CHANGES IN COLLAGEN SUFFERING CALIFORNIA RABBIT RACE AFTER THE SLAUGHTER

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ABSTRACT

The objective in this research was the characterization of the changes that occur in collagen of rabbit after slaughter of the rabbit to 96 h later. The methodology used, including the extraction and quantification of total collagen and its fractions (soluble and insoluble) *Quadriceps femoris* muscle of thirty rabbits of 70 ± 3 days old race California, as well as obtaining the electrophoretic profiles of fractions and collagen fibers, under denaturing or reducing conditions. The pH in the *Quadriceps femoris* muscle at baseline was 6.640±0.07 and during the first 24 h fell to 5.57±0.10 and after 48 h to 5.478±0.12. After 72 h and 96 h the pH value increases 5.873±0.0437 and 5.924±0.1315, respectively. *Quadriceps femoris* muscle in average was obtained 2.24±0.245 mg collagen total/g, insoluble collagen 1.84±0.186 mg/g and soluble collagen 0.34±0.072 mg/g. According to the behavior of these fractions respect time was observed that the collagen total was not statistically different, while the soluble and insoluble fractions were statistically different ($P<0.05$) mainly in *post mortem* at 96h. The changes observed by electrophoresis in fractions of collagen according to results obtained in this experiment, may be related to the enzymatic activity of metalloproteinases and collagenases as well as changes in pH according to previous reports in literature. The amount of soluble collagen is related to the kinetics of pH at 96 h *post-mortem.* The pH was near 6 and the highest amount of soluble collagen was obtained. The collagen fibers and soluble and insoluble fractions obtained under denaturing and reducing conditions, showed and profile electrophoretic, characteristic of

subunits α_1, α_2, β_{11}, β_{12} of collagen. To determine the concentration of collagen subunits over 10 µg/µL were distributed between the γ subunit and the high molecular mass polymers. The use of reducing agent in the electrophoresis confirmed the presence of type III collagen. Methodologies of extraction of collagen fibers and their soluble and insoluble fractions affect the sensitivity of the results although uniformity in the methods used for quantification of total collagen and its fractions. The highest percentage of soluble collagen was obtained at 96 h *post mortem*.

Key words: Total collagen, soluble collagen, insoluble collagen, meat rabbit, SDS-PAGE

INTRODUCTION

Collagen is fibrous protein and water-insoluble for to their amino acid composition, is characterized by the presence of triplet Xaa Yaa Gly, where Xaa and Yaa position are often proline and hydroxyproline, constitutes 95% of connective tissue and has function as the support and protection of skeletal muscle (Shoulders *et al*., 2009; Kadler *et al*., 1996). Although we have identified more than 25 types of collagen in skeletal muscle were found collagen type I and III mainly distributed in the *epimysium*, *perimysium* and *endomysium* (Shoulders *et al*., 2009, Purslow, 2005). The amount of muscle collagen depends on genetics, nutrition, animal age and anatomical location of muscles. During the transformation of muscle into meat, this protein undergoes structural and mechanical changes that may favor the development of quality characteristics such as tenderness, which is part of the selection criteria or preference by the consumer. However, such modifications and depends on several parameters including the type of covalent bonds (amines, pyrrol and pyridoline) forming collagen fibers (Nishimura, 2010; Purslow, 2005; Bailey 1985, Eyre *et al*., 2005).

OBJECTIVES

The objective in this research was the characterization of the changes that occur in total collagen and their fractions (soluble e insoluble) of Quadriceps femoris muscle of California rabbit race 70±3 days old through spectrophotometric and electrophoretic methods, after the slaughter through 96 hours, through quantitation of hydroxy proline

METHODS

The methodology used, including the extraction and quantification of total collagen and its fractions (soluble and insoluble) of *Quadriceps femoris* muscle of thirty rabbits of 70 ± 3 days old, race California, divided into 5 groups (0, 24, 48, 72 and 96 hours *post mortem*), at 4 °C until use. Each hind limb, *Quadriceps femoris* muscles were dissected, they withdrew the fascia, the pH was measured and homogenized. Were quantified total collagen and fractions (soluble and insoluble) (AOAC, 2006) for construction of a standard curve at 558 nm Hyp. After total collagen hydrolysis (105 ° C for 17 h) and fractions (soluble and insoluble) (120 ° C for 16 h) in 3.5 M sulfuric acid and 6 N hydrochloric acid was performed (AOAC, 2006 and Hill, 1966). The

collagen fibers and fractions were extracted, with protocols of Field (1970) and Nalinanoon *et al*. (2007). The collagen fibers with 1 M PBS, 1.1 M potassium chloride and 0.9% sodium chloride, the fractions were digested with pepsin (50 mg / g of tissue) and subsequent dialysis of the soluble fraction with 0.05 M acetic acid The extracts were frozen at -50 ° C and lyophilized at 0.06 mbar and -49 ° C. Each extract was prepared as Nalinanoon *et al* (2007) and the electrophoretic profile was obtained (Laemmli, 1970), gels (6 and 4%, separator and concentrator, respectively) 15 □L samples with a concentration of 5 □g/□L. The conditions used during the shift were 20 mA / gel for 120 min. Stained with 1 % Coomassie Blue G 250 (Bio-Rad) n acetic acid, methanol and water (1:4.5:4.5) (v/v/v) or 30 min and washed out 30 min with acetic acid, methanol and water in proportions (1:1:8) (v/v/v). Comparing extracts from fibers and fractions in a gel for each of the five groups.

RESULTS AND DISCUSSION

After sacrifice, the pH value decreases rapidly during the first 24 hours *post-mortem* (5.75±0.10), phenomenon due to the formation of lactic acid from the muscle glycogen through anaerobic glycolysis, 48 h (5.47±0.12) 72 h (5.47±0.12) y 96 h (5.92±0.13). The total collagen showed similar values with respect to time ($p<0.05$). When the sum of the soluble and insoluble fractions, the different values of total collagen (CT) as it was called calculated total collagen (CTc) were obtained, both showed similar trends ($p<0.05$). The CI at 72 h was not significantly different ($P < 0.05$); but at 96 h is decreased corresponding to the trend of CS. The CS increases in the first 24 h and then decreased until 72h. At that time in the meat system occurring biochemical changes associated with maturation (enzyme activity) and increased the fraction of collagen. In general, the proportion of soluble collagen or soluble fraction increases with time, but the mechanical, physical, chemical and structural changes undergone collagen fibers *postmortem* may be related to the enzyme activity (Nishimura, 2010, Mills 1989), which is primarily regulated by pH.

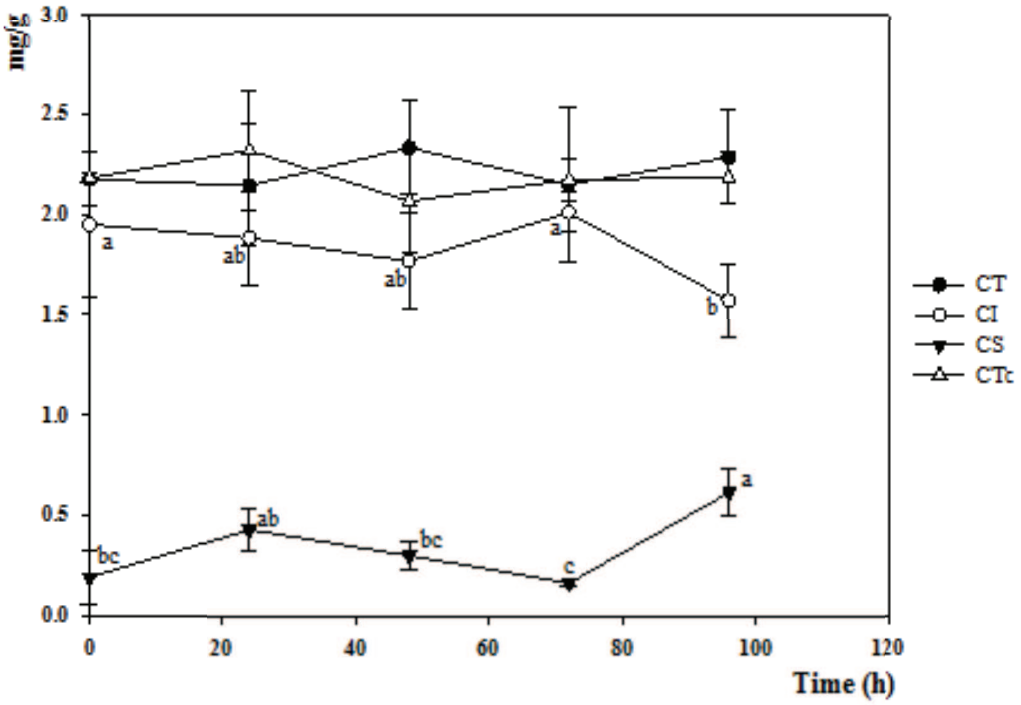

Figure 1. Behavior of total collagen and its fractions Quadriceps femoral muscle of rabbits California race 70 ± 3 days old, during the 96 h *post-mortem (*CT: Total collagen. CTc: Total calculated collagen. CI: Insoluble collagen. CS: Soluble collagen) ($^{a-c}$ Test Tukey significantly different between types of collagen (p>0.05))

The collagen fibers and soluble and insoluble fractions obtained under denaturing and reducing conditions, showed an electrophoretic profile with the characteristics of the collage α_1 subunits (around 140 kDa), α_2 (around 123 kDa), β_{11} (around 240 kDa), β_{12} (around 225 kDa), γ and the high molecular weight polymers. However, in denaturing gels of collagen fibers β_{22} presence of chain (217.62±4.59 kDa) insoluble collagen gels identified the presence of a band which probably $\alpha_{1(III)}$ (141.53±1.16 kDa) was observed.

The difference in electrophoretic mobility and the molecular masses derived from the α_1 and α_2 chains is probably due to the content of amino acids (Pro e Hyp) de according to Furtmayr *et al*. (1971).

In the gels of collagen fibers and soluble and insoluble extracts with respect to time have no statistical difference in their behavior, in general, the melting of the samples and structural modifications are related to the use of agents denaturing (SDS) reducing (β-mercaptoethanol) and chaotropic (urea).

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CONCLUSIONS

When analyzing the behavior of the pH with the soluble collagen is observed that there are modifications that can be related to the activation of enzyme systems with collagenolytic activity. However, research is needed about the changes generated by these enzymes during transformation of muscle into meat.

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