FOCUS : JSMTV

Nutritional properties of meat peptides and proteins: impact of processing

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RÉSUMÉ
Propriétés nutritionnelles des peptides et protéines de la viande : impact des procédés de transformation
En raison de leur teneur et leur bon équilibre en acides aminés indispensables, les protéines de la viande présentent une qualité nutritionnelle élevée. L’apparition de nouveaux critères d’évaluation de cette qualité, tels que la vitesse de digestion et la potentialité des protéines alimentaires à libérer des peptides bioactifs au cours de la digestion ont relancé l’intérêt pour les produits carnés. En effet les protéines de la viande, qui peuvent être considérées comme des protéines à digestion rapide, pourraient aider à lutter contre la fonte musculaire chez les personnes âgées. De plus, la présence dans la viande de peptide bioactifs natifs, tels que la carnosine et le glutathion, ainsi que la libération pendant la digestion de peptides antihypertensifs, pourraient se révéler très intéressants dans un contexte de nutrition préventive. Avant sa consommation, la viande subit une série de réactions biochimiques, et de traitements technologiques, conduisant à la dénaturation des protéines (perte de leur structure tertiaire et quaternaire), leur oxydation, et la formation d’agrégats. Ces transformations peuvent affecter la vitesse de digestion, la libération des peptides et la biodisponibilité des acides aminés. L’importance de ces modifications et leur impact sur la qualité nutritionnelle des protéines de la viande nécessite d’être approfondi.

Mots clés
viande, protéines, peptides bioactifs, procédés de transformation.

SUMMARY
Because of their high content of well-balanced indispensable amino acids, and their high digestibility, proteins from meat have a high nutritional quality. New criteria for the evaluation of this quality, such as digestion rate and potentiality to release bioactive peptides, have led to a renewed interest in meat products. Indeed meat proteins, which can be considered as a source of rapidly digested proteins, could help to counteract muscle wasting in the elderly. Furthermore, the
occurrence in meat of native bioactive peptides, such as carnosine and glutathione, but also the release of antihypertensive peptides during digestion could be of interest in the context of preventive nutrition. Before being consumed, meat undergoes a series of biochemical reactions, and technological treatments, leading to protein denaturation (loss of quaternary and tertiary structure), oxidation, aggregation which may affect the amino acid bioavailability, the release of peptides, but also the digestion rate. The significance of these modifications and their impact on the nutritional quality of meat proteins require further investigations.

Keywords
Meat, proteins, bioactive peptides, processing.

1 – INTRODUCTION

For many years, the research effort on the quality of meat has focused on the sensory and technological dimensions of quality with special emphasis on the search of biological determinants, accelerated by the recent development of tools for functional genomics (Hocquette, 2005). Since the recent crises which have occurred in bovine, porcine and avian industries, the consumer insists on sanitary traits of meat products. In addition, most consumers are now aware of the impact of food on their health. Thus, in addition to sanitary and sensorial aspects, the nutritional quality becomes an important factor in the choice of food, especially in a context of an increasing demand of more elaborated food products. In accordance with public health policies, the quality of lipids in meat have been extensively studied (Scollan, 2005). All these studies should not make us forget that meat is primarily an important source of proteins for humans. Recent advances in the field of protein nutrition have brought new criteria for the evaluation of the quality of dietary proteins, which led to a renewed interest in meat products. The meat is a complex type of food which undergoes numerous transformations before being consumed (maturation, preservation, mincing, cooking...), technological processes which may alter the biochemical composition and structure of proteins, and hence may change their nutritional properties.

2 – NUTRITIONAL PROPERTIES OF PROTEINS OF MEAT PRODUCTS

The classic criteria for evaluating the quality of a protein source are based on amino acids composition and digestibility of the protein fraction. These basic criteria can only assess the ability of a food to globally provide amino acids. We now know that the definition of the quality of dietary proteins needs to integrate new concepts such as:

- The capacity of dietary proteins to release, during the digestion, peptides having a local or systemic biological impact;
- The rate of digestion which may, in some cases, have a direct influence on whole body assimilation of amino acids.
2.1 Classical criteria for evaluating the quality of protein sources

The meat is a heterogeneous food that varies according to the origin of the flesh (ruminants, other herbivores, monogastrics such as pig or poultry), the type of muscles, which are themselves complex in terms of structural and biochemical properties, and the preparations used to process them into various dishes. Yet, meats present some common nutritional features (Biesalski, 2005). They are rich in protein (50-80% of energy content) and (together with fish), are the fresh foods that present the highest protein content. In addition, these proteins which are particularly rich in indispensable amino acids, notably lysine and histidine, present a balance in essential amino acids supply close to Human requirements, from child to adult. This means that proteins from meat products are used with great efficiency to increase or renew whole body proteins because it is not necessary to supply huge amounts of proteins to cover the requirements in each indispensable amino acid, contrarily to what occurs when the diets are imbalanced in indispensable amino acids supply relatively to the requirements. Furthermore, the high concentrations in lysine in meat-proteins are useful to improve the quality of other proteins such as proteins from cereals. In addition, these proteins that are well digested in the small intestine (Silvester 1995) do not induce any notable reaction in the digestive tract which could increase endogenous losses, as might occur with high-fiber feedstuff.

Because of their favourable balance in indispensable amino acids, their very high digestibility, and the high bioavailability of their amino acids, meat proteins have a high biological value (Young, 1975). Thus, as part of a well balanced diet, the meat and fish consumption does not need to exceed 120 g/d for healthy adults without any specific requirements. In these conditions, the part of the daily supply which is provided by the meat is variable according to nutrients. It can exceed 60% for some indispensable amino acids, vitamin B12 and zinc, 40% for vitamin B3 and cholesterol, 20% for iron, selenium, riboflavin, vitamin B6 and pantothenic acid as well as saturated fatty acids.

2.2 Bioactive peptides

More and more data have proven the physiological effects of certain food-derived peptides on the activity of the digestive tract and other physiological functions (antihypertensive, opioid, immunomodulatory, antianxiolitic activities). Among the peptides found in foods, some are already present, and abundant, in the original feed product and their synthesis does not follow the classical metabolic pathways of protein synthesis and degradation; these are for instance carnosine and glutathione in meat products. Other peptides are present in proteins and can be only released during the various processes of meat transformation or during the intestinal digestion.

2.2.1 Carnosine

Carnosine is a dipeptide (β-alanine-L-histidine) only present in animal tissues. It is particularly abundant in brain and skeletal muscle from mammals. Its concentration is more elevated in muscles with glycolytic activity (Aristoy, 1998; Cornet, 1999); it can vary according to animal species (Crush, 1970), age and/or food intake (Watanabe, 2004; Purchas, 2005). The carnosine concentration in meat is not really affected by the type of ageing or cooking processes (Savary-Auzeloux, 2006; Bauchart, 2007).

The main biological activity of carnosine appears to be its buffering activity (Abe, 2000) that allows, for instance, offsetting the decrease in intracellular pH linked to
lactic acid production in muscles where anaerobic glycolysis is particularly active. Thus, in sportsmen, an increase in carnosine concentration in muscles seems able to attenuate fatigue after intensive exercise (Hill, 2007). However, the effect of carnosine on sport performance itself is controversial (Hoffman, 2006; Kendrick, 2008). Carnosine also has antioxidant properties by its ability to bind divalent metallic ions and its ability to trap free radicals (Guitto, 2005). Moreover, it seems able to reduce aldehydes formed from unsaturated fatty acids during an oxidative stress. It would also play a prominent role in protection against glycation and crosslinking of proteins (Hobard, 2004; Lee, 2005). Protein crosslinking interferes with tissue function and can lead to aggregation of cellular material in the form of plates. Thus, carnosine may play an important role in prevention against secondary complications in diabetes (Lee, 2005) and the protection against neurodegenerative disorders such as Alzheimer’s disease (Hipkiss, 2007). Carnosine-rich diets could hence become increasingly interesting in old age (Hipkiss, 2006).

Due to the specific position of the amino group of \( \beta \)-alanine, carnosine is not degraded by dipeptide hydrolases but by two specific enzymes called aminoacyl-histidine dipeptidases (or carnosinases). One is present in great quantities in many tissues such as kidney, liver and lungs and to a lesser extend in skeletal muscle and small intestine (Lenney, 1976), and the other one is specifically present in serum (Jaskson, 1991). Despite carnosinase activity in small intestine and serum, ingestion of bovine meat induces a rapid increase in plasma concentration of carnosine in humans (Park, 2005). A recent study on animal model suggests that 1/5 of ingested carnosine (after a meal of meat) is actually absorbed and released in the blood (Bauchart, 2007b). This absorption is associated with increased nonbicarbonate buffering capacity (Susuki, 2006), and antioxidant capacity (Antonini, 2002; Bauchart, 2007b) of the serum. Moreover, even if the serum carnosinase hydrolyses an important part of the absorbed carnosine, the availability of its precursors (ie histidine but more importantly \( \beta \)-alanine) could be sufficient to ensure an increase in carnosine synthesis and concentration within the skeletal muscle in humans (Harris, 2006).

### 2.2.2 Glutathion

Glutathion is a tripeptide (GSH: L-\( \gamma \)-Glutamyl-L-cysteinyl-glycine) whose concentration is very high in the liver but also important in the skeletal muscle. Contrarily to carnosine, GSH is not specific to animal products. GSH is also present in significant quantities in vegetables such as broccoli and spinach (Wierzbiak, 1989). Because of the thiol function of cystein radical, glutathion exists as reduced (GSH) or oxidised (GSSG) forms. GSH is the major hydrosoluble antioxidant in animal cells. It is an efficient free radicals scavenger, protecting cells from reactive oxygen species (ROS) attacks. Any changes in GSH and GSSG concentrations directly reflect alterations of their redox state. Glutathion also plays a role in xenobiotics detoxification, metabolism of various molecules (leucotriens, prostaglandins, formaldehyde, methlyglyoxal, nitric oxide,...) and the regulation of expression and/or activation of transcriptional factors “oxidation-sensitive” and necessary for the antioxidant response (Wu, 2004). Glutathion deficiencies contribute to the oxidant stress which plays a key role in the ageing process and the establishment of various pathologies (Alzheimer, Parkinson, inflammation of the digestive tract...). Studies in humans and animals show that an adequate protein intake in essential to maintain glutathion homeostasis. It seems that a portion of the dietary glutathion can be absorbed intact (Hagen 1990), and participate in the intracellular glutathion store in peripheral tissues (Favelli, 1997). Moreover, GSH plays an important role in maintaining the integrity of the intestinal mucosa (Mårtensson, 1990). Finally, dietary GSH, in addition to biliary GSH, participate in the reduction of lipid peroxides present in the intestinal lumen (Yee, 1992).
2.2.3 Antihypertensive peptides

Very few studies are available on the ability of meat proteins to be potential interesting sources of bioactive peptides. The most studied biological activity is the antihypertensive activity based on the inhibition of the angiotensine-converting enzyme (ACE). This activity seems to occur in normal feeding conditions and studies in humans have shown a significant decrease in arterial pressure compared to a control group following the repeated ingestion of fermented milk containing antihypertensive peptides (Hata, 1996; Seppo, 2003).

Several ACE-inhibiting peptides have been evidenced in controlled muscle proteins hydrolysates: from a skeletal muscle hydrolysed with thermolysine (Arihara, 2001), from myosin hydrolysed with thermolysine (Nakashima, 2002), from troponin C hydrolysed with pepsin (Katayama, 2008), and from sarcoplasmic proteins hydrolysed with a mixture of thermolysine, proteinase A and protease type XIII (Jang, 2005). An in vivo study on animal model shows that, following the ingestion of beef meat, numerous peptides are reproducibly released during the digestive process (table 1) and that many of them contain an amino acid sequence known to present an ACE-inhibiting potential (Bauchart, 2007a). To be active at the peripheral level, these sequences will still need to be released intact by mucosal peptidases, to enter the blood circulation and to be resistant to the peptidases present in plasma. The possibility of an absorption of antihypertensive peptides has been demonstrated in humans after an oral administration of the dipeptide Val-Tyr (Matsui, 2002) but the occurrence (and significance) of such an absorption following the ingestion of dietary proteins containing bioactive sequences has so far never been reported. However, it was shown that a partial substitution of dietary carbohydrates by red meat can lower blood pressure in hypertensive patients (Hodgson, 2006).

2.3 Digestion rate

It has been clearly shown that the kinetics of the digestion of dietary protein determines the effectiveness of their assimilation; optimal kinetic is not necessarily the same for all subjects. For instance, for elderly people, it seems preferable to concentrate the daily protein supply on a single meal, or to ingest rapidly digested proteins, in order to accentuate the postprandial increase in plasma amino acids and stimulate protein synthesis (Mosoni, 2003). From these observations, nutritional strategies are now developed to counteract aging related muscle wasting (sarcopenia). The meat in this context could be a very interesting food because of its high concentrations in highly digestible proteins allowing the concentration of the protein supply on one meal.

As in the field of bioactive peptides, many studies on protein digestion kinetics have been carried out with milk proteins (caseins, lactoserum proteins), and few data are available regarding the proteins of meat products. A recent study in old subjects has shown that meat can be considered as a source of rapidly digested proteins (Rémond, 2007). However this property depends on the chewing capacity of elderly people, and more precisely on the level of bolus disruption before swallowing. Slower in subjects with reduced chewing efficiency (with a complete denture for example) than among subjects of the same age normally toothed, it induces a lesser increase in postprandial protein anabolism. The interest of meat consumption to counteract muscle wasting in the elderly, especially when associated with physical activity (Campbell, 1999), would require to take into account the decline in chewing efficiency with ageing and to develop new and adapted forms of meat, allowing the meat to show its full nutritional potential.
Table 1
Peptides reproducibly released in duodenum and jejunum digestive contents during digestion of cooked beef meat in pigs (TL = top loin; PP = Pectoralis profundus; S = shoulder). Bioactive sequences within each peptide are notified. (Bauchart, 2007 and unpublished data).

<table>
<thead>
<tr>
<th>Parent protein</th>
<th>Meat</th>
<th>Fragment position</th>
<th>Fragment sequences</th>
<th>m/z (M+H+)</th>
<th>Bioactive sequences</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duodenum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>TL, PP, S</td>
<td>96-106</td>
<td>LRVAPEEHPTL</td>
<td>1261.70</td>
<td>VAP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP, S</td>
<td>97-106</td>
<td></td>
<td>RVAPVEHPTL</td>
<td>1146.61</td>
<td>VAP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP</td>
<td>24-33</td>
<td></td>
<td>AGDAPRAVF</td>
<td>1018.50</td>
<td>PR, VF</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP</td>
<td>171-180</td>
<td></td>
<td>YALPHAIMRL</td>
<td>1184.66</td>
<td>YALPHA, ALPHA, RL</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>TL, PP, S</td>
<td>171-178</td>
<td></td>
<td>YALPHAIM</td>
<td>915.48</td>
<td>YALPHA, ALPHA</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP</td>
<td>181-191</td>
<td></td>
<td>DLAGRODLDY</td>
<td>1251.62</td>
<td>YL</td>
<td>Antihypertensive, Opioïd</td>
</tr>
<tr>
<td>PP</td>
<td>31-41</td>
<td></td>
<td>AVFPSIVGRPR</td>
<td>1198.71</td>
<td>IVGRPR, GRP, VF, FP, PR, RP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>Myosin</td>
<td>PP</td>
<td>326-333</td>
<td>YKIKPLL</td>
<td>1021.85</td>
<td>IKP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphate aldolase</td>
<td>TL</td>
<td>19-31</td>
<td>IAHRIVAPGKIL</td>
<td>1344.85</td>
<td>VAP, PG</td>
<td>Antihypertensive, Antithrombotic</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>PP</td>
<td>193-202</td>
<td>LFDPVSPLL</td>
<td>1128.67</td>
<td>VSP, LF</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>TL, PP, S</td>
<td>193-201</td>
<td></td>
<td>LFDPVSPLY</td>
<td>902.5</td>
<td>VSP, LF</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>GA3PDH</td>
<td>TL, PP, S</td>
<td>231-240</td>
<td>FRVPTPVNSV</td>
<td>1115.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>S</td>
<td>111-124</td>
<td>AIHVLHAKFPSDF</td>
<td>1584.87</td>
<td>LH</td>
<td>Antioxydant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147-154</td>
<td>YKVLGFGH</td>
<td>920.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Jejunum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>TL, PP, S</td>
<td>31-41</td>
<td>AVFPSIVGRPR</td>
<td>1198.71</td>
<td>IVGRPR, GRP, VF, FP, PR, RP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP, S</td>
<td>32-41</td>
<td></td>
<td>VFPSIVGRPR</td>
<td>1127.67</td>
<td>IVGRPR, GRP, VF, FP, PR, RP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>S</td>
<td>33-41</td>
<td></td>
<td>FPSIVGRPR</td>
<td>1028.60</td>
<td>IVGRPR, GRP, VF, FP, PR, RP</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>GA3PDH</td>
<td>TL, PP, S</td>
<td>231-240</td>
<td>FRVPTPVNSV</td>
<td>1115.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>PP</td>
<td>193-201</td>
<td>LFDKPVSPY</td>
<td>1015.58</td>
<td>VSP, LF</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>PP</td>
<td>194-201</td>
<td></td>
<td>FDPKVSPY</td>
<td>902.50</td>
<td>VSP</td>
<td>Antihypertensive</td>
</tr>
</tbody>
</table>

3 – IMPACT OF PROCESSING ON NUTRITIONAL PROPERTIES OF MEAT PROTEINS

The meat we eat is the result of a series of biochemical reactions triggered at slaughter, that take place in muscle cells. The rate and extent of these reactions impacts on protein functionality, and thus on the taste, texture, eating quality and nutritional quality of the meat. In structural terms, actomyosin complex forms, and once all fuel sources have been depleted, the result is that muscle tissue loses its
flexibility and its structures stiffen. In biochemical terms, changes in ionic balance together with the post mortem acidification of muscle tissue under anaerobic conditions upset the prooxidant/antioxidant balance of the cell, thus promoting the generation of reactive oxygen species that will trigger chain oxidation reactions damaging unsaturated fatty acids and proteins. Local acidification will also promote the release of lysosomal proteases and their proteolytic action in synergy with the calcium-dependent proteolytic system.

Alongside these post mortem biochemical transformations, there is the co-action of refrigerating the carcasses, which is one of the first technological processing treatments designed to ensure meat hygiene.

The meat then generally goes through one or more technological treatments before being consumed by the end-customer. The wide range of technological treatments applied to meat can be classified into three broad categories: mechanical treatment, where the product is broken down then reassembled and reshaped; chemical treatment, where solutes are used to chemically modify the tissue structure and composition; heat treatment, which has different affects depending on target temperature. The most widely-used treatment is heating, except for steak tartare and carpaccio, where the products are eaten raw after being finely chopped and mixed with onions and spices (steak tartare) or simply marinated in an acidic oil-based solution (carpaccio).

Different processes tend to predominate depending on the animal used as meat. Mechanical treatment is extensively used for processing beef, notably as a means of upgrading forequarter muscle into hamburger meat, which accounts for 40% of consumer purchases. Around 70% of pork meat undergoes at least one technological processing treatment. The flagship product is cooked ham, which is treated chemically (salt injection), mechanically (churning) and thermally. Processed poultry products currently account for almost half of poultry sales volumes.

Each of these processes clearly interacts with the components of the raw ingredients and thus plays a role in developing the taste and texture and nutritional qualities of the end-product foods. However, there are surprisingly few literature reports on the impact of these processes on the structural and biochemical characteristics of the meat proteins, and consequently on the nutritional quality of the final meat.

3.1 Molecular and structural changes in proteins

A protein’s native structure, particularly its secondary, tertiary and quaternary structure, confers the protein physiological, metabolic, enzymatic, structural roles, among others. These roles become altered through the environmental changes induced by the processing treatments. Polypeptide chain folding involves four main types of interactions: Hydrophobic bonding, ionic bonding, hydrogen bonding and disulfide bridging. Furthermore, proteins also interact with the lipids in muscle tissue water, underpinning three important functional properties: water-binding capacity, emulsifying and foaming capacity, and viscoelastic properties.

3.1.1 Denaturation

A protein is described as denatured when it can no longer perform its biological function due to changes in its quaternary and tertiary structure. The first effect of an increase in temperature is to break the weak hydrogen bonds binding the functional groups in the chain. Similarly, most proteins become denatured in strong acids or strong alkalis or in media with high electrolyte concentrations. The hydrophobic
regions then open up to the outside of the molecule while the hydrophilic regions aggregate at the centre. This leads to a loss of protein solubility, protein precipitation, and – for some – protein gelation. Staburski (1984), employing differential scanning calorimetry on pork, showed that the myosin presented structural transition states indicative of conformational changes at temperatures of 58°C and 66°C, whereas actin peaked at 78°C. Hydrophobic probes such as ANS (8-anilino-1-naphtalene sulfonic acid), Bromophenol Blue or Nile Red can also be used (Chelh, 2006) to assess the protein hydrophobicity induced by processing treatments. The hydrophobicity of proteins increases sharply when they are first subjected to heat, before stabilizing for the remaining duration of the heat treatment (Santé-Lhoutellier, 2008a).

3.1.2 Oxidation

In order to combat oxidative stress in vivo, muscle cells can mobilize liposoluble and hydrosoluble antioxidant systems to inhibit or eliminate free-radical reactions. However, post mortem, the balance between the prooxidant and antioxidant systems leans towards oxidation during the phases of meat aging, and the phenomenon strengthens lastingly during processing treatments. Heating, grinding or adding solutes all promote the formation of oxygen free radicals (singlet oxygen \(1{O_2}\), superoxide radical \(O_2^\cdot\) and hydroxyl radical \(OH^\cdot\)). These radicals have one or more unpaired electrons that lend them strong chemical reactivity with the cell components with which they will exchange an electron, and they can also trigger a cascade of free radical reactions. Astruc (2007) used immunochemical DNPH labeling on tissue slides to demonstrate that protein oxidation started in cell membranes, and that the process gained in strength at longer heating durations and higher temperatures (figure 1). Free radical-induced protein oxidation involves three classes of amino acids: basic amino acids, sulfur-containing amino acids, and aromatic amino acids. Basic amino acids (particularly lysine and arginine) undergo oxidative deamination in the presence of hydroxyl radical, thereby generating a carbonyl group. The oxidation of cysteine leads to the formation of disulfide bridges. The oxidation of methionine yields methionine sulfone and methionine sulfoxide. Finally, the hydroxyl radical-induced oxidation of aromatic amino acids converts phenylalanine into tyrosine, and then dityrosine, and converts tryptophan into hydroxytryptophan. The formation of dityrosine molecules has been demonstrated as a discriminatory marker of myofibrillar oxidation (Morzel, 2006).

![Figure 1](image.jpg)

**Figure 1**

Microscopic images of protein carbonyl distribution achieved with the CY3 fluorescent probe (\(\lambda_{ex} = 550\) nm \(\lambda_{em} = 565\)nm) \(b^*\) is the yellowness measured inside the cell and \(bp^*\) is the yellowness measured at the periphery of the cell.

(1 to 3) microscopic images of protein carbonyl distribution from bovine Rectus abdominis muscle after a 100°C cooking for 0 min (1), 15 min (2) and 30 min (3). (4) Microscopic images of protein carbonyl distribution from bovine Rectus abdominis muscle after a 270°C cooking for 1 min.
3.1.3 Protein aggregation

Protein aggregation results from new interactions between polypeptide chains stemming from oxidation of conformational changes. The formation of carbonyl groups, disulfide bridges or dityrosine, the changes in overall system charge and the increasing hydrophobicity at the protein surface all trigger polymerization and protein aggregation (Davies, 1987; Stadtman, 1990, 1993; Grune, 2004). Furthermore, advanced glycation end-products (products of the Maillard reaction), which form at an increasingly large rate by heating strongly in the presence of sugars (Koschinsky, 1997), also participate in the formation of these protein aggregates. In living tissue, chaperone proteins (HSPs, the “heat shock protein” family) play a pivotal role in protein folding and conformation to prevent protein aggregation (Lindquist, 1988; Glover, 1998). It has been demonstrated under supraphysiological temperatures that α-cristallin (HSP of 22 kDa) acts to maintain the enzymatic activity of myosin and prevent myosin aggregation (Melkani, 2006). However, HSP stability and activity have not yet been studied under post mortem conditions or during meat processing treatments. In muscle tissue, myofibrillar proteins and especially myosin tend to establish intermolecular interactions in response to the action of antioxidants (Liu, 2000; Kamin-Belski, 1996; Santé-Lhoutellier, 2007) or following heat treatment (Liu, 2000; Lefèvre, 2008). The characterization and analysis of the mechanisms underlying the formation of aggregates reveal that the oxidation of myosin leads to the formation of high-molecular-weight structures that conventional polyacrylamide gel-based protein separation techniques are unable to identify. Nevertheless, the size and form of these structures can be studied via techniques based on turbidity and light scattering measurements or microscopy (figure 2).

![Figure 2](image_url)

**Figure 2**
Pig myofibrils observed by scanning electronic microscopy before and after heating.

3.2 2. Nutritional impacts

Meat does a priori present beneficial nutritional properties due to its essential amino acid composition, the biologically-relevant peptides it contains, and its high digestibility. Nevertheless, there is a strong rationale for reassessing the changes induced by processing treatment that affect proteins in terms of their impact on the nutritional quality of meat proteins, as new knowledge comes to light. These post mortem changes (aging, processing treatments, etc.) in meat structure can decrease the bioavailability of essential amino acids, not only by changing the type of digestive end-products (more or less resistant peptides) produced but also by affecting digestion rate.
A wealth of research, for the most part conducted on milk proteins, has demonstrated that processing treatments, and notably heating, can have deleterious effects on amino acid absorption resulting from the formation of products of the Maillard reaction, lysinoalanine and disulfide bridges, or even the isomerization of L-form amino to D-form amino acids (Finot, 2005). In practice, these modifications appear to remain limited in meat products, largely due to the temperature scales involved.

Older research based on measurements of digestive rate through the rat digestive tract shows that overall meat protein digestibility is very high and relatively unaffected by heat treatment (Laser-Reutersward, 1982). However, the measurements carried out cannot be used to differentiate digestion in the small bowel, which supplies amino acids, from microbial degradation in the large intestine, which can lead to the production of toxin by-products such as nitroso compounds (Bingham, 2002) and sulfides (Magee, 2000). The small bowel digestion of meat proteins has rarely been measured, and there is no data available for assessing the impact of processing treatments on this parameter. However, recent in vitro research suggests that the impact could be substantial, as it has recently been shown that the degree of protein hydrophobicity influences protein-protein recognition by proteases (Davies, 2001; Santé-Lhoutellier, 2008b). Thus, proteolytic enzymes operate at a slower rate in the digestive tract or even lose their ability to digest the protein if the protein has not been recognized by the active site of the enzyme (figure 3). Similarly, pepsin, trypsin and chymotrypsin display reduced activity when in the presence of oxidized myosin and in the form of molecular aggregates, and the digestates contain heterogeneous protein fragments of higher molecular weight than the non-oxidized controls (Kamin-Belski, 1996; Liu, 2000). Hence, structural and biochemical changes in the proteins could not only impact on digestibility in the small bowel (bioavailability of amino acids vs. colonic fermentation) but also impact on the type of end-products (effect on the release of bioactive peptides) and their rate of digestion.

Figure 3

Effect of cooking duration (100°C) on myofibrillar proteins proteolysis rate by pepsine. The black bar corresponds to a 270°C cooking for 1 min.
4 – CONCLUSIONS

New insights into the field of bioactive peptides make it possible to re-examine current conceptions on the nutritional quality of dietary proteins, which to date have been based on essential amino acid composition and digestibility, by integrating other properties liable to have an impact on health, and therefore to revise the position of these foods within the framework of a balanced diet. Thus, through its carnosine content and the potential of meat proteins to release bioactive peptides, especially antihypertensive peptides, meat could offer new nutritional benefits. Furthermore, the high protein content and fast rate of digestion of meat make it a key point in nutritional strategies designed to counteract aging-related sarcopenia. Digestion in the small bowel (rate and end-products), which to date has been relatively unexplored, remains a key point to deeper insight into whether nutritional quality is positively or negatively impacted by the biochemical and structural changes in proteins that are caused by food processing procedures.

REFERENCES


