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Proteolysis and Lipolysis in Flavour Development of Dry-cured Meat Products

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ABSTRACT

Numerous proteolytic and lipolytic reactions are involved in the generation of flavour and/or flavour precursors in meat and meat products. Most of these reactions are known to be due to endo-/exo-peptidases and lipases, respectively. The origin of these enzymes may be either from muscle and/or from microorganisms, although their relative relevance for a given meat product strongly depends on the manufacture and distribution. In this paper, the postmortem proteolysis and lipolysis is described with particular reference to dry-cured ham, a typical meat product naturally ripened by endogenous enzymes. © *1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

The development of flavour in meat products is a very complex process, not yet fully understood due to the high number of reactions involved. In general, flavour compounds may result from either enzymatic action or chemical reactions such as lipid oxidation, Maillard reactions, Strecker degradations, etc. There are many factors, such as raw meat properties, additives, processing conditions... affecting the flavour quality of a meat product; control is possible with a good biochemical knowledge of each factor involved. This would allow a better standardization of the processing and/or enhancement of the flavour quality of the product. Proteolysis and lipolysis constitute the main biochemical reactions in the generation of flavour or flavour precursors. Both groups of reactions are due to proteases and lipases, respectively, although the degree of contribution of either endogenous enzymes or those of microbial origin naturally present in the product or added as starter cultures will mainly depend on the type of process (Berdagué *et al.*, 1993; Molly *et al.*, 1997). Dry-cured ham constitutes an interesting exception since proteolysis and lipolysis are mainly attributed to the endogenous enzymatic systems in view of the low microbial counts found inside the hams, the difficult conditions for microbial growth (Toldrá and Etherington, 1988) and the low microbial enzyme activity levels (Molina and Toldrá, 1992). So, the lack of overlapping of microbial enzymes with those from muscle

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facilitates the study of flavour development in dry-cured ham as a model system and this will be the object of this work.

The typical dry-curing process consists of the following stages: salting with dry salt, washing, post-salting for salt equalization and ripening-drying (Flores and Toldrá, 1993). The quality of dry-cured ham thus depends on the raw materials and the ripening conditions. For instance, Iberian dry-cured ham, that typically shows a high degree of marbling, firm texture and exquisite flavour is produced in 18 to 24 months from an autochthonous pig, found in the southwest region of Spain, fed and fattened with acorns. On the other hand, Serrano dry-cured ham is produced in 9 to 12 months from different crossbreedings of white pigs but presents a cross section with lower marbling, firm texture and a variable flavor depending on the length of ripening (Toldrá *et al.*, 1997*a*). There is a great variety of dry-cured hams in the Mediterranean area, with some of the most important being the Spanish Iberian and Serrano hams, the Italian Parma and San Daniele hams and the French Bayonne ham. Other dry-cured hams, such as country-style ham in the USA and Westphalia ham in Germany, are smoked and cooked before consumption. In all cases, the process involves complex biochemical reactions with the participation of dozens of muscle enzymes, mainly of proteolytic and lipolytic nature, that generate non-volatile and volatile compounds which will finally contribute to the development of flavour (Toldrá, 1992; Toldrá *et al.*, 1997*b*; Toldrá and Flores, 1998).

GENERATION OF NON-VOLATILE COMPOUNDS

Peptides and free amino acids have been reported to contribute to meat taste during ageing (Nishimura *et al.*, 1988; Kato *et al.*, 1989; Aristoy and Toldrá, 1995) and/or cooking (Spanier *et al.*, 1988; Spanier and Miller, 1993). The effect is even more pronounced in a long ripened product like dry-cured ham (Toldrá and Flores, 1998). A high increase in the amounts of peptides and free amino acids has been reported in dry-cured hams (Toldrá *et al.*, 1995). These compounds are taste-active and may also exert a strong influence on the final flavour. In fact, several amino acids such as glutamic acid, aspartic acid, histidine, arginine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan and lysine have been found to be strongly correlated with the length of the drying process of Spanish Serrano ham and with both the cured and pork flavour (Flores *et al.*, 1997*a*,*b*). Lysine and tyrosine have been related to an improvement in the aged taste of Parma ham and glutamic acid to saltiness (Careri *et al.*, 1993). Furthermore, phenylalanine and isoleucine contributed possitively and tyrosine negatively to the acid taste. However, a small effect of these protein compounds on flavour development has been reported in French-type dry-cured ham (Buscailhon *et al.*, 1994*a*). Finally, it should be mentioned that an excess of protein hydrolysis (proteolysis index higher than 29–30%) is not always beneficial because it can be associated with unpleasant tastes such as bitter-like or metal aftertaste (Careri *et al.*, 1993; Virgili *et al.*, 1995).

In addition to the direct contribution to taste, free amino acids also constitute a source of volatile aromatic compounds such as 2-methyl propanal and 2-methyl butanal, sulfide compounds or thiols from Strecker degradations and, although in a very low extension, pyrazines from Maillard reactions with sugars (Toldrá and Flores, 1998).

GENERATION OF VOLATILE COMPOUNDS

There are many reports about flavor of meat but only a few on the aroma of dry-cured meat products, especially in ham. Most of the volatile compounds are the result of chemical

or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids. Other volatile compounds result from Strecker degradation of free amino acids and Maillard reactions as previously mentioned. A good number of volatile compounds have been reported in Spanish Serrano (Flores *et al.*, 1997*c*), and Iberian (García *et al.*, 1991; López *et al.*, 1992) dry-cured hams, Italian Parma hams (Barbieri *et al.*, 1992; Hinrichsen and Pedersen, 1995; Bolzoni *et al.*, 1996), French dry-cured hams (Berdagué *et al.*, 1991, 1993; Buscailhon *et al.*, 1993) and Country-style American hams (Ockerman *et al.*, 1964; Lillard and Ayres, 1969; Piotrowski *et al.*, 1970). Today, more than 260 volatile compounds have been detected in dry-cured ham (Flores *et al.*, 1998). Some of the most important are hydrocarbons (alkanes and methyl-branched alkanes) that may come from autooxidation of the lipids (Loury, 1972), aldehydes with more than six carbon atoms resulting from free fatty acids oxidation, alcohols, ketones which are the products either of β-keto acid decarboxylation or of fatty acid β-oxidation (Berdagué *et al.*, 1991), free fatty acids resulting from the hydrolysis of triglycerides and phospholipids (Motilva *et al.*, 1992), γ-lactones coming from the dehydration and cyclization of the γ-hydroxyacids (Berdagué and García, 1990), esters resulting from the esterification of the various alcohols and carboxylic acids (Shahidi *et al.*, 1986) and other compounds such as benzene derivatives, amines and amides. Thus, the typical aroma of dry-cured ham is mainly associated with the generation of these volatile compounds during the process, especially in the latest stages (Buscailhon *et al.*, 1993, 1994; Careri *et al.*, 1993; Flores *et al.*, 1997*b*).

Thus, proteolytic and lipolytic enzymatic reactions play an important role in the generation, directly or indirectly, of non-volatile and volatile flavour compounds. The importance of these enzymatic reations are now described.

PROTEOLYSIS

Meat proteins are known to experience an intense degradation during postmortem ageing due to the action of calpains and cathepsins resulting in an increase in meat tenderness (Koohmaraie, 1996; Valin and Ouali, 1992). The main changes are associated with the fragmentation of myofibrils through the Z-disc, the degradation of desmin, titin and nebulin and the appearance of two polypeptides with molecular mass of 95 and 30 KDa (Koohmaraie, 1994).The long processing of dry-cured ham (up to 24 months) allows a more intense action of muscle proteases, according to the scheme shown in Fig. 1, and results in an extensive protein breakdown (Flores *et al.*, 1984; Bellatti *et al.*, 1985; Astiasaran *et al.*, 1988; Aristoy and Toldrá, 1991; Toldrá, 1992; Toldrá *et al.*, 1992*a*, 1993*a*) and marked ultrastructural changes (Monin *et al.*, 1997). The electrophoretic patterns of muscle proteins show very interesting changes along the process such as the progressive disapearance of myosin heavy chain and light chains 1 and 2, troponins C and I and the simultaneous appearance of several fragments with 150, 95 and 16 KDa (Toldrá *et al.*, 1993*a*). Other fragments are formed in the 50–100 and 20–45 KDa ranges (Toldrá *et al.*, 1992*a*). A comparison of densitograms between proteins from raw and dry-cured ham, as an example of these changes, is shown in Fig. 2a and b. A recent study with Bayonne ham reports relatively similar changes (Monin *et al.*, 1997). Cathepsins B, H and L show good stability during the dry-curing process (Toldrá and Etherington, 1988) and a residual 5–10% activity is usually found even after 15 months of process (Toldrá *et al.*, 1993*a*). The activity of cathepsin D however tends to disappear around the 6th month of processing (Rico *et al.*, 1991) while the contribution of calpains is limited to the initial two weeks due to poor stability (Sárraga, 1992; Rosell and Toldrá, 1997). Cathepsins are partly inhibited by myoglobin (Rosell *et al.*, 1996) and salt (Rico *et al.*, 1990, 1991; Toldrá

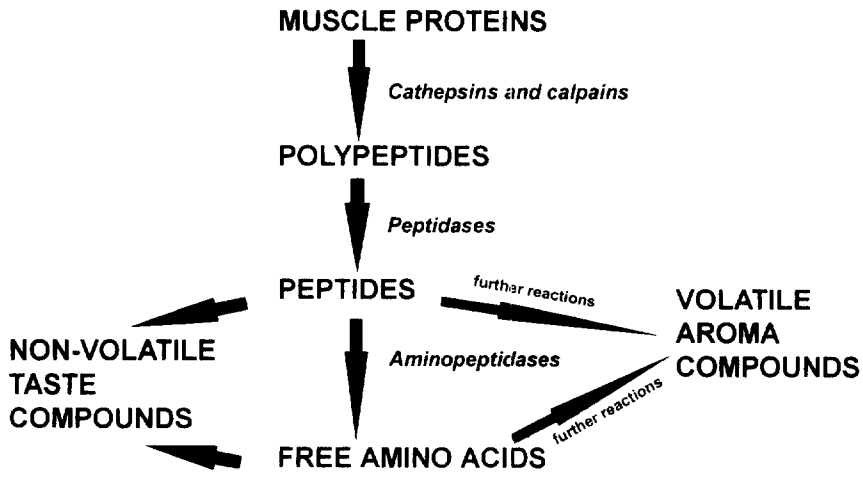

Fig. 1. Flow chart showing the major steps in post-mortem muscle proteolysis.

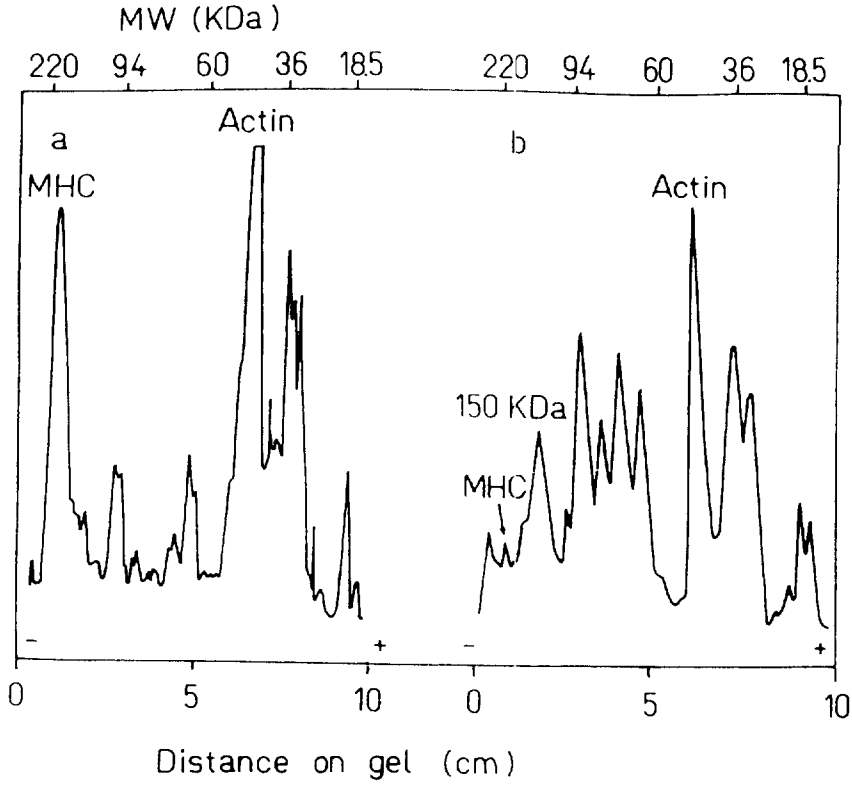

Fig. 2. Densitograms of electrophoretograms of total protein extracts from (a) raw and (b) Spanish Serrano dry-cured ham. Adapted from Toldrá *et al.* (1992*a*).

et al., 1992*b*) while m-calpain remains unaffected or even enhanced by salt (Rosell and Toldrá, 1997).

Numerous peptides resulting from protein breakdown (see Fig. 1), some of them associated to specific tastes (Aristoy and Toldrá, 1995), are usually detected during the processing of dry-cured ham. Capillary electrophoretograms show numerous peptides, especially in the I and III zones, generated in dry-cured ham [see Fig. 3(b)] as compared to the initial raw ham [see Fig. 3(a)]. Peptide mappings obtained through reverse-phase HPLC show the appearance or increase of numerous peaks as can be observed in Fig. 3(d) vs 3(c). A noticeable accumulation of free amino acids, as a final confirmation of the proteolysis (as indicated in Fig. 1), is also detected in all types of dry-cured hams (Aristoy and Toldrá, 1991; Buscailhon *et al.*, 1994*b*; Toldrá *et al.*, 1995; Schivazzappa *et al.*, 1995; Monin *et al.*, 1997) suggesting an important role of muscle aminopeptidases (Toldrá, 1992; Toldrá *et al.*, 1992*c*). The high concentrations of glutamic acid, alanine, leucine, lysine, valine and aspartic acid, as shown in Fig. 4, are particularly important. Muscle aminopeptidases are also inhibited by myoglobin (Rosell *et al.*, 1996) and salt (Toldrá *et al.*, 1993*b*; Flores *et al.*, 1997*d*) with aminopeptidase B being an exception since it is a chloride-activated enzyme (Flores *et al.*, 1993).

In general, an excess of proteolysis results in a poor firmness associated with poor ratings by sensory panelists and consumers (Parolari *et al.*, 1994). It also results in a high concentration of low molecular weight nitrogen compounds (peptides and free amino acids), sometimes so excessive that they may impair the typical flavor of dry-cured ham by exagerating the bitter and metallic taste (Virgili *et al.*, 1995). Furthermore, differences in proteolytic activities have been reported among porks from different breed types and/or ages (Flores *et al.*, 1994; Toldrá *et al.*, 1996; Rosell and Toldrá, 1998).

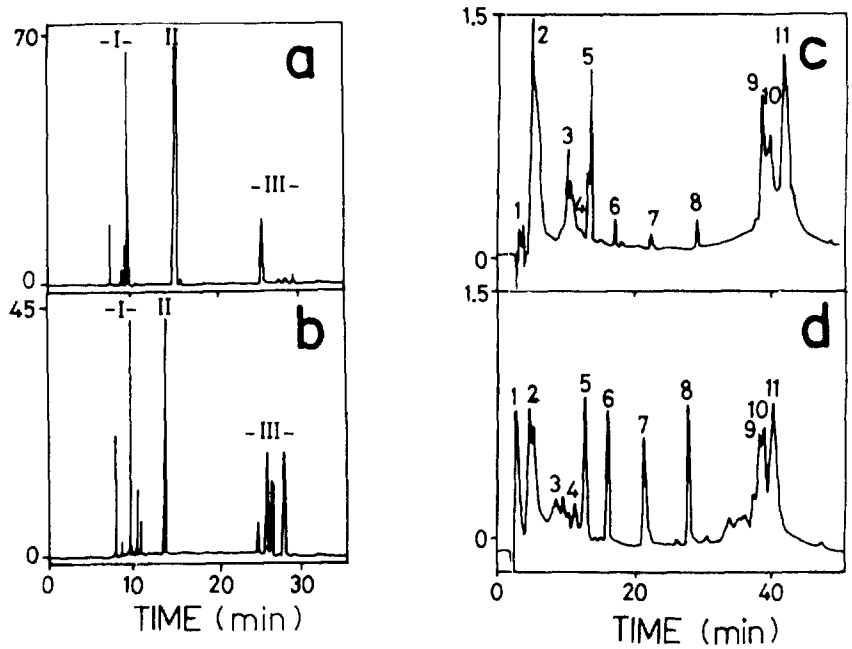

Fig. 3. Capillary electrophoretograms and RP-HPLC chromatograms from raw (a and c, respectively) and Spanish Serrano dry-cured ham (b and d, respectively). Adapted from Rodríguez-Nuñez *et al.* (1995) and Aristoy and Toldrá (1995).

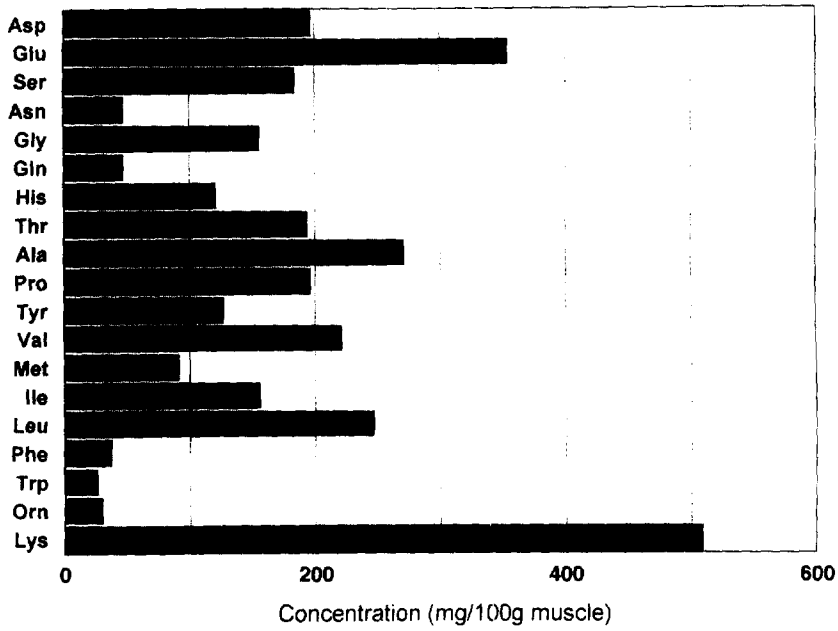

Fig. 4. Typical profile of free amino acids generated in Spanish Serrano dry-cured ham. Adapted from Toldrá and Aristoy (1993).

LIPOLYSIS

An intense lipolysis is observed during the processing of dry-cured ham, especially in the initial five months of process. A great percentage of the generated free fatty acids occur as a result of phospholipid hydrolysis, indicating a major role of phospholipases according to the scheme shown in Fig. 5, while triglycerides remain almost intact (Motilva *et al.*, 1993*a*; Buscailhon *et al.*, 1994*c*). Free fatty acids accumulate as the process progresses up to 10 months, then some of the free fatty acids start to decrease due to the higher susceptibility to oxidation when in the free form. The resulting oxidised compounds can act as flavor precursors of a great number of volatile compounds as previously described. An example of the composition in free fatty acids at the end of the processing of a Spanish Serrano dry-cured ham is given in Fig. 6. Acid muscle lipases are active in salty and lower water activity environments (Motilva and Toldrá, 1992) which favours its action during dry-curing. In addition, the generation of short chain free fatty acids is almost negligible (Motilva *et al.*, 1993*a*), suggesting a minor role of esterases.

Triglycerides from adipose tissue also undergo an intense lipolysis during the salting and post-salting stages, and a substantial increase in free fatty acids has been reported (Pezzani *et al.*, 1988; Motilva *et al.*, 1993*b*). An example is shown in Fig. 6. Neutral and basic lipases from the adipose tissue (Belfrage *et al.*, 1984) are very active during both stages although only the neutral enzyme remains active during the ripening/drying period (Motilva *et al.*, 1993*b*). Myristic, linolenic and oleic acids are those generated in greater amounts (Motilva *et al.*, 1993*b*) although the generation rate, especially in mono- and polyunsaturated fatty acids depends on the previous feeding of the pigs (Toldrá *et al.*, 1996). Neutral lipase is inhibited by salt (Motilva and Toldrá, 1992). Similar levels of lipolysis are observed at the end of the process when using frozen/thawed hams as raw material (Motilva *et al.*, 1994).

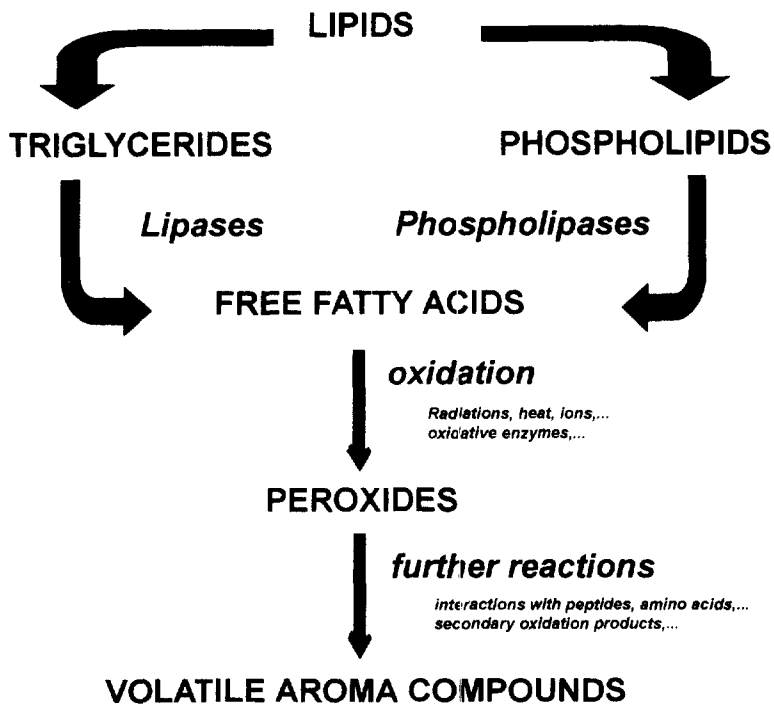

Fig. 5. Flow chart showing the major steps in postmortem muscle lipolysis and oxidation to flavour compounds.

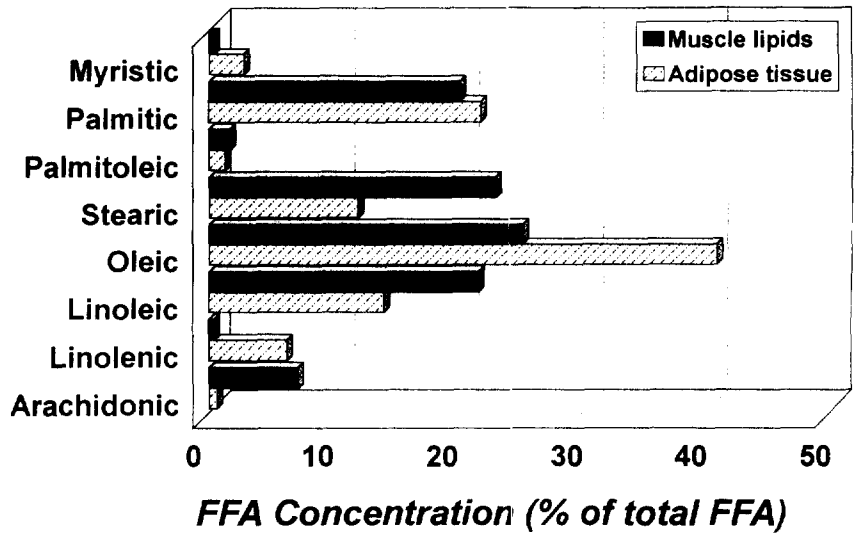

Fig. 6. Typical profiles of the most important free fatty acids (expressed as a % of total free fatty acids) generated in muscle lipids and adipose tissue from Spanish Serrano dry-cured hams. Adapted from Motilva *et al.* (1993*a*,*b*).

CONTROL OF ENZYMATIC ACTIVITY

The control of proteolytic and lipolytic activity in the hams can be achieved through different ways. The easiest way is by controlling the relative humidity and temperature in the curing rooms since they have an important effect on enzymatic activity (Toldrá *et al.*, 1997*b*; Toldrá and Flores, 1998). In particular, proteolysis and neutral lipolysis can be controlled by adding an excess of salt because of its proved inhibitory effect on cathepsins (Toldrá *et al.*, 1992*b*), most aminopeptidases (Toldrá *et al.*, 1993*b*; Flores *et al.*, 1997*d*) and neutral lipases (Motilva and Toldrá, 1992). The age and genetics of pigs also exert a clear influence on the muscle enzymes systems (Flores *et al.*, 1994; Toldrá *et al.*, 1996; Rosell and Toldrá, 1998) and this can have important consequences for the final quality of the meat product. In fact, further research on the biochemical characteristics of pork muscle is being carried out in our Laboratory in view of its importance for the final sensory quality when used as raw material for the processing of cured meats.

Thus, research is continuing in this area because the ability to control the complex reaction systems in the ham is very important from an economical point of view in order to obtain products of constant high quality (Toldrá and Verplaetse, 1995). In fact, there is today a great variety of meat products having specific colour and flavour depending on the different customs and habits of each country as well as consumer demands (Flores and Toldrá, 1993).

CONCLUSIONS

The muscle enzyme system, especially of proteolytic and lipolytic nature, is of primary importance for the development of typical sensory characteristics of dry-cured meat products. Raw meat (genotype, age, sex, ante and postmortem treatment) and process technology have a decissive influence on the activity of muscle enzymes. In addition, the existence of endogenous and/or added enzyme inhibitors have been reported although in many cases their stability during ripening processes and mode of interaction remains still unknown. Further research needs to be developed in this particular area. In addition, the presence of exogenous enzymes from microbial origin, which is the case in dry fermented sausages, also contribute to modulate the final flavour of the product. Production parameters are also known to exert an important influence on the enzyme activity and stability and further research is also needed. Thus, a better knowledge of the biochemical mechanisms involved during the processing of dry-cured meat products will ensure a good development of flavour and a correct standardization of the quality.

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