Food Chemistry 151 (2014) 7–14

Contents lists available at ScienceDirect

Food Chemistry

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

Building phenomenological models that relate proteolysis in pork muscles to temperature, water and salt content

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ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form 23 October 2013
Accepted 30 October 2013
Available online 13 November 2013

Keywords:
Proteolysis
Pork muscle
Water and salt content
Temperature
Doehlert design
Response surface methodology
Multiple linear regression

ABSTRACT

Throughout dry-cured ham production, salt and water content, pH and temperature are key factors affecting proteolysis, one of the main biochemical processes influencing sensory properties and final quality of the product. The aim of this study was to quantify the effect of these variables (except pH) on the time course of proteolysis in laboratory-prepared pork meat samples. Based on a Doehlert design, samples of five different types of pork muscle were prepared, salted, dried and placed at different temperatures, and sampled at different times for quantification of proteolysis. Statistical analysis of the experimental results showed that the proteolysis index (PI) was correlated positively with temperature and water content, but negatively with salt content. Applying response surface methodology and multiple linear regressions enabled us to build phenomenological models relating PI to water and salt content, and to temperature. These models could then be integrated into a 3D numerical ham model, coupling salt and water transfers to proteolysis.

1. Introduction

During dry-cured ham production, the main biochemical mechanism affecting the tenderization of meat and the quality of the final product (texture, flavour and appearance) is proteolysis, resulting from the action of proteolytic endogenous enzymes, such as cathepsins and calpains, which remain active for a long time (Arnau, Guerrero, & Sárraga, 1998; Garcia-Garrido, Quiles-Zafra, Tapiador, & Luque De Castro, 2000; Tabilo, Flores, Fiszman, & Toldra, 1999; Toldrá & Etherington, 1988; Toldrá & Flores, 2000; Virgili, Parolari, Schivazappa, Bordini, & Borri, 1995; Zhao et al., 2008). A literature review showed that many factors influence proteolytic activity, such as temperature, salt content, water content and pH. The effect of temperature has been described in previous studies (Arnau, Guerrero, & Gou, 1997; Parolari, Virgili, & Schivazappa, 1994, among others). Morales, Serra, Guerrero, and Gou (2007) showed that letting biceps femoris (BF) muscle age at 30 °C increased PI significantly compared with hams aged at 5 °C. It is well known that temperature rise promotes cathepsin B and L activity and it has been observed that high temperatures during the drying-ageing stage favour the formation of non-protein nitrogen compounds, which increases PI. However, at the same time, a high enzyme activity results in uncontrolled hydrolysis, leading to an anomalous texture or even an undesirable colour in the product. The effect of NaCl content has also been widely demonstrated (Garrido, Domínguez, Lorenzo, Franco, & Carballo, 2012; Rico, Toldrá, & Flores, 1991; Sárraga, Gil, Arnau, Monfort, & Cussó, 1989). Studies report that PI increases in hams as salting time is shortened. A high concentration of NaCl may inhibit the enzymes responsible for proteolytic activity. For example, cathepsin D, the most active cathepsin throughout the dry-curing of ham, loses more than 70% of its activity at a salt concentration of 5%. However, the reduction of salt may pose a major problem in terms of colour formation and stability, as reported by Benedini, Parolari, Toscani, and Virgili (2012). High salt concentration significantly affected the yellow colour of fat as a result of a high rate of autoxidation, and the red colour of meat as a result of greater penetration of nitrate and nitrite, generating nitrosomyoglobin, responsible for the characteristic pink colour of cured meats. Martin, Cordoba, Antequera, Timon, and Ventanas (1998) related pasty texture to the amount of salt adsorbed during the salting stage. Garrido et al. (2012) also related increased hardness of meat to an increase in salt content. Studies have often found a relation between salt concentration and water content, another factor affecting proteolysis and texture. High water content increased water activity (a_w) and in turn, the proteolytic activity. Serra, Ruiz-Ramirez, Arnau, and Gou (2005) highlighted a negative non-linear relationship between hardness and water content, but a positive relationship with cohesiveness and springiness. Time is also important for proteolysis. Some

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http://dx.doi.org/10.1016/j.foodchem.2013.10.164

studies have shown that one month storage at 30 °C augments proteolysis, thereby increasing the pastiness of BF muscle (Arnau et al., 1997), whereas at the same temperature after one week, pastiness, adhesiveness and softness decrease in BF, without obviously affecting a_w or proteolysis (Morales et al., 2007). Finally, the activity of proteases, like that of all enzymes, depends on an optimal pH. In dry-cured ham, since the pH rarely exceeds 6.2, only proteases acting in slightly acid pH will be active, such as cathepsins B, D, H and L (Garcia-Garrido, Quiles-Zafra, Tapiador, & Luque De Castro, 2000). By contrast, several studies found greater hardness in hams with $pH_{24} < 5.8$ than in hams with $pH_{24} > 6.2$, whereas others highlighted harder dry-cured hams with pH_{24} between 5.6 and 6 than with $pH_{24} < 5.6$ (Arnau et al., 1998). In addition to these physico-chemical factors (pH, temperature, water and salt content), the type of muscle may also affect the time course of proteolysis during the ham dry-curing process, since the percentage of myofibrillar and sarcoplasmic proteins differs from one muscle to another. Studies have reported that the above factors act on proteolysis in meat. However, the interaction between these factors has not yet been elucidated. Also, no study describes the time course of proteolysis as a function of temperature, salt content, water content and muscle type.

Response surface methodology (RSM) is commonly defined as a collection of mathematical and statistical techniques that explore the relationships between several independent variables, termed input or explanatory variables, and one or more response (or output) variables. RSM is generally based on fitting a polynomial equation to the experimental data obtained through a designed sequence of experiments. Many applications of RSM can be found in the literature, e.g., in analytical chemistry (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008), and in meat science. For example, Zhao et al. (2005) evaluated the effects of temperature, salt content, pH and nitrite content on the activities of cathepsin B and L, using RSM based on a Box-Behnken design, and calculated the actual activities of these cathepsins during Jinhua ham processing. Jakobsen, and Bertelsen (2000) developed a response surface model to predict the effects of temperature, storage time and oxygen partial pressure on the colour stability and lipid oxidation of fresh beef muscle. They concluded that RSM was a very promising tool for modelling chemical quality changes in meat stored under various conditions, providing that the broad biological variability among animals could be controlled. From the combined use of a RSM approach and a full factorial design with six factors, MØller et al. (2003) found that the interactions between packaging and storage conditions were crucial to limiting light-induced oxidative discoloration of cured ham packaged in a modified atmosphere during the 14 days of chill storage. More recently, Jin et al. (2012) used RSM coupled with central composite design to investigate the effects of temperature (from 15 to 35 °C) and sodium chloride content (from 0.5% to 4.0%) on lipid oxidation in minced pork muscle, demonstrating that both temperature and NaCl content had significant pro-oxidant effects, and also extremely significant interaction for lipid oxidation.

In the present study, RSM was investigated in a way similar to that detailed in Zhao et al. (2005) and Jin et al. (2012) to spotlight the interactive effect of the different factors affecting proteolysis time course in dry-cured ham, and so build phenomenological models that map proteolysis throughout the process. The objective of this study was to quantify the effects of salt content, temperature, water content and muscle type, together with their interactions, on proteolytic activity in laboratory-prepared, dried, salted small pork meat samples, and thereby to determine models to quantify PI as a function of these factors. In future work, these models could be incorporated into a global finite-element model coupling salt and water transfers with proteolysis in a 3D "numerical" ham that is cured and dried for several months.

2. Materials and methods

2.1. Preparation of the laboratory pork meat samples

This study required a very large number of pork meat samples to be prepared rapidly under pre-defined, accurate temperature, salt content and moisture values. To do this, we decided to work from fresh pork muscles rather than from samples taken directly from dry-cured hams, from which it is very difficult to obtain samples with the desired salt and water content.

Fig. 1 details the experimental protocol followed to prepare and condition the small salted and/or dried pork meat samples, before quantifying proteolysis. Every stage in this figure, i.e., decontamination, cutting, salting, drying and proteolysis quantification, required much preliminary experimental work to determine the best way to proceed. Eight fresh hams of average weight 10.6 ± 0.75 kg were selected at a local slaughterhouse. Five different muscles, biceps femoris (BF), semitendinosus (ST), semimembranosus (SM), rectus femoris (RF) and gluteus medius (GM) were extracted from each ham four days *post mortem*. Their moisture content and pH were respectively, 74.7 ± 1.7% and 5.74 ± 0.13. Entire muscles were vacuum-packed in plastic bags, frozen in an ultra-low temperature freezer (Bio Memory, Froilabo) at −80 °C and stored for later use. Muscle surfaces were decontaminated twice under an extractor fan by treatment with 0.1% peracetic acid for 3 min followed by 1 min rinsing-out with sterile water. Samples were then cut into small slabs (5 × 4 × 0.3 cm) after discarding the superficial muscle layers damaged by the acid treatment. These operations were performed under sterile air conditions using sterile tools. Samples were prepared so as to obtain a final mass greater than 5 g per sample in order to perform all the biochemical tests; samples were then vacuum-packed in bags and frozen at −80 °C until needed. The small pork meat samples were then thawed and salted by covering the surface of the piece with a 300 g L^{-1} NaCl solution using a pipette (Eppendorf AG, multipette plus, Hamburg, Germany) adjusted to 4 μl per spot. The thinness of the samples allowed the salt to diffuse rapidly, in a few hours, and homogeneously. The quantity of salt added was calculated on the basis of the salt concentration (1–15% of the dry matter – DM) and the water content (50–75% of the total matter – TM) required in the final pork meat sample. The uncertainty of salting of these small pork meat samples was evaluated from preliminary experiments; it was found to range from 0.1% DM for the least salted (1.1% DM) samples to 0.8% DM for the most salted (14.9% DM) samples. The next step was drying; this was carried out under sterile air conditions at 15–16 °C for different periods of time (up to 22 h for the most thoroughly dried samples) until each sample reached the weight corresponding to the selected water content. Applying this simple procedure allowed the uncertainty of drying to be maintained below 1% TM, whatever the final desired water content (from 56.3% to 68.5% TM). Samples were then vacuum-packed in plastic bags and kept in controlled-temperature chambers (Model 14 D-78532, Binder GmbH, Tuttlingen, Germany) at various temperatures (3, 13 and 24 °C) in order to study the proteolysis kinetics.

Preliminary tests gave an estimation of 35, 25 and 13 days, respectively, for the shelf-life of these small samples at these three temperatures, without observing any microbiological growth, so that only proteolysis resulting from endogenous enzyme activity occurred, i.e. that taking place inside hams during the drying and curing processes, and not the degrading action of some microorganisms, which leads to an exponential increase in PI and an increasingly unbearable odour. Once prepared, the samples were finally stored at −80 °C before final analysis (PI, salt and water contents). Four samples were prepared for each processing condition.