INFLUENCE OF SALT CONTENT AND HEATING ON PHYSICOCHEMICAL CHARACTERISTICS OF EXUDATE FROM PORK'S TUMBLING

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Abstract – Exudates from *rectus femoris* pork's tumbling were characterized by quantification of proteins, lipids and indicators of bonds between proteins (hydrophobicity, protein carbonyl groups, free thiols). Effect of salt content (112, 165 and 220 g/l in the brine) and cooking time were studied. No influence of salt was observed on protein content and composition, on lipids and on free thiol groups. Protein surface hydrophobicity was dependent on salt content, with a maximum with 165g/l of salt in brine. Anti-oxidant effect of salt on carbonyl content appeared when exudate was heated. Heating exudate led to an increase in protein hydrophobicity and carbonyl content and to a decrease in free thiols.

Key Words – binding capacity, cooked ham, gelation, hydrophobicity, protein oxidation.

I. INTRODUCTION

Over consumption of salt (sodium chloride) is responsible for improving risk of cardiovascular disease or development of hypertension [1]. In industrialized countries, the daily consumption of salt was estimated between 8 and 10 g [1], which is higher than recommendation: between 5.75 and 8 g/d of sodium chloride according to Health Canada and to French Agency for Food, Environmental and Occupational Health and Safety (ANSES). With 20 % of the salt consumption due to meat industries [1], reducing salt in meat products is a clue to diminish salt in human diet.

In cooked ham, salt provides technical and organoleptic properties. In particular, salt is involved in binding capacity. Indeed, salt is responsible for protein solubilisation during tumbling, which leads to the formation of a sticky exudate. Then, during heating, gelation occurs in this exudate which enables to bind muscles and to obtain one unique piece of ham from several muscles. It is well documented in literature that salt improves protein solubilisation and influences gelation [2], but relation between salt content and binding capacity is poorly studied. Some studies have proved that binding capacity of proteins is improved with salt content [3, 4], but they were performed with protein extract and not directly with exudate from tumbling. The aim of the present study is to determine the effect of salt content on the physicochemical characteristics of exudate from pork's tumbling, and more particularly to quantify chemical bonds implicated in gelation.

II. MATERIALS AND METHODS

Experiments were made with pork's *rectus femoris* vacuum-packed and frozen at -20 °C seven days *post mortem*. Water content of muscles was 3.36 ± 0.13 kg/kg dry mass.

3x3x3 cm cubes were cut in frozen meat, put per five in plastic bags and thawed in water. 10 % (by mass) of brine was then added. Brine was composed of water, sodium chloride (0; 5.3 or 10.8 g/l), 11.2 g/l of salt with 0.98 % of sodium nitrite and 3 g/l of Yam (purchased from La Bovida, France). Total sodium chloride content was respectively 112, 165 and 220 g/l in brine. Samples were vacuum-packed and tumbled 15 h with massaging cycles of 5 min. rotation and 10 min. time off. Rotation speed was 7.5 revolutions/min. (STALE, Austria, with 25 l tank volume). Exudate was collected at the end of tumbling and divided in four samples: one was kept raw, and the other ones were heated at 70 °C during 30, 60 and 120 min. respectively. Cooling was made in two steps: firstly 1 h in ambient temperature and secondly 1 h at 4 °C. This thermal treatment is supposed to be representative of cooked ham industry. Samples were then stored at -80 °C for biochemical investigations.

Cured meat samples were also collected at the end of massaging for salt content measurement. Salt content is deduced from measurement of chloride

anion with ionic chromatography (Metrohm 850 professional IC): 0.5 g of cured meat was homogenised in 10 ml of pure water, centrifugation (13 000 rpm, 10 min.) was performed and solution was collected and diluted before measurement of chloride content.

Protein surface hydrophobicity was determined by using a hydrophobic probe (bromophenol blue, BPB) according to the method of Chelh *et al.* [5].

Protein carbonyl groups were measured by the method of Morzel *et al.* [6]. Carbonyl groups were detected by reactivity with 2,4 dinitrophenylhydrazine (DNPH) to form protein hydrazones.

Free thiols were measured by a modification of Ellman's method using 2,2'-dithiobis (5-nitropyridine) DTNP [6].

SDS-Polyacrylamide gel electrophoresis was performed according to the method of Laemmli [7]. Protein intensity was evaluated directly on the gel by using Quantity One.

Analyses of variance (ANOVA) were performed with R2.12.2 to determine if there were any significant influence of salt content, heating time and interactions between salt and time. Where significant effect was detected, Student t-test was used to determine the levels of statistical significance between groups.

III. RESULTS AND DISCUSSION

As expected, salt content in meat was 1.1 ± 0.2, 1.5 ± 0.1 and 2.0 ± 0.1 % respectively with 112, 165 and 220 g/l of sodium chloride in brine. 2.0 % correspond to the mean salt content in ham in France. Salt content was slightly higher in the exudate than in the meat, respectively 1.4 ± 0.2, 2.0 ± 0.3; and 2.6 ± 0.3 %.

Protein level was 69 ± 4 mg/ml whatever was salt content ($p = 0.35$). Siegel *et al.* [8] found that protein content can reach 14 %, which is a greater value than the one we found. Nevertheless, the comparison with literature is difficult because tumbling parameters greatly affect ham characteristics [9] and thus can lead to differences in protein content in exudate.

Electrophoresis revealed the nature of proteins in the exudate (Fig. 1): 14 proteins were identified. Myosin and actin were predominant in the exudate. Protein composition of exudates was representative of the meat protein composition: for example, actomyosin and myoglobin contents were respectively 49.6 ± 2.1 % and 2.0 ± 0.2 % in exudate's proteins and 35-45 % and 2.0 % in meat's proteins according to literature [10]. Protein composition was not dependent on salt level: in our conditions, protein extraction from meat to the exudate seems to be more dependent on massaging conditions than on salt content. Similarly, Siegel *et al.* [8] found that salt is no more influent on protein composition, contrary to polyphosphate (which is a forbidden ingredient in the main category of cooked ham in France).

Before heating, protein surface hydrophobicity in exudates was significantly dependent of brine salt content ($p = 0.01$) (Fig. 2). Firstly, increasing salt content from 112 to 165 g/l led to protein denaturation with an increase in surface hydrophobicity from 66 to 73 %. Decrease in protein surface hydrophobicity (from 73 to 54 %) observed for the highest salt level can be attributed to the salting out effect: protein aggregation which can mask the hydrophobic sites. During heating, hydrophobicity increased rapidly ($p = 0.00$) to reach a maximal value which was dependent on salt level, as usually happen when meat is heated [5]. Salt effect was reduced when exudates were heated, but hydrophobicity was still significantly lower

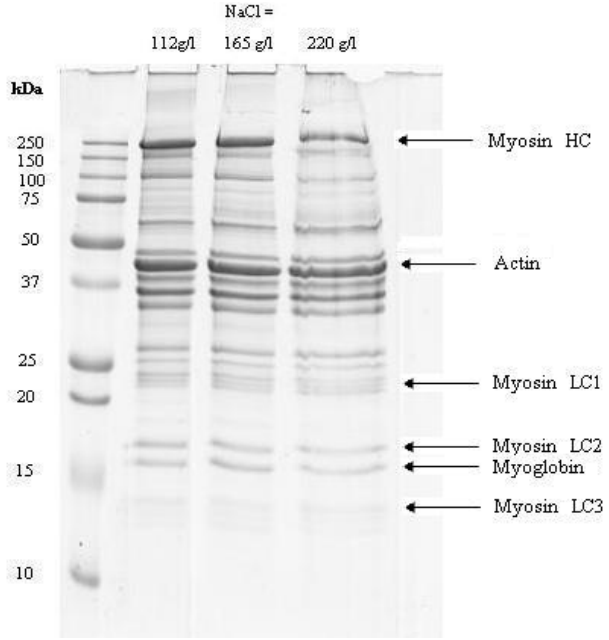

Figure 1. SDS-PAGE of exudates obtained from tumbling pork's *rectus femoris* with 112, 165 or 220g/l of salt in brine (HC: heavy chain; LC: light chain).

with 220 g/l of salt in brine. Hydrophobic bonds are involved in gelation: reducing salt from 2.0 to 1.5 % may improve binding capacity, but a more important reduction may be not beneficial in term of adhesion.

Salt did not influence carbonyl content in raw exudates (Fig. 3). During heating, an increase in carbonyl content was observed with increasing time ($p = 0.00$) and with salt reduction ($p = 0.01$) (Fig. 3). The observed anti-oxidant effect of high NaCl concentrations may result from a decreased solubility of oxygen in high ionic strength solutions [11]. Carbonyl groups could interact with free amino groups to form amide bonds, which are strong covalent bonds. Thus, by increasing carbonyl groups, reducing salt in ham may improve binding capacity.

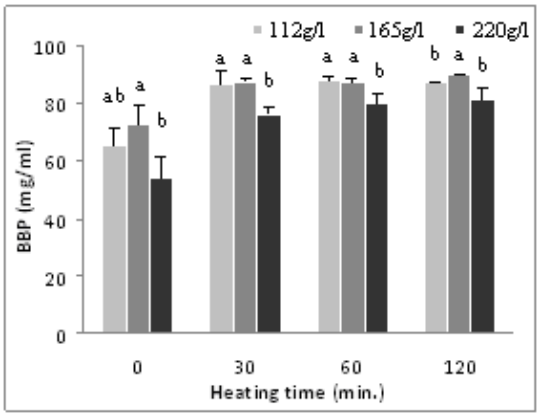

Figure 2. Effect of salt and heating time on protein surface hydrophobicity of exudates obtained from tumbling pork's *rectus femoris* with 112, 165 or 220 g/l of salt in brine. Salt effect: values not bearing common superscripts differ significantly ($p < 0.05$).

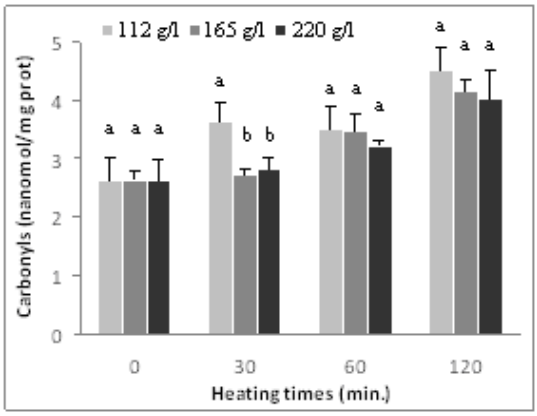

Figure 3. Effect of salt and heating time on carbonyl content of proteins in exudates obtained from tumbling pork's *rectus femoris* with 112, 165 or 220 g/l of salt in brine. Salt effect: values not bearing common superscripts differ significantly ($p < 0.05$).

Free thiol group content was not dependent on salt content ($p = 0.25$): 35 ± 1 nmol/mg of proteins in raw exudate whatever salt content. They decreased during heating ($p = 0.00$) down to 25 ± 2 nmol/mg of proteins after 2 hours (Fig. 4). Thus, heating leads to disulfide bridges formation, taken place in gelation, but these bridges are not affected by salt content.

As well as proteins, lipids can also be involved in gelation by interaction of proteins with aldehydic products of lipid oxidation. Total lipid content was then investigated and no influence of salt was observed ($p = 0.25$): total lipid content was 1.1 ± 0.1 % in the exudate, a value which is representative of fat content in *rectus femoris* (1.85 % according to Porcine Myology [12]).

IV. CONCLUSION

During our tumbling of pork's *rectus femoris* muscles, salt content in the brine did not affect protein composition and concentration in the exudate. Lipid content in the exudate remained also unchanged. Moderate reduction of salt content has increased carbonyl content and protein hydrophobicity in the exudate which should lead to a better binding of muscles during thermal treatments. However further investigations are needed to extend these results to exudates coming from industry and obtained from other pork

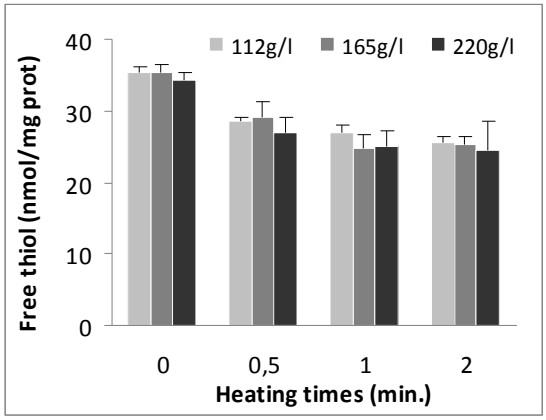

Figure 4. Effect of salt and heating time on free thiol group content in exudates obtained from tumbling pork's *rectus femoris* with 112, 165 or 220 g/l of salt in brine.

muscles or under other tumbling conditions. It is also important to connect present results on the physicochemical analysis of exudate to measurements of binding forces between muscles in cooked ham.

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REFERENCES

1. Desmond, E. (2006). Reducing salt: A challenge for the meat industry. Meat Science 74: 188-196.

2. Sun, X. D. and Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety 10: 33-51.

3. Siegel, D. G. and Schmidt, G. R. (1979). Ionic, pH, and temperature effects on the binding ability of myosin. Journal of Food Science 44: 1686-1689.

4. Macfarlane, J. J., Schmidt, G. R., and Turner, R. H. (1977). Binding of Meat Pieces - Comparison of myosin, actomyosin and sarcoplasmic proteins as binding agents. Journal of Food Science 42: 1603-1605.

5. Chelh, I., Gatellier, P., Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. Meat Science. 74: 681-683.

6. Morzel, M., Gatellier, Ph., Sayd, T., Renerre, M., & Laville, E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. Meat Science, 73: 536-543.

7. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680.

8. Siegel, D. G., Theno, D. M., Schmidt, G. R., and Norton, H. W. (1978). Meat massaging - Effects of salt, phosphate and massaging on cooking loss, binding strength and exudate composition in sectioned and formed ham. Journal of Food Science 43: 331-333.

9. Olkiewicz, M., Senik, I., and Tyszkiewicz, I. (1995). Pork meat tumbling, binding ability of exudate. In Proceedings 41st Annual International Congress of Meat Science and Technology (pp 491-492), 20-25 August 1995, San Antonio, Texas, U.S.A.

10. Pearson A.M. and Young, R.B. (1989). Muscle and meat biochemistry in Food Science and Technology. Academic Press, INC. London.

11. Mabrouk, A.F., and Dugan, L.R. (1960). A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsion in presence of sodium chloride. Journal of the American Oil Chemist's Society, 37: 486-490.

12. Porcine Myology: http://porcine.unl.edu/porcine2005/pages/showMuscle.jsp?ID=79