HISTOLOGICAL CHARACTERISATION OF THE EFFECT OF ADDED SALT IN FRESH BEEF SAUSAGE

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Introduction

The EU regulation (EC) N° 853 /2004 on the hygiene of products of animal origin which applies from January 1st, 2006 assigns meat preparations to meat if the product has undergone a process sufficient to modify the internal muscle fibre structure of the meat. Therefore histology was used in this study to characterise changes in the muscle fibre structure of fresh beef sausages due to the addition of salt and to try to assign products to the EU-definitions.

Materials and Methods

Minced meat with 3 levels of sodium chloride: 0, 0.8 and 1.6% was prepared from various cuts of beef forequarters. Three samples of each mince (1x1x1cm^3) were frozen in isopentane chilled by liquid nitrogen (-160°c). Frozen sections (10 µm thick) were stained using Hematoxylin Eosin Safran (HES) coloration to visualise general structure, and picro-Sirius red coloration (Flint and Pickering, 1984) which reveals the collagen of perimysium and endomysium. Immunohistology was done using antibodies against myosin and laminin. Histological sections were studied with a Polyvar Reichert microscope, Sony CCD video camera, computer with a Matrox image acquisition card and image analysis software (Noesis, France).

To quantify muscle structures, sections were analysed with an optic microscope using a 25x lens, by systematic vertical then horizontal scanning. One random field of every four was photographed. Fifty images were captured per section with triplicate repeats by level of salt (150 images per treatment group). Each image was visually assessed and assigned/ ranked to a class as: 0 class (all fibres of the field are intact, Figure 1A), 25 class (1 to 25% of the field is damaged), 50 class (26 to 50% of the field damaged), 75 class (51 to 75 % of the field damaged, Figure 1B), 100 class (76 to 100% of the field damaged, Figure 1C).

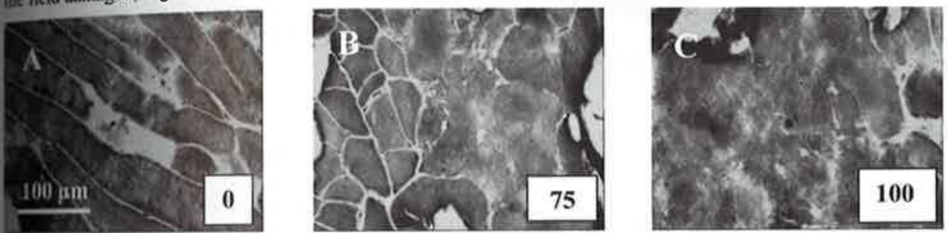

Figure 1: Distribution in classes in relation with % damaged fibres (1A 0%; 1B 75%; 1C 100%).

Statistical comparison of values of % damage for the three levels of salt was done using ANOVA and comparison of means with the "Linear Model" procedure (Statistical Analysis System).

Results and Discussion

As a first approach, suggested by a previous study (Ripoche *et al.*, 2001), to evaluate the effect of salt on muscle structure, in response to an EU request allowing characterization of minced meat, we decided to evaluate the sarcomeric A band as a potential indicator structure. Sarcomeric striations were evident in sections colored with HES or immunohistology (Figure 2, staining of myosin to show sarcomeres). Visualization depends on the sarcomeres being chosen from longitudinal cuts to the fibres. In fact sarcomere orientation was too random in minced meat to allow quantification. Therefore we next evaluated sarcolemma to sarcomere attachment as an index of salt and grinding. We also used a scoring of general fibre structure as an index of meat integrity.

Using the criteria of general fibre integrity and fibre to fibre attachment as our damaged fibre index it is apparent that salt added to mince at both 0.8 and 1.6% causes extensive loss of normal fibre structure. It is evident in these sections, as reported by Offer and Knight (1988), that the perimysium is a barrier of salt diffusion in minced meat, as shown by the fibre damage being limited by the perimysium (Figure 3). The endomysium was not a damage barrier. Quantification of fibre damage (Figure 4) shows that virtually all fibres in control samples were classified as intact, whereas 0.8 and 1.6% salt resulted in 49% and 18% of fibres remaining intact respectively. 1.6% salt was necessary to cause extensive fibre damage (42% of fibres) with 0.8% salt causing intermediate damage. Statistical analysis indicated a significant difference (P<0.05) between three levels of salt.

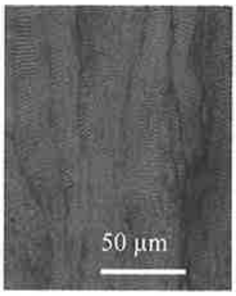

Figure 2 : Antibody staining of myosin.

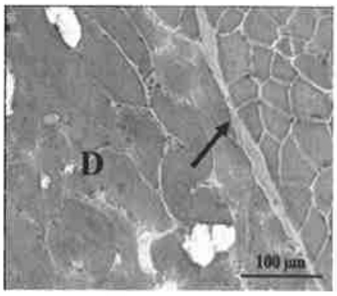

Figure 3: Damaged fibres (D) HES coloration Perimysium: arrow.

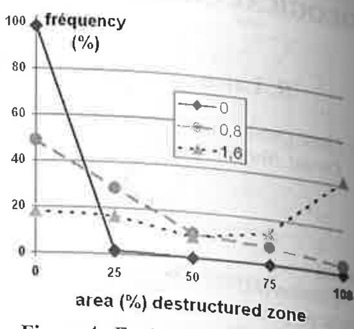

Figure 4 : Evolution of % fibre damage in relation to salt concentration.

We also evaluated other staining methods as tools to characterise fibre damage. Antibody staining of laminin, basal lamina's constituent, was used. As shown in Figure 5A, red sirius staining of connective tissue, also tested, highlights the endomysium sufficiently to see that it is continuous around intact fibres but not at damaged fibres. Image analysis was applied to determine if automatic quantification was possible.

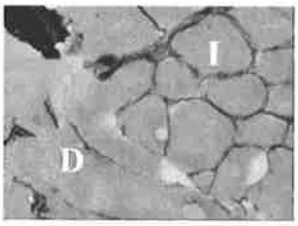

Figure 5A: Red sirius staining of endomysium.

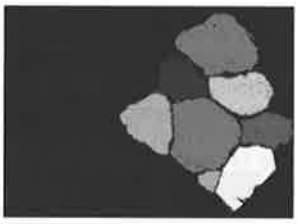

Figure 5B: Image analysis.

As shown in Figures 5A and 5B, intact fibres (I) can be identified and the boundaries determined but for damaged regions (D) we were not able to mark boundaries which by visual inspection corresponded to these regions. However, since this test should be applied to determine industry standards, an automated method is desirable.

Conclusions

These results demonstrate that meat structure standards can be developed using standard microscopy techniques. HES staining of frozen sections coupled with visual classification of fibre destruction clearly distinguishes intact control samples from altered muscle fibres in ground sausages with added salt. However, due to the variability of fibre orientation and damage, automated image analysis with and without specific antibody stains cannot by applied routinely. The perimysium is the barrier limiting salt penetration in meat samples, so further investigation on the relationship of meat beef standards should consider connective tissue content as an important variable.

References

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