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Dry-cured ham flavour: enzymatic generation and process influence

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The processing of dry-cured ham is very complex and involves numerous biochemical reactions that are reviewed in this paper. Muscle proteins undergo an intense proteolysis resulting in a great number of small peptides and high amounts of free amino acids. The enzymes responsible of these changes are proteinases (cathepsins B, D, H and L and, to a less extent, calpains) and exopeptidases (peptidases and aminopeptidases). Muscle and adipose tissue lipids are also subject to intense lipolysis generating free fatty acids by the action of lipases that, in a second stage, are transformed to volatiles as a result of oxidation. Sensory profiles of dry-cured ham are strongly affected by these enzymatic reactions. In addition, the activity levels of the muscle enzymes significantly depend on the properties of raw ham, such as age and crossbreeding as well as the process conditions such as temperature, time, water activity, redox potential and salt content. Thus, the control of the muscle enzyme systems, mainly proteases and lipases, is essential for the optimal standardisation of the processing and/or enhancement of flavour quality of dry-cured ham. © 1997 Elsevier Science Ltd

INTRODUCTION

The origin of dry-curing, initially used as a preservation process for times of scarcity, is lost in ancient times. Today, salting has lost importance as a preservation method due to the spread use of refrigeration techniques, but has been modified or improved to dry-curing where there is an incorporation of additives and adjuncts such as nitrates and ascorbic acid to the salt (Flores & Toldrá, 1993). The result is a flavourful and attractive meat product, so famous that it is present in the most prestigious restaurants and the most remarkable banquets and ceremonies.

The quality of dry-cured hams depends on the raw materials and the ripening conditions. In Spain there are two main types of dry-cured hams, Iberian and Serrano. Iberian dry-cured ham is produced from an autochtonous pig, fed and fattened with acorn (*Quercus Ilex* L.) which is found in the south west region of Spain. A cross section of this ham shows a high degree of marbling, firm texture and exquisite typical flavour. Serrano dry-cured ham is produced from different crossbreedings of white pigs and presents a cross-section with lower marbling, firm texture and a typical flavour that can be more or less intense depending on the length of the ripening. There are other dry-cured hams in the Mediterranean area; some of the most important are Italian Parma and San Daniele hams, French Bayonne ham.

Complex biochemical reactions, which are described in this review, take place during the ripening period. The result is an important contribution to the development of a characteristic and typical flavour (Toldrá *et al.*, 1996*a*).

TECHNOLOGY FOR THE PROCESSING OF DRY-CURED HAM

The process for dry-cured ham, based on traditional procedures, mainly consists of several stages which are briefly described (Toldrá, 1992; Flores & Toldrá, 1993):

1. reception and classification of hams, and then presalting where a mixture of curing ingredients (salt, nitrate and/or nitrite) and additives (ascorbic acid) are rubbed onto the lean muscle surface of the ham;
2. salting, where hams are then placed fat side down, entirely surrounded by salt and arranged in single layers without touching each other. There is no addition of water so that curing agents slowly diffuse into the ham solubilized by the original moisture in the meat. This period usually takes 8–10 days (1–1.5 days Kg^{-1} of weight) at temperatures between 2 and 4°C;

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3. during the next stage, named post-salting, a complete salt equalization takes place. The temperature is kept below 4°C for a period of time not less than 20 days but not exceeding 2 months;
4. the last and more complex stage is the ripening/drying stage. The hams are placed in natural or air-conditioned chambers and subjected to different time-temperature-relative humidity cycles. Temperature is usually held between 14 and 20°C with a relative humidity decreasing from 90 to 70%. Hams ripening or aging might take from 6 to 18 months.In fact, this last stage has the most important variations in process conditions. Thus, the ripening period for Serrano ham is about 9–12 months while in the case of Iberian ham it can be extended up to 18–24 months. The external part of the ham, which mainly corresponds to the muscle *Semimembranosus* (SM), dries up during the process due to the dehydration. On the other hand, internal muscles such as *Biceps femoris* (BF) present higher residual moisture content that allows a better enzyme activity for longer periods of time and thus increased biochemical changes.

ACTIVITY OF THE MUSCLE ENZYMES IN THE PROCESSING OF DRY-CURED HAM

Proteins and lipids constitute the major chemical components of meat and are the main subject of action of the muscle enzyme systems. Since they are involved in many reactions affecting the flavour quality of a meat product (Spanier, 1992), proteolysis and lipolysis will constitute the main focus of discussion.

Proteolysis

An intense proteolysis expressed as an increase in non protein nitrogen has been reported especially during the initial period of maturation (Bellatti *et al.*, 1985; Toldrá *et al.*, 1992*a*; Astiasarán *et al.*, 1988). This change should be of an endogenous origin since low numbers of micro-organisms are found inside the hams (Toldrá & Etherington, 1988). Furthermore, no proteolytic activity was detected on myofibrillar and sarcoplasmic proteins (Molina & Toldrá, 1992) by *P. pentosaceus* and *S. xylosus* which are the most important micro-organisms found in dry-cured ham (Molina *et al.*, 1989*a*,*b*). Thus, most of the recent research has been focused on the muscle enzyme systems.

Lysosomal proteinases

Lysosomes contain a great variety of enzymes including proteinases and inhibitors such as cystatins. The main lysosomal proteinases, which are also the best characterized, are cathepsins B, D, H and L. These enzymes are small, in the range 20–40 KDa, and active at acidic pH values. Cathepsins B, H and L are cysteine proteinases while cathepsin D is an aspartic proteinase. They have shown the ability to degrade different myofibrillar proteins when in *in vitro* environments. Thus, Cathepsin D has been found especially active against myosin heavy chains, titin and also on M and C proteins, tropomyosin and troponins T and I (Zeece & Katoh, 1989). Actin is degraded but at a slower rate (Schwartz & Bird, 1977). Cathepsin B also degrades myosin and actin but has no effect on myosin light chains and troponin C (Schwartz & Bird, 1977). Cathepsin L has been demonstrated to degrade myosin heavy chain, actin, tropomyosin, α-actinin and troponins T and I but not troponin C (Matsukura *et al.*, 1981). This enzyme has been found extremely active in degrading both titin and nebulin (Etherington, 1987). Finally, cathepsin H posseses the properties of both endo and aminopeptidase so that Okitani *et al.* (1981) proposed it to be classified as an aminoendopeptidase.

Toldrá *et al.* (1993*a*) compared the electrophoretic patterns of different stages in the processing of dry-cured ham using a specific extraction buffer (Toldrá *et al.*, 1992*a*). An intense proteolysis is observed with a progressive disappearance of myosin heavy chain and light chains 1 and 2, troponins C and I and the appearance of 150, 95 and 16 KDa fragments as well as numerous fragments in the 50–100 KDa and 20–45 KDa regions. Thus, cathepsins would play an important role in all these proteolytic events collaborating with the initial degradation by calpains in hydrolyzing muscle proteins, especially those not degraded by calpains, such as myosin, actin and α-actinin (Toldrá & Etherington, 1988; Toldrá *et al.*, 1993*a*). The role of endogenous inhibitors (cystatins) during dry-curing is unknown as yet.

There are some peptides isolated from meat, resulting from endogenous proteolysis, that show specific tastes (Spanier & Miller, 1993). Peptide mappings, carried out along the complete process, revealed a strong proteolysis

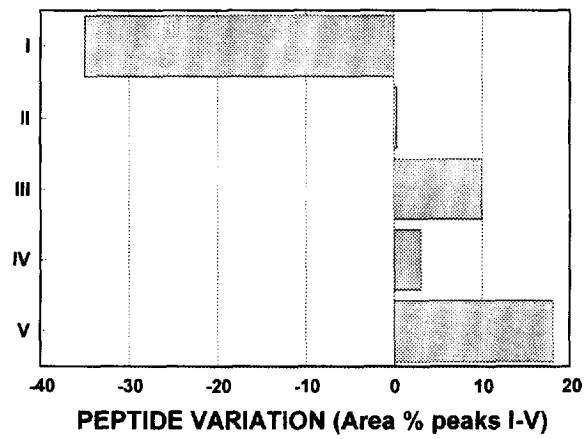

Fig. 1. Peptide accumulation, expressed as area percentage, in dry-cured ham after 15 months of processing. Ranges I to V indicate the order of ranges of molecular mass after gel filtration HPLC. Range I: 4500–2700 Da, range II: 2700–1200, range III: 1200–500 Da, range IV: 500–375 Da and range V: 375–160 Da. Adapted from Rodriguez *et al.* (1995).

(Rodriguez *et al.*, 1995) associated with specific tastes (Aristoy & Toldrá, 1995). Figure 1 shows the increase of small peptide fractions (below 1,200 Da) at the end of the process while those of intermediate size decrease (2700–4500 Da). Further fractionation of the peptides with Mr below 2700 by gel filtration revealed that the savoury fractions, corresponding to Mr between 1500 and 1700, mainly contained serine, glycine, alanine, arginine, threonine and leucine (Aristoy & Toldrá, 1995).

Cathepsins B, H and L are quite stable and show activity along the entire process (see Table 1) with recoveries of 5–10% of the initial activity after 15 months of processing (Toldrá *et al.*, 1993*a*). However, cathepsin D is not so stable and its activity disappears between 6 and 10 months of process (Rico *et al.*, 1991; Toldrá *et al.*, 1993*a*).

Neutral proteinases: calpains

Calpains are a group of cystein endopeptidases, known in the literature by a variety of names such as calcium-activated neutral proteinases, calcium-dependent proteases or calcium-activated factor, requiring calcium ions for activity. These enzymes, that seem to be widely distributed in the cytosol and in the Z-disc region, show maximal activity at neutral pH, around 7.5. Calpain I requires 50–70 μM of Ca^{2+} for activation while calpain II requires 1–5 mM of Ca^{2+}. However, calpains have little activity at pH 6.0, usual in hams, and only show 25% of their optimal activity at pH 5.8. Another limitation for calpain activity in post mortem muscle is the existence of the endogenous inhibitor, calpastatin, although when compared to other species, pork muscle has the lowest level (Ouali & Talmant, 1990). However, calpains have been reported to degrade troponin T and I, tropomyosin, C-protein, filamin, desmin and vinculin as well as titin and nebulin (Goll *et al.*, 1983; Koohmaraie, 1994). No effect has been detected on myosin, actin, α-actinin and troponin C. In any case, calpains have a restricted activity so that proteins are cleaved to large peptides (Goll *et al.*, 1983, 1991), even though there is evidence for *in vitro* degradation to small peptides or amino acids (Harris *et al.*, 1995). The contribution of calpains to proteolysis is very limited because its activity is lost after the salting stage (Sárraga, 1992; Rosell & Toldrá, 1996). In any case, they would collaborate with cathepsins in the initial breakdown of myofibrillar proteins during the salting and early post-salting stages.

Muscle exopeptidases

The role of exopeptidases in protein and peptide degradation has received relatively little attention in meat although these enzymes are involved in the latter stages of proteolytical degradation (Toldrá & Verplaetse, 1995). A substantial amount of work on the purification and characterization of dipeptidyl peptidases and tripeptidyl peptidases is being carried out at the author's laboratory so that their role during dry-curing will be clarified in the near future. On the other hand, information regarding aminopeptidases is already available and is described below.

Muscle aminopeptidases

Aminopeptidases appear to be metallo-proteins with a wide variability in molecular weights and a complex structure. Aminopeptidase B, leucyl, alanyl, and pyroglutamyl aminopeptidases have been localized in the cytosol. These enzymes are named on the basis of their preference or requirement for a specific N-terminal amino acid. All of them are active at neutral pH (Flores *et al.*, 1993) except leucyl aminopeptidase at basic pH. Alanyl aminopeptidase is the most important since it accounts for as much as 86% of the total aminopeptidase activity in the cytosolic fraction of skeletal muscle (Lauffart & Mantle, 1988). This enzyme has a broad substrate specificity towards aromatic, aliphatic and basic aminoacyl-bonds. Aminopeptidases have shown good stability along the processing of dry-cured ham (see Table 2) with activity still recovered after more than 8 (Toldrá, 1992) or even 12 months (Toldrá *et al.*, 1992*b*)

There is an important relationship between aminopeptidase activity, especially alanyl aminopeptidase, and the generation of free amino acids. In fact, a noticeable increase in the concentration of free amino acids during the processing of dry-cured ham, as shown in Fig. 2, has been reported (Aristoy & Toldrá, 1991; Toldrá *et al.*, 1995). Glutamic and aspartic acids, alanine, leucine, lysine and valine appear to be some of the amino acids experiencing higher increases (Toldrá & Aristoy, 1993). The combination of all these free amino acids and small peptides contribute to the characteristic taste of dry-cured ham (Aristoy & Toldrá, 1995). However, an excess of proteolysis (proteolysis index higher than 29-30%) might impair this typical flavour by exaggerating the bitter and metallic taste (Virgili *et al.*, 1995). Other

Table 1. Typical conditions for the processing of Serrano dry-cured ham. Adapted from Toldrá *et al.* (1996*a*)

Stages	Temperature (°C)	Relative humidity (%)	Time (days)
Reception/pre-salting	0–4	85–95	1–2
Salting	0–4	75–95	8–12
Post-salting	0–6	70–95	40–60
1st phase dry-curing	6–16	70–95	>45
2nd phase dry-curing	16–24	70–95	>35
3rd phase dry-curing	24–34	70–95	>30
4th phase dry-curing	12–20	70–95	>35

Table 2. Stability of the muscle enzymes in dry-cured ham, expressed as percentage of residual activity, after 8 and 12 to 15 months of processing. Adapted from Motilva *et al.* (1993*b*); Toldrá *et al.* (1992*c*, 1993*a*)

Enzymes	Residual activity (%), eight months	Residual activity (%), 12–15 months
Cathepsin B	20–25	5–10
Cathepsin B + L	20–30	5–10
Cathepsin H	15–20	5–10
Cathepsin D	0–5	0
m-calpain	0	0
Alanyl aminopeptidase	20–25	20–25
Aminopeptidase B	10–15	5–10
Leucyl aminopeptidase	90–95	15–20
Pyroglutamylaminopeptidase	20–25	0–5
Adipose neutral lipase	8–12	0
Acid lipase	40–45	5–10
Neutral lipase	40–45	10–15
Acid esterase	60–65	2–5
Neutral esterase	80–100	10–15

amino acids are correlated with specific ham tastes such as glutamic acid with saltiness, tyrosine and lysine with aged taste and leucine with acid taste (Careri *et al.*, 1993). These compounds may also act as flavour precursors in the generation of volatiles.

Lipolysis

Lipids of subcutaneous adipose tissue show an intense lipolysis during dry-curing. The onset of changes has been reported to occur during the first processing steps, salting and post-salting (Pezzani *et al.*, 1988) where a substantial increase in free fatty acids is reported (Motilva *et al.*, 1993*a*). In the case of muscle lipids, most of the lipolysis takes place during the initial five months (Motilva *et al.*, 1993*b*). As in the case of proteolysis, the muscle enzyme systems play an important role in the generation of free fatty acids.

Adipose tissue lipases

Adipose tissue contains three important lipolytic enzymes (Belfrage *et al.*, 1984): lipoprotein lipase, hormone-sensitive lipase and monoacylglycerol lipase. Optimal pH for these enzymes is in the neutral/basic range (Motilva *et al.*, 1992). The lipoprotein lipase is specific for primary esters so that fatty acids at position 1 have preference over those at position 3. Unsaturated monoacylglycerols are faster hydrolyzed than saturated compounds (Miller *et al.*, 1981). The hormone-sensitive lipase, which has an optimum of activity at neutral pH, hydrolyses the ester-bond in triacylglycerols and the resulting diacylglycerols. The last enzyme is the monoacylglycerol lipase that hydrolyses 1 or 2 monoacylglycerols with no positional specificity. These enzymes are active during salting and post-salting but only the neutral enzyme remains active during the ripening/drying (Motilva *et al.*, 1993*a*) as shown in Table 2.

An intense lipolysis, as a result of triglycerides hydrolysis, has been detected in the first stages of the process with an important generation of free fatty acids which accumulate as shown in Fig. 3. So, myristic, heptadecanoic, linolenic and arachidonic acids are those generated in greater amounts.

Muscle lipases

Lysosomal acid lipase is the major lipolytic enzyme in muscle. It is located in the lysosomes and hydrolyses tri, di and monoacylglycerols at acid pH (4.5–5.5) (Imanaka *et al.*, 1984), although it has a marked preference for primary ester bonds of triacylglycerol. However, only a slight degradation of triglycerides has been observed in dry-cured ham (Motilva *et al.*, 1993*b*; Buscailhon *et al.*, 1994) even though the enzyme is stable through the entire process. Acid phospholipase A2 catalyses the hydrolysis of the 2-acyl ester of sn-3-phosphoglycerides at the lipid/water interface (Yuan *et al.*, 1990). This enzyme plays an important role in the biochemical pathways involving phospholipids degradation. This is

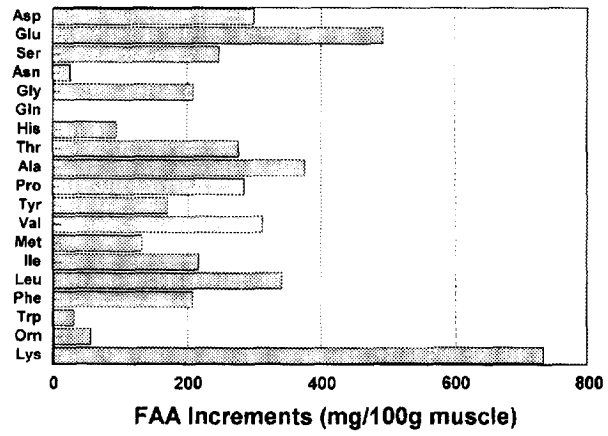

Fig. 2. Accumulation of free amino acids (FAA) in dry-cured ham after 15 months of processing. Adapted from Toldrá *et al.* (1995).

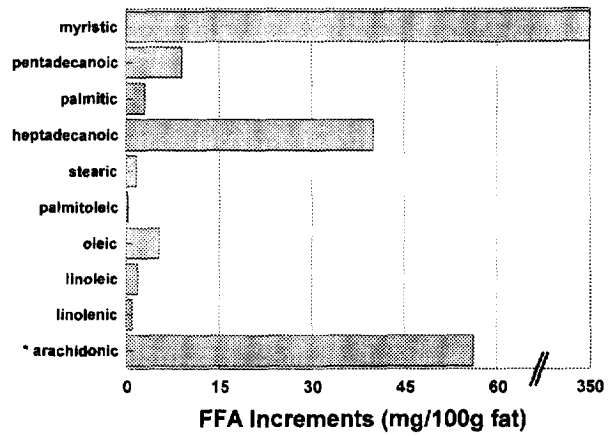

Fig. 3. Accumulation of free fatty acids (FFA) in the adipose tissue of dry-cured ham after 15 months of processing. Adapted from Motilva *et al.* (1993*a*).

also the case in dry-cured ham since most of the generated free fatty acids proceed from phospholipid degradation (Flores *et al.*, 1985; Buscailhon *et al.*, 1994). These free fatty acids accumulate as the process progresses up to 10 months where they reach a maximum (see Fig. 4) or even decrease. It should be taken into account that muscle lipases are still active after 15 months of processing (see Table 2). On the other hand, the generation of short chain free fatty acids is very low suggesting a minor role of muscle esterases even though they are quite stable (see Table 2) and active (Motilva *et al.*, 1993*b*).

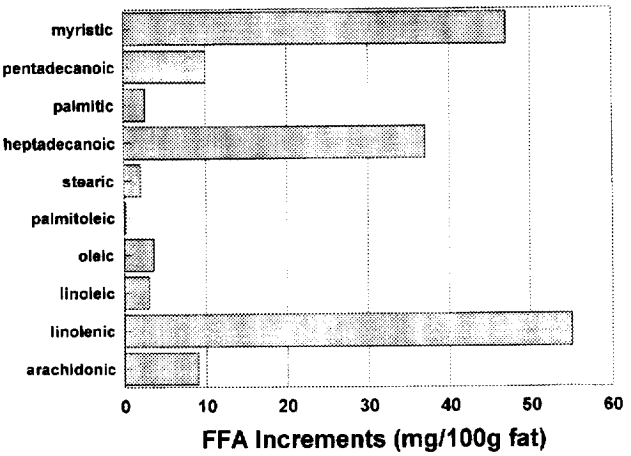

Fig. 4. Muscle accumulation of free fatty acids (FFA) dry-cured ham after 15 months of processing. Adapted from Motilva *et al.* (1993*b*).

Generation of volatiles

The typical aroma of dry-cured ham is correlated with the initiation of lipid oxidation (Flores *et al.*, 1985) and the subsequent generation of volatile compounds during the process, especially during the latest stages (Buscailhon *et al.*, 1993*a*). There are dozens of compounds, even though the particular extraction methodology used in each laboratory may give slight differences, already identified in the volatile fraction of Iberian (García *et al.*, 1991; López *et al.*, 1992) and French (Berdagué *et al.*, 1991; Buscailhon *et al.*, 1993*b*) dry-cured hams. The major groups are hydrocarbons (Loury, 1972), aldehydes — hexanal being the most abundant — and alcohols (García *et al.*, 1991). There are other compounds like ketones (Berdagué *et al.*, 1991), free fatty acids (Motilva *et al.*, 1992), γ-lactones (Berdagué & García, 1990), esters (Shahidi *et al.*, 1986) and other compounds such as benzene derivatives, amines and amides. Further discussion on volatiles is reviewed by other authors in this issue.

PROCESS INFLUENCE

Sensory profiles of dry-cured ham are strongly affected by the hydrolysis and oxidation patterns which can significantly differ depending on the raw ham properties and the manufacturing technique.

Effect of raw meat

Some differences in proteolytic activity, especially in cathepsin L and H and some aminopeptidases, have been detected among porcine breed types (Flores *et al.*, 1994) and, in fact, further research on pork genotypes and enzyme activity is currently being developed at the author's laboratory. On the other hand, no clear relationships have been found between muscle lipases and aminopeptidases and the metabolism of the muscles (Flores *et al.*, 1996) even though Buscailhon *et al.* (1993*b*) observed a favourable correlation between pigment concentration and sensory quality.

DFD (dark, firm and dry) hams have been reported to have lower proteolytic activity than normal hams while PSE (pale, soft and exudative) have a higher activity (Sárraga, 1992). High cathepsin B activity in raw hams has been statistically correlated with higher moisture content and lower protein levels (Schivazzappa *et al.*, 1992). Thus, a higher level of cathepsin B activity may result in an excess of softness as observed in deffective Parma hams (Parolari *et al.*, 1994). In a recent work, light hams (12.5 Kg average weight) from 11 months old pigs have been found to contain higher activities of cathepsins B and B + L and lower of pyroglutamylaminopeptidase and dipeptidylpeptidase IV than heavy (10.5 Kg average weight) hams from 7–8 months old pigs (Toldrá *et al.*, 1996*b*). In general, heavy hams showed a kind of trend characterised by a greater peptidase to proteinase ratio and a higher lipase activity (Toldrá *et al.*, 1996*b*).

Influence of process parameters

Dry-curing is a mild process where no high temperatures are used. Temperature is kept below 4°C during the salting and post-salting stages so that low enzyme activity is expected. In fact, most of the biochemical changes become significant in the drying/ripening stage where temperature rises to 14–16°C, or even to 20–24°C for a short period at the end of the process. These temperatures are adequate for a good activation of the enzymes.

Water activity decreases along the process down to values of 0.80 or even lower at the end. The enzyme activities progressively decrease as water activity is reduced (Toldrá *et al.*, 1992*c*, 1993*b*) except acid lipase and acid esterase that remain unaffected (Motilva & Toldrá, 1992).

Redox potential ranges from −150 mV to −250 mV during the processing of dry-cured ham, facilitating the activity of a great number of muscle proteases, such as cathepsins and some aminopeptidases, that need reducing conditions for activity (Motilva *et al.*, 1993*c*).

The pH experiences a slight increase along the process, starting at values around 5.6 and finishing at 6.3–6.5. This narrow pH range is however adequate for most of the muscle enzymes. Only cathepsin D, an acid proteinase, presents low activity (below 20%) at the pH typical of dry-cured ham (Rico *et al.*, 1990). It has to be taken into account that the time of processing is very long, between nine and 24 months depending on the final quality, and this allows a noticeable enzyme action even for those enzymes not working in optimal conditions.

Effect of curing agents and adjuncts

Salt and nitrate constitute the main curing ingredients while ascorbic acid is added as a curing adjunct (Flores & Toldrá, 1993). Salt plays an important role in controlling the enzyme activity. Thus, neutral lipase and acid esterase are inhibited by salt (Motilva & Toldrá, 1992) while cathepsins and aminopeptidases are strongly inhibited (Rico *et al.*, 1990, 1991; Toldrá *et al.*, 1992*c*, 1993*b*) except aminopeptidase B that shows an enhancement in activity because it is a chloride activated enzyme (Flores *et al.*, 1993). Acid lipase (Motilva & Toldrá, 1992) and m-calpain (Rosell & Toldrá, 1996) are also activated at low salt concentrations. Salt can help in controlling some defects such as the reported soft deffective Parma hams where the activity of cathepsin B is too high (Parolari *et al.*, 1994). In that case, an additional amount of salt would help in preventing that problem by inhibiting the excess of cathepsin B activity. Nitrate and ascorbic acid do not significantly affect the enzyme activities (Toldrá, 1992). Only m-calpain has shown inhibition by ascorbic acid (Rosell & Toldrá, 1996).

CONCLUSIONS

In summary, muscle proteases and lipases are very important for the development of typical sensory characteristics of dry-cured ham. A better knowledge of these endogenous enzymes as well as the effect of raw meat and process conditions is very important. Manipulation of process parameters or curing agents might give hams with different quality levels. Thus, the control of the muscle enzyme systems is essential for the optimal standardization of the processing and/or enhancement of flavour quality of dry-cured ham.

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REFERENCES

Aristoy, M.-C. & Toldra, F. (1991). Deproteinization technique for HPLC amino acids analysis in fresh pork muscle and dry-cured ham. *J. Agric. Food Chem.*, **39**, 1792–1795.

Aristoy, M.-C. & Toldrá, F. (1995). Isolation of flavor peptides from raw pork meat and dry-cured ham. In *Food flavors: generation, analysis and process influence*, ed. G. Charalambous. Elsevier Science Publ. B.V. Amsterdam, The Netherlands. pp. 1323–1344.

Astiasarán, I., Beriain, M. J., Melgar, J., Sanchez-Monge, J. M., Villanueva, R. & Bello, J. (1988). Estudio comparativo de las características de jamones curados de cerdo blanco elaborados con distinta tecnología. *Rev. Agroquim. Tecnol. Aliment.*, **28**, 519–528.

Belfrage, P., Fredrikson, G., Stralfors, P. & Tornqvist, H. (1984). Adipose tissue lipases. In *Lipases*, eds. B. Borgström & H. L. Brockman. Elsevier, Amsterdam, The Netherlands. pp. 365–416.

Bellatti, M., Dazzi, G., Chizzolini, R., Palmia, F. & Parolari, G. (1985). Modifications chimiques et physiques des proteines an cours de la maturation du Jambon de Parme. *Viandes Produits Carneés*, **6**, 142–145.

Berdagué, J. L., Denoyer, C., Le Queré, J.-L. & Semon, E. (1991). Volatile components of dry-cured ham. *J. Agric. Food Chem.*, **39**, 1257–1261.

Berdagué, J. L. & García, C. (1990). Les composants volatils du jambon sec. *Viandes Produits Carneés*, **11**, 319–320.

Buscailhon, S., Berdagué, J. L. & Monin, G. (1993*a*). Time-related changes in volatile compounds of lean tissue during processing of french dry-cured ham. *J. Sci. Food Agric.*, **63**, 69–75.

Buscailhon, S., Berdagué, J. L., Bousset, J., Cornet, M., Gandemer, G., Touraille, C. & Monin, G. (1993*b*). Relations between compositional traits and sensory qualities of french dry-cured ham. *Meat Sci.*, **37**, 229–243.

Buscailhon, S., Berdagué, J. L. & Monin, G. (1994). Time-related changes in intramuscular lipids of French dry cured ham. *Meat Sci.*, **37**, 245–255.

Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R. & Parolari, G. (1993). Sensory property relationships to chemical data of Italian-type dry-cured ham. *J. Food Sci.*, **58**, 968–972.

Etherington, D. J. (1987). Conditioning of meat factors influencing protease activity. In *Accelerated processing of meat*, eds., A. Romita, C. Valin & A. A. Taylor. Elsevier Applied Sci., London, 21–28.

Flores, M., Aristoy, M.-C. & Toldrá, F. (1993). HPLC purification and characterization of porcine muscle aminopeptidase B. *Biochimie*, **75**, 861–867.

Flores, M., Aristoy, M.-C. & Toldrá, F. (1996). Activity of aminopeptidase and lipolytic activity enzymes in five skeletal muscles with various oxidative patterns. *J. Sci. Food Agric.*, **70**, 127–130.

Flores, J., Nieto, P., Bermell, S. & Miralles, M.-C. (1985). Cambios en los lípidos del jamón durante el proceso de curado, lento y rápido, y su relación con la calidad. *Rev. Agroquim. Tecnol. Aliment.*, **25**, 117–124.

Flores, M., Romero, J., Aristoy, M.-C., Flores, J. & Toldrá, F. (1994). Differences in muscle proteolytic activities among pork breed types. *Sci. des Aliment.*, **14**, 469–474.

Flores, J. & Toldrá, F. (1993). Curing: Processes and applications. In *Encyclopedia of Food Science, Food Technology & Nutrition*, eds., R. Macrae, R. Robinson, M. Sadler & G. Fullerlove. Academic Press. London. 1277–1282.

García, C., Berdagué, J. L., Antequera, T., López-Bote, C., Córdoba, J. J. & Ventanas, J. (1991). Volatile components of dry-cured ham. *Food Chem.*, **41**, 23–32.

Goll, D. E., Otsuka, Y., Nagainis, P. A., Shannon, J. D., Sathe, S. K. & Mugurama, M. (1983). Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.*, **7**, 137–177.

Goll, D. E. (1991). Role of proteinases and protein turnover in muscle growth and meat quality. *Proc. Reciprocal Meat Conf.*, **44**, 25–36.

Harris, A. L., Gregory, J. S., Maycock, A. L., Graybill, T. L., Osifo, I. K., Schmidt, S. J. & Dolle, R. E. (1995). Characterization of a continuous fluorogenic assay for calpain I. Kinetic evaluation of peptide aldehydes, halomethyl ketones and (acyloxy)methyl ketones as inhibitors of the enzyme. *Bioorg. Med. Chem. Letters*, **5**, 393–398.

Imanaka, T., Amanuma-Muto, K., Ohkuma, S. & Takano, T. (1984). Characterization of lysosomal acid lipase purified from rabbit liver. *J. Biochem.*, **96**, 1089–1101.

Koohmaraie, M. (1994). Muscle proteinases and meat ageing. *Meat Sci.*, **36**, 93–104.

Lauffart, B. & Mantle, D. (1988). Rationalisation of aminopeptidase activities in human skeletal muscle soluble extract. *Biochim. Biophys. Acta*, **956**, 300–306.

López, M. O., de la Hoz, L., Cambero, M. I., Gallardo, E., Reglero, G. & Ordoñez, J. A. (1992). Volatile compounds of dry hams from Iberian pigs. *Meat Sci.*, **31**, 267–277.

Loury, M. (1972). Possible mechanisms of autooxidative rancidity. *Lipids*, **7**, 671–675.

Matsakura, U., Okitani, A., Nishimura, T. & Kato, H. (1981). Mode of degradation of myofibrillar proteins by an endogenous protease, cathepsin L. *Biochim. Biophys. Acta*, **662**, 41–47.

Miller, C. H., Parce, J. W., Sisson, P. & Waite, M. (1981). Specificity of lipoprotein lipase and hepatic lipase toward monoacylglycerols varying in the acyl composition. *Biochim. Biophys. Acta*, **665**, 385–392.

Molina, I., Silla, M. H., Flores, J. & Monzó, J. L. (1989*a*). Study of the microbial flora in dry-cured ham. II. Micrococaceae. *Fleischwirtsch.*, **69**, 1433–1434.

Molina, I., Silla, M. H., Flores, J. & Monzó, J. L. (1989*b*). Study of the microbial flora in dry-cured ham. III. Lactic acid bacteria. *Fleischwirtsch.*, **69**, 1709–1710.

Molina, I. & Toldrá, F. (1992). Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *J. Food Sci.*, **57**, 1308–1310.

Motilva, M.-J., Rico, E. & Toldrá, F. (1993). Effect of redox potential on the muscle enzyme system. *J. Food Biochem.*, **16**, 207–215.

Motilva, M.-J., Toldrá, F. & Flores, J. (1992). Assay of lipase and esterase activities in fresh pork meat and dry-cured ham. *Z. Lebensm. Unters Forsch.*, **195**, 446–450.

Motilva, M.-J., Toldrá, F., Aristoy, M.-C. & Flores, J. (1993). Subcutaneous adipose tissue lipolysis in the processing of dry-cured ham. *J. Food Biochem.*, **16**, 323–335.

Motilva, M.-J. & Toldrá, F. (1992). Effect of curing agents and process parameters on pork muscle lipolityc activity. *Z. Lebens Unters. Forsch.*, **196**, 228–232.

Motilva, M.-J., Toldrá, F., Nieto, P. & Flores, J. (1993). Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chem.*, **48**, 121–125.

Okitani, A., Nishimura, T. & Kato, H. (1981). Characterization of hydrolase H, a new muscle protease possesing aminoendopeptidase activity. *Eur. J. Biochem.*, **115**, 269–274.

Ouali, A. & Talmant, A. (1990). Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Sci.*, **28**, 331–348.

Parolari, G., Virgili, R. & Schivazzappa, C. (1994). Relationship between cathepsin B activity and compositional parameters in dry-cured hams of normal and defective texture. *Meat Sci.*, **38**, 117–122.

Pezzani, G., Barbuti, S. & Ghisi, M. (1988). Modificazione del tessuto adiposo durante la stagionatura del prosciutto. *Ind. Conserve*, **63**, 338–342.

Rico, E., Toldrá, F. & Flores, J. (1990). Activity of cathepsin D as affected by chemical and physical dry-curing parameters. *Z.Lebensm.Unters. Forsch.*, **191**, 20–23.

Rico, E., Toldrá, F. & Flores, J. (1991). Effect of dry-curing process parameters on pork muscle cathepsins B, H and L activities. *Z. Lebensm. Unters. Forsch.*, **193**, 541–544.

Rodríguez-Nuñez, E., Aristoy, M.-C. & Toldrá, F. (1995). Peptide generation in the processing of dry-cured ham. *Food Chem.*, **53**, 187–190.

Rosell, C.-M. & Toldrá, F. (1996). Effect of curing agents on m-calpain activity through the curing process. *Z. Lebensm. Unters. Forchs.*, **203**, 320–325.

Sárraga, C. (1992). Meat proteinases and their relation with curing. In *New technologies for meat and meat products*, eds., F. J. M. Smulders, F. Toldrá, J. Flores & M. Prieto. Audet, Nijmegen, The Netherlands, pp. 233–246.

Schivazzappa, C., Virgili, R. & Parolari, G. (1992). Proteolytic enzymes in raw ham. II. Correlation with chemical parameters of fresh muscle. *Ind. Conserve*, **67**, 413–416.

Schwartz, W. N. & Bird, J. W. C. (1977). Degradation of myofibrillar proteins by cathepsins B and D. *Biochem. J.*, **167**, 811–820.

Shahidi, F., Rubin, L. J. & d'Souza, L. A. (1986). Meat flavor volatiles: A review of the composition, technique of analysis and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.*, **24**, 219–227.

Spanier, A. M. (1992). Current approaches to the study of meat flavour quality. In *Food science and human nutrition*, ed. G. Charalambous. Elsevier Science Publ. B.V. Amsterdam, The Netherlands. pp. 695–709.

Spanier, A. M. & Miller, J. A. (1993). Role of proteins and peptides in meat flavor. *ACS Symp. Series* **528**, Washington, DC. pp. 78–97.

Toldrá, F. (1992). The enzymology of dry-curing of meat products. In *New Technologies for meat and meat products*, eds F. J. M. Smulders, F. Toldrá, J. Flores & M. Prieto. Audet, Nijmegen, The Netherlands. pp. 209–231.

Toldrá, F. & Aristoy, M.-C. (1993). Availability of essential amino acids in dry-cured ham. *Int. J. Food Sci. Nutr.*, **44**, 215–219.

Toldrá, F., Aristoy, M.-C., Cerveró, M.-C., Rico, E., Part, C., Motilva, M.-J. & Flores, J. (1992*c*). Muscle and adipose tissue aminopeptidase activities in raw and dry-cured ham. *J. Food Sci.*, **57**, 816–818, 833.

Toldrá, F., Cerveró, M.-C. & Part, C. (1993*b*). Porcine aminopeptidase activity as affected by curing agents. *J. Food Sci.*, **58**, 724–726, 747.

Toldrá, F. & Etherington, D. J. (1988). Examination of cathepsins B, D, H and L activities in dry-cured hams. *Meat Sci.*, **23**, 1–7.

Toldrá, F., Flores, M. & Aristoy, M.-C. (1995). Enzyme generation of free amino acids and its nutritional significance in processed pork meats. In *Recent developments in food science and Nutrition*, ed., G. Charalambous. Elsevier Science Publ. B.V. Amsterdam, The Netherlands. pp. 1303–1322.

Toldrá, F., Miralles, M.-C. & Flores, J. (1992*a*). Protein extractability in dry-cured ham. *Food Chem.*, **44**, 391–394.

Toldrá, F., Flores, M., Navarro, J.-L., Aristoy, M.-C. & Flores, J. (1996*a*). New developments in dry-cured ham. In *Chemistry of novel foods*, eds H. Okai, O. Mills, A. M. Spanier & M. tamura. Allured Pub. Co., Carol Stream, IL. in press.

Toldrá, F., Flores, M., Aristoy, M.-C., Virgili, R. & Parolari, G. (1996*b*). Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *J. Sci. Food Agric.*, **71**, 124–128.

Toldrá, F., Rico, E. & Flores, J. (1992*b*). Activities of pork muscle proteases in cured meats. *Biochimie*, **74**, 291–296.

Toldrá, F., Rico, E. & Flores, J. (1993*a*). Cathepsin B, D, H and L activities in the processing of dry-cured ham. *J. Sci. Food Agric.*, **62**, 157–161.

Toldrá, F. & Verplaetse, A. (1995). Endogenous enzyme activity and quality for raw product processing. In *Composition of meat in relation to processing, nutritional and sensory quality*, eds., K. Lundström, I. Hansson & E. Wiklund. ECCEAMST, Utrecht, The Netherlands. pp. 41–55.

Virgili, R., Parolari, G., Schivazzappa, C., Bordini, C. & Volta, R. (1995). Effects of raw material on proteolysis and texture of typical Parma ham. *Ind. Conserve*, **70**, 21–31.

Yuan, W., Quinn, D. M., Sigler, P. B. & Gelb, M. H. (1990). Kinetic and inhibition studies of phospholipase A2 with short chain substrates and inhibitors. *Biochem.*, **29**, 6082–6094.

Zeece, M. G. & Katoh, K. (1989). Cathepsin D and its effect on myofibrillar proteins: a review. *J. Food Biochem.*, **13**, 157–178.