

Influence of cooking process on protein fractions in cooked ham and mortadella

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RIASSUNTO – Influenza del processo di cottura sulle frazioni proteiche di prosciutto cotto e mortadella – *E' stato valutato l'effetto del trattamento termico su prodotti cotti derivati da carne suina mediante uno studio della frazione proteica. La solubilità proteica è stata valutata mediante determinazione della concentrazione proteica negli estratti della frazione sarcoplasmatica e miofibrillare di muscolo suino, prosciutto cotto e mortadella. Inoltre, l'estratto proteico in condizioni denaturanti e riducenti di ogni singolo campione, analizzato mediante elettroforesi bidimensionale, ha consentito di rivelare gli effetti del trattamento termico sul prodotto finito. I primi risultati della ricerca evidenziano l'elevato stato di denaturazione delle proteine dovuto alla fase di cottura; tale stato è particolarmente evidente nei campioni di prosciutto cotto.*

KEY WORDS: two dimensional gel electrophoresis, cooked ham, mortadella, protein denaturation.

INTRODUCTION – The mortadella is a pork meat sausage (in natural or artificial bowel) accurately trituated and mixed with little backfat cubes, salt, sodium nitrate and nitrite, spices and peppercorns, and then cooked in oven for many hours. The cooked ham is obtained from an anatomically completed piece of meat; the working process provides the addition of salt and spices, the brine, the bones removal, the churning and the pressing, so the cured meat is first packed in a mould provided for this purpose, then cooked and after cooled and packed.

The meat cooking is the last step in the cooked sausage production technology, and let us obtain a stable and eatable product. The effect of the heat and the length of processing are the main responsables for modifications in water- and salt-soluble protein fractions. Indeed myofibrils denature themselves after cooking and consequently their solubility decreases; particularly the denaturation begins over 30°C in the myosin chain, instead the actin solubility begins to decrease over 60°C, being the actin more stable than myosin (Barbieri *et al.*, 1997).

2-D electrophoresis sorts proteins according to two independent properties in two discrete steps: the first, isoelectric focusing (IEF), separates proteins according to their isoelectric points (pI); the second, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), separates proteins according to their molecular weight (Klose *et al.*, 1995). Each spot on the resulting 2-D array corresponds to a single protein species.

This work wants to characterize salt-soluble protein fraction, by means of 2D-electrophoresis, that are directly implicated in the creation of some important organoleptic properties, like flavour and juiciness, and that serve as molecular markers (Toldrà *et al.*, 1997; Geay *et al.*, 2001).

MATERIAL AND METHODS – Three samples of fresh pork meat, of expensive (1) and cheaper (2) mortadella, and of cooked hams were freed from the adipose tissue by a lance; after 100 g of each sample were homogenized with distilled water for 5 min at 0°C. The homogenate is centrifuged three times at 0°C (5000 rpm for 30 min, 5000 rpm for 20 min and 13000 rpm for 15 min) and the supernatant was filtered (0.45 µm) and then used for sarcoplasmic protein concentration determination by Bio-Rad test (Bredford *et al.*, 1976). The pellet obtained was dissolved in 50 ml of distilled water and then centrifuged

(500 rpm for 20 min) until the best part of water-soluble fraction was removed. After the pellet is lyophilized. The dried sample (30 mg) is re-suspended in 1 ml of extraction buffer (0.3M NaCl, 0.1M NaH₂PO₄, 50mM Na₂HPO₄, 1mM EDTA) to extract myofibrillar proteins. These proteins are recovered in the supernatant after shaking for 2 h and centrifugation at 12000 rpm for 5 min, filtered (0.45 µm), and used for Bio-Rad assay. Two-dimensional electrophoresis was performed by isoelectric focusing in immobilized pH gradient (IPG) 4-7 in the first dimension and by an alkaline electrophoresis in polyacrylamide gradient gel (9-18%) in presence of sodium dodecil sulphate in the second dimension, according to Bjellquist *et al.* (1993). The gel is stained using Coomassie Blue R-250.

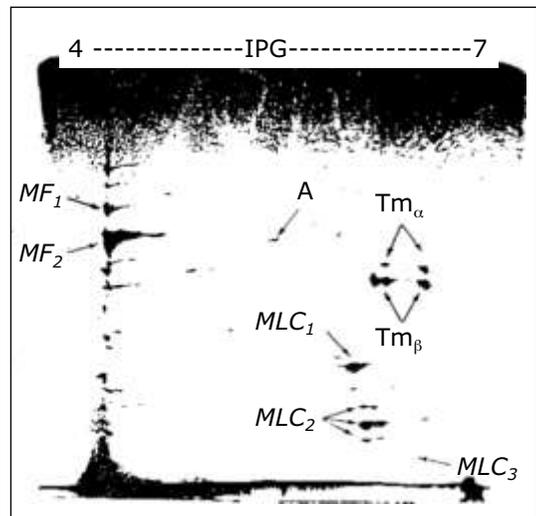
RESULTS AND CONCLUSIONS – In Table 1 is reported the protein assay of sarcoplasmic and myofibrillar proteins.

Table 1. Protein concentration of sarcoplasmic and myofibrillar fractions.

	Pork meat	Mortad.1	Mortad.2	Cooked ham
Sarcoplasmic prot. (mg/g)	7.05 ± 0.25	0.54 ± 0.08	0.29 ± 0.06	0.32 ± 0.09
Myofibrillar prot. (mg/g)	0.91 ± 0.01	0.7 ± 0.01	0.06 ± 0.01	0.09 ± 0.01

A lower concentration of saline-soluble proteins was to be expected, being these proteins components of muscle myofibrils. Furthermore, it is evident that protein fractions of cooked products are less extractible in native conditions. The 2-D map of pork meat myofibrillar proteins (Fig. 1), extracted in denaturing and reducing conditions (Di Luccia *et al.*, 1992), shows eight main spots that are identified with the α and β tropomyosin, the actin, light chains of 1, 2 and 3 myosin (MLC1,2,3) and myosin fragments (MF1,2), in accordance with both molecular weights and isoelectric points (Di Luccia *et al.*, 1992; Pernelle *et al.*, 1988; Lametsch *et al.*, 2002). The 2-D map of cooked ham (Fig. 2) points out the spots related to myosin fragments 1 and 2, tropomyosins, actin and MLC1, 2 and 3. We can also see many spots in the acid and alkaline zone of IEF that are not present in the 2-D map of pork meat and that can be related to sarcoplasmic proteins, denaturated during cooking and so not more water-soluble but extractible in reducing conditions.

Figure 1. 2-D map of myofibrillar proteins of pork meat.



Into the 2-D electrophoresis of mortadella (Fig. 3) we can notice the spots of MF2 and MLC1 and 2. It's also important to notice the very small number of spots after cooking, but, on the other hand, we also must remember that the amount of pork muscle in the mixture is less than the amount of backfat cubes and other components. Surprisingly we observe the absence of Tm, more abundant of MLCs, that let us suppose the lost of these proteins during technological process.

Figure 2. 2-D map of myofibrillar proteins of cooked ham.

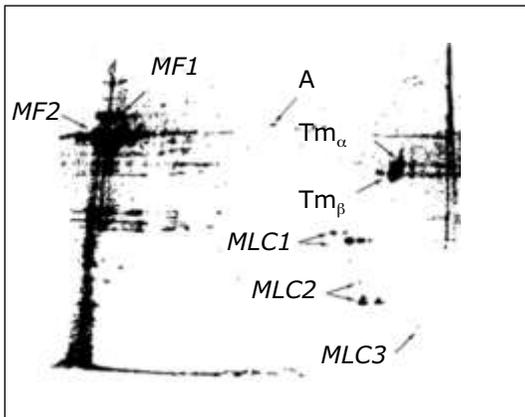
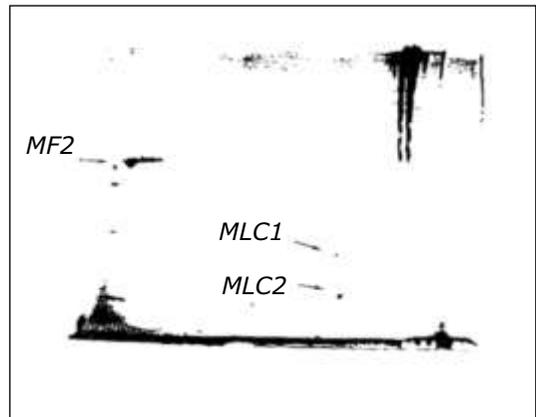


Figure 3. 2-D map of myofibrillar proteins of mortadella.



Concluding, work results point out an evident denaturation of proteins produced by the cooking process of meat; this denaturation is essentially reflected in the protein solubility so the protein assay results show that proteins extracted in native conditions are not present in cooked products. On the contrary the 2-D maps of these products, particularly of cooked ham, show more spots than pork meat map, revealing the presence, in cooked ham, of denaturated water- and salt-soluble proteins, extractible in reducing condition.

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