TEXTURE CHARACTERIZATION OF DRY-CURED HAM USING X-RAY MULTI ENERGY SPECTROMETRY

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Abstract – Application of X-ray multi energy technology in meat products is based on the different X-ray attenuation depending of tissue density. In this study, feasibility of this device to detect changes in dry-cured ham slices after inducing proteolysis by means of a proteolytic enzyme was evaluated. Classification ability of commercial samples according to its proteolysis index (defective texture level) was also tested. Results show a decrease of the attenuation spectrum when proteolysis induction times increased ($p<0.0001$). Also, attenuation decreases with water content, while it increases with salt content. A correct classification score in the validation exhibit a limited discrimination power (41.1%) showing a necessity of more studies before their implementation in the food industry.

Key Words – non-destructive, quality evaluation, proteolysis.

I. INTRODUCTION

Chemical and physical changes occurring during dry-cured ham elaboration process define the final texture of the product which is one of the main factors influencing consumer's acceptability. At the industry, the correct development of dry-cured ham texture is difficult to control and texture defects such as pastiness (which is highly related to the proteolysis intensity of the samples) occurs in an important percentage of the dry-cured ham production. Therefore, from a practical point of view, it would be interesting to develop online rapid methods able to detect defective textures before commercialization and discard those defective samples.

Several non-invasive technologies have potential for the determination of textural features of dry-cured meat products [1]. Near infrared and microwave spectroscopy have been described to be able to discriminate dry-cured hams with pastiness from those with normal texture [2-4] However, these technologies are difficult to implement at industrial conditions. Laser backscattering imaging was also related to proteolysis index, but many factors interfered on the estimation of the proteolysis index [5].

The aim of this work was to evaluate the feasibility of X-ray multi energy spectrometry to detect changes in dry-cured ham slices after inducing proteolysis by means of proteolytic enzyme. Different energy bands were analyzed for the estimation of different proteolysis intensities. The interference caused by salt and water contents was estimated. The ability to classify commercial samples depending on their proteolysis level was also assessed.

II. MATERIALS AND METHODS

Prototype device with multi energy X-ray detector

An X-ray prototype system with a multi energy detector (MXV-PACK 4010, Multiscan Technologies, Cocentaina, Spain) was used to scan the packed samples at a speed of 10 m/min. The energy of the transmitted X-rays was measured at the upper part of the device using the mentioned detector. The system acquired an image of the sample with each pixel containing

an X-ray energy spectrum of 128 channels (from 20 to 160 keV), so the size of the acquired information was a 3D matrix (1000 x 256 pixels x 128 channels). The emission conditions were 110kV and 1.5mA.

Data treatment: mean spectra and energy bands

In order to analyze the images, specific regions of interest (ROI) from each sample (*Biceps femoris* muscle) were selected on each image. These selected ROIs were analyzed using a Matlab script written in house (MATLAB, Ver. 7.7.0, The Mathworks Inc., Natick, MA, USA). The mean X-ray attenuation (S_a) for the energy channel *a* of the selected ROI was calculated after background correction and logarithmic transformation as described in equation 1.

$$S_a = \frac{-\sum_{i=1}^{p} \ln\left(\frac{I_f^{a,i}}{I_o^{a,i}}\right)}{p} \qquad \text{(Eq 1)}$$

Where I_f is the intensity of the transmitted radiation and I_o is the energy of the incident radiation at each pixel *i* of the ROI which contains *p* pixels. The calculation was done for each energy channel *a* that ranges from 1 to 128. According to Eq 1, an increase of S_a value represents an increase of the X-ray attenuation. Spectral pre-treatments before the statistical analysis were performed when needed in order to minimize spectral variations and improve the predictive ability of the models.

Different Energy bands (EB) of the energy spectra were selected. Energy band attenuation was calculated as the average attenuation channels included in energy band of the mean attenuation S_a and it was calculated as follows (Eq 2);

$$EB_{x-y} = \frac{\sum_{a=x}^{y} S_a}{y-x+1} \qquad \text{(Eq. 2)}$$

x and *y* correspond to the first and last energy channel *a* of the energy band considered. Energy bands investigated contained 20 channels.

Effect of induced proteolysis on multi energy spectra and energy bands

Twenty-two commercial dry-cured ham packages with 12 slices each were used. Proteolysis was induced by spreading 0.125 mL of a proteolytic enzyme (Delvolase®, DSM Food Specialties, France) on all the faces of each slice of the package. After, slices were immediately placed one on top of the other and vacuum packaged again. Samples were kept vacuum packaged for 48 h at 25ºC to induce proteolysis and they were scanned after several exposure times (0h, 2h, 4h, 6h, 8h, 24h and 48h). According to Rubio *et al*. [4], different proteolysis induction times can be related to a given pastiness intensity or defective level. From each acquired image, the mean X-ray attenuation for each channel and for each energy band were calculated. Afterwards, salt and water contents were determined analytically.

In order to evaluate the effect of proteolytic induction time (tightly related to proteolysis and pastiness intensity) on the X-ray multi energy spectra, a two-way ANOVA was carried out including proteolysis induction times and sample as fixed factors using XLSTAT (Addinsoft, Paris, France). Differences between proteolysis induction times were tested by means of Tukey test. In order to study the influence of salt and water contents on the evaluation of proteolysis intensity, an ANCOVA analysis was performed including proteolysis induction times as a fixed factor and salt and water contents as covariables.

Ability of the technology to classify commercial samples

Two cm thick slices from 80 hams were scanned. Afterwards, instrumental texture, salt and water contents and proteolysis index of *Biceps femoris* muscle of these samples were determined. Then, samples were distributed in different groups according to their proteolysis indexes (low/standard proteolysis, PI < 33%; moderate proteolysis, 33% < PI < 37%; and high proteolysis, PI > 37%) which are known to be related to the defective textural level [6].

A partial least square regression coupled with a discriminant (PLS-DA) analysis was used to determine the capacity of the model to distribute samples into different groups according to their proteolysis index level and thus, to analyze the feasibility to separate high defective from less defective textural samples.

III. RESULTS AND DISCUSSION

Effect of induced proteolysis on multi energy spectra and energy bands

Figure 1 shows the mean attenuation spectra for *Biceps femoris* muscle obtained from sliced dry-cured ham after different proteolysis induction times and the incident energy spectrum. Most of the total incident energy (>75%) was recorded from channel 1 to 48.

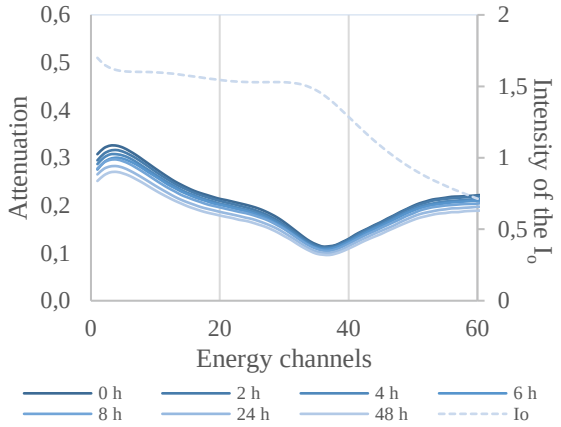

Figure 1. Mean energy spectra after different proteolysis induction times (0, 2, 4, 6, 8, 24 and 48 hours). Energy spectrum of incident energy (I_o) is also presented.

A similar attenuation curve and a maximum peak of attenuation between energy channel 32 and 40 (55 - 63 keV) for all the proteolysis induction times was observed.

A decrease in the attenuation when increasing proteolysis intensity was detected. The observed variations were attributed to the severe degradation of the tissue which increases with proteolysis induction times (or proteolysis intensity) [5]. At energies below 140 kV, attenuation of X-rays was mainly due to the photoelectric absorption and only a small part is scattered (Compton Effect). The probability of photoelectric absorption of X-rays by an atom is dependent on the effective atomic number Z (number of protons and electrons) of the sample and the density [7]. In a degraded sample, a decrease of density is expected what could explain the decrease of X-ray attenuation.

Statistical analysis shows a significant decrease of attenuation when increasing proteolysis induction times ($p<0.0001$) for all the studied energy bands of the spectra (Table 1).

Table 1. Mean and standard deviation of the attenuation for different energy bands of the spectra after different proteolysis induction times (n=22).

Hours	EB_{1-20}		EB_{21-40}		EB_{41-60}	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
0 h	0.297^{a}	0.062	0.179^{a}	0.028	0.221^{a}	0.032
2 h	0.289^{b}	0.060	0.175^{b}	0.027	0.218^{a}	0.032
4 h	0.282^{c}	0.059	0.170^{c}	0.027	0.212^{b}	0.032
6 h	0.278^{c}	0.060	0.169^{c}	0.028	0.210^{b}	0.033
8 h	0.270^{d}	0.058	0.163^{d}	0.027	0.202^{c}	0.031
24 h	0.258^{e}	0.057	0.156^{e}	0.027	0.195^{d}	0.032
48 h	0.248^{f}	0.056	0.150^{f}	0.026	0.188^{e}	0.031

^{abc} Different letters indicate significant differences ($p<0.05$) between proteolysis induction times within each calculated energy band. SD: Standard deviation; EB: Energy band.

Effect of dry-cured ham composition on the X-ray attenuation has been reported previously for both X-ray sensors [8] and multi energy X-ray sensors [10]. Therefore, the compositional differences of the samples used in the study (salt and water contents) will probably influence the attenuation.

No significant correlation was found between salt and water contents of dry-cured ham samples ($r=0.283$). Analysis of the three energy bands studied through proteolytic induction time using water and salt contents as co-variables showed a significant effect for both parameters (Table 2). In all energy bands, a negative slope for water was observed indicating that attenuation of spectra was negatively influenced by this parameter (an increase of water produced a decrease of attenuation). In contrast, attenuation was positively influenced by salt (an increase of salt produced an increase of attenuation). Because the slope value of salt content was higher than that of water content, the influence of salt content on the spectra will be probably more relevant. Slope of salt content was 5.9, 3.2 and 2.7 times higher than slope of water content for EB_{1-20}, EB_{21-40} and EB_{41-60}, respectively. However, it must be taken into account that standard deviation of water content was 2.5 times higher

than standard deviation of salt content what reduces the differences between them. In all the cases, slope was higher at low energies what suggest that influence of composition will be higher at EB_{1-20} than at EB_{41-60}.

Table 2. Slope, standard error and significance of water and salt contents used as co-variables when studying the effect of proteolysis induction time on mean attenuation spectra (n=22).

		Slope (1/%)	SE (1/%)	p
Water content	**EB_{1-20}**	-0.0024	0.0007	0.0005
	EB_{21-40}	-0.0016	0.0004	0.0003
	EB_{41-60}	-0.0020	0.0005	<0.0001
Salt content	**EB_{1-20}**	0.0143	0.0018	<0.0001
	EB_{21-40}	0.0052	0.0011	<0.0001
	EB_{41-60}	0.0054	0.0013	<0.0001

Detection of textural defects will be affected by sample composition. Besides, the proteolysis intensities obtained by using a proteolytic enzyme are much higher than those found in commercial samples. For these reasons, in order to determine the feasibility of this technology to characterize texture and/or detect textural defects, a validation using sliced dry-cured ham commercial samples is needed.

Ability of the technology to classify commercial samples

X-ray multi energy spectrometry was used to classify samples in different groups according to their proteolysis indexes. Using a PLS-DA model, the overall correct classification score in the validation was 41.1 % showing a limited discrimination power. At this moment, more sophisticated algorithms are being tested with promising results.

IV. CONCLUSION

X-ray multi energy spectrometry was able to detect changes caused by induced proteolysis in sliced dry-cured ham. Because of the important interference of sample's composition on the X-ray attenuation, more experimental work and sophisticated data treatment to classify the commercial samples according to proteolysis index level are needed.

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