Food Chemistry 172 (2015) 447–455

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Protein modifications in cooked pork products investigated by a proteomic approach

Aldo Di Luccia [a,*], Barbara la Gatta [a], Annarita Nicastro [a], Giovanni Petrella [a], Carmela Lamacchia [a], Gianluca Picariello [b]

[a] *Department of Sciences of Agricultural, Food and Environmental, University of Foggia, Via Napoli, 25, 71122 Foggia, Italy*
[b] *Institute of Food Sciences – National Research Council, Via Roma, 64, 83100 Avellino, Italy*

ARTICLE INFO

*Article history:*
Received 8 May 2014
Received in revised form 22 August 2014
Accepted 17 September 2014
Available online 28 September 2014

*Keywords:*
Cooked hams
Emulsion sausages
Heat treatments
Proteomics
Muscle protein oxidation

ABSTRACT

To evaluate process-induced protein modifications in cooked ham and emulsion sausages, the proteomes of whole-cut (Parma and "Praga" cooked hams) and comminuted pork (mortadella and würstel) products were compared to raw pork using two-dimensional gel electrophoresis (2-DE) coupled to image analysis and mass spectrometry (MS). Other than heat-induced breakdown of part of the myosin heavy chains, the 2-DE pattern of cooked ham was substantially similar to that of raw pork. However, the MS-based analysis showed minor modifications, including the extensive oxidation of methionines. In contrast, likely due to emulsification, comminuted sausages were characterized by an abundant insoluble protein fraction (IPF). Interestingly, tropomyosin and myosin light chains in comminuted sausages were exclusively found in the IPF. Our results indicate that the protein aggregation systems of cooked hams and emulsion sausages reflect the processing conditions and are definitely different, the former being characterized mainly by disulphide bridges and the latter by additional covalent inter-protein links.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

Cooking meat and meat products is commonly practiced to obtain safer products and to develop sensory attributes (Mottram, 1998). In cooking procedures, the heat treatment induces modifications to the structural setting of the main meat constituents (Barbera & Tassone, 2006; Tornberg, 2005), especially proteins, as a consequence of degradation, denaturation, oxidation and polymerization events (Barbieri & Rivaldi, 2008; Gatellier, Santé-Lhoutellier, Portanguen, & Kondjoyan, 2009). Proteins (~20% of the muscle) represent the primary constituents of meat. Skeletal muscle proteins include the salt-soluble structural components, (myofibrillar proteins; ~45–55%), the water-soluble metabolic enzymes which constitute the sarcoplasmic fraction (~30–35%), and the connective tissue (or stroma) proteins (~10–15%), which envelope fibres (holding them together and are soluble in dilute acidic solutions). Upon cooking (37–80 °C), the muscle protein fractions undergo conformational, chemical, and physical modifications to a variable extent.

Structural proteins of thick (myosin heavy chain) and thin filaments (actin, tropomyosins, and troponins) and Z-disk proteins (desmin and α-actinin) respond differently to cooking temperatures in the 40–80 °C range; α-actinin is the most heat sensitive and becomes insoluble at 50 °C, myosin at 55 °C, actin between 70 and 80 °C, and tropomyosins (TPMs) and troponin at higher temperatures, greater than 80 °C (Cheng & Parrish, 1979). Denaturation of other sarcomeric proteins, such as titin, occurs at 73 °C (Fritz, Dietrich, & Greaser, 1992), while nebulin withstands cooking at 80 °C (Locker, 1984). Most sarcoplasmic proteins undergo aggregation between 40 and 60 °C, even though, for some of them, coagulation can occur up to 90 °C (Laakkonen, 1973; Tornberg, 2005). Collagen, the predominant connective protein, denatures at about 65 °C (Laakkonen, 1973) and gelatinizes at 80 °C. These events trigger transversal and longitudinal shrinkage as well as the flaking of muscle fibres and connective tissue, as a consequence of the heat treatment (Palka & Daun, 1999). Thus, each fraction affects the organoleptic traits of cooked meat products in a way that is strictly related to its structure, and that in turn is determined by the cooking process.

In the processing of cooked pork products, which includes the production of a large variety of traditional meat preparations worldwide, whole anatomical cuts, generally deriving from pork legs, or assembled muscle pieces, are salted, spiced, rubbed with

* Corresponding author at: Department SAFE – Università degli Studi di Foggia, Via Napoli, 25, 71122 Foggia, Italy. Tel.: +39 0881 589 243.
*E-mail address:* aldo.diluccia@unifg.it (A. Di Luccia).

http://dx.doi.org/10.1016/j.foodchem.2014.09.099
0308-8146/© 2014 Elsevier Ltd. All rights reserved.

specific aromas, and injected with brine. However, to produce emulsioned sausages, meat pieces from different cuts are finely comminuted, salted, flavoured, and cased.

Muscle fibres of cooked ham undergo biochemical and shape modifications similar to those in cooked meat (transversal and longitudinal shrinkage, aggregation and gel formation of the sarcoplasmic proteins; Tornberg, 2005). For this reason, a ham press mould is used to prevent shape loss and to adhere muscle fibres during the cooking phase; this step is simply called "forming" or "stuffing".

The salting step and subsequent heating of the comminuted emulsion sausages, (such as mortadella and "frankfurter", also called würstel), promote the effective solubilization of myofibrillar proteins (Di Luccia et al., 2005; Trani et al., 2010), which form a dense gel protein network that retains water by capillary force (Tornberg, 2005). The type of gel matrix is related to the balance between disperse or aggregated muscle proteins prior to this.

The cross-linking of proteins in the gel network is triggered by free radicals (Promeyrat et al., 2010) and by the conformational transitions (Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008) that affect several properties of the proteins, including their solubility, hydrophobicity, and emulsion stabilizing power (Sun, Zhou, Zhao, Yang, & Cui, 2011). The physico-chemical properties of proteins can also be altered due to covalent coupling of reactive amino acid side chains (e.g. lysine, cysteine) to the secondary products of lipid peroxidation, especially carbonyl derivatives such as malondialdehyde and 4-hydroxynonenal (Gardner, 1979).

Myosin denaturation involves a two-step gel formation that occurs in different temperature ranges, between 30 and 50 °C and above 50 °C, respectively, resulting in further aggregation.

In all of these events, the use of varying recipes, salt concentration, and pH determine the properties of the protein networks, which are what primarily govern the overall sensory traits of the cooked meat products (Tornberg, 2005).

Cooked hams are produced by heating in an oven to a core temperature of 70 °C, following brine injection and tumbling. Generally, a cooking time of 1 h per kg of meat product is required, which means an overall time of 10–12 h. The origin of cooked ham dates back to the mid-fifteenth century, as quoted noted in *Libro de coquinaria art* by Martino of Como (about 1465), where it was obtained from the hind limb of a pig, from which the fat was removed, cut, boned, massaged, processed and, finally, steamed. "Praga" ham is a related specialty from the Trieste area of central Europe, which formerly belonged to the Austro-Hungarian Empire. For this style of ham, the cooking stage is carried out in special hot-air ovens rather than by exposure to wet steam. Following this, the smoking stage is entirely natural and is based exclusively on beech wood.

The comminuted meat products analysed in this study are mortadella di Bologna, a typical Italian cooked sausage, and würstel, a sausage manufactured worldwide.

The origin of the word "mortadella" is somewhat controversial, but one of the most reliable hypotheses is that it derives from the late Latin "mortarium", which described the pestle and mortar, in which the friars in Bologna (Italy) prepared the mixture of pounded meat mixed with fat and spices. Nowadays, comminution is accomplished by grinders that reduce the granulometry of the meat to less than 0.9 mm, and the cooking is performed in stages (drying, pre-cooking, firing, and second firing) with temperatures as high as 80 °C for 19–20 h overall (Barbieri, Bergamaschi, Barbieri, & Franceschini, 2013).

The würstel is linked to the butcher Johann Georg Lahner, who in 1807, invented the frankfurter sausage (today's frankfurters) that gradually spread to the entire Austro-Hungarian Empire (Lahner, 1969). Würstels are cooked at variable times and temperatures, typically for about two hours or until the entire product reaches a temperature of 70 °C. Following this, the sausages are traditionally smoked with beech wood, to give them a characteristic flavour.

In this paper, we compared the proteomes of cooked salami obtained from whole cuts or comminuted meat as determined by different processing technologies, and in particular, investigated heat-induced protein modifications with respect to raw pork.

## 2. Materials and methods

### 2.1. Sample preparation

Traditional meat products, manufactured according to well-defined process guidelines, were selected in this study. Three samples each of Parma cooked ham, "Praga" cooked ham, mortadella di Bologna, würstel, and raw pork were purchased at a local supermarket. The samples (50 g) were mixed with a 0.03 M phosphate buffer (pH 7.2) to obtain a homogeneous slurry; they were then centrifuged at 8000 rpm for 20 min at 4 °C. The supernatant, containing the sarcoplasmic fraction, was recovered for electrophoresis analysis. Each resulting pellet, about 1 g, was suspended in 10 ml of extraction buffer (8 M urea, 2 M thiourea, 2% NP-40, 10% dithiothreitol, DTT), stirred for 4 h, and centrifuged at 10,000 rpm for 10 min. The supernatant, which contained the myofibrillar fraction, was recovered and used for successive electrophoresis analyses. Total meat proteins (myofibrillar and sarcoplasmic proteins) were extracted by homogenizing the cooked and raw products with a denaturing and reducing buffer (DRB; 8 M urea, 2 M thiourea, 2% NP-40, 10% DTT) or with a nonreducing buffer (DB; 8 M Urea, 2 M thiourea, 2% NP-40). The homogenates were magnetically stirred for 12 h prior to clarification (by centrifugation, 12,000*g* for 20 min). The resulting supernatant (S1) was recovered, and the resulting pellet was dried in an oven until it reached a stable weight. It was then weighed and resuspended in 2.5 ml of both DRB and DB, sonicated 3 times for 10 min each, centrifuged at 13,000 rpm at 4 °C; and the resulting supernatant (S2) was analysed by 2-DE. The pellet was resuspended three times with distilled water, centrifuged after each washing, and dried in an oven until it reached a stable weight, which was then recorded.

### 2.2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The protein sample (1:1 vol:vol) was diluted with 10 mM Tris–HCl, 1 mM EDTA, 2.5% SDS, 1% DTT, and 0.01% bromophenol blue (BPB), pH 8.0, and loaded onto a 8–18% polyacrylamide gel gradient (10 μl after 3 min heating in a boiling water bath).

Separation was performed using a Multiphor II (GE-Healthcare Bio-Sciences, Little Chalfont, U.K.), at 200 V, and 20 mA for the stacking gel, and 600 V, 50 mA for the resolving gel. The gels were stained with G-250 Coomassie (blue silver).

### 2.3. Two-dimensional gel electrophoresis (2-DE)

The two-dimensional gel electrophoresis of protein extracts were carried out in triplicate. Briefly, a sample volume equivalent to 100 μg of total protein extract, quantified with the Bradford assay, was loaded onto immobilized pH gradient (IPG) strips, pH 3–10, 13 cm long (GE-Healthcare Bio-Sciences, Little Chalfont, U.K). Following rehydration (7 M urea, 2 M thiourea, 2% w/v CHAPS, 2% carrier ampholyte, 0.001% BPB), isoelectrofocusing (IEF) was carried out at 20 °C using a unit Ettan IPGphor 3 (GE-Healthcare Bio-Sciences, Little Chalfont, U.K.); the voltage was increased stepwise to 8000 V, reaching a total of 40,000 Vhrs.

Download English Version:

https://daneshyari.com/en/article/7594093

Download Persian Version:

https://daneshyari.com/article/7594093

Daneshyari.com