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Control of bioflavour and safety in fermented sausages: first results of a European project☆

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Abstract

Four types of fermented sausages were prepared: two using Northern technology (Norway and Belgium) and two using Mediterranean technology (Belgium and Italy). Mediterranean sausages showed higher pH values and highest residual amounts of myosin and actin. Free fatty acid concentrations reflected the nature of the raw material, rather than the ripening period. Italian sausages contained the highest amounts of hexanal. Norwegian sausages contained the highest amounts of both free fatty acids and free amino acids. Putrescine concentration could be related to initial contamination of raw materials. Mediterranean sausages were characterised by a "pop corn" odour, identified as 2-acetyl-1-pyrroline. Proteolytic activity of pork *Triceps brachii* was found to be related to animal sex. *Staphylococci* and lactic acid bacteria were investigated. Leucine metabolism involving aldehyde production was found to be strain specific and very sensitive to pH and the presence of nitrite. Bacteria showed anti-oxidant activity, enhanced by the presence of manganese. Bacteriocin production by *L. casei* CTC 494 was studied and results incorporated into a mathematical model.

Keywords: Dry fermented sausage; Flavour; Lipolysis; Lipid oxidation; Proteolysis; Bacteriocins; Amines

1. Introduction

The fermentation and drying of a comminuted mixture of mainly meat, fat, salt and spices, stuffed into a casing is a traditional means of meat conservation. The production technology allows for many but imprecise variations, yielding a variety of different "fermented sausages", presenting a considerable challenge to standardisation and management of quality. Northern type products contain beef and pork and are characterised by relatively short ripening periods, up to about 3 weeks and involving clearly separated fermentation and drying periods. Rapid acidulation to final pH values below 5 and smoking rather than drying ensure safety and shelf life. Mediterranean type products are predominantly pure pork products and involve longer ripening periods, up to several months, often without clear separation between fermentation and drying. Smoke is not applied and acidulation to final pH values above 5 is slower. Shelf life is mainly determined by drying and lowered water activity.

Several recent excellent reviews have underlined the complexity of changes determining flavour and safety, the major quality characteristics of these products

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(Dainty & Blom, 1992; Montel, Masson & Talon, 1998; Verplaetse, 1994). Northern and Mediterranean products are each characterised by specific flavours and safety risks and industry is obviously interested to understand the control mechanisms affecting flavour development and safety in both "Mediterranean" and "Northern" products: producing the former in the shorter production periods of the latter is one aspect of obvious economic interest. Therefore, the European Research Project "Control of bioflavour and safety in Northern and Mediterranean fermented meat products" (FAIR CT97 3227) was initiated. The project was a continuation of an earlier one (AAIR-CT94-1517), during which the relative importance of endogenous and microbial metabolism during sausage fermentation was established and factors affecting microbial amine production were investigated. The new project has three main objectives or tasks:

1. Characterisation of raw materials and finished products for Northern and Mediterranean fermented sausages, prepared using well defined processing technology. Characteristics are to be evaluated for variability and differences related to processing technology.
2. To elucidate pathways leading to desirable and undesirable compounds in fermented meat products.
3. To improve quality, safety and process time of fermented meat products in pilot-scale experiments.

The first results of the project, related to tasks 1 and 2 are reported in this paper. As the elucidation of pathways is closely associated with the characterisation of the raw materials meat, fat and bacteria, the latter will mainly be dealt with under task 2. Results were obtained using three experimental models mainly: sausages prepared under well-defined conditions, aseptic incubations of meat and fat and cultures of isolated bacteria.

2. Materials and methods

2.1. Preparation of sausages

Four types of fermented sausages were prepared by three partners of the project: two Mediterranean types in Italy and Belgium (Belgium S), respectively and two Northern types in Norway and Belgium (Belgium N), respectively. Five batches of each type were prepared between November 1998 and February 1999. Five sausages of each batch were wrapped in aluminium foil, vacuum packed, frozen, packed on dry ice and distributed by courier to the various partner laboratories for analysis. For each type of sausage, detailed information on processing technology is available. Sausages mainly differ, however, in size, the presence of beef (Northern types), the use of fungi starters (Mediterranean types), smoking (Northern types) and ripening time (Table 1).

2.2. Aseptic meat incubations

At Ghent University, pork (M. *Triceps brachii*) and pork back fat, obtained 24 h post-mortem from cooled pig carcasses, were frozen and later aseptically sampled by superficial burning, followed by removal of surface cuts using sterile knives. Samples were vacuum packed and stored for exactly 4 months at −18°C. The procedure resulted in total bacterial counts below 10^3 CFU/g. After thawing, samples were mixed with glucono-delta-lactone (1%), colouring salt (3% of NaCl containing 0.6% $NaNO_2$) and an antibiotic cocktail (1000 U penicillin, 200 μg amphotericin B and 1 mg streptomycin sulphate) for 1 min in a sterilised mincer. Fifty g of the mixture was then vacuum packed and incubated for 3 days at 25°C.

2.3. Incubation of isolated bacteria

In Theix, several species and strains of *Staphylococci* and *Lactobacilli* were incubated in MC media (meat extract 10g/l; yeast extract 5g/l; Na_2HPO_4 2g/l; NaCl 5 g/l; glucose 1g/l; agar 3g/l) at pH 6.0. After sterilisation, either melted pork fat, oleic acid, linoleic acid or linolenic acid was added (0.5 g/l). The media were emulsified with an ultra Turrax T25 (100,000 rpm, 1 min) distributed in small flasks, inoculated at approximately 10^6 cells/ml and incubated at 25°C for up to 20 days (Talon, Walker & Montel, 1998). Cell suspensions of a strain of *S. carnosus 833* were incubated with L-leucine (Larrouture, Masson, Talon & Montel, 1998). At the university of Brussels, environmental effects on growth and bacteriocin (sakacin K) production by *Lactobacillus sakei* CTC 494 were studied in a computer controlled laboratory fermentor containing 10 l of MRS broth as described elsewhere (Leroy & De Vuyst 1999, in press).

2.4. Analyses

Fermented sausage samples were used for a large number of analyses in the different laboratories. As for proximate composition, dry matter and crude protein content were determined according to the methods ISO-1442-1973 (E) and AOAC 24.038, respectively. Crude fat determination (Soxhlet, diethyl ether extract) was carried out according to the EU norm ISO 1444-1973. The pH in the sausage was measured using a Knick Portamess 654 pH metere with an Ingold LoT406-M6-DXK-S7/25 electrode. Other kinds of analyses were SDS-PAGE quantification of meat polypeptides (Claeys, Uytterhaegen, Buts & Demeyer, 1995), free and peptide-bound α-NH_2–N (based on Oddy, 1974),

Table 1
Main production parameters of the four types of dry fermented sausage

Parameter	Norway	Belgium N	Belgium S	Italian
Diameter (mm)	92	90	60	50–60
Weight	3 kg	1 kg	0.8 kg	0.5 kg
Meat species	Pork/beef, 1/1	Pork/beef, 1/1	Pork only	Pork only
Lean meat cuts	Miscellaneous	Pork shoulder + beef	Shoulder	Shoulder
Fat tissue	Pork back fat	Pork back fat	Pork back fat	Pork throat fat
Meat/fat ratio	2/1	2/1	2/1	2/1
LAB starter	+	+	+	+
Fungi starters	–	–	+	+
Particle size	1–2 mm	1–2 mm	1–2 mm	3.5 mm
Smoking	+	±[a]	–	–
Total ripening time	3 weeks	2 weeks	4 weeks	40 days

[a] Sausages were contaminated with smoke due to the use of production fermentation chambers (where smoking is normally carried out).

non-protein tryptophan (Messineo & Musarra, 1972), acid lipase (Motilva, Toldra & Flores, 1992), cathepsin D (based on Barret & Kirschke, 1981), collagen (ISO/DIS 3496.2), acetate (Boehringer, 1995), lactate (Conway, 1957), ammonia (Conway, 1957), residual sugars (Herbert, Philips & Strange, 1971), reversed phase high performance liquid chromatography of an 80% v/v ethanol non-protein N extract with peak identification by relative absorbances at 214, 254 and 280 nm and molecular weight estimation using gel permeation chromatography; quantitative separation of biogenic amines by reversed phase HPLC of dansyl derivatives (Eerola, Hinkkanen, Lindfors & Hirvi, 1993); fatty acids and lipid class composition by TLC (Molly, Demeyer, Civera & Verplaetse, 1996); bacteriocin production (Leroy & De Vuyst, 1999, in press); gas chromatography-olfactometry (GC-O) and aroma analysis by gas chromatography-mass spectrometry (GC-MS) of dynamic head space compounds collected on tenax tubes (Stahnke, 1995); aldehydes by reversed phase liquid chromatography as 2,4-dinitrophenyl hydrazones (Reindl & Stan, 1982) and free amino acids by LC of o-phtalaldehyde derivatives (Brückner, Wittner & Godel, 1991; Blankenship, Krivanek, Ackermann & Cardin, 1989); bacteriological counts (cfu); TBARS, cholesterol and cholesterol oxides (Chizzolini, Zanardi & Dorigoni, 1999). Discrimination by electronic nose sensory analysis (Eklov, Johannson, Windquist & Lundström, 1998) was compared with classic sensory analyses (Matforsk and Imperial Meat Products).

3. Results and discussion

Only a brief summary of a selection of the main results can be presented. Detailed results are reported in Individual and Consolidated Progress EU Reports, whereas some of the work has been reported at conferences (Harnie, Claeys, Raemaekers & Demeyer, 1998; Lambregts, Raemaekers & Demeyer, 1998; Stahnke, Sunesen & De Smedt, 1999; Talon, Walker et. al., 1998) or published (Leroy & De Vuyst, 1999, in press; Talon, Chastagnac, Vergnais, Montel & Berdagué, 1998; Talon, Walter, Chartier, Barrière & Montel, 1999).

3.1. Characterisation of dry fermented sausages

Analyses of the four types of sausage (five batches each) are nearly complete and involve a whole series of compounds. For presentation, results are grouped in four classes:

1. proximate composition and biochemistry, the latter involving major end products or groups of end products of carbohydrate, protein and lipid metabolism;
2. peptides, free amino acids, free fatty acids and aldehydes, considered to be desirable compounds;
3. amines and cholesterol oxides, considered to be undesirable compounds and
4. flavour compounds, together with results of GC–O and sensory analyses, as far as available.

For some results, data were subjected to statistical analyses of variance, in order to separate within and between batch variability.

3.1.1. Proximate composition and biochemistry

The data presented in Table 2 illustrate that Mediterranean technology produces sausages with higher pH values, but not necessarily higher dry matter contents. Besides length of drying period, differences in drying rate must be involved. The clearly higher protein content of the Italian sausage is probably related to the use of skimmed milk powder in its formulation. The differences in protein content are not reflected in different collagen contents (between 3.8 and 4.8 g/100 g DM for all types) and may be due to the use of different meat cuts. Values for pH are significantly lower in Northern type sausages, reflecting higher amounts of sugar added

Table 2
Proximate composition and end products of biochemical changes in Northern and Mediterranean types of sausages[a]

Sausage type[d]	Norway[e]	Belgium N[e]	Belgium S[e]	Italy[e]
Dry matter (%)	63 (3)b[b]	57 (4)d	67 (3)a	61 (2)c
pH	4.9 (1)a	4.8 (1)a	5.5 (2)b	5.7 (1)b
% in DM				
Crude protein	28 (7)c	31 (7)b	28 (9)c	35 (8)b
Crude fat	57 (3)	61 (7)	61 (8)	46 (3)
NaCl	8.5 (4)	5.3 (12)	6.1 (11)	6.8 (3)
mmol/100g DM				
Lactate	20 (16)b	21 (12)a	17 (17)c	17 (17)c
Acetate	0.94 (10)a	1.0 (14)a	0.86 (19)a,b	0.74 (11)b
Sugars	0.83 (18)b	0.56 (23)b	0.40 (18)c	9.3 (20)a
mg N/g N				
Peptide α-NH_2-N	25 (26)c	30 (13)a	27 (16)b	28 (17)b
Free α-NH_2-N	37 (14)a	23 (10)c	37 (13)a	31 (9)b
Ammonia - N	6 (35)b	3 (22)c	10 (18)d	14 (12)a
μg BSAeq/mgCP[c]				
Myosin (200 kDa)	14 (14)d	18 (30)b	25 (12)c	32 (25)a
HMM (150 kDa)	25 (16)	24 (21)	24 (8)	23 (26)
Actin (46 kDa)	31 (10)d	35 (14)b	39 (10)c	48 (21)a
38 kDa	17 (6)c	15 (7)b	15 (7)b	10 (10)a
mg/g TFA[c]				
Free fatty acids	46 (11)b	27 (4)a	37 (11)b	37 (11)b

[a] See Table 1. Mean values of 25 determinations per type (5 batches and 5 sausages per batch). For acetate, SDS-PAGE electrophoresis and free fatty acid determination (TLC) of Northern type sausages from Norway and Italy, only 1 sample per batch was used.
[b] 0 = coefficient of variation i.e. S.D. as % of the mean values.
[c] BSAeq = Bovine serum albumin equivalents determined by semi-quantitative SDS-PAGE; CP = crude protein; TFA = Total fatty acids.
[d] Belgium N and Belgium S indicate sausages produced in Belgium following Northern and Mediterranean technology, respectively.
[e] Different letters indicate significant differences ($P < 0.05$) between columns.

and probably different fermentation conditions. The higher pH values are consistent with lower and higher amounts of lactate and ammonia respectively (Demeyer, Vandekerckhove & Moermans, 1979). A constant lactate/acetate ratio is apparent (20.0 SD 0.8), suggesting a similar heterofermentative fermentation in all types.

One would expect however that a difference in sausage diameter would affect oxygen supply and thus fermentation pattern (Demeyer, Verplaetse & Gistelinck, 1986). Acetate may also be derived from lipid oxidation. The non-protein N fraction in total N is higher in Mediterranean sausages, mainly because of the higher ammonia contents and thus probably reflecting more bacterial activity. Slight and non consistent differences in free and peptide-bound α-NH_2–N are apparent, although clear differences were observed in myofibrillar protein contents. For the latter, higher residual amounts of myosin and actin were determined in the Mediterranean sausages with lower amounts of some proteolytic degradation products such as a 38 kDa fragment. Interpretation of these combined data is difficult however as results for peptide-bound α-NH_2–N only cover peptide molecular weights up to about 1000 Dalton, whereas SDS-PAGE does not cover peptide molecular weights below 10.000 Dalton. Differences seem to be related to the higher values of pH and DM content in Mediterranean types, and probably reflect pH and water activity effects on muscle cathepsin D activity (Lambregts et al., in preparation). Free fatty acid content was obviously determined by both length of ripening period and raw materials used. The latter may explain the highest value found in Norwegian sausage. Data for dry matter, proximate analysis and pH show variation coefficients below 10% and, for dry matter content, below 5%. Variation coefficients for the concentration of end products of biochemical changes however exceed 15% and reach 35%. Variance was mainly due to within batch variability for ammonia and peptide nitrogen concentration (52–77% of variability) except for the Norwegian sausages showing most of the variability between batches (60–69 % of variability). The latter may reflect variability in raw material characteristics, rather than in process technology.

3.1.2. Desirable compounds

It is clear from Table 3, that the total lipid fraction of the Belgian sausages was more unsaturated than the Norwegian and the Italian sausages. Such difference obviously reflects differences in the origin of the pork fat used, in relation to both age and feeding of the animals.

Comparison of the composition of the free fatty acid fraction with that of the total lipids confirms earlier reports of specific release of (poly-)unsaturated fatty acids. Muscle fibre enzyme activity is probably involved as suggested by the preferential hydrolysis of phospholipids (Hierro, de la Hoz & Ordóñez, 1997; Molly et al., 1996; Navarro, Nadal, Izqujerdo & Flores, 1997).

Results for aldehyde analysis show considerable variation: variation coefficients for all sausage types are between 50 and 100%. Nevertheless, Mediterranean sausages obviously contain higher amounts of 6–10 carbon straight chain aldehydes, with an outspoken difference for hexanal, a typical product of linoleic acid oxidation (de Man, 1992). Octanal and nonanal are oxidation products of oleic acid according to the same author.

In accordance with the data in Table 2, the highest free amino acid concentrations were found for the Norwegian type sausage. In all sausages a strikingly high concentration of free arginine is observed (for all sausages: 27% S.D. 6% of total free amino acids) as well as of free lysine (17% S.D. 2%). Similar concentrations were reported earlier for arginine, but not for lysine (Eerola et al., 1996). Some differences were observed for the relative amounts of the individual amino acids. The Norwegian sausage contained significantly higher amounts of alanine, whereas the amounts of tyrosine reflect the presence of tyramine (Table 4). An 80% v/v ethanol extract of the sausages allowed quantification of 10 chromatography peaks mainly containing small peptides (MW < 500 D). The peak surface distribution was type specific (Lambregts et al, 1998).

Table 3
Concentrations of desirable compounds in Northern and Mediterranean types of sausages[a]

Sausage type	Norway[f]	Belgium N[f]	Belgium S[f]	Italy[f]
Fatty acid[b]				
16:0	26.6b[c]	24.7a	24.2a	25.0
	(19.4)[d]	(18.5)	(16.2)	(17.7)
18:0	14.5b	12.7a	12.7a	12.6a
	(10.0)	(11.2)	(8.9)	(9.3)
18:1	42.0a,b	41.9a,b	41.3a	43.5b
	(45.3)	(44.5)	(50.3)	(48.2)
18:2	9.4a	13.2b	13.9b	11.9a,b
	(13.4)	(13.1)	(13.6)	(13.7)
18:3	0.9b	1.2c	1.2c	0.6a
	(1.3)	(2.3)	(1.2)	(0.3)
Aldehydes[e] *(μg/kg)*				
Hexanal	330	160	12,000	8000
Heptanal	25	17	360	160
Octanal	39	34	390	230
Nonanal	128	130	460	550
Decanal	77	140	69	120
Free amino acids (g/kg)[e]				
Total	7.26	5.61	6.79	6.59
glutamic acid	0.83	0.70	0.68	0.52
arginine	1.75	1.27	1.81	2.35
alanine	0.75	0.37	0.38	0.39
tyrosine	0.14	0.11	0.07	0.04
cysteine	0.41	0.31	0.27	0.26
leucine	0.41	0.52	0.56	0.47
lysine	1.20	0.85	1.32	1.06

[a] See Tables 1 and 2.
[b] Shorthand notation for palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids.
[c] % w/w in total fatty acids.
[d] ( ) = concentration in the corresponding free fatty acid fraction.
[e] Mean values of 5 samples/type.
[f] Different letters indicate significant differences ($P < 0.05$) between columns.

3.1.3. Undesirable compounds

Except for spermidine and spermine, data for amine contents display considerable variability (Table 4). This difference in variability probably reflects the natural presence of the polyamines spermidine and spermine in the meat and considerable environmental effects (pH, temperature,...) on bacterial amine production (Bover-cid, Schoppen, Izquierdo-Pulido & Videl-Carou, 1999). The main amine found is tyramine (38.5–61.5 % of total amines) and its concentration in the Mediterranean sausages reaches the limits reported as unsafe by some authors: 100–800 mg/kg of food (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font & Videl-Carou, 1997). Its formation is due to bacterial decarboxylation of tyrosine and may be a reflection of the presence of tyramine forming lactic acid bacteria

Table 4
Concentrations of undesirable compounds in Northern and Mediterranean types of sausages[a]

Sausage type	Norway	Belgium N	Belgium S	Italy
Amines (μg/g)[a]				
Tryptamine (38)[b]	14	18	56	24
Phenyl-ethylamine	1	5	22	35
Putrescine (74)	< 1	28	100	2
Cadaverine (52)	1	< 1	6	3
Histidine (60)	1	2	36	1
Tyramine (30)	17	70	160	160
Spermidine (13)	< 1	4	5	5
Spermine (9)	4	30	30	30
Cholesterol oxides (μg/g)	0.42 (19)[b]	0.95 (13)	1.42 (68)	0.52 (48)
% Cholesterol oxidation	0.06	0.13	0.17	0.06
% 5,6α-epoxycholesterol	55	81	73	25
TBARS[c] (mg/kg)	0.154	0.077	0.106	0.333
	2.382[d]	0.152	0.190	0.846

[a] See Table 1, mean values of five batches per type.
[b] ( ) = average variation coefficient (%) over different sausage types.
[c] TBARS = thiobarbituric acid reactive substances expressed as mg malondialdehyde equivalents/kg.
[d] Values obtained after vacuum packed exposure at 4°C to fluorescent light for 45 days.

(Eerola, Maijala, Roig-Sangués, Salminen & Hirvi, 1996) although both *Lactobacillus* and *Enterococcus* were shown to produce tyramine (Montel et al., 1998). As the contaminating meat flora, such as enterobacteria, would mainly be involved in the production of cadaverine and putrescine (Roig-Sangués & Eerola, 1997), the presence of putrescine in both Belgian sausages may be a reflection of the hygienic quality of the raw materials used. Indeed, *Enterobacteriaceae* were present on the Belgian raw materials, in contrast to the other types (Montel, personal communication).

Results on lipid oxidation products reveal some interesting differences: levels of cholesterol oxides are higher in the Belgian sausages, both in absolute amounts and relative proportion of cholesterol oxidised, although they clearly show low levels for TBARS values, in line with the widespread use of vitamin E in the Belgian pig diet (60 ppm added) and in the Italian pig diet (no vitamin E added at that time). Also, oxidation patterns clearly differ as 7-ketocholesterol is the major oxidation product in the Italian sausage, in contrast to 5,6α-5,6-epoxycholesterol, the major end product in the other types. It should be stressed that the values found for cholesterol oxidation products are about 100 times lower than those required for toxicity in vivo with laboratory animals (Bösinger, Lut & Brandl, 1993), whereas the most toxic cholestanetriol and 25-hydroxycholesterol could never be detected. Thiobarbituric acid reactive substance (TBARS) concentrations were always below the suggested threshold for the appearance of rancidity off flavours in fresh pork (0.5 mg MDA/kg; Lanari, Schaefer & Scheller, 1995). The lowest concentrations were found in the Belgian sausages. These also show more resistance to lipid oxidation following exposure to fluorescent light. The striking contradiction of low TBARS levels and high levels of cholesterol oxides in Belgian sausages indicates the different oxidation susceptibility among cholesterol at one side and unsaturated fatty acids at the other. Each group may act differently under different processing conditions (raw materials, additives, mincing, temperature, oxygen pressure...).

3.1.4. Flavour compounds and sensory analyses

Flavour is a complex sensory reaction involving taste, smell (odour) and texture of a product. Odour or aroma is by far the most important component, because of the high sensitivity of the nasal receptors for the numerous volatile components released during chewing and ingestion. Flavour studies often involve analyses of volatile compounds in a (dynamic) headspace by GC–MS (Stahnke, 1995). Analyses after separation of volatile compounds by (steam) destillation is claimed to be subject to lower variability (Dirinck, Van Opstaele & Vandendriessche, 1997; Schmidt & Berger, 1998). Obviously, method of isolation affects the number and relative proportion of the volatile compounds, but, in general a major proportion is derived from fatty acid oxidation and includes alkane, alkene, aldehyde, ketone and carboxylic acid families, with low threshold values for the last three. The dry sausage aroma is associated with the dominance of 2-methyl ketones, whereas a rancid aroma is associated with hexanal (Montel et al., 1998).

The branched-chain aldehydes 2- and/or 3-methylbutanal are important components of the dry sausage aroma (Stahnke, 1995). Bacterial metabolism of L-leucine, mainly by *Staphylococcus*, and to a less extent by *Lactobacilli*, is generally held responsible for the production of 3-methyl butanal (Berdagué, Monteil, Montel & Talon, 1993; Masson, Hinrichsen, Talon & Montel, 1999; Møller, Hinrichsen, Andersen, 1998).

Ethyl esters of carboxylic acids are always present in fermented meat products, have low sensory threshold values and contribute a fruity note to the flavour. They are both produced (Talon, Chastagnal et al., 1998) and hydrolysed (Talon & Montel, 1997) by *Staphylococcus*.

Acids derived from carbohydrate fermentation induce an acid taste (D-lactate) or aroma (acetic acid), whereas diacetyl and acetoin, again associated with *Staphylococcus*, impart a buttery flavour (Montel et al., 1998).

Although compounds derived from spices and smoking (Northern types) dominated, compounds derived from metabolism are important for the specific sausage flavour. Recently, Schmidt and Berger (1998) identified 126 different compounds from salami produced in France, Italy, Spain and Germany. Most of the sensory active compounds occurred in all sausages, but often in very different proportions. Diallyl disulphide and eugenol, compounds derived from garlic and nutmeg respectively, had the highest odour activities, but were followed by 3-methylbutanoic and acetic acid, end products of metabolism and/or oxidation.

The volatile compounds, isolated by dynamic head space extraction of sausages, have been separated, identified and quantified by GC-MS yielding 106 different structures in the sausages (Stahnke et al., 1999). The experimental sausages were also used as reference materials in a Eureka project. Table 5 presents a sample of preliminary data and illustrates difficulties in interpretation of apparent differences in the composition of the volatile compounds derived from metabolism and/or oxidation, both for different sausages and different laboratories. Although data are often subject to enormous variability (variation coefficients between 50 and 100%) some tentative interpretation may be ventured: lipid oxidation, reflected in hexanal concentration, seems to be more important in Mediterranean sausages. The same conclusion is valid for amino acid metabolism, reflected in the presence of branched chain aldehydes and alcohols. The latter finding may be related to the strong inhibitory effects of pH values below 5.4, the presence of 0.01% nitrite and low temperature on the

Table 5
Volatile compounds representative of metabolism in dynamic headspace samples of Northern and Mediterranean types of sausages (mean values only of 2–5 sausages)

Type/compound	Norway		Belgium N		Belgium S		Italy	
	(1)[a]	(2)[b]	(1)	(2)	(1)	(2)	(1)	(2)
Hexanal	266	1104	123	2836	227	11568	263	4461
3-Me butanal	97	875	44	856	32	941	18	505
3-Me butanol	366	889	355	580	315	1015	188	561
2-Me butanal	34	183	7	198	7	225	14	181
2-Me butanol	91	0	22	0	47	0	17	0
Ethyl esters	247	82	221	0	82	625	100	0
Diacetyl + acetoin	4246	562	3052	311	681	167	503	1599

[a] As nmol 4-methyl, 2-pentanon/kg (Stahnke et al., 1999).
[b] ng/kg (Dirinck, personal communication).

initial metabolism of leucine by *Staphylococci* (Larrouture et al., 1998). Such conditions are indeed typical for the Northern sausages. Relative amounts of aldehydes and alcohols however are very variable. Catabolites from microbial metabolism of isoleucine and valine, such as 2-methyl-1-butanol and 2-methyl-1-propanol, respectively, were found to characterise the four types of sausages. However, both compounds were present in higher ratios in Norwegian and in Belgian Mediterranean type, compared to Italian and Belgian Northern type of sausages.

Most ethyl esters are found in the Belgian Mediterranean type. Acetic acid is absent in all analyses, yet Northern types were characterised also by vinegar odours, associated with acetic acid in static headspace analysis by GC–O. The Northern types are characterised by a higher proportion of the dairy type metabolites acetoin and diacetyl. It is clear that more detailed analyses of all data using e.g. PCA can be used to specify flavour differences (Stahnke, 1995). Only limited amounts of nitrogen-containing compounds are found in the volatiles, yet, extent of proteolysis as reflected in non-protein N and free amino acid concentrations, is directly related to flavour evaluation (Demeyer, 1992). The typical flavour of fermented sausages containing a surface mould growth was found to be proportional to the degree of proteolysis (Lücke, 1999) and surface inoculation with mould strains were found to enhance proteolysis, especially in sausages filled in natural casings (Toledo, Selgas, Cusa, Ordoñez & Garcia, 1997). Such findings suggest that most of the compounds carrying the characteristic aroma of fermented meat are derived from the protein fraction of the sausage. However, intensity of proteolysis may liberate peptides affecting taste, rather than aroma (Nishimura, Rhue, Okitani & Kato 1988). Also, the non-protein nitrogen fraction will affect sausage pH (Demeyer et al., 1979) and sausage pH may affect liberation of aroma determining acid compounds during chewing (Dainty & Blom, 1992) (Dirinck, personal communication). It may also be instructive to remember that amounts of extractable volatile compounds decrease with ripening time (Viallon et al., 1996). In line with the importance of mould protein metabolism, GC–O characterised Mediterranean sausages by a "pop corn" odour note, identified as 2-acetyl-1-pyrroline, possibly originating from mould metabolism (Stahnke et al., 1999). Diacetyl, acetic acid and hexanal were associated with buttery, vinegar and green odour notes, respectively. Sensory analyses have to be obtained from panels trained in Mediterranean and Northern flavours both. Such panel characterised the Norwegian sausages as most acid and the Mediterranean types as most "mature". The panel only detected taste differences between the Mediterranean types for the spice-related descriptors "pepper" and "spicy". Response of semiconductor gas sensors to volatile compounds, without analysis and separation has been proposed as a principle for rapid and robust identification of fermented sausages as well other meat products and bacterial strains (Eklöv et al., 1998; Vernat-Rossi, Garcia, Talon, Denoyer & Berdagué, 1996). Preliminary results indeed indicate that the matrix of the responses of 16 sensors of an electronic nose could be correlated with the matrix of the raw data of sensory analyses: $r^2 = 0.75$–0.95 (Hagen & Holck, unpublished data).

3.2. Characterisation of raw materials and bacteria

It is now clear that metabolism during dry sausage ripening is a complex interaction between residual enzyme activity of muscle and/or fat tissue and bacterial and/or fungal metabolism. The former produces free fatty acids, peptides and free amino acids as substrates for the production of flavour and other compounds through microbial and non-biological reactions, the latter mainly involving lipid oxidation (Demeyer, 1992; Molly, Demeyer, Johansson, Raemackers, Gristelinck & Greenen, 1997; Molly et al., 1996; Verplaetse, Gerard, Buys & Demeyer, 1992).

3.2.1. Muscle metabolic potential

Meat protease and lipase activities may be increased by freezing and comminution, respectively. But muscle, genetic and sex related differences in proteolytic and lipolytic enzymes have been reported for pork (Armero, Barbesa, Taldrà, Baselga & Pla, 1999; Flores, Alasnier, Aristoy, Navaro, Gandemer & Toldrà, 1996), whereas carcass conformation is known to affect muscle proteolytic activity and thus beef tenderness (Uytterhaegen et al., 1994). Similar differences may affect the rate of substrate supply for bacterial metabolism and for lipid oxidation during dry sausage ripening. We, therefore, investigated the effect of carcass conformation and sex on cathepsin D and acid lipase activity of a muscle and its metabolism in aseptic conditions simulating the sausage environment (Harnie et al., in preparation). Ten barrows and 10 sow carcasses were used, with meat percentages varying between 50 and 68%. Cathepsin D and acid lipase activities showed variation coefficients of 17 and 14%, respectively. The activities were related to peptide and free fatty acid production respectively. Cathepsin D activity was higher for sows than for barrows (Fig. 1). Whether such differences are important in sausage ripening remains to be established through study of flavour development using different meats.

3.2.2. Effects of bacteria on leucine metabolism and on lipid oxidation

In continuation of earlier work, Laroutture et al. (1998) showed that all strains of Lactic acid bacteria and *Staphylococci* that can be present in fermented meat are able to catabolise leucine to 3-methyl butanoic acid. Only *Carnobacterium* and some *Staphylococci* however are able to produce 3-methyl-butanal, a flavour compound with much lower threshold value (Møller, Hinrichsen & Andersen, 1998). The highest overall production of leucine metabolites by *Staphylococcus carnosus* was observed in the absence of nitrate (Masson, Hinrichsen, Talon & Montel, 1999).

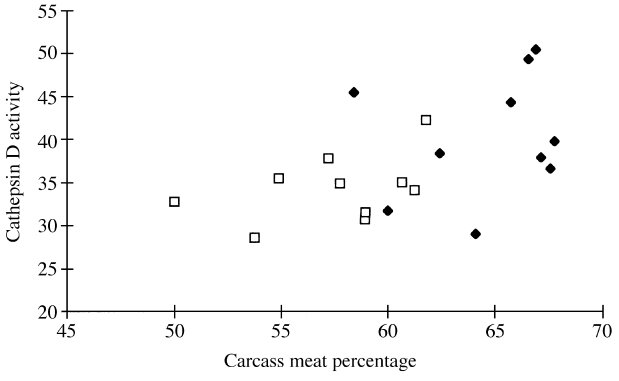

Fig. 1. Cathepsin D activity (U/g) in M. *triceps brachii* in relation to carcass meat percentage for barrows (□) and sows (◆).

Bacteria interfere with the oxidation of lipids and the production of carbonyls (Smith & Alford, 1969). Bacterial lipases show very low activity under conditions found in fermented sausages (Hierro et al., 1997; Kenneally, Leuschner & Arendt, 1998; Navarro et al., 1997) and anti- or pro-oxidant activity is probably a more important selection criterion for starter cultures than lipolytic activity. The interaction of *Staphylococci* and *Lactobacilli* often used as starter cultures with the oxidation of free poly-unsaturated fatty acids was therefore studied in pure culture (Talon & Walker et al., 1998). A representative sample of results illustrates that no strains had pro-oxidant activity. Several strains of *Staphylococci* inhibit oxidation of linoleic acid but not of linolenic acid (Table 6). In the presence of Mn (0.05 g/l) in the medium *S. xylosus* and *carnosus* strongly inhibit linolenic acid oxidation. Lactic acid bacteria had no such effect in the absence of Mn. In the presence of Mn, several species of *Lactobacillus* inhibited linoleic acid oxidation. The antioxidant properties of *Staphylococci* can be explained by their synthesis of cytosolic catalase and Mn superoxide dismutase. With *Lactobacillus*, scavenging of superoxide radicals is probably brought about by high intracellular Mn concentrations.

3.2.3. Bacteriocin production

According to Lücke (1999), the most important mechanism to ensure microbial safety of fermented sausages appears to be the production of lactic acid. The effect of bacteriocins on the overall safety of meat products is limited because of the resistance of Gram-negative bacteria such as *Escherichia coli* to them, their inactivation in the meat and the possibility of resistance development in the target organisms. In fact, outbreaks of cases of diarrhoea caused by *E. coli* 0157:H7 by consumption of dry-cured salami have been laboratory confirmed and not all types of dry sausage types meet criteria of pH and a_w for safety (Lee & Stylliadis, 1996). Nevertheless, although the growth potential of *List. monocytogenes* in fermented sausage is low and no report has been made of an outbreak of listeriosis associated with the consumption of fermented meat products, bacteriocin–producing lactic acid bacteria could be used to lower the level of this pathogen in fermented

Table 6
Effect of *Staphylococci* and *Lactobacilli* on free fatty acid oxidation (μmol TBARS/g substrate)[a]

	Sterile	Control	*Staphylococcus* sp.	*C. divergens*	*L. sakei*
C18:2	15.51		2.82	13.19	14.99
C18:2 + Mn	17.68			12.94	6.28
C18:3	31.73		26.21		
C18:3 + Mn	15.41		4.39		

[a] From Talon, Walker et al. (1998a).

sausage by one or two log units, compared to non-bacteriocin producing lactic acid bacteria (Hugas, Garriga, Aymerich & Montfort, 1995; Lücke, 1999).

It was therefore judged worthwhile to investigate environmental effects on *L. sakei* CTC 494, an isolate from fermented sausage. It was found that the temperature and pH conditions prevailing during the fermentation stage of sausage ripening are optimal for production of its bacteriocin sakacin K. The addition of salt and, to a less extent, nitrite reduces specific bacteriocin production. Mn and lactic acid production have stimulatory and inhibitory effects on cell growth respectively. All results could be incorporated successfully into a mathematical model (Leroy & De Vuyst, 1999, in press).

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References

Armero, E., Barbosa, J.-A., Toldrá, F., Baselga, M., & Pla, M. (1999). Effects of the terminal sire type and sex on pork muscle cathepsins (B, B + L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. *Meat Science, 51*, 185–189.

Barrett, A. J., Kirschke, H., & Lorand, L.,. Methods of enzymology, Vol. 80, part C: proteolytic enzymes (pp. 567). New York: Academic Press.

Berdagué, J. L., Monteil, P., Montel, M. C., & Talon, R. (1993). Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat Science, 35*, 275–287.

Blankenship, D. T., Krivanek, A. M., Ackermann, B. L., & Cardin, A. D. (1989). High sensitivity amino acid analysis by derivatization with o-phtalaldehyde and 9-fluorenylmethyl chloroformate using fluorescence detection: application in protein structure determination. *Analytical Biochemistry, 178*, 227–232.

Boehringer, M. (1995). *Methods of enzymatic bioanalysis and food analysis*. Mannheim, Germany: Boehringer Mannheim GmbH Biochemicals (pp. 6–9).

Bösinger, S., Luf, W., & Brandl, E. (1993). Oxysterols: their occurrence and biological effects. *International Dairy Journal, 3*, 1–33.

Bover-Cid, S., Schoppen, S., Izquierdo-Pulido, M., & Videl-Carou, M. C. (1999). Relationship between biogenic amine contents and the size of dry fermented sausages. *Meat Science, 51*, 305–311.

Brückner, H., Wittner, R., & Godel, H. (1991). Fully automated high performance liquid chromatographic separation of DL-amino acids derivatized with o-phtaladehyde together with N-isobutyryl-cysteine. Application to food samples. *Chromatography, 32*, 383–388.

Chizzolini, R., Zanardi, E., & Dorigoni, V. (1999). Antioxidants and oxidative processes of lipids and cholesterol in a dry fermented meat product (*Salame Milano*). *Proceedings of the 45th International Congress on Meat Science and Technology, Barcelona, 2*, 662–663.

Claeys, E., Uytterhaegen, L., Buts, B., & Demeyer, D. (1995). Quantification of Beef Myofibrillar Proteins by SDS-PAGE. *Meat Science, 39*, 177–193.

Conway, E. J. (1957). *Microdiffusion analysis and volumetric error* (4th ed.). London: Crosby, Lockwood and Son Ltd.

Dainty, R. H., & Blom, H. (1992). Flavour chemistry of fermented sausages. In G. Campbell-Platt, & P. E. Cook, *Fermented Meats* (pp. 176–193). London: Blackie Academic and Professional.

de Man, J. M. (1992). Chemical and physical properties of fatty acids. In C. K. Chow, *Fatty acids in foods and their health implications* (pp. 17–47). New York: Dekker.

Demeyer, D. I. (1992). Meat fermentation as an integrated process. In F. J. M. Smulders, F. Toldrá, J. Flores, & M. Prieto, *New technologies for meat and meat products* (pp. 21–36). Nijmegen: ECCEAMST/Audet tijdschriften.

Demeyer, D. I., Vandekerckhove, P., & Moermans, R. (1979). Compounds determining pH in dry sausage. *Meat Science, 3*, 161–167.

Demeyer, D. I., Verplaetse, A., & Gistelinck, M. (1986). Fermentation of meat: an integrated process. *Proceedings of the 32nd European Meetings of Meat Research Work*. (pp. 241–246).

Dirinck, P., Van Opstaele, F., & Vandendriessche, F. (1997). Flavour differences between Northern and Southern European cured hams. *Food Chemistry, 59*, 511–521.

Eerola, S., Hinkkanen, R., Lindfors, E., & Hirvi, T. (1993). Liquid chromatographic determination of biogenic amines in dry sausages. *Journal of AOAC International, 76* (3), 575–577.

Eerola, S., Maijala, R., Roig-Sangués, A.-X., Salminen, M., & Hirvi, T. (1996). Biogenic amines in dry sausages as affected by starter culture and contaminant amine-positive *Lactobacillus*. *Journal of Food Science, 61*, 1243–1246.

Eklöv, T., Johansson, G., Windquist, F., & Lundström, I. (1998). Monitoring sausage fermentation using an electronic nose. *Journal of the Science of Food and Agriculture, 76*, 525–532.

Flores, M., Alasnier, C., Aristoy, M.-C., Navarro, J.-L., Gandemer, G., & Toldrà, F. (1996). Activity of aminopeptidase and lipolytic enzymes in five skeletal muscles with various oxidative patterns. *Journal of the Science of Food Agriculture, 70*, 127–130.

Harnie, E., Claeys, E., Raemaekers, M., & Demeyer, D. (1998). Lipolysis and proteolysis in aseptic meat-fat model systems. 12th Forum for Applied Biotechnology, Brugge, 24–25 September, 1998. *Mededelingen faculteit landbouwkundige en toegepaste biologische wetenschappen, Universiteit Gent, 63* (4b), 1535.

Herbert, D., Phipps, P. J., & Strange, R. E. (1971). Chemical analysis of microbial cells. In J. R. Norris, & D. W. Ribbons, *Methods in microbiology*, (Vol. 5 B). London: Academic press.

Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Mariné-Font, A., & Videl-Carou, M. C. (1997). Biogenic amine and polyamine contents in meat and meat products. *Journal of Agricultural and Food Chemistry, 45*, 2098–2102.

Hierro, E., de la Hoz, L., & Ordóñez, J. A. (1997). Contribution of microbial and meat endogenous enzymes to the lipolysis of dry fermented sausages. *Journal of Agricultural and Food Chemistry, 45*, 2989–2995.

Hugas, M., Garriga, M., Aymerich, M. T., & Montfort, J. M. (1995). Inhibition of Listeria in dry fermented sausages by the bacte-riocinogenic *Lactobacillus sake* CTC494. *Journal of Applied Bacteriology, 79*, 322–350.

Kenneally, P. M., Leuschner, R. G., & Arendt, E. K. (1998). Evaluation of the lipolytic activity of starter cultures for meat fermentation purposes. *Journal of Applied Microbiology, 84*, 839–846.

Lambregts, L., Raemaekers, M., & Demeyer, D. (1998). Peptides involved in flavour development of dry fermented sausage. 12th Forum for Applied Biotechnology, Brugge, 24-25 September, 1998. *Mededelingen faculteit landbouwkundige en toegepaste biologische wetenschappen, Universiteit Gent, 63* (4b), 1537.

Lanari, M. C., Schaefer, D. M., & Scheller, K. K. (1995). Dietary vitamin E supplementation and discoloration of pork bone and

muscle following modified atmosphere packaging. *Meat Science, 41*, 337–350.

Larrouture, C., Masson, F., Talon, R., & Montel, M. C. (1998). Production of aromatic compounds from leucine by lactic acid bacteria and *Staphylococci*. *Proceedings of the 44th International Congress on Meat Science and Technology, Barcelona, 2*, 792–793.

Lee, M. B., & Styliadis, S. (1996). A survey of pH and water activity levels in processed salamis and sausages in Toronto. *Journal of Food Protection, 59*, 1007–1011.

Leroy, F., & De Vuyst, L. (1999). The temperature and pH conditions that prevail during fermented sausage production are optimal for the production of the bacteriocin sakacin K. *Applied and Environmental Microbiology, 65*, 974–981.

Leroy, F., & De Vuyst, L. (in press). The presence of salt and curing agents reduces the bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation. *Applied and Environmental Microbiology*.

Lücke, F.-K. (1999). Utilization of microbes to process and to preserve meat. *Proceedings of the 45th International Congress on Meat Science and Technology, Yokohama (Japan), 2*, 538–547.

Masson, F., Hinrichsen, L., Talon, R., & Montel, M. C. (1999). Factors influencing leucine catabolism by a strain of *Staphylococcus carnosus*. *International Journal of Food microbiology, 49*, 173–178.

Messineo, L., & Musarra, E. (1972). A sensitive spectrophotometric method for the determination of free or bound tryptophan. *International Journal of Biochemistry, 3*, 700–704.

Møller, J. K. S., Hinrichsen, L. L., & Andersen, H. J. (1998). Formation of amino acid (L-leucine, L-phenylalanine) derived volatile flavor compounds by *Moraxella phenylpyruvica* and *Staphylococcus xylosus* in cured meat model systems. *International Journal of Food Microbiology, 42*, 101–117.

Molly, K., Demeyer, D., Civera, T., & Verplaetse, A. (1996). Lipolysis in a Belgian Sausage: relative importance of endogenous and bacterial enzymes. *Meat Science, 43*, 235–244.

Molly, K., Demeyer, D., Johansson, G., Raemaekers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. First results of a European project. *Food Chemistry, 59*, 539–545.

Montel, M. C., Masson, F., & Talon, R. (1998). Bacterial role in flavour development. *Meat Science, 49*(Suppl.1), S111–S123.

Motilva, M.-J., Toldrá, F., & Flores, J. (1992). Assay of lipase and esterase activities in fresh pork meat and dry-cured ham. *Zeitschrift für Lebensmittel Untersuchung Forschung, 195*, 446–450.

Navarro, J. L., Nadal, M. I., Izquierdo, L., & Flores, J. (1997). Lipolysis in dry cured sausages as affected by processing conditions. *Meat Science, 45*, 161–168.

Nishimura, T., Rhue, M. R., Okitani, A., & Kato, H. (1988). Components contributing to the improvement of meat taste during storage. *Agricultural and Biological Chemistry, 52*, 2323–2330.

Oddy, V. H. (1974). A semiautomated method for the determination of plasma alpha amino nitrogen. *Clinica Chimica Acta, 51*, 151–156.

Reindl, B., & Stan, H.-J. (1982). Determination of volatile aldehydes in meat as 2,4-dinitrophenylhydrazones using reversed-phase high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 30*, 849–854.

Roig-Sangués, A., & Eerola, S. (1997). Biogenic amines in meat inoculated with *Lactobacillus sakei* starter strains and an amine-positive bacterium. *Z. Lebensm. Unters. Forsch. A, 205*, 227–231.

Schmidt, S., & Berger, R. G. (1998). Aroma compounds in fermented sausages of different origins. *Zeitschrift für Lebensmittel Untersuchung Forschung, 31*, 559–567.

Smith, J. L., & Alford, J. A. (1969). Action of microorganisms on the peroxides and carbonyls of fresh lard. *Journal of Food Science, 34*, 75–82.

Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels-Part II. *Volatile components. Meat Science, 41*, 193–209.

Stahnke, L. H., Sunesen, L. H., & De Smedt, A. (1999). Sensory characteristics of European, dried, fermented sausages and the correlation to volatile profile. Invited lecture, 13th Forum for Applied Biotechnology, Gent, 22–23 September, 1999. *Mededelingen faculteit landbouwkundige en toegepaste biologische wetenschappen, Universiteit Gent, 64*, 559–566.

Talon, R., & Montel, M. C. (1997). Hydrolysis of esters by *Staphylococci*. *International Journal of Food Microbiology, 36*, 207–214.

Talon, R., Chastagnac, C., Vergnais, L., Montel, M. C., & Berdagué, J. L. (1998). Production of esters by *Staphylococci*. *International Journal of Food Microbiology, 45*, 143–150.

Talon, R., Walker, D., & Montel, M. C. (1998). Effect of *Staphylococci* and lactic acid bacteria on the oxidation of unsaturated free fatty acids. *Proceedings of the 44th International Congress on Meat Science and Technology, Barcelona, 2*, 796–797.

Talon, R., Walter, D., Chartier, S., Barrière, C., & Montel, M. C. (1999). Effect of nitrate and incubation conditions on the production of catalase and nitrate reductase by *Staphylococci*. *International Journal of Food Microbiology, 52*, 47–56.

Toledo, V. M., Selgas, M. D., Casa, M. C., Ordóñez, J. A., & Garcia, M. L. (1997). Effect of selected mould strains on proteolysis in dry fermented sausages. *Zeitschrift für Lebensmittel Untersuchung Forschung, 204*, 385–390.

Uytterhaegen, L., Claeys, E., Demeyer, D., Lippens, M., & Fiems, L. O., Boucqué, Ch. V., Van de Voorde, G., & Bastiaens, A. (1994). Effect of double-muscling on carcass quality, beef tenderness and myofibrillar protein degradation in Belgian Blue bulls. *Meat Science, 38*, 255–267.

Vernat-Rossi, V., Garcia, C., Talon, R., Denoyer, C., & Berdagué, J.-L. (1996). Rapid discrimination of meat products and bacterial strains using semiconductor gas sensors. *Sensors and actuators, B37*, 43–48.

Verplaetse, A. (1994). Influence of raw meat properties and processing technology. *Proceedings of the 40th Int. Congress on Meat Science and Technol*ogy (pp. 45–65). The Hague.

Verplaetse, A., Gerard, S., Buys, E., & Demeyer, D. (1992). Endogenous and Bacterial proteolysis in dry sausage fermentation. *Proceedings of the 38th International Conference on Meat Science and Technology* (Vol. 4, pp. 851–854). Clermont-Ferrand, France.

Viallon, C., Berdagué, J. L., Montel, M. C., Talon, R., Martin, J. F., Kondjoyan, N., & Denoyer, C. (1996). The effect of stage of ripening and packaging on volatile content and flavour of dry sausage. *Food Research International, 29*, 667–674.