	-	-	-
c	0.09	0.18	0.19
a + b	75.7	49.5	42.6
final value	68.7	45.3	43.1
α-NH_2-N[2]			
Initial value	74.6	67.6	69.4
t_o	0.66	0.84	0.18
c	0.05	0.17	0.13
a + b	299.4	182.5	161.6
final value	223.0	163.7	159.0

[1] mmoles/100 g DM [2] mgN/100 g DM

The same conclusions are apparent form a study of overall fermentation stoichiometry (Demeyer, 1982). Table 4 shows that starter sausage does not change the contribution of amino acid fermentation and total oxidation in metabolism, whereas starter culture decreases these relative contributions by more than 50 %. This shift in metabolism is also reflected in the lower amounts of free α-NH_2-N accumulating in the sausage (table 3).

Table 4. Effect of starter sausage or culture on stoichiometry[1]

	Control	S1	S2
Metabolized			
Hexose	14.46	14.34	16.58
Amino Acid[2]	3.28(18)[3]	2.40(14)	1.54(9)
Total	17.74	16.74	18.12
Formed			
Lactate	9.42	9.53	15.47
Acetate	2.95(16)[3]	1.25(7)	1.29(7)
Substrate oxidized[4]	5.37(30)[3]	5.96(35)	1.36(7)

[1] All data expressed as mmoles pyruvate equivalents per 100 g DM and calculated from analyses on days 0 and 21

[2] Calculated from NH_3-N formed assuming that 1 mmole of NH_3 formed is equivalent to 1 mmole of amino acid metabolized

[3] ( ) = % of total

[4] calculated as (Total) - [(lactate) + (acetate)]

Fermentation stoichiometry as calculated here does not take into account metabolism of glycerol, liberated from lipolysis. From earlier work (Demeyer et al., 1974) it can be estimated that glycerol may contribute about 3 mmoles/100 g D.M. of pyruvate equivalents to metabolism. Lipid metabolism was not studied here but may be partially reflected in the formation of carbonyl compounts. Values show a typical increase / decrease/increase pattern (Demeyer et al., 1974) and the presence of starter sausage is associated with lower values in the last period of ripening (Demeyer et al., 1983).

3.3. Sensorial evaluation.

The addition of starter sausage did not result in a significant preference for general acceptability over the control. Only for firmness preference was statistically significant. Starter culture addition however resulted in significant preference over the control for firmness, acid taste and general acceptability (Demeyer et al., 1983).

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