Food Chemistry 166 (2015) 522–530

Contents lists available at ScienceDirect

Food Chemistry

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

Quantitative study of the relationships among proteolysis, lipid oxidation, structure and texture throughout the dry-cured ham process

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ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 30 April 2014
Accepted 4 June 2014
Available online 21 June 2014

Keywords:
Dry-cured ham
Proteolysis
Structure
Texture
Polynomial regression model

ABSTRACT

Temperature, salt and water contents are key processing factors in dry-cured ham production. They affect how proteolysis, lipid oxidation, structure and texture evolve, and thus determine the sensory properties and final quality of dry-cured ham. The aim of this study was to quantify the interrelationships and the time course of (i) proteolysis, (ii) lipid oxidation, (iii) five textural parameters: hardness, fragility, cohesiveness, springiness and adhesiveness and (iv) four structural parameters: fibre numbers, extracellular spaces, cross section area, and connective tissue area, during the dry-cured ham process. Applying multiple polynomial regression enabled us to build phenomenological models relating proteolysis, salt and water contents to certain textural and structural parameters investigated. A linear relationship between lipid oxidation and proteolysis was also established. All of these models and relationships, once combined with salt penetration, water migration and heat transfer models, can be used to dynamically simulate all of these phenomena throughout dry-cured ham manufacturing.

1. Introduction

Dry-cured ham can be an important source of essential amino acids for humans. Proteolysis, one of the main biochemical reactions during dry-cured ham processing, is considered to be the source of many of these free amino acids. Proteolysis generally refers to endogenous enzyme activity (Cordero & Zumalacarregui, 2000), such as that of cathepsins B, L, H and D, calpains, peptidases and cytosolic enzymes (Luccia et al., 2005). Proteolytic activity depends on many factors. Zhao et al. (2005) found that potential activities of cathepsin B and L were reduced during processing to only 9.3% and 13.7% of their original potential activity, as a result of the inhibitory role of salting. According to Morales, Serra, Guerrero, and Gou (2007), temperature also has a strong influence on these enzymes. They showed that ageing biceps femoris (BF) muscle, at 30 °C, increased proteolysis intensity significantly compared with hams aged at 5 °C. It has also been observed that high temperatures during the drying–ageing stage promote the formation of non-protein nitrogen compounds and, in turn, affect the course of proteolysis. Many authors have found that proteolysis rate is affected by several processing parameters, such as temperature, relative air humidity and salt content (Arnau, Gou, & Comaposada, 2003; Ruiz-Ramirez, Arnau, Serra, & Gou, 2006; Toldrá, Flores, & Sanz, 1997). High water content has been found to increase proteolytic activity as a result of high water activity (a_w) values (Serra, Ruiz-Ramírez, Arnau, & Gou, 2005). In addition, some studies have shown that proteolysis remains stable during one week of storage at 30 °C and increases after one month of storage under the same conditions (Arnau, Guerrero, & Gou, 1997; Morales et al., 2007). The anatomic location of muscles inside the ham, whether external, e.g. semimembranosus (SM), or internal, e.g. BF, also plays a major role in the time course of proteolysis during the dry-cured ham production process, owing to different salt and water transfer kinetics in each muscle. Very recently, Harkouss, Safa, Gatellier, Lebert, and Mirade (2014) have developed phenomenological models allowing proteolysis (through a proteolysis index) to be quantified, in five different pork muscles, as a function of temperature and water and salt contents. Using these statistical models, these authors predicted that the highest increase in proteolysis occurred during the last stage of the dry-cured ham process (ageing), with a mean monthly PI augmentation of 2–2.5%. It is well-known that proteolysis impacts the final texture (hardness, cohesiveness) of the product, and is considered

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http://dx.doi.org/10.1016/j.foodchem.2014.06.013
0308-8146/

as a crucial parameter for obtaining good quality sensory characteristics at the end of the process (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Toldrá, 1998; Zhao et al., 2008). Thus, a better understanding and control of proteolysis rates can favour an optimised texture in dry-cured ham.

In parallel, lipid oxidation, which is an important biochemical phenomenon that occurs in foods during processing and storing, strongly affects (negatively, but also positively) their quality and chemical safety (Jin et al., 2012). Lipid oxidation is also an essential factor and has a positive impact on the development of the typical aroma of dry-cured ham. Many research studies have characterised lipid oxidation in food products or examined the effect of NaCl and temperature on it (Aidos, Lourenco, Van der Padt, Luten, & Boom, 2002; Jin et al., 2012; Rhee, Smith, & Terrell, 1983, among others). Indeed, in minced pork muscles, Rhee et al. (1983) showed that NaCl inhibited lipid oxidation at concentrations higher than 2%, but promoted it at lower concentrations. Aidos et al. (2002) found that elevating temperature could accelerate the degradation of hydroperoxides in crude herring oil. Recently, Jin et al. (2012) reported that, even at 4% salt content, lipid oxidation gradually increased in minced pork muscles, but this varied according to the process temperature; in other words, lipid oxidation is strongly affected by both salt content and temperature, and probably by their interaction. Jin et al. (2012) confirmed that, as the temperature increased, so the threshold value of NaCl content affecting lipid oxidation in pork muscle progressively decreased.

In general, the sensory quality of dry-cured ham is evaluated through flavour, appearance and texture criteria. Although colour and flavour are highly important for reaching a high final product quality, texture remains a major sensory characteristic to be tested. Texture problems, such as softness, pastiness and crusting, frequently impede slicing and give a mouth-coating sensation, underlining the important role of texture for both retailer and consumer acceptability. Hams are usually classified into four texture types: very pasty, pasty, soft, and normal, each identified by various properties. It has been shown that textural defects are closely related to anomalous proteolysis (Virgili, Parolari, Schivazappa, Soresi-Bordini, & Borri, 1995). Studies have shown that non-protein nitrogen (NPN) values are highest in pasty hams and lowest in soft hams. Ruiz-Ramirez, Serra, Gou, and Arnau (2005) observed that high proteolysis rates led to poor cohesiveness and sliceability, as a result of abnormal softness. The role of several process factors (e.g. salt content, temperature) on texture has also been investigated. Gou, Morales, Serra, Guàrdia, and Arnau (2008) studied the effect of salt, and observed that, as NaCl content decreased, so texture problems increased; these results were in line with those of Desmond (2006), who showed that limiting salt concentration tended to decrease fibre swelling, leading to poor texture. Also, Sforza et al. (2001) showed that high temperature in the final ageing period played an important role in lowering ham dryness. These results were confirmed by Morales, Arnau, Serra, Guerrero, and Gou (2008), who observed a decreased pastiness in BF with no increased hardness in SM muscle, after one week of storage at 30 °C. Most texture problems in dry-cured hams could be related to a short processing time and low salt content. Consequently, studies have to be more narrowly focussed on processing factors (time, temperature, salt amount), in association with textural properties (e.g. hardness) and biochemical changes (proteolysis, oxidations, lipolysis) to ensure a dry-cured ham of high final quality. To achieve this aim, structural properties must also be thoroughly studied and combined with the properties stated above.

Few studies have been carried out to study the main biochemical changes influencing the structural proteins, especially those related to the thick and thin filaments, the Z-disks and the costamere proteins (Fritz, Mitchell, Marsh, & Greaser, 1993). Electron microscopy highlighted the effect of endogenous proteolytic enzymes on muscle ultrastructure during dry-cured ham processing. Using this technique, in combination with gas chromatography, Larrea, Perez-Munuera, Hernando, Quiles, and Lluch (2007) related dry-cured ham quality to its microstructure, and observed modifications in the structural proteins that could explain the texture and flavour time course of the final product. They reported that, after the salting stage, the Z-disks inside the myofibrils were no longer in line, and a marked degradation of the cell membranes was detected; in the last stage of ripening, several accumulated proteolysis products were observed. This brief literature review shows that little quantitative information about the interrelationships between proteolysis, texture and/or structure is available.

Here we aim to study lipid oxidation and devise phenomenological models, quantitatively, relating proteolysis to several textural and/or structural parameters, through a statistical analysis of experimental results obtained on samples extracted from industrial dry-cured ham at five key times during their manufacture. These phenomenological models will be implemented, at the end, into a numerical finite-element model dedicated to predicting heat and mass transfer phenomena and coupling them with proteolysis index (PI) calculation, using other phenomenological models recently published (Harkouss et al., 2014), with the objective of simulating all that happens in a 3D dry-cured ham during its elaboration process.

2. Materials and methods

2.1. Extraction of samples of Bayonne dry-cured hams

The work described here evaluates changes in the time course of proteolysis, lipid oxidation, texture and structure that occur in PDO (Protected Designation of Origin) Bayonne dry-cured hams during their manufacture. In total, 15 Bayonne dry-cured hams were selected, on the basis of an initial pH value in the range 5.6–5.9, corresponding to three hams removed from the process at five different processing times: (i) four days *post mortem* (“fresh hams”); (ii) at the end of the resting period (11 weeks); (iii) at the end of the drying period (21 weeks); (iv) at mid-period (35 weeks);and (v) at the end of the ageing period (12 months). The five processing times were chosen in order to obtain five very different levels of proteolysis. These hams were purchased from Pyragena (Arzacq, Pyrénées-Atlantiques, France), an experimental centre producing and working on Bayonne dry-cured hams. At a distance of 10 cm from the coaxial bone, a 3 cm-thick cross sectional area section was cut on each of the 15 hams (Fig. 1a). On each section, two slabs ($2 \times 3 \times 5$ cm) of muscle were cut: the first 2 cm from the bottom in the BF muscle, and the second 2.5 cm from the top of the ham in the SM muscle (Fig. 1b). In this way, we expected to have similar salt and water contents in all the samples obtained from each ham for the various experimental measurements, and to keep constant the geometrical position where the samples were cut for the 15 dry-cured hams. Each muscle slab was then divided into various samples: 2×1 g for the quantification of proteolysis ($n = 2 \times 3 = 6$, per muscle per time), 1 g for the quantification of lipid oxidation ($n = 3$, per muscle per time), 1 g for the determination of salt content ($n = 3$, per muscle per time), about 2 or 3 g for the determination of water content ($n = 3$, per muscle per time), one sample of $2 \times 3 \times 0.5$ cm dedicated to structural analysis and the rest of the slab was cut into two large samples dedicated to texture analysis ($n = 2 \times 3 = 6$, per muscle per time). All the samples were then vacuum-packed in plastic bags and frozen at -80 °C until needed, except the samples dedicated to structural analysis that were treated separately (Section 2.3.1) and those dedicated to water content measurement that were placed directly in a laboratory controlled-temperature chamber, after being weighed.