AN ULTRASTRUCTURAL OBSERVATION ON CHARQUIS, SALTED AND INTERMEDIATE MOISTURE MEAT PRODUCTS

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Keywords: hurdle technology, microscope, myofibril proteins, extracellular matrix

Introduction

Charqui is a typical Brazilian salted meat and is an important source of animal protein, particularly in those regions where refrigeration facilities are limited. A derivative has recently been introduced to the market known as Jerked beef (JB) which differs from traditional charqui by having sodium nitrite in the NaCl solution and being vaccum packed. Charqui and its derivatives are tropical products formulated using hurdle technology, a concept described by Leistner (1). Salt, sodium nitrite, dehydration and packaging are hurdles applied in sequence to inhibit deteriorating microorganisms as well as possible selecting for desirable flora. Biochemical and physico-chemical changes occur during charqui processing (2,3) and the intermediate moisture nature of the product has recently been established (2).

Objectives. The present work describes the microscopic changes in structure of a meat product exposed to a relatively high salt concentration followed by solar dehydration known as Jerked Beef.

Material and Methods.

Samples. Control and Jerked Beef (JB) samples were prepared from post-rigor bovine *Sternomandibularis* muscles from 4 to 5 years old animals kindly donated by Industrias Allyson, Santana de Parnaiba, SP. Processing closely followed the method described in (2). The major difference was the injection of a saline solution of 25°Baumé containing sodium nitrite as the first salt treatment followed by application of rock salting at less than 15°C for five days.

Histological studies. Light Microscope. Samples (≅ 5.0mm) were obtained and fixed in Bouin solution, dehydrated in ethanol solution and embedded in paraplast, stained with hematoxilin and eosin and observed with a Nikkon photomicroscope

Electron Microscope. Samples were cut and fixed in 2% glutaraldehyde, 2% paraformaldehyde in 0.1M sodium cacodylate buffer, pH 7.2, for 2h at room temperature, washed several times in buffer and fixed with 1% osmium tetroxide. Then these samples were dehydrated in a graded series of ethanol solutions and embedded in Spurr type resin. Ultrathin sections (= 50 nm) were stained with 2% uranyl acetate and lead citrate and observed with a JEOL 100CX II electron microscope. For quantitative measurement, cell number and area occupied by those cells were measured using a digitilizing tablet (JANDEL Scientific, Corte Madera, CA, USA) with a software SIGMA-SCAN).

Results

Light Microscope. Fig 1A shows the typical fluid filled channels at the endomysial and perimysial levels in control samples described by Offer et al. (4). Differences are observed in JB samples (Fig. 1B) despite the harsh processing conditions the myofibers were not completely destroyed. Clear spaces can be seen between the muscle cells and endomysium. Moreover, fewer collagen fibers are observed within the perimysium (Fig 1B). Table 1 shows that cell numbers in a specific area diminished by 20-30% and the area occupied by these cells decreased by 30-40 % in JB, as a consequence of sample shrinking.

Electron microscope.Intracellular compartments. Fig 2A and 2B show a longitudinal section of muscle fibers. In the JB samples, the typical dark and light banding patterns seen in the controls were not evident and Z-lines appeared to be more pronounced. In addition, the M- band was not clear and the T-tubules system appeared to be dilated. These differences are more clearly seen in Fig 3 where the border between A and I bands is not clear, and the Z-lines are seen to have lost their homogeneity and are fragmented.

Extracellular compartments. The typical banding pattern of collagen fibers were unaffected by processing (Fig 4B). The compact fibers in the control samples were embedded with electrondense components which appeared to be proteoglycans (Fig 4A). In JB samples (Fig 4B) collagen fibrils seemed to be shrunk and devoid of electrondense material.

Discussion

Both intracellular and extracellular components were affected by the harsh conditions of JB processing. The high salt concentration, app. 15-20% and also the drying conditions at the temperatures in the range 30-40°C for 5 days caused structural changes in *Sternomandibularis*. To understand the water movement process during JB preparation at least two events should be considered. First, in the initial drying phase, injected NaCl is absorbed by muscle and uniform salt distribution is reached during the addition of coarse salt. The first reaction would be abstraction of water to dilute the salt followed later by swelling. Since the NaCl concentration is between 2.0 - 3.0 M (≈15% as final concentration), the A-band would be extracted and myosin would no longer would retain water. The osmotic and physical pressures associated with depolymerisation of myosin would encourage water to move from the myofibrillar to interfibrillar compartments then to the extracellular compartments and finally drip would be formed on the meat surface. On a dry weight basis, it was estimated that the total protein lost was around 35-40% and solubilized collagen represented 40-45% of this amount. Consequently, the intercellular space shrunk by 20-30% (Table 1) and conversely, the extracellular space increased which would aid drainage of saline solution. Secondly, as the temperature during processing reached 35-40°C, would denature myosin and consequently water would be drained further until an equilibrium Aw= 0.70-0.75 was reached. Moreover, in the control samples, collagen fibrils were combined with a strong electrodense material (proteoglycans) which was not seen in JB samples (Fig 4A,B). Presumably proteoglycans were solubilized by the saline solution which would aid dehydration since proteoglycans are very hydrophilic. In conclusion, the results of these ultrastructural studies reflect the several events which take place during JB preparation, notably extraction of A-bands, disaggregation of myofibrils and partial solubilization of extracellular matrix components.

References

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Fig. 1- Photomicrograph of *Sternomandibularis* muscle. (A) cross sections of control and (B) Jerked Beef. In B the amound of connective tissue of the perimysium (P) is lower than observed in control samples. Note the shrinkage of muscle fibers as well as an increase of endomysium spaces arrow. H & E 64X.

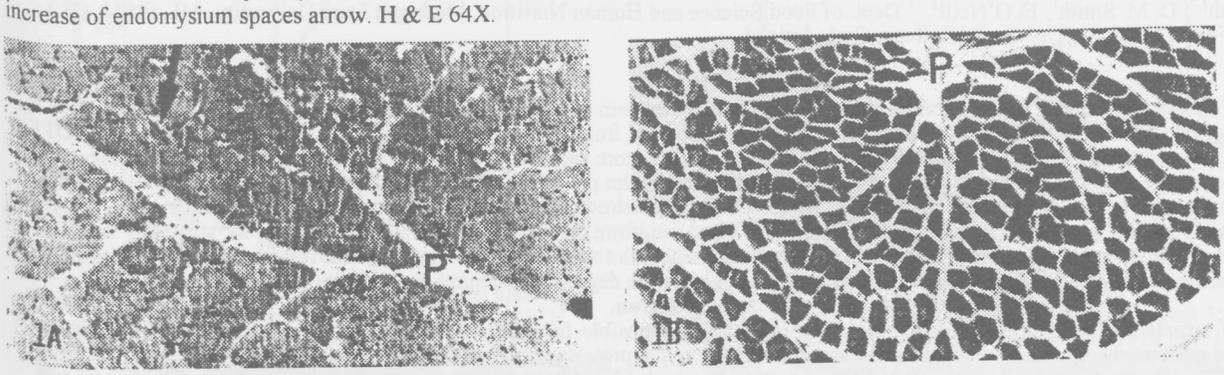

Fig. 2- Eletronmicrograph of *Sternomandibularis* muscle. (A) control (B) Jerked Beef. Note in Fig. B a disorganization of A and I banda. The M band is not very well defined. 15,500 X.

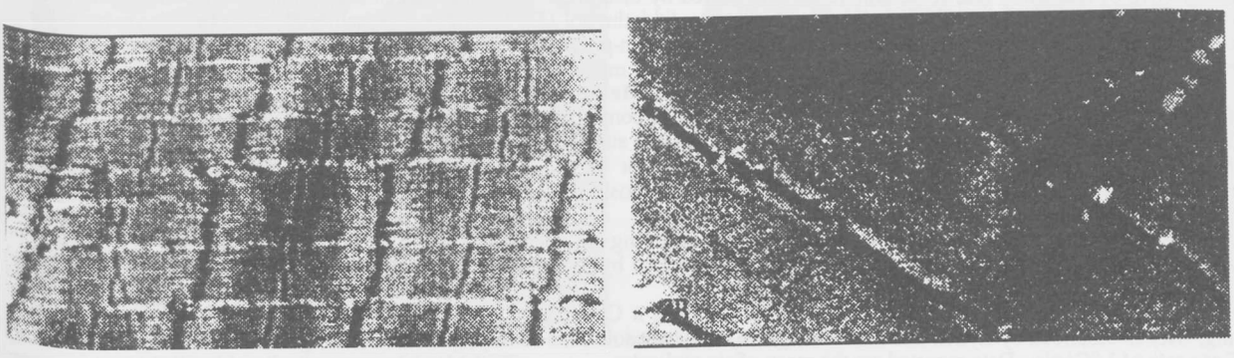

Fig. 3- High magnification of Jerked Beef. The disorganization of sarcomere structure is evident. Z-Z-line; M-M-band 45,000 X.

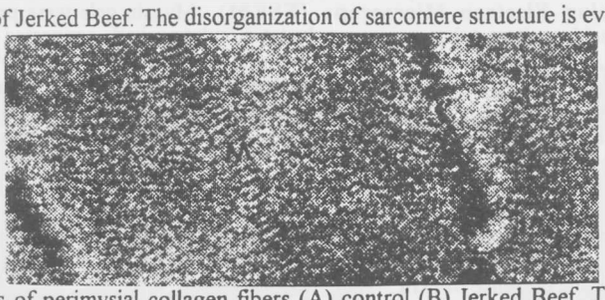

Fig. 4- Electronmicrographs of perimysial collagen fibers (A) control (B) Jerked Beef. The typical banding pattern is present in both samples. Observe the presence of electrondense material which surrounds the fibers in A (arrows) and is not present in B (*); C-collagen fiber. 66,000 X.

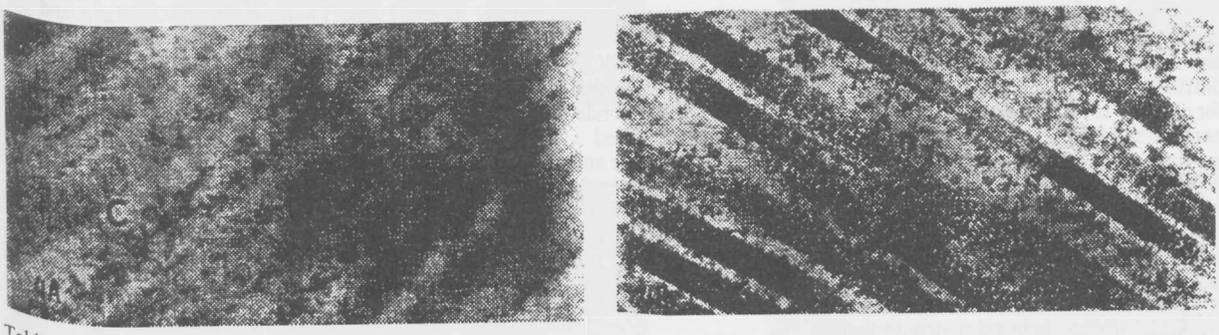

Table 1 - Number of muscle cells and area occupied by these cells in Jerked Beef (*Sternomandibularis*).

Samples	Cells number	Area occupied by Cells (μm^2)	F
Control	160	31,232.98	11,52*
Jerked Beef	123	20,103.14	

(*) - significant at $p < 0.01$ level.