

The Electrophoretic Profile Myofibrillar Proteins Extracted From Camel Muscles, Kept in Various Modes

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Abstract— Changes in electrophoretic profiles of myofibrillar protein (MFP) in the *Longissimus thoracis* (LD) of young camels (2 to 4 years), preserved by refrigeration has been treated or not by lactic acid solution 4% or citric acid 1%, were followed during the post-mortem time at the following times: 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours. The cold preservation for 48 hours has not shown any particular distinctions in the protein profiles of this muscle. Changes related to the type of treatment were recorded during the storage time. Proteolysis of the myofibrillar fraction was earlier in this muscle in the case of treatment with one of two solutions of organic acids used, particularly in the case of using lactic acid. Indeed, these changes have affected at the first hour after slaughter the proteolysis of the myofibrillar proteins. Fragments of low molecular weight (42, 36, 33, 26, 23, 18, 16, 14 and 13 kDa) have been identified. The electrophoretic analysis showed that during refrigeration, LD treated with a solution of lactic acid is more sensitive to disruption phenomena and muscle protein proteolysis that lots of this muscle that even in the case of preservation by refrigeration only or by refrigeration after treatment with a solution of citric acid.

Keywords— Camel, citric acid, lactic acid, *Longissimus thoracis*, refrigeration, protein electrophoresis.

I. INTRODUCTION

Meat is the product of transformation of muscle after animal slaughter [1]. Organoleptic quality is extremely important in the red meat industry [2, 3]. Tenderness is the first criteria for selection by consumers of meat and they are willing to pay more for this quality [4, 5, 6]. The main problem facing meat industry are the variabilities due to complex factors rising from variations during animal growth as well as factors ante and post mortem.

Tenderness is the most difficult criterion to control or predict [7, 8], the tenderness of the meat depends on two tissue structures, the myofibrillar proteins and connective tissue. The first is strongly influenced by meat's storage conditions while the second is directly linked to livestock characteristics of the animal at time of slaughter [9]. Indeed, after slaughter, myofibrillar muscle structure undergoes profound changes that are largely dependent on enzymatic activity and physicochemical characteristics of the fibers.

After the death of the animal, the tenderizing processes are started. They are the cause of the rupture of the myofibrils and leads to tenderizing meat [10, 11]. There are few studies on the comparison of the effect of the refrigeration or refrigeration after treatment with a solution of lactic or citric acid on the myofibrillar proteins of dromedary muscle. The objective of this work is to study the evolution of *Longissimus thoracis* myofibrillar profiles (LD) of camels aged two to four years within 48 hours of refrigeration. These are subjected to a prior treatment with one of two organic acid solutions (the 4% lactic acid or citric acid 1%). And searching for the effect of variation of the method of preservation on these profiles.

II. MATERIALS AND METHODS

1.1. Biological material

To study the evolution of proteolysis of the myofibrillar proteins, the *Longissimus thoracis* (LD) from camels ages two to four years of the Sahrawi race were used. The animals were slaughtered according to the Muslim rituals at the slaughter house of Ouargla, Algeria.

Longissimus thoracis (dorsal Long) is the larger muscles of the body. It forms the most important muscle of the thoracolumbar region; it extends along the vertebral gutter bro-costal from the sacrum to the base of the neck (Figure 1).

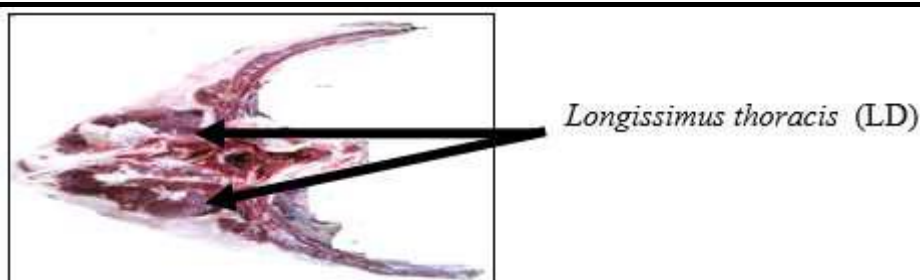


Fig.1: Location of the studied muscle at the last vertebra.

1.2. Preservation (organic acids)

Conservation of the muscles studied, is accomplished by the combination of two methods, one physical (refrigeration), while the other is chemical using two organic acid solutions (lactic acid and citric acid, at concentrations of 4% and 1%, respectively).

Samples of muscle were taken after the gutting and cutting of carcasses. Triplicate samples of each muscle were individually wrapped in polythene bags and then transported to the laboratory where they were boned and trimmed of external fat. The removal of lipids before protein extraction is desirable to avoid formation of an emulsion preventing protein extraction [12, 13]. These were divided into three groups; a control, one treated with 4% lactic acid, the third was subjected to treatment with 1% citric acid. Each sample was individually packaged in sealed sterile plastic bags and placed in a refrigerator at 4°C. These muscles were followed up proteolysis myofibrillar proteins at specific time intervals.

1.3. Extraction of myofibrillar proteins

The extraction of myofibrillar proteins was achieved using the method described by Gagaoua et al., [14]. To avoid activation of proteases, samples were maintained on ice throughout the extraction procedure. 200 mg of sample was incubated on ice with an extraction buffer for 10 minutes with constant agitation. These were then ground and homogenized by polytron for 15 to 20 seconds and reincubated under the same conditions for a further 5 minutes. The mixture was then centrifuged for 15 min at 5000g. The supernatant containing the sarcoplasmic proteins was removed. The myofibrils pellet obtained was reconstituted in the extraction buffer and homogenized using a vortex. These samples were stored at -20 ° C for ulterior use.

1.4. Electrophoresis on polyacrylamide gel in the presence of SDS (SDS-PAGE)

Myofibrillar protein electrophoresis was performed under denaturing conditions as described by Laemmli, [15]. Protein separation was based solely on their molecular weights. The estimate of the degree of proteolysis of the myofibrillar proteins was performed at different time points post mortem by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl Sulfate (SDS-

PAGE) from myofibrillar proteins obtained at the end of extraction. The protein sample was treated with a reducing agent, the β -mercaptoethanol, a compound which has a denaturing effect on proteins by disruption of their three dimensional structure. Migration in the gel is thus affected only by molecular weight [16]. Samples were fractionated by electrophoresis on 12% polyacrylamide gel. All protein fragments separated according to their molecular weight which has been determined by protein markers (reference protein). The standard proteins used are low molecular weight markers containing the β phosphorylase (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30kDa), trypsin inhibitor (20, 1 kDa), and α -lactalbumin (14,4 kDa). Protein bands were revealed using the stain with Coomassie blue R-250. This colouration starts with a fixing step in a solution containing 30% methanol, 5% acetic acid for 20 min, followed by staining in the same solution containing 0.12% Coomassie Blue R-250 for 1 hour. Discoloration was carried out by the same solution as that used for fixing, it may last overnight. Finally, the gels were scanned to be studied.

III. RESULTS AND DISCUSSION

According to Jia et al., [17]; Zapata et al., [18]; Kemp et al., [19] and Ouali et al., [20], *post-mortem* maturation of muscles is an enzymatic process that leads to degradation of myofibrillar structures and to lesser extent collagens by endogenous proteolytic enzymes, which will condition tenderizing red meat. Proteolysis of the myofibrillar proteins was evaluated in the LD muscle preserved by refrigeration or treated with citric acid or 1% lactic acid 4% before being refrigerated. The effect of different treatments was well highlighted. The electrophoretic profiles of myofibrillar fraction muscle protein in *Longissimus thoracis* from camels aged 2 to 4 years preserved by refrigeration having undergone previous treatment with a citric acid solution 1%, lactic acid 4%, are represented respectively in figures 2, 3, 4.

According to the electrophoretic profile of the myofibrillar fraction in refrigerated LD or previously treated by a solution of citric acid 1%, several bands were observed in protein extracts (Figures 2, 3 and 4). The

bands identified one hour after slaughter were had molecular weights of 66, 53, 42, 36, 33, 18, 16 and 14 KDa. According to the work of Delbarre-Ladrat et al., [21] and Bond et al., [22], these bands were identified as tropomyosin, desmin, actin (AC), tropomyosin (TMP), Troponin I, Troponin C and two light chains myosin (MLC), respectively. Starting from hours post-mortem, the strips 66 and 48 kDa disappeared as well as the bands of relative molecular weights of the order of 42, 36, 33, 26 and 23 kDa, have been identified and the molecular weight bands 13, 14, 16, 18 and 97 kDa. The intensity of these bands is variable. Observing these protein profiles showed no major differences 6 hours after slaughter for LD preserved by refrigeration having ,or not, undergone previous treatment with a 1% citric acid solution (Figures 2 and 3). This is probably due to a similar proteolysis during this period. Similar results were observed in sheep [23]. However, the small differences in band intensity can be attributed to the difference in the relative amount of protein loaded on the gel electrophoresis as was noted by Martinez et al., [24].

Pretreatment with a 4% lactic acid solution strongly influenced the proteolysis of the myofibrillar proteins, the disappearance of the bands corresponding to molecular weight fragments greater than 66 kDa was observed starting from the first hour following slaughter (Figure 4). The disappearance of high molecular weight protein in citric acid treated samples took at least eight hours. The appearance of bands at 33 kDa indicator of tenderness were also reported by Ho et al., [25] and Zamora et al., [26] on beef and Smili, [27] on camel meat. The lower molecular weight bands at 23 kDa for the muscles having undergone or not before refrigeration acid treatment consistent with the results of Chobert et al., [28]; Cho, [29]; Barany et al., [30], Delbarre-Ladrat et al., [21] and Ouali et al., [20] on beef. The use of organic acids (citric and lactic acids) accelerated proteolysis myofibrillar proteins, thus leading to early proteolysis, which can induce a tenderizing meat. The same effect of the organic acids has been demonstrated by Cannon et al., [31] and Erthbjerg et al., [32]. Early onset fragments whose molecular weight varies between 33 and 30 kDa, associated with the maturation of meat confirms that the rate of maturation of the treated muscles was faster than the muscles which did not undergo any treatment before refrigeration . This suggests an early proteolysis, which can be explained by lower pH thus promoting the activity of proteases. Bands whose molecular weight is in the order of: 13, 14, 16, 18, 23, 26, 33, 36, 42 and 53kDa, persisted in the electrophoretic profile of refrigerated muscles or having previously been treated by one organic acid solutions used, between 24 hours and 48 hours *post-*

mortem. Intensity was more remarkable at the end of the experiment (48 hours) (Figure 2).

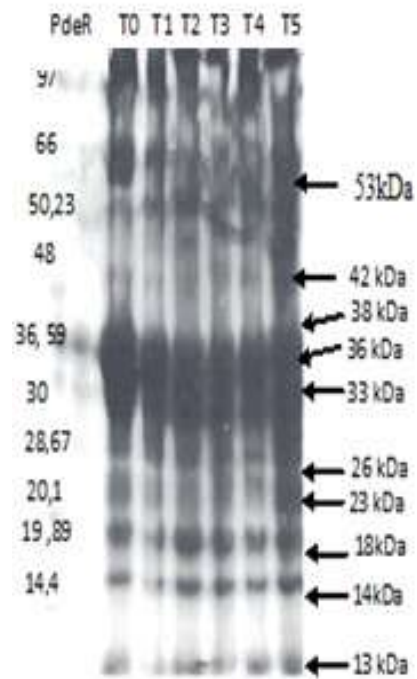


Fig.2: Electrophoretic profile of the myofibrillar fraction in refrigerated LD

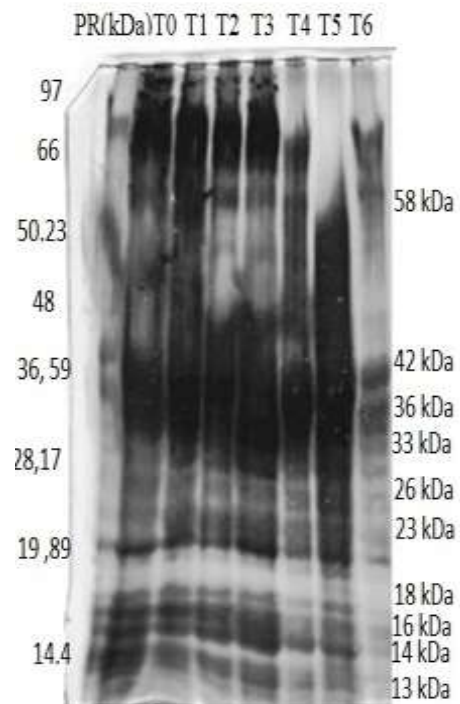


Fig.3: Electrophoretic profile of the myofibrillar fraction in refrigerated LD previously treated by a solution of citric acid 1%

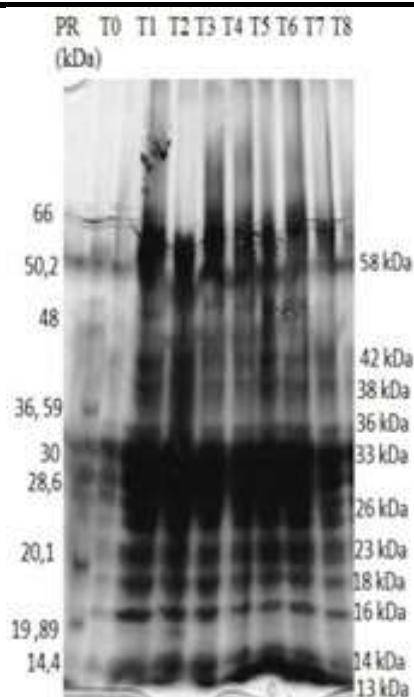


Fig.4: Electrophoretic profile of the myofibrillar fraction in refrigerated LD previously treated by a solution of lactic acid 4%

IV. CONCLUSION

During refrigeration for 48 hours, the profiles of myofibrillar proteins of muscle *Longissimus*

During refrigeration for 48 hours, the profiles of myofibrillar proteins of muscle *Longissimus thoracis* (LD) of dromedary have shown relative stability and have displayed only small changes in the intensities of several protein bands for the same treatment suffered. This indicated a low proteolysis of myofibrils. However, variations affecting these proteins were detected between different lots of the muscle according to the retention mode. Art refrigeration supplemented with a treatment with an organic acid has proven adequate to accelerate the maturation of muscle LD dromedary. But other analyzes of other qualitative parameters, including the enzyme level is needed to better determine the chilling period and the dose of organic acids which allow preserving the nutritional quality of the meat.

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