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Bacterial Role in Flavour Development

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ABSTRACT

The role of bacteria in the production of non volatile and volatile compounds involved in the fermented meat flavour is discussed. Lactic acid bacteria produce D-lactic and acetic acids which may give a sour note. By reducing the pH, they also modulate the other biochemical bacterial activities. In muscle tissue proteins are degraded into peptides and lipids into fatty acids mainly by endogenous enzymes. In fermented meat products with a high pH lipases from very lipolytic species of Staphylococcus could increase lipolysis. Bacteria could also play a role in the production and degradation of free amino acids. Staphylococcus and to a lesser extent, lactic acid bacteria could participate in the production of methyl-branched aldehydes and their corresponding alcohols and acids from branched-chain amino acids. By their nitrate reductase and catalase Staphylococcus species limit fatty acid oxidation and aldehyde production. Staphylococcus could contribute to the ester content as they can produce or hydrolyse esters in vitro. *© 1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

The characteristic flavour of fresh meat, fermented meat products or dry cured ham is a subtle balance between non-volatile compounds with taste properties and volatiles which both interact with each other and with proteins and lipids. The flavours distinguishing meat products are associated with variations in the type of these different components and an imbalance among them could generate off flavours. The tasty and aromatic components come from the raw material, or, in the case of fermented meat products or cured products, from the ingredients (for example, garlic, wine, spice). They also result from carbohydrate, protein and lipid degradation, but, above all, from amino acids, fatty acids and nucleotides. The processes are linked to endogenous and microbial enzyme activities, and also to chemical reactions dependent on the technological processing. The relative contribution of microbial flora is governed by the identity and the number of micro-organisms, their intrinsic metabolic activities and the expression of these activities in the products. These activities depend on the meat composition, any added ingredients such as sugars, salt, nitrate, nitrite, and on the technological variables such as the pH, the

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temperature, the gaseous environment and the extent of drying. Dainty and Mackey (1992) reviewed the relationship between meat off-flavour and the production of volatile compounds by bacteria, from which we shall only give a few examples. On fresh meat stored in air, excessive growth of *Pseudomonas fragi* increases the production of ethyl esters which are responsible for a fruity odour or sulphur containing compounds which can cause putrid and sulphury odours. Contamination by *Brochothrix thermosphacta*, which produces diacetyl and acetoin, may lead to dairy odours. The sour taste of certain vacuum packed meats has been attributed to the production of methanethiol and dimethyldisulfide by *Enterobacteriaceae* or by certain lactic bacteria rather than to the production of acids. One of the ways to improve meat flavour is to control spoilage flora. The role of microbial flora in the development of the characteristic flavour of dry-cured ham is seldom taken into account (Berdagué *et al.*, 1991; Garcia *et al.*, 1991; Barbieri *et al.*, 1992) except to impute off-flavour to micro-organisms (Vidal-Aragón *et al.*, 1994).

On the contrary, in fermented meat products, microbial flora might well participate to the development of their characteristic flavours. The inside bacterial flora is dominated by lactic acid bacteria, often *Lactobacillus sake*, *Lactobacillus curvatus* and *Lactobacillus plantarum*, which exceed 10^6/g at the end of the fermentation period, whether the products are inoculated or not. The species *Pediococcus acidilactici* and *Pediococcus pentosaceus* are also inoculated in the starter culture. *Kocuria varians* (ex *Micrococcus varians*) and *Kocuria kristinae* (ex. *Micrococcus kristinae*), and especially *Staphylococcus warneri*, *Staphylococcus saprophyticus*, *Staphylococcus carnosus* and *Staphylococcus xylosus* could be present, but only the last two species are used as a starter culture (Nychas and Arkoudelos, 1990). Their count in meat products varies according to the technology and, in particular the pH. They are absent in certain Nordic products and generally stabilize at around 10^6/g. *Enterococcus* could also be present, but most often below 10^4/g. Yeasts and moulds comprise external flora for the products.

The high count of lactic acid bacteria and *Micrococcaceae* in fermented meat products has led us to concentrate, in this presentation, on their role in the flavour of these products. The precise role of this bacterial flora is difficult to quantify, since it may complement, or superimpose on product activities and chemical processes. Their role can be estimated from *in-vitro* metabolic activities but is better understood using aseptic models into which specific flora are inoculated. Unfortunately there are difficult to prepare as it is impossible to pasteurize or to sterilize the product. Paucimicrobial or sterile products were obtained through the addition of antibiotics (Verplaetse *et al.*, 1992; Molly *et al.*, 1996) or by decontamination of the raw material with the right hygienic conditions for manufacturing (Ordóñez *et al.*, 1989; Johansson and Borch, 1993). We shall examine in order the potential and real effects of lactic acid bacteria or *Micrococcaceae* on the catabolism of carbohydrates, proteins and amino acids, lipids and fatty acids, and show their importance in flavour production.

CARBOHYDRATE CATABOLISM

The acid taste is one of the components of the overall taste of fermented meat products. It is often appreciated and sought after in Northern Europe, whereas it may be rejected in the South of Europe when it can become too strong, masking the much more popular dry sausage aroma. The acid taste is positively correlated to the D-lactate content (Bucharles *et al.*, 1984; Ramihone *et al.*, 1988). Dry sausages which are considered too acid have about 5mg D-lactate/g, which is 4 times higher than in normal dry sausages. The sour taste of German salami would also appear to correspond to its 5-fold higher D-lactate concentration compared with Italian salami (Marchesini *et al.*, 1992). The production of

acids in dry sausage depends on the type and concentration of sugars added to the meat mixture, the diameter of the dry sausage and other technological factors (Flores and Bermell, 1996). But it is particularly dependent on the bacterial species. Lactic acid bacteria (*L. sake*, *L. curvatus*, *L. plantarum*, *P. pentosaceus*, *P. acidilactici*) have a strong influence on the production of L-lactate, but especially of D-lactate, an acid of purely bacterial origin. *Leuconostoc* produces only D-lactate and following inoculation produce an acid off-taste (Ramihone *et al.*, 1988). *S. warneri*, a significant producer of D-L lactate in laboratory medium increases, D-lactate in sausages which were subsequently considered to be more acidic (Montel *et al.*, 1993)

The acid odour is often weakly correlated with acetic acid or lactate (Bucharles *et al.*, 1984) although Stahnke (1995*b*) found that acetic acid gave a sour note to salami. Acetic acid contributes to dry sausage aroma (Berdagué *et al.*, 1993) and may be produced by homofermentative lactic acid bacteria and *Staphylococcus* (Kandler, 1983; Schleifer, 1986; Arkoudelos and Nychas, 1995) and also by fatty acid oxidation and by alanine catabolism.

The contribution of other acids such as, formic acid or other acids originating from fatty acids or amino acids, to the acid taste is still unclear (Girard and Bucharles, 1988).

The production of acids is primarly responsible for the decrease in the pH in products and is counterbalanced by ammonia production (Demeyer *et al.*, 1979). This acidification is very important for texture and also for flavour development (Flores and Bermell, 1996). It is also one of the determining factors in the development of *Staphylococcus* species and limits the metabolism of bacteria, as we shall see later.

The buttery or dairy aroma which is noted in certain dry sausages is also associated with pyruvate catabolism and has been associated with high desorption of diacetyl and acetoin after inoculation of *S. saprophyticus* and *S. warneri* (Berdagué *et al.*, 1993). The lowest desorption was associated with a dominant dry sausage aroma when *S. carnosus* and *S. xylosus* were inoculated. These desorption differences between products inoculated with different *Staphylococcus* correlated with those noted in laboratory medium from pyruvate catabolism (Montel *et al.*, 1996). *P. acidilactici* and *P. pentosaceus* produced more acetoin than *L. sake* (Lücke and Hechelmann, 1987) in laboratory media, but their low production in products was similar (Berdagué *et al.*, 1993).

PROTEOLYSIS

The contribution of peptides and amino acids to the taste of dry sausage has not yet been clearly determined. They appear to have a direct influence on the taste, but they also act in synergy with each other and with other constituents. Individual amino acids and certain peptides in aqueous solution have their own taste with generally high threshold ($> 50\,mg\,l^{-1}$ for glutamate, up to $2000\,mg\,l^{-1}$ for leucine) (Kato *et al.*, 1989). Henriksen and Stahnke (1997) have identified amino acids and peptides which would appear to contribute to the taste.

Strong, rapid increases in amino acid concentrations following proteinase addition has no marked impact on the final flavour but accelerates maturation (Naes *et al.*, 1995; Diaz *et al.*, 1996, 1997). Similarly, products which had different amino acids composition, after the inoculation of *P. pentosaceus* or *P. acidilactici* in association with *K. varians*, had identical sensory characteristics (De Masi *et al.*, 1990).

In fermented meat products, protein degradation into peptides is due to endogenous enzymes since the addition of antibiotics, which inhibit bacteria growth, does not reduced degradation of actin, myosin or troponin (Verplaetse *et al.*, 1992; Verplaetse, 1994). Lactic acid bacteria have only weak proteolytic activities and would appear to contribute to

proteolysis by decreasing the pH, which reduces protein solubility (Klement and Cassens, 1974) and increases tissular cathepsin D activity (Verplaetse *et al.*, 1992; Molly *et al.*, 1997). *Micrococci* are slightly proteolytic even though certain strains were capable of hydrolysing gelatin and sarcoplasmic proteins (Selgas *et al.*, 1988; Miralles *et al.*, 1996).

The inoculation of starter cultures leads to an increase in amino acid concentration (Dierick *et al.*, 1974). Dry sausages containing antibiotics had lower amino acid concentrations than those inoculated by micrococci (Sajber *et al.*, 1971). Verplaetse (1994) attributed 40% of the degradation of peptides into amino acids to micro-organisms.

Studies of the peptidasic activities of lactic bacteria in meat juice (Reuter, 1975) or on synthetic substrates (Montel *et al.*, 1991) also gives an indication of their role in products. However meat pH lower than 6 inhibits their activity which is optimal generally around neutrality (Montel *et al.*, 1995). *S. carnosus*, *S. xylosus*, *S. warneri* had weaker peptidasic activities than *K. varians* or *K. roseus* (Hinrichsen *et al.*, 1994; Montel *et al.*, 1996). Nevertheless products inoculated with *S. carnosus* did have a stronger dry sausage aroma than products inoculated with other species (Montel *et al.*, 1996).

The amino acid profile is not solely the result of the tissular and bacterial degradation of the peptides, but is also dependent on interconversions between amino acids, their interactions with other molecules and their degradation.

AMINO ACID CATABOLISM

Amino acids can be broken down into amines, ammonia or aromatic components. Volatile amines have been little studied in the fermented products although biogenic amines are often detected and should be avoided because of their potential toxicity. There is very little published work on the sensory properties of biogenic amines. However polyamines in an aqueous solution appear to have unpleasant odours whose threshold value is apparently 22 ppm for putrescine and 190 ppm for cadaverine (Wang *et al.*, 1975). Sensory qualities is not correlated with amine content in fermented meat products. The production of biogenic amines is mainly of bacterial origin. *Enterobacteriaceae* can be involved in the production of histamine, cadaverine and *Pseudomonas* in the production of putrescine (Edwards *et al.*, 1987). Tyramine and phenylethylamine can be produced by *Enterococcus*, *Carnobacterium divergens*, *Carnobacterium piscicola* and some strains of *L. curvatus* (Straub *et al.*, 1994; Masson *et al.*, 1996; Roig-Sagues *et al.*, 1996).

The degradation of amino acids into volatile molecules apparently plays an important role in the characteristic flavour of dry sausage. Aldehydes, alcohols and acids resulting from the degradation of branched-chain amino acids (leucine, isoleucine, valine), phenylalanine or methionine which have very low threshold values (Table 1).

Dimethyldisulfide and carbon disulfide must be avoided as they give putrid odours in both fresh and processed meats (Dainty and Mackey, 1992). On the contrary, 2- or 3-methylbutanol, and above all 2- or 3-methylbutanal and 2-methylpropanal, are important components for dry sausage aroma (Halvarson, 1973; Berdagué *et al.*, 1993; Stahnke, 1995*a*,*b*; Montel *et al.*, 1996). The addition of an extract containing proteinase from *Lactobacillus casei* seems to accelerate the synthesis of 3-methylbutanal, accelerating aroma development (Hagen *et al.*, 1996). The stimulatory effect observed may be also due to the presence of manganese in the extract (Hagen, pers. comm.). The 2-methyl propionic and 3-methyl butanoic acids with a cheesy note participate to dry sausage aroma (Stahnke, 1995*a*,*b*; Montel *et al.*, 1996).

The catabolism of branched-chain amino acids into aromatic compounds could involve the Strecker reaction (Barbieri *et al.*, 1992; Ventanas *et al.*, 1992; Hinrichsen and Pedersen, 1995).

TABLE 1

Non Volatile and Volatile Compounds Involved in the Fermented Meat Flavour. Potential Role of Bacteria According to Their Biochemical Activities *in Vitro*

Compounds	*Flavour note*	*Threshold (ppm)*	*Reaction enzymes*	*Bacteria involved*
From carbohydrate fermentation				
D L Lactate			Homofermentation	*L. sake*$^{+++}$, *L. curvatus*$^{++}$, *L. plantarum Pediococcus*
Acetate	Vinegar, pungent	22 to 54		*S. warneri, S.carnosus*$^{+}$
Ethanol			Heterofermentation	*Leuconostoc*
Acetaldehyde	Green		Pyruvate catabolism	*L. plantarum*
2, 3-butanedione (acetoine)	Buttery	2·5		*S. saprophyticus*$^{+++}$, *S. warneri*$^{++}$,
			Pyruvate catabolism	*S. xylosus*, *S. carnosus*
2, 3-butanediol	Buttery	0·002		*Pediococcus*$^{+}$, *L. plantarum*$^{+}$
From amino acid metabolism				
3-methyl butanal (leucine)	Malty, bit fruity	0·06	Amino transferase	
2-methyl butanal (isoleucine)	Malty, bit fruity	0·1	Ketoacid decarboxylase	
2-methyl propanal (valine)		0·1		*S. carnosus*$^{++}$
Phenylacetaldehyde	Floral			*S. xylosus*$^{++}$
3-methyl butanol	Fruity	4·75		*S. saprophyticus*$^{++}$, *S. warneri*$^{++}$
2-methyl butanol	Socks chitney		Alcohol dehydrogenase	*C. piscicola*$^{+++}$
2-methyl propanol	Alcohol			*L. sake*$^{+}$, *L. curvatus*$^{+}$, *L. plantarum*$^{+}$,
Benzaldehyde	Almond, acre	0·35		
3-methyl butanoic	Sweat socks	0·07		*P. acidilactici*$^{+}$
2-methyl butanoic	Socks, cheesy		Aldehyde or	
2-methyl propanoic	Cheesy		α-ketoacid dehydrogenase	
Phenylacetate	Floral			
Dimethyl disulfide (methionine)	Cauliflower	0·12		
Acetaldehyde (threonine)				
From lipid and fatty acids				
Butyric acid	Rancid, cheesy	0·3 to 0·7	Limit auto-oxidation by	
Hexanoic acid	Pungent, cheese	5 to 15		
Octanoic acid	Wax, soap, goat,	10 to 350		
	Rancid, fruity	5 to 15	• Nitrate reductase	*S. xylosus*$^{+++}$, *S. carnosus*$^{+++}$

(continued)

Table 1—*contd*

Compounds	*Flavour note*	*Threshold (ppm)*	*Reaction enzymes*	*Bacteria involved*
Pentanal	Fruity			
Hexanal, heptanal	Rancid, green leaves	0·015	• Catalase	*Staphylococcus*$^{+++}$, *L. sake*$^{+}$, *L. plantarum*$^{+}$, *Pediococcus*$^{+}$
Nonanal, decanal	Pelargonium, rancid		• Anti-oxidant material from bacteria	
1-pentanol, 1-nonanol	Fruity			
octene-2-ol	Green	0·018		
1-octene-3-ol	Mushroom			
2-pentanone	Fruity, acetone	22		
2-hexanone	Floral, fruity	4·7		
2-heptanone	Fruity, cheesy	0·15 to 1	β oxidation of fatty acid	Yeast, moulds
2-octanone, 2-nonanone	Fruity, musty	0·23 to 0·77		
3-octene-2-one	Mushroom	0·0009		Bacteria?
From glucide, carbohydrate, amino acid–fatty acid metabolism				
Ethyl butanoate	Fruity, strong	5		
Ethyl acetate	Pineapple	0·009		
Ethyl pentanoate	Fruity			*S. saprophyticus*$^{+++}$, *S. warneri*$^{+++}$, *S. xylosus*$^{++}$, *S. carnosus*$^{+}$
Ethyl 2- or 3-methyl butanoate	Fruity, apple		Esterases	
Ethyl 2-methyl propanoate	Fruity apple			*Lactobacillus*?
2-methyl propyl acetate	Fruity			
2-methyl butyl acetate				

Compounds were selected from literature data cited in the text.
Threshold values were according to Molimard and Spinnler (1995).

Nevertheless, methylaldehydes could also be produced by bacterial catabolism. In Parma ham their production could be linked to the presence of *Micrococcaceae* (Andersen and Hinrichsen, 1995). Hinrichsen and Andersen (1994) associated 3-methylbutanal in bacon to the presence of a strain of *Vibrio* sp., *S. warneri*, *S. saprophyticus*, *S. xylosus*, to a lesser extent *S. carnosus* and *Corynebacterium callunae* which produce 3-methylbutanol in a 'bacon model' (Pedersen and Hinrichsen, 1996). Production of metabolites from branched chain amino acids in dry sausage can be modulated by the type of flora inoculated. *Staphylococcus* would seem to play a more important role than *L. sake*, *P. acidilactici* or *P. pentosaceus* in the production of these volatile compounds (Berdagué *et al.*, 1993). Dry sausages inoculated with *S. carnosus* contained higher quantities of 2 or 3-methyl-butanal and 3-methyl-butanol, than those inoculated with *S. warneri* and *S. saprophyticus* (Berdagué *et al.*, 1993; Montel *et al.*, 1996). In laboratory medium, the same strains of *S. warneri*, *S. saprophyticus*, *S. carnosus* and *S. xylosus*, produced mainly 3-methylbutanoic acid and smaller quantities of 3-methylbutanal and 3-methylbutanol from leucine (Larrouture *et al.*, 1998). Under the same conditions *L. sake*, *L. curvatus*, *L. plantarum* and *P. acidilactici* produced the same molecules, but in smaller quantities, and particularly for 3-methylbutanal. High 3-methylbutanal production was demonstrated with strains of *C. piscicola* (Larrouture *et al.*, 1998). However we do not know if they are produced in products since metabolites from leucine was not produced after the inoculation of *S. warneri* and *L. sake* (Montel *et al.*, 1996). For all strains, the leucine degradation seems to involve chain reactions which commence by transamination producing a ketoisocaproic, subsequent non-oxidative decarboxylation into 3-methylbutanal which is then oxidized or reduced to acids or alcohols. 3-methylbutanoic acid may also be formed directly from α-ketoisocaproate (Larrouture *et al.*, 1998; Masson, 1998).

In dry sausage the degradation of leucine by bacteria seems limited by acid pH, since transamination is higher at pH 8 than at pH 5·8 (Masson, 1998). Decarboxylation and dehydrogenation would appear to occur with a pH around 5·8. Nitrate in dry sausage could also be a limiting factor since nitrite also inhibits the production of 3-methylbutanoic acid by *S. carnosus* and *S. xylosus*.

LIPOLYSIS

Lipolysis in dry sausage has been widely studied (see review of Dainty and Blom, 1995); it entails the liberation of fatty acids whose direct role in flavour has not yet been demonstrated. Short chain fatty acids have sour tastes, but their sensory characteristics diminish with increasing chain length (Forss, 1972). The addition of exogenous lipases significantly and rapidly increases free fatty acid concentration, thus reducing the time necessary to mature products but without systematically improving the flavour (Zalacain *et al.*, 1995, 1997*b*). The more intense odour has been associated with an increase in acetic, butyric and propionic acids after the addition of *Aspergillus* lipase and a starter culture (Zalacain *et al.*, 1997*a*) or aldehydes when the pancreatic enzyme was added (Fernandez *et al.*, 1991). Bearing in mind the diverse origins of these molecules it is difficult to attribute them directly to lipolysis.

Initial research into dry sausages considered that micro-organisms played an important role (see review Dainty and Blom, 1995). The evidence was based on the coincidence between the increase in free fatty acid content and the strong growth of lipolytic micro-organisms. *Lactobacillus* species are weakly lipolytic (Reuter, 1975; Papon and Talon, 1988). Lipolytic bacteria belong to the genus *Micrococcus* or *Staphylococcus* (Cantoni *et al.*, 1967; Debevere *et al.*, 1976). Strains of *S. saprophyticus* and *S. warneri* have more pronounced lipolytic activities on pork fat than strains of *S. carnosus* or *S. xylosus*, even

though some strains of the latter species do have some activity (Talon *et al.*, 1992). The lipases of these bacteria have most activity at pH values above 7·5 (Sorensen and Samuelsen, 1996; Talon *et al.*, 1995). Yeasts and moulds are also lipolytic (Nestorov *et al.*, 1983).

The role of bacteria in the lipolysis of products was subsequently reduced. The inoculation of highly lipolytic strains in dry sausage caused only a very slight increase in free fatty acid content (Garcia *et al.*, 1992; Montel *et al.*, 1993; Stahnke, 1994). Similarly Johansson (1996) only considered that only 30% of the lipolysis came from a highly lipolytic *S. xylosus* strain inoculated into a sterile mixture of fat and lean pork. Hierro *et al.* (1997) observed that lipolysis was not modified by the simultaneous inoculation of the highly lipolytic *Staphylococcus* sp and *L. plantarum*. However inoculation of the *Staphylococcus* sp alone, without lactic bacteria increased the free fatty acid content 5-fold in comparison with sterile controls. They explained these results through the acidification (pH close to 5·3) by lactic flora which inhibits the lipolytic activity of the *Staphylococcus*. The inhibition of bacterial flora through the addition of antibiotics did not reduce the free fatty acid content in dry sausages (Molly *et al.*, 1996). It would thus appear that lipolysis in dry sausage comes mainly from the pork fat lipases and muscle phospholipases (Molly *et al.*, 1997). Moreover no extra bacterial lipolytic activity seems necessary to develop an intense dry sausage aroma. Very weakly lipolytic strains of *S. carnosus* or *S. xylosus* were capable of generating a strong dry sausage odour in dry sausage model (Montel *et al.*, 1996).

FATTY ACID OXIDATION

Even if long chain fatty acids have only a limited impact on flavour, certain oxidation products are predominant in flavour. Oxidation products from fatty acids belong to the alkane, alkene, aldehyde, alcohol, ketone and acid families. Although production of these components in sausages is very low, of the order ppm, their low sensory threshold, except for the alkanes and alkenes which are odourless, means that they have a real effect (Berger *et al.*, 1990; Stahnke, 1995*a*,*b*). In sausages without spices they represent 60% of the volatile fraction (Berdagué *et al.*, 1993).

Dry sausages can give rise to various aromatic notes depending on variations in quantity of these molecules following different bacterial inoculations. Thus, besides the methylaldehydes previously mentioned, dry sausage aroma is also due to the dominance of 2-methyl ketones (2-pentanone, 2-hexanone, 2-heptanone) (Berdagué *et al.*, 1993; Stahnke, 1995*a*,*b*; Montel *et al.*, 1996). They have been associated with the presence of *S. carnosus* and *S. xylosus*. Rancid aroma on the other hand, is apparently due to the dominance of alkanal (hexanal, nonanal) or certain alcohols (1-pentene-3-ol, 1-octene-3-ol). They can be perceived in the absence of microbial flora or with *S. warneri* and *S. saprophyticus*. Hexanal concentration (0·03 mg kg^{-1}) in cured products is lower than that (12 mg kg^{-1}) in non-cured products (Ramarathnam *et al.*, 1991).

Fatty acids are oxidised into aldehydes, alkanes, alcohols and ketones by chemical (auto-oxidation) or enzymatic (β oxidation) reactions (Table 1). Bacteria would seem to change the auto-oxidation (Talon *et al.*, 1998). Lactic acid bacteria produce more hydrogen peroxide than micrococci (Wahlroos and Niinivaara, 1969). Hydrogen peroxide can be broken down by catalase activities systematically found in *Staphylococcus* and *Kocuria* (ex *Micrococcus*) (Schleifer, 1986; Barrière *et al.*, 1998). Catalase activities could also be present in *L. sake*, *L. plantarum* but not in *L. curvatus*. They are heme-dependent and reduced by NaCl and nitrite (Wolf *et al.*, 1991; Mares *et al.*, 1994). Certain yeasts and moulds contain catalase and superoxide dismutase (Clarkson *et al.*, 1991). Nitrite has

strong anti-oxidising properties (Erduran and Hotchkiss, 1995) and therefore the nitrate reductase activity of the bacteria via nitrite appears to limit oxidation. Strains of *S. carnosus* and *S. xylosus* display high nitrate reductase activity whereas strains of *S. warneri* and certain strains of *S. saprophyticus* do not (Miralles *et al.*, 1996; Montel *et al.*, 1996). The high concentrations of aldehydes in products inoculated with *S. saprophyticus* and *S. warneri* would appear, in part, to be due to their lack of nitrate reductase (Montel *et al.*, 1996). Lactic acid bacteria (*Leuconostoc*, *Pediococcus*, *L. plantarum*) have nitrite reductase activities (Wolf *et al.*, 1990). It is also possible that bacteria have non enzymatic anti-oxidant material (Smith and Alford, 1970).

β-oxidation breaks down saturated fatty acids into acetylCoA and propionylCoA, and could result in the liberation of the corresponding fatty acids. β-oxidation can also be directed towards the production of methyl ketones or secondary alcohols. Indeed, ester β-ketoacyl-CoA can be broken down to its β-keto acid and then into methyl ketone under the respective actions of thiolhydrolase and fungal decarboxylase (Yagi *et al.*, 1991; Creuly *et al.*, 1992). Secondary alcohols are subsequently formed by the reduction of methyl ketones. There was no suggestion of the intervention of bacterial enzymes.

ESTER PRODUCTION

Ethyl esters are present in fermented meat products and their aromatic characteristics contribute to the fruity note of the products (Stahnke, 1994; Montel *et al.*, 1996).

But the origin of these molecules is unknown. They have been detected in raw ham ripened for a long time with a low count in bacterial flora, and their origin could be chemical. In dry sausages they could be of bacterial origin. In laboratory media *S. warneri* and *S. saprophyticus* had more intense esterase activities for hydrolyzing and forming esters than *S. xylosus* and the weakest activity was by *S. carnosus*. (Talon *et al.*, 1996; Talon and Montel, 1997). These studies do not tally with results obtained on the products. The esters were associated with *S. carnosus* and *S. xylosus* strains, but not with *S. warneri* and *S. saprophyticus* strains (Stahnke, 1994; Montel *et al.*, 1996). The production of esters in dry sausage is reduced when *Pediococcus* is present, probably because of a decrease in pH (Stahnke, 1995*a*). The esterase activities of *Staphylococcus* were inhibited when pH values were below 5·5 (Talon *et al.*, 1996).

CONCLUSION

The enzymatic potential of bacteria is a valuable asset for the rapid reproduction of the characteristic flavour of meat products and could also lead to greater diversification in the flavour of the products. For the time being this potential has been exploited by the inoculation of bacterial mixtures as starter cultures in fermented meat products and perhaps it will be in other meat products in the future. In this presentation we set out selection criteria for efficient strains in order to improve the flavour. Acidification from carbohydrate catabolism by lactic acid bacteria contributes to the acid taste. It seems to be a limiting factor in the development of aroma since pH values below 5 do not generally favour bacterial metabolic activities. The anti-oxidizing activities of the bacteria through their nitrate reductase and catalase are important in controlling the oxidation of fatty acids. The proteolytic and lipolytic activities of the bacteria play a lower role that previous thought. They are low at the pH values during ripening sausages. Tissue proteolysis and lipolysis are preponderant and can be sufficient. Amino acid and fatty acid contents are not limiting factors in the production of aromatic molecules. Quantities of

volatile compounds, in the region of ppm, produced from amino acids and fatty acids are low in comparison to their concentration (g/100 g).

Identification of the volatile molecules in dry sausage has established which metabolites are important in the aroma of meat products. These studies have led us to include that the ability to catabolize amino acids or to produce esters is important in our choice of ferments, and has not been considered sufficiently. The possible production of ketones by bacteria also needs to be explored. The catabolism of nucleotides by bacteria could also be important since 5′ mononucleoside and monophosphate guanine are taste enhancers (Welsh *et al.*, 1989). Other important metabolisms may well emerge as analytical methods and our understanding of flavour chemistry progress.

Aldehydes, alcohols, acids, branched chain amino acid by-products and esters all play a role in the characteristic aroma of products, but their production must be controlled. Indeed, if these molecules can enhance flavour as a result of their strong aromatic characteristics, they can also alter it when produced in too high quantity. Initial research into the catabolism of amino acids or ester production by lactic bacteria and the *Staphylococcus* of meat products must be pursued as our knowledge of these metabolisms is insufficient to understand how they are produced in meat products. In several species (*L. sake, L. curvatus, S. carnosus* and *S. xylosus*) which have been shown to be effective in these products, the enzymes responsible for this catabolism should be characterised in order to establish their role in the flavour of fermented meat. In a similar way, for any bacterial strain studied, different mutants lacking the enzymes involved in aroma production should be drawn up, and their effect on the flavour of products tested. Only when their precise role is known it will be possible to increase or diminish its activity through genetic manipulation. The coding gene for this enzyme (transaminase, decarboxylase ketoacid, esterase) could then be transferred into another species which is considered more competitive in the products for other criteria. In the similar way to the promising initial trials to reduce ripening times by adding proteases and lipases, the important bacterial enzymes could be added to products.

Although bacteria are a precious asset to improve the aroma of these products, we should not forget that their activities depend on the characteristics of the raw material and processing technology. In future microbiologists, biochemists, chemists and aromaticians and technologists should coordinate their activities to formulate aroma using the emerging technology.

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