DIFFERENCES IN THERMOSTABLE PROTEIN PROFILE OF GOAT SKELETAL MUSCLE AS OBSERVED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Abstract – The effects of different temperature treatments, (1) chilled at 4°C for 30 min, (2) boiled at 100°C for 30 min and (3) autoclaved at 121°C at 15 psi for 20 min on the protein profile of goat *longissimus* muscle were observed using two-dimensional gel electrophoresis. We detected 221, 153 and 46 protein spots in the chilled, boiled and autoclaved samples, respectively using Coomassie Brilliant Blue staining. Protein identification of autoclaved samples using the MALDI-TOF/TOF mass spectrometry revealed that myosin light chain (MLC), actin, tropomyosin-1 (TPM1) and troponin-T (TnT), creatine kinase and myoglobin are among the putative thermostable proteins which remained after the heat treatment. The heat treatments have resulted in visible differences of spot intensities of actin, troponin T, myoglobin and creatine kinase with no changes noted in the other proteins.

Key Words – goat meat, heat treatment, *longissimus*, myofibrillar protein, MALDI-TOF/TOF

I. INTRODUCTION

The consumption of goat meat is increasingly popular among other types of red meat [1] and this is explained by the increased of goat production from 4.90 million tons in 2008 to 4.99 million tons in 2011 [2]. It could also be resulted from growing research work demonstrating its advantages in nutritional and health attributes [3].

Meat and meat products have been subjected to processing for the maintenance or improvement in palatability, stability, convenience and safety. However, processing and cooking can adversely affect the nutritional and sensory qualities of meat [4, 5]. This process affects whole and individual protein in the meat and meat products. Although thermal denaturation of meat has been studied by using differential scanning calorimetry [6, 7], ultraviolet difference spectroscopy and circular dichroism [8], proteomics data on the thermostable protein in chevon is still limited. Thus, the present experiment was conducted to determine the effects of heat treatments on goat skeletal muscle protein particularly myofibrillar proteins as well as to characterize and identify the thermostable protein using two-dimensional gel electrophoresis and mass spectrometry approaches. Hypothetically, autoclaved meat will exhibit greater reduction in proteins compared to the boiled and chilled samples.

II. MATERIALS AND METHODS

Samples collection

A total of 10 crossbred Boer goats with average body weight of 35 kg were humanely slaughtered at a research abattoir in the Department of Animal Science, Universiti Putra Malaysia. The entire slaughtering and processing procedure were carried out according to MS1500: 2009 (Department of Standards Malaysia, 2009). Samples of *longissimus* muscle were specifically taken from between the 12th and 13th rib region and plunged in liquid nitrogen within 1 hr postmortem. The samples were pulverized in liquid nitrogen and kept in -80°C until subsequent analysis.

2-Dimensional electrophoresis

Approximately 1 g of pulverized muscle sample was subjected to following heat treatments: (1) boiled at 100°C for 30 min, and (2) autoclaved at 121°C, 15 psi for 20 min, with the (3) chilled samples served as control. The heat treated muscle was extracted with 7M Urea, 2M thiourea, 50mM DTT (dithiothreitol), 4% (w/v) 3-[(3-

cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.4% (v/v) carrier ampholytes (pH 3-10, BioRad, USA) and 50 µL of protease inhibitor cocktail (Calbiochem, USA). The resulted supernatant was subjected to 2-dimensional gel electrophoresis following the BioRad (USA) protocol. Protein spots were visualized by Coomassie Brilliant blue R-250 staining (Merck, Germany). The images were analyzed with PDQuest® 2DE image analysis software (Bio-Rad, USA).

MALDI-TOF/TOF mass spectrometry analysis
Spots of interest were excised and identified with MALDI-TOF/TOF mass spectrometer (ABI 4800 plus, Applied Biosystems, USA). The peptide mass spectra obtained were processed and analyzed by the Global Protein Server Explorer 3.6 software (Applied Biosystems, USA). The internal MASCOT (Matrix Science, UK) program was used for matching MS and MS/MS data against database information. The data obtained were screened against mammalian databases downloaded from the Swiss-Prot/TrEMBL at http://www.expasy.ch/sprot

III. RESULTS AND DISCUSSION

The present experiment was designed to examine the effects of heat (temperature) solely on goat *longissimus* muscle without considering the influence of post mortem changes like pH over aging. Therefore, all the skeletal muscle samples collected were immediately frozen in liquid nitrogen to avoid proteolytic degradation.

Decreasing number of spots was consistently observed across ten replicate gels. There were 211 spots detected in the chilled muscle extracts alone. A total number of 153 protein spots were obtained in the boiled samples, while only 46 protein spots were observed in the autoclaved samples. The representative gel shown in Figure 1 indicates the protein present in goat meat after denaturation by heat which could have resulted in decreased spots intensities of some proteins.

Thirteen spots with high optical density from the autoclaved meat were chosen for protein identification using MALDI-TOF/TOF mass spectrometry (Table 1). Molecular weight and pI of the proteins were further confirmed with mass spectrometry analysis and compared with theoretical pI. However, most of the database sources were found to be referring to other species of animal as there was very little or limited protein database in goats.

Most of these putative thermostable proteins have been linked with the myofibrillar proteins which are principally involved in maintaining the structural integrity of skeletal muscle such as myosin light chain 2 and 3 (MLC2 and MLC3), actin and tropomyosin (TPM). A myofibril contractile component, troponin T (TnT) was also less disrupted by the heat. Abundance of myofibrillar proteins in the muscle and higher number of acto-myosin complex could explain why these proteins are less susceptible to heat [6, 8].

Meanwhile, other protein spots like myoglobin and creatine kinase were grouped as sarcoplasmic proteins. Most of the soluble sarcoplasmic proteins are easily purged out of meat at high temperature [6]. However, among the proteins identified, these myoglobin and creatine kinase are less influenced by the heat. Thermal stability of creatine kinase could be due to its association with skeletal muscle in generating energy during the conversion of ATP to ADP [9]. Myoglobin is a protein which determines meat color changes during cooking. The process depends on three forms of myoglobin; metmyoglobin, oxymyoglobin and deoxymyoglobin which differ in their sensitivity to heat denaturation. The fragmentation of myoglobin into several spots influence color changes of meat from red to brown following heating process.

In general, the proteins presented in this study may also reflect those which are usually present in meat and meat products after cooking. However we believed that the amount of protein will be lesser from those reported here, particularly when cooking is done at higher temperature and longer duration, as these may result in greater denaturation and more severe damage to proteins and polypeptides.

IV. CONCLUSION

The present results demonstrated changes in the myofibrillar proteins following the heat treatments applied. Differences in thermal stability of actin and TnT proteins with increasing temperature were also observed. In contrast, the heat treatments did not cause any visible reduction in the spot intensities of TPM1, MLC3F, and MLC2F proteins and this merits their candidature as possible thermostable proteins. The results generated through this study appeared to be the first in reporting the effects of heat treatments as well as identification of thermostable proteins in goat skeletal muscle.

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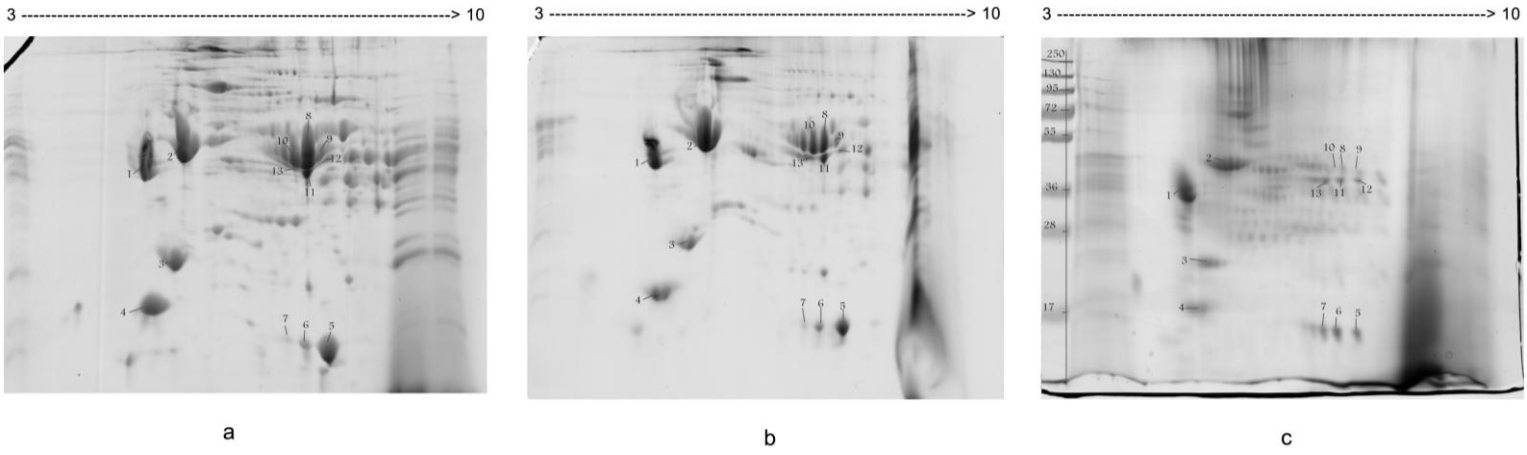

Figure 1. Images of representative 2-DE gels of (a) chilled, (b) boiled, and (c) autoclaved skeletal muscle proteins. Spots numbered indicate the proteins remained after 121 °C, 15 psi heat and consistently present across three gels.

Table 1. Identified proteins from *longisimus* muscle of Boer goat by MALDI-TOF/TOF mass spectrometry.

Spot no	Protein name	Accession no[a] (source)	Experimental pi/MW[b]	Theoretical pi/MW	Matched peptides[d]/ sequence coverage (%)[e]
1	Tropomyosin-1 alpha chain (TPM1)	gi|20522240 (Mouse)	8.2/39.7	4.69/32.661	10/14
2	Actin	gi|124007203 (Bovine)	6.1/46	5.23/41.992	17/28
3	Myosin light chain 3, skeletal muscle isoform (MLC3F)	gi|127130 (Rabbit)	5.8/23.1	4.62/16.647	2/5
4	Myosin regulatory light chain 2, skeletal muscle isoform (MLC2F)	gi|2829841 (Mouse)	5.6/17.6	7.72/35.130	4/5
5	Myoglobin	gi|127638 (Bovine)	8.9/15.3	6.90/17.067	8/35
6	Myoglobin	gi|127638 (Bovine)	8.5/15.6	6.90/17.067	10/28
7	Myoglobin	gi|127638 (Bovine)	8.5/15.6	6.90/17.067	5/22
8	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.9/39.7	5.63/33.014	14/20
9	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.2/39.5	5.63/33.014	16/21
10	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.6/39.6	5.63/33.014	4/9
11	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.6/44.2	6.63/42.962	20/25

[a]Protein name and accession numbers were derived from National Center for Biotechnology Information database.
[b]Isoelectric point and Molecular weight of spot.
[c]Mowse scores greater than or equal to 67 are significant ($p<0.05$).
[d]Percentage of coverage of the entire amino acid sequence.
[e]The number of matched peptides in the database search.