PHENOMENOLOGICAL MODELS RELATING PROTEOLYTIC EVOLUTION OF SMALL PORK MEAT SAMPLES TO TEMPERATURE AND WATER AND SALT CONTENTS

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Abstract – In dry-cured ham, proteolysis evolution is a major issue since it influences not only the sensory qualities and product characteristics, but also the ease and quality of the slicing process in industry. Many physicochemical parameters affect this biochemical phenomenon, such as salt content, water content, pH and temperature. The aim of this study was to quantify the effect of these various physicochemical parameters (except pH) on the evolution of the proteolysis rate of laboratory-prepared pork meat samples, with the objective of building a function allowing the proteolysis index (PI) to be calculated according to these different factors. Based on Doehlert-type experimental design, samples of five different types of pork muscles have been prepared, salted, dried and placed under different temperature, and taken at different times, before determining proteolysis kinetics. Experimental results and statistical analyses show that PI is positively correlated to the temperature and the water content, but negatively correlated to the salt content.

Key Words – Dry-cured ham, Proteolysis, Salt content, Statistical models.

I. INTRODUCTION

Dry-cured ham is an important traditional product in the daily food intake in the world. One main factor affecting final product quality in dry-cured ham is the proteolytic activity, which impacts the flavour and the texture (Arnau *et al.* [1], Toldra *et al.* [2]). Proteolysis is related to pH, temperature, water and salt contents, and the drying conditions (Martin *et al.* [3], Ruiz-Ramirez *et al.* [4], Arnau *et al.* [5]). Our study is divided in two parts: first, to quantify the effect of temperature, and water and salt contents on the evolution of proteolysis in laboratory-prepared pork meat samples; second, to obtain phenomenological models describing proteolysis evolution as a function of these factors and time, in each type of muscles, by means of statistical analyses applied on biochemical measurements already obtained. These phenomenological models would be used (in another study) in combination with other models (salt and water diffusions, heat transfer), in order to obtain a 3D multi-physical model describing numerically the proteolysis evolution while simulating ham drying and curing.

II. MATERIALS AND METHODS

Five types of muscles were studied: Semitendinosus (ST), Semimembranosus (SM), Biceps femoris (BF), Rectus femoris (RF) and Gluteus medius (GM). They were extracted from green pork hams and prepared according to the experimental protocol detailed in Figure 1. This experimental protocol allowed a rapid conditioning in temperature and water and salt contents of these small laboratory pork meat samples.

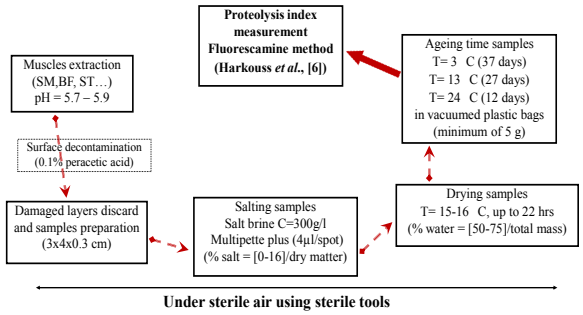

Figure 1. Experimental protocol of samples preparation

To reduce the number of samples to analyze, a Doehlert-type experimental design was built (Table 1), on the basis of 3 factors: temperature with 3 levels [2-26°C], salt content with 5 levels [0-16 % of dry matter (DM)] and water content with 7 levels [50-75 % total matter (TM)]. pH

values of all muscles were measured and ranged from 5.6 to 5.9. This experimental design allowed the number of kinetics to be reduced from 105 to 13 per muscle. The center, i.e. the first point of Table 1 was repeated twice, to take into account the effect of the inter-animal variability. Finally, 15 kinetics of 10 samples repeated 4 times for each muscle were obtained, that is a total of 3000 measurements for the 5 muscles.

Table 1 Doehlert-type experimental design

	T (°C)	Salt (% DM)	Water (% TM)
1 (center)	13	8	62.5
2	13	8	75
3	13	14.9	68.5
4	24	10.3	68.5
5	13	8	50
6	13	1.07	56.25
7	3	5.68	56.25
8	13	1.07	68.5
9	3	5.68	68.5
10	13	14.9	56.25
11	3	12.6	62.5
12	24	10.3	56.25
13	24	3.38	62.5

The proteolysis index (PI) of these small laboratory-prepared samples was determined using the fluorescamine method (Harkouss *et al.* [6]) detailed in Figure 2, which is a powerful and rapid technique to determine the proteolysis intensity. This method could be used directly in industry on small amount of meat without defecting the dry-cured ham product.

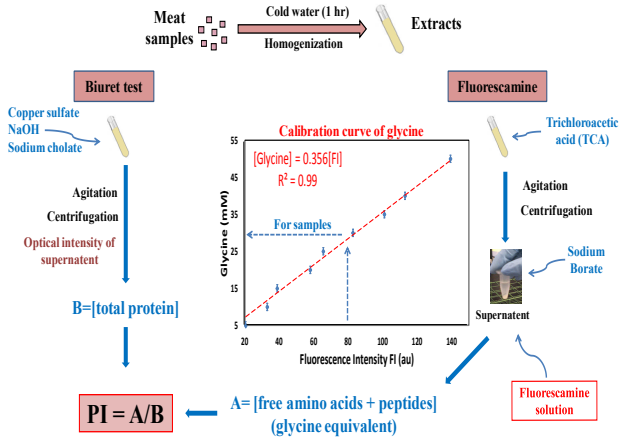

Figure 2. Fluorescamine method protocol

The statistical analyses of the different kinetics obtained by the biochemical measurements were performed using the version "2.12.11" of the software R. An analysis of variance (ANOVA) was made on all the slopes of all kinetics obtained for the five muscles SM, BF, ST, GM and RF. The purpose was to study and to quantify the effect of each studied factor, as well as the effect of the muscle type. Furthermore, a multiple linear regression was realized, to build models of proteolytic evolution relating temperature, water content, salt content and their interactions, for each type of muscles.

III. RESULTS AND DISCUSSION

Biochemical results obtained on the 5 muscles obtained showed that, at a given temperature, salting and drying reduced proteolysis intensity. As an illustration, Figure 3 shows the effect of salting and drying for SM muscle, at 13°C during 27 days.

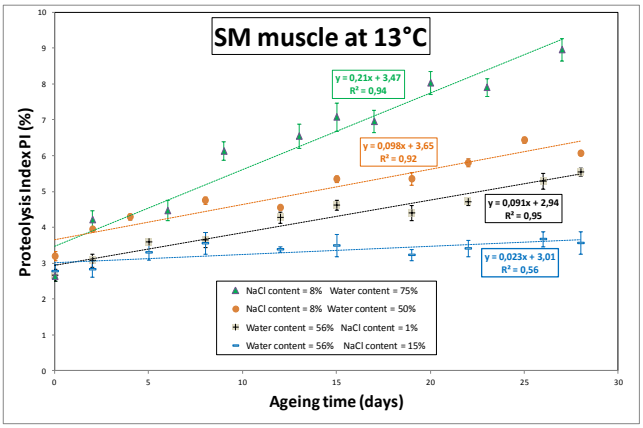

Figure 3. Proteolysis kinetics in SM muscle

The green and orange curves, referring to the same salt content (8g salt/100g of DM) but to different water content (75% and 50% of TM, respectively), show that by drying 25% more, the proteolysis evolution inhibited approximately to the half; proteolysis velocity are 0.21 and 0.098, respectively. The black and blue curves, correspondin to the same water content (56% of TM) but to different salt concentration (1% and 15% of DM), show that reducing salt content quietly favored proteolysis. Results also showed that temperature is an important factor for the proteolysis evolution. By rising temperature to 24°C for only 12 days, proteolysis velocity highly increased since high temperature favored proteolytic enzymes activity. At low temperature

(3°C), the degradation of proteins was very slow and required about 35 days to show a notable proteolysis. These results were in line with those generally reported in literature demonstrating a decrease in proteolysis activity with increasing salt content, and with decreasing temperature or water content, since water activity which affects proteolysis is highly dependent to these factors.
Figure 3 also shows that kinetics of proteolysis could be represented by straight lines and thus characterized by slopes (equivalent to proteolysis velocity). Table 2 contains the slopes of all proteolysis kinetics of this study, corresponding to the Doehlert-type experimental design.

Table 2 Slopes of all kinetics for the 5 muscles

	Slopes				
	SM	BF	ST	RF	GM
Repeated kinetics of 1 (center)	0,086	0,06	0,098	0,088	0,055
	0,1	0,065	0,09	0,09	0,05
	0,092	0,077	0,082	0,095	0,0695
Mean average of 1	0,0927	0,06733	0,09	0,091	0,0582
2	0,081	0,098	0,13	0,22	0,084
3	0,11	0,11	0,1	0,098	0,08
4	0,31	0,17	0,25	0,37	0,35
5	0,053	0,061	0,05	0,063	0,05
6	0,056	0,068	0,082	0,13	0,084
7	0,043	0,045	0,053	0,055	0,043
8	0,13	0,12	0,157	0,186	0,13
9	0,034	0,065	0,08	0,116	0,057
10	0,023	0,047	0,038	0,024	0,028
11	0,035	0,053	0,092	0,043	0,044
12	0,124	0,175	0,133	0,24	0,17
13	0,25	0,21	0,33	0,27	0,35

The values of slopes ranged from 0.023 to 0.37. Those of the repeated kinetics, i.e. the center of Doehlert experimental design, were very close, which proves that there was no very marked inter-animal effect. The statistical analyses were thus made on the values of these slopes. The ANOVA showed that the temperature had a highly significant effect ($p < 0.001$), the salt content had a very significant effect ($p<0.01$) and the water content had a significant effect ($p<0.05$) on proteolysis evolution. On the other hand, macroscopically speaking, the muscle type had no significant effect on proteolysis, when considering SM, BF and ST muscles ($p>0.05$). After that, by means of a multiple linear regression, proteolysis evolution models were built for all muscles.

Table 3 contains all the coefficients statistically calculated to build the proteolysis velocity models, relating the studied factors and their interactions.

Table 3 Multiple linear regression coefficients calculated from proteolysis velocity for all muscles

	SM	BF	ST	RF	GM
Intercept	3,45E-02	-7,99E-03	4,84E-03	-2,80E-02	2,53E-02
T	-1,41E-02	-2,01E-03	-9,18E-03	-1,57E-02	-5,73E-03
S	-5,83E-03	3,56E-03	-7,28E-04	3,46E-03	-5,88E-03
W	-4,26E-04	2,76E-04	1,69E-04	5,68E-04	-9,77E-05
T S	-1,57E-04	-1,15E-04	-3,92E-04	-3,84E-04	-3,45E-04
T W	3,68E-04	1,52E-04	3,25E-04	4,56E-04	2,57E-04
S W	9,23E-05	-6,00E-05	5,21E-06	-4,46E-05	8,91E-05

As an example, the model of proteolysis evolution of the SM muscle fits this equation:
Slope (PI of SM) = $3.45.10^{-2}$ - $1.41.10^{-2}$ T - $5.83.10^{-3}$ S - $4.26.10^{-4}$ W - $1.57.10^{-4}$ T.S + $3.68.10^{-4}$ T.W - $9.23.10^{-5}$ S.W; where T is the temperature, S is the salt content and W is the water content.

Fig. 3 shows the response surface of proteolysis evolution of SM muscle at 70% water content (% TM), as function of salt content and temperature.

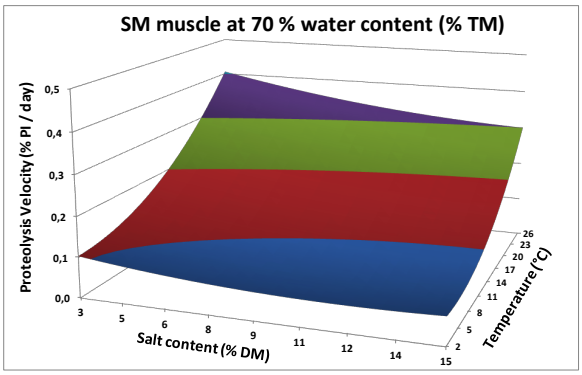

Figure 3. Proteolysis velocity of SM muscle at 70% water (TM)

At low temperature, the values of slopes of proteolysis kinetics were low and the salting effect was not so clear. When temperature approached to 13°C, proteolysis progressed but still moderately. And by rising temperature from 13°C to 24°C, the rate of proteolysis quickly progressed, especially when the salting content was low; at this stage, it is obvious that salting reduced proteolysis velocity. Through these proteolysis evolution models, the rate of protein degradation would be estimated in any conditions of temperature, time, water and salt contents for any of these five muscles.

IV. CONCLUSION

The experimental results confirmed that temperature, water and salt contents are key processing factors highly influencing proteolysis during dry-cured ham elaboration. Temperature favored proteolysis evolution, where salting and drying slowed it down. Statistical analyses reinforced the highly significant effect of temperature on proteolysis, proved a very high significant effect of water content and showed a high significant effect of salting. On the other hand, the muscle type did not have any significant effect on proteolysis, especially for the most studied muscles in dry-cured ham (BF, SM and ST muscles). Different phenomenological models were built, relating proteolysis to temperature and water and salt contents for each type of muscles. These models will be then integrated in a 3D numerical model to predict proteolysis evolution, salt/water diffusions and heat transfers throughout dry-cured ham process.

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