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Review

Proteolysis follow-up in dry-cured meat products through proteomic approaches

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ABSTRACT

Dry-cured meat products experience an intense proteolysis phenomenon during their processing. Products like dry-cured ham, that experience processing times longer than 10 months, show an extensive breakdown of major proteins and the generation of a high number of small peptides and finally, large amounts of free amino acids. Proteomic techniques have been successfully applied to the identification of the generated peptides and their sequencing. These data are essential for a better understanding of proteolytic enzymes and their reaction during processing. This approach should reveal key biomarker peptides controlling the process and establish strategies to drive and optimise enzyme reactions for the production of optimal quality products.

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1. Introduction

Traditional dry-cured ham is a high-quality product with a typical and characteristic texture and flavour. These parameters require a long process of production involving salting, post-salting, and ripening steps, which could last up to 24 months or even more. Many biochemical reactions take place during this process and are responsible for its final characteristic texture and flavour properties (Toldrá, 2002).

Proteolysis, that consists in the degradation of the muscle proteins as a result of the action of endogenous muscle peptidases, is by far the most studied biochemical phenomena during the dry-curing process (Toldrá, 1998; Toldrá & Flores, 1998). Techniques such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), two dimensional gel electrophoresis (2-DGE), or high performance liquid chromatography (HPLC) (Di Luccia et al., 2005; Rodríguez-Núñez, Aristoy, & Toldrá, 1995; Sforza et al., 2001), have been the most used techniques to study the protein changes during this period as well as to separate the protein fragments generated for their subsequent identification. Most of these studies have been focused on the description of the gross changes occurring in the protein profile, but the sequences corresponding to the proteolysis products have been published only in recent years, due to the difficulties in the identification of small size and naturally generated peptides. The availability of advanced proteomic techniques such as tandem mass spectrometry has prompted the identification and sequencing of such generated peptides.

This review gives a description of the proteomic strategies carried out up to date to study the changes occurring in proteins during dry-curing as well as to identify the naturally generated peptides.

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2. Dry-cured ham process

Dry-cured ham processing has for many centuries used salt as a means of preservation and subsequently drying. However, current goals have changed and the main purpose is to obtain a high added value product, safe, flawless, and much appreciated for its sensory characteristics. The product obtained at the end of the process is conditioned by the raw material and the technological process (Toldrá, 2002). Thus, there are different possible conditions of dry-cured ham processing but in essence they are all focused on the stabilization of the product to keep it safe for human consumption and facilitate the development of their sensory characteristics.

Fig. 1 shows the typical workflow of dry-cured ham processing including the optimal conditions applied at each stage of the process. Salt penetrates during the salting and then diffuses through the ham during post-salting reaching the inner part by the end of such stage. The conditions of temperature and humidity applied during ripening and drying facilitate the development of biochemical reactions responsible for its final characteristic properties of texture, colour and flavour (Parolari, 1996; Toldrá, 2002).

The main biochemical reactions that occur during the processing of dry-cured ham are enzymatic, including the hydrolysis of the muscle proteins (proteolysis), the hydrolysis of triacylglycerols and phospholipids (lipolysis) components, the hydrolysis of glucose (glycolysis) and the transformation of nucleotides (Toldrá & Flores, 1998). Chemical reactions such as Maillard reactions, Strecker degradations and oxidative reactions, also take place during the process contributing to the development of the characteristic flavour of these products (Gandemer, 2002; Toldrá & Flores, 1998).

3. Proteolysis during dry-curing

One of the most important biochemical changes that occur during dry-curing is the intense proteolysis that takes place as a result of the action of endogenous muscle peptidases. It is assumed that the main enzymes responsible for the degradation of muscle proteins during this period are endopeptidases, mainly calpains and cathepsins, and certain groups of exopeptidases (Toldrá, 1998; Toldrá, 2002). Endopeptidases degrade the muscle protein structure by cleaving intact proteins, giving rise to large polypeptides which are further degraded by exopeptidases. In this way, the action of muscle endopeptidases such as cathepsins and calpains is of great importance during processing because they are directly responsible for changes in postmortem muscle texture (Lametsch et al., 2003). Regarding the action of these endopeptidases during the dry-curing process, cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), and L (EC 3.4.22.15) have been reported to be stable during dry-cured ham processing, showing activity even after 15 months of processing (Toldrá, Flores, & Sanz, 1997; Toldrá, Cerveró, & Part, 1993), whereas cathepsin D (EC 3.4.23.5) activity disappears after 6–10 months of processing (Rico, Toldrá, & Flores, 1991; Toldrá, Rico, & Flores, 1993). Cathepsins have a clear effect on texture that can be damaging for sensory quality in case of an excess of activity (Virgili, Parolari, Schivazappa, Bordini, & Borri, 1995). Calpains (EC 3.4.22.17) can participate in the post-mortem muscle proteolysis only during the first stages of the curing processes since its stability is rather poor and the activity disappears in near 2 weeks (Toldrá & Flores, 1998).

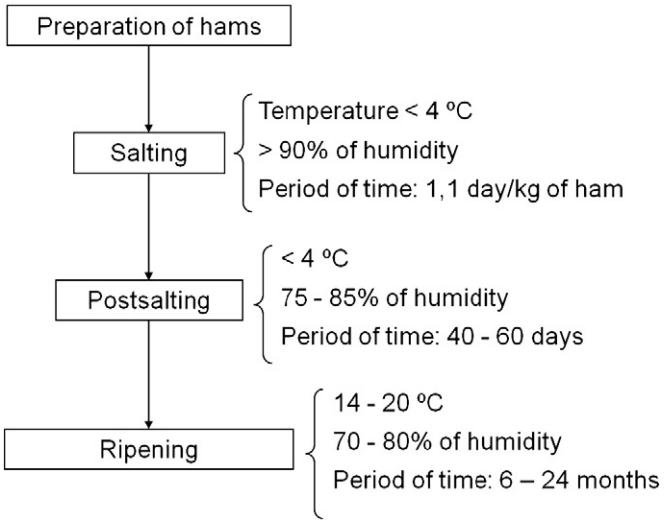

Fig. 1. Typical experimental workflow of dry-cured ham processing for the identification of the complete sequence of naturally generated peptides.

Exopeptidases are proteolytic enzymes that degrade the large polypeptides generated by endopeptidases, giving rise to small peptides and free amino acids. In this sense, dipeptidyl peptidases (DPP I, II, III, and IV) are a group of enzymes able to release different dipeptides from the N-terminal site of peptides. The stability of DPP II would be restricted during salting and resting times due to its poor activity at low temperatures and optimum at relatively acid pH, so its activity would be relevant only from the end of the post-salting period (50 days) up to 240 days, when its activity decreases. The contribution of DPP IV activity during dry-curing is also expected although in a lower degree, as it expresses a low percentage of activity at pH around 6.0 and its partial inhibition by NaCl (Sentandreu & Toldrá, 2001). Its activity decreases to very low values at 240 days, although it remained active until the end of the process.

On the other hand, the accumulation of free amino acids relevant for the development of the characteristic dry-cured flavour of dry-cured ham, has been attributed to muscle aminopeptidases, responsible for the release of amino acids from the N-terminus of peptides and proteins. Aminopeptidase activity has been detected in meat products even after more than 12 months of processing, suggesting that these enzymes are involved in the later stages of protein degradation (Toldrá, Aristoy, & Flores, 2000). Many factors, such as curing agent or the presence of other peptides, can modulate the activity of these enzymes (Gianelli, Flores, Moya, Aristoy, & Toldrá, 2000; Toldrá, Cerveró, & Part, 1993).

Carboxypeptidases and peptidyl dipeptidases constitute other groups of exopeptidases responsible for the hydrolysis of amino acids and dipeptides, respectively, from the C-terminal side of the protein fragments. These groups of enzymes have not been so well studied in dry-cured ham as aminopeptidases and the effect of curing agents on their stability during curing period remains unknown.

4. Proteomics applied to the identification of naturally generated peptides in dry-cured ham processing

4.1. Separation of peptides

The fractionation and isolation of the naturally generated peptides for their subsequent mass spectrometry analysis are crucial. The SDS-PAGE has been the method of choice for the separation of the proteins in meat and its extracts. In this sense, the structural alteration and progressive disappearance of muscular proteins during maturation and dry-cured processing have also been extensively studied. Some authors used SDS-PAGE gels and observed the progressive disappearance of myosin heavy chain, myosin light chains 1 and 2 (MLC 1 and MLC 2), and troponins C and I, as well as an appearance of numerous smaller fragments in the 50–100 and 20–45 kDa regions (Monin et al., 1997; Toldrá, Cerveró, & Part, 1993). More recently, an intense proteolysis of actin, tropomyosin, and myosin light chains extracted in the myofibrillar fraction of dry-cured hams with different ripening times was also reported (Di Luccia et al., 2005). These authors concluded that after 12 months of ripening, most myofibrillar proteins were completely hydrolyzed. Troponin T is a myofibrillar