Effect of Freezing and Thawing on the Histology and Ultrastructure of Buffalo Muscle

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ABSTRACT : Histology and transmission electron microscopy studies were carried out on buffalo muscles that were subjected to repeated freeze-thaw cycles at -10 and -18°C. In the first freeze thaw cycle (-10°C) structures of muscle showed slight change and closely resembled to those of normal muscle. There were frequent gaps in the half way across the fibres and some cracks in individual fibre were also noticed in second freeze thaw cycle. In the muscle frozen at -18°C, more pronounced shrinkage with extensive damage of fibres with tearing was observed. The interfibrillar gaps were wider, shrinkage and tearing of the fibres were more distinct after second freeze-thaw cycle. After the second cycle, the interior portion showed large scale degradation of the ultrastructure. Our studies of buffalo muscle showed that under the proper condition, little structural damage takes place in the meat histology and ultrastructure under repeated freeze-thaw conditions. This study adds continued weight to the evidence that limited freeze-thaw cycles will not deteriorate the quality of meat. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1291-1295)***

Key Words : Freezing, Buffalo Muscle, Thawing, Histology, Ultrastructure

INTRODUCTION

In India, exportation of frozen buffalo meat is the most important item in international meat trade, especially for Middle East countries. Freezing involves the removal of heat accomplished by a phase change, converting water to ice. Meat while in remaining unfrozen portion, the concentration of dissolved substances increases and water activity of product decreases. The rate of freezing determines the position and size of ice crystals. In slow freezing, ice crystals are formed intercellular (Grujic et al., 1993). In general, fast freezing is conducive to the formation of small ice crystals which are located intracellular but cause little physical damage to meat components. On the other hand, a slow freezing process favors large ice crystals to form, extracellularly, which results in disruption of muscle cells and cause exudation.

Meat shows a gradual deterioration in quality with frozen storage (Miller et al., 1980; Ziauddin et al., 1993). The value of meat as an export commodity is adversely affected by product deterioration during transportation and frozen storage. A substantial portion of the weight losses is due to formation of fluid, reddish exudates called drip (Strange, 1987) which contains valuable nutrients (Sen and Sharma, 2004).

Most of studies to date have focussed on changes in physical, chemical or sensory properties of the frozen meats (Sakata et al., 1995; Farouk and Swan, 1998; Hildrum et al., 1999; Boles and Swan, 2002). Very little work has been devoted to microscopic or ultra structural investigation of these systems. One histological study (Bevilacqua et al., 1979) centered on the formation of ice crystal in frozen meat and was able to relate, qualitatively, ice crystal damage to other deleterious effects of frozen storage. The first electron microscopy study to appear was an ultrastructural study on frozen cod (Jarenback and Liljemark, 1975) by transmission electron microscopy of both intact fish muscle and extracted myofibrils. Carroll et al. (1981) examined the ultrastructure of bovine muscle as a function of long term frozen storage as well as of repeated freeze-thaw cycles. The ultrastructural differences were also observed after thawing pork muscles stored at the higher temperatures (Feldhusen et al., 1992; Ngapo et al., 1999).

In the present study, we examined the histological changes in buffalo muscles due to freezing and thawing. We also utilized transmission electron microscopy (TEM) to examine the ultrastructure of frozen and thawed buffalo muscle. Our objectives were to determine structural changes that take place as a function of freeze-thaw cycles.

MATERIALS AND METHODS

Adult female Murrah buffaloes (About 10 years of age) were slaughtered at the buffalo slaughter house of Bareilly Municipal Corporation. *Biceps femoris* muscles were collected within one hr of slaughter, packed in low density polyethylene (LDPE) bags and brought to the meat technology Laboratory of LPT Division, IVRI, UP. Eight muscles from different animals were collected and the best were selected from the photographs. For the cyclic freeze-thaw experiments buffalo muscles were frozen at -10°C and

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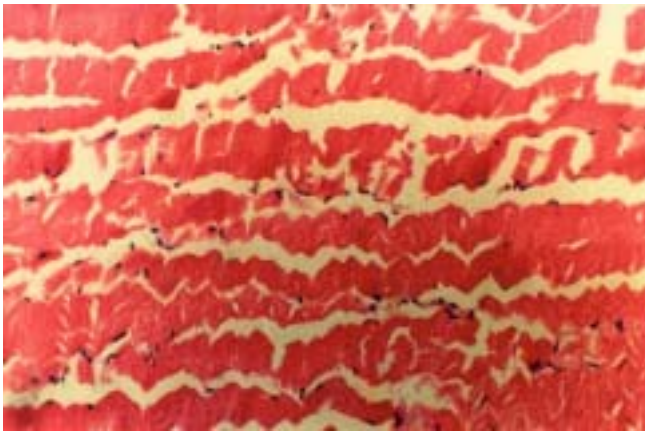

Figure 1. Section of muscle (normal) showing no change (H&E ×100).

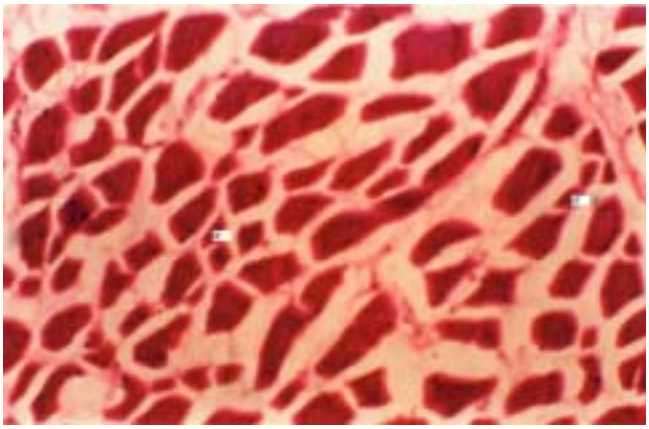

Figure 2. Section of muscle (after one freeze thaw cycle -10°C) showing shrinkage of fibre with nuclei towards periphery (H&E ×100).

-18°C for five days and thawed at refrigerated temperature (4±1°C) for 24 h. In this way, freezing and thawing was continued for two cycles. For microscopic studies, samples were taken from both the temperature but for ultrastructure, samples were taken only frozen at -10°C.

Histological

Samples of fresh and frozen-thawed meat of 0.5 mm thickness were fixed in 10% formal saline for 48 h. The formal saline fixed tissues were processed and embedded in paraffin bocks. The paraffin section of 4-5 µm were cut by conventional methods. The microsections were stained by haematoxylin and eosin, examined under microscope and typical locations of all samples were photographed (Disbrey and Rack, 1970).

Electron microscopy

Electron microscopy was done as per the procedure of Hyat (1981). Small pieces (1 mm^3) of fresh and frozen-thawed (-10°C) buffalo muscle was collected and fixed the

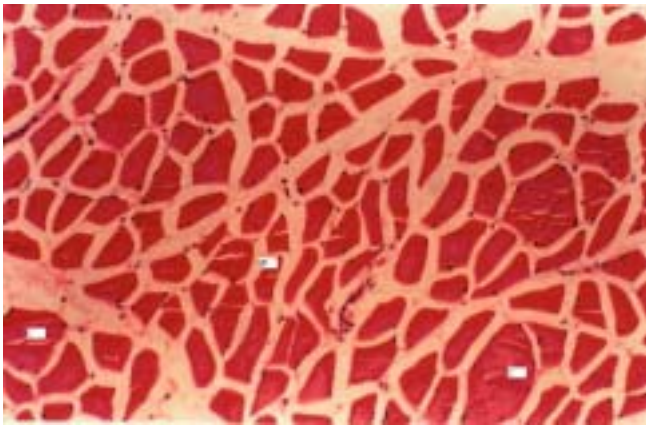

Figure 3. Section of muscle (after two freeze thaw cycles at -10°C) showing shrinkage of fibre (H&E ×100).

tissue in 2.5% glutaraldehyde for overnight at 4°C. Following fixation the tissues were washed in 0.1 M phosphate buffer, pH 7.2. The tissues were then post fixed in 1% osmium tetraoxide for 2 h at 4°C and again washed in phosphate buffer.

The tissue samples were subsequently dehydrated using dry acetone. Later these were changed to epoxy propane to remove the acetone and infiltrated with the liquid resin at room temperature. The processed tissue blocks were embedded in the liquid Araldite medium and kept at 50°C in a special oven of 12-24 h for polymerization. The temperature is then raised to 60°C and the embedded tissues are kept for 24-48 h. Then thick section (0.5 µm) were cut and stained with 1% toludene blue. Ultra thin sections (70-80 nm) were cut in ultramicrotome (ultra cut E) and lifted on copper grids. Then stained with saturated solution of uranyl acetate in alchohol for 15 min and lastly counter stained with lead citrate for 10 minutes. These processed ultrathin sections were observed in Transmission Electron Microscope (Philips CM-10 model).

RESULTS AND DISCUSSION

In the histological sections of normal muscle, fibres were intact without any significant gap in between the fibres (Figure 1). In the first freeze thaw cycle (-10°C) structures of muscle showed slight change and closely resembled to those of normal muscle. Muscle fibres revealed mild shrinkage with some gaps in between them (Figure 2) the fibres were separated from one another by approximately equal spaces. Like wise Caroll et al. (1981) also showed some compaction of the structure in bovine muscle after freezing and thawing but most parts had the open structure indicative of well-preserved muscle. On the other hand in our similar type of experiment, buffalo meat quality could be preserved after four freeze thaw cycles

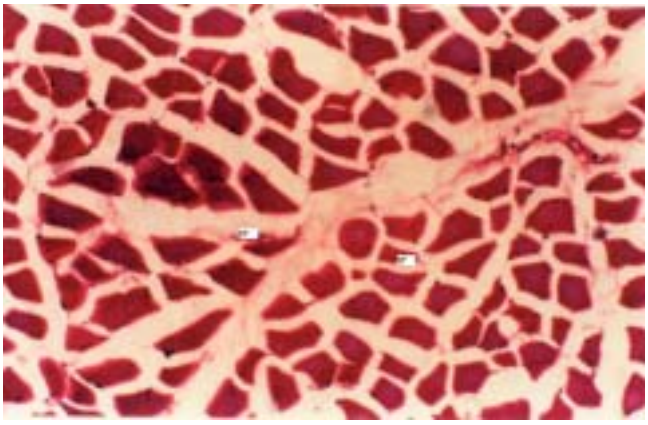

Figure 4. Section of muscle (after on freeze thaw cycle at -18°C) showing mild shrinkage of fibre (H&E ×100).

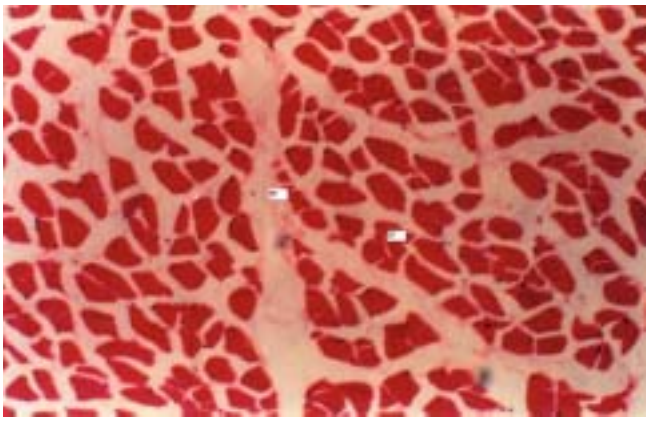

Figure 5. Section of muscle (after two freeze thaw cycles -18°C) showing extensive damage of muscle fibre (H&E ×100).

without any health hazards (Sen and Sharma, 1999). Muscle fibre after second freeze-thaw cycle at -10°C were markedly damaged with pronounced shrinkage and nucleus was pushed towards periphery; there were frequent gaps in the middle of fibres and some cracks in individual fibre were also noticed (Figure 3).

In the muscle frozen at -18°C, more pronounced shrinkage with extensive damage of fibres with tearing was observed (Figure 4). The interfibrillar gaps were wider, shrinkage and tearing of the fibres were more distinct after second freeze-thaw cycle (Figure 5). The histological changes in muscles due to repeated freezing and thawing can be compared with the findings of Rahelic et al. (1985). In general, the critical aspect of the preservation of structure in repeated freezing of meat seems to be in the time/temperature of thaw cycle. Although it is not applicable in our experiment because our thawing conditions were similar. The markedly damage of the muscle fibre in the second freeze-thaw cycle could be explained by the fact that, in such muscle, large ice crystals are formed. The crystals formed between fibres will

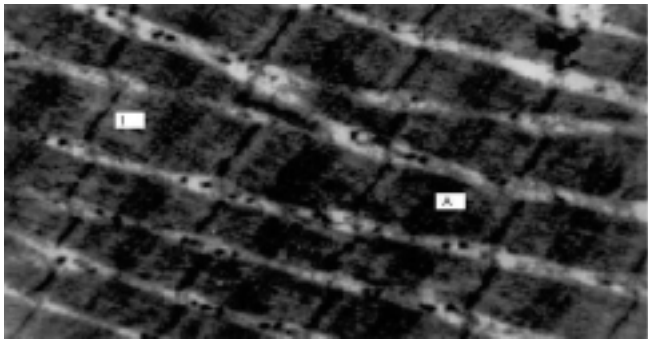

Figure 6. Electron micrograph fo normal muscle tissue (×3,400) showing ‘A’ band, ‘I’ band, ‘Z’ line, ‘M’ line and ‘H’ zone.

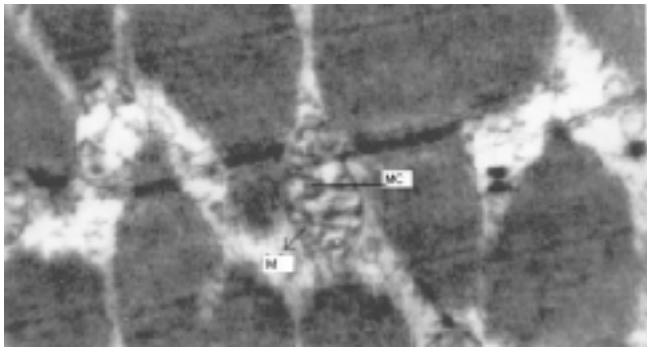

Figure 7. Electron micrograph of normal muscle tissue (×6,300) showing mitochondria in intermyofibrillar space.

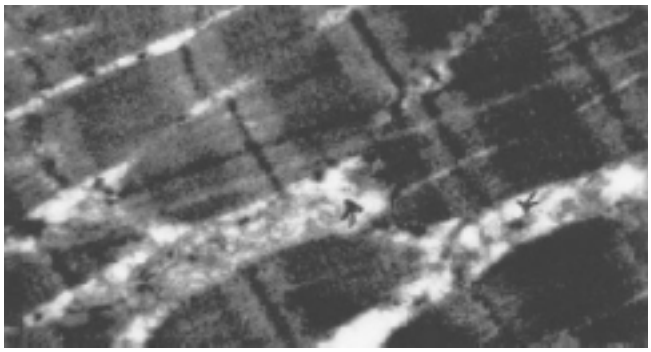

Figure 8. Electron micrograph of muscle tissue (×4,600) after one freeze thaw cycle (-10°C) showing extensive damage of muscle fibre.

generate pressure which will separate fibres.

Electron micrograph of normal muscle (Figure 6) tissue showed well distinguished ‘A’ and ‘I’ bands, ‘Z’ lines, ‘H’ zones and ‘M’ lines. Myofibrils are straight and parallel and separated from each other by narrow spaces with several nuclei (N). The sarcomeres are clearly bordered by ‘Z’ lines. The myofillaments are separated and are apparently seen as individual formations. Figure 7 showed the normal mitochondria (M) in intermyofibrillar space. Mitochondrial structure was particularly taken into consideration to ascertain evolved enzymatic methodology to differentiate between fresh and frozen thawed muscle (Sen and Sharma, 2003).

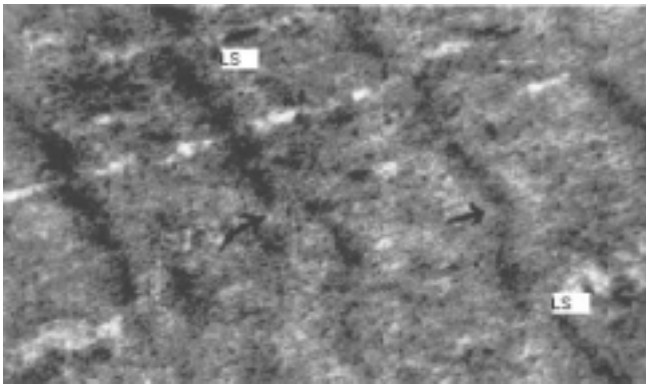

Figure 9. Electron micrograph of muscle tissue (×6,300) after two freeze thaw cycles (-10°C) showing cracks in 'Z' line and no intermyofibrillar space.

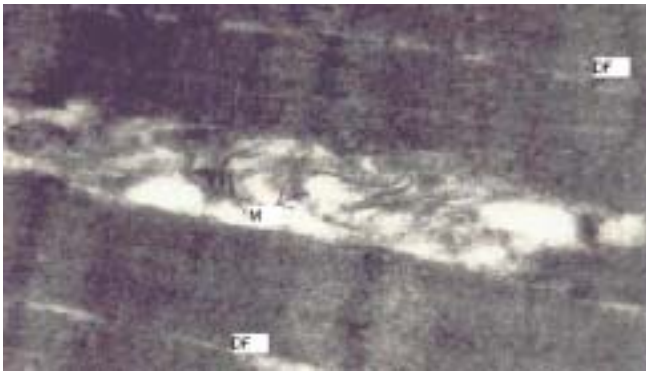

Figure 10. Electron micrograph of muscle tissue (×8,400) after two freeze thaw cycles (-10°C) showing mitochondrial destruction.

Some fibrils were damaged at the edges due to freeze thaw cycles (Figure 8, arrow head). The fibrils were attached with each other and no distinct intermyofibrillar space (Figure 9). There was extreme lysis of the sarcomeres and wavy, crack Z-lines (arrow). The 'A' band was not discernible. Some fibrils were torn by oblique fissures (F). The Z-lines were markedly denser. Figure 10 showed the mitochondrial membrane destruction (M) after second freeze thaw cycle of muscle. No distinguished mitochondrial cristae were observed. After the second cycle, the interior portion showed large scale degradation of the ultrastructure. However, in the freezing of meat tissues the problem is complicated because the solutions are contained inside and outside the fibres. As a consequence, the ice crystal can be intra or extracellular giving rise to different configuration of the tissue depending on the freezing temperature. The more structural damage in the present electron microscopy study may be due to slow freezing which promotes the formation of large ice crystals (Bevilacqua et al., 1979) which would be thought to create more structural damage than small ones. This type of ultrastructural changes was also noticed in pork muscles (Ngapo et al., 1999) frozen at -20°C.

CONCLUSION

Our studies of buffalo muscle showed that under the proper condition (-10°C and repeated freezing and thawing) little structural damage takes place in the meat histology and ultrastructure under repeated freeze-thaw conditions. This study adds continued weight to the evidence that one or two freeze-thaw cycles will not deteriorate the quality of meat.

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