CHANGES IN LIPIDS BOUND TO MYOFIBRILS AND I-Z-I FRACTION RELATED TO MYOFIBRILLAR ATPases ACTIVITIES AND MFI OF OVINE *Longissimus dorsi* STORED AT 2-4 °C.

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Background.

Lipids play a central role in preserving protein stability (Busalmén *et al.*, 1995; Roura *et al.*, 1993). Actomyosin and myofibril preparations usually contain lipids that bind to the complex and produce significant effects on the constitutive protein properties (Hamada *et al.*, 1982). Previous reports (Taguchi, 1970; Ishizaki *et al.*, 1995) have shown that the addition of lecithin, the main lipid component usually found in actomyosin preparation, produce an increase in the Mg^{2+}- and Ca^{2+}-ATPase activities of fish actomyosin and in its substrate affinity. Our group reported that in hake (*Merluccius hubbsi*) the relative percentage of phospholipids present in actomyosin preparations influence actomyosin ATPases activities (Busalmén *et al.*, 1995). In other way, Shimada *et al.* (1998) have recently reported that the weakening of the Z-disk, measured as an increase in the Myofibrillar Fragmentation Index (MFI), could be related to the liberation of phospholipids from the amorphous matrix of the Z-disk during the aging of chicken meat.

Objective.

The purpose of this work was to investigate the changes in the lipids of myofibrils related to myofibrillar ATPase activities and the changes in the phospholipids of the I-Z-I fraction in relation to MFI, in post-rigor ovine *Longissimus dorsi* during the aging.

Methods.

Lamb *L. dorsi* muscles from 16 animals of about 12-13 months of age were used. After slaughtering, animals were eviscerated and dressed. The carcasses were allowed to age up to 48 hrs post-mortem at 2-4 °C. Meat samples corresponding to post-rigor muscle were taken (vertebrae 12-13). From each sample, portions were taken for myofibril preparations at 0, 2, 5, and 10 days of storage at 2-4 °C.

The preparation of myofibrils and the Ca^{2+}- and Mg^{2+}-ATPase activity determinations were carried out by the procedures previously described (Ojeda *et al.*, submitted for publication). The MFI was determined by the method of Takahashi *et al.* (1967). The I-Z-I fraction was obtained from myofibrils sedimented at 900 x g 15 min treated with a modified Hasselbach-Schneider solution containing 0.6 M KCl, 10 mM $Na_4P_2O_7$, 1 mM $MgCl_2$, and 0,1 M potassium phosphate buffer, pH 6.4. Treated myofibrils were centrifuged at 10000 x g 20 min. This extraction was repeated twice more. The final resultant pellet constitutes the I-Z-I fraction. Proteins were determined by the method of Lowry *et al.* (1951).

Total lipids from myofibrils sedimented at 900 x g 15 min were extracted with chloroform:methanol (2:1, v/v) and purified by the procedure of Folch *et al.* (1957). The procedure for lipid extraction from the I-Z-I fraction sedimented at 10000 x g 20 min was the same one used for myofibrils. Thereafter, for exhaustive acidic phospholipid extraction, Folch´s residues of the I-Z-I fraction were extracted three times at 37 °C for 20 min with four times its packed volume of chloroform:methanol (2:1, v/v) containing 0.25% (v/v) of conc. HCl by the procedure of Dawson and Eichberg (1965). After mineralization, the lipidic phosphorous content in lipids of both myofibrils and I-Z-I fraction was determined by the method of Chen *et al.* (1956). Phospholipid contents were obtained from phosphorus values multiplied by 23.5 (Geigy Tables, 1975). Neutral lipids were calculated by difference between total lipids and phospholipids.

The statistical significance of differences among mean values was determined by analysis of variance, using Duncan´s new multiple range test (Steel and Torrie, 1960).

Results and discussion.

As it can be seen in Fig. 1, both Ca^{2+}- and Mg^{2+}-ATPase activities showed a significant ($p<0,01$) increment between the beginning and the 5th day of storage. This increase had been previously reported for bovine and rabbit muscles (Robson *et al.*, 1967; Goll and Robson, 1967; Nishiwaky *et al.*, 1996). No changes were observed thereafter up to the 10th day.

TL of myofibrils showed a significant ($p<0,01$) decrease between the 2nd and the 5th day of cold storage (Fig.2). Because the PL fraction remained unchanged during storage, this change could be attributed to a decrease in the NPL fraction. Because of that, an increase in the relative percentage of the PL ([PL/TL] x 100) of the myofibrils between the 2nd and the 5th day of cold storage was observed. The results in Figs. 1 and 2 suggest that the increment in myofibrillar ATPases activities could be related to an increase in the relative percentage of phospholipids bound to myofibrils. A similar influence of phospholipids on ATPases activities of actomyosin had been previously reported (Busalmén *et al.*, 1995).

The TL, PL, and NPL contents of the I-Z-I fraction from myofibrils of stored *L. dorsi* are shown in Fig. 3. Non-significant changes were observed in the different lipid fractions of I-Z-I extracts. These results disagree with those of Shimada *et al.* (1998) who reported a decrease in I-Z-I phospholipids during aging. An additional and exhaustive extraction by the Dawson and Eichberg method in I-Z-I-Folch extracted residues was done to determine if this discrepancy could be due to an inefficient extraction of acidic phospholipids by Folch´s method in our experiments. The liberation of acidic phospholipids from the I-Z-I fraction increased significantly ($p<0,01$) during storage (Fig. 4). As it can also be seen in Fig. 4, MFI showed a significant ($p<0,01$) increase during storage. The results in Fig. 4 suggest that the release of acidic phospholipids from I-Z-I could be related to the MFI evolution, during post-rigor aging.

Conclusions.

- The enhancement of Mg^{2+}- and Ca^{2+}-ATPase activities in myofibrils from stored muscles could be related to an increase in the relative percentage of phospholipids.
- The acidic phospholipids were released from the I-Z-I fraction during post-rigor aging. This change could be related to the MFI evolution.

Relevant literature.

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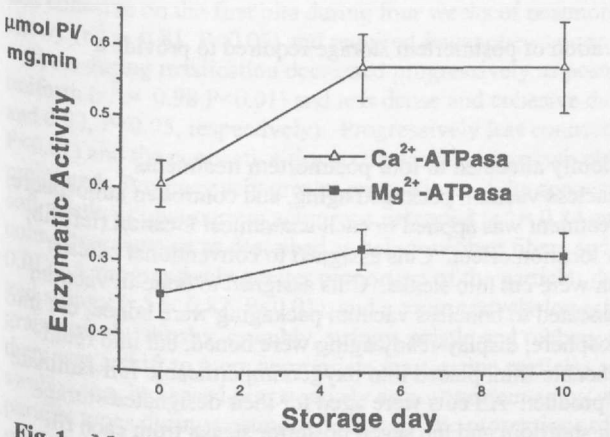

Fig 1.- Myofibrillar ATPase activities from ovine *L. dorsi* stored at 2-4 °C. Each point is the mean of sixteen samples. Bars indicate standard deviations.

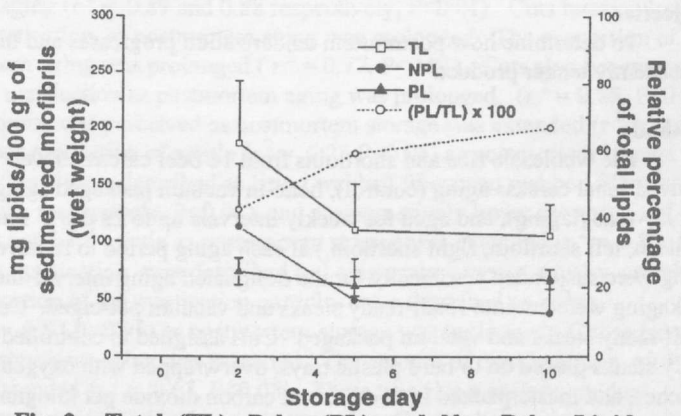

Fig 2.- Total (TL), Polar (PL) and Non Polar Lipids (NPL) contents in myofibrils from ovine *L. dorsi* stored at 2-4 °C. Each point is the mean of eight samples. Bars indicate standard deviations. Some bars were omitted for clarify the figure.

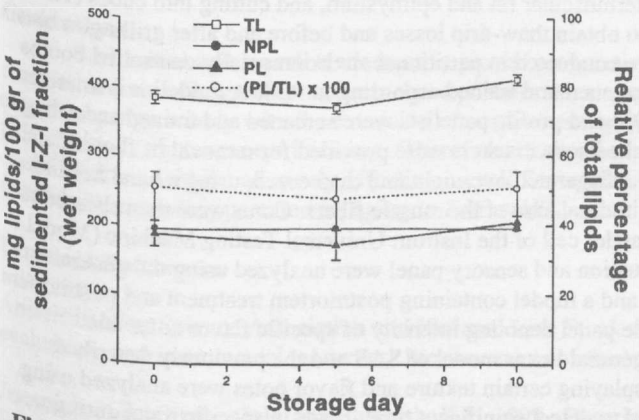

Fig 3.- Total (TL), Polar (PL) and Non Polar Lipids (NPL) contents of I-Z-I brushes from ovine *L. dorsi* stored at 2-4 °C. Each point is the mean of four samples. Bars represent standard deviations. Some bars were omitted for clarify the figure.

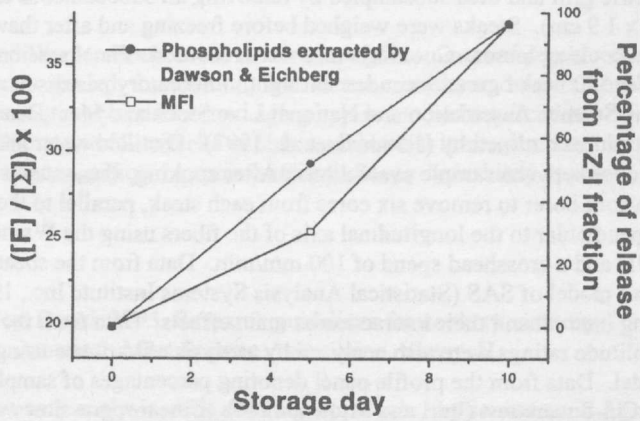

Fig 4.- Myofibrillar Fragmentation Index (MFI) and percentage of released acidic phospholipids from I-Z-I fraction during storage of ovine *L. dorsi* at 2-4 °C. Percentage of released acidic phospolipids was calculated by substracting the remaining amount of acidic phospholipids from the original amount in I-Z-I fraction (100%). Each point is the mean of four samples. For MFI each point is the mean of sixteen samples.