Biochemical and Structural Changes promoted by Beef Meat Marination

Sharedeh, D., Gatellier, P., Peyrin, F., Astruc, T. and Daudin J.D.

UR370 Qualité des Produits Animaux, INRA, 63122 Saint-Genès-Champanelle, France

Abstract—**Lipid and protein oxidations, protein denaturation and histological modifications promoted by marination were assessed on thin samples of beef meat tissue which pH (6.5, 5.4 and 4.3) and NaCl content (0.9 and 2.1 % w/w) were set by immersion. The biochemical changes which can affect nutritional quality and the increase in extra cellular space were mainly due to pH decrease. Increasing pH had no effect on the biochemical measurements and promoted swelling of cells especially marked when salt content was 2.1 %.**

Keywords— **Marination, Beef.**

I. INTRODUCTION

Meat marination has been shown to improve tenderness [1, 2], cooking yield [3] and shelf life [4]. So it is expected that new beef products from low value cuts could be developed using this process. During marination ingredients migration rates are different; this leads to steep gradients of pH and ions contents within meat [5]. Thus, it is important to quantify how much the local physicochemical properties affect both protein state and histological structure since they are related to water holding capacity and cooking yield and probably affect final juiciness. Moreover, little is known on the effect of this process on the nutritional quality.

The aim was to evaluate the biochemical and structural modifications in beef meat tissue promoted by pH and NaCl content in well controlled conditions.

II. MATERIALS AND METHODS

A. Meat samples

To focus on marinating effect without confusing effect due to animal variability all the experiments were performed on the same muscle: one *Semitendinosus* from one two years old Charolais. The muscle was removed from the carcass 24h after animal slaughter, vacuum packaged, stored 12 days at 4°C and cut in large pieces which were frozen at -18°C. Before marination, one piece was thawed until -2°C and cut into thin 6 slices : 15 g and 2mm in thickness.

B. Marination and marinated samples

Marination was performed by immersion of meat tissue slices during 20 h at 10°C to achieve equilibrium between marinade and samples. A laboratory bioreactor (Labfors, 3L) that can control the pH of the solution (HCl and NaOH) was used. The soaking conditions were defined so that the combined effects of three different meat pH (6.5, 5.4 and 4.3) and two NaCl meat contents (0.9 or 2.1 % w/w) could be tested (Table1).

After marination all the samples were cut in two halves. Six halves were used for the biochemical measurements. Four halves were used for histological analysis; they were frozen in cooled isopentane chilled by liquid nitrogen (-160 °C). Three samples of 1g each were randomly taken and grinded to control the final pH and NaCl content (Table 1). Non incubated slices were used as control.

Table 1: Immersion conditions, sample pH and salt content.

	pH		NaCl	
	target	sample	Solution	sample
Trial			g/L	% (w/w)
Control	-	5.43	-	-
1	6.5	6.53	11	0.8
2	6.5	6.48	27	2.3
3	5.4	5.42	11	0.9
4	5.4	5.38	27	2.0
5	4.3	4.39	11	1.0
6	4.3	4.31	27	2.0

C. Biochemical measurements

Lipid oxidation was evaluated on whole meat extracts by measurement of the TBARS [6]. The results were expressed as mg of malondialdehyde (MDA) per kg of meat (TBA units).

Oxidation and surface hydrophobicity of proteins were evaluated on myofibrillar proteins purified from the samples [7]. Protein oxidation:

• Protein carbonyl groups were measured by the method of Oliver *et al.* [8]. The results were expressed as nanomoles of dinitrophenylhydrazine (DNPH) fixed per milligram of protein.

• Cysteine oxidation was evaluated by the decrease of free thiols [9]. The results were expressed as nanomoles of free thiols per milligram of protein.

Surface hydrophobicity of myofibrillar proteins was determined by using a hydrophobic probe (bromophenol blue, BPB) according to the method of Chelh et al. [10]. The results were expressed as the percentage of BPB bound to protein.

Protein aggregation was evaluated on meat extracts with a granulometer (Sysmex FPIA-3000) using the method of *Promeyrat et al.*[11]. The two following descriptors were assessed:

• The equivalent circle diameter (EC) which is the diameter of the circle having the same projected area as the particle image,

• The circularity which is the ratio between the circumference of a circle with the same area as the projected area and the peripheral length of the projected image (0.4 for a fibre and 1 for a sphere).

D. Histological analysis

For each marinating condition, four sections (10 µm thick) were cut from each of the 4 samples using a cryostat at -16 °C (Microm, HM 560), mounted on slides and air dried for about 15 min at room temperature. Sections were cut so that they were approximately orthogonal to the mean meat fibres direction and stained using Hematoxylin Eosin Safran.

Colour images were acquired using an Olympus BX61 transmission white field microscope coupled to a digital camera. The mean area of the fibre cross section (CSA, μm^2) and the ratio of the extra-cellular space to the total image area (ECS, percent area) were estimated by image analysis (Image J).

One image in each section was analysed. Its size corresponded to a number of whole cells which varied from 70 to 100.

The main steps of the image analysis process were: correction of luminosity by comparison to a white image, extraction of the green channel image, binarisation using a defined threshold, delimitation of all the fibres. The calculations were automated but the latter step was manually supervised.

E. Statistical analyses

For each condition, values are reported as the mean ± standard error on mean (SEM): (1) 6 meat extracts for lipid oxidation and protein aggregation, (2) 6 myofibrillar protein purifications for protein oxidation and surface hydrophobicity and (3) 16 histological images for CSA and ECS.

Data were analysed by a two-way variance analysis (ANOVA). Where a significant effect of pH or NaCl was detected, the Student t-test was used to determine the levels of statistical significance between groups.

III. RESULTS

A. Biochemical Changes

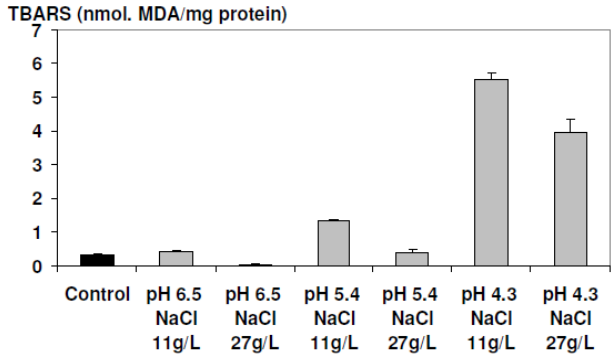

Figure 1: Effect of pH and NaCl content of marinade on lipid oxidation (TBARS; means ± SEM of 6 determinations)

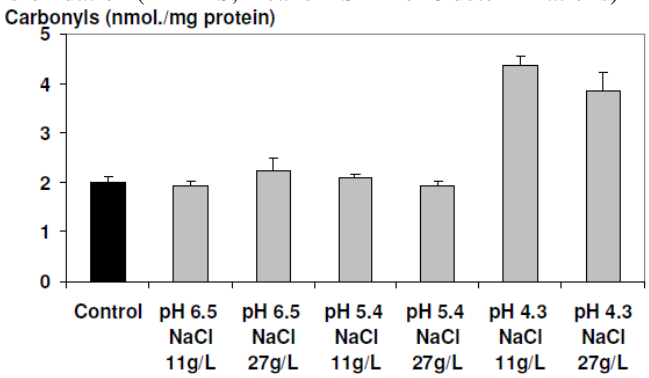

Figure 2: Effect of pH and NaCl content of marinade on protein oxidation (means ± SEM of 6 determinations)

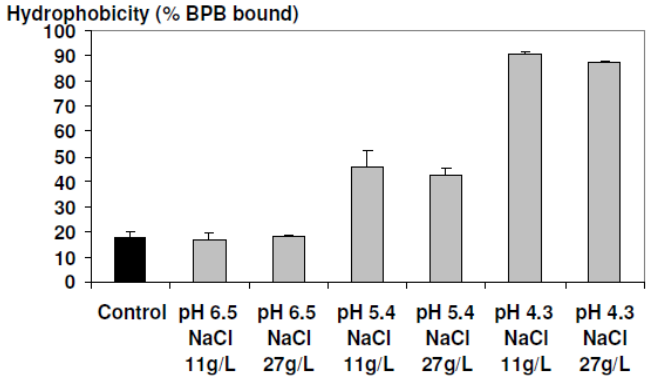

Figure 3: Effect of pH and NaCl content of marinade on protein surface hydrophobicity (means ± SEM of 6 determinations)

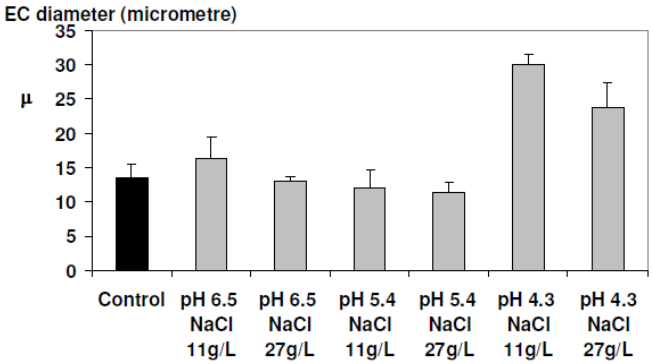

Figure 4: Effect of pH and NaCl content of marinade on aggregation evaluated by the diameter of equivalent circle of particles (means ± SEM of 6 determinations)

ANOVA indicated a significant effect of pH and salt content ($p<0.001$) on lipid oxidation with slight interaction between the two parameters. Comparison of the different marinated meats among themselves showed an important increase of lipid oxidation with decreasing pH from 6.5 to 4.3 while increasing salt content led to a decreased lipid oxidation (figure 1).

Carbonyl groups reveal oxidation of amino acids, especially essential amino acids, which decreases meat nutritional value. ANOVA shows a significant pH effect on carbonyl content ($p<0.001$) but no significant salt effect. A two fold significant increase ($p<0.001$) was observed between the group at pH 4.3 and all the other samples (figure2). ANOVA shows no significant effect of pH and NaCl on the level of free thiols.

Oxidation of lipids and proteins at low pH can be due to myoglobin oxidation, with a release of HO_2• and O_2•$^-$ radicals, and by release of "free iron" which can react with hydrogen peroxide to give OH•.

Protein surface hydrophobicity reveals protein denaturation. ANOVA showed a significant effect of pH ($p<0.001$) but no salt effect on this value. When compared with control meat, incubation at either 5.4 or 4.3 increased significantly ($p<0.001$) this value (figure 3). Surface hydrophobicity and carbonyls were highly correlated ($r = 0.866$, $p<0.001$) and promoted by acidic marination; this suggests that oxidation could be implicated in the protein structural change.

ANOVA indicated a significant effect of pH ($p<0.001$) but reveals no significant salt effect on EC. In addition, no significant effect of pH or NaCl level was observed on the mean circularity of particles. When compared with control meat, only incubation at pH 4.3 affected EC significantly (figure 4). Correlations between EC and oxidation parameters ($r = 0.682$, $p<0.001$, for TBARS and $r = 0.713$, $p<0.001$, for carbonyls) and between EC and protein surface hydrophobicity ($r = 0.615$ $p<0.001$) suggest that, by generating covalent or non-covalent inter-chain bridges, changes in the physicochemical state of proteins induced by acidic marination could promote protein aggregation.

B. Structural Changes

Figure 5 presents one representative histological image of the control sample and of all the marinating conditions tested. It is clear that all the immersion treatments strongly affect the meat tissue structure in comparison to the control. The main features that can be assess visually is that the distance between the cells increase with pH decrease and that the structure is similar at pH 4.3 for the 2 salt contents but very different at pH 6.5. For CSA and ECS, ANOVA indicates a significant effect of both pH and NaCl content ($p<0.001$) and reveals a significant interaction between pH and salt content. When compared with the control sample (i) CSA of all the treated samples were significantly different ($p<0.01$ or 0.001) and (ii), except for the case (pH = 5.4 and 27 g/L) for which there was no significant difference, all ECS values were significantly different ($p<0.001$). Figure 5 shows that CSA decreased while ECS increased when pH decreased from 6.5 to 4.3 at either low or high NaCl salt content but these variations were more pronounced in the latter case.

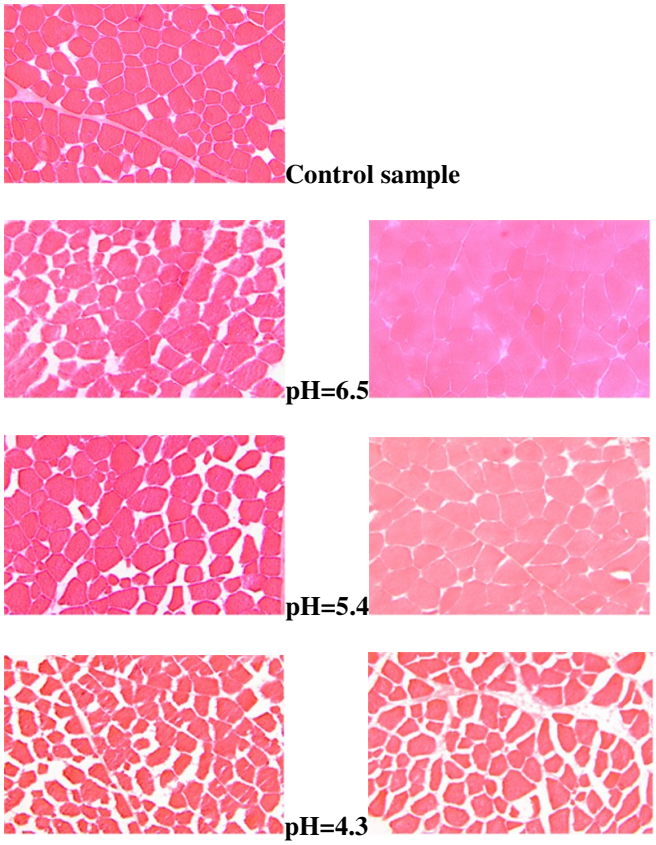

Figure 5: Examples of histological images (2290x1720 µm). Solution NaCl concentration 11 g/L (left) or 27 g/L (right).

The basic marinated meat at the usual 2% salt content corresponds to the highest CSA and to the lowest ECS, suggesting that in this case marinade uptake by meat tissue is mainly due to swelling of cells.

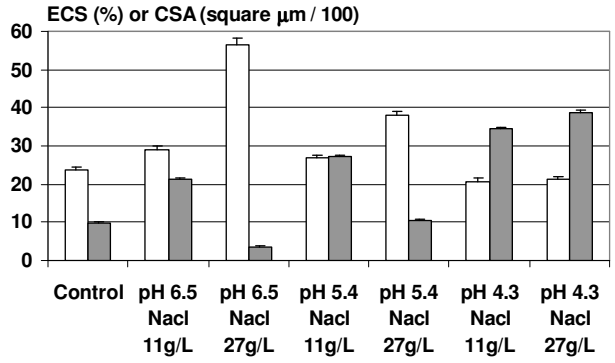

Figure 6: Effect of pH and NaCl content of marinade on CSA (white) and ECS (grey); means ± SEM of 16 images, minimum 1200 cells.

For acidic marination, CSA is just slightly lower than that of the control. In parallel ECS is about 4 times higher, showing that marinade uptake is not the result of the swelling of the myofibrillar part.

IV. CONCLUSIONS

Decreasing pH induced an important oxidation of lipids and proteins and increased the protein surface hydrophobicity. These protein changes are implicated in aggregation. Structural modifications depend on both pH and salt content. When meat pH is 6.5, marination promotes swelling of the cells which is more marked when the salt content is 2%. Acidic marination favours the increase in inter cellular spaces and salt content has little effect.

ACKNOWLEDGMENT

This work was supported by a grant awarded as part of the ProSafeBeef project under the 6th Framework Programme of the European Union.

REFERENCES

1. Burke RM, Monahan FJ (2003) Meat Sci. 63, 161-168
2. Sheard PR, Tali A (2004) Meat Sci. 68,305-311
3. Ke S, Huang Y, Decker EA, Hultin HO (2009) Meat Sci. 82, 113-118
4. Drosinos EH, Mataragas M, Kampani A, Kritikos D, Metaxopoulos I (2006) Meat Sci. 73,75-81
5. Daudin JD, Lebert A (2009) 55th ICoMST, Copenhagen, Denmark, PS 4.07.
6. Lynch SM, Frei B (1993) J Lipid Res. 34, 1745-1751.
7. Martinaud A, Mercier Y, Marinova P, Tassy C, Gatellier P, Renerre M (1997). J Agric. Food Chem. 45, 2481-2487.
8. Oliver CN, Alin BW, Moerman EJ, Goldstein S, Stadtman ER (1987) J Biol. Chem. 262, 5488-5491.
9. Morzel M, Gatellier P, Sayd T, Renerre M, Laville E, (2006) Meat Sci. 73, 536-543.
10. Chelh I, Gatellier P, Santé-Lhoutellier V (2006) Meat Sci. 74, 681-684.
11. Promeyrat A, Gatellier P, Lebret B, Kajak-Siemaszko K, Aubry L, Santé-Lhoutellier (2010). Food Chem. 121, 412-417.