Salt (NaCl) diffusion and distribution in rat skeletal muscle

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Abstract— **In order to understand the mechanisms involved in salt transfer through the skeletal muscle, we determine the *in situ* distribution of the Na^+ and Cl^- ions in salted skeletal muscles of rat to learn about the role of the connective tissue layers in salt diffusion.**

Keywords— **X-Ray microanalysis, sodium chloride, rat *Gastrocnemius* muscle.**

I. INTRODUCTION

Overconsumption of sodium chloride markedly increases the risk of hypertension and cardiovascular diseases as well as the incidence of stomach cancer, kidney stones and osteoporosis in humans [1,2,3]. Decreasing the salt consumption is thus an important recommendation of the World Health Organization. In this manner, the decrease of salt quantities in muscle-based products becomes very necessary because they contribute for nearly 15% to the sodium chloride dietary intake. However, in meat products, the NaCl is not simply a savour enhancer. It increases shelf life, conserves water and improves the juiciness and the texture of the end products. Thus, to optimize salting processes and salt addition, and to preserve sensory and technological qualities of meat products, it is necessary: 1) to understand the mechanisms involved in ion transfers through the different skeletal muscle compartments (muscle fibres, adipocytes, connective tissue layers); and 2) to establish the links between the local content of these ions and the tissue structure changes. Due the soluble nature of sodium chloride, there are no current histochemical techniques able to stain and identify sodium (Na^+) and chlorine (Cl^-) at high concentration in tissues. Then, the objectives of this study were to determine Na and Cl distribution in salted rat skeletal muscles by X-ray microanalysis and to evaluate the role of connective tissue layers on salt diffusion.

II. MATERIAL AND METHODS

A. Animals and samples

Five rats (*Rattus norvegicus*) were euthanized at the experimental facilities of INRA Theix, France. Then, the ten *Gastrocnemius* (GN) muscles were dissected from the hind legs in order to be used for two different salting procedures: brining and dry salting.

After 5h post-mortem the GN muscles were immersed in a 1M NaCl brine solution during 0 (control), 5, 10, 20 or 30 minutes. In the dry salting procedure the GN muscles were covered with NaCl crystals during the same period of time, i.e. 5, 10, 20 or 30 minutes.

Samples were cross-sectioned, frozen in cooled isopentane chilled by liquid nitrogen (-160°C), freeze dried and carbon sprayed.

B. Elemental X-ray Microanalysis and data processing

The samples were fixed on a plate cross area section on the top, and introduced into the chamber of a scanning microscope (SEM, Jeol JSM-5910) equipped with a system of X-ray microanalysis (EDS diode Si[Li], Princeton Gamma-Tech) to obtain elementary maps.

The data were processed using Matlab and ImageJ software.

III. RESULTS AND DISCUSSION

Results obtained after 30 min of brining or dry salting of rat GN muscles are shown in Fig 1 and 2 respectively, with the photomicrographs of the visible field of view (1), chlorine ion map (2), K, Cl, Na and

S relative value along sample profile (3). The extent of sodium and chlorine distribution was greater and more homogenous in brined samples than in dry salted samples. A higher concentration of Na and Cl was observed close to the edge of the samples than in the core after brining. Moreover, the levels of these elements increased slightly and constantly, over the entire surface of the sample section, during the increase of the brining time from 5 to 30 minutes. This indicates the relative permeability of muscle structures (connective tissue layers and membrane cells) to salt in solution.

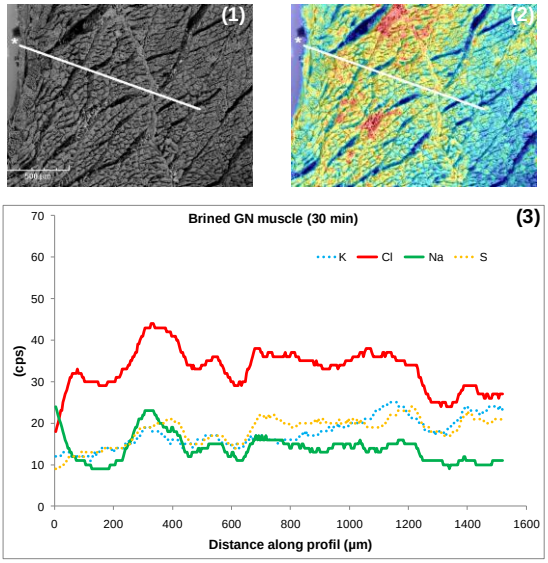

Fig. 1 Sodium and chlorine diffusion in rat *Gastrocnemius* muscle after 30 min brining. 1) visible field of view, 2) chlorine diffusion map, 3) K, Cl, Na and S relative value along sample profile.

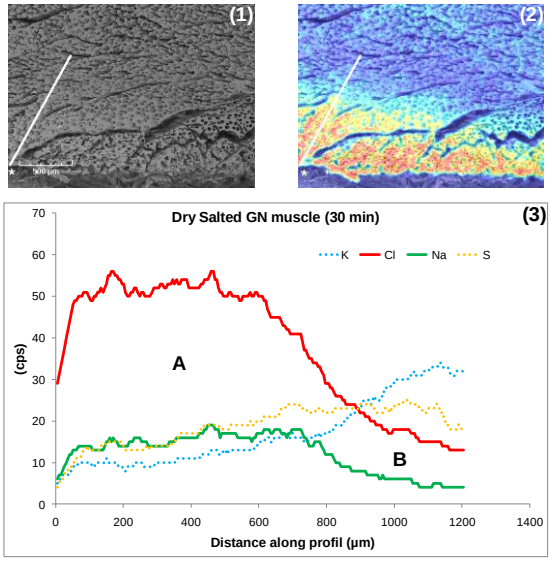

Fig. 2 Sodium and chlorine diffusion in rat *Gastrocnemius* muscle after dry salting. 1) visible field of view, 2) chlorine diffusion map, 3) K, Cl, Na and S relative value along sample profile (A) sample edge with high concentration of Na and Cl; and (B) central muscle area with basal concentration of Na and Cl.

On the other hand, dry salting has generated a massive and marked concentration of Na and Cl at the edge of muscle samples, and a basal level of these ions in the centre of the salted samples, regardless to the incubation time, giving rise to a biphasic curve of salt penetration (Fig. 2). As a result of the high concentration of NaCl on the surface of muscle during dry salting, the structure of myofibres and the organization of muscle tissue located in the periphery of the muscle were significantly affected, because of the denaturation and solubilisation of muscle proteins related to the high ionic strength [4]. This phenomenon increases with the length of dry salting.

The *Gastrocnemius* muscle of rat is known to contain important layers of dense connective tissues. The figure 3 shows the presence of connective tissue layers inside the sample and their influence in salt diffusion. In the present study we observed that the layers of connective tissue seems to attract and facilitate salt diffusion through muscle samples by acting as a carrier of the brine from the edge to the centre of muscle. This effect was quite evident in brined muscle samples, but less obvious when the dry

salting procedure was applied. The connective tissue layers could be implicated in the salt diffusion by the interaction between their own constitutive electrically charged residues of proteins and the sodium and chlorine ions of the brine. However, further investigation is needed to confirm this hypothesis, improving the sample preparation in order to better preserve the cells morphology and the soluble ions localization. It is also relevant to explore the physicochemical mechanisms involved in the affinity between ions and ions transfer in biological tissues. Our laboratory is currently working to clarify these questions.

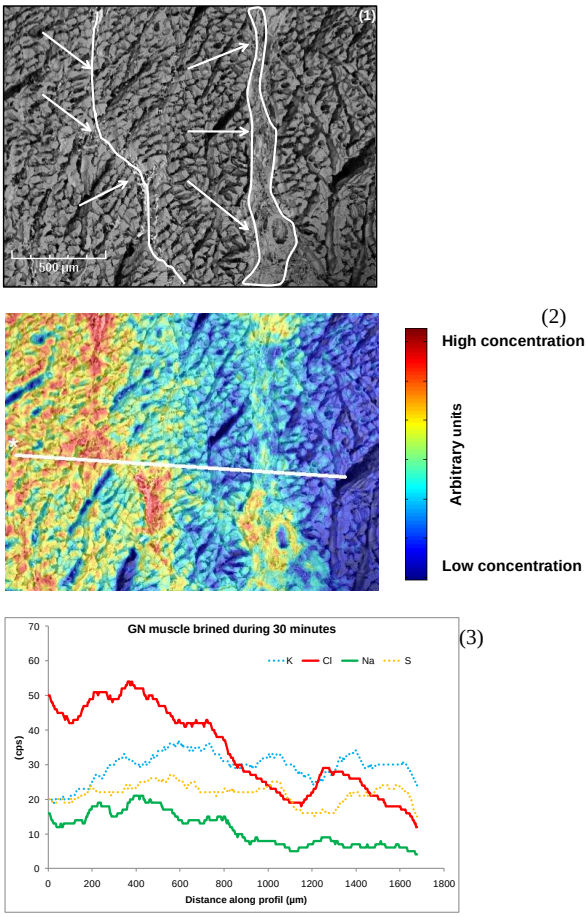

Fig. 3 Effect of connective tissue layers on sodium and chlorine diffusion after 30 min brining. 1) visible field of view, arrows indicates connective tissue layers 2) chlorine diffusion map, 3) K, Cl, Na and S relative value along sample profile.

IV. CONCLUSIONS

Methodologically, the X-ray microanalysis is particularly suitable for the detection and localization of elements chlorine and sodium in a biological sample. However, our equipment requires a dehydratation of the sample. Although lyophylization limit the relocation of water-soluble elements it significantly alters the tissue ultrastructure. The use of cryofixed biological material and Environmental Scanning Electron Microscope equipped with a cryo stage should enhance the quality of elementary maps by preserving both the structure of the tissue and the initial location of the ions.

Our preliminary results indicate that the brine penetrates more rapidly and more evenly than dry salt. The frames of connective tissue appear to play the role of drains and concentrate the sodium ions and chlorine.

Previous work suggested that the frames of connective tissue could act as a barrier to the diffusion of salt [4]. It is possible that the composition of these perymysium frames and in particular their content in adipocytes, and therefore in lipids, affects the salt diffusion. On the other hand, the structural organization of these frames, more or less loose, and the level of crosslinking of collagen which composes them, could influence the parameters of transfer of salt in animal tissues.

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