INFLUENCE OF RAW MEAT PROPERTIES AND PROCESSING TECHNOLOGY ON AROMA QUALITY OF RAW FERMENTED MEAT PRODUCTS

VERPLAETSE A.

R&D department
Ter Beke NV, Waarschoot,
Belgium.

SUMMARY

Sensory quality aspects of raw fermented meat products can be divided into four main categories : product safety, texture, appearance and aroma. This contribution focuses on aroma production in raw and fermented meat products. This sensory attribute is the most important quality trait of meat products because it is this feature that convinces the consumer to buy the product again. In this review a description will be given of the major compounds that contribute to smell and taste in these meat products and where they originate from. Conversion of raw meat with nearly no flavour into meat products with a distinct recognizable aroma involves a system of interacting microbial, physical and (bio)chemical reactions. The role of endogenous meat enzymes and microbial enzymes in this complex system of chemical and physical reactions will be discussed. Proteolysis in dry sausages will be dealt with in more detail. Through the use of antibiotics and specific inhibitors it is possible to clarify the proteolytic processes involved. It becomes evident that the effect of endogenous meat proteinases and lipases on aroma in meat products have clearly been underestimated in the past. In meat products, in contrast to tenderness of raw meat where the calpain system is recognized to be the active proteolytic enzyme system, cathepsins are the predominant active enzyme family. Of great importance to meat producers is the possibility to steer the production process so that a constant quality is always obtained. An overview will be given of steering actions which can be taken to obtain other aromas or more stable aromas. The influence of different meat species, sugar concentrations, starter cultures and added enzymes on aroma formation in dry sausages and raw hams will be described.

INTRODUCTION

Conversion of raw meat with nearly no flavour (Gorbatov & Lyaskovskaya, 1980 ; Mottram, 1991) into meat products with a distinct recognizable aroma involves a system of interacting microbial, physical and (bio)chemical reactions. The relative importance of these reactions in the development of quality traits like texture, colour, taste and safety is not easy to determine. The final quality and kind of product obtained is dependent on the sort of reactions involved as well as the ratio of reaction rates. The manufacturer has the possibility to manipulate and steer this complex system of interacting changes to get different products or different quality levels (Demeyer & Verplaetse, 1985 ; Demeyer, 1992). Steering can be done through internal or external parameters (Rödel, 1985). Internal parameters are usually chemical or microbiological parameters as e.g. adding of starter cultures, different sugars or spices to the meat batter or curing brine. External parameters are nearly all physical variables as e.g. temperature and humidity. Large changes in these parameters give rise to different existing families of raw and fermented meat products. For instance the difference between low and high acid fermented sausages is primarely the result of adding sugars and lactic acid bacteria to a meat batter in combination with different ripening conditions. A similar difference exists in dry hams where low and high salt products can be produced. High salt products areusually produced by using brine, low salt products are normally dry salted.

The ability to steer this complex reaction system will be important from an economical point of view. It determines the difference between a succesful company and one in trouble, because reproducible and steerable production processes are the only way to have products of constant high quality. The latter is important when economical growth is envisaged. In most cases the production processes used for speciality production such as dry sausage and raw ham are very empirical. This gives rise to dry sausage fermentations and raw ham productions with a certain variability which is reflected in product quality and a relatively high amount of quality costs due to rework and faulty productions. In this paper aroma formation in dry sausage and raw ham will be discussed in more detail. Aroma and flavour will be used as synonyms in this review and aroma is defined as the combination of taste and smell. Mouthfeel which is normally included in the definition of flavour (Piggott, 1988) is not included here. The perception of flavour is a fine balance between the sensory input of desirable and undesirable flavours. Final sensory perception or response to the food is regulated by the action and interaction of flavour compounds on the smell and taste systems. The major food flavour components involved in the initiation and transduction of the flavour response are the food's proteins, lipids and carbohydrates as well as their reaction products (Spanier & Miller, 1993). Since proteins and lipids constitute the major chemical components of meat products, they will be the major focus of discussion in this review. Our own market research shows that aroma is one of the most important sensory attributes of meat products because unlike other quality traits which initiate the purchase of a product for the first time (e.g. appearance) or the rejection of a product (e.g. texture) is aroma the feature that convinces the consumer to buy the product again. The same market study also revealed that some light meat products were not succesfull because during product development attention was paid only to the energy value of the products and the aroma was disregarded to a great extent. Therefore, food technologists require a thorough knowledge of how aroma is produced and how flavour deteriorates if they want to prepare products that consumers will buy repeatedly . This knowledge is particularly important in meat products, since production and deterioration of the aroma of meat products is a serious and continual process that involves the formation and loss of desirable flavour components and the production of off flavour compounds (Spanier & Miller, 1993). In these flavour processes breakdown of proteins and lipids will play a vital role. 5'-Nucleotides present in meat may also play a role in aroma of meat products through flavour enhancement of aroma components (Mottram, 1991). Excellent reviews on flavour formation in raw and fermented meat products are given by Dainty & Blom (1993) and Buscailhon & Monin (1994). In both reviews the authors state that despite the progress that has been made in recent years we are still a long way from understanding the different pathways involved in this flavour production. In the last years a lot of research has been done on cataloguing flavour volatiles and non volatiles, steering processes with enzymes and micro-organisms and clarifying several pathways. The relative importance of the proteolytic and lipolytic processes involved has been studied. The role of microbial and endogenous enzymes in these processes will be discussed.

AROMA DEVELOPMENT IN DRY SAUSAGE

As already mentioned above dry sausage fermentation is a lactic acid solid state fermentation where a microbiological process interacts with a physical process (acid gelation of meat proteins and drying) and a biochemical process (enzymatic breakdown of meat proteins and lipids) (Demeyer et al., 1986). The typical flavour of dry sausage is due to products originating from fermentation of carbohydrates, lipolysis and lipid oxidation, proteolysis, seasonings and curing salts. A differentiation can be made between the volatile compounds closely related to smell and the non-volatile products which influence taste.

Volatile aroma compounds

In the volatile flavour fraction of dry sausages different classes of chemical compounds can be recognized. The following chemical families are found in dry sausage : alkanes & alkenes, aromatichydrocarbons, alcohols, carboxylic acids, esters, ketones, aldehydes, terpenes, sulfur compounds, phenols, furans, amines, pyrazines and chloride compounds (Berger et al., 1990 ; Stahnke & Zeuthen, 1992 ; Berdagué et al., 1993b ; Johansson et al., 1993 ; Verplaetse, 1994 ; Mottram, 1991). A list of identified compounds is shown in table 1. This indicates that the volatile flavour of salami is not comprised of a single impact component. Some compounds will be more dominant than others depending on sausage type. In Italian and Spanish types of salamis four important types of chemical compounds are found : terpenes, ketones, aldehydes and esters (Berger et al., 1990 ; Stahnke & Zeuthen, 1992 ; Dainty & Blom, 1993). The apolar fraction containing monoterpenes originates from added spices and more especially from pepper. The distribution of monoterpenes present in the essential oils of pepper resembles very closely the one found in dry sausages. This fraction will also have a typical peppery odour. Terpenes are not present in the volatile fraction when spices are intentionally ommitted (Berdagué et al., 1993b). Only a single terpene, limonene was detected. This was presumably a contaminant (Dainty & Blom, 1993). According to Melgar et al. (1990) the polar fraction of aldehydes and ketones is derived from lipids through lipolysis and lipid oxidation. This fraction, with a clear salami smell (Berger et al., 1990), will have the greatest impact on flavour due to their low flavour threshold in comparison to the hydrocarbons, alcohols, furans,.. For each of these classes of compounds, flavour thresholds decrease with an increase in chain length (Spanier, 1992). Berger et al. (1990) however concluded that lipid oxidation and lipolysis could not be the only pathway for ketone and aldehyde formation because the typical autoxidation intermediates, the seven-to-ten alkenals and alkadienals, were not detected. These authors stated that as well as normal lipolysis and lipid oxidation, breakdown of monoterpenes was also responsible for production of these compounds. The absence of dienals was also interpreted as evidence against autoxidation and for microbial oxidation of fatty acids. The odour profile of dry salami results from the well balanced actions of microorganisms on lipid and pepper constituents. In these low acid products proteolysis seems to have no significant effect on the odour properties of dry sausage as no N-containing volatiles were detected. Certain low acid products like salchicon, chorizo and some kinds of italian salami contain in addition to aldehydes, ketones and a relatively large number of alcohols, a series of esters. These are mainly ethyl esters of saturated, unsaturated and branched chain acids (Dainty & Blom, 1993 ; Stahnke & Zeuthen, 1992). Medium acid products (pH = 5.1-5.3) show as well as an important fraction of aldehydes and ketones (= 60% of total volatile mass extracted) the production of furans, sulfur compounds, pyrazines and amines. Carbohydrate fermentation can, depending on the starter

cultures used, also yield some volatile compounds. In addition to the normal production of lactic acid, volatile products with butter or yoghurt aroma can be produced e.g. diacetyl, acetoin, 2,3-butanediol and 1,3-butanediol (Berdagué et al., 1993b). The latter products represent 27% of the total volatiles. Proteolysis in these products is not important for the production of volatiles. Only 6 % of the volatiles are derived from proteins (Berdagué et al., 1993b). Johansson et al. (1993) showed that in high acid products (pH<5.0) as well as terpenes, aldehydes and ketones, sulfur compounds can become dominant. They consisted of a fraction of 20% of the total compounds present in the headspace. These sulfur compounds as well as the methyl-aldehydes and methyl-alcohols may be formed from amino acids liberated by proteolysis. Verplaetse (1994) found in Belgian salami the same spice related terpenes, aldehydes and ketones, but only found limited amounts of sulfur compounds such as dimethyldisulfide and methanethiol.

Non-volatile compounds

The non-volatile fraction of dry sausage aroma is formed by fermentation of carbohydrates to acids, proteolysis and lipolysis. The non volatile compounds play an important role in the taste impression of the product. Glycolysis results in production of organic acids. The major products derived from carbohydrates are lactate and acetate. These products are often considered as the most important contributors to acid taste of fermented sausages. In our view a constant lactic acid production is very important to have stable production processes with no quality costs. It determines the development rate of texture, which is linked to the globalproduction time. However too much acid leads to non desirable acid products where all other aromas are suppressed by an acid taste. Hence, an optimum has to be found between production efficiency and taste. Lipolysis as well as proteolysis may originate from endogenous meat enzymes or from starter cultures. Free fatty acids are produced through hydrolyzing triglycerides. As well as free fatty acids 1,2-diglycerides are formed. These products are subject to acyl migration with 1,3-diglycerides as the result. This happens spontaneously as the 1,3 form is thermodynamically more stable (Johannson et al., 1993). Demeyer et al. (1974) confirm these findings that large amounts of diglycerides are formed together with some minor amounts of mono-glycerides. They also state that the outer position is attacked first. The fatty acids on this position are normally unsaturated. This agrees with findings that the more unsaturated fatty acids will be preferentially released and subjected to lipid oxidation with the formation of oxidation products such as aldehydes and ketones (Samelis et al., 1993). This lipolytic process will be very important for the volatile aroma fraction because it delivers the precursors for aldehyde and ketone formation (Melgar et al., 1990). However for taste free fatty acids seem to be less important. Dierickx (1991) showed that free fatty acids do not have a definite impact on dry sausage taste. Sausages with high amounts of free fatty acids are described as spicy sausages. However the author indicates that the concentration of free fatty acids has to be very high to see significant differences between sausages. Fernandez et al. (1991) and Wierinck (1993) did not observe any differences in sensory analysis between control sausages and lipase added sausages. Also in "chorizo" type sausages a similar finding was obtained that free fatty acids are not responsible for sensory improvements in dry sausages (Arboles & Julia, 1992).

Biochemical pathway lipolysis

Lipolysis in dry sausage is to a great extent of endogenous origin (Montel et al., 1993). This is confirmed by Civera (1992), who found no difference in free fatty acid production between control sausages and sausages prepared with antibiotics to suppress microbial growth. Toldra (1992) showed that in meat an endogenous acid lipase is active during dry sausage ripening. Addition of lipolytic micro-organisms only has a limited impact on the formation of taste compared to endogenous meat enzymes (Montel et al., 1993 ; Civera, 1992 ; Arboles & Julia, 1992 ; Wierinck, 1993). Wierinck (1993) showed that only 20% of the total lipolytic activity is accounted for by micro-organisms. The rest is of endogenous origin. Johansson & Borch (1993a) confirmed these findings in sterile meat-fat mixtures. In their experiments they saw only small differences between not inoculated controls and meat-fat mixtures inoculated with different bacteria such as *L. pentosus*, *P. pentosaceus* and *S. xylosus*. However once free fatty acids are produced it appears that starters can have a great influence on the volatile composition of dry sausage flavour. This shows that sausage smell can be modulated by microbial combinations (Berdagué et al., 1993b). Lipolysis and lipid oxidation have to be seen as two different processes where micro-organisms only have a substantial impact on the formation of oxidation products. Addition of lipase to sausages can in some cases be used to accelerate aroma development. These enzymes can bring about a more extensive turn over of unsaturated fatty acids to serve as aroma-precursors. The overall effect is a more rapid development of taste maturity, taste intensity and acidity. If adding lipase had an effect on the aroma of the final product was not indicated (Naes et al., 1992).

Biochemical pathway proteolysis

Proteolytic breakdown of meat proteins results in an increase of non protein nitrogen concentration (NPN). This liberation of free amino acids and peptides is of great importance to taste development in dry sausages (Dierickx, 1991 ; Lücke, 1986 ; Van Hoye et al., 1988). Van Hoye et al. (1988) and Dierickx (1991) showed a clear correlation ($r > 0.8$) between free amino acid and peptide concentrations present in the sausage environment and taste descriptors such as spicy, beefy, sweet, bitter and astringent. High amounts of small peptides give sausages a spicy taste, which can evoluate to a negative taste impression such as bitter and astringent when they become too high. Low amounts of small peptides result in beefy and sweet sausages. As well as exo- and endopeptidase activity desaminase activity is alsopresent in the sausages. This results in an accumulation of ammonia (Demeyer et al., 1979) which can also have a clear influence on taste development in dry sausage. Which proteins are broken down to provide these peptides is dependent on the acidity of the sausages. Low acidity sausages show low proteolytic activity and no major meat proteins are broken down (Verplaetse, 1992). This is confirmed by Lois et al. (1987) and Garriga et al. (1986). In medium and high acidity sausages myosin and actin are clearly degraded to fragments of 135kDa and 38kDa respectively (Verplaetse et al., 1989 ; Verplaetse et al., 1994b). The proteolytic pattern observed is similar to the one produced by endogenous cathepsins. Through the use of antibiotics to completely inhibit microbial growth and metabolism we were able to show that this breakdown is 100% endogenous (Verplaetse et al., 1992). The only parameter which controls the proteolysis of these major meat proteins is the pH. Addition of Glucono-_-lacton to antibiotica sausages to a pH below 5.0 increased proteolysis. At a high pH of 5.8 no breakdown was observed, which indicates that acid loving proteinases are active in this process (Verplaetse et al., 1994c ; Verplaetse, 1992).

The formation of small peptides during dry sausage production was studied using reversed phase HPLC. Peptides produced are both hydrophilic as hydrophobic. The hydrophilic peptides are dominant and show the most changes in composition during ripening (Opsomer, 1989). Changes in taste can be correlated with these changes in the peptide fraction. Bitter taste can be a consequence of too high amounts of hydrophobic peptides. The resemblance with peptides formed during cooking of meat is striking. In this case hydrophilic residues are also associated with desirable flavour while hydrophobic peptides are associated with off-flavours. Degradation of the hydrophilic residues during refrigerated storage of the cooked meat results in an increase of sour and bitter notes of the meat sample (Spanier, 1992). This author reports also a delicious tasting meaty flavoured octapeptide in cooked meat. Enzymatic cleavage of two amino-terminal residues yields a hexapeptide with bitter taste.

In dry sausages breakdown of polypeptides to smaller peptides and free amino acids is partly of microbial origin (40%) and partly endogenous (60%). This can be established through the use of antibiotics where enzyme activities as desaminase, exopeptidase and endopeptidase decreased to 60% of the control when micro-organisms were not able to grow or metabolize (Verplaetse et al., 1992 ; Verplaetse et al., 1994a). Identification of the proteolytic enzyme system active during proteolysis was done with specific inhibitors (EDTA, PMSF, E64, Leupeptin, Pepstatin, Soy trypsin inhibitor, Egg trypsin inhibitor, Ebelacton B) for the different proteinase-families. No activity was observed for metallo-proteinases and serine proteinases. Cysteine proteinases were active only on actin. Acid proteinases were responsible for both breakdown of actin and myosin. From the proteolytic pattern it was possible to identify cathepsin D as the enzyme responsible for breakdown of myosin. 50% of the cleavage of actin is carried out by cathepsin D and 50% by cysteine proteinases such as cathepsin B, H or L (Verplaetse, 1992 ; Verplaetse et al., 1994a). Toldra (1992) confirms these findings, however, the author rules out cathepsin H activity due to an unfavourable acid pH in the sausage environment. During ripening the cathepsin D activity remains completely constant (Civera, 1992) and curing agents such as salt and nitrate do not inhibit enzyme activity because the concentration is too low (Toldra, 1992). Identification of peptidase activity was more difficult because it involves microbial as well as endogenous enzymes. Endopeptidase activity mainly belonged to the acid peptidase family and is probably also related to cathepsin D. Identification of exopeptidases was not conclusive and, in addition to acid and cysteine peptidases, serine peptidases are also clearly active (Verplaetse, 1992 ; Civera, 1992). Because pH is a very important parameter in proteolysis and since several European countries have different processing technologies resulting in products with different acidities, four of the major companies of four European countries (Belgium, France, Germany and the Netherlands) were sampled. The sampling was carried out three times with an interval of 1 month between the samples. The most acidic sausages were found in the Netherlands (mean pH = 4.65) and Belgium (mean pH = 4.70), followed by the less acidic German sausages (mean pH = 4.90) and the non-acidic French sausages (mean pH = 5.80). The high acid sausages are characterized by low ammoniaproduction and high peptide formation. French sausages show high ammonia concentrations and low free amino acid and peptide production. German sausages combine high ammonia and free amino acid concentrations with low amounts of peptides. French sausages showed no breakdown of myosin and actin. Dutch and Belgian sausages had a clear degradation of myosin and actin. German products were intermediate. Their different processing technologies clearly have an impact on proteinase as well as peptidase

activity (Verplaetse et al., 1990 ; Verplaetse et al., 1994c). Interaction between lipolysis and proteolysis becomes evident from work done by Spanier et al. (1992b). Generation of free radicals by lipid oxidation has a significant effect on cathepsin D activity. A reduction in enzyme activity of 33% was observed.

Effect raw meat characteristics

Effect of different meat species on proteolysis were studied by Demeyer et al. (1992). Their results indicated that horse meat had a significantly higher exo- and endopeptidase activity when compared to beef and pork meat. In horse sausages and to a lesser extent in beef sausages more myosin and actin degradation was observed than in pork sausages. This was correlated with a higher cathepsin D activity in these meat species compared to pork meat. The differences were apparent mainly during the fermention period.

Effect processing technology enzymes

Addition of proteinases to dry sausages represents a way to manipulate the aroma development. Naes et al. (1991) and Naes & Nissen-Meyer (1992) purified and characterized a bacterial serine proteinase of *L. paracasei subsp. paracasei* to be used in dry sausages. Also the gene encoding for the proteinase was located on the chromosome of the micro-organism. This gene was cloned, sequenced and brought to expression in *L. plantarum* (Holck & Naes, 1992). Use of this proteinase in fermented sausages (Naes et al., 1992 ; Naes et al., 1993) results in an increase in lactic acid production with a more extensive pH drop and a more rapid colour and texture development as a consequence. Probably the formation of certain peptides stimulates the growth of the starter cultures. For lactobacilli it is known that small peptides can have a strong growth stimulating effect (Chopin, 1993 ; Pritchard & Coolbear, 1993). Addition of proteinases has an effect on various sensory attributes. Naes et al. (1992) show that it increases the rate of aroma development e.g. taste intensity and maturity, but due to the increased amount of D-lactic acid in the sausage it also has a more acidic taste. Also a difference in bitter taste is observed between the control and the proteinase added sausages. The latter giving the most bitter sausages. The positive effects seen after 14 days diminish after 35 days of ripening because then the texture and colour differences in the control and the proteinase treated sausages not only disappear, but texture even tends to be worse in the proteinase sausages. These texture changes in proteinase treated sausages are confirmed by Diaz et al. (1993) and Diaz et al. (1992). These authors stated that their taste panel considered the remarkable softening of the products as objectionable. From our own experience it is clear that most proteinases can only be used in very small amounts because otherwise you certainly will run into serious texture problems. Those very low enzyme concentrations will have no or only a slight impact on aroma development. The latter is confirmed by Diaz et al. (1993). Maybe some of these problems can be overcome with entrapment of proteinases and lipases in liposomes. In cheese these encapsulation systems are already used to accelerate ripening (El Soda, 1993).

Effect processing technology microorganisms

Another way to manipulate the aroma development of the sausages is through the use of different micro-organisms with lipolytic and proteolytic enzyme activities. A lot of papers have been written about enzymatic activities of micro-organisms isolated from different meat

products (Talon et al., 1992 ; Montel et al., 1992 ; Comi et al., 1992 ; Larpent-Gourgaud et al., 1994). The impact of the starter cultures on aroma formation has been studied less. Stahnke & Zeuthen (1993) demonstrated that the addition of a *S. xylosus* resulted in production of several esters in the volatile fraction which were not found in the control sausages. The sausages with *S. xylosus* were preferred over the control sausages because of a more pleasantodour. Johansson & Borch (1993a) and Johansson et al. (1993) showed that proteolysis can be manipulated with certain bacteria e.g. *L. pentosus, P. pentosaceus, S. xylosus*. De Masi et al. (1990) and Verplaetse (1992) confirmed that changes in NPN fraction can be dependent on the starter cultures used. These variations in free amino acids and peptides did not affect sensory scores. Berdagué et al. (1993b) showed that the composition of the volatile fraction can be changed with addition of starter cultures. When the bacterial influence on proteolysis is studied, it is of the utmost importance to separate the effect of pH from the real bacterial metabolism. As already explained above endogenous proteolysis will be very pH dependent. A lower pH will automatically give a higher amount of NPN, even if only endogenous proteinases are working. Therefore it is very important when studying proteolytic processes with acidifying micro-organisms to compare them with controls of the same pH. We can only say this fact is not taken into account often enough in most publications dealing with bacterial proteolysis.

AROMA DEVELOPMENT IN DRY CURED HAM

Dry hams can be divided into two main categories, low salted dry cured hams with long ripening and drying periods (6 months or more) and high salted brine cured hams with short ripening and drying periods (less than 3 months). On dry cured hams e.g. the Italian Pharma ham, French Bayonne ham and Spanish Serrano ham, a lot of research has been done in recent years (Toldra & Etherington, 1988 ; Rico et al., 1990 ; Rico et al., 1991a ; Rico et al., 1991b ; Rico et al., 1991c ; Toldra, 1992 ; Toldra et al., 1992 ; Berdagué et al., 1993a ; Berdagué et al., 1991a ; Berdagué et al., 1991b ; Buscailhon & Monin, 1994a ; Garcia et al., 1991 ; Barbieri et al., 1993).

The production processes of dry cured hams involve long ripening and drying periods during which complex reactions on proteins and lipids take place and the typical flavour compounds are formed. For the high salted hams which are normally smoked, flavour development is not only limited in time but will also be dominated by a saltier and smoked taste. Examples of those hams are the Belgian Jambon d'Ardenne and the German Coburger Schinken. Optimizing production processes of raw hams involves firstly the control of the salt diffusion into the hams. Too low salt concentrations will give microbiological and textural problems and too high salt concentrations results in loss of aroma quality of the ham.

Aroma as already mentioned above is one of the quality parameters that convinces the consumer to buy a certain product again. Therefore to control the aroma quality of a raw ham it is necessary to know how the major constituents of meat, proteins and lipids are contributing to the final flavour of dry ham. What biochemical processes are involved and how they are influenced by raw meat characteristics and processing technology.

Volatile aroma compounds

The volatile fraction of dry ham is very similar to the one produced in fermented sausages. The same chemical families are found : alkanes and alkenes, aromatic hydrocarbons, alcohols, carboxylic acids, esters,

ketones, aldehydes, terpenes, furans, lactones, sulfur compounds, nitrogen compounds as pyrazines, chlorides compounds (Berdagué et al., 1991a ; Berdagué et al., 1991b ; Berdagué et al., 1993a ; Garcia et al., 1991 ; Barbieri et al., 1993 ; Buscailhon et al., 1993a ; Buscailhon et al., 1993b ; Lopez et al., 1992 ; Verplaetse, 1994). In hams of the northern European type, in addition to these compounds, a lot of smoke related compounds are found e.g. phenols and guaiacols (Verplaetse, 1994 ; Mottram et al., 1991). In table 2 a list of identified compounds in raw-ham is given. Most of the volatiles recovered from raw hams are lipid related. Through lipolysis and lipid oxidation alkanes, alkenes, alcohols, aldehydes, ketones, furans and lactones are formed. Formation of lactones is the result of a cyclisation of hydroxyacids produced from lipids (Buscailhon & Monin, 1994a ; Mottram, 1991). Proteolysis resulting in the production of small peptides and free amino acids delivers in this way precursors for certain volatile compounds. Branched alcohols, aldehydes and ketones with less than 6 carbon atoms can be derived from branched amino acids. Amino acidswith an aromatic group can result in the production of aromatic compounds e.g. phenylethanol. Sulfur containing amino acids give rise to sulfur compounds such as dimethyl-disulfide (Berdagué et al., 1991b ; Garcia et al., 1991 ; Verplaetse, 1994). Several other compounds such as toluene, xylene, terpenes and branched alkanes are thought to originate from the smoking process as well as from animal feed additives and contaminants, which have accumulated in the meat and fat tissue of the animal (Buscailhon & Monin, 1994a ; Verplaetse, 1994).

The rate with which certain compounds are formed was studied by Buscailhon et al. (1993b). These authors indicate that 20% of the volatiles extracted from raw hams are not found in raw meat. In particular compounds originating from amino acids and proteins are only formed during aging of dry ham. They noted also that the rate with which compounds are formed is different. Certain compounds will show a maximum concentration after 2 to 6 months of production although the total production time is 9 months.

Lipolysis and lipid oxidation are considered to be the major processes in the volatile flavour production of dry cured ham (López-Bote et al., 1990). Flores et al. (1985) show that formation of a typical raw ham flavour coincided with the beginning of lipid oxidation. They found that carbonyl compounds were at a maximum between the second and fourth month of maturation. Buscailhon et al. (1993a) found a relationship between markers of lipid oxidation such as ketones, aldehydes and alcohols and intensity of typical ham odour. There is an optimum for the concentrations of these products, because when their concentrations rise too high a rancid odour is experienced.

Non-volatile compounds

The non-volatile aroma fraction in dry cured ham is comparable to dry sausage. Here also proteolysis and lipolysis result in the libration of resp. free amino acids, peptides and free fatty acids. The difference between both products is that the amount produced in dry cured ham is much higher. For amino acids an increased concentration is observed which is 3 to 5 times higher in dry cured ham than in fermented sausages (Vandekerckhove, 1978 ; Aristoy & Toldra, 1991). Also for free fatty acids a substantial increase is observed, with 9 to 16% of the fatty acids present liberated during the ripening and drying period (Flores et al., 1985). In dry sausage 3 to 5% of the fatty acids are subjected to lipolysis (Demeyer et al., 1974 ; Wierinck, 1993). Mc Cain et al. (1968) observed a highly significant correlation between organoleptic measurements of aged taste and the concentration of free amino acids and peptides. Maggi et al. (1977) indicated that in Italian Parma hams limited proteolysis was observed, which was mainly confined to

sarcoplasmatic proteins. These authors reported also a small decrease in myosin and actin. In more recent work on Spanish Serrano hams an intense proteolytic breakdown of myosin was observed during the production of these cured hams with the appearance of a 150 kDa fragment as well as numerous fragments in the 50-100 kDa region (Toldra, 1992). In northern European hams a similar protein breakdown is seen. During the production process myosin is degraded and the formation of a 135kDa fragment appears.

Long storage of these products (> 8 months up to 8 years) results in a complete breakdown of myosin, a degradation of actin by more than 50% and a large production of 135kDa, 38kDa and 25kDa fragments. Minor amounts are produced in the 50-100kDa region (Verplaetse, 1994).

Biochemical pathway proteolysis/ lipolysis

According to Toldra & Etherington (1988) only low amounts of micro-organisms are found in the interior of dry cured hams suggesting that the lipolytic and proteolytic changes observed would have to be of an endogenous meat origin. This is confirmed by Molina & Toldra (1992) who observed no proteolytic activity on myofibrillar and sarcoplasmatic proteins by P. pentosaceus and S. xylosus. These strains were isolated from dry cured ham and identified as being the most important micro-organisms in dry cured hams (Molina et al., 1989a ; Molina et al., 1989b ; Silla et al., 1989 ; Carrascosa & Cornejo, 1992 ; Cornejo & Carrascosa, 1991). Toldra & Etherington (1988) found that even after 8 months of ripening significant enzyme activities of cathepsin B, D, H and L could be detected. As well as proteinases, exopeptidases, acid lipases and esterases also remained active for many months in these hams (Toldra, 1992). Although the enzymes stay active in theham, in vitro experiments show that salt can be a potent inhibitor for most of these enzymes. Of the proteolytic enzymes present, only cathepsin B and L are not affected by salt (Sarraga et al., 1989). Acid lipases show an activation in vitro, possibly due to an increase in enzyme stability. From these in vitro results it was concluded that mainly cathepsin B and L are responsible for the proteolytical changes seen during ham production. The rising of the pH at the end of ripening can result in an activation of cathepsin H. This means that cathepsin H will only be active in the drying stage and not in the salting period. The activity of cathepsin D was considered to be very low in dry cured hams due to salt inhibition. However in vitro results were contradicted by findings of Melo et al. (1974), Toldra & Etherington (1988) and Sarraga et al. (1989). These authors all showed that cathepsin D stays active even after several months of curing. Melo et al. (1974) and Toldra & Etherington (1988) reported a decrease in activity during curing time. However, even after 8 months the cathepsin D activity was not negligible. Toldra and Etherington (1988) suggested that curing salts might have a stabilizing effect on the enzyme activity. Sarraga et al. (1989) showed that in certain cases even after 200 days of curing no decrease in cathepsin D activity is observed. Certain exopeptidases with leucyl hydrolysing activity present in raw meat stay very active during the complete production process in contrast to other peptidase activities which will decrease in activity due to salt inhibition. Active aminopeptidases were recovered from 7 month old dry hams. The increase in free amino acids in dry hams is believed to be strongly related to this aminopeptidase activity (Toldra et al., 1992). In vitro experiments contradicted these results because an almost complete inhibition of aminopeptidase by salt was observed (Toldra et al., 1993). Also for these enzymes the meat environment must have a stabilizing effect. Lipolytic enzymes as acid lipases and

acid esterases are in vitro both influenced by salt (Toldra, 1992). However, the activity of lysosomal acid lipase stays high enough to produce considerable amounts of free fatty acids. In vitro acid esterase seems to be completely inhibited by salt. During dry curing of hams lipases stay active for several months. Motilva et al. (1994) stated that the most intense lipolysis occurs between 0 and 5 months. After that period the non volatile free fatty acid fraction reached a plateau.

Other curing agents like KNO_3, glucose and ascorbate seem to have nearly no effect on enzyme activities, although some enzymes like cathepsin B are slightly activated by glucose. Environmental factors such as pH also play a significant role in the activity of the different enzymes. The typical ham pH (pH >6) is very near to the optimal pH for cathepsins B, H and L resulting in high activity. For cathepsin D the ham pH will be too high for optimal activity. Ham conditions of high salt concentration and high pH are not optimal for cathepsin D. Hence high activity of this enzyme in dry cured hams will be less probable (Toldra, 1992).

The role of calpains in dry ham is limited because their activity is completely lost due to salting. No residual activity is found in hams after the salting period (Sarraga et al., 1992). Also other curing agents such as nitrate, nitrite and phosphate interfere with calpain activity and result in 100% inhibition.

Effect raw meat characteristics

The effect of raw meat characteristics on aroma quality in dry cured hams was shown by Gil et al. (1989), Sarraga (1992) , Sarraga et al. (1988), Parreno et al. (1990), Flores et al. (1993), Lopez et al. (1992), Berdagué et al. (1993a) and Buscailhon et al. (1991). Meat of different qualities (normal, PSE and DFD) was used to see what influence this would have on the proteolytic process in dry cured hams. It appeared that the observed differences in meat quality had no influence on cathepsin D activity in crude acid extracts. So although salt penetration is more rapid in PSE meat and cathepsin D is inhibited by salt, no effect was seen from this higher diffusion rate. In crude alkaline extracts significant proteolytic activity was observed. This activity increased even after the salt equalisation step. Alkaline proteolytic activity was clearly influenced by meat quality. Compared to normal hams, DFD hams had a lower and PSE hams a higher activity. This difference in activity in alkaline crude extracts was not due to calpain activity, because after the salting step no activity could be detected for this enzyme irrespective of meat quality (Gil et al., 1989 ; Sarraga, 1992 ; Parreno et al. , 1990).Differences in proteolytic activity among pork breed types are described by Flores et al. (1993). These authors indicated that only cathepsin B, cathepsin D and a leucyl hydrolyzing aminopeptidase were not altered by breed type. Cathepsin L, cathepsin H and some aminopeptidases were clearly influenced by pork breed type. Buscailhon et al. (1991) observed that the concentration of pigment is favourably correlated with sensory qualities, whereas glycogen and lactic acid were unfavourably related to these qualities. Muscle tissue containing more red fibres gives hams with better sensory qualities. The latter can be the result of an acceleration of the lipid oxidation process catalyzed by a higher myoglobin concentration with higher ketone and aldehyde formation as a consequence (Spanier et al., 1992b ; Kanner et al., 1992). Berdagué et al. (1993a) studied the effect of pig crossbreed on the flavour of dry cured ham. These authors indicated that crossbreed had a restricted effect on the content of volatile compounds and ham flavour. Small differences were observed in aromatic compounds like 1-octen-3-ol (mushroom), 2,3-butadione and acetoin (butter-like). However based

on the existence of two groupes of statistically independent variables related to resp. lipid oxidation and amino acid catabolism, they suggested that it may be possible to modulate flavour of dry cured ham by acting separately on these two catabolic pathways via controlled modifications of the raw material or processing technology. López et al. (1992) studied the effect of diet on the composition of volatiles in dry hams. The qualitative composition was unchanged by diet. The same compounds were found in all hams.

However the quantitative differences were very significant. Pigs fed on a diet of only acorns and pasture had a significantly higher concentration of aldehydes, alcohols and short chain fatty acids than hams from pigs fed on a commercial diet. The authors indicated that the differences are related to differences in fat composition between batches. In particular C18:1 is more abundant in the acorns fed pigs (López et al., 1990).

Effect processing technology enzymes and microorganisms

Manipulation of ham aroma with enzymes or micro-organisms is not extensively studied. Of course micro-organisms can be isolated from dry cured hams and their proteolytic and lipolytic activity tested *in vitro* (Molina et al., 1989a ; Molina et al., 1989b ; Nieto et al., 1989 ; Cornejo & Carrascosa, 1991 ; Molina et al., 1991 ; Motilva Casado et al., 1992 ; Bermell et al., 1992 ; Carrascosa & Cornejo, 1992). Production time can be shortened by adding starter cultures e.g. *P. cerevisiae*. No difference in aroma development was observed by taste panel evaluation (Bartholomew and Blumer, 1977a ; Bartholomew and Blumer, 1977b). Results from model systems indicate that the main micro-organisms isolated from dry hams (*P. pentosaceus, S. xylosus*) show no proteolytic activity (Molina & Toldra, 1992). They can however have a remarkable lipolytic effect, which can influence the amount of sensory volatiles produced (Nieto et al., 1989 ; Molina et al., 1991 ; Bermell et al., 1992).

CONCLUSION

Aroma development in fermented sausages and dry cured hams show a lot of similarities. Lipolysis and lipid oxidation is believed to be one of the major processes involved in the production of aroma volatiles (or odour) in both products. Taste formation in these products is clearly correlated with the breakdown of proteins. Proteolysis in both products was shown to be similar. Cathepsin activity plays an important role in the non-volatile aroma production. Salt concentration, acidity and the presence of significant amounts of micro-organisms are parameters which are clearly different in dry hams and fermented sausages. It is believed that these parameters are responsible for the differences observed in aroma development between both types of meat products. What the relative importance of each parameter is and how the various aroma processes and aroma compounds interact is not known. Although some progress has been made in recent years especially where proteolytic processes in dry hams and sausages is concerned, research is needed to unravel this complex system of biochemical reactions involved in aroma production. Sensory analysis is necessaryto establish what the importance is of the different compounds listed in tables 1 and 2 for the ham and sausage aroma. Gaining a better knowledge of the biochemical processes involved will give meat producers the possibility to steer their different production processes more accurately.

Standardizing processing conditions will not only result in products with a constant aroma quality but gives also the opportunity to food technologists to develop different aromas in these "ancient" meat products.

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