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A PROTEOMIC BASED ASSESSMENT ON CHANGES IN MYOFIBRILLAR PROTEINS OF GOAT *LONGISSIMUS* MUSCLE AS AFFECTED BY HEAT TREATMENTS

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ABSTRACT

The present study examined the effect of different heat treatments; (1) chilled, (2) boiled at 100°C for 30 min, and (3) autoclaved at 121°C at 15 psi for 20 min, on the expression of goat skeletal muscle proteins using two-dimensional gel electrophoresis. The molecular weight (MW) and isoelectric point (pI) of heat stable proteins were characterised followed by identification of the proteins by MALDI-TOF/TOF mass spectrometry. There were 153 protein spots obtained in the boiled samples, while only 46 protein spots were observed in the autoclaved samples. Thirteen spots that exhibited high intensity of protein were chosen from the autoclaved sample for MALDI-TOF/TOF mass spectrometry analysis. The putative heat stable proteins identified were myosin light chain (MLC), actin, tropomyosin (TPM), troponin T (TnT), myoglobin, and creatine kinase. The Proc-GLM analysis revealed that the heat treatments have resulted in significant differences in spot intensities of actin, troponin T (TnT), myoglobin, and creatine kinase with no significant changes noted in other proteins.

Key words: goat meat, heat treatments, myofibrillar protein, 2-dimensional electrophoresis, MALDI-TOF/TOF.

INTRODUCTION

The earlier trend of goat meat consumption was rather lower than its counterparts from cattle and sheep. Nowadays, the consumption of goat meat is increasingly popular compared to other types of red meat (Webb *et al*., 2011), which was proven by the increase in goat meat production from 4.90 million tons in 2008 to 4.99 million tons in 2011 (FAO, 2011). This could be due to the influence from the growing research findings demonstrating its advantages in nutritional and health attributes (Anaeto *et al*., 2010).

Meat has been commonly subjected to processing for its palatability, stability, convenience, and safety. Despite all the advantages of thermal processing, the harsh condition caused by cooking and sterilisation temperature during processing can adversely affect the nutritional and sensory qualities of meat (Gatellier *et al*., 2009; Horn and Voit, 2009; Oluwaniyi *et al*., 2010), which in turn may result in either lower or absence of protein in meat and meat products. Protein denaturation by heat treatment occurs through depolymerisation and solubilisation of skeletal muscle proteins (Cheftel and Culioli, 1997). More recently, studies have also reported the effect of heat on solubility and mechanical properties of myofibrillar proteins (Christensen *et al*., 2000) that proved these proteins are also susceptible to protein denaturation.

Proteomics has become one of the major tools in studying protein expression and degradation in meat and meat products under the influence of pH, water holding capacity, and temperature. Proteomic studies on thermostable species marker protein (Chen and Hsieh, 2002) and protein oxidation (Promeyrat *et al*., 2011) in porcine have been investigated. In addition to that, species specific peptide biomarkers in beef, pork, chicken, and turkey meats were also identified after digestion and hydrolysis of myofibrillar proteins (Ballin *et al*., 2009; Primrose *et al*., 2010; Montowska and Pospiech, 2011) using proteomics approach. Most of the studies in goat meat were confined to the determination of amino acid composition (Sheradin *et al*., 2003), fat cell, and microstructure (Yarmand and Homayouni, 2009). To the best of our knowledge, data on the effects of heat on goat skeletal muscle associated with proteomics are still unavailable. Thus, the present experiment was conducted to determine the effects of heat treatments on goat skeletal muscle proteins particularly myofibrillar proteins as well as to characterize and identify the heat stable proteins 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.

MATERIALS AND METHODS

Slaughtering and sampling procedures: The animal

handling and slaughtering were carried out following the guidelines of Research Policy of the Universiti Putra Malaysia on animal ethics and Malaysian Standard 1500:2009 on *halal* food production, preparation, handling, and storage respectively. 10 male crossbred Boer goats at 35 ± 2 kg body weight were humanely slaughtered at a research abattoir in the Department of Animal Science, Universiti Putra Malaysia. Samples of *longissimus* (LD) muscle were specifically taken between 12^{th} and 13^{th} rib region and snap frozen in liquid nitrogen within 1 hr post-mortem. The accessibility, size, and economic importance are among the criteria leading to the selection of this muscle type in the study. All samples were pulverized in liquid nitrogen and kept at –80°C until subsequent analysis.

Heat treatment of samples: Two different heat treatments were employed: (1) boiled at 100°C for 30 min and (2) autoclaved at 121°C at 15 psi for 20 min. The chilled samples (3) served as control. One gram of frozen, pulverized muscle tissue was placed individually in a heat stable container (capped glass tube) and allowed to thaw before being subjected to both heat treatments (100°C for 30 min and 121°C at 15 psi for 20 min). For boiling, the meat samples were heated by fully immersing the container in a boiling water bath (Memmert, Germany) for 30 min until the internal temperature reached 99 ± 0.7°C. On the other hand, the temperature of the autoclave machine was allowed to reduce until 90°C before removal of the samples. Once removed, the samples were immediately chilled in ice before extraction to prevent any protein loss.

Extraction of proteins: Approximately 1 g of pulverised muscle tissue was homogenised (Wiggen Hauser D500, USA) in 3 mL of ice cold extraction buffer containing 7 M Urea, 2 M Thiourea, 50 mM 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 (Catalogue No. 535140, Calbiochem, USA). The homogenate was then centrifuged at 12,000 g for 20 min at 4°C. The resulted supernatant was collected and subsequently subjected to clean-up procedure using ReadyPrep 2D Cleanup Kit as outlined in the manufacturer's protocol (BioRad, Hercules, USA) followed by storage at –80°C until subsequent two-dimensional electrophoresis. Total extractable protein concentration was determined using the Bradford protein assay kit (Bio-Rad, Hercules, USA).

Two-Dimensional Gel Electrophoresis (2DE): An equivalent of 100 µg of proteins in rehydration buffer [7 M Urea, 2 M Thiourea, 50 mM DTT, 4% (w/v) CHAPS, and 0.4% (v/v) carrier ampholytes (pH 3-10, BioRad)] was loaded onto a 7 cm, pH 3-10 immobilised pH gradient strip (IPG, BioRad, USA). The active rehydration process was performed at 50 V for 12 h using the Protean IEF cell system (BioRad, USA). The first dimension of protein separation (isoelectric focusing) was carried out at low voltage, where 250 V was applied initially followed by a stepwise increase to 8,000 V. The IPG strips were focused at 15,500 V/h. The focused strips were then pre-equilibrated in reducing buffer for 15 min [6 M Urea, 20% (v/v) glycerol, 0.375 M Tris-HCl pH 8.8, 2% sodium dodecyl sulfate (SDS), and 1% DTT]. This was followed by alkylating buffer, also for 15 min [6 M Urea, 20% (v/v) glycerol, 0.375 M Tris-HCl pH 8.8, 2% SDS, and 1% iodoacetamide (IAA)]. The excess buffer was drained out before continuing with the second dimension electrophoresis. The IPG strips were placed on 15% polyacrylamide gel and sealed with 1% agarose. The second dimension separation was performed by initially applying 10 mA/gel current for 15 min followed by 15 mA/gel for the subsequent 90 min. Protein spots were visualised using Coomassie Brilliant blue R-250 staining (Merck, Germany). All gels were produced in duplicates.

Image and statistical analysis of gels: The gels were first visualised using QuantityOne® software (Bio-Rad, USA). Image analysis was then performed using PDQuest® 2DE image analysis software (Bio-Rad, USA). For comparative image analysis, the images were grouped, after which the intensity of individual spots were analysed and compared within and between the image groups. The matches suggested by automated image analysis were individually inspected and confirmed. Following background subtraction, protein spots were automatically defined and the volume of each spot in a gel was normalised as a percentage of the total volume of all spots detected on the gel.

To test the effect of heat treatment, the resulting protein spot volume data were imported to the SAS software (SAS/STAT® Version 9.2, 2009). The mean of spot intensities were subjected to analysis of variance using general linear model procedure with SAS software and comparison was made among the chilled, boiled, and autoclaved sample. The aim was to check if the expression of the analysed heat stable proteins differs significantly among the heat treatments. Any differences among the protein spots were considered statistically significant at 95% confidence level.

In-gel tryptic digestion: The protein spots of interest were cut out from the preparative gels using pipette tips and extracted from the gels according to the published method (Dahlan *et al*., 2011). Briefly, gel pieces were washed repeatedly with 50 µL of 50 mM ammonium bicarbonate/50% acetonitrile (ACN), 1:1 (v/v) for 15 min at ambient temperature until completely destained. This was followed by incubation in 150 µL of 10 mM DTT/100 mM ammonium bicarbonate for 30 min at 60 °C, and alkylation in 150 µL of 55 mM IAA/100 mM ammonium bicarbonate for 20 min at room temperature

(25°C) in the dark. The gel plugs were then washed twice with 50% (v/v) ACN/100 mM ammonium bicarbonate. They were then incubated in 100% ACN for 15 min and dried in vacuum centrifuge (SpeedVac, Thermo Scientific, Savant DNA 120).

In-gel tryptic digestion was performed with 25 µL of 6 ng/µL trypsin digestion buffer (Promega trypsin gold) overnight at 37°C. Tryptic peptides were then extracted using 50% ACN for 15 min, followed by 100% ACN for 15 min. The extracted solutions were then pooled into a single tube and dried in a vacuum concentrator and solubilised with 10 µL of 10% ACN/40 mM ammonium bicarbonate.

Protein identification with MALDI-TOF/TOF mass spectrometry: Extracted peptides were first desalted using ZipTip C18 (Millipore, USA) according to protocols described by the manufacturer. The final elution volume following ZipTip cleanup was 1.5 µL. The peptide samples were then mixed (1:1) with a matrix consisting of a saturated solution of CHCA (α-cyano-4-hydroxycinnamic acid, Sigma) prepared in 50% ACN/0.1% TFA. Aliquots of samples (0.6 µL) were spotted onto stainless-steel sample target plates. Peptide mass spectra were obtained on a MALDI-TOF/TOF mass spectrometer (ABI 4800 plus, Applied Biosystems, USA) in the positive ion reflector mode. For precursor ion selection, all fractions were measured in single MS before MS/MS was performed. For MS/MS spectra, the peaks were calibrated by default. The 20 most abundant precursor ions per sample were selected for subsequent fragmentation by high-energy CID. The collision energy was set to 1 keV and air was used as the collision gas. The criterion for precursor selection was a minimum S/N of 5. The mass accuracy was within 50 ppm for the mass measurement and within 0.1 Da for CID experiments. The other parameters for searching were of trypsin, 1 missed cleavage, variable modification of carbamidomethyl and oxidation of methionine, peptide charge of 1+, and monoisotopic. For database searches, known contamination peaks such as keratin and autoproteolysis peaks for trypsin were removed before searching. Spectra were processed and analysed by the Global Protein Server Explorer 3.6 software (Applied Biosystems, USA). This software uses an internal MASCOT (Matrix Science, UK) program for matching MS and MS/MS data against database information. The data obtained were screened against mammalian databases downloaded from the Swiss-Prot/TrEMBL homepage (http://www.expasy.ch/sprot).

RESULTS

In the present experiment, boiled, autoclaved, and chilled protein extracts from goat *longissimus* muscle were analysed by gel-based proteomics followed by tandem mass spectrometry analysis. The effect of heat treatment on proteins was examined by 2-dimensional electrophoresis whereby heat was the only factor allowed to influence the denaturation. Hence, it was crucial to ensure that the skeletal muscle samples collected were immediately frozen in liquid nitrogen to prevent further proteolytic degradation associated with early post-mortem changes. In addition, meat samples pulverised in liquid nitrogen, were used instead of meat cubes as we aimed to observe the effect of heat thoroughly onto the meat. The heat treatment employed during ordinary cooking of meat cubes usually results in the variability of internal core temperature and this may consequently leave some proteins unaffected with the regime of heat applied.

Irrespective of the type of heat treatment, most of the proteins were found to be present within the mass region of 10-200 kDa and isoelectric point (pI) range from 4 to 9. Apparently, abundant proteins were consistently present in the chilled samples across all animals. There were 211 spots detected in the chilled muscle extracts alone. The lower number of detected proteins may be resulted from the use of Coomassie Brilliant Blue (CBB) staining. The elimination of the use of silver staining in the present work was justified by the abundance of myofibrillar proteins known to be present in most skeletal muscles. Furthermore, the use of CBB had also reduced artefacts.

On the other hand, the results from PDQuest® analysis illustrated that 153 protein spots were resolved in the boiled samples, whereby 110 proteins were found to be reduced, and this was suggestive of protein denaturation that has occurred during the heating process. In addition, 29 proteins were found to be overexpressed and this could be induced by the heat treatments while the remaining proteins in the boiled sample were unaffected by the heat treatments. Meanwhile, only 46 spots were found to be present in the autoclaved samples, and these spots were also consistently encountered in the chilled and boiled samples. Of all the 46 spots, only those with high volume/optical density across the 10 animals were further analysed by MALDI-TOF/TOF-MS. As presented in Figure 1, the numbered spots in each representative gel indicate proteins remained after the heating process.

Proteins separated by 2DE gels were subsequently identified by MALDI-TOF/TOF-MS. The identity and information related to the spots are as shown in Table 1. These proteins were categorised into 7 types, namely; tropomyosin-1 alpha chain (TPM1), actin - alpha cardiac muscle 1 (ACTC), myosin light chain 3 - skeletal muscle isoform (MLC3F), myosin regulatory light chain 2 - skeletal muscle isoform (MLC2F), myoglobin, troponin T - fast skeletal muscle (TnTf), and creatine kinase M chain (M-CK). Overall, most of the identified proteins were grouped as myofibrillar proteins, which responsible in structural integrity of skeletal muscle while

creatine kinase and myoglobin were grouped as sarcoplasmic proteins. Most of the identity of proteins was obtained from MASCOT (Matrix Science, UK) database which the animal sources were referred to the closest animal species such as bovine as there is limited protein database for goats.

The expression profiles of the 7 identified proteins are as presented in Figure 2. The General Linear Model analysis of spots intensity revealed that the heat treatment employed in this study could have resulted in significant reduction in actin, troponin-T, myoglobin, and creatine kinase, while others (myosin light chain 2, myosin light chain 3, and tropomyosin-1) showed no significant differences among the heat treatments.

Table 1: Identified proteins from *Longisimus ~~dorsi~~* 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	Mowse score[c]	Matched peptides[d]/ sequence coverage(%)[e]
	Chilled (untreated samples)					
1	Tropomyosin-1 alpha chain (TPM1)	gi|20522240 (Mouse)	5.3/39300	4.69 / 32661	476	22/ 36
2	Actin, alpha cardiac muscle 1 (ACTC)	gi|124007203 (Bovine)	6.10 / 42700	5.23 / 41992	462	15 / 29
3	Myosin light chain 3, skeletal muscle isoform (MLC3F)	gi|127130 (Rabbit)	5.80 / 24000	4.62 / 16647	115	5/ 19
4	Myosin regulatory light chain 2, skeletal muscle isoform (MLC2F)	gi|2829841 (Mouse)	5.40 / 19200	4.82 / 18943	188	7/ 20
5	Myoglobin	gi|127638 (Bovine)	8.80 / 15300	6.90 / 17067	127	8/ 28
6	Myoglobin	gi|127638 (Bovine)	8.4 / 15800			
8	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.4 / 36800	5.63 / 33014	65	7/ 14
11	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.4 / 40100	6.63/ 42962	410	19 /37
12	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.6 / 40500	6.63/ 42962	409	20/40
13	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.2 / 39.7	6.63/ 42962	772	24/37
	Boiled samples					
1	Tropomyosin-1 alpha chain (TPM1)	gi|20522240 (Mouse)	5.3/39300	4.69 / 32661	405	15/20
2	Actin, alpha cardiac muscle 1 (ACTC)	gi|124007203 (Bovine)	6.10 / 42700	5.23 / 41992	478	14/23
3	Myosin light chain 3, skeletal muscle isoform (MLC3F)	gi|127130 (Rabbit)	5.80 / 24000	4.62 / 16647	92	4/12
4	Myosin regulatory light chain 2, skeletal muscle isoform (MLC2F)	gi|2829841 (Mouse)	5.40 / 19200	4.82 / 18943	140	9/25
5	Myoglobin	gi|127638 (Bovine)	8.80 / 15300	6.90 / 17067	168	4/ 16
6	Myoglobin	gi|127638 (Bovine)	8.4 / 15800	6.90 / 17067	253	6 / 25
8	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.9 / 39700	5.63 / 33014	203	12 / 20
9	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.2 / 39500	5.63 / 33014	94	17 / 22
11	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.6 / 40500	6.63 / 42962	116	12/17
	Autoclaved samples					
1	Tropomyosin-1 alpha chain	gi|20522240 (Mouse)	8.2 / 39.7	4.69/32661	156	10/ 14
2	Actin, alpha cardiac muscle 1	gi|124007203 (Bovine)	6.1 /46000	5.23/ 41992	471	17/28
3	Myosin light chain 3, skeletal muscle isoform (MLC3F)	gi|127130 (Rabbit)	5.8/23100	4.62/16647	72	2/5
4	Myosin regulatory light chain 2, skeletal muscle isoform (MLC2F)	gi|2829841 (Mouse)	5.6 / 17600	7.72/35130	80	4/5
5	Myoglobin	gi|127638 (Bovine)	8.9 / 15300	6.90 / 17067	196	8 / 35
6	Myoglobin	gi|127638 (Bovine)	8.5 / 15600	6.90 / 17067	328	10 / 28
7	Myoglobin	gi|127638 (Bovine)	8.5 / 15600	6.90 / 17067	165	5 / 22
8	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.9 / 39700	5.63 / 33014	223	14 / 20
9	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.2 / 39500	5.63 / 33014	294	16 / 21
10	Troponin T, fast skeletal muscle (TnTf)	gi|1717774 (Rabbit)	8.6 / 39600	5.63 / 33014	105	4/9
11	Creatine kinase M-type (M-CK)	gi|109940091 (Bovine)	8.6 / 44200	6.63 / 42962	239	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.

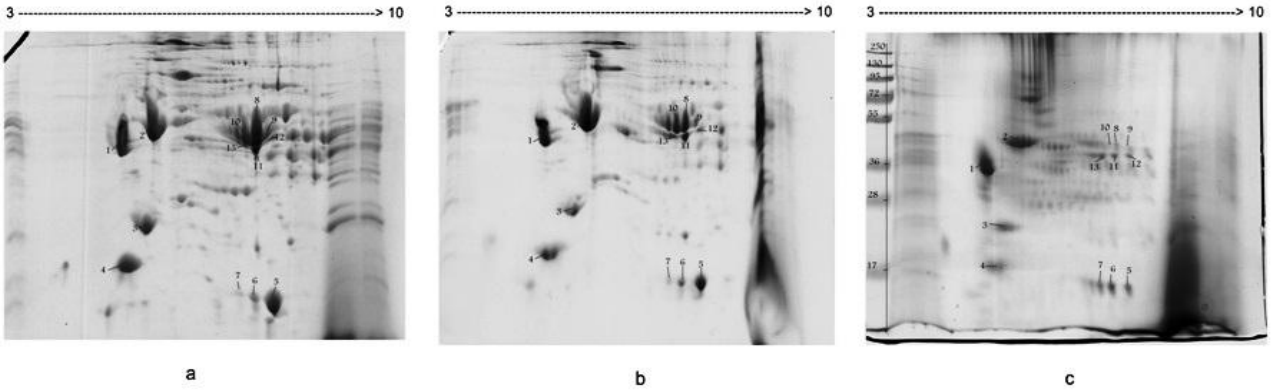

Figure 1: Images of representative 2-DE gels of (a) chilled (b) boiled and (c) autoclaved skeletal muscle proteins. The remaining protein spots following to heating at 121 °C, 15 psi heat are numbered.

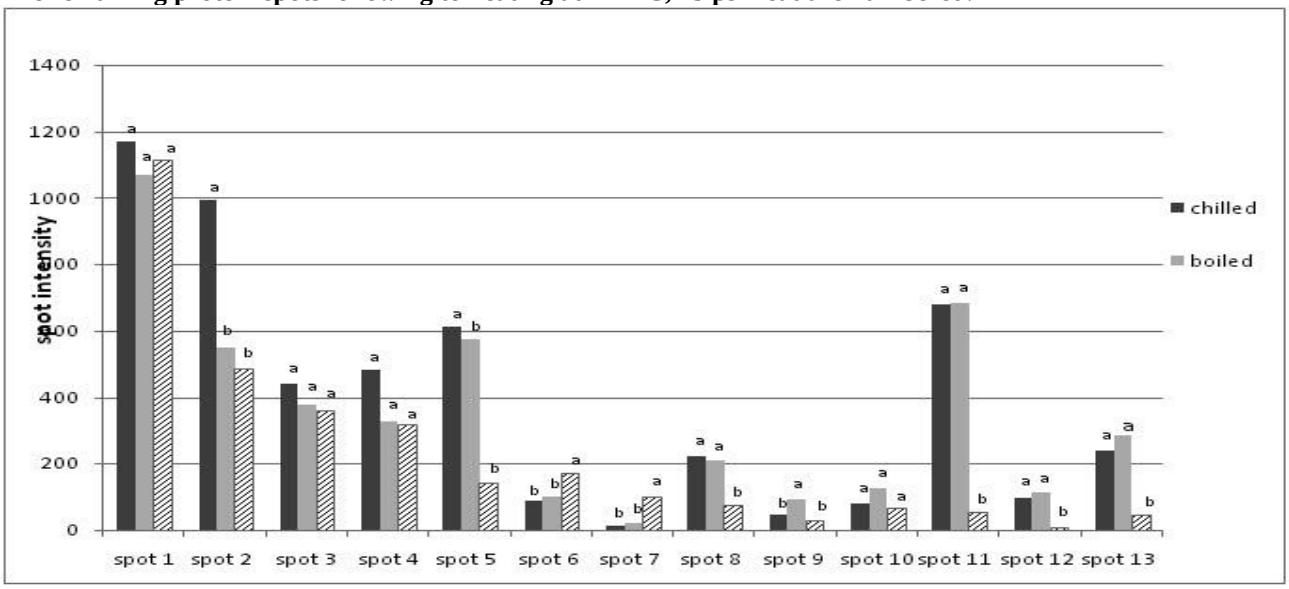

Figure 2: The spot intensities of heat stable proteins in goat skeletal muscle after subjected to heat treatment. The mean for each spot of each treatment was derived from 10 replicates. Different alphabets indicate significant differences ($p < 0.05$) in terms of spot intensity.

DISCUSSION

It has been well-documented that heating process leads to protein denaturation (Tornberg, 2005). The first reaction is the unfolding of tertiary structure of the proteins followed by aggregation and coagulation of protein chains (Promeyrat *et al*., 2010). Changes in protein solubility caused by heat also associated with changes in water-holding capacity of meat (Bouton and Harris, 1972). This process is commonly associated with the breaking of hydrogen or electrostatic bonds, which in turn may result in water loss in meat (Tornberg, 2005). The severity of protein denaturation increases with time and temperature, whereby less amount of water is entrapped within the protein structures that are normally held by capillary forces. The above mechanism explains the reason most of the soluble sarcoplasmic proteins are easily purged out of meat at the temperature between 40°C to 60°C.

The susceptibility of meat proteins to thermal denaturation depends on their structure, predominantly the number of cross-links. Myofibrillar proteins undergo less thermal denaturation as there is great amount of actomyosin-complexes required to be broken down by the heat. The mass spectrometry result in autoclaved samples obtained from spot number 3 and 4 (Figure 1) indicates the presence of MLC2F and MLC3F isoforms at 5.6 and 5.8 kDa with pI 17.6 and 23.1, respectively. Myosins are composed of one or two heavy chains and four or more light chains. MLC fast isoforms are expressed in fast-twitch fibers (Schiaffino and Reggiani, 1996), and the presence of MLC2F and MLC3F in the protein extracts confirmed that *longissimus* muscle is fast-twitch muscle. No native myosin molecule and myosin heavy chain (MHC) were left and only myosin monomers were present in the meat and these are in agreement with the previous work by Tornberg (2005),

which reported a prolonged heating has changed the helix structure of the myosin. Moreover, the denaturation of myosin has been linked with the dissociation of other adjacent proteins such as actin, troponin, and tropomyosin within the acto-myosin cross bridge complex. This is in line with the observed similar reductions in spot intensities of the actin, troponin, and tropomyosin in our study.

In the case of spot number 1 (Figure 1) that corresponds to tropomyosin-1 alpha chain (TPM1), the presence of salt bridges, side chain hydrogen bonds, and a large proportion of residues in α-helical conformation (Oe *et al*., 2007) could explain the increase in their thermal stability as observed in this study. However, despite the characteristics mentioned earlier, decreases in TPM1 spots intensities were clearly noted when the samples were subjected to both heat treatments (Figure 2). This finding was supported by the mass spectrometry results, which indicated the sequence coverage of TPM1 in chilled, boiled, and autoclaved samples as 36%, 20%, and 14%, respectively. As TPM1 is a coiled-coil protein that interacts with actin in regulating skeletal muscle contraction as well as stabilising the actin filament (Choi and Kim, 2009), similar reducing spot intensity was also observed in spot number 2 (actin protein), of which the sequence coverage was 29%, 23%, and 28%, respectively.

In the current experiment, the constant presence of TnT in samples from all treatments highlighted its possible stability to heat. The appearance of 39 kDa troponin-T (TnT) (as spot number 8, 9, and 10) was noted at pI 8.9, 8.2, and 8.6, respectively. It indicated that the thermal degradation of TnT was in agreement with the previous findings by Muroya *et al*. (2007) that the shift in pI from 5.7-9.6 could be due to their phosphorylation and degradation. The present study has applied heat treatments that could have altered the N-terminal variable regions, which in turn may have caused a shift towards the basic region. This basic TnT fragments have been suggested as a potential biomarker in beef aging (Muroya *et al*., 2007) and also a thermostable biomarker in other species (Chen *et al*., 2002; Ballin *et al*., 2009).

There were differences in spots intensities of protein numbered 5, 6, and 7 among the treatments that corresponded to myoglobin protein. In the case of spot number 5, the greatest intensity was observed in the chilled samples and appeared to be less intense in boiled and autoclaved samples. In contrast, the intensities of spots number 6 and 7 were markedly increased in the boiled and autoclaved samples. The decreased intensity of spot 5 and increased intensities of spots 6 and 7 illustrate the fragmentation of myoglobin protein when subjected to heat treatments. The native protein (spot number 5), which was visibly reduced in the gels may be due to the increased protein fragmentation that has been further fragmented with increasing temperature (100°C to 121°C). In addition, the conversion of ferrohaemochrome could also have resulted in the fragmentation of myoglobin observed in this study. Myoglobin consists of globin protein and a haeme group. It is an oxygen-binding protein in muscles that is easily affected by autoxidation, which then results in loss of stability (Promeyrat *et al*., 2011; Suman *et al*., 2009). When subjected to heat, the haeme ion loss its affinity to the myoglobin structure resulting in the conversion of ferrohaemochrome (red myoglobin) to ferrihaemochrome (brown myoglobin), a phenomenon that usually occurs during cooking (King and Whyte, 2006). The phenomenon could explain the fragmentations of myoglobin into several spots following heating process.

In general, the proteins studied reflect those usually present in meat and meat products after cooking. However we believe that the amount of protein may be lesser than that 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.

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 significant reduction in the spot intensities of TPM1, MLC3F, and MLC2F proteins and this merits their candidature as possible heat stable 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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