CHARACTERISTICS OF SKELETAL MUSCLE COLLAGEN OF WUZHUMUQIN SHEEP FROM NATURAL GRAZING

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I. INTRODUCTION

Meat tenderness and biological value are reduced by high collagen content and high mechanical stability of collagen fibers. Concerning meat quality, not only collagen content but also age-related cross-linkages must be considered. The aging process increase stiffness of the connective tissues and change physical and biological characteristics of the collagens by increasing the amount of cross-linking between and within the collagen fibrils.[1] Tropocollagen molecules are stabilized by inter- and intramolecular covalent cross-links. These cross-links increase the mechanical and thermal stability of collagen fibers. Currently, there are more than about 27 different types of collagen. Among these, I, III collagen mainly influence the meat quality. Wuzhumuqin sheep, a type of Mongolian sheep was employed in the study. In general, Wuzhumuqin sheep are accustomed to voluntary movements and a high amount of free-feeding (also called natural grazing) for their entire lifespan. The objective of the present study was to investigate the thermal stability, secondary structure of collagen I and III in skeletal muscles obtained from naturally grazing Wuzhumuqin sheep during postnatal development.

II. MATERIALS AND METHODS

MATERIALS: Wuzhumuqin sheep (male) aged at 6, 9, 12 and 18 months old (n=10) were selected from the Original Breeding Farm of Wuzhumuqin Sheep in East Wuzhumuqin, Xilin-gol, Inner Mongolia, China. Semitendinosus muscle was collected immediately after slaughter, and the samples were snap-frozen in liquid nitrogen and then stored at -20°C until further analysis.

1, Preperation of collagen Ⅰ and Ⅲ: Collagen Types I and III were prepared from skeletal muscle following pepsin digestion according to method of Fujii K. and Murota K. [2]

2, SDS-PAGE analysis: SDS-PAGE of the collagen was performed by the method described by Laemml. [3] Each sample was loaded onto a polycrylamide gel composed of 8% separating and 5% stacking gel. The gels were stained using Coomassie Brilliant Blue R-250 and destained using an acetic acid and methanol mixture to visualize the bands.

3, FTIR spectra: FTIR spectra were recorded on potassium bromide (KBr) disks using a FT-IR spectrophotometer (Tensor 27, Bruker Corp., Germany). Spectra of the samples were obtained in the 500 to 4000 cm^{-1} range.

4, Differential scanning calorimetry (DSC) analysis: DSC was performed by DSC20F3 (Maia Corp., Germany). Thermal contraction temperatures were estimated using a DSC thermogram.

III. RESULTS AND DISCUSSION

SDS-PAGE patterns of collagen I and III from semitendinosus muscle are shown in Figure 1a and 1b. The collagen is composed largely of α-chain along with considerable amounts of inter and intra molecular crosslinked components of α-chain; β (dimmer). Electrophoresis of the collagen I (1a) protein showed four clear bands: β1, β2, α1 and α2 chains. The collagen III (1b) protein showed two clear bands: β and α chains. β chain with a molecular weight of about 210 KDa; and α chain with molecular weights of about 130 KDa.

The infrared spectra of collagen from semitendinosus muscle are shown in Figure 2a and 2b. The peak of amide A (N-H stretch, coupled with hydrogen bond) of collagen I and III from Semitendinosus muscle aged 6, 9, 12 and 18 months wuzhumuqin sheep was detected at 3308, 3307, 3303 and 3300 cm^{-1}, respectively. The amide B peak (CH_2 asymmetric stretch) was at 2943 cm^{-1}. The amide I peak (C=O stretch/hydrogen bong coupled with COO-) was observed at 1652 cm^{-1}. The amide II band (NH bend coupled with CN stretch) was found at 1550 cm^{-1}.[4] The characteristic peak of the amide III (NH bend coupled with CN stretch) was observed at 1240 cm^{-1}. [5]

Thermal stability of collagen from semitendinosus muscle are shown in Figure 3a and 3b. The shrinkage temperature of collagen I were detected at 40.70, 41.30, 44.80 and 48.70°C, collagen III were detected at 41.90, 42.60, 43.60 and 45.00°C, at aged to 6, 9, 12 and 18 months wuzhumuqin sheep, respectively. During the aging of Wuzhumuqin sheep, thermal stability of both collagen I and III were increased gradually (P<0.01). Thermal stability of collagen III was higher than that of collagen I of the same age. The amount of imide acid (proline and hydroxyproline) in collagen played a key role in thermal stability. Proline and hydroxyproline contain tetrapyrrole rings which caused a higher thermal shrinkage temperature.

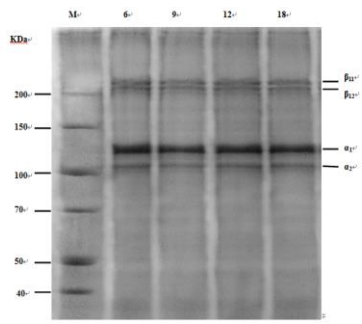

Figure 1a

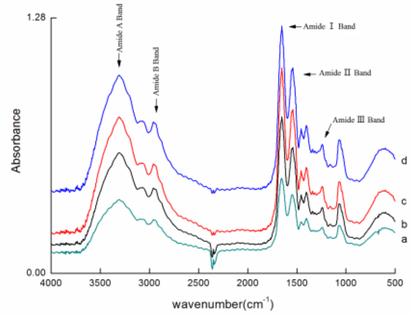

Figure 2a

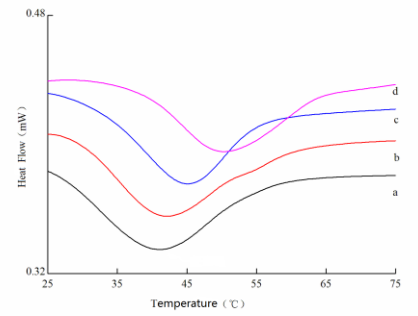

Figure 3a

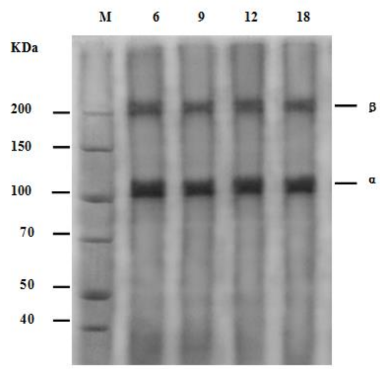

Figure 1b

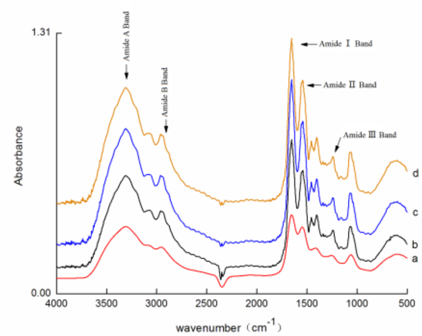

Figure 2b

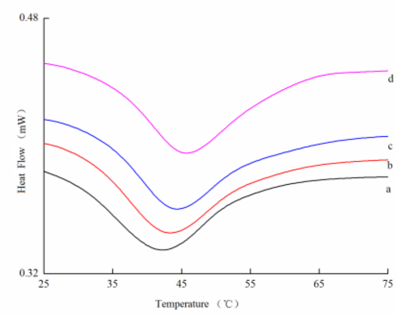

Figure 3b

Figure 1. SDS-PAGE analysis of collagen Ⅰ and Ⅲ in intramuscular connective tissue of semitendinosus muscle.
M: marker; 1a: collagen Ⅰ, 1b: collagen Ⅲ

Figure 2. FTIR spectra of collagen Ⅰ and Ⅲ in intramuscular connective tissue of semitendinosus muscle.
a: 6 months, b: 9 months, c: 12 months, d: 18 months; 2a: collagen Ⅰ, 2b: collagen Ⅲ

Figure 3. DSC analysis for thermal characteristics of collagen Ⅰ and Ⅲ in intramuscular connective tissue of semitendinosus muscle. a: 6 months, b: 9 months, c: 12 months, d: 18 months; 3a: collagen Ⅰ, 3b: collagen Ⅲ

IV. CONCLUSION

Collagen Ⅰ and Ⅲ were successfully extracted from *Semitendinosus Muscle* of Wuzhumuqin sheep under different aged. SDS-PAGE and FTIR showed that the extracted substances were typical collagen I and Ⅲ. There was also a difference in thermal stability.

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