MUSCLE TRAITS FOR LONG MATURED DRIED MEATS

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ABSTRACT

The distinctive flavour of aged pork products is a complex mix of taste, smell and even touch. To meet increasing consumer's preference for ethnic foods or foods addressing linkage with specified geographic origin or complying with traditional manufacturing and breeding guidelines, efforts should be made to encourage selection of processing techniques and raw materials targeting desired end product qualities. Thanks to research carried out with sensory analysis and chemical and physical characterisation of raw and matured pork, as well as breeding and genetics, improved knowledge is now available relating dried meat properties to raw matter or manufacturing. The achievement of the distinctive taste and texture properties of aged products through genetics (animal selection for the enhancement or the removal of some muscle traits), breeding (effect of diet and slaughtering age on pork properties) with reference to current findings about this type of products is discussed.

Keywords: Aged products, Sensory analysis, Proteolytic enzymes, Meat quality, Heritability

1. INTRODUCTION

In countries where agriculture mainly relies on pig production and processing of meat into high added value typical products (mainly dry-cured hams), a branch of pork quality research has been addressed to improve quality of end products by genetic selection, breeding techniques and manufacturing practices.

The scientific approach to matured pork products during the last decades was mainly focussed on the development of a comprehensive vocabulary of sensory descriptors and the research of analytical parameters related to product properties.

By means of these studies, the role of such green muscle traits as pH, proteolytic enzymes, fat content and morphology as related to origin crossbreed, were investigated for their effects on pork muscle at various stages of the process, including salting, drying, and maturing under controlled temperature and relative humidity conditions.

The influence of pH (fall rate and ultimate value) on drip-loss, colour, protein status, tenderising process of fresh meat has been widely investigated and established, while fewer studies are available on the effect of this muscle parameter on sensory and chemical properties of dry-cured ham.

Muscle proteolytic activity has been extensively studied for tenderness and flavour promotion in fresh meat, with major emphasis on the calpain-calpastatin system whose activity is exhausted in the short term of meat conditioning. In the case of dry cured muscle products like matured hams a key role for cathepsin and exopeptidase long-term activities has been postulated to explain taste and texture as affected by non-volatile low molecular weight nitrogen molecules and their changes during maturation.

Crossbreeds selection was aimed at improving growth rate, carcass traits, lean meat yield and meeting consumer demand for tender, juicy, acceptably red and adequately marbled pork, but these achievements may have lowered the sensory and technological quality of cuts to be processed into dry-cured, aged products.

It is the purpose of this communication to discuss current knowledge of muscle traits as related to technological processes adopted for long matured pork meats, and provide an overview of data or tools available to manufacturers willing to improve sensory quality of end products by selection or control of raw matter.

While it is beyond the aim of these authors to discuss all mechanisms and processes relating to flavour of dried products, this work will deal essentially with taste and texture of long-matured meats.

2. TASTE AND TEXTURE SENSORY PROFILING OF DRY CURED HAM

Several studies have been reported in the past decade dealing with sensory properties of dry cured ham by descriptive analysis (Virgili, 1994; Flores et al, 1997a; Ruiz et al., 1998), hedonistic testing (Rousset-Akrim et al., 1996; Virgili et al., 1997; Lozano et al., 1999), and instrumental-to-sensory relationships (Careri et al., 1993; Buscailhon et al., 1994).

In Table 1 a number of sensory and related analytical parameters are listed. The reported attributes were found related with fresh or/and aged muscle parameters and were able to describe specified taste or texture perceptions for a variety of dry-cured ham types.

Regardless of differences in dry-cured hams due to manufacturing techniques based on geographic habits or tutelary guidelines, main suggestions may be obtained from Table 1:

- a number of muscle measurements are available to track undesired defective traits occurring in dry-cured hams, while fewer measurements are related to accepted sensory properties;
- attributes describing accepted aged taste appear to be dependent on ham type and panel origin. Such geographic bias is also confirmed by affective tests showing best consumer preference for domestic hams or hams with known or customary sensory properties (Rousset-Akrim et al., 1996; Virgili et al., 1997; Lozano et al., 1999); in contrast,
- attributes describing less desired taste and texture (saltiness, bitterness, pastiness, and firmness), show relationships with aged or fresh muscle properties common to all ham types.

Therefore, the perception of valuable sensory descriptors of taste and texture seems to be mainly due to the achievement of:

- selected proteolysis products;
- proper dehydration, firmness and marbling degree of aged muscle;
- ham morphology from more traditional crossbreeds.

On the other hand, unwanted sensory descriptors (bitterness, pastiness) may be ascribed to:

- uncontrolled extent of proteolysis and release of low-weight nitrogen molecules;
- high activity of some proteases;
- high pH_{24h};
- ham morphology from heavily muscled crossbreeds;
- incorrect dehydration (poor or very high).

For raw muscle, endogenous proteolytic enzymes, meat quality and carcass traits with particular emphasis to pH and origin crossbreed were charged for playing a main role in the onset of aged muscle properties.
Therefore, management of over mentioned muscle traits through genetic, breeding and other means affecting pork quality, will likely affect dry-cured ham outcome.

Table 1. Aged or raw (italics) muscle parameters enhancing perception of reported sensory descriptors in several dry-cured ham types

Sensory attribute	Ham type	Muscle trait	Reference [a]
Aged/dry-cured taste	Parma	Tyr and Lys	Careri et al., 1993
	Parma	NaCl, total nitrogen, hardness [b]	Virgili et al., 1995
	French	Dry matter, glycogen, Cl^-	Buscailhon et al., 1995
	Spanish	*Duroc line (high intramuscular fat)*	Guerrero et al., 1996
	Serrano	Most FAAs and unidentified peptides (MW<3000)	Flores et al., 1997b
	Parma	Glu-Tyr	Sforza et al., 2001
Acid taste/sour	Parma	Phe and Ile, NPN [c]	Careri et al., 1993
	French	dry-matter, glycogen, lactic acid, Cl^-	Buscailhon et al., 1995
	Serrano	Asp and Glu	Flores et al., 1997a
Salty taste	Parma	Glu and NaCl	Careri et al., 1993
	Spanish	*F1 pig line*	Guerrero et al., 1996
	Spanish	*PSE*	Banon et al., 1998
	Iberian	NaCl	Ruitz et al., 1999
Bitterness	Parma	NPN, *cathepsin B*, white film	Virgili et al., 1995
	Parma	NPN, *cathepsin B*, white film, DPPI, DPPII, NPN, FAAs, Asn, Ile	Virgili et al., 1998
	Serrano	Asn, unidentified peptide	Flores et al., 1997b
	Iberian	hydrophobic peptides	Ruiz et al., 1999
	Parma	NPN, Gln, Phe, Ile, Leu, Asn, GlyLeu(Ile), Leu(Ile)Leu(Ile), GlyPhe	Sforza et al., 2001
Pastiness/ mellowness/ mushy texture	Spanish	NPN	Arnau, 1991
	French	pH_{24h}	Buscailhon et al., 1994
	Spanish	*Duroc sire*	Gou et al., 1995
	Parma	moisture, NPN	Virgili et al., 1995
	Spanish	*heavily muscled pig*, NPN	Guerrero et al., 1996
	Spanish	weight losses, Tyr, white film	Arnau et al., 1997
	Spanish	pH_{24h}, moisture	Arnau et al., 1998
	Spanish	NPN, white spots, moisture	Garcia-Garrido et al., 1999
	Spanish	pH_{24h}	Guerrero et al., 1999
	Spanish	cathepsin B, B+L	Garcia-Garrido et al., 2000
Hardness/ Firmness	Spanish	*Large White sire*	Gou et al., 1995
	Italian	*L*, hue angle*	Chizzolini et al., 1995
	Spanish	NaCl	Arnau et al., 1997

[a] extensive references are given in the text and in the reference list.
[b] mechanical measurement made on m. *biceps femoris* by means of compression test.
[c] per cent ratio between nitrogen soluble in 5% trichloroacetic acid and total nitrogen of ham.

3. PROTEOLYTIC ENZYMES

Several muscle proteolytic enzymes were investigated for their significance in the proteolytic pathway leading to aged meat products. Studied were focussed on those enzymes whose optimum pH is consistent with the muscle pH that, from chilling phase to fully matured ham can span from 5.3 to 6.5. Table 2 reports most such peptidases. Proteolytic enzymes are hierarchically grouped into major catalytic types, clans and families with reference to the chemical nature of the group responsible for catalysis, similarity in three dimensional structure and amino acid sequence responsible for proteolytic activity, respectively. This classification (Barrett, 1997), based on the molecular structures of the enzymes and reflecting evolutionary relationships, updates the one previously released by Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB 1992), where an enzyme was classified by the substrates it hydrolyses.
Many studies are available on activity, specificity, structural chemistry, biological aspects and distinguishing features of reported proteolytic enzymes (for a comprehensive overview of peptidases see Barrett et al., 1998), but, with reference to their effect on dry-cured products, the following points can be regarded as milestones:

- large variability in proteolytic enzymes reported in Table 2 was found in pork muscles;
- fresh to aged muscle properties together with dry-curing manufacturing practices allow these proteolytic enzymes to keep partly their activity up to the end of maturing (up 2 years);
- a significant link has been established between the activity of some peptidases in fresh muscle, the degree and/or pattern of dry-cured ham proteolysis and sensory descriptors;
- muscle enzyme activities are affected by muscle pH and by sodium chloride diffusion inside the muscle.

Table 2. List of proteolytic enzymes more widely investigated in raw matter to be processed into aged meat products

Name and NC-IUBMB enzyme classification	Catalytic type	Clan	Family	pH optimum	Location
Cathepsin B (EC.3.4.22.1)	Cysteine peptidase	CA	C1	3.5-6.0	lysosomes
Cathepsin L (EC.3.4.22.15)	Cysteine peptidase	CA	C1	3.0-6.5	lysosomes
Cathepsin H (EC.3.4.22.16)	Cysteine peptidase	CA	C1	5.5-6.5	lysosomes
Cathepsin D (EC.3.4.23.5)	Aspartic peptidase	AA	A1	2.5-5.0	lysosomes
Dipeptidyl-peptidase I or cathepsin C (EC.3.4.14.1)	Cysteine peptidase	CA	C1	5.0-6.0	lysosomes
Dipeptidyl-peptidase II (EC.3.4.14.2)	Serine peptidase	SC	S28	5.0-6.0	lysosomes
Aminopeptidase Puromycin-Sensitive (PS) or alanyl aminopeptidase (EC.3.4.11.14)	Metallopeptidase	MA	M1	6.5-7.5	cytosol
Aminopeptidase B (EC.3.4.11.6)	Metallopeptidase	MA	M1	6.5-7.0	cytosol

These peptidases release from muscle proteins and peptides non-volatile low-weight nitrogen molecules responsible for the development of dry-cured ham taste. Recent studies allowed the FAAs and the sequence of some peptides positively affecting dry-cured ham flavour to be identified (Table 1); on the other hand, abnormal proteolysis degree and increase of specified FAAs and peptides, due to high activities of endogenous cathepsins were charged with drawbacks of taste and texture in Serrano and Parma hams.

While for cathepsins B and L a direct role in dry-cured ham sensory properties and proteolysis has been reported (see Table 1), for exopeptidases a debittering role was evidenced in β-casein hydrolysates but not for meat products (Barry et al., 2000). Aminopeptidase PS is the major cytosolic aminopeptidase in mammalian cells, but its role in enhancing proteolysis seems to be secondary to endopeptidase protein beakdown. Hiroi et al. (1992) reported that degradation of hemoglobin by a purified endopeptidase was significantly enhanced by the addition of aminopeptidase PS, which by itself didn't have protein degrading activity. In case of dry-cured hams, green hams with low cathepsin B activity and high exopeptidase activities resulted in hams with low proteolysis degree (Virgili et al., 1998). The added salt proved to have a stronger inhibitory action on aminopeptidase PS (Flores et al., 1997c) than on cathepsins B and L (Toldrà et al., 1993), and an activating role for aminopeptidase B. Possible effects of selective inhibitory action of sodium chloride may be found in proteolysis profile of Parma ham, where the per cent ratio of FAAs to NPN and salt amounts of dry-cured hams were related (Toscani et al., 2000). As shown in Fig. 1, FAAs contribution to NPN is significantly influenced by salt in such a way that FAAs release (mainly ascribed to aminopeptidase PS) is lowered by sodium chloride increase. Salt addition proves a tool for affecting NPN pattern besides NPN amount (Virgili et al., 1999). Among FAAs released during dry-cured ham ageing, lysine is one of the more abundant (Flores et al., 1997b; Virgili et al., 1999) and, as shown in Table 1, lysine is one of parameters enhancing the perception of "dry-cured ham" flavour. Aminopeptidase B is strictly selective for removing lysine and arginine residues and its activity is enhanced by chloride anions in the range 150 mM (Cadel et al., 1995). A relationship ($r = 0.73$, $p<0.001$) was found between per cent lysine in FAAs and sodium chloride amount in dry-cured ham (Toscani et al., 2000). Since salt increases don't meet nutritional and taste requirements, a higher activity of aminopeptidase B, might be a tool for improving "dry-cured ham" perception by means of the increase of lysine.

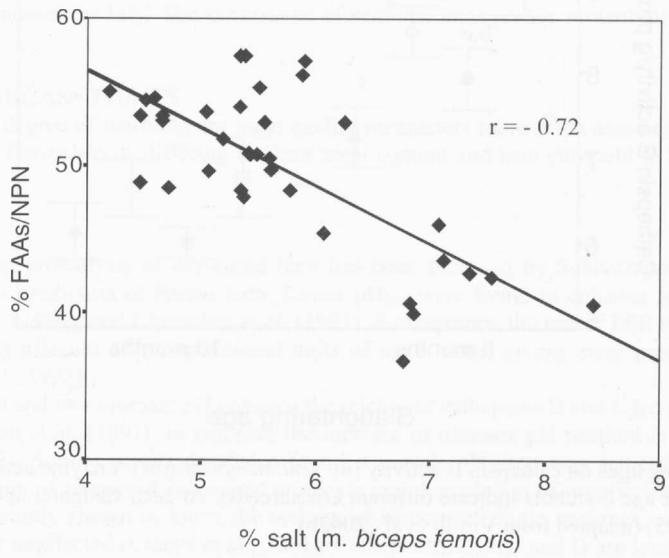

Fig. 1. Correlation between salt content and FAA-to-NPN ratio in 13-month-old Parma hams. FAAs in g/100g protein (*adapted from Toscani et al., 2000*).

Further studies are needed to relate exopeptidase activities of raw matter to pattern and degree of proteolysis of aged muscle, taking into account the influence of processing parameters and other muscle proteolytic enzymes.

Changes in dried muscle composition (salt, fat and moisture) may be an additional source of variability for the perception of pleasant or unwanted tastes attributable to proteolysis: type and amount of molecules imparting bitterness and pastiness to Parma ham, may be associate to accepted dry-cured ham flavour in more dried, salty and firm ham classes. In Table 3 an overview is given for composition of more known types of European dry-cured hams.

More investigations are needed to detect the proteolytic pathway involved in the generation of molecules with positive influence on taste and to assess the muscle proteolytic pattern more suitable for the production of dry-cured ham. The aim is managing raw matter properties to meet dry-cured ham requirements for a valuable sensory profile.

In this direction works are being been carried out to put into evidence possible sources of variability of muscle proteolytic enzymes, in order to achieve the best enzymatic profile with regard to ham type, salt adjuncts, and dehydration through genetics and breeding.

Table 3. Mean proximate chemical components of some types of European hams (taken from Virgili et al. 1999)

Components [a]	IBE	CV [b]	SER	CV	COR	CV	BAY	CV	ICS	CV	PAR	CV
Moisture	53.3^{b}	4.8	57.5^{c}	5.6	45.2^{a}	8.3	60.8^{d}	2.3	61.0^{d}	4.1	60.8^{d}	2.0
NaCl	5.3^{a}	15.6	6.6^{b}	23.6	9.2^{d}	24.4	$6.8^{b,c}$	10.3	7.6^{c}	16.8	$6.1^{a,b}$	16.2
Protein	27.8^{a}	9.1	27.6^{a}	10.5	32.5^{c}	15.9	29.3^{b}	13.2	26.5^{a}	6.8	26.8^{a}	9.8
NPN	40.8^{e}	8.5	34.0^{d}	9.7	30.4^{c}	29.6	$27.3^{a,b}$	14.5	25.1^{a}	13.9	$28.9^{b,c}$	13.3
M:P	1.9^{b}	10.0	2.1^{c}	9.7	1.4^{a}	22.3	2.1^{c}	13.3	2.3^{d}	9.7	2.3^{d}	6.1

Means among ham classes having the same superscript are not significantly different (P>0.05). Ham abbreviations: IBE=Iberian, SER=Serrano, COR=Corsican, BAY=Bayonne, ICS=Italian Country-style, PAR=Parma.

[a] As percentage of meat (w/w). NPN as per cent ratio between nitrogen soluble in 5% trichloroacetic acid and total nitrogen of ham. M:P = moisture-to-protein ratio.

[b] Coefficient of variation.

3.1. Pork age, genetics and feeding as source of variability for muscle peptidases

3.1.1. Age effect

Sarraga et al. (1993) found significantly higher calpain, cathepsin B, L and D activities in Large White barrows slaughtered at 90 kg than those slaughtered at 130 kg. This result is in agreement with the role of proteolytic enzymes in protein turnover and muscle growth. *In vivo* protein turnover can be regarded as consisting of two contributions: a turnover associated with the maintenance of cell function and a turnover associated with growth. The latter is close to zero in the mature individual (Reeds, 1989) which may account for lower proteolytic activities of older pigs, according to findings from Toldrà et al. (1996) and Armero et al., (1999a) for cathepsin B, B+L and H. Virgili et al., (2002b) confirmed these results by means of a trial performed with Italian heavy pigs (4 crosses, divided into gilts and barrows, given the same dietary regimen) slaughtered at 8 and 10 months. As displayed in Fig. 2 for cathepsin B, age increase proved to be a tool both for lowering proteolytic activity and differences among crossbreeds. Opposite results were found for cytosolic aminopeptidase PS, higher in heavier and older pigs (Toldrà et al., 1996; Rosell & Toldrà, 1998), and in pork with lower cathepsin B and L activities (Virgili et al. 1998).

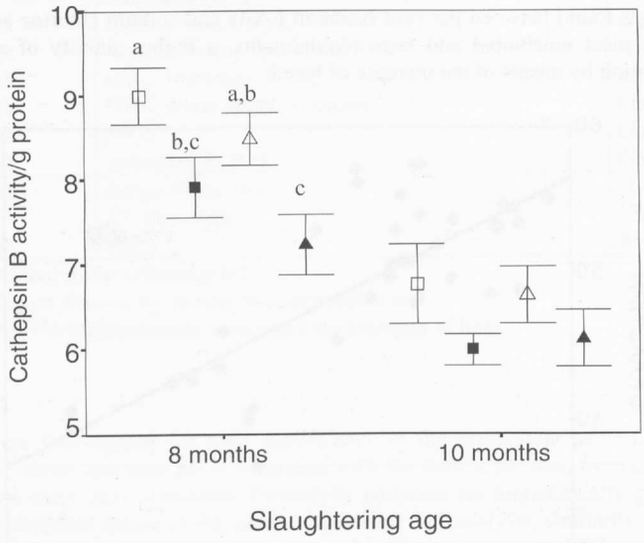

Fig. 2. Effect of crosses and slaughtering ages on cathepsin B activity (m. *semimembranosus*). Enzyme activity (mean ± SEM) is reported as average of each cross at each slaughter age (symbols indicate different crossbreeds). At each slaughter age, the means denoted by different letters are significantly different (p<0.05) (adapted from Virgili et al., 2002b).

3.1.2. Genetics effect

As to proteolytic enzymes, the role of pork genetics was investigated, to evaluate if:

- changes in muscle proteolytic pattern may be ascribed to different genetic type;
- heritabilities of assayed proteolytic enzymes allow a genetic selection to be carried out for influencing muscle peptidase activities.

Armero et al. (1999a) and Armero et al., (1999b) found significant effects of sire types on muscle endo- and exopeptidases, reporting lower activities for more heavily-muscled crossbreeds like Belgian Landrace, than for Large White and Duroc sire types. Schivazappa et al. (2002), examining over 400 from Italian purebreeds Large White, Landrace and Duroc, found higher cathepsin B activity in fresh muscles from Duroc hence higher NPN in corresponding dry-cured hams. Armero et al. (1999c) reported a higher sensory score for the descriptor "tyrosine crystals" (a marker of muscle proteolysis during aging) for dry-cured Spanish ham obtained from Duroc sired pigs.

Though in these studies some significant differences were found among crossbreeds, the data collected by Schivazappa et al. (2002) put into evidence a large variability within the same breed, while between full-sibs from the central Sib-Test station (groups of two to three animals), a major homogeneity was found.

A first attempt to investigate the opportunity of affecting muscle proteolytic activity by means of genetic selection, was carried out by Russo et al. (2000), who reported a moderate heritability (0.23-0.28) for cathepsin B (m. *semimembranosus).*
Additional researches are in course to update this first trial using only purebred Italian Large-White (189 groups of two full sibs sired by 121 boars). Studies address testing of pH_{24h}, cathepsin B and B+L, aminopeptidase PS and cysteine proteinases inhibitor (anonymous, 2001). The "*Single Trait*" linear model was applied, to calculate heritabilities of over mentioned enzymes and inhibitor:

$$Y_{(i,j,k,l)} = M + SD_{(i)} + S_{(J)} + b1*SAGE + b2*CONS + A_{(k)} + E_{(i,j,k,l)}$$

where M = general mean, SD = day of sampling, S = sex, b1 = reg. part. coefficient for age at slaughtering (SAGE), b2 = reg. part. coefficient for consanguinity (CONS), A = animal, E = error.
First results from this study give a good h^2 for cathepsin B and for cystatin endogenous inhibitors (0.66 and 0.43 respectively). No other literature is available on this issue for proteolytic enzymes, and a comprehensive report of the research will be published. A previous study was carried out by Shakelford et al. (1993), to estimate h^2 of bovine post rigor calpastatin activity, finding that this activity was a highly heritable trait (h^2=0.65) and selection against calpastatin activity should be a suitable mechanism for improving meat tenderness.

3.1.3. Diet effect

Few data are available about the influence of dietary regimen on muscle proteolytic enzymes; Van den Hemel-Grooten et al. (1997) showed a significant decrease of mRNA levels for μ-calpain for the groups of barrows fed with a protein-free diet. Aminopeptidase PS and aminopeptidase B activities of heavy pigs fed with a diet characterised by low lysine/energy ratio (2.28g/Mcal net energy average value in the range 40-160 kg), were significantly lower than activities of the groups fed with higher ratios (Della Casa, personal communication). A more recent approach has been reported by Kristensen et al. (2001), who achieved a significantly higher μ-calpain activity and lower Warner-Bratzler shear force for m. *longissimus dorsi* of pigs undergoing 2 months restricted and 2 months ad libitum feeding (40 to 110 kg weight range). This treatment caused an accelerated growth (compensatory growth) during ad libitum feeding, and the result in terms of tenderness improvement emphasizes the difference between fast- and slow-growing muscle as to proteolytic potential of calpain-calpastatin system. A similar treatment was applied to domestic heavy pigs (5 months restricted feeding followed by 3-4 months of standard feeding), in a trial performed at a test station (Virgili 2001, unpublished results). The group of more restricted pigs, if compared with control group, were significantly higher in cathepsin B and B+L, and lower in aminopeptidase PS activity. Based on these results, the possibility of affecting muscle proteolytic potential by means of dietary regimen, may be envisaged. Accordingly, dietary treatments need to be fitted to pig type and use: those to be processed into aged product would avoid feed restriction during the first breeding phase followed by a quick growth, while in case of pork for fresh consumption, this treatment may be a tool for improving meat tenderness.

3.2. Other known sources of variability for muscle proteolytic enzymes

Like fertility, body growth, backfat thickness, muscle proteolytic enzymes also exhibit significant seasonal changes. Seasonal changes for cathepsin B were detected by means of a patterned sampling from breeding houses, taking the same number of samples once a season, over four years. The rhytmometric analysis of time series data of cathepsin B (Virgili et al., 2002a), supported the hypothesis of significant seasonal change of the enzyme activity, yielding a fitted period corresponding to the year (circannual), reaching a maximum predicted value at the half of January and a minimum in July. The occurrence of seasonal changes for proteolytic enzymes was reported in mackerel too (Matsumiya et al., 1990).

4. MEAT QUALITY AND CARCASS TRAITS

As reported in Table 1, pH and degree of marbling are meat quality parameters more often associated with aged muscle sensory properties. As to carcass traits, suitability of crossbreeds differing for lean meat content and lean cut yield to be processed into dry-cured hams, are a matter of investigation.

4.1. Meat quality: pH

The role of pH_{24h} in influencing proteolysis of dry-cured ham has been focussed by Schivazappa et al. (2002) by means of a multiple regression model for proteolysis prediction of Parma ham. Lower pH_{24h} were found to enhance proteolysis in dry-cured ham, supporting previous findings of Arnau et al. (1994), and Chizzolini et al. (1995). Furthermore, the use of PSE meat (pH_{1h}<6.0 in m. *semimembranosus*) in dry-cured ham manufacturing affected all compositional traits of aged hams, giving more proteolysed (Tabilo et al., 1999) salty and dehydrated products (Banon et al., 1998).
High rate of post-mortem pH fall and low ultimate pH enhance the release of cathepsins B and L from lysosomes and their activity on muscle proteins, as shown by O'Halloran et al. (1997). In contrast, the increase of ultimate pH resulted in a lower release of cathepsin B+L from lysosomes (Ertbjerg et al., 1999). Similar results were also found if muscle pH drop was achieved by means of post-mortem lactic acid injections, yielding an acceleration of release of lysosomal proteases and degradation of myofibrillar proteins (Berge et al., 2001). A faster pH post-mortem decline was recently shown to lower the activity of some proteolytic enzymes, as a result of protein denaturation, while cathepsins (B, B+L and D) were unaffected (Claeys et al., 2001). Cathepsins B, B+L and D are located within lysosomes, where pH is low, and have been shown to be rapidly inactivated at neutral pH (Turk et al., 1995). In these respect, intact cathepsin activities, enhanced leakage from lysosomes and breakdown susceptibility of denatured protein may account for higher proteolysis found in low pH dry-cured hams.
A research project (FAIR-CT-97-9517) was carried out to evaluate if variability of pH_{24h} together with variability of cathepsin B and L activities, might be an explanation for unhomogeneity found in dry-cured ham production in terms of texture and proteolysis (Magraner et al, 2002). To minimize the influence of weight, salt amount and manufacturing technique, and focus the effect of meat quality on dry-cured ham features, raw hams were selected within a narrow weight range, and cured with a very low salt amount (final salt 4-5%) at the same plant. For the processing of Italian hams, over 600 thighs (heavy and light hams) were tested for pH_{24h}, cathepsin B and L in order to have a satisfactory number of samples for several categories of meat quality. According to values of pH and cathepsin activity (m. *semimembranosus*), the hams were grouped and compared as shown in Fig. 3 for Parma hams. NPN is a rough index of proteolysis but proves to be associated with sensory properties of dry-cured hams (see Table 1) like bitterness and pastiness. Data displayed in Fig. 3 show that pH of raw matter plays a main role in affecting the final proteolysis of aged hams and accounts for NPN differences between hams with the same levels of cathepsin B activity. As reported in Table 1, an increase of NPN affects ham taste and texture: a study of texture profile of

Spanish dry-cured hams (Tabilo et al., 1999) evidenced lower springiness, cohesiveness and chewiness for dry-cured hams from PSE meat than normal quality meat (RFN).
As displayed in Fig. 3, the increase of pH_{24h} (DFD-type meat) is associated with lower NPN, in agreement with results of Guerrero et al., (1999) and Schivazappa et al., (2002). Similar findings were given by the analysis of peptide fractions and FAAs released in pork 2 hours post-mortem, (Flores et al., 2000) showing lower peptide areas and FAAs amount for DFD meat. However, the possibility of limiting excessive proteolysis by higher meat pH_{24h} proves unsuitable, because dry-cured hams from high pH thighs, were found to be impaired by a poor texture as witnessed both by sensory and mechanical data (Guerrero et al., 1999). A decrease of hardness was also reported by Schivazappa et al. (1997) for Italian coppas (a product consisting of cervical muscles, filled in natural casings and aged for 4-6 months) where more proteolysed aged product came along with high meat pH_{24h}. Such texture impairment was not due to lower salt intake, that was similar or even higher in high pH hams (Buschailon et al., 1994, Arnau et al., 1998, Schivazappa et al., 2002), but might be partly ascribed to the higher water-holding capacity of high pH meat, leading to lower muscle dehydration.

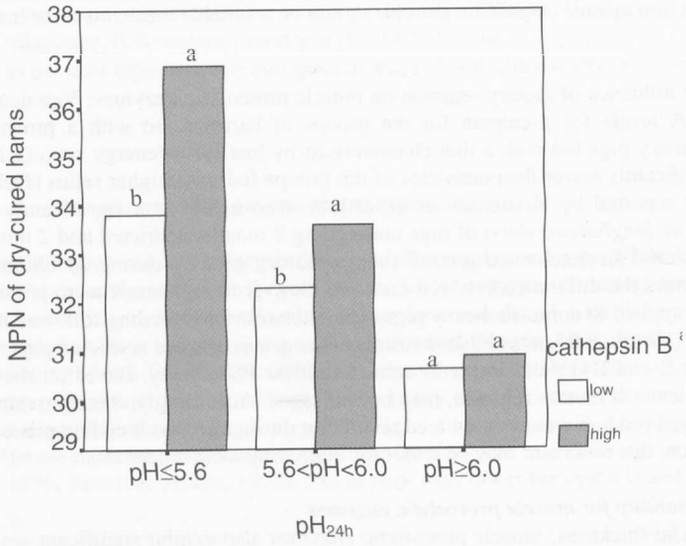

Fig. 3. Comparison between NPN of Parma hams in the same range of pH_{24h} and different levels of cathepsin activity (cathepsins B and L). (adapted from Magraner et al., 2002). Low and high cathepsin activity were fixed according to cathepsin B, because cathepsins B and L changed accordingly (r=0.802) as reported by Schivazappa et al. (1992). Enzyme activity is expressed as nmol AMC·min^{-1}· g $protein^{-1}$. Mean ± S.D. of cathepsin B of all assayed raw hams = 6.54 ± 1.50. Samples ≤ 5.5 (low) and ≥ 7.0 (high) underwent processing into Parma hams Means in each pH category with a different superscript letter differ (P<0.05).

4.2. Pork age, genetic and feeding as sources of variability for muscle pH

4.2.1.Age

With reference to Italian heavy pig to be processed into typical hams, mostly ranging between 9-and 12-months at slaughtering, the occurrence of ultimate pH beyond or below the normal range (5.6-6.0) was different in frequency. On the basis of the large monitoring of raw matter carried out in the last 5-6 years, DFD hams (m. *semimembranosus*) were found to be in the range 2-4%, while PSE frequency was found to be lower, i.e. about 1-2%, as reported by Chizzolini et al. (1993). A helpful contribution to the lowering of PSE in Italian heavy pig was the selection program against the carriers of the halothane positive gene using the PCR test (Russo et al., 1993).
More remarkable is the frequency of pH_{24h} in the low range (pH<5.6), being on average about 20% overall tested green hams (Centro Ricerche Produzioni Animali & Virgili 2001, unpublished results). This phenomenon deserves further investigation, because the frequency of low pH_{24h} values was found to be higher for older and heavier pigs (Virgili et al., 2002b; Cisneros et al., 1994; Beattie et al., 1999), whereas a low ultimate pH is commonly ascribed to an excess of muscle glycogen (Sellier and Monin, 1994).
For pigs yielding heavier carcasses, bigger muscles and higher fat thickness, a slower rate of chilling might yield a faster glycolysis and pH fall (Cisneros et al., 1994). The conversion of glycogen to lactic acid continues until the glycolytic enzymes become inactivated, while the ultimate pH is related to the muscle glycogen content by a non-linear relationship. The pH decreases according to the increase of glycolitic potential fitting a curvilinear regression up to a threshold, from which pH doesn't change regardless of glycolitic potential (plateau) (Larzul et al., 1999). To explain the higher frequency of low pH_{24h} for heavier and elder pigs, a greater slope in pH fall might be postulated and ascribed to the slower temperature decrease in the inside. A faster pH decline in muscles keeping higher temperature was reported by Milligan et al. (1998). A significant effect of different chilling temperature and rate during the first post-mortem hours on pH_{24h} was reported by Hildrum et al., 2000. In case of fresh meat, post-mortem treatments or muscle properties associated with a higher pH fall (slow chilling, electrical stimulation, or increased glycolytic potential) yielded more tender meat, favouring fast proteolysis of muscle fibres. In these respect, low muscle pH, through a more extensive proteolysis during processing, can impair texture of aged muscle.

4.2.2. Genetics

Several studies were performed to establish if the genetic selection might be a means to affecting ultimate pH, for changes in this trait are known to affect technological yield of cooked pork; furthermore, current research in the field of aged pork underlined the strong effect of raw matter pH upon the properties of dry-cured hams, and the control of this parameter should be an effective way to quality improvement of processed meats.
Removing unfavourable pH values by genetic selection proved to be scarcely effective because the heritability value for pH_{24h} was generally as low as 0.07-0.34 (Sellier and Monin 1994).

Recent attempts to perform a selection experiment for reduced muscle glycolitic potential in Large White pigs didn't give significant increase in ultimate pH (Larzul et al., 1999), even if the same authors found that for m. *biceps femoris* h^2 of ultimate pH raised to 0.39. This result may be relevant for dry-cured ham, since the inner m. *biceps femoris* is a muscle more strongly affected by softness blemishes than outer dryer muscles like m. *semimembranosus*.

4.2.3. Diet

Leheska et al. (2002), investigated the effects of high-protein/low carbohydrate diet given to barrows for a time period before slaughtering, but this treatment was not effective in reducing the decrease of both muscle $pH_{45'}$ and pH_{24h}. The lack of any possible control for pH_{24h} was ascribed to the high protein intake as a maintenance source for glycolytic potential in muscles, and to the lack of exercise for pigs. High energy supply at slaughter, when practiced to improve preslaughter procedures by minimizing stress hence DFD incidence, seemed to play a negative role on ham muscles, because it may predispose muscles to PSE-type defects if associated with a mild chilling (Barton-Gade & Blaabjerg, 1989). These findings are in agreement with the over mentioned high frequency of low pH_{24h} in raw hams. Effective chilling, proper diets and lairage times may improve this situation.

4.3. Other known sources of variability for muscle pH

A muscle ultimate pH increase was achieved by means of ante-mortem injections of epinephrine plus exercise (Ertbjerg et al., 1999) and the addition of β-agonists to the feed (Dazzi et al., 1991). Post-mortem electrical stimulation of carcasses, resulted in a lower $pH_{22'}$ and increase of cathepsin B+L in the myofibrillar fraction (Maribo et al., 1999). On the basis of the remarks reported in previous sections, these treatments are not suitable for pork undergoing long ageing times. A significant change of ultimate pH (m. *longissimus dorsi*) was due to the stunning procedures as reported by Velarde et al. (2001): the electrical stunning resulted in lower ultimate pH if compared with CO_2 stunning.

4.4. Meat quality: intramuscular fat (IMF)

Table 1 puts into evidence a possible role for origin breed in affecting dry-cured ham properties. The usage of the Duroc line for dry-cured ham yielded dry cured hams with IMF in the range 9-12% (m. *biceps femoris*), twice the amount commonly found in dry-cured hams from other crossbreeds (Parolari et al., 2002). This is a reason why Duroc purebred is not allowed for Italian typical ham manufacturing, but it is frequently used as a terminal sire, both for its good performance in resistance and growth rate and because IMF is regarded as an index of meat quality for fresh consumption and dry-curing.

Crosses with 0.50 Duroc inclusion resulted in better eating quality, due to its higher level of IMF (Blanchard et al, 1999), and a remarkable effect of IMF on texture was reported by Carrascal et al. (2000), showing a contrasting effect between IMF content and the onset of excessive hardness and fibrousness in highly dehydrated and aged Iberian hams. Nevertheless, in mildly dehydrated dry-cured ham, poor texture was related to high marbling of Duroc and Duroc-sired pigs (Parolari et al., 1988; Gou et al., 1995). This contradictory role may explain why, in association with different product traits, IMF could be either a blemish or a positive attribute, and the optimal range should be established in agreement with final outcome traits.

4.5. Pork age, genetics and feeding as source of variability for muscle IMF

4.5.1. Age

Čandek-Potokar et al. (1998), reported that a simultaneous increase of age and weight at slaughter (range 5-8 months and 100-130 kg respectively) gave an increase of IMF; Virgili et al. (2002b) found a 11% higher IMF in m. *longissimus dorsi* of 10- vs 8- months old heavy pig. However, Mayoral et al. (1999), working with Iberian pigs fed ad libitum with natural resources (fattening period with acorns and pasture up to 16 months and 150 kg) didn't find a real increase of IMF, even if deposition of subcoutaneous fat was enhanced by pig age.

4.5.2. Diet

Restricted feeding (Candek-Potokar et al., 1998) as well as high protein diets (Essen-Gustavson et al., 1994), reduced muscle IMF content. On the other hand, a feeding regimen based on low protein/high energy diet, gave higher IMF content (Blanchard et al., 1999).

4.5.3. Genetics

Reduction or increase of IMF by means of genetic selection proved to be efficacy, since estimates of IMF heritabilities ranged from moderate to high (0.26-0.86) and a strong breed effect was found for this parameter, traditionally associated with the Duroc line. Knapp et al.(1997), found a marked variability in estimates of IMF heritabilities due to breed type.

IMF proved to be scarcely influenced by covering fat thickness (Armero et al., 1999c; Lo Fiego et al., 2000), as confirmed by low genetic correlations between backfat thickness, intramuscular fat (less than 0.20), carcass lean content (-0.20) reported by Sellier and Monin (1994). Since genetic correlations between IMF, daily liveweight and lean growth rate were found to be more consistently positive (range 0.14-0.61 and 0.25-0.32 respectively), the increased lean tissue and daily gain and the decreased fat deposition characterizing heavily muscled pigs, could be associated with high IMF (Sellier and Monin 1994).

4.6. Carcass traits: effect of lean meat increase

Hams from heavily muscled pigs were reported to result in higher pastiness and lower saltiness in spite of higher weight losses (Guerrero et al., 1996). In the case of heavy pigs for typical dry-cured hams, the increasing diffusion of commercial hybrids selected on the basis of performances during breeding (daily gain, feed conversion) and at slaughtering (% lean meat) was regarded as a possible source of dry-cured ham loss of quality. Pigs referred to as hybrids are characterised by excellent growth performances, by remarkable increase in lean meat and reduction in fat. Trombetta et al. (1994), found a remarkable frequency of hams impaired by poor texture, taste and colour during the assessment of dry-cured hams obtained from 10 types of commercial heavy hybrids commonly used for dry-cured hams.

Significant differences in mechanical texture of dry-cured hams from 3 types of commercial hybrids and 3 types of traditional crossbreeds (based on different crosses of Duroc and Large White breeds) were found by Cerioli, 2001 (Fig. 4). Hams from traditional crosses proved better associated with hardness, cohesiveness and Young modulus than hams from commercial heavy hybrids, more characterised by higher % deformation, adhesiveness and springiness.

Guerrero et al. (1996) found low saltiness in dry-cured hams from heavily-muscled pigs, attributable to the greater thickness of muscles resulting in lower salt uptake.

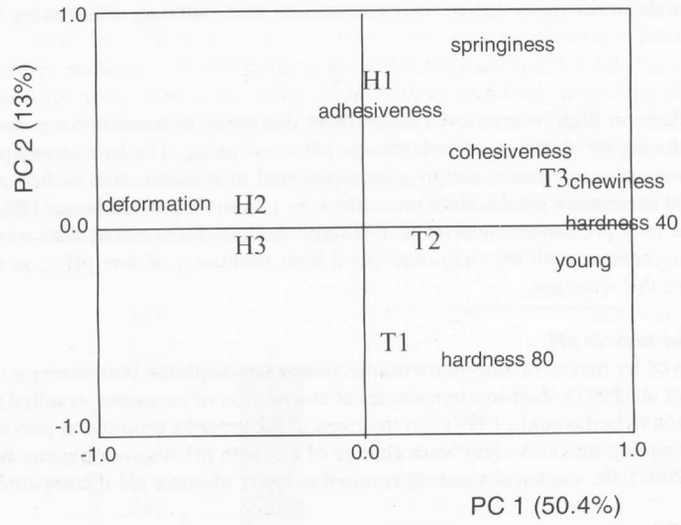

Fig. 4. Principal Component Analysis of texture measurements of hams grouped according to pig crossbreeds (ham groups are represented by their barycentres). T1, T2, T3 label the hams from traditional crosses, H1, H2, H3 hams from commercial hybrids
Uniaxal compression test: hardness 80= force required to achieve 80% deformation of the sample.
TPA test: hardness 40=force required to achieve 40% deformation of the sample during the 1st compression cycle; cohesiveness=ratio of the energy required during the 2nd cycle to the energy required during the 1st cycle; springiness=ratio of the time recorded between start and probe reversal of the 2nd cycle and time recorded between start and probe reversal of the 1st cycle; chewiness=hardness × cohesiveness × springiness; adhesiveness=negative area between the cycles; young=young modulus or ratio strain/deformation computed in the linear part of the curve (10% deformation); deformation=% deformation of the sample compressed for 30'' with a force of 80 newton during the 1st cycle (*adapted from Cerioli 2001*).

4.7. Pork age, genetics and feeding as source of variability for lean meat

4.7.1. Age

An extended pig age at slaughter causes reduction of lean content and increase of backfat thickness (Candek-Potocar et al., 1998). For pigs slaughtered in the range 105-135 kg, an increase of 10 kg in live weight means a decrease of lean meat content by approx. 1%, while backfat increases significantly (Albar et al., 1990). A study carried out by Mayoral et al. (1999) with free ranged Iberian pigs fed ad libitum with acorns and pasture, investigating changes in carcass traits from birth up to 16 months (approx. 150 kg) evidenced a sharp increase of fat with age and a decrease of per cent lean content of hams and shoulders. In case of heavy pigs slaughtered at 8 and 10 months, the muscularity index (% ratio of longissimus dorsi area to carcass weight), together with ham yield decreased for older pigs (Virgili et al., 2002b).

4.7.2. Diet

A lower feed consumption resulted in reduced subcutaneous and intramuscular fat, and in highest lean meat content (Affentranger et al., 1996); feeding in conformity with organic farming (AGOL guidelines) with reduced intensity of fattening dietary regimen, increased meat lean content up to 3%, with a significant increase of polyunsaturated fatty acids (PUFA) in the backfat (Fisher, 2001).

4.7.3. Genetics

Ham leanness was mainly determined by genotype (Affentranger et al., 1996), in agreement with moderate to high heritability estimates reported for lean meat content (h^2: 0.75 by Cameron, 1990 and h^2: 0.40-0.53 by Knapp et al., 1997), m. *longissimus dorsi* area (h^2: 0.46-0.80, Lo et al., 1992), lean meat weight of the ham (h^2: 0.38, Hermesh et al., 2000).
Positive relationship between increased lean meat content and PUFA was previously shown by Cameron 1990, who reported that selection for increased meat content results in backfat increased moisture and PUFA and in muscle pH decrease. In this respect, Trombetta et al. (1997), assessing the traits of hams from commercial hybrids characterised by good growth performances and increased lean meat content, found a remarkable frequency of high iodine number (exceeding the limit of 70 set by the tutelary Consortia of typical Italian hams) and of low pH values. Higher PUFA content and lower pH are to be regarded as potentially harmful for pork undergoing a long ageing because of increased fat susceptibility to rancidity and oiliness, and enhanced muscle proteolysis.

5. CONCLUSION

Extended knowledge of the product in terms of sensory profile, chemical markers and physical properties is needed to define on a scientific basis selection practices and breeding procedures for animals to be used in the manufacturing of matured dried products. Advances in technological equipments and processing techniques should not be intended as the sole way to achieve high quality products from any kind of raw matter (an approach that has been questioned for possible drawbacks in terms of sensory qualities), but as a means to enhance green meat as a source of unique functional properties. Therefore, selection of raw matter should be made to allow the most desired features of matured products to be obtained. The link between raw matter and processing may enhance at the same time typicality and consistency.
Such parameters involved with the development of the sensory profile of aged products as endogenous proteolytic enzymes, intramuscle fat, and lean meat content proved to be partly heritable and partly affected by dietary regimen and animal age. The muscle pH, by far less influenced by crossbreed, diet or selection, can be more easily measured on-line or evaluated by means of recordable measurements like conductivity and meat colorimetric indices.

Further studies to define range of analytical markers in aged products in agreement with quality categories and sensory properties and according to current knowledge in field of genetics and breeding would be of value.

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2. Making use of model organisms : in order to identify potential genes involved in meat quality, a model organism like *Caenorhabidtis elegans* could help identify all the genes involved in the development of the muscle tissue since the complete genome is now sequenced and cell differentiation is well known.

Finally, as new tools and methodologies are now available to researchers for the study of the genetic determinants of specific traits and phenotypes, particular efforts have to be made on the characterisation and the dissection of specific phenotypes into simple biological units for which the genetic determinants are easier to identify : a complex trait would then be the interaction of several "simple" biological units.