HISTOCHEMICAL AND BIOCHEMICAL CHARACTERISTICS OF FOUR MAJOR MUSCLES OF THE HAM

Carlier M.[1], Martin J.L.[1], Vautier A. [2], Gault E. [2], Bombrun L. [1,3], Burton O. [4], Loison O.[3], Danon J. [3], Santé-Lhoutellier V. [3] and Astruc T.[3]

[1]IFIP, French institute for pig and pork industry, F-94700 Maisons-Alfort, France,

[2]IFIP, French institute for pig and pork industry, F-3500 Le Rheu, France,

[3]INRA, UR370 Qualité des Produits Animaux, 63122 Saint Genès Champanelle, France

[4]ADIV, process engineering, technology and products quality department, F-63039 Clermont−Ferrand Cedex, France

Abstract – Reduction of salt content in processed food is an important issue for both human nutrition and industry. Ham is composed of different muscles and the impact of salt reduction on each of them is unknown. To analyze and understand the effect of salting on the evolution of ham, it is essential to know the characteristics of muscles before applying any technological treatment. Muscles *semi-membranosus*, *biceps femoris*, *rectus femoris* and *gluteus medius* were selected on their physiological differences. These muscles were finely characterized in their structure and biochemical composition. Each muscle was then cured and cooked with two brines, which brought respectively 1.3% and 1.8% of sodium chloride in the meat. Cooking yield was determined. Significant differences were observed between muscles for physical, biochemical or histological parameters of non-cured muscles and between muscles and salt contents for cooking yield. Thus, muscle characteristics have to be taken into account in any study on the optimization of salting meat.

Key Words – cooking yield, histology, meat quality, muscle composition

I. INTRODUCTION

Premium cooked ham is a major product for the French pork meat industry. In 2010, 203 007 t of “superior cooked ham” were manufactured in France, accounting for 21% of total volume of French manufactured pork meat products. Nevertheless, in France, premium cooked ham is one of the least salted pork meat products, with a sodium chloride mean content of 2.09 g/100g. Furthermore addition of phosphates is not allowed. The Fédération Française des Industriels Charcutiers Traiteurs engaged its members through a Charter for volunteer nutritional progress, signed with the Health Minister to go on with lowering sodium in premium cooked ham, as in some other pork meat products. The knowledge of the raw material, in terms of biochemical, physical and histological properties is the first step of a large project to be able to develop a mass transfer mathematical model in ham with reduced salt content.

From the 26 muscles, which are partially or completely included in the ham fabrication, 4 muscles, *semi-membranosus* (SM), *biceps femoris* (BF), *rectus femoris* (RF) and *gluteus medius* (GM) were chosen for both economical significance and various metabolic muscle properties [1,2].

The objective was to characterize finely these four muscles and imitate the manufacturing process at laboratory to establish the relationship between structure, composition and evolution of the product with variable salt levels. Biochemical and structural data gave a solid characterization of the muscles. These results allowed the study of cooking loss evolution according to muscles characteristics.

II. MATERIALS AND METHODS

8 pork gilt carcasses (Large-White x Landrace female and Pietrain male), 6 month old were selected on the basis of post slaughter weight (90.4 kg to 99.2 kg), pHu in the SM (5.60 to 5.80) and lean yield (56% to 62% lean meat). One day after slaughter the muscles (BF, GM, RF, and SM) were collected from the carcass, trimmed from fat and connective tissue, weighed and sampled for 24 h post mortem measurements. After sampling, the muscles were individually vacuum packed and stored at 4°C for 24 h before processing.

48 h post mortem muscles were used for cured and cooked process. Two different brines were prepared and added to muscles before vacuum packed in a plastic bag. Brine 1 (B1) and brine 2 (B2) respectively delivered 1.8% and 1.3% of sodium chloride in the final salted muscle. Then the muscles with brine were tumbled at 4°C for 15 h, molded and steam-cooked in oven until the core temperature reached 66°C. After heating the samples were cooled at room temperature and stored at 4°C.

The following measurements were performed on 24 h post mortem samples: drip loss [3], color (CIE L*a*b*), moisture, total phosphorus, crude protein and collagen contents. Glycogen and lactate in the muscles were determined by enzymatic method after storage at –80 °C. Glycolytic potential (GP) was calculated according to [4]: GP = 2 x glycogen + lactate, expressed in µmol of lactate / g of meat.

Structure, connective tissue, fiber types and adipocytes distribution were assessed by histology. Cryofixed serial sections (10µm thick) were stained using Hematoxylin Eosin Safran coloration [5] to visualise general structure, picro-Sirius red coloration [6] which reveals the collagen of perimysium and endomysium and red oil to highlight adipocytes [7].
Fiber type identification was based on the pH sensitivity of myofibrillar ATPase [8]. Image analysis software (ImageJ) was used to evaluate the fiber cross sectional area, and the extracellular space area. For each muscle category, about 10000 fibers were counted and analyzed.

pH measurements were performed with a pH-meter (Schott-Gerate CG 819) equipped with a Xerolyt© electrode (LoT type, Mettler Toledo).
After 4 days of storage at 4°C cured-cooked products were strained, dried with paper towels and re-weighed. Cooking yield was calculated as a percentage of final weight based on the starting weight.

Morphometrical data acquired by image analysis were expressed as mean ± SEM. Statistical analysis were conducted under 8.02 SAS software version (SAS Institute, USA), by one-way analysis of variance (ANOVA) using the GLM procedure and the unpaired Student t-test. Variance analysis, adjusted means comparison and Bonferroni tests were carried out on the other measured parameters.

III. RESULTS AND DISCUSSION

Differences between muscles are presented in table 1, figures 1 and 2. RF showed a highest pH, lowest drip loss, and lowest GP, which is in accordance with results of previous studies [1, 9].
Color parameters (lightness L*, redness a*) differed between the 4 muscles. GM had the highest and RF the lowest lightness values, while BF and RF both had high redness values. These differences appeared to be more marked than those reported in Porcine Myology [10]. In the present study, GM had the highest drip loss.

BF had the higher level of intramuscular free fat and the lowest level for total phosphorus. Connective tissue, as measured by collagen content was higher in BF but the difference with RF and SM was not significant. GM and SM had the highest level of protein content and BF the lowest one. These chemical results were very similar to those of Porcine Myology data [10].

RF presented the highest pH and the lowest glycolytic potential, which affected positively its drip loss. As expected, less salt reduced the cooking yield, so brine B1 gave higher cooking yields than brine B2. Differences were significant only for BF and GM muscles.

Cross sectional area of fibers and connective tissue area varied with muscle category (figures 1 & 2). The percentage of the different fiber types were:
- RF type I 22%, IIA 18%, IIX/IIB 60%,
- GM type I 7%, IIA 7%, IIX/IIB 86%,
- RF type I 33%, IIA 35%, IIX/IIB 32%,
- SM type I 2%, IIA 6%, IIX/IIB 92%.

Highest connective tissue area in BF, was coherent with biochemical result. RF, was the more oxidative had the lowest glycolytic potential, the highest pH and redness score (a*), and the lowest lightness (L*). All these results are in accordance with previous data.
Extra cellular space areas (figure 2), the results were not statistically different for GM and RF.

They were the same for SM and BF too, but larger. Cross sectional areas were dependent on muscle ($p<0.01$) and RF fibers were the most circular ($p<0.01$).

Glycolytic muscles typically show higher cooking losses than oxidative muscles [11]. Similarly, high lipid content reduces cooking losses [12, 13]. But our results indicate that BF, is significantly more oxidative and two times richer in fat than GM and SM, presents highest cooking losses. This result suggests that metabolic type and lipid content do not necessarily explain the lower cooking yield. This observation could be due to the lower salt penetration in BF muscle fibers. Complementary sodium measurements will be performed to assess salt content in muscles. Our results showed that a decrease of 5 g of salt / kg resulted in a loss in a 3% loss of cooking yield whatever the muscle considered. It therefore appears that in our conditions, the biochemical and structural characteristics of the muscles have little effect on the evolution of cooking yields after salting.

IV. CONCLUSION

Biochemical and histochemical properties differed largely between the four studied muscles. Histochemistry results were in accordance with chemical data and gave information about repartition of intramuscular fat tissue, collagen and fiber typing.. Variations of instrumental measures (color, drip loss, cooking yield) were mostly explained by composition and metabolic type of the muscles. Cooking loss were dependent of muscle but interestingly decreasing salt reduce the cooking yield of a similar value whatever the muscle. The *biceps femoris* muscle had the highest cooking loss despite its metabolic and fat content features. Further studies are needed to relate these properties to the physical-chemical state of the proteins due to processing.

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Table 1. Results by muscles

	biceps femoris	*gluteus medius*	*rectus femoris*	*semi-membranosus*	P-value
n	8	8	8	8	
Muscle weight (g)	1787 a	891 c	563 d	1204 b	< 0.001
L*	44.1 bc	51.4 a	40.9 c	46.3 b	< 0.001
a*	13.5 a	8.0 b	12.4 a	8.6 b	< 0.001
b*	4.3 a	3.5 ab	3.0 b	3.4 ab	0.01
pH	5.84 b	5.72 b	6.11 a	5.74 b	< 0.001
Drip loss (%)	1.7 b	3.8 a	0.3 c	1.8 b	< 0.001
Glycogen (µmol lactate / g meat)	14.6 ab	16.5 a	12.8 b	16.1 a	0.01
Lactate (µmol lactate /g meat)	75.5 a	84.0 a	57.9 b	82.7 a	< 0.001
Glycolytic potential (µmol lactate /g meat)	105 a	117 a	84 b	115 a	< 0.001
Moisture (%)	74.1 b	74.8 ab	76.2 a	75.1 ab	< 0.001
Free fat (%)	4.5 a	2.4 b	1.7 b	2.0 b	< 0.001
Protein (%)	21.0 b	22.1 a	21.4 ab	22.3 a	< 0.001
Collagen (%)	0.8 a	0.6 b	0.7 ab	0.7 ab	0.05
Phosphorus (% P_2O_5)	0.484 b	0.513 a	0.508 a	0.505 a	0.01
Cooking yield, brine B1: 18 g salt /kg	84.4 c	89.7 a	88.3 ab	89.3 ab	< 0.05
Cooking yield, brine B2 : 13 g salt /kg	81.2 d	87.0 bc	85.8 bc	86.8 bc	

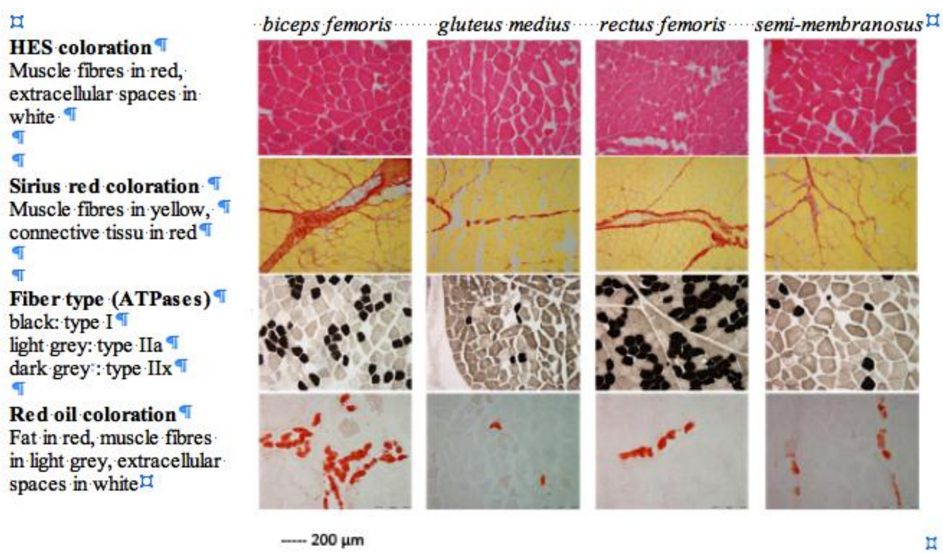

Figure 1. Histological colorations of the 4 studied muscles

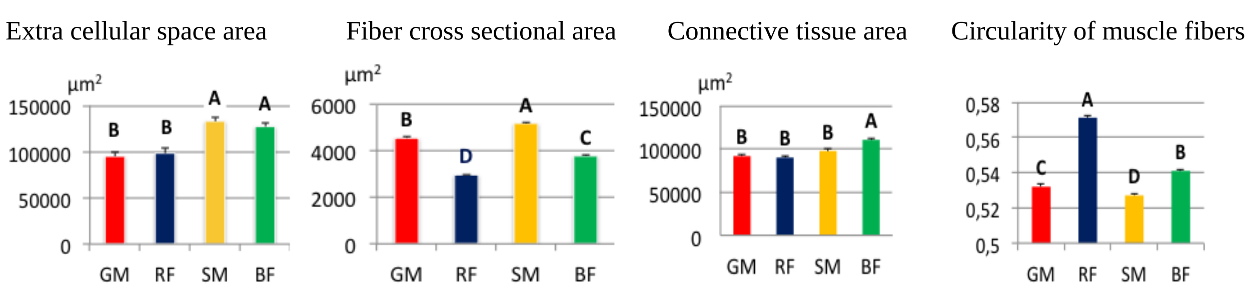

Figure 2. Morphometric data in the 4 studied muscles