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Proteolysis in fermented and not fermented dry cured salumi

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Proteolisi in salumi stagionati fermentati e non fermentati

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Summary
Muscle proteins undergo an intense proteolysis from conversion of muscle in meat to cured processed pork. Throughout this period is produced a great number of small peptides and high amounts of free aminoacids that characterize aroma of dry cured products. The enzymes responsible of these changes can be only endogenous muscle proteinases (cathepsins B, D, H and L and calpains) or both endogenous muscle proteinases and exogenous microbial proteolytic enzymes. Proteolysis generated by endogenous and exogenous enzymes typify fermented salami and not fermented processed pork resulting from entire anatomic cuts. Proteomic approach was applied to study the effect of enzymatic action on different processed pork products. Two dimensional gel electrophoresis (2DGE) maps of dry cured salumi, ripened for 1 month, and ham, ripened 12 months, describe protein evolution at final occurrence of proteolysis.

Riassunto
Le proteine muscolari subiscono una intensa proteolisi durante la conversione del muscolo in carne di maiale elaborata. Durante questo periodo si produce un grande numero di piccoli peptidi e elevate quantità di aminoacidi liberi che caratterizzano l'aroma dei salumi secchi. Gli enzimi responsabili di questi cambiamenti possono essere sia solo le proteasi endogene muscolari (catepsine B, D, H e L e calpains) o le stesse accompagnate da enzimi proteolitici microbici e esogeni. Le proteolisi generate dagli enzimi endogeni ed esogeni caratterizzano sia i salami fermentati che quelli non fermentati ma derivanti da pezzi anatomici interi di carne di maiale. Per lo studio dell'effetto dell'azione enzimatica su diversi salumi trasformati è stato applicato un approccio proteomico. Gli elettroferogrammi in seconda dimensione (2DGE) di salume secco crudo, stagionato per 1 mese, e prosciutto crudo, stagionato 12 mesi, mostrano l'evoluzione delle proteine sino alla completa proteolisi.

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Introduction

Meat tenderness is the major quality trait of dry cured productand the ability to optimize it depends on a detailed understanding of the mechanisms behind this process. Specifically, the conversion of muscles in meat through the tenderization is the result of the proteolysis of Z-lines by Calpains, cytoplasmic enzymes calcium dependent named m- and m-calpains according to m- and millimolar calcium concentration, causes tenderization weakening myofibrils (1-12).

The fall in pH during post-mortem glycolysis weakens the walls of lysosomes (13), which consequently causes the release of lysosomal proteinases, named cathepsins B, H and L, that have pH optimal at around 5.5-6.5 (13-15). Both calpains and lysosomal proteinases degrade troponin T, troponin I, tropomyosin, C-protein, desmin, titin and nebulin, while myosin heavy chain, myosin light chains, a-actinin, troponin C and actin appear to be sensitive to the action of lysosomal proteinases, especially to cathepsins D, B and L (12, 16-18).

In general, muscle proteases are quite stable except calpains, that are restricted to the initial days and cathepsin D, which fully inactivates by 6 months of process (19). The rest of cathepsins and peptidases are very stable during the full process (20). The final products of the proteolytic chain result from the action of exopeptidase and consist in small peptides and free amino acids. However, some authors found that also micro-organisms may contribute to the proteolysis which take place on hams. Among these biochemical events muscle endogenous enzymes play a crucial role in the ripening and maturation of pork products deriving from entire anatomic cuts such as ham.

In the case of fermented dry-cured salami are obtained after a short ripening time (max 60-90 days) in which both endogenous and microbial enzymes play a decisive role in these reactions (21, 22). Thus sarcoplasmic and myofibrillar proteins undergo deep proteolytic changes that influence the texture and flavour of the products (23).

This paper aims highlighted proteolysis event in ripening process of different dry cured salami by proteomic approach of proteins of pork meat.

For this purpose we considered fermented and not fermented meat products: 12 months ripened dry cured ham, as not fermented products, and salami as dry fermented sausages, with 1 month of ripening.

Material and methods

Dry cured ham three months seasoned and salame have been bought in an ordinary market.

1 mg of lyophilized sample was dissolved in 400 l of rehydration solution (8M Urea, 0,5-4% Chaps, 0,4% DTT, 2% Ampholine 3-10 and bromophenol blue). IPG strips (Immobiline Dry-Strip, pH 3-10 L, 18 cm; Pharmacia) were left in contact overnight,carefully covered with Cover fluid. After rehydration, strips were focused in "2117 Multiphor II Electrophoresis Unit" (Pharmacia Biotech) for 17 hours with a two steps program: 1 hour at 1 mA, 1000V and 5 W, 16 hours at 1 mA, 3500 V and 5W.

Then IPG strips were equilibrated incubating at room temperature for 12 minutes in 10 ml of Tris-HCl (50 mM) pH 6,8; Urea (6 M); Glycerol (30%); SDS (2%) and DTT (2%) and thenfor 5 minutes in 10 ml of Tris-HCl (50 mM) pH 6,8; Urea (6 M); Glycerol (30%); SDS (2%); Iodoacetamide (2.5%). Glycerol and Urea are useful to reduce streaking effect due to protein migration. IPG strip were loading at the top of vertical second dimension gel (SDS-PAGE gradient 10-20%) with 0,5% (w/v) agarose in 25 mMTris, 0,192 mMglicine and 0,1% (w/v) SDS (running buffer) and running conditions were 1 hour at 500V, 10000W/gel and 5-7 hours at 500V, 20000W/gel, until the bromophenol blue reached the bottom of the gel. Second dimension were performed in electrophoretic cell"Investigator 2-D Electroforesis System"(Casting System, ESA).

Gels were stained with 0,25% Coomassie Brilliant Blue R-250.

Results and discussion

It is worthy of note that fermented products, salami, after 30 days showed the least protein concentration. This result suggested that a faster diminution of protein concentration in fermented product could be due to microbial proteolysis and a faster emulsion between proteins and fats; in order to verify this hypothesis an alkaline gel electrophoresis in presence of SDS was performed.

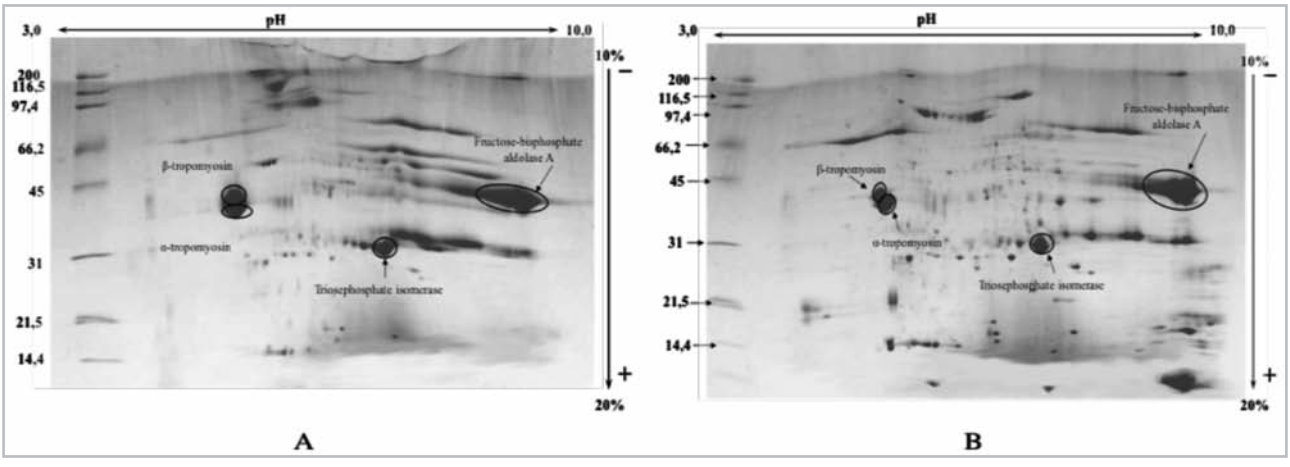
Figure 1 showed two-dimensional maps of 3 months ripened Salami Naples and 12 months dry-cured ham respectively. In first dimension, proteins were separated for their isoelectrical point in an immobilized pH gradient (IPG) while in second dimension on the basis of their molecular weight (SDS-PAGE). Most of proteins is focused in the basic pH part of first dimension and is included in an area of about 3.5 pH units (from 6 to 9) and of about 20 kDa (from 43.000 to 17.000 Da) of 2D map; in this figure Creatine Kinase, Enolase B, Fructose-bisphophatealdolase A, Triosephosphateisomerise, Glyceraldheyde 3-phosphate dehydrogenase and Myoglobin can be recognised.

Dry-cured ham and Salami Naples maps did not reveal great differences between them, showing Fructose-bisphophatealdolase A and Triosephosphate isomerise yet, that were not hydrolyzed during ripened time, according to Picariello et al. (22), and lacking of Enolase B, Creatine Kinase, Glyderaldheyde 3-phosphate dehydrogenase and Myoglobin, hydrolysed proteins.

The presence of salt-soluble proteins in water extract ham was already reported by Di Luccia et al. (24), which studied the evolution of myofibrillar and water soluble proteins through ripening process of dry cured ham by a proteomic approach. These authors suggested that salting process would cause the solubilization of myofibrillar proteins in dry-cured ham by virtue of their solubility in saline solution. A similar event was also observed by Thorarinsdottira et al. (25).

Fermented products were characterized by the major bacterial concentration and, at the end of seasoning, predominant microorganisms were Lactobacilli and Micrococcaceae. This result was consistent with data widely discussed in literature. It is undoubtedly that these strains define and influence final organoleptic attribute of products.

Figure 1 - Bidimensional map IPG/SDS-PAGE of Salame Napoli (A) and 12 months dry-cured ham (B).



Conclusion

So we can prove to schematize proteolysis phenomenon as follow: in fermented products, myofibrillar proteins were hydrolysed by endogenous enzyme to produce peptides and free aminoacids, in turn hydrolysed by bacterial enzymes to produce amines; sarcoplasmic proteins, instead, seem to be hydrolysed especially by bacterial enzymes. In not fermented meat products, all proteins fraction were probably hydrolysed by endogenous enzymes, to release free aminoacids.

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