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

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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 (Arnaud, Guerrero, & Sarraga, 1998; Garcia-Garrido, Quiles-Zafra, Tapiador, & Luque De Castro, 2000; Tabilo, Flores, Fitzman, & Toldra, 1999; Toldra & Etherington, 1988; Toldra & Flores, 2000; Virgili, Parolari, Schivarzappa, 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 (Arnaud, Guerrero, & Sárraga, 1998; Garcia-Garrido, Quiles-Zafra, Tapiador, & Luque De Castro, 2000; Tabilo, Flores, Fitzman, & Toldra, 1999; Toldrá & Etherington, 1988; Toldrá & Flores, 2000; Virgili, Parolari, Schivarzappa, Bordini, & Borri, 1995; Zhao et al., 2008). A literature review showed that many factors influence proteolytic activity, such as temperature, salt content, 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.

 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.

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studies have shown that one month storage at 30 °C augments proteolysis, thereby increasing the pastiness of BF muscle (Arnau et al., 1998), whereas at the same temperature after one week, pastiness, adhesiveness and softness decrease in BF, without obviously affecting aw 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<5.8 than in hams with pH>6.2, whereas others highlighted harder dry-cured hams with pH<5.6 (Arnau et al., 1998). In addition to these physicochemical 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 (Brezerra, 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 in ham, 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, Möller 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 (1–1.5% 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, Tuttingen, 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.
2.2. Quantification of the proteolysis

In parallel to the preparation of the laboratory pork meat samples, to cope with the large number of samples to be analysed, a rapid, accurate, fluorometry-based procedure was developed to determine sample PI. In this procedure, the new PI is defined as the percentage of the ratio of the N-terminal α-amino group content (A) to the total protein content (B). The content (A), which reflects the real intensity of proteolytic activity occurring during the process, was measured by the fluorosamine method (Harkouss, Mirade, & Gatellier, 2012), and content (B) by the biuret method (Gornall, Bardawill, & David, 1949). This procedure is more rapid, more specific and more economical in raw materials than classical methods, e.g., the classic nitrogen method, and could also be easily used in industry. Full information on this powerful technique can be found in Harkouss et al. (2012).

Preliminary tests of quantification of proteolysis performed on some small laboratory-prepared pork meat samples revealed that over the periods of time investigated (i.e., 13, 25 and 35 days, depending on temperature) proteolysis kinetics could be approximated by straight lines, and thus characterised by a slope representing a rate of protein degradation.

2.3. Response surface methodology and Doehlert design

In the present study, a response surface methodology based on a Doehlert design was used to investigate the interaction between temperature, water and salt content on the proteolysis time course in small laboratory pork meat samples, with the final objective of building phenomenological models allowing proteolysis to be quantified in pork muscles as a function of these factors.

A Doehlert design (Doehlert, 1970) was implemented on the basis of three factors for each muscle: temperature, water content and salt content. This particular design offers a uniform distribution of points over the whole experimental region, arranged in a rhombohedral figure. In the case of three-variable designs, a cuboctahedron is produced geometrically. It is important to note that in a Doehlert design, the number of levels is not the same for all variables: in a three-factor problem, the first factor is studied at three levels, the second at five levels and the third at seven levels. Although pH is also a key factor for the proteolysis time course, pH was not included in the present Doehlert design. The pH of green muscles had been previously measured and found to range between 5.6 and 5.9. The selection of the independent variables and their levels was made in accordance with values commonly found in industrial production of dry-cured ham. The different levels of each factor were chosen as: five levels for water content, centred at 62.5% (50–75% TM), seven levels for salt content, centred at 8% (0–16% DM) and three levels for temperature, centred at 13 ºC (approximately 2 to 26 ºC). This design allowed the number of manipulations to be decreased from 105 to 13 experiments per muscle (Table 1). Also, the centre of this experimental design was repeated twice to take into account animal variability; at this particular design point, kinetics of proteolysis were recorded on ten small samples prepared from muscles belonging to a different animal each time. Finally, 15 proteolysis kinetics were determined for each muscle, with 10 points (samples) per plot and four measurements per sample, i.e., a total of 3000 experimental proteolysis quantification items for all five muscles.

For each muscle investigated, the quadratic polynomial regression equation was taken as given by RSM:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2^2 + \beta_3 X_3 X_4 + \lambda_i,
\]

where \(Y\) is the response variable (proteolysis), \(\beta_0\) is the constant coefficient (intercept), \(\beta_i\) is the linear coefficient, \(\beta_2\) is the quadratic coefficient, \(\beta_{ij}\) is the two-factor interaction coefficient, and \(\lambda_i\) is the random error. Eq. (1) describes the linear, quadratic and interaction effects of the three variables \(T\) (temperature), \(W\) (water content) and \(S\) (salt content) on the response value (proteolysis rate).

2.4. Statistical analysis

All statistical analyses were performed using R 3.0.1 software (R Development Core Team, 2013), the statistical packages 'car' (Fox & Weisberg, 2011), ‘HH’ (Heiberger, 2013), 'leaps' (Lumkey, 2009) and the graphical package 'rgl' (Adler & Murdoch, 2013). A three-way analysis of variance (ANOVA) was performed on all the proteolysis kinetics obtained for the five muscles SM, BF, RF and GM. The objective was to assess the effect of each factor (temperature, water and salt content) on PI. The effect of muscle type was also studied. Post hoc procedures were used when the F-test was significant (\(p < 0.05\)). Multiple comparisons among means were examined by the Fisher’s least significant difference (LSD) test, for temperature, water and salt content. Multiple linear regressions were performed in order to find the best model of proteolysis time course as a function of the factors studied for each muscle, and their interactions. As advocated by Fox, and Weisberg (2011), regression diagnostics such as ‘Component+Residual’ plots were performed to investigate non-linearity problems, hat values, Studentized residuals and Cook’s distances to identify outlier points, and finally model factors selection by exhaustive search, forward or backward stepwise, or sequential replacement (Mallows’s Cp)
and linearly with time (\( R^2 = 0.93 \)) in the laboratory-prepared pork meat samples, at 3 °C. In general, at this temperature protein degradation of the different muscles was very slow (0.06 ± 0.02% PI/day). Similar results were obtained by Costa-Corredor, Serra, Arnau, and Gou (2009), who indicated that increasing post-resting temperature to above 5 °C reduced processing time. At low temperature, proteolysis requires more time to progress. In the present study, at 3 °C, the proteolysis indices were generally low; more than 35 days being required to increase PI from 2.97 ± 0.59% to 4.95 ± 0.99%. The same results have been reported in several previous studies, thus confirming that PI at low temperature (≤5 °C) is lower than at higher temperatures (Martin et al., 1998). Fig. 2a shows that there was also a slight effect of drying on proteolytic activity of the enzymes, clearly visible from day 20 (Fig. 2a). Similar results were reported by Toldra (2006) in his review, denoting that drying decreased water activity values, which affected the muscle proteolytic enzymes activity. The averages of proteolysis kinetics slopes for all muscle types at 5.7% DM salt content, and 68.5% TM and 56.3% TM water content were 0.07 ± 0.03% PI/day and 0.05 ± 0.01% PI/day, respectively (Experiments 7 and 9, Table 1). This could be because at low temperature there is not enough energy to sufficiently activate the enzymes (cathepsins, calpains, etc.) responsible for protein degradation. Unfortunately, the effect of salt at 3 °C could not be investigated directly from graph analysis because the Doehlert design did not provide for experiments at this temperature (Table 1). The interpretation of salt effect will therefore be discussed later when analysing the 3D response surface plots. The overall conclusion is that all muscles behaved in approximately the same way at 3 °C in terms of the proteolysis time course.

3.2. Time course of proteolysis at moderate temperature (13 °C)

As at low temperature, proteolysis index increased markedly and linearly with time (\( R^2 = 0.95 \)), at 13 °C (Fig. 2b). Generally, the progress of proteolysis at moderate temperature was faster and stronger than at low temperature; the average of slopes of all the muscle proteolysis kinetics at 13 °C were globally higher than at 3 °C (0.11 ± 0.04% PI/day), except for kinetics with high salt content combined with low water content, where low values of slope were obtained, e.g., for the 14.9% DM salt-56.3% TM water curve in Fig. 2b (Experiment 10, Table 1). When pork meat samples were weakly dried and salted (i.e., the 1.1% DM salt-68.5% TM water curve of Fig. 2b, corresponding to Experiment 8 in Table 1), measurements showed that values of PI could double in 25 days. Unlike experiments performed at low temperature, proteolysis kinetics recorded at 13 °C allowed simultaneous assessment of the effect of drying and salting on the time course of proteolysis owing to the high number of proteolysis kinetics plotted at this temperature (Experiments 1–3, 5–6, 8 and 10 in Table 1). Analysing Fig. 2b, which shows the time course of three different proteolysis kinetics in the case of ST muscle, allows these effects to be assessed. Comparing the 1.1% DM salt-68.5% TM water curve with the 1.1% DM salt-56.3% TM water curve of Fig. 2b, shows that a 12% increase in drying in weakly salted pork meat samples clearly slowed down protein degradation, and the proteolysis rate decreased from 0.14% to 0.08% PI/day. Costa-Corredor, Muñoz, Arnau, and Gou (2010) reported similar results, which could be explained by the inhibitive effect of drying on proteolytic enzymes, leading to a slowed proteolysis time course. Fig. 2b also shows that on adding about 14% salt (comparison of the 1.1% DM salt-56.3% TM water curve with the 14.9% DM salt-56.3% TM water curve), PIs fell even more for pork meat samples that were already well-dried. This confirms that salting is a key factor during the ham dry-curing process to control proteolysis. These results are consistent with those of Hutton (2002), who indicated that at constant temperature, adding salt and losing water decrease \( a_w \), which is a key physicochemical variable for biochemical reactions and enzyme activity, and so slows the proteolysis time course.

3.3. Time course of proteolysis at high temperature (24 °C)

Table 1


<table>
<thead>
<tr>
<th>List of experiments of Doehlert design</th>
<th>Temperature (°C)</th>
<th>Salt content (% dry matter)</th>
<th>Water content (% total mass)</th>
<th>Muscle SM (% PI/day)</th>
<th>Muscle BF (% PI/day)</th>
<th>Muscle ST (% PI/day)</th>
<th>Muscle RF (% PI/day)</th>
<th>Muscle GM (% PI/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1a (centre)</td>
<td>13</td>
<td>8.0</td>
<td>62.5</td>
<td>0.09</td>
<td>0.06</td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Exp. 1b (centre)</td>
<td>13</td>
<td>8.0</td>
<td>62.5</td>
<td>0.10</td>
<td>0.07</td>
<td>0.09</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>Exp. 1c (centre)</td>
<td>13</td>
<td>8.0</td>
<td>62.5</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Exp. 1 (mean)</td>
<td>13</td>
<td>8.0</td>
<td>62.5</td>
<td>0.09</td>
<td>0.07</td>
<td>0.09</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>13</td>
<td>8.0</td>
<td>75.0</td>
<td>0.08</td>
<td>0.10</td>
<td>0.13</td>
<td>0.22</td>
<td>0.08</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>13</td>
<td>14.9</td>
<td>68.5</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Exp. 4</td>
<td>24</td>
<td>10.3</td>
<td>68.5</td>
<td>0.31</td>
<td>0.17</td>
<td>0.26</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>Exp. 5</td>
<td>13</td>
<td>8.0</td>
<td>50.0</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Exp. 6</td>
<td>13</td>
<td>1.1</td>
<td>56.3</td>
<td>0.06</td>
<td>0.08</td>
<td>0.08</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Exp. 7</td>
<td>3</td>
<td>5.7</td>
<td>56.3</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Exp. 8</td>
<td>13</td>
<td>1.1</td>
<td>68.5</td>
<td>0.13</td>
<td>0.12</td>
<td>0.16</td>
<td>0.19</td>
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</tr>
<tr>
<td>Exp. 9</td>
<td>3</td>
<td>5.7</td>
<td>68.5</td>
<td>0.03</td>
<td>0.06</td>
<td>0.08</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Exp. 10</td>
<td>13</td>
<td>14.9</td>
<td>56.3</td>
<td>0.02</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Exp. 11</td>
<td>3</td>
<td>12.6</td>
<td>62.5</td>
<td>0.04</td>
<td>0.05</td>
<td>0.09</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Exp. 12</td>
<td>24</td>
<td>10.3</td>
<td>56.3</td>
<td>0.12</td>
<td>0.18</td>
<td>0.13</td>
<td>0.24</td>
<td>0.17</td>
</tr>
<tr>
<td>Exp. 13</td>
<td>24</td>
<td>3.4</td>
<td>62.5</td>
<td>0.25</td>
<td>0.21</td>
<td>0.33</td>
<td>0.27</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Even at high temperature, proteolysis kinetics could be well-represented linearly with time (\( R^2 = 0.96 \)). The time course of protein degradation was, as expected, faster than at lower temperatures. Experiment 4 in Table 1 plotted in Fig. 2c led to PIs that increased from approximately 2.5% to 3.5% in only 13 days, even for pork meat samples salted at 10.3% DM. Comparing the plots in Fig. 2c and a shows that the proteolysis rates were multiplied by 3 or 4 when temperature increased from 3 to 24 °C, even for a
higher salt content (10.3% DM vs. 5.7% DM). These results could be explained by the fact that increasing temperature favours the degradation of muscular proteins by proteolytic enzymes, the activity of which is increased due to the greater amount of energy available in the medium. Many studies mention that when temperature increases proteolysis also increases (Martin et al. (1998), among others).
others). Fig. 2c also shows that drying played an inhibitor role in proteolysis, thus confirming the results obtained at 13 °C for lower salt content. This inhibitor role was more clearly and obviously visible at 24 °C than at the previous temperatures of 13 and 3 °C, as seen from the fourth day (Fig. 2c). Also, in spite of a salt content increased by 50% at 24 °C, similar PI s were reached in 10 days at this high temperature against 35 days at 3 °C, for identical water contents. The effect of drying has often been described in several reports, and findings similar to these results have been reported (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006; Toldra (2006); Toldrá, Cerveró, & Part, 1993). For the same reasons as at low temperature, the effect of salting could not be analysed at this temperature.

3.4. Statistical analysis

Table 1 shows the slopes of all the proteolysis kinetics plotted corresponding to the Doehlert design for each of the five muscles investigated: SM, BF, ST, RF and GM. Globally, the values of the slopes ranged from 0.02% to 0.37% PI/day. Those of the repeated kinetics, i.e., the centre point of the Doehlert design, were very close: mean values of 0.09 ± 0.01% PI/day for SM; 0.07 ± 0.01% PI/day for BF; 0.09 ± 0.01% PI/day for ST; 0.09 ± 0.003% PI/day for RF; 0.06 ± 0.01% PI/day for GM, showing that there was no very marked inter-animal effect (standard deviation values lower than 0.01% PI/day). Statistical analyses were then carried out on the values of these slopes.

For each muscle, ANOVA showed that the temperature had a highly significant effect (p < 0.001), the salt content a very significant effect (p < 0.01) and the water content a significant effect (p < 0.05) on the proteolysis time course. On the other hand, macroscopically, the muscle type had no significant effect on proteolysis, especially considering SM, BF and ST muscles (p > 0.05). A Fisher LSD test confirmed the difference between the three temperatures used in this study. This test also showed that there was no difference between the proteolysis rates of the muscles, which could thus be lumped together, except for the GM muscle, which displayed specific behaviour (data not shown).

By means of multiple linear regressions, proteolysis time course models were built for all the muscles. ‘Component+Residual’ plots indicated that the effect of factors was linear. No outlier point was identified by the examination of hat values, Studentized residuals and Cook’s distances. Table 2 contains all the coefficients statistically calculated to build the proteolysis rate models, relating the factors studied and their interactions. Also, as stated above, considering that all the muscles exhibited approximately the same behaviour (except for the GM muscle), a global phenomenological model was also built in which all these muscles were considered together.

In the present study, only the proteolysis time course model found for the ST muscle is reported. The R-squared coefficient determined in this case was high (0.94), which means that the model that gives the time course of proteolysis rate (RatePI-ST) fits the experimental data closely:

\[
\text{Rate}_{\text{PI-ST}} = 4.84 \times 10^{-3} - 9.18 \times 10^{-3}T - 7.28 \times 10^{-4}S + 1.69 \times 10^{-4}W - 3.92 \times 10^{-5}TS + 3.25 \times 10^{-4}TW + 5.21 \times 10^{-5}SW, 
\]

where T is the temperature (°C), S is the salt content (% DM) and W is the water content (% TM).

As an example, Fig. 3 shows the response surface plot of the proteolysis time course for ST muscle, at 4% DM salt content. At low temperature, the values of the slopes of proteolysis kinetics are low, and the inhibitor effect of drying is not clearly visible in this figure. Gradually, as the temperature increases, protein degradation also increases, until at high temperature (24 °C) the inhibitor effect of the drying is clearly visible (Fig. 3). Results also show that increasing salt content slows down the proteolytic activity, especially at low water content. On the basis of all the phenomenological models built, the coefficients of which are listed in Table 2, the rate of protein degradation could be quantified as a function of time, for any of the five muscles studied, and for any of the conditions of temperature, water and salt content.

4. Discussion

During their production process, French dry-cured hams are generally subjected to a variable temperature regime that can be summarised as: about 11 weeks at 4 °C (salting and post-salting stages) optionally followed by 1 week at 23 °C (pre-ripening stage) and from the 13th week until the end, at 12 °C (drying and ageing

![Fig. 3. Response surface plot of the combined effect of temperature and water content on the proteolysis rate in an ST pork muscle, at 4% sodium chloride content (% dry matter).](image-url)
Calculation of proteolysis indices (PIs) and their variation as a function of temperature, water and salt contents during each main stage of the dry-cured ham production process using the phenomenological models built in this study, for three different pork muscles (SM: semimembranosus, BF: biceps femoris and RF: rectus femoris).

<table>
<thead>
<tr>
<th>Stages of dry-cured ham production process</th>
<th>Salting and post-salting</th>
<th>Pre-ripening</th>
<th>Drying</th>
<th>Ageing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (day)</td>
<td>77</td>
<td>7</td>
<td>70</td>
<td>150</td>
</tr>
<tr>
<td>Air temperature (°C)</td>
<td>4</td>
<td>23</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

### Muscle SM

<table>
<thead>
<tr>
<th>Mean water content (% TM)</th>
<th>66.0</th>
<th>61.5</th>
<th>58.0</th>
<th>55.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean salt content (% TM)</td>
<td>3.4</td>
<td>3.2</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Mean proteolysis rate (%/day)</td>
<td>0.04</td>
<td>0.17</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>PI (%)</td>
<td>5.9a</td>
<td>1.2</td>
<td>5.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Xw, PI (%)</td>
<td>1.3</td>
<td>0.1</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>XNaCl, PI (%)</td>
<td>0.3</td>
<td>0.1</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Xw, Mean PI (%)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Muscle BF

<table>
<thead>
<tr>
<th>Mean water content (% TM)</th>
<th>71.0</th>
<th>68.0</th>
<th>66.0</th>
<th>62.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean salt content (% TM)</td>
<td>1.9</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mean proteolysis rate (%/day)</td>
<td>0.04</td>
<td>0.18</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>PI (%)</td>
<td>5.5d</td>
<td>1.2</td>
<td>6.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Xw, PI (%)</td>
<td>1.3</td>
<td>0.1</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>XNaCl, PI (%)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.15</td>
<td>0.3</td>
</tr>
<tr>
<td>Xw, Mean PI (%)</td>
<td>0.15</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

### Muscle RF

<table>
<thead>
<tr>
<th>Mean water content (% TM)</th>
<th>68.0</th>
<th>65.0</th>
<th>62.0</th>
<th>57.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean salt content (% TM)</td>
<td>3.2</td>
<td>4.2</td>
<td>4.2</td>
<td>4.9</td>
</tr>
<tr>
<td>Mean proteolysis rate (%/day)</td>
<td>0.06</td>
<td>0.23</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>PI (%)</td>
<td>7.2c</td>
<td>1.6</td>
<td>8.0</td>
<td>13.7</td>
</tr>
<tr>
<td>Xw, PI (%)</td>
<td>1.8</td>
<td>0.1</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>XNaCl, PI (%)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Xw, Mean PI (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

TM: total mass.

- a Impact of a 2 °C variation in air temperature on the variation of PI over the considered stage.
- b Impact of a 2% TM variation in water content on the variation of PI over the considered stage.
- c Impact of a 0.5% TM variation in salt content on the variation of PI over the considered stage.
- d A 2.5% initial value was considered when calculating PI at this particular stage. This initial value corresponds to the mean value of PI measured for the laboratory-prepared pork meat samples before salting and drying, i.e., for a few days-old “fresh” pork meat.

**5. Conclusion**

The present results confirm that temperature, water and salt contents are key processing factors, strongly influencing proteolysis during the ageing of laboratory-prepared pork meat samples mimicking dry-cured ham production. Temperature rise favoured proteolysis, whereas salting and drying slowed it down. Statistical analyses supported the highly significant effect of temperature on proteolysis, highlighting a very high significant effect of water content and high significant effect of salting. On the other hand, the muscle type had no significant effect on proteolysis, especially for the most often studied and voluminous muscles in dry-cured ham, i.e., BF, SM and ST. To the authors’ knowledge, several phenomenological models were built here for the first time. These models relate proteolysis...
to temperature and water and salt content for five types of muscle. Some of them were used to predict PIs and to assess the impact of some variations in the control of temperature, drying and salting on PI values at the end of the main stages of the production process: salting and post-salting, pre-ripening, drying and ageing. It was highlighted that introducing a one-week-long pre-ripening stage during the process had very little influence on the PI time course. However, the great advantage of these phenomenological models is that they can be easily integrated into a 3D multi-physical-numerical model to predict simultaneously the time course of proteolysis as a function of salt diffusion, water migration and heat. Further studies are needed to validate these models with real-time data from industrial processes.

Acknowledgements

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References