# PRE-FREEZING AFFECTS TEXTURE OF COOKED HAMS: POTENTIAL INFLUENCE OF PROTEIN OXIDATION

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*Abstract*— The effect of using fresh vs. frozen raw material on the colour and texture of cooked-hams was studied in relation to the occurrence of protein oxidation. Thus, twenty Iberian fresh hams were selected and subsequently randomly divided into two groups (n=10). UF-hams were manufactured using fresh (unfrozen) hams whereas F-hams were manufactured from hams kept frozen at -40 º C for 5 months. At the end of the process, all the cooked-hams were vacuum-packed and refrigerated stored (+4 ºC) for three months until their analysis. Three muscles, namely, *biceps femoris* (*BF*), *semimembranosus* (*S*) and *quadriceps* (*Q*) from UF- and F-hams were instrumentally analyzed for colour and texture and for the amount of protein oxidation markers, α-aminoadipic semialdehyde (AAS) and γ-glutamic semialdehyde (GGS), by high performance liquid chromatography (HPLC).

Texture of cooked hams was significantly affected by pre-freezing as *S*-muscles from F-hams were harder, gummier and chewier than the UF-counterparts. Consistently, the total amount of protein carbonyls, particularly AAS, was higher in *S*-muscles from F-cooked hams than in the UF-counterparts, whereas no significant differences were detected with respect to *BF* and *Q* muscles. Significant correlations between protein oxidation and texture parameters support the possible implication of protein oxidation on the texture changes observed in cooked hams elaborated from frozen material. *S*-muscle suffered the most intense oxidative and texture deterioration as a likely result of its higher exposure to oxygen. Consequently, the protein carbonylation occurred during freezing of raw hams could have influenced the final quality of cooked hams.

*Keywords*— **pre-freezing, texture, protein oxidation.**

## I. INTRODUCTION

Cooked ham is a delicatessen meat product mainly sold in Europe, being France, Spain and Italy its major consumers.

The traditional processing of cooked hams involves the use of brine, which can be injected or infused through soaking, followed by the application of thermal treatment.

The final quality of cooked ham depends on several factors but the most important are the raw material characteristics and the processing conditions. Regarding this matter, the selection of the raw material is essential in order to improve the sensory quality of the final product (*1*).

Refrigerated hams or frozen/thawed hams could be employed in the manufacture of cooked ham. Nowadays, the use of frozen hams could offer slight advantages. For instance, the storage of the hams at frozen temperatures could lead to more homogeneous batches with sufficient quantify of product to be processed industrially. Nevertheless, chill storage could be promoted changes in physical (drip loss, texture modifications) chemical (lipolysis and lipid oxidation, protein denaturation and aggregation, changes in color) and sensory properties in the final product (*2*).

Muscle proteins are affected by freezing temperatures due to the denaturation and their proteolitic degradation that occur during chill storage. However, recent studies have demonstrated that meat proteins are also susceptible to oxidative reactions leading to the formation of carbonyl compounds during frozen storage (*3*).

This fact has been confirmed in several meat products (pork, beef, poultry, turkey and rhea), which showed an increase on the total amount of protein carbonyls after frozen storage at -18ºC. In addition, the modifications induced by protein oxidation under chill storage could cause loss of colour, flavours and nutritive value and limit the shelf-life of the meat product (*4*).

The occurrence of protein oxidation in frozen/ thawed raw material in relation with its effect on the colour and texture parameters has never been studied in cooked meat products.

Recent studies employed a specific methodology (liquid chromatography) to analyze specific protein carbonyls, namely α-aminoadipic and γ- glutamic semialdehydes (AAS and GGS, respectively) derived from oxidized proteins (*5*).

Consequently, the aim of this study was evaluated the effect of using fresh vs. frozen raw material on the texture and colour parameters of cooked-hams in relation to the occurrence of protein oxidation.

## II. MATERIALS AND METHODS

### *A. Processing of the cooked hams*

The hind legs of ten Iberian pigs, weighing between 130-140 Kg (live weight) were used as the manufacture of frozen cooked hams (F). After slaughter, the legs were frozen at -18º C during five months. Afterwards, ten hind legs from Iberian pigs with the same characteristics than the aforementioned were selected and used for the unfrozen hams elaboration (UF). Before elaboration F hams were thawed. Subsequentely, UF/F hams were injected with the corresponding brine. This operation was performed in a commercial plant. After the completion of the tumbling operation, UF and F cooked hams were vacuum packed and kept at least for three weeks at 4ºC.

After chilled storage, *Semimembranosus (S)*, *Biceps femoris* (*B*) and *Quadriceps (Q)* muscles from UF/ F cooked hams were extracted and vacuum packed and storage at -80 ºC until the corresponding analysis.

### *B. Colour analysis*

Instrumental colour (CIE L*, a*, b*; CIE, 1976) was measured on the surface of the *Semimembranosus*, *Biceps femoris* and *Quadriceps* muscles using a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ). Before each measurement the chromameter was calibrated on the CIE colour space system using a white tile. D65 illuminant and 0º standard observer angle were used. Colour measurements were made at the end of the processing of UF/F cooked hams in quadruplicate and at room temperature (~ 22 ºC).

Three colour indices were obtained: L* (lightness), a* (redness) and b* (yellowness) values. Saturation index and Hue angle (hº) values were obtained by the following equations: saturation index = (a*2 + b*2)0.5; hº = arctg b*/a*.

### *C. Texture measurements*

Texture profile analysis (TPA) was performed at room temperature with a Texture Analyser TA-XT2i (Stable Micro Systems, Surrey, UK). Four cylindrical samples (1.5 x 1.5 mm) were taken from *Semimambranosus*, *Biceps femoris* and *Quadriceps* muscles in each UF/F cooked ham sample and subsequently were subjected to a two-cycle compression test. The samples were compressed to 35% of their original height with a cylindrical probe of 5 cm diameter and a cross-head speed of 5 mm/s. Texture profile parameters were determined following descriptions by Bourne (1978) (*6*) and the SMS manual (Stable Micro Systems, Surrey, UK). All analyses were performed in triplicate.

### *D. Analysis of AAS and GGS using LC–ESI-MS*

Standard AAS and GGS were synthesised in vitro from N-acetyl-L-lysine and N-acetyl-L-ornithine using lysyl oxidase activity from egg shell membrane as described by Estévez et al. (2010) (*7*). AAS and GGS from UF/F cooked ham samples were analysed following a derivatisation procedure and a HPLC-FLD technique described by Utrera et al. (2011) (*8*).

### *E. Statistic analysis*

Data obtained from analysis were used as variables and evaluated by one-way Analysis of Variance (ANOVA). Tukey's test was performed when ANOVA revealed significant ($p< 0.05$) differences. Pearson's correlation coefficients were also calculated to establish relationships between AAS/GGS and texture parameters measurements. SPSS (v. 12.0) software (1998) was used to carry out all statistic test.

## III. RESULTS AND DISCUSSION

As can be observed in Tables 1-3, texture parameters of cooked hams was significantly affected by pre-freezing. In this regard, *S*-muscles from F-hams were harder, gummier and chewier than the UF-counterparts, whereas no significant differences were detected with respect to *Q*-muscle (see Tables 1-2). On the contrary, *BF*-muscles from UF-hams were more adhesive, cohesive and also showed higher resilience values than F-hams (see Table 3).

Table 1. Texture parameters ± standard deviation measured from *S*-muscle in UF/F cooked hams.

| | **Semimembranosus** | |
|---|---|---|
| | **UF Cooked-hams** | **F Cooked-hams** |
| Hardness (g) | $1740.80^{b} \pm 350.74$ | $2799.70^{a} \pm 440.91$ |
| Adhesiveness (g.s) | $-7.53 \pm 10.58$ | $-10.24 \pm 15.02$ |
| Springiness | $0.78 \pm 0.09$ | $0.82 \pm 0.09$ |
| Cohesiveness | $0.5 \pm 0.06$ | $0.5 \pm 0.06$ |
| Gumminess (g) | $880.57^{b} \pm 247.82$ | $1394^{a} \pm 244.61$ |
| Chewiness (g) | $694.36^{b} \pm 216.89$ | $1141.39^{a} \pm 257.24$ |
| Resilience | $0.27 \pm 0.07$ | $0.25 \pm 0.08$ |

Values with different letter $^{(a-b)}$ within the same row are significantly different ($p< 0.05$).

The increase of harder and other texture parameters in *S*-muscle during chill storage (F-hams) has been previously

reported and related to the process of oxidative damage of meat proteins. The oxidative damage of meat proteins has an impact on protein solubility leading to aggregation and complex formation due to cross links which could explain the increase in hardness of F-cooked hams (*4*).

Table 2. Texture parameters ± standard deviation measured from *BF*-muscle in UF/F cooked hams.

| | Biceps femoris | |
|---|---|---|
| | **UF Cooked-hams** | **F Cooked-hams** |
| Hardness (g) | 2662.58 ± 637.17 | 2970.63 ± 611.4 |
| Adhesiveness (g.s) | $-14.73^{a}$±138 | $-6.56^{b}$±954 |
| Springiness | 0.95± 0.02 | 0.89± 0.1 |
| Cohesiveness | $0.62^{a}$ ±0.04 | $0.57^{b}$± 0.05 |
| Gumminess (g) | 1666.7± 465.54 | 1703.91± 411.64 |
| Chewiness (g) | 1589.68± 442.46 | 1543.31 ± 491.82 |
| Resilience | $0.45^{a}$± 0.04 | $0.37^{b}$± 0.06 |

Values with different letter $^{(a-b)}$ within the same row are significantly different ($p< 0.05$).

Table 3. Texture parameters ± standard deviation measured from *Q*-muscle in UF/F cooked hams.

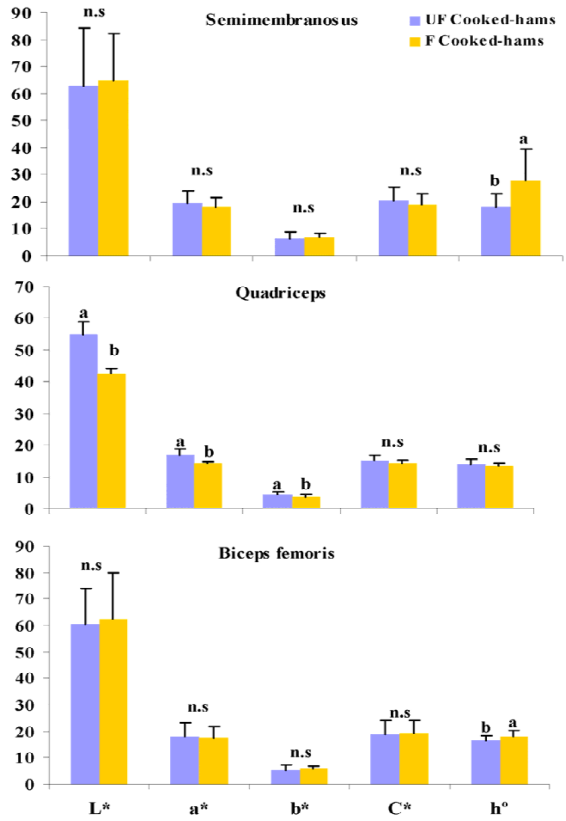
| | Quadriceps | |
|---|---|---|
| | **UF Cooked-hams** | **F Cooked-hams** |
| Hardness (g) | 2286.75 ± 846.2 | 2940.25 ± 983.39 |
| Adhesiveness (g.s) | -6.87 ± 8.02 | -9.39 ± 1.07 |
| Springiness | 0.93 ± 0.05 | 1.09 ± 0.42 |
| Cohesiveness | 0.58 ± 0.07 | 0.6 ± 0.12 |
| Gumminess (g) | 1357.24 ± 603.03 | 1820.46 ± 741.05 |
| Chewiness (g) | 1278.18 ± 613.96 | 1959.03 ± 962.19 |
| Resilience | 0.39 ± 0.1 | 0.44 ± 0.11 |

Values with different letter (a-b) within the same row are significantly different ($p< 0.05$).

CIE L*, a*, b* and chroma and hue angle measured in UF/F cooked hams are shown in Figure 1. UF/F-cooked hams presented different colour characteristics respect to *Q*-muscle, as suggested by the parameters measured. Thus, UF- hams exhibited more intense redness and yellowness values than F-hams in the *Q*-muscle. The red-brownish colour of cooked meat products is determined by the presence of denatured-globin hemochromes formed as a result of high temperatures. Additionally, the redder showed by the UF-hams in the *Q*-muscle could due to an increase on the formation of coloured Maillard products on heating, the physico-chemical state of proteins and other meat components. Moreover, an intense red colour is generally preferred by cooked meat consumers.

On the contrary, no significant differences were found in *S/BF* muscles between batches (UF/F). Only, an increase of hue angle in F-hams was observed. This fact has been previously described in raw and cooked meats subjected to refrigerated and frozen storage (*9*).

Figure 1. Instrumental colour measured from *S*, *BF* and *Q*-muscle in UF/F cooked hams.



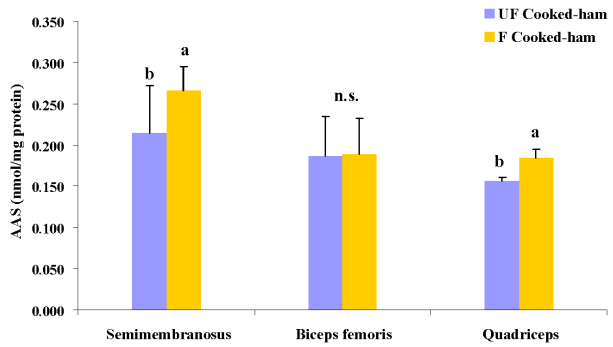

$^{a-b}$ Different letters on the bars denote statistical differences amongst products. n.s: not significant differences ($p> 0.05$).

The total amount of protein carbonyls in *S*, *BF* and *Q*-muscles from UF/F-muscles are shown in Figures 2 and 3, respectively. The total amount of AAS was higher in *S*-muscle from F-cooked hams than in the UF-counterparts, whereas no significant differences were detected with respect to *BF* and *Q* muscles. The present results confirm that muscle proteins undergo oxidative reactions during frozen storage of hams, which leads to the formation of AAS from specific amino acid residue, namely lysine. In addition, the enhanced pro-oxidant effect of iron and myoglobin as a result of the cryoconcentration around myofibrillar proteins would explain the considerably large amounts of AAS (*10*).

On the contrary, UF-cooked hams showed higher GGS values in the *S*-muscle than in the UF-counterparts.

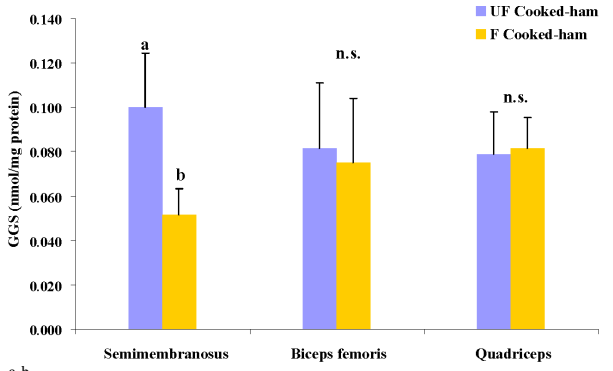
However, no significant differences were found between UF/F cooked hams in the *BF* and *Q* muscle.

Figure 3. AAS content in the *S*, *BF* and *Q* muscle from UF/F cooked hams.




[a-b] Different letters on the bars denote statistical differences amongst UF/F cooked hams.

Figure 4. AAS content in the *S*, *BF* and *Q* muscle from UF/F cooked hams.




[a-b] Different letters on the bars denote statistical differences amongst UF/F cooked hams.

Significant correlations between protein oxidation and texture parameters support the possible implication of protein oxidation on the texture changes observed in cooked hams elaborated from frozen material. *S*-muscle suffered the most intense oxidative and texture deterioration as a likely result of its higher exposure to oxygen.

## IV. CONCLUSIONS

The effect of muscle type and technological operations such as pre- frozen storage, had a great impact on the carbonylation of meat proteins. In fact, the differences between UF/F cooked hams for the relative amounts of the AAS and GGS semialdehyde were only significantly in case of *S*-muscle. According to the present results, cooked hams elaborated from frozen material presented a higher AAS content and a lower GGS content, than those elaborated from raw material. Additionally, the carbonylation of meat proteins during frozen storage seems to be linked to the occurrence of hardness and is affected by the type of muscle. Therefore, the protein carbonylation occurred during freezing of raw hams could have influenced the final quality of cooked hams.

## ACKNOWLEDGMENT

This work was supported by the CDTI (IDI 20090264) of Spain. Mario Estévez thanks the Spanish Ministry of Science and Innovation for the contract through the "Ramón y Cajal (RYC-2009-03901)" program and the support through the project "Protein oxidation in frozen meat and dry-cured products: mechanisms, consequences and development of antioxidant strategies; AGL2010-15134". Mario Estévez thanks the European Community for the economical support from the Marie Curie Reintegration Fellowship (PERG-GA-2009-248959-Pox-MEAT). Mónica Armenteros thanks the University of Extremadura (Uex) for the post-doctoral grant (Human Resources Recruitment Program "D action"). The technical assistance by the owners, managers and staff from Consorcio de Jabugo, S.A. is greatly acknowledged.

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