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SCIENCE DIRECT

Meat Science 74 (2006) 44–58

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Revisiting the conversion of muscle into meat and the underlying mechanisms

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Received 24 February 2006; received in revised form 27 April 2006; accepted 8 May 2006

Abstract

The conversion of muscle into meat is a complex process in which all mechanisms responsible for the development of meat qualities are very likely interdependent. Colour and flavour are thus both dependent on oxidative mechanisms. Oxidation and proteolysis are probably two processes involved in the development of meat tenderness. This paper reviewed the consequences of programmed cell death or apoptosis on muscle cells structure and biochemistry and on meat qualities as well. We therefore look at different new hypothesis susceptible to highlight the meat science field and provide new supports for a more dynamic meat research. One of them which would have appeared evident for our purpose since a decade, deals with the fact that, after animal bleeding, muscle cells have no other alternative to only enter the programmed cell death procedure or apoptosis. If we introduce an early phase corresponding to apoptosis, taking place before the rigor onset and overlapping it, we will see that the known consequences of that process bring forward possible answers to still unexplained observations. After an overview of the actual state-of-the-art in meat science, we will introduce the programmed cell death and its underlying mechanisms. We then described the strong analogies between the known consequences of apoptosis and the postmortem changes affecting a set of different muscle characteristics.
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Keywords: Meat qualities; Muscle; pH; Calcium; Caspases; Apoptosis; Cell death

1. Introduction

Storage of meat at low temperature is a prerequisite for the development of the major eating qualities including tenderness and flavour. Whether increasing storage length will be profitable for tenderness and flavour, it will have a rather deleterious effect on juiciness and colour. Hence, it will be necessary to find out the best compromise between these adverse time effects and/or to apply storage technologies enabling a preservation of these qualities.

For decades, consumers considered tenderness as the most important quality attribute of meat. In a review of factors influencing consumption, selection and acceptability of meat purchases, Jeremiah (1982) concluded that the most common cause of unacceptability in beef was toughness and that this was a common problem in pork and lamb. In contrast to mammals, fish flesh often undergoes very rapid softening rendering it unappealing to consumers (Ashie & Simpson, 1997; Crapo, Himelbloom, Pfutzenreuter, & Chong, 1999; Jiang, 2000). Despite some progress, this is still an acute problem for meat industry and fisheries. Actually, the meat tenderising process is unanimously recognized to be enzymatic in nature and the most studied proteolytic systems were cathepsins, calpains and, although more recently, the 20S proteasome (Sentandreu, Coulis, & Ouali, 2002). However, the major peptidases of concern are

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0309-1740/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2006.05.010

not identified yet in an unquestionable way and this question is still strongly debated.

Besides meat tenderness, juiciness and flavour are two other organoleptic qualities the consumers are looking for when eating meat especially red meats.

Despite its close relationship to overall meat desirability, juiciness has received limited research attention. Variability in juiciness is commonly recognized in various meat animal species but, to our knowledge, there is still no clear explanation for this phenomenon. Meat juiciness is assumed to be directly related to the progressive intracellular water efflux occurring during the acidification of postmortem muscle, a change in good agreement with the parallel increase in the extra-cellular space (Guignot, Vignon, & Monin, 1993; Monin & Ouali, 1991; Offer & Knight, 1988a, 1988b). Attempts to find out muscle characteristics susceptible to explain this biological variability led to highly controversial findings and much remains to be learnt about meat juiciness and muscles water holding capacity as well, two variables probably inter-related (Ouali, 1991).

Flavour is an important part of the eating quality of foods, including meat, and complaints of blandness are often levelled against modern lean meat from most animal species. Attempts to improve this quality with enhanced nutritional characteristics were therefore carried out (Moloney, Mooney, Kerry, & Troy, 2001; Wood et al., 1999). Flavour compounds are highly variable in nature. Peptides (Sentandreu et al., 2003), amino acids, lipids derivatives are indeed important determinants of meat flavour. On the other hand, radical reactions are known to constitute a central process in the oxidation of these compounds even if the nature and the origin of the radicals remain still unclear (Renerre, 1999). In addition, cooking conditions are essential in the flavour development through the Maillard reaction taking place, upon heating, between carbohydrates and proteins and their derivatives (Mottram, 1992). Apart from the management of its intensity through modulating the end-point temperature or the heating length, control of the Maillard reaction is difficult to achieve.

Meat colour is the first quality taken into account by consumers when purchasing the meat, brown colour being synonymous of bad hygienic quality for consumers. Meat colour is defined by the extent of myoglobin oxygenation and the oxidative status of the haem iron. Oxygenated myoglobin gives an appealing light red coloured meat and a dark red colour in the absence of oxygen. Oxidation of the haem iron from a ferrous (Fe^{2+}) to a ferric (Fe^{3+}) state give rise to the brown colour and is often associated to the release of oxygen radical (Satoh & Shikama, 1981; Wallace, Houtchens, Maxwell, & Caughey, 1982). The concentration of residual oxygen and the radical formation are believed to be the major cause of colour deterioration in stored meat but the underlying mechanisms are still unclear.

The conversion of muscle into meat as a whole and the postmortem development of the eating quality are far from being understood. Improvement of our knowledge about the underlying mechanisms is particularly faced to the large biological variability of these qualities and to the non-identification of their major determinants.

Since about a decade, intensive researches have been developed on programmed cell death (PCD) (Kerr, Wyllie, & Currie, 1972) in relation with important pathologies like cancers (cells never died), neurodegenerative disorders including Alzheimer disease (cells died before their complete differentiation), etc. (Hengartner, 2000; Majino & Jons, 1995; Nicholson, 2000; Sandri & Carraro, 1999; Shi, 2002; Tews, 2005). The major form of PCD is apoptosis (''suicide'' program), a process finely regulated and initiated by either the central nervous system or by the target cell itself. After animal bleeding, all cells will be in anoxia and will receive no more nutriments. In such conditions, each cell can decide to die by initiating the apoptotic process. Up to date, the conversion of muscle into meat is assumed to occur through three steps: the pre-rigor step which is not well defined yet, the rigor step and the tenderising step. If we assumed that postmortem muscle cells will commit to suicide, the apoptotic process will start immediately and progress as long as the enzymes of concern remain active. Apoptosis induced a series of biochemical and structural changes in dying cells which will be very likely found in postmortem muscle. The extent of these changes will be however more limited than *in vivo* where the cell content totally disappear, since the dynamic of the process will be faced to unfavourable environmental modifications (pH, ionic strength, low energy availability, etc.).

In this review, we revisited the conversion of muscle into meat after the integration of an additional phase before the rigor process corresponding to the apoptotic phase. After a rapid overview of the state-of-the-art, we will describe the mechanisms and the consequences of the programmed cell death process and, in the last part of this review paper, we will try to see how the known effects of cell death on the cellular structures and proteins can constitute a new approach to the muscle conversion into meat and provide possible original answers to some still unexplained observations reported in postmortem muscle.

2. Current concept of the conversion of muscle into meat

Animal are slaughtered, dressed, deboned and muscles then stored at refrigerated temperature for one week or more depending on the current national practice and/or regulations before selling. Storage of muscles for a reasonable length of time is a perquisite for the development of the organoleptic qualities of the final product namely meat.

2.1. Tenderness

Postmortem improvement in meat tenderness results from a softening of the myofibrillar structure by endogenous peptidases (Ouali, 1992; Sentandreu et al., 2002). For several decades, attention of meat scientists has been mainly focused on the two best known enzymatic systems,

i.e. cathepsins and calpains. Three currents ways of thinking about that process however exist:

– those who are prone to think that calpains are the only proteases responsible for meat tenderisation,
– those who suggest that the two quoted systems take part in this process,
– a third group of researchers, to which we belong, who propose a multienzymatic process implying these systems and also probably others which function, in postmortem muscle, is less clear (proteasomes, caspases, etc.).

Actually, the meat tenderising process is unanimously recognized to be enzymatic in nature and the most studied proteolytic systems are:

– the cathepsins, a system discovered in the years 1950 by De Duve, Pressman, Gianetto, Wattiaux, and Appelmans (1955).
– the calpains which are calcium-dependent peptidases highlighted for the first time in rat brain by Guroff (1964).
– the proteasomes discovered more recently by Wilk and Orlowski (1980).

The cathepsins were the first enzymatic system considered in the studies focusing on the mechanisms of meat tenderisation. Later, calpains received much more attention than cathepsins mainly because of their ability to alter the Z-line density, a modification often observed postmortem, even if this change is not correlated with tenderness (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). More recently, several sets of evidence supported a potential role of the 20S proteasome in this process. Based on different approaches, all results reported clearly showed that proteasome could contribute to tenderisation of stored meat (Dutaud, 1998; Lamare, Taylor, Farouta, Briand, & Briand, 2002; Matsuishi & Okitani, 1997; Otsuka et al., 1998; Ouali, 1999; Thomas et al., 2004).

The most current actual concept suggested a major role of calpains and more specially of calpain 1 or μ-calpain (Veiseth and Koohmaraie, 2005). However, this statement has never been definitely proven and the main reason is very likely our very limited knowledge about calpains, their *in situ* regulation and their exact biological functions. As compared to proteasomes, a proteolytic complex discovered much more recently, we can affirm that our knowledge of the biological function and the operating mode of the calpain system in the cell is much less advanced. This statement is supported by recent findings indicating that calpain 1 is concentrated on the N1 and N2 line region of titin and this might constitute a reservoir of calpain 1 for the cell (Fernandez et al., 2005).This further suggest a special regulation of the release of calpain 1 from its binding sites and the existence of a finely regulated equilibrium between the amounts of free and bound enzyme. On the other hand, calpastatin, the specific inhibitor of ubiquitous calpains, is often considered as a single protein. In fact, as already suggested in the 1990s (Ouali & Talmant, 1990), this calpain inhibitor is a family of at least four different isoforms (Raynaud et al., 2005) some of which being differently expressed in slow-twitch and fast-twitch skeletal muscles (Ouali & Talmant, 1990). Their properties and tissue expression are still unknown. Therefore, it must be emphasized that our understanding of the calpain system is actually very restricted and most remains to be learned about the peptidases themselves, their activity regulation and their specific inhibitor.

Calpains are inhibited by calpastatin, a highly polymorphic protein. Cathepsins have their own inhibitors designed cystatins (Dubin, 2005; Sentandreu et al., 2002). The term "cystatin" refers to a group of homologous and evolutionary related cysteine peptidase inhibitors (Barrett, 1987; Muller-Esterl et al., 1985; Turk et al., 1986) which are inactive against other classes of peptidases (serine-, aspartyl- and metallopeptidases). On the basis of their primary structure, there are four distinct families of cysteine peptidase inhibitors, which are recognized as belonging to the cystatin superfamily (Rawlings & Barrett, 1990).

– *Family 1 cystatins*, also designated *stefins*, are low molecular weight- proteins (10–14 kDa) containing one copy of the basic inhibitory structure but no intramolecular disulfide bridges. The most well characterised members of this family are stefin A and stefin B. As stefins lack the signal sequence present in secreted proteins, they are generally found intracellularly.
– *Family 2 cystatins*, also designated *cystatins*, are low molecular weight proteins (10–14 kDa) containing one copy of the basic inhibitory structure and at least one intramolecular disulfide bridge. Cystatins are predominantly extracellular. Cystatin C and chicken cystatin are the most representative of this cystatin family.
– *Family 3 cystatins*, also designated kininogens, are high Mr cystatins (>50 kDa) containing generally three copies of the basic inhibitory structure and several disulfide bonds. The kininogen family comprises three subclasses referred to as low Mr (LMW), high Mr (HMW) and T-kininogens differing by their molecular masses and their specificity towards cysteine peptidases. They are generally present in body fluids.
– *Family 4 cystatins* are glycosylated protein inhibitors of intermediate Mr containing two copies of the basic inhibitory structure and several intramolecular disulfide bridges.

All members of the cystatin superfamily inhibit cysteine peptidases such as papain and the major lysosomal peptidases including cathepsins B, H and L. In addition, kininogens have been shown to inhibit the ubiquitous calpains, which are calcium dependent cysteine peptidases (Ishiguro et al., 1987).

In muscle tissue, several low Mr cystatin-like proteins have been described (Bige, Ouali, & Valin, 1985; Schwartz

& Bird, 1997) but as no sequence was available, it was and is still impossible to certify that these are really members of the cystatin superfamily. Indeed, their identification as cystatin was only based on their closely similar molecular mass and inhibitory properties. This statement is true for most of the other cysteine peptidase inhibitors isolated from skeletal muscles of various animal species (Ouali et al., 1995; Zeece, Woods, Keen, & Reville, 1992). Importance of cystatins in postmortem and living muscles is supported by their identification as potent predictor of meat tenderness (Barnier, 1995; Shackelford et al., 1991) and their possible use as biological markers of different pathologies in humans (Dubin, 2005; Kos & Lah, 1998; Strojan et al., 2000).

Each one of us will be therefore able to note that, a rapid and objective analysis of the literature published to date in this field clearly indicates that the process of meat tenderisation results probably from the synergistic action of several endogenous enzymatic systems, even if the major peptidases of concern are not identified yet in an unquestionable way.

2.2. Juiciness

When meat is tender, juiciness is the next quality of interest for consumers especially for red meats, whiter meat cuts being generally considered as dry. Decrease in juiciness was often associated with water holding (WHC) capacity of muscles. However, the ultimate pH which is one of the major determinants of WHC seems to have negligible effect on meat juiciness (Bouton, Carroll, Fisher, Harris, & Shorthose, 1973). Relationship of juiciness with various biological parameters is unclear and data reported often highly controversial. Valin, Touraille, Vigneron, and Ashmore (1982) thus noted a significant correlation between muscle type and meat juiciness confirming the empiric thought that red meats are more juicy than white meats. No valuable biological determinants were however identified so far. A correlation with muscle fat content was also mentioned (Owens & Gardner, 1999) but, since years this point led to controversial findings as already analysed in the 1970s by Purchas and Davies (1974). Nevertheless, all scientists agree that juiciness strongly depends on the movement of intracellular water towards the extracellular space assuming that, as the pH becomes more acidic and closer to the p*I* of myofibrillar proteins, these will release their bound water (Bertram, Schafer, Rosenvolda, & Andersen, 2004; Lawson, 2004; Offer & Knight, 1988a, 1988b).

By analogy with pork where a mutation of the PRKAG3 gene encoding the γ_3 subunit of the AMP dependent kinase (AMPK) was responsible for the PSE character of the meat, one can thought that mutations of the same gene could be responsible for the biological variability in drip loss. The AMPK, comprising three subunits (α, β,γ), has been pointed out as one of the main actors in the regulation of intracellular energy metabolism (Carling, 2004). As AMPK is activated by a drop in the energy status, it is usually considered as a cellular fuel gauge. Its activation switch off energy-using pathways and switch on energy-generating pathways, thus helping to restore the energy balance within the cell. Numerous mechanisms of AMPK action on lipid and carbohydrate metabolism have been proposed (Ferre, Azzout-Marniche, & Foufelle, 2003; Hardie, Scott, Pan, & Hudson, 2003). The corresponding cattle gene has been recently sequenced and was shown to be highly polymorph and to exhibit several mutations leading to different possible alleles (Roux et al., 2006). The consequences of these mutations on muscle WHC, meat juiciness and drip loss are however still unknown. Investigation on the AMPK gene function and its polymorphic allelic expression would be an alternative way to provide answers to carcasses, muscles and meat exudation. In a longer term, these studies would probably be also very helpful for the genetic selection of animals expressing the most suitable isoform of the corresponding AMPK subunit. In addition, this gene is very likely common to all meat animal species and findings obtained can be therefore extended to any species.

2.3. Meat flavour and colour

Although important for the consumers, our purpose will not be to overview in detail these qualities, their biological variability and the underlying mechanisms. Our objective will be just to emphasize the importance of peroxidation in the time course evolution of these characteristics.

Major contributors to meat flavour are lipids peroxidation together with amino-acids and peptides generated by proteolysis (Campo et al., 2006; Gorbatov & Lyaskovskaya, 1980; Sentandreu et al., 2003). Similarly, colour degradation during storage related to metmyoglobin formation also results from oxidative processes. Metmyoglobin indeed is generated by a spontaneous oxidation of the haem iron from the ferrous state ($Mb–Fe^{2+}$) to the ferric state ($Mb–Fe^{3+}$) which cannot bind O_2 (reviewed by Bekhit & Faustman, 2005).

For both meat qualities and tenderness as well (Warner, Dunshea, Ponnampalam, & Cottrell, 2005), oxidation in muscle systems is initiated as a free-radical autocatalytic chain mechanism in which prooxidants, especially oxygen and related radicals will generate free radicals ensuring the autocatalytic propagation of the oxidative chain (Kanner, 1994).

3. Programmed cell death or apoptosis

The concept of programmed cell death or apoptosis is much older than suggested in most reports. Indeed, according to Clarke and Clarke (1995), naturally occurring cell death, including the concept of apoptosis, was a flourishing subject at the end of the 19th century. The first report that cells die naturally in development was published by Carl Vogt in 1842 (cited by Clarke & Clarke, 1995).

Programmed cell death is a physiological mechanism naturally occurring in living organisms that eliminates

excessive, damaged or potentially dangerous cells from an organism without damaging surrounding cells (Fidzianska, Kaminska, & Glinka, 1991; Kerr et al., 1972). Such systematic cell clearance is necessary for both the normal development of a multicellular organism during embryogenesis and the maintenance of tissue homeostasis in adults (Dirks & Leeuwenburgh, 2005; Meier, Finch, & Evan, 2000).

Selective elimination of the cells is ensured by a process called *apoptosis*. The word apoptosis refers to the programmed fall of leaves at autumn time: apo for distance and ptosis for fall. The very strict regulation of this program is essential to make sure that it is activated only in the cell concerned and at the right time. Deregulation of the apoptotic process is associated with various pathologies such as cancer, auto-immune and degenerative diseases (Hengartner, 2000; Kerr et al., 1972; Majino & Jons, 1995; Nicholson, 2000; Sandri & Carraro, 1999; Shi, 2002; Tews, 2005).

Apoptosis is usually mediated by a complex machinery which is becoming well-known in humans (Fuentes-Prior & Salvesen, 2004; Green & Amarante-Mendes, 1998; Hengartner, 2000; Zimmermann, Bonzon, & Green, 2001; Zimmermann & Green, 2001). On the other hand, although researchers agree that programmed cell death also exists in plants, relatively little is known about mediators of the process in plants. However, its role in plant development and morphogenesis is regarded as essential. It is thus admitted that deregulation of apoptosis is very often associated with diverse disturbances in development but also with a strong lethality (Sanmartın, Jaroszewski, Raikhel, & Rojo, 2005). Apoptosis is mediated by a particular group of cysteine peptidases called caspases.

Apoptosis is an evolutionary highly conserved process from monocellular organisms to mammals (Driscoll, 1996; Yuan, 1996). To illustrate this fact, we must keep in mind that the biological function of caspases, the main enzymes responsible for programmed cell death, was discovered in the nematode *Caenorhabditis elegans* (Yuan, Shaham, Ledoux, Ellis, & Horvitz, 1993). It is in this nematode that the first gene responsible for cellular death, the gene encoding for CED3, was identified: CED3 is a cysteine peptidase very homologous with ICE (Interleukin-1 Converting Enzyme), an enzyme discovered in humans shortly before (Cerretti et al., 1992; Thornberry et al., 1992). These last findings constitute the starting point of many works developed thereafter on these peptidases and their regulators. These extensive investigations led very quickly to a better understanding of the apoptotic process.

3.1. Necrosis and apoptosis

The concept of apoptosis was introduced in 1972 by Kerr et al. to indicate a form of cellular death totally different from necrosis, from both the morphological and biochemical points of view. Comparative analysis of muscle cell death led to the same conclusion (Fidzianska et al., 1991).

Necrosis is regarded as a "disordered" cellular death. During necrosis, cells swell up with water to the point where lysis of their plasmic membrane occurs. It is a genuine cellular explosion which leads to the release of the cytoplasmic contents in the surrounding medium. Cellular organites also inflate and are emptied of their contents. The nuclear ADN is degraded in a "random" way by endonucleases activated in particular by serine peptidases. The size of the DNA fragments generated is very heterogeneous. Necrosis of a cell affects other cells by the action of the released intracellular enzymes and of blood leucocytes which come in reinforcement to clear the site (local inflammation). The result is that a whole area of an organ will have to be regenerated after total destruction of the damaged cells (Buja, Eigenbrodt, & Eigenbrodt, 1993; Fidzianska et al., 1991; Majino & Jons, 1995).

In opposition to necrosis, apoptosis is regarded as an "ordered" cellular death, proceeding through various phases (Bratton et al., 1997; Buja et al., 1993; Gavrieli, Sherman, & Ben-Sasson, 1992; Majino & Jons, 1995; Martin et al., 1995; Matsura et al., 2005; Wyllie, 1980; Youle & Karbowski, 2005)

- first of all, cells in apoptosis are isolated by loss of contacts with neighbouring cells;
- an important condensation of the nucleus and of the cytoplasm induces a significant reduction in cellular volume;
- mitochondria of the apoptotic cell undergo several major modifications: release of cytochrome *c* in the cytoplasm, reduction in the membrane potential and deterioration of the membrane permeability with opening of specialized pores and diffusion of diverse proapoptotic proteins;
- after condensation of the nucleus, chromatin is cleaved in regular fragments of approximately 180 pairs of bases;
- sometimes, the plasmic membrane buds and forms apoptotic bodies, containing some of the cell cytoplasm;
- in order to facilitate recognition of the apoptotic bodies by phagocytes, the cell signals its apoptotic state by a change of localisation of phosphatidylserines molecules, from a cytoplasmic orientation to an extracellular orientation.

Programmed cell death is a rapid process (from a few minutes to a few hours) (Green, 2005). Compared to necrosis, one of the key properties of apoptosis is that the plasmic membrane is not entirely destructed during the process, thereby avoiding discharge of the cellular contents and consequent damage inflicted to neighbouring cells.

3.2. The caspases

Because caspases (structure and functions) and apoptotic regulation will be only succinctly overviewed hereafter, readers must refer to the review of Fuentes-Prior and Salvesen (2004) for more information.

3.2.1. Caspases, a new family of peptidases

Apoptosis-generating peptidases are cysteine peptidases with a strict specificity of cleavage after an aspartic acid residue (Asp or D). In a new nomenclature suggested by Alnemri et al. (1996), all apoptosis-generating peptidases are designated *caspase*. The first letter of the name (*C*) stands for the cysteine of the active site; *asp* defines the strict specificity of cleavage after an aspartic acid residue and *ase* is the suffix common to all enzymes. ICE, (interleukin-1b-converting enzyme) which was chronologically the first caspase characterised, was naturally renamed caspase 1. To date, 14 caspases have been identified but there is no doubt that this list is not exhaustive (Fig. 1). Some caspases seem specific to an animal species. Thus, caspase 11 was found only in the mouse and the rat whereas caspase 13 seems to be expressed only in bovines. Caspase 12 may be present only in the mouse. Structure of those three caspases (caspases 11, 12 and 13) is less documented than others (caspases 1–10).

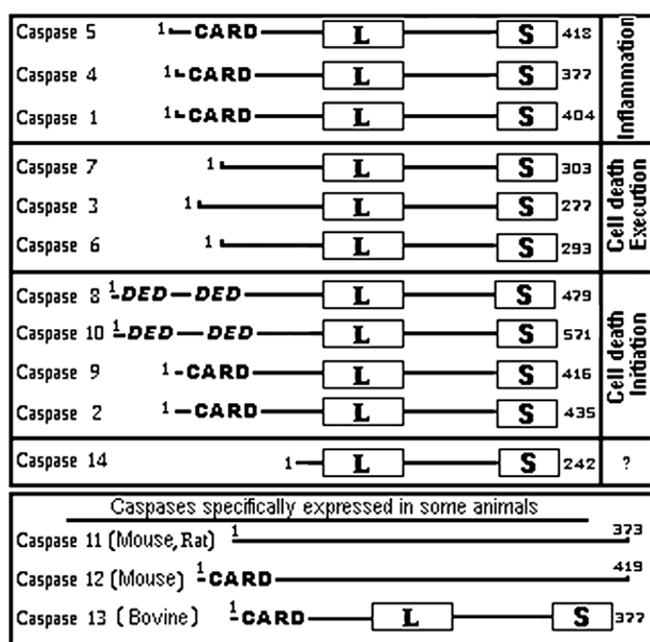

Fig. 1. *Schematic structure of caspase* precursors identified in humans or expressed only in some animal species. Human caspases have been grouped on the basis of their similarity of sequence. Homologies of sequence divide the caspases 1–10 into three subfamilies, according to their physiological function and their implication in the inflammatory processes, in the phase of initiation or execution of apoptosis. The caspase 14 is separated from others because it is expressed only in the skin and because it is cleaved at sites not containing a residue aspartate when activated. Active caspases are made up of a large (L) and a small (S) subunits. Activation of the caspases require cleavages which eliminate N-terminal ends and the fragment associating S and L subunits, except for caspases 3, 10, 14 and 13. The N-terminal regions of several caspases contain various domains essential for the interactions with activator complexes at the time of activation (DED, Death Effector Domain and CARD, CAspase Recruitment Domain). Numbers correspond to those of amino acids from N- to C-terminal ends. (Adapted from Fuentes-Prior and Salvesen, 2004).

3.2.2. Schematic structure of caspases

All caspases have a highly conserved structure (Fig. 1) including:

- an N-terminal prodomain, varying in size and having a prominent role in protein–protein interactions, in particular with regulators of apoptosis,
- a second domain which will become, after cleavage, the large subunit (**L** for large) carrying the active site of the enzyme with a cysteine (C) and a histidine (H) residue,
- a third domain which will become, after cleavage, the small subunit (**S** for small) with a conformational role.

Precursor enzymes undergo maturation to the active form by N-terminal prodomain elimination and subsequent formation of the heavy and light chains which associate to form one enzyme molecule. Two molecules then join to form a dimer having two active sites in head-to-tail position. Catalytic sites can become active only when a dimer is formed (Fuentes-Prior & Salvesen, 2004).

3.2.3. Three classes of caspases

According to Fuentes-Prior and Salvesen (2004), on the basis of their potential biological function and their role in apoptosis, three different groups of caspases can be distinguished (Fig. 1):

- caspases involved in *inflammatory processes* (caspases 1, 4, 5). Knowledge on the precise function of these caspases is less developed than for the two other classes;
- caspases involved in the phase of *apoptosis initiation* (caspases 8, 9, 10). They are characterised by large prodomains often containing essential areas for their interactions with other proteins. For example, the prodomains of caspases 8 and 10 contain Death Effector Domains: DEDs. Such structures allow binding of caspases to regulatory molecules (activators or inhibitors) carrying similar domains, through DED–DED interactions. Other caspases (caspases 1, 2, 4, 5 and 9) have a Caspase Recruitment Domain: CARD. Like DEDs, CARDs are responsible for the interaction of caspases with a large variety of regulating molecules (activators or inhibitors) through CARD–CARD interactions;
- *effector's caspases* disrupting the cell once activated (caspases 3, 6 and 7). Compared to initiating caspases, those generally have small prodomains.

3.3. General outline of apoptosis and regulation

3.3.1. General outline

Apoptosis proceeds according to a relatively precise program, characterised successively by a phase of initiation, implicating initiator caspases (caspases 8, 9 and 10) and dependent on the nature of the stimulus and on the type of cell, followed by execution via the effector's caspases (caspases 3, 6 and 7).

The stimulus at the origin of the process of apoptosis can be extrinsic, via activation of death receptors, or intrinsic in response to critical conditions for cell survival (Fig. 2). Initiating caspases are then activated within different complexes and those in turn activate the effector's caspases which will start the cell disruption phase (Fuentes-Prior & Salvesen, 2004).

3.3.2. Regulation of the apoptotic process

The regulation mode of apoptosis will depend on the nature of the initial stimulus (Fuentes-Prior & Salvesen, 2004). On this basis, three main pathways of cellular death development can be distinguished (Fig. 2).

- *Pathway 1* corresponds to a stimulus involving extracellular cell death receptors which can bind either activator (ligand +) or inhibitor (ligand −) effectors. Binding of an activator will activate an intracellular complex activating initiating caspases (caspases 8 and 10) through DEDs interaction (Fig. 2). To control this step, cells will synthesise proteins containing DEDs domains which will act as a competitive inhibitor of the activator complex for caspases binding. Excess of such proteins will divert the caspases from their complex activator.
Active caspase 8 or 10 can also be inactivated by IAPs (Inhibitors of APoptosis) which are protein inhibitors binding close to the caspase active sites and blocking therefore its access to protein substrates. If this is not the case, the quoted caspases can activate effector's caspases (caspases 3 and 7) responsible for cell disruption.
- *Pathway 2* corresponds to particular situations where the cell as no other solution but suicide (intrinsic stimulus). In this pathway, mitochondria are the central element of the process (Kroemer, Dallaporta, & Resche-Rigon, 1998; Mishra & Kumar, 2005; Mohamad et al., 2005; Petit et al., 1997). This process implies a membrane deterioration of the mitochondria by Bax-type proapoptotic proteins. Mitochondria membrane potential is lost, their external membrane becomes permeable leading to the release of cytochrome *c* (proapoptotic factor) in the cystosol. This alteration results from the binding of a complex containing Bax on the mitochondrial membrane. It should be noted that Bcl2, a protein of the same family as Bax has an antiapoptotic activity and will tend to preserve the mitochondrial membrane. The concentration ratio between the two antagonistic apoptotic proteins will determine the speed of cytochrome *c* release into the cytosol. Free cytochrome *c* then will form a complex called apoptosome, composed, among others, of a protein called Apaf-1 (apoptotic protease activating Factor-1) and caspase 9. Caspase 9 will be activated within the apoptosome and can itself activate the effector's caspases (caspases 3 and 7). This activation of caspase 9 can be blocked by inhibitors of the IAP family mentioned above (Inhibitors of Apoptosis).
- *Particular case of stress (Pathway 3)*: whatever its nature, stress induces synthesis of protective proteins called heat shock proteins (HSP), which function is to preserve cellular proteins against denaturation and possible loss of function (Kultz, 2003).
The many known Heat Shock Proteins are generally classified in subfamilies on the basis of their size (molecular weight): Hsp 90, Hsp 70, Hsp 40, Hsp 27, etc. Such proteins appear as soon as the cell is in danger. They have an essential role since they will contribute to proper conformation of proteins and preservation of their biological function. One can therefore expect that stress proteins will have, at the time of programmed cellular death, an antiapoptotic role (Arrigo, 2005; Arrigo et al., 2002; Beere, 2001, 2004, 2005; Flower, Chesnokova, Froelich, Dixon, & Witt, 2005).
In the apoptotic process, HSPs may therefore have diverse antiapoptotic actions which can be summarised as follows:

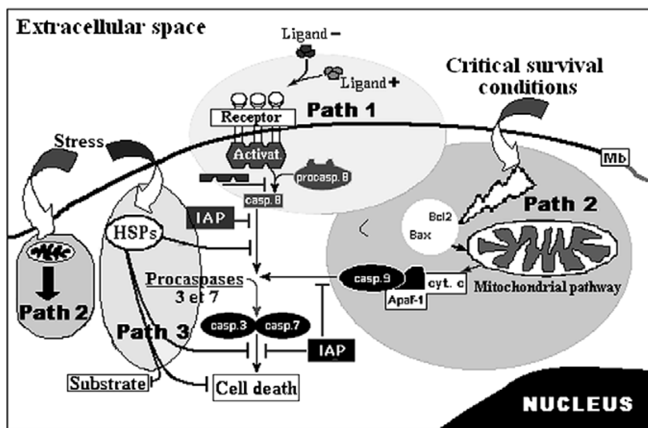

Fig. 2. *Regulation of the apoptotic process. Pathway 1 or extrinsic way:* after activation of death receptors by their proapoptotic effectors, receptors will associate with several proteins to form an intracellular membrane complex that can activate the procaspase 8 by specific interactions. The process is controlled by inhibitors carrying the same caspase 8-binding motifs as those present on the activating complexes. Inhibitors compete with activators to bind procaspases 8 and 10. If the initiating caspases are activated, the cell has inhibitors of apoptosis (IAP) able to slow down or even interrupt the process by inactivating the caspase 8. IAP would be also able of inactivating the effector caspases (caspases 3 and 7). *Pathway 2 or intrinsic way:* Under the impulse of Bax, a proapoptotic protein, a complex is formed and bound on the external membrane of mitochondria. This will deteriorate the membrane with a concomitant accumulation of calcium in mitochondria and release of cytochrome *c* which will contribute to the formation, with other proteins, of a complex responsible for the activation of the procaspase 9. This action of Bax can be blocked by Bcl-2, a protein with an antiapoptotic activity. If the complex is formed, the process can still be blocked by the inhibitors of apoptosis (IAP) which can bind to procapase 9 and prevent its activation. *Pathway 3 or stress pathway:* HSP's will exhibit their antiapoptotic activity through different ways affecting initiating and executing capases themselves and their target substrates. Intense stress can also lead to apoptosis activation through pathway 2 involving mitochondria and cytochrome *c*. *Abbreviations:* Apaf-1, APoptose activating factor-1; IAP, inhibitor of apoptosis; Bax and Bcl2: two proteins of the same family with opposite effects; Activat., complex of activation of the initiating caspases; Cyt *c*, cytochrome *c*; HSP, heat shock proteins or stress proteins. Thick lines ended by a vertical or horizontal T shape are inhibition pathways. Thin lines ended by an arrow head correspond to activation pathways.

– Formation of a complex with active caspases (initiators or effectors) thus hindering their function.
– Protection of target proteins (substrates) of effector caspases preventing or delaying their degradation by these enzymes.
– Attempt to re-established the initial and active structure of proteins having undergone structural damage following either the stress itself or the initiation of apoptosis.

Through HSPs, stress will therefore generate actions of an antiapoptotic nature. In the case of intense stress, however, it can induce cellular death by the mitochondrial pathway (pathway 2 previously described).

4. Cell death and meat qualities

As for most other aspects of cell biology, skeletal muscle tissue is far from being a current model for apoptosis investigations. In humans, apoptosis in muscle tissue have been mainly studied in relation with either pathologies including neuromuscular disorders, myosistis or muscle atrophy (Leeuwenburgh, 2003; Liu & Ahearn, 2001; Primeau, Adhihetty, & Hood, 2002; Sandri & Carraro, 1999; Sandri, 2002; Tews, 2002; Tews, 2005; Yuan, Wang, & Murrell, 2003).

In all meat animal species and whatever the technology of stunning used, the last phase of the slaughter process is bleeding. Consequently, all cells and tissues will be irreversibly deprived of nutrients and oxygen. Under these very harmful environmental conditions, muscular cells will have no alternative but engage towards "suicide", with all the consequences described above. Under similar conditions, such cell behaviour is currently observed in living organisms. Hence why this would not be the case in postmortem tissues and cells?

All following considerations relate mostly to cattle, but, because of the ubiquitous character of apoptosis, the reported observations can be extended to all meat animal species. The objective will be to consider the main cellular changes associated with apoptosis and to establish a link with modifications observed in muscle during meat ageing, in relation to organoleptic qualities and more particularly tenderness, the major quality attribute for the consumer.

4.1. Inversion of the membrane polarity

4.1.1. Rapid overview of the process in vivo

In vivo, cellular membranes have a well defined polarity dependent on the distribution of phospholipids. The electronegative phosphatidylserine groups, are on the inner leaflet of the cellular plasmatic membrane whereas the electropositive phosphatidylcholine and phosphatidylethanolamine groups, are on the outer leaflet. When the process of apoptosis begins, an inversion of the phospholipids distribution occurs: phosphatidylserine switching to the external leaflet of the membrane by a well-known flip-flop process, while the reverse happens for the other phospholipids (Martin et al., 1995). This change isolates the apoptotic cell from surrounding cells and signals its suicide status. In apoptotic cells, the membrane remains however impermeable to avoid diffusion of the intracellular components in the extracellular environment. Transfer of phosphatidylserine groups to the external leaflet of the membrane constitutes also a sign of recognition by macrophages which will, *in vivo*, take part in the degradation of dying cells. The phospholipids translocation is ensured by different types of translocases which can be divided into three classes: bidirectional "scramblases" and energy-dependent transporters that move phospholipids toward ("flippases") or away ("floppases") from the inner surface of the membrane (Bevers, Comfurius, & Zwaal, 1996). Among them, the most important active class of translocases in postmortem muscle will be more likely scramblases which are bidirectional translocases with a wide specificity. Scramblases are calcium but not energy dependent (Bratton et al., 1997).

Cell death is not coordinated meaning that, each individual cell can initiate apoptosis independently. That is particularly true since, within a muscle, fibres are very heterogeneous (Pette & Staron, 1990).

4.1.2. Consequences on postmortem muscle pH fall

What are the consequences of this inversion of polarity in *postmortem* muscle? Within the cells, acidic components are replaced by others of a rather basic nature. One can thus expect a partial neutralization of protons generated by glycolysis and, consequently, a deceleration of the acidification process. This was what we observed in a recent study involving nearly 180 bovine animals of different age, sex and breed. The experiment was carried out on *Longissimus* muscle. The vast majority of these animals (98%) present one or two transient pH stability steps appearing quickly after slaughter (within 1–8 h postmortem) and persisting for a variable time length ranging from 2 to 6 h (Fig. 3). Note that a similar polyphasic pH drop has been observed in four different lamb hindleg muscles (unpublished data). Similarly to beef, one or two pH stability steps were observed in lamb muscles in the time range of 1–6 h suggesting that this feature is not dependent on the length of the rigor process which is shorter in lamb. Preliminary analysis of the bovine data set indicates that *Longissimus* muscle of animals showing two transient steps tends to be tougher than those showing only one step. However, clarification of the exact meaning of these transient pH stability steps calls for further investigations.

Selected animals [two 19-month-old Charolais young bulls (Mr-CH-19M-1 and 2) and a five-years-old Charolais cull cow] represent the various scenario encountered for most animals. Only some rare animals do not present this transient pH stability step in the first postmortem hours. For the three profiles presented in Fig. 3, the average rate of pH drop over the whole rigor process varies between

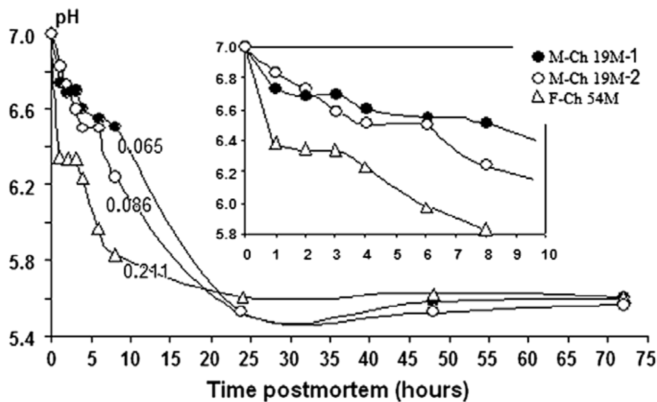

Fig. 3. pH evolution in the *Longissimus* muscle of three animals (charolais). The three animals are two 19-month-old young bulls (Mr.-CH-19M-1 and -2) and a 54 month-old cull cow (F-CH-54M). pH was measured every hour during the first 8 h after slaughter then at 24, 48 and 72 h. The pattern is presented for each animal with the average rate of pH fall indicated on each curve and expressed in Unit pH per hour (UpH h^{-1}). In the insert, evolution of this parameter during the first 8 h post-slaughter is presented. Each point is the mean of three independent determinations. For each point, the coefficient of variation is generally lower than 1%.

0.065 and 0.21 pHunit/hour (UpH h^{-1}), the Charolais cull cow exhibiting the fastest pH fall. Refined analysis of the pH drop profile during the first 8 h following slaughter (insert Fig. 3) shows a discontinuity in the pH fall, with the presence of one (F-CH-54M, Mr.-CH-19M-2) or two pH stability steps (Mr.-CH-19M-1). The time length of these steps varied between 2 and 5 h. For the first Charolais young bull (Mr.-CH-19M-1), a first pH stability step is observed between 1 and 3 h followed by a second between 4 and 8 h. For the second Charolais young bull (Mr.-CH-19M-2), only one pH stability step appears between 4 and 6 h. For the Charolais cull cow, only one pH stability step is observed between 1 and 3 h. Note the very fast pH drop in this first phase (0–3 h). Unfortunately, in the available literature, it was difficult to find out a detailed analysis of the pH drop in the first hours postmortem which limit the discussion and the extension of our conclusions to the results so far published.

Postmortem, when phosphocreatine stores are exhausted, the required energy is mainly produced through degradation of glycogen by glycolysis. The rate of the process depends on the type of muscle considered but, in all cases, it persists as long as enzymes are not inhibited by acidic pH. Therefore, the discontinuity in pH fall observed here cannot be explained by a transient reduction in the activity of phosphocreatine kinase and other enzymes of the glycolytic pathway but rather by a modification of either the buffering capacity and/or the charge distribution within muscle cells. Replacement of acidic components (phosphatidylserine) by basic components (phosphatidylcholine and phosphatidylethanolamine) in the intracellular compartment, accompanied by a redistribution of ions, could explain the existence of these transient pH stability steps. Because such transient pH stability occurs between 1 and 8 h postmortem, inversion of polarity of the plasmic membrane probably take place during the first 8 h postmortem when pH ranged between 6.4 and approximately 6.8. This observation is comforted by the change observed, in the same pH range, in the conductivity of muscle tissue assessed by impedancemetry (Bertram et al., 2004; Damez, Lepetit, Desneux, Clerjon, & Favier, 2002, in press).

4.1.3. Consequences on muscle thrombin activation

The large heterogeneity of muscle tissue reflects its high degree of functional specialization and is the basis of its functional plasticity and adaptability (Pette & Staron, 1990). This plasticity concerned to a similar extent the neuromuscular junction since the synapse must be constantly adapted to the contractile properties and the electrical activity of each fibre (Glazner et al., 1997). In the 1990s, extravascular cellular functions mediated by thrombin in the process of neural development have been also identified. Since then, thrombin, a serine peptidase extensively studied in the vascular system where the enzyme is known to play a key role in the maintenance of haemostasis (Fenton, 1986), the suspected new extravascular functions of this peptidase have received much attention. In muscle cells, thrombin has been shown to be involved in synapse elimination and remodelling at the neuromuscular junction (Liu, Fields, Festoff, & Nelson, 1994; Zoubine, Ma, Smirnova, Citron, & Festoff, 1996) as well as in inflammatory pathologies affecting skeletal muscles (Akaaboune et al., 1998; Mbebi, Hantai, Jandrot-Perrus, Doyennette, & Verdiere-Sahuque, 1999). The recruitment of activated thrombin from blood in the absence of vascular injury during these events is unlikely. In support of this scenario, the expression of prothrombin mRNA has been demonstrated in brain and neural cell lines (Dihanich, Kaser, Reinhard, Cunningham, & Monard, 1991; Weinstein, Gold, Cunningham, & Gall, 1995), as well as in rodent skeletal muscle and primary skeletal muscle cultures (Glazner et al., 1997; Zoubine et al., 1996). Thrombin has been thus found to be located at the neuromuscular junction in the vicinity of the plasma membrane (Akaaboune et al., 1998; Mbebi et al., 1999). Muscle cells expressed thrombin may be activated to act locally (Citron, Smirnova, Zoubine, & Festoff, 1997; Kim, Buonanno, & Nelson, 1998). As shown in Fig. 4, activation of thrombin is facilitated by flip-flop exposure of phosphatidylserine on the outer membrane leaflet (Boon, Lambert, Sisson, Davis, & Smith, 2003). The membrane itself is not sufficient for thrombin activation suggesting that the presence of phosphatidylserine at the surface of the membrane is an absolute requirement for its activation (Majumder, Weinreb, & Lentz, 2005). Postmortem apoptotic induced phosphatidylserine exposure at the surface of the cell membrane would therefore lead to thrombin activation at the neuromuscular junction. The consequence will be a rapid alteration of the synapse and of the electrical conduction towards the cell. This will probably affect also the nerves themselves (Grand, Grabham, Gallimore, & Gallimore, 1989; Gurwitz & Cunning-

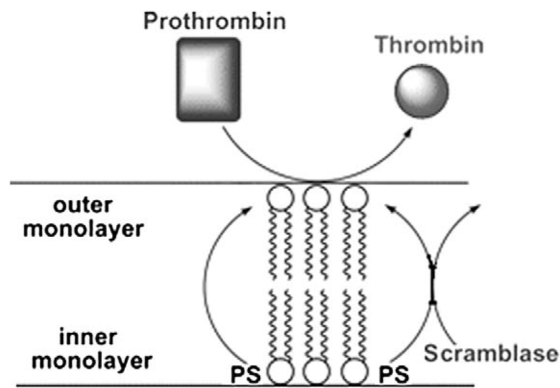

Fig. 4. Thrombin activation needs the translocation of phosphatidylserine to the outer leaflet of the membrane (adapted from Boon et al., 2003).

ham, 1988; Jalink & Moolenaar, 1992; Suidan, Stone, Hemmings, & Monard, 1992; Tews, 2002).

Does this can explain the rapid time dependent efficiency loss of low voltage electrical stimulation which essentially uses the nervous system for the electrical field conduction? It is indeed well recognized that low voltage electrical stimulation of carcasses is efficient only if applied within few minutes following animal stunning and bleeding. As illustrated in Fig. 5, decrease in the rate of pH decline is significantly reduced when low voltage stimulation (100 V, 2 min) is applied 15 min postmortem as compared to the effect of similar stimulation applied 2 min postmortem. After 2 min, the kinetic of the time course pH drop is wholly comparable to the curve obtained upon high voltage stimulation (750 V, 2 min) applied 30 min postmortem.

4.2. Calcium and meat ageing

Since the 1960s, we know that injection of calcium in meat accelerates the tenderising process (Khan & Kim, 1975). The action of calcium is generally attributed to an activation of calpains, the calcium-dependent peptidases. Because of the numerous roles of this cation in cell signalling pathways, other potential functions of calcium have received very few attentions from meat scientists. However, if we now consider that, after slaughter, cells have no other alternative but engage towards suicide or apoptosis, we have to reconsider some of these functions. Calcium is indeed a crucial effector for triggering and controlling apoptosis (Orrenius, Zhivotovsky, & Nicotera, 2003; Szabadkai & Rizzuto, 2004). In *postmortem* muscle, calcium concentration increases gradually in the cytoplasm during the *rigor mortis* onset while the sarcoplasmic reticulum is emptied of its contents (Vignon, Beaulaton, & Ouali, 1989).

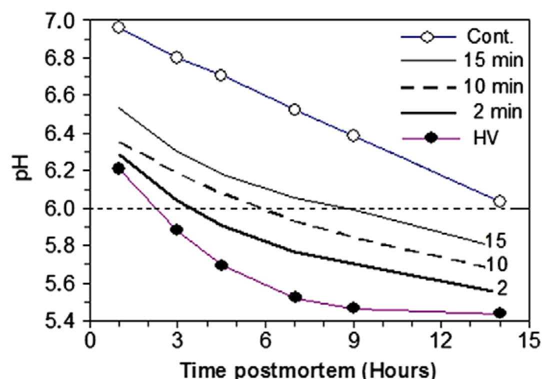

Fig. 5. pH drop profile in the *Longissimus* muscle friesen cull cows. Open circles, control animals; close circles, high-voltage (HV) stimulated animals; curves 2, 10 and 15 correspond to low-voltage (LV) stimulated animals 2, 10 and 15 min postmortem. Sampling times for LV stimulated animals are the same than for the control and the HV stimulated animals. Each point is the mean for three animals (5–6-years-old friesan cull cows). The horizontal dashed line corresponds to the pH limit for prevention of cold shortening, i.e. pH 6. The current voltage was 750 and 100 V for HV and LV stimulation, respectively. (adapted from Valin, 1986).

We now know that this cation is a central element of the apoptotic process, inducing swelling and extensive alteration of mitochondria. It contributes also to the release of cytochrome *c* together with other proapoptotic proteins. This process ends by the activation of caspase 9 which in turn, will activate the effector caspases. Since the process of apoptosis is irreversible, once engaged, it continues during all the shelf-life of refrigerated meat. As for other intracellular peptidases active at neutral pH, one of the major limitations to the activity of caspases will be the acidic pH. Unfortunately, as no data are so far available on the pH effect on caspases activity, this point remains to be analysed.

4.3. Variation of intra- and extracellular spaces in postmortem muscle

Over the past decades, much work was devoted to the *postmortem* evolution of intra- and extracellular spaces in relation with water movements in the muscle and with water holding capacity (see reviews of Offer & Knight, 1988a, 1988b). It was generally recognized that the main cause of these changes was the distribution, between the two compartments, of water which accounts in weight for approximately 75% of muscle tissue. Acidification of muscle decreases protein charges and increase their hydrophobicity, thereby reducing water retention. This is confirmed by the very high correlation observed between the increase in extracellular space and muscle pH (Guignot et al., 1993). The only point which remained unexplained was the early increase in extracellular space, starting immediately after slaughter, whereas pH was still very close to neutrality. Events associated with cell death provide an explanation since a cell entering in apoptosis is dissociated from others and “shrinks”. The consequence will be a reduction in intracellular space and a parallel increase in extracellular space.

We have shown above that *postmortem* pH profile was polyphasic and presented one or two steps of relative stability in the first 8 h following slaughter. By analogy, Guignot et al. (1993) showed that extracellular space reached its maximum value approximately 10 h *postmortem*. Thus retraction of cells related to cellular death coincides with

the period of polyphasic pH decrease and with progressive increase in extracellular space. All these results would suggest that cellular retraction and probably also inversion of membranes polarity, two major consequences of cellular death, reached their ultimate point approximately 8–10 h post-slaughter.

4.4. Deterioration of mitochondria and cellular oxidation

Mitochondria are a central element in the apoptotic process and this explains the large number of interesting scientific reviews published over the last decade on the role of this cellular organelle in cell death (Bras, Queenan, & Susin, 2005; Gottlieb, 2000; Granville & Gottlieb, 2002; Green & Amarante-Mendes, 1998; Gulbins, Dreschers, & Bock, 2003; Haeberlein, 2004; Parone, James, & Martinou, 2002; Ravagnan, Roumier, & Kroemer, 2002). This is particularly true as *postmortem*, the stimulus does not consist in an activation of cellular death receptors but rather from the cell itself in response in the harmful environmental conditions. In addition to the respiratory chain losing its capacity to oxidize molecular oxygen, the mitochondrial external membrane becomes permeable to all protein compounds localised in the intermembrane space, including cytochrome *c*, a central caspase 9 activator. Other proteins with proapoptotic activity are also released in the cytosol (Youle & Karbowski, 2005). In parallel, calcium from the endoplasmic reticulum is transferred to the mitochondria. Mitochondria become overloaded with calcium causing an irreversible alteration of their internal membrane. Molecular oxygen, not oxidized anymore by the respiratory chain, will form free oxygen radicals able to oxidize all cellular compounds (lipids, proteins, etc.).

As apoptosis will start within few minutes after death, the first oxygen radicals will be generated by mitochondria and this will initiate the autocatalytic process which will go on over the whole storage period even at low temperature including freezing. This essential initiating phase must be taken into account in studies dealing with the identification of the major determinants of meat flavour and colour evolution.

4.5. Stress and apoptosis

It is well recognized that stress impairs the meat ageing process, leading generally to tougher meat. Probably the best illustration of this phenomenon is the case of exsudative pig meat.

Confronted to any type of stress, living organisms react by the emission of signals directed to the cells, the first chronological signals being hormones. If the stress is particularly intense (e.g. oxidative stress), the cells receive apoptosis-inducing signals via the receptors of cellular death. If the stress is not as severe, cells prepare their defence as quickly as possible. Among available means, the most described is the synthesis of various protective proteins known as HSPs (heat shock proteins). These proteins help in the protection of intracellular components and structures against hazards associated with loss of their biological functions.

In relation to the subject of cellular death, we saw that HSPs have an anti-apoptotic activity (Beere, 2004). They will consequently slow down the process of cellular death and will constitute an obstacle to good meat ageing. Once again, the concept of apoptosis being the first stage of meat ageing provides an answer to the question of the relationship nature between animal stress and unacceptable meat tenderisation.

4.6. Peptidases and proteolysis

Tenderisation of meats results very likely from the softening of the myofibrillar structure by the synergistic action of endogenous peptidases including mainly cathepsins, calpains and proteasome (Sentandreu et al., 2002).

Up to date, calpains are considered as the major proteolytic system responsible for meat tenderisation and most researchers are prone to think that this system explained most changes in postmortem muscle (Veiseth & Koohmaraie, 2005).

If we reconsider meat tenderisation through introduction of programmed cell death, the first active peptidases after animal bleeding would be undoubtedly caspases. These peptidases are in a better position than others to alter cellular structures since this is their primary function *in vivo* (Creagh & Martin, 2001). The common occurrence of sequences that match the preferred inherent substrate specificity of caspases in intracellular proteins would suggest a multitude of substrates *in vivo* – somewhere in the order of several hundred. Indeed, the list of proteins that are cleaved by caspases either *in vivo* or *in vitro* is ever growing (for detailed analysis of caspase substrates, see Earnshaw, Martins, & Kaufmann, 1999; Fischer, Jänicke, & Schulze-Osthoff, 2003; Nicholson, 1999). However, only a few of these proteins have been rigorously established as biologically relevant and many of them may represent just 'innocent bystanders (Denault & Salvesen, 2002). These findings are comparable to those reported for calpains for which a great number of potential muscle protein substrates have been reported for calpains (Goll, Thompson, Li, Wei, & Cong, 2003). Because capases are specialized in cell destructuration, they probably first degrade the key proteins involved in the complex spatial organisation of myofibrils within muscle cells and the hydrolysis of cellular components and organelles will then go on with the probable contribution of other proteolytic systems including very likely cathepsins, calpains, proteasomes and else. The same process will very likely take place similarly in postmortem muscle and, because of the changes in physicochemical conditions occurring in muscle after animal bleeding, the extent of cellular deterioration will be less extensive.

Caspases are neutral cysteine peptidases and their activity will be affected by muscle acidification to a similar

extent than calpains and proteasomes (Sentandreu et al., 2002). Unfortunately, as no data are available in the literature on the effect of pH on the stability and the hydrolytic activity of these peptidases, no development in our comments can be done on this point. Meat scientists would have to explore the effect of postmortem physico-chemicals conditions on these particular peptidases.

It is worthy to note that the implication of caspases would contribute to explain the often reported assertion that the first hours following slaughter are essential for a satisfactory meat ageing process.

5. Conclusion

Programmed cell death, a process investigated in more detail and at a molecular level since 1995, is now relatively well-known. Although all mediators are not identified yet, knowledge of the mechanisms of apoptosis is sufficient to be integrated in meat tenderisation studies. This prospective review attempted to emphasize that apoptosis can constitute a new thinking way about the conversion of muscle into meat. Entry of muscular cells in apoptosis is indeed difficult to dispute, taking into account the environmental conditions which exist after animal bleeding. It is the unique possible route for cells and tissues of a dead animal and for all animal species considered. On the basis of the knowledge acquired since 1995 on apoptosis especially in humans, analysis of the consequences of apoptosis on cells engaged in programmed cell death brings possible answers to many questions facing scientists who study the conversion of muscle into meat. Even if the participation of caspases does not explain everything, it is probably an essential element facilitating the action of other intracellular proteolytic systems, i.e. cathepsins, calpains, proteasomes and may be also other proteolytic enzymes not considered so far as potential effector of meat softening.

It is therefore necessary to integrate the available knowledge in our future investigations on meat tenderisation, including the search of predictive markers of this essential quality for the consumer. The search of biological predictors of tenderness and other qualities of the meat is an absolute necessity, in order to enhance valorisation of carcasses by directing them shortly after slaughter towards an optimal use on the basis of their potential qualities. It is further a prerequisite for the introduction of meat quality in the genetic selection programs.

In the traditional view of the conversion of muscle into meat, it would be therefore necessary to add a step before the *rigor mortis* (Fig. 6). This step would correspond to the setting of cell death and apoptosis, with all the consequences overviewed above and their impact on the rigor and meat tenderising phases.

This prospective review is the primary step for new considerations about the meat ageing process and we hope that it will incite new experimental approaches in this field. This hypothesis will very likely trigger intense discussions and we are sure that if some of you will support this assumption, many others will probably suggest that we are in the wrong track.

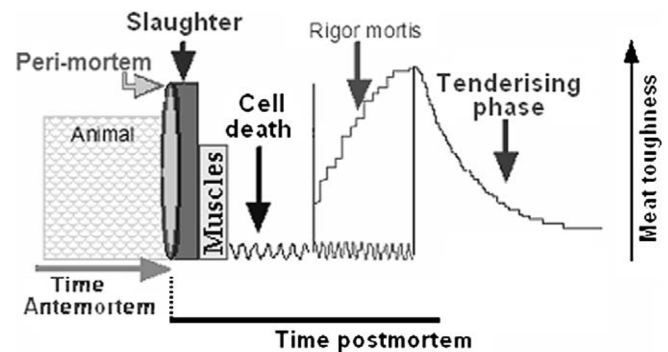

Fig. 6. Different phases of the conversion of muscle into meat. In addition to the phases of rigor and ageing, it would be necessary to add an earlier step corresponding to the phase of initiation of cellular death and its biochemical and structural consequences on the cell.

References

Akaaboune, M., Hantai, D., Smirnova, I., Lachkar, S., Kapsimali, M., Verdiere-Sahuque, M., et al. (1998). Developmental regulation of the serpin, protease nexin I, localization during activity-dependent polyneuronal synapse elimination in mouse skeletal muscle. *Journal Comparative Neurology, 397*, 572–579.

Alnemri, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W., et al. (1996). Human ICE/CED-3 protease nomenclature. *Cell, 87*, 171.

Arrigo, A. P. (2005). In search of the molecular mechanism by which small stress proteins counteract apoptosis during cellular differentiation. *Journal of Cellular Biochemistry, 94*, 241–246.

Arrigo, A. P., Paul, C., Ducasse, C., Manero, F., Kretz-Remy, C., Virot, S., et al. (2002). Small stress proteins: novel negative modulators of apoptosis induced independently of reactive oxygen species. *Progress in Molecular and Subcellular Biology, 28*, 185–204.

Ashie, I. N. A., & Simpson, B. K. (1997). Proteolysis in food myosystems – a review. *Journal of Food Biochemistry, 21*, 91–123.

Barnier, V. M. H. (1995). Determinants and predictors of beef tenderness. PhD Thesis, University of Utrrech, The Netherlands.

Barrett, A. J. (1987). The cystatins: a new class of peptidase inhibitors. *Trends in Biochemical Sciences, 12*, 193–196.

Beere, H. M. (2001). Stressed to death: regulation of apoptotic signaling pathways by the heat shock proteins. *Science's Signal Transduction Knowledge Environment, 93*, RE1.

Beere, H. M. (2004). The stress of dying: the role of heat shock proteins in the regulation of apoptosis. *Journal of Cell Science, 117*, 2641–2651.

Beere, H. M. (2005). Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *Journal of Clinical Investigation, 115*, 2633–2639.

Bekhit, A. E. D., & Faustman, C. (2005). Metmyoglobin reducing activity. *Meat Science, 71*, 407–439.

Bertram, H. C., Schafer, A., Rosenvolda, K., & Andersen, H. J. (2004). Physical changes of significance for early post mortem water distribution in porcine M. longissimus. *Meat Science, 66*, 915–924.

Bevers, E. M., Comfurius, P., & Zwaal, R. F. (1996). Regulatory mechanisms in maintenance and modulation of transmembrane lipid asymmetry: pathophysiological implications. *Lupus, 5*(5), 480–487.

Bige, L., Ouali, A., & Valin, C. (1985). Purification and characterization of a low molecular weight cysteine proteinase inhibitor from bovine muscle. *Biochimica et Biophysica Acta, 843*, 269–275.

Boon, J. M., Lambert, T. N., Sisson, A. L., Davis, A. P., & Smith, B. D. (2003). Facilitated phosphatidylserine (PS) flip-flop and thrombin activation using a synthetic PS scramblase. *Journal of the American Chemical Society, 125*, 8195–8201.

Bouton, P. E., Carroll, F. D., Fisher, A. L., Harris, P. V., & Shorthose, W. R. (1973). Effect of altering ultimate pH on bovine muscle tenderness. *Journal of Food Science, 38*, 816–820.

Bras, M., Queenan, B., & Susin, S. A. (2005). Programmed cell death via mitochondria: different modes of dying. *Biochemistry (Moscow), 70*, 231–239.

Bratton, D. L., Fadok, V. A., Richter, D. A., Kailey, J. M., Guthrie, L. A., & Henson, P. M. (1997). Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. *Journal of Biological Chemistry, 272*, 26159–26165.

Buja, L. M., Eigenbrodt, M. L., & Eigenbrodt, E. H. (1993). Apoptosis and necrosis. Basic types and mechanisms of cell death. *Archives of Pathology and Laboratory Medicine, 117*, 1208–1214.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*, 303–311.

Carling, D. (2004). The AMP-activated protein kinase cascade—a unifying system for energy control. *Trends in Biochemical Science, 29*, 18–24.

Cerretti, D. P., Kozlosky, C. J., Mosley, B., Nelson, N., Van Ness, K., Greenstreet, T. A., et al. (1992). Molecular cloning of the interleukin-1∼converting enzyme. *Science, 256*, 97–100.

Citron, B. A., Smirnova, I. V., Zoubine, M. N., & Festoff, B. W. (1997). Quantitative PCR analysis reveals novel expression of prothrombin mRNA and regulation of its level in developing mouse muscle. *Thrombosis Research, 87*, 303–313.

Clarke, P. G. H., & Clarke, S. (1995). Historic apoptosis. *Nature, 378*, 230.

Crapo, C., Himelbloom, B., Pfutzenreuter, R., & Chong, L. (1999). Causes for soft flesh in giant grenadier (*Albatrossia pectoralis*) fillets. *Journal of Aquatic Food Production Technology, 8*, 55–68.

Creagh, E. M., & Martin, S. J. (2001). Caspases: cellular demolition experts. *Biochemical Society Transactions, 29*, 696–702.

Damez, J. L., Clerjon, S., & Abouelkaram, S., (in press). Mesostructure assessed by alternating current spectroscopy during meat ageing. In *51st international congress of meat science and technology*, 2005, Baltimore, August 7–12.

Damez, J. L., Lepetit, J., Desneux, L., Clerjon, S., & Favier, R. (2002). Non destructive assessment of meat ageing using electrical impedance anisotropy. *48th ICOMST. Rome, Italie, 1*, 802–803.

De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., & Appelmans, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochemical Journal, 60*, 604–617.

Denault, J. B., & Salvesen, G. S. (2002). Caspases: keys in the ignition of cell death. *Chemical Reviews, 102*, 4489–4500.

Dihanich, M., Kaser, M., Reinhard, E., Cunningham, D., & Monard, D. (1991). Prothrombin mRNA is expressed by cells of the nervous system. *Neuron, 6*, 575–581.

Dirks, A. J., & Leeuwenburgh, C. (2005). The role of apoptosis in age-related skeletal muscle atrophy. *Sports Medicine, 35*, 473–483.

Driscoll, M. (1996). Cell death in *C. elegans*: molecular insights into mechanisms conserved between nematodes and mammals. *Brain Pathology, 6*, 411–425.

Dubin, G. (2005). Proteinaceous cysteine protease inhibitors. *Cellular and Molecular Life Sciences, 62*, 653–669.

Dutaud, D. (1998). Quantification et caractérisation du proteasome 20 S de muscle de bovin en relation avec l'attendrissage de la viande bovine. PhD Thesis, Blaise Pascal University, Clermont-Ferrand, France.

Earnshaw, W. C., Martins, L. M., & Kaufmann, S. H. (1999). Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annual Review of Biochemistry, 68*, 383–424.

Fenton, J. W. II, (1986). Thrombin. *Annals of the New York Academy of Sciences, 485*, 5–15.

Fernandez, E., Raynaud, F., Coulis, G., Aubry, L., Vignon, X., Bleimling, N., et al. (2005). Calpain 1–titin interactions concentrate calpain 1 in the Z-band edges and in the N2-line region within the skeletal myofibril. *FEBS Journal, 272*, 2578–2590.

Ferre, P., Azzout-Marniche, D., & Foufelle, F. (2003). AMP activated protein kinase and hepatic genes involved in glucose metabolism. *Biochemical Society Transactions, 31*, 220–223.

Fidzianska, A., Kaminska, A., & Glinka, Z. (1991). Muscle cell death. Ultrastructural differences between muscle cell necrosis and apoptosis. *Neuropatologia Polska, 29*, 19–28.

Fischer, U., Jänicke, R. U., & Schulze-Osthoff, K. (2003). Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differentiation, 10*, 76–100.

Flower, T. R., Chesnokova, L. S., Froelich, C. A., Dixon, C., & Witt, S. N. (2005). Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. *Molecular Biology, 351*, 1081–1100.

Fuentes-Prior, P., & Salvesen, G. S. (2004). The protein structures that shape caspase-activity, specificity, activation, and inhibition. *Biochemical Journal, 384*, 201–232.

Gavrieli, Y., Sherman, Y., & Ben-Sasson, S. A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *Journal of Cell Biology, 119*, 493–501.

Glazner, G. W., Yadav, K., Fitzgerald, S., Coven, E., Brenneman, D. E., & Nelson, P. G. (1997). Cholinergic stimulation increases thrombin activity and gene expression in cultured mouse muscle. *Developmental Brain Research, 99*, 148–154.

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiological Reviews, 83*, 731–801.

Gorbatov, V. M., & Lyaskovskaya, Y. N. (1980). Review of the flavour-contributing volatiles and water-soluble non-volatiles in pork meat and derived products. *Meat Science, 4*, 209–225.

Gottlieb, R. A. (2000). Role of mitochondria in apoptosis. *Critical Reviews in Eukaryotic Gene Expression, 10*, 231–239.

Grand, R. J., Grabham, P. W., Gallimore, M. J., & Gallimore, P. H. (1989). Modulation of morphological differentiation of human neuroepithelial cells by serine proteases: independence from blood coagulation. *EMBO Journal, 8*, 2209–2215.

Granville, D. J., & Gottlieb, R. A. (2002). Mitochondria: regulators of cell death and survival. *Scientific World Journal, 2*, 1569–1578.

Green, D. R. (2005). Apoptotic pathways: ten minutes to dead. *Cell, 121*, 671–674.

Green, D. R., & Amarante-Mendes, G. P. (1998). The point of no return: mitochondria, caspases, and the commitment to cell death. *Results and Problems in Cell Differentiation, 24*, 45–61.

Guignot, F., Vignon, X., & Monin, G. (1993). Post mortem evolution of myofilament spacing and extracellular space in veal muscle. *Meat Science, 33*, 333–347.

Gulbins, E., Dreschers, S., & Bock, J. (2003). Role of mitochondria in apoptosis. *Experimental Physiology, 88*, 85–90.

Guroff, G. (1964). A neutral, calcium-activated proteinase from the soluble fraction of rat brain. *Journal of Biological Chemistry, 239*(January), 149–155.

Gurwitz, D., & Cunningham, D. D. (1988). Thrombin modulates and reverses neuroblastoma neurite outgrowth. *Proceedings of the National Academy of Sciences of the United States of America*, 3440–3444.

Haeberlein, S. L. (2004). Mitochondrial function in apoptotic neuronal cell death. *Neurochemical Research, 29*, 521–530.

Hardie, D. G., Scott, J. W., Pan, D. A., & Hudson, E. R. (2003). Management of cellular energy by the AMP-activated protein kinase system. *FEBS Letters, 546*, 113–120.

Hengartner, M. O. (2000). The biochemistry of apoptosis. *Nature, 407*(6805), 770–776 (Review).

Ishiguro, H., Higashiyama, S., Namikawa, C., Kunimatsu, M., Takano, E., Tanaka, K., et al. (1987). Interaction of human calpains I and II with high molecular weight and low molecular weight kininogens and their heavy chain: mechanism of interaction and the role of divalent cations. *Biochemistry, 26*, 2863–2870.

Jalink, K., & Moolenaar, W. H. (1992). Thrombin receptor activation causes rapid neural cell rounding and neurite retraction independent of classic second messengers. *Journal of Cell Biology, 118*, 411–419.

Jeremiah, L. E. (1982). Factors affecting consumption, selection and acceptability of meat purchases. *Journal of Consumer Studies and Home Economics, 6*, 137–154.

Jiang, S. T. (2000). Effect of proteinases on the meat texture and seafood quality. *Food Science and Agricultural Chemistry, 2*, 55–74.

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Science, 36*, 169–189.

Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer, 26*, 239–257.

Khan, A. W., & Kim, Y. K. (1975). Effect of calcium on isometric tension, glycolysis and tenderness of poultry breast meat. *Journal Food Science, 40*, 1119–1121.

Kim, S., Buonanno, A., & Nelson, P. G. (1998). Regulation of prothrombin, thrombin receptor, and protease nexin-1 expression during development and after denervation in muscle. *Journal of Neuroscience Research, 53*, 304–311.

Kos, J., & Lah, T. T. (1998). Cysteine proteinases and their endogenous inhibitors: target proteins for prognosis, diagnosis and therapy in cancer. *Oncology Reports, 5*, 1349–1361.

Kroemer, G., Dallaporta, B., & Resche-Rigon, M. (1998). The mitochondrial death/life regulator in apoptosis and necrosis. *Annual Review of Physiology, 60*, 619–642.

Kultz, D. (2003). Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. *Journal of Experimental Biology, 206*, 3119–3124.

Lamare, M., Taylor, R. G., Farouta, L., Briand, Y., & Briand, M. (2002). Changes in proteasome activity during postmortem aging of bovine muscle. *Meat Science, 61*, 199–204.

Lawson, M. A. (2004). The role of integrin degradation in post-mortem drip loss in pork. *Meat Science, 68*, 559–566.

Leeuwenburgh, C. (2003). Role of apoptosis in sarcopenia. *Journal of Gerontology A. Biological Sciences and Medical Sciences, 58*, 999–1001.

Liu, C. C., & Ahearn, J. M. (2001). Apoptosis of skeletal muscle cells and the pathogenesis of myositis: a perspective. *Current Rheumatology Reports, 3*, 325–333.

Liu, Y., Fields, R. D., Festoff, B. W., & Nelson, P. G. (1994). Proteolytic action of thrombin is required for electrical activity-dependent synapse reduction. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 10300–10304.

Majino, G., & Jons, I. (1995). Apoptosis, oncosis and necrosis. An overview of cell death. *American Journal of Pathology, 146*, 3–15.

Majumder, R., Weinreb, G., & Lentz, B. R. (2005). Efficient thrombin generation requires molecular phosphatidylserine, not a membrane surface. *Biochemistry, 44*(51), 16998–17006.

Martin, S. J., Reutelingsperger, C. P., McGahon, A. J., Rader, J. A., van Schie, R. C., LaFace, D. M., & Green, D. R. (1995). Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *Journal of Experimental Medicine, 182*, 1545–1556.

Matsuishi, M., & Okitani, A. (1997). Proteasome from rabbit skeletal muscle: some properties and effects on muscle proteins. *Meat Science, 45*, 451–462.

Matsura, T., Togawa, A., Kai, M., Nishida, T., Nakada, J., Ishibe, Y., et al. (2005). The presence of oxidized phosphatidylserine on Fas-mediated apoptotic cell surface. *Biochimica et Biophysica Acta, 1736*, 181–188.

Mbebi, C., Hantai, D., Jandrot-Perrus, M., Doyennette, M. A., & Verdiere-Sahuque, M. (1999). Protease nexin I expression is up-regulated in human skeletal muscle by injury-related factors. *Journal of Cellular Physiology, 179*, 305–314.

Meier, P., Finch, A., & Evan, G. (2000). Apoptosis in development. *Nature, 407*, 796–801.

Mishra, N. C., & Kumar, S. (2005). Apoptosis: a mitochondrial perspective on cell death. *Indian Journal of Experimental Biology, 43*, 25–34.

Mohamad, N., Gutierrez, A., Nunez, M., Cocca, C., Martin, G., Cricco, G., et al. (2005). Mitochondrial apoptotic pathways. *Biocell, 29*, 149–161.

Moloney, A. P., Mooney, M. T., Kerry, J. P., & Troy, D. J. (2001). Producing tender and flavoursome beef with enhanced nutritional characteristics. *Proceedings of the Nutrition Society, 60*, 221–229.

Monin, G., & Ouali, A. (1991). Muscle différentiation and meat quality. *Developments in Meat Science, 5*, 89–157.

Mottram, D. S. (1992). Meat flavour. *Meat Focus International,* June issue, 81–92.

Muller-Esterl, W., Fritz, H., Kellermann, J., Lottspeich, F., Machleidt, W., & Turk, V. (1985). Genealogy of mammalian cysteine proteinase inhibitors. Common evolutionary origin of stefins, cystatins and kininogens. *FEBS Letters, 191*, 221–226.

Nicholson, D. W. (2000). From bench to clinic with apoptosis-based therapeutic agents. *Nature, 407*, 810–816.

Nicholson, D. W. (1999). Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differentiation, 6*, 1028–1042.

Offer, G., & Knight, P. (1988a). The structural basis of water-holding in meat. I. General principles and water uptake in meat processing. *Developments in Meat Science, 4*, 63–171.

Offer, G., & Knight, P. (1988b). The structural basis of water-holding in meat. II. Drip losses. *Developments in Meat Science, 4*, 173–243.

Orrenius, S., Zhivotovsky, B., & Nicotera, P. (2003). Regulation of cell death: the calcium-apoptosis link. *Nature Reviews Molecular Cell Biology, 4*, 552–565.

Otsuka, Y., Homma, N., Shiga, K., Ushiki, J., Ikeuchib, Y., & Suzukib, A. (1998). Purification and properties of rabbit muscle proteasome, and its effect on myofibrillar structure. *Meat Science, 49*, 365–378.

Ouali, A. (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie, 74*, 251–265.

Ouali, A. (1991). Sensory quality of meat as affected by muscle biochemistry and modern technologies. In L. O. Fiems, B. G. Cottyn, & D. I. Demeyer (Eds.), *Animal biotechnology and the quality of meat production* (pp. 85–105). Amsterdam: Elsevier Science Publishers B.V..

Ouali, A. (1999). Structure and biochemistry of muscle as related to meat texture. In *Proc. 14th European symposium on the quality of poultry meat*, Bologna, (Vol. 1, pp. 91–121).

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

Ouali, A., Rouchon, P., Zabari, M., Berri, M., Zamora, F., & Tassy, C. (1995). Natural serine and cysteine proteinase inhibitors in bovine skeletal muscle. In A. Ouali, D. Demeyer, & F. J. M. Smulders (Eds.), *Expression of tissue proteinases and regulation of protein dégradation as related to meat quality* (pp. 173–198). Utrecht, NL: ECCEAMST.

Owens, F. N., & Gardner, B. A. (1999). Ruminent nutrition and meat quality. *Proceedings of the Annual Reciprocal Meat Conference, 52*, 25–36.

Parone, P. A., James, D., & Martinou, J. C. (2002). Mitochondria: regulating the inevitable. *Biochimie, 84*, 105–111.

Petit, P. X., Zamzami, N., Vayssiere, J. L., Mignotte, B., Kroemer, G., & Castedo, M. (1997). Implication of mitochondria in apoptosis. *Molecular and Cellular Biochemistry, 174*, 185–188.

Pette, D., & Staron, R. S. (1990). Cellular and molecular diversities of mammalian skeletal muscle fibers. *Reviews of Physiology Biochemistry and Pharmacology, 116*, 1–76.

Primeau, A. J., Adhihetty, P. J., & Hood, D. A. (2002). Apoptosis in heart and skeletal muscle. *Canadian Journal of Applied Physiology, 27*, 349–395.

Purchas, R. W., & Davies, L. H. (1974). Meat production of Friesian steers: the effect of intramuscular fat on palatability and the effect of growth rates on composition changes. *Australian Journal of Agricultural Research, 25*, 667–677.

Ravagnan, L., Roumier, T., & Kroemer, G. (2002). Mitochondria, the killer organelles and their weapons. *Journal of Cellular Physiology, 192*(2), 131–137.

Rawlings, N. D., & Barrett, A. J. (1990). Evolution of proteins of the cystatin superfamily. *Journal of Molecular Evolution, 30*, 60–71.

Raynaud, P., Gillard, M., Parr, T., Bardsley, R., Amarger, V., & Leveziel, H. (2005). Correlation between bovine calpastatin mRNA transcripts and protein isoforms. *Archives of Biochemistry and Biophysics, 440*, 46–53.

Renerre, M. (1999). Biochemical basis of fresh meat colour. *Proceedings of the 45th ICoMST. Yokohama, Japan, 2*, 344–353.

Roux, M., Nizou, A., Forestier, L., Ouali, A., Leveziel, H., & Amarger, V. (2006). Characterization of the bovine PRKAG3 gene: structure, polymorphism, and alternative transcripts. *Mammalian Genome, 17*, 83–92.

Sandri, M., & Carraro, U. (1999). Apoptosis of skeletal muscles during development and disease. *International Journal of Biochemistry and Cell Biology, 31*, 1373–1390.

Sandri, M. (2002). Apoptotic signaling in skeletal muscle fibers during atrophy. *Current Opinion in Clinical Nutrition and Metabolic Care, 5*, 249–253.

Sanmartın, M., Jaroszewski, L., Raikhel, N. V., & Rojo, E. (2005). Caspases. Regulating death since the origin of life. *Plant Physiology, 137*, 841–847.

Satoh, Y., & Shikama, K. (1981). Autoxidation of oxymyoglobin. A nucleophilic displacement mechanism. *Journal of Biological Science, 256*, 10272–10275.

Schwartz, W., & Bird, J. W. C. (1997). Degradation of myofibrillar proteins by cathepsins B and D. *Biochemical Journal, 167*, 811–820.

Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology, 13*(12), 400–421.

Sentandreu, M. A., Stoeva, S., Aristoy, M. C., Laib, K., Voelter, W., & Toldra, F. (2003). Identification of small peptides generated in Spanish dry-cured ham. *Journal of Food Science, 68*, 64–69.

Shackelford, S. D., Koohmaraie, M., Whipple, G., Wheeler, T. L., Miller, M. F., Crouse, J. D., et al. (1991). Predictors of beef tenderness: development and verification. *Journal of Food Science, 56*, 1130–1135.

Shi, Y. (2002). Mechanisms of caspase activation and inhibition during apoptosis. *Molecular Cell, 9*, 459–470.

Strojan, P., Budihna, M., Smid, L., Svetic, B., Vrhovec, I., Kos, J., & Skrk, J. (2000). Prognostic significance of cysteine proteinases cathepsins B and L and their endogenous inhibitors stefins A and B in patients with squamous cell carcinoma of the head and neck. *Clinical Cancer Research, 6*, 1052–1062.

Suidan, H. S., Stone, S. R., Hemmings, B. A., & Monard, D. (1992). Thrombin causes neurite retraction in neuronal cells through activation of cell surface receptors. *Neuron, 8*, 363–375.

Szabadkai, G., & Rizzuto, R. (2004). Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighbourhood. *FEBS Letters, 567*, 111–115.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science, 73*, 1351–1367.

Tews, D. S. (2002). Apoptosis and muscle fibre loss in neuromuscular disorders. *Neuromuscular Disorders, 12*, 613–622.

Tews, D. S. (2005). Muscle-fiber apoptosis in neuromuscular diseases. *Muscle and Nerve, 32*, 443–458.

Thomas, A. R., Gondoza, H., Hoffman, L. C., Oosthuizen, V., Ryno, J., & Naude, A. (2004). The roles of the proteasome, and cathepsins B, L, H and D, in ostrich meat tenderisation. *Meat Science, 67*, 113–120.

Thornberry, N. A., Bull, H. G., Calaycay, J. R., Chapman, K. T., Howard, A. D., Kostura, M. J., et al. (1992). A novel heterodimeric cysteine protease is required for interleukin-1∼ processing in monoctes. *Nature, 356*, 768–774.

Turk, V., Brzin, J., Kotnik, M., Lenarcic, B., Popovic, T., Ritonja, A., et al. (1986). Human cysteine proteinases and their protein inhibitors stefins, cystatins an kininogens. *Biomedica Biochimica Acta, 45*, 1375–1384.

Valin, C., Touraille, C., Vigneron, P., & Ashmore, R. (1982). Prediction of lamb meat quality traits based on muscle biopsy fibre typing. *Meat Science, 6*, 257–263.

Valin, C. (1986). Caractéristiques qualitatives et technologiques des viands bovines: influence des conditions d'abattage et de la technologie. In D. Micol (Ed.). *Production de Viande Bovine* (pp. 85–97). Paris: INRA.

Veiseth, E. & Koohmaraie, M. (2005). Beef tenderness: significance of the calpain proteolytic system (pp. 111–126). In Hocquette, J. F., Gigli S., (Eds.), EAAP Publ. 112, Wageningen, The Netherlands: Wageningen Academic Publishers.

Vignon, X., Beaulaton, J., & Ouali, A. (1989). Ultrastructural localization of calcium in post-mortem bovine muscle: a cytochemical and X-ray microanalytical study. *Histochemical Journal, 21*, 403–411.

Wallace, W. J., Houtchens, R. A., Maxwell, J. C., & Caughey, W. S. (1982). Mechanism of autooxidation for hemoglobins and myoglobins. Promotion of superoxide production by protons and anions. *Journal of Biological Science, 257*, 4966–4977.

Warner, R. D., Dunshea, F. R., Ponnampalam, E. N., & Cottrell, J. J. (2005). Effects of nitric oxide and oxidation in vivo and post-mortem on meat tenderness. *Meat Science, 71*, 205–217.

Weinstein, J. R., Gold, S. J., Cunningham, D. D., & Gall, C. M. (1995). Cellular localization of thrombin receptor mRNA in rat brain: expression by mesencephalic dopaminergic neurons and codistribution with prothrombin mRNA. *Journal of Neuroscience, 15*, 2906–2919.

Wilk, S., & Orlowski, M. (1980). Cation-sensitive neutral endopeptidase: isolation and specificity of the bovine pituitary enzyme. *Journal of Neurochemistry, 35*(5), 1172–1182.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I., & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society, 58*, 363–370.

Wyllie, A. H. (1980). Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature, 284*, 555–556.

Youle, R. J., & Karbowski, M. (2005). Mitochondrial fission in apoptosis. *Nature Reviews Molecular Cell Biology, 6*, 657–663.

Yuan, J., Wang, M. X., & Murrell, G. A. (2003). Cell death and tendinopathy. *Clinical Sports Medicine, 22*, 693–701.

Yuan, J. (1996). Evolutionary conservation of a genetic pathway of programmed cell death. *Journal of Cellular Biochemistry, 60*, 4–11.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M., & Horvitz, H. M. (1993). The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin1-converting enzyme. *Cell, 75*, 641–652.

Zeece, M. G., Woods, T. L., Keen, M. A., & Reville, W. J. (1992). Role of proteinases and inhibitors in postmortem muscle protein degradation. *Proceedings of the Annual Reciprocal Meat Conference, 45*, 51–61.

Zimmermann, K. C., Bonzon, C., & Green, D. R. (2001). The machinery of programmed cell death. *Pharmacology Therapy, 92*, 57–70.

Zimmermann, K. C., & Green, D. R. (2001). How cells die: apoptosis pathways. *Journal of Allergy and Clinical Iummunology, 108*(Suppl. 4), S99–S103.

Zoubine, M. N., Ma, J. Y., Smirnova, I. V., Citron, B. A., & Festoff, B. W. (1996). A molecular mechanism for synapse elimination: novel inhibition of locally generated thrombin delays synapse loss in neonatal mouse muscle. *Developmental Biology, 179*, 447–457.