

Proteome Changes in *biceps femoris* Muscle of Iranian One-Humped Camel and Their Effect on Meat Quality Traits

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Summary

In this study physicochemical and quality traits of *biceps femoris* and *longissimus thoracis* muscles of male and female Iranian one-humped camel were determined during 14 days of refrigeration storage. Analysis of variance of the results showed that only shear force and temperature were affected by the gender ($p < 0.05$). Anatomical location of the muscle influenced the meat properties except for drip loss ($p < 0.05$). Also, except for cooking loss, ageing influenced the physicochemical and quality properties of meat; during 14 days of storage, proteolysis resulted in an increase of L^* and b^* values, drip loss and myofibrillar fragmentation index, and the decrease of a^* value, expressed juice, shear force and sarcomere length. Proteome changes (myofibrillar proteins) during storage were investigated. Gel analysis revealed that 19 protein spots were significantly changed during 24, 72 and 168 h *post-mortem*. Fifteen spots were identified by MALDI-TOF/TOF mass spectrometer. Correlation analysis revealed significant correlations of actin, troponin T, capping protein, heat shock proteins (HSP) and desmin with physicochemical and quality properties of meat ($p < 0.05$). Actin might be a potential protein marker for colour, tenderness and water-holding capacity, and HSP27 and desmin are good candidate markers for colour and tenderness, respectively.

Key words: camel, *biceps femoris*, *longissimus thoracis*, proteolysis, proteomics

Introduction

The one-humped camel (*Camelus dromedarius*) is the most useful domestic animal species for animal production in the arid and semi-arid regions (1). In comparison with other livestock species, fat content of camel carcasses is lower and meat from camel muscles has relatively high polyunsaturated fatty acids and low cholesterol contents (2). Also, camel meat is used for treating diseases like hypertension, hyperacidity, pneumonia and respiratory disease, as well as an aphrodisiac (2). Recent attention to organic and health-promoting diets has led to an increase in the demand for camel meat (1). Since camels inhabit

mainly the deserts of developing countries and are generally used by nomads, research into improving meat characteristics is lacking (1).

The conversion of muscle into meat and the underlying mechanisms are complex processes with many poorly characterized pathways (3). Prominent characteristics of meat such as water-holding capacity and tenderness are considerably influenced by the rate and extent of *post-mortem* metabolic changes (4). During the last decade, proteomics has been applied to better explain the mechanisms behind meat quality traits (3). Proteomics appears as a promising technique to establish the link between protein changes occurring *post-mortem* and sensory or

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technological characteristics of meat (5). Numerous studies into the proteome of porcine (4–13) and bovine (3,14–21) muscles have indicated that many cellular defence/stress proteins, metabolic enzymes and structural proteins change during *post-mortem* ageing.

In the recent years, many investigations have been carried out of camel meat characteristics and more information has been gathered (1,22–24). It was of interest to understand what happens in camel meat proteome during storage and how breakdown of proteins relates to meat quality. The aims of this study are to investigate the changes that occur in the protein profiles of camel muscle during 7 days of *post-mortem* storage and to determine the relationship between proteolysis and physicochemical and quality properties.

Materials and Methods

Chemicals

All chemicals and reagents used were of analytical grade. Glutaric dialdehyde, ethylenediaminetetraacetic acid disodium salt dehydrate (Titriplex® III), potassium phosphate (KH_2PO_4), sodium azide, bovine serum albumin (BSA), Tris, sucrose, DL-dithiothreitol (DTT), Bromophenol Blue, sodium dodecyl sulphate (SDS), HCl, colloidal Coomassie Brilliant Blue G-250, phosphoric acid, ammonium sulphate and acetic acid were purchased from Merck KGaA (Darmstadt, Germany), potassium chloride (KCl), magnesium chloride (MgCl_2) and glycerol from Acros Organics (Geel, Belgium), urea and methanol from Panreac (Barcelona, Spain), boric acid (H_3BO_3) from AppliChem (Darmstadt, Germany), and porcine trypsin, EDTA, CHAPS, thiourea, iodoacetamide and agarose from Sigma-Aldrich Chemie GmbH (Schnellendorf, Germany), unless otherwise noted.

Camels

Eight 4- to 6-year-old dromedary camels (four males and four females with live mass of (512 ± 52) and (453 ± 37) kg, respectively) were carried to the slaughterhouse in Mashhad (Iran). Handling and transportation of the camels were done with maximum possible welfare and afterwards maintained in a lairage for 2 days. Slaughtering and dressing of camels were carried out according to Islamic standards. The average slaughter mass of male and female carcasses was (306 ± 33) and (281 ± 24) kg, respectively. The temperature of slaughterhouse varied between 25 and 27 °C at slaughter time.

Meat samples

The required amounts of *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles were cut from the left side of carcasses after 24 h *post-mortem*. Connective tissue and exterior fat of the muscles were removed. After packaging in zipped plastic bags, meat samples were placed in an insulated cool box and moved to the meat laboratory at Ferdowsi University of Mashhad and kept in a refrigerator (4–5 °C) before running quality measurements on days 1, 3, 5, 7 and 14 *post-mortem*. Samples of approx. 1 g were taken from the BF muscle 1, 3 and 7 days *post-mor-*

tem, flash frozen in liquid nitrogen and subsequently kept at –80 °C until proteomic analysis.

Physicochemical and quality trait measurements

Muscle pH decrease

The pH decrease in LT and BF muscles from the left side of each carcass was measured by a portable pH meter (Testo 230; Testo AG, Lenzkirch, Germany) fitted with a glass electrode and a thermometer probe. Measurements were done 1, 3, 6, 12, 24 h and 3, 5, 7 and 14 days *post-mortem* at the depth of 1.5 cm in the muscle.

Colour measurements

Changes of the muscle colour (L^* , a^* and b^* values) were measured by a CR-410 chromameter (Minolta, Kyoto, Japan) on freshly cut surfaces of each muscle after blooming for 60 min at room temperature.

Expressed juice

Expressed juice was measured according to the method of Pohja and Niinivaara (25) with some modifications. A mass of 0.3 g of ground meat was placed on Whatman No. 1 filter paper (GE Healthcare Life Sciences, Maidstone, UK), put between two plastic plates and a 2-kg load was placed on top of them for 5 min. Then, ground meat was separated from the filter paper and the paper was weighed. Expressed juice was calculated using the following equation:

$$w(\text{juice}) = \left(\frac{m_2 - m_1}{m_s} \right) \cdot 100 \quad /1/$$

where m_s is the mass of the ground meat, m_1 is the initial mass of the filter paper, and m_2 is the mass of the paper after ground meat separation.

Drip loss

For the drip loss measurement, cubic samples of approx. 40 g were placed in plastic nets and hanged in a glass container. The containers were maintained in a refrigerator for 24 h. Then, the meat samples were blotted dry with filter paper, reweighed and the drip loss was calculated as the mass loss expressed as a percentage of the original mass of the sample (26).

Cooking loss

Cooking loss was assessed by a method described by Honikel (26) with some modifications. Meat samples with approximate thickness of 1 cm were weighed, put in zipped plastic bags, heated in a water bath at 80 °C for 60 min. The bags containing cooked samples were then removed from the water bath, cooled under running tap water, blotted dry with absorbent paper and reweighed. Cooking loss was calculated as the difference in the sample mass before and after cooking, expressed as a percentage of the initial sample mass.

Shear force

For evaluating meat tenderness the same samples that were used for determination of cooking loss were taken. After cooking loss assay the samples were placed in zipped plastic bags, stored in a refrigerator overnight and after equilibration at room temperature used for

shear force assay. To analyse instrumental tenderness, three cylindrical cores were cut from each cooked sample using a 1.27-cm diameter tube-shaped sharp metal mould. Samples were randomly removed from the centre of each muscle. A TA.XTplus texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) provided with a Warner-Bratzler shear force blade was employed to measure tenderness. Maximum shear force values (in N) required to shear a cylindrical core of cooked muscle, perpendicular to the longitudinal orientation of the muscle fibres at a crosshead speed of 200 mm/min (27), were recorded for each sample and the mean of three replications was calculated for each muscle.

Sarcomere length

Sarcomere length was measured using the method of Botha *et al.* (27). The samples were cut from the centre of the muscle slices (1 cm thick). Meat cubes of approx. 1 cm³ were immediately fixed in 2.5 % (by mass per volume) glutaric dialdehyde solution A (2.5 % glutaric dialdehyde, 0.1 M KCl, 0.039 M H₃BO₃ and 5 mM Titriplex III) and stored at 4 °C for 24 h. Then, the meat cubes were transferred to 2.5 % glutaric dialdehyde solution B (2.5 % glutaric dialdehyde, 0.25 M KCl, 0.29 M H₃BO₃ and 5 mM Titriplex III) and stored at 4 °C. The samples were homogenised in fresh 2.5 % glutaric dialdehyde solution B with an IKA T25 Ultra-Turrax[®] digital homogeniser (IKA, Staufen, Germany) at 17 000 rpm for 30 s. The homogenised samples were stored at 4 °C until microscopic analysis. Muscle fibre from individual muscle samples was transferred to glass slides and covered with a glass coverslip. Moisture of the samples was maintained with the addition of 2.5 % glutaric dialdehyde solution B. Images of two individual muscle fibre samples from each glass slide were taken with a digital camera (model DP71; Olympus, Tokyo, Japan) connected with a microscope (Olympus BH2 microscope, Tokyo, Japan). Sarcomere length was measured with the use of Adobe Photoshop, v. 6.0 (Adobe, San Jose, CA, USA) by counting three sets of three consecutive sarcomeres from each fibre image. The mean sarcomere length for each muscle fibre image was then calculated.

Myofibrillar fragmentation index determination

Myofibrillar fragmentation index (MFI) was determined as described by Culler *et al.* (28). The frozen samples were minced in a cutter after all visible fat and connective tissue had been removed. A mass of 4 g of minced muscle was homogenised at 4 °C for 30 s in 10 % (by volume per mass) of isolation solution (MFI buffer) consisting of 100 mM KCl, 20 mM KH₂PO₄, 1 mM EDTA, 1 mM MgCl₂ and 1 mM sodium azide. The homogenate was sedimented at 1000×g for 15 min and the supernatant was decanted. The sediment was resuspended in 10 % (by volume per mass) of isolation solution using a stirring rod, sedimented again at 1000×g for 15 min and the supernatant was decanted. The sediment was resuspended in 2.5 % (by volume per mass) of isolation solution and passed through a cotton strainer to remove connective tissue and debris. An additional 2.5 % (by volume per mass) of the solution was used to facilitate the passage of myofibrils through the strainer. Protein concentrations of the myofibril suspensions were determined by the biuret method described by Gornall *et al.* (29). Briefly, 0.25 mL of each

suspension was placed into glass tubes and 0.75 mL of MFI buffer was added, and finally, 4 mL of biuret reagent were added and vortexed. Glass tubes were placed in the dark at room temperature for 30 min. The absorbance was read at 540 nm using UV-2601 double-beam UV-Vis spectrophotometer (Beijing Rayleigh Analytical Instrument Corp., Beijing, PR China). BSA was used to establish a standard curve. An aliquot of the myofibril suspension was diluted in isolation solution to a protein concentration of (0.5±0.05) mg/mL. Protein concentration was again determined by the biuret method. The diluted myofibril suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give a MFI.

Proteomic analysis

Extraction of muscle proteins

Myofibrillar protein from muscle was extracted using a slightly modified procedure of Hollung *et al.* (6). Frozen BF muscle tissue (approx. 300 mg) was ground under liquid nitrogen and homogenised in 1 mL of TES buffer (10 mM Tris, pH=7.6, 1 mM EDTA and 0.25 M sucrose) using a Heidolph SilentCrusher M homogeniser (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 10 000 rpm for 60 s. Subsequently, the homogenate was sedimented (at 8800×g for 30 min) at 4 °C and the supernatant was discarded. This step was repeated twice in order to remove all TES buffer-soluble proteins (*i.e.* sarcoplasmic proteins). Finally, the pellet was homogenised in urea buffer (1 % DTT, 7 M urea, 2 % CHAPS and 2 M thiourea) using a Heidolph SilentCrusher M homogeniser at 10 000 rpm for 60 s. The homogenate was forcefully agitated for 1 h at room temperature followed by sedimentation at 8800×g for 30 min to separate any insoluble components. The Bradford assay was utilised to quantify myofibrillar protein concentration using BSA as a standard (6).

Two-dimensional gel electrophoresis

For first-dimension separation, 1000 µg of protein were added to a rehydration buffer containing 0.2 % (by mass per volume) DTT, 7 M urea, 2 % (by mass per volume) CHAPS, 2 M thiourea, 0.2 % (by volume) carrier ampholyte (40 %, pH=5–8; Bio-Lyte[®] Ampholyte, Bio-Rad, Hercules, CA, USA) and 0.002 % (by mass per volume) Bromophenol Blue. The protein was then loaded onto immobilized pH gradient (IPG) strips (17 cm, linear, pH=5–8; Bio-Rad) by in-gel rehydration for 16 h at room temperature, and afterwards, focusing steps were conducted on a Protean IEF cell apparatus (Bio-Rad). Low voltage (250 V) was applied for 1 h in the first step. The voltage was raised stepwise to 8000 V, and kept at this level until a total of 47 000 V·h was achieved. Immediately after focusing, the IPG strips were transferred to a freezer (–20 °C) and stored until second-dimension separation. Before the second-dimension separation, the IPG strips were reduced for 15 min in an equilibration buffer containing 20 % (by volume) glycerol, 6 M urea, 2 % (by mass per volume) SDS, and 2 % (by mass per volume) DTT, in 375 mM Tris-HCl, pH=8.8, and then alkylated for 15 min in an alkylating buffer containing 20 % (by volume) glycerol, 6 M urea, 2 % (by mass per volume) SDS, and 2.5 % (by mass per volume) iodoacetamide, in 375 mM Tris-HCl, pH=8.8. The strips were loaded onto vertical 12.5 % SDS-PAGE, sealed with heated agarose solution and run on PROTEAN[®] II xi Cell apparatus (Bio-Rad) for 30 min at 16 mA,

then for 8 h at 24 mA and 20 °C, until the Bromophenol Blue front reached the end of the gel. A protein standard (10–250 kDa; SinaClon protein ladder, Tehran, Iran) was used to estimate the molecular mass of separated proteins.

Gel staining and analysis

Visualisation of proteins was performed by staining of the gels with Coomassie Brilliant Blue (0.1 % colloidal Coomassie Brilliant Blue G-250 in 3.6 % phosphoric acid, 17 % ammonium sulphate and 34 % methanol) for 24 h followed by destaining with 1 % acetic acid for 24 h. Stained gels were scanned using a GS-800 imaging densitometer (Bio-Rad) and images were then analysed using the Image Master Platinum v. 6.0 software (GE Healthcare, Chicago, IL, USA). The spots were automatically matched with the spots of a master gel used as the reference. Errors and mismatch spots were removed manually. Each detected and matched spot was normalised by expressing its intensity relative to the total intensity of all spots. For one sample and one spot, the mean value was calculated over three gels, and the resulting average spot intensities were analysed by one-way analysis of variance (ANOVA) using SPSS for Windows, v. 16.0 (SPSS Inc, Chicago, IL, USA). The value of $p < 0.05$ was regarded as statistically significant.

In-gel digestion, mass analysis and database search for protein identification

Candidate protein spots were excised and transferred to microtubes and sent to the Centre of Excellence in Mass Spectrometry at University of York, York, UK, for in-gel digestion, identification and characterization by Ultraflex III MALDI-TOF/TOF (matrix-assisted laser desorption ionization-time of flight analyser; Bruker Daltonics, Bremen, Germany). The gel pieces were destained, digested with porcine trypsin and analysed by a MALDI-TOF/TOF mass spectrometer according to the Centre protocols. The flexAnalysis software v. 3.3 (Bruker Daltonics, Billerica, MA, USA) was used to perform the spectral processing

and peak list generation for both the MS and MS/MS spectra. Tandem mass spectral data were submitted to database searching using a locally running copy of the Mascot program v. 2.1 (Matrix Science Ltd., London, UK), through the ProteinScape interface v. 2.1 (Bruker Daltonics).

Statistical analysis

The statistical effects of gender, muscle type and *post-mortem* storage on physicochemical and quality traits of camel meat were analysed for significance at the 5 % level by one-way ANOVA using the general linear models (GLM) procedure of SPSS (SPSS Inc). The statistical effect of sampling time on spot quantity was tested by ANOVA (at 5 % level). Spots whose intensity differed significantly at 24, 72 and 168 h were identified. Duncan's multiple range test was used to evaluate the differences between groups at the 5 % significance level. Pearson's correlations between meat characteristics and significantly changed proteins were performed.

Results and Discussion

Effects of gender on meat quality

The average values of meat quality characteristics of Iranian one-humped male and female camels are presented in Table 1. Results showed that gender had a significant ($p < 0.05$) effect on the carcass temperature variations and Warner-Bratzler shear force. The carcass temperature of female was higher than of male camels. The reason for slower rate of temperature drop in the carcass of female camels was higher content of carcass fat in comparison with male camels (30), because heat transfer coefficient of fat is lower. Muscle shear force in male camels was significantly higher than in females. The more force needed, the tougher the meat is. This may be attributed to low expression of type I collagen in the female camels. Collagen types I and III are two of four major proteins of perimysium

Table 1. Meat quality characteristics (mean value±standard error) of the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles of male and female camel carcasses

Parameter	<i>biceps femoris</i>		<i>longissimus thoracis</i>		Significance		
	Male	Female	Male	Female	Gender	Muscle	Time
Temperature/°C	18.49±10.13	19.31±9.36	17.10±8.39	16.87±11.45	0.03	0.00	0.00
pH	6.03±0.31	6.04±0.27	5.92±0.34	5.97±0.29	0.09	0.00	0.00
pH _u	5.74±0.09	5.78±0.10	5.63±0.09	5.67±0.03	–	–	–
L*	34.27±2.25	34.35±1.78	34.92±0.98	34.82±1.53	0.84	0.00	0.00
a*	14.39±1.11	14.21±1.12	12.75±0.77	12.82±0.70	0.10	0.00	0.00
b*	6.54±0.36	6.53±0.41	6.48±0.25	6.55±0.41	0.45	0.65	0.00
w(expressed juice)/%	39.92±2.75	40.31±2.98	36.71±3.02	37.13±2.14	0.43	0.00	0.00
w(drip loss)/%	2.15±0.47	1.93±0.36	1.92±0.37	1.81±0.41	0.17	0.14	0.00
w(cooking loss)/%	34.72±4.05	34.66±3.42	31.43±3.50	32.09±3.14	0.75	0.00	0.48
Shear force/N	51.82±7.93	48.67±8.71	44.09±8.16	42.81±8.65	0.02	0.00	0.00
MFI	77.96±12.77	75.82±11.84	88.11±12.27	86.13±13.25	0.18	0.00	0.00
l(sarcomere)/µm	1.62±0.14	1.64±0.09	1.74±0.12	1.75±0.11	0.08	0.00	0.00

pH_u=ultimate pH value, MFI=myofibrillar fragmentation index

um, which has a rigid structure (31). In this respect, Hakimov *et al.* (32) showed that estrogen, female sex hormone, reduces types I and III collagen synthesis in porcine muscles. Many researchers have also reported that the meat from male species is tougher than from female species (33–35).

Effects of muscle type on meat quality

The average values of physicochemical properties of the LT and BF muscles are presented in Table 1. Anatomical location of a muscle had an influence on the meat properties except for drip loss and b^* value ($p < 0.05$). The mean temperature of the BF during 24 h *post-mortem* was 2 °C higher than that of the LT ($p < 0.05$). The pH of the LT muscle decreased to lower values in comparison with the BF during *post-mortem* storage in a refrigerator. This may be related to slightly higher glycogen content in the LT in comparison with the BF (36). In this study, the LT muscle was slightly lighter than the BF (higher L^* value) and redness (a^* value) of the LT was greater than that of the BF ($p < 0.05$). This could be partly attributed to differences in muscle myoglobin content and its chemical state, and muscle fibre type differences (2). Furthermore, higher intramuscular fat content of the LT (data not presented) resulted in increased lipid oxidation, which could be a promoter of (oxy)myoglobin oxidation, and decrease in a^* value (37). Low water-holding capacity of the BF led to enhancement in the juice extraction ($p < 0.05$). Similarly, Kadim *et al.* (23) reported that anatomical location of the muscle in the camel carcass influenced the expressed juice mass fraction. These variations were described by the differences in muscle activity, pH, intramuscular fat, composition of muscle fibre and water to protein ratio. In the present study, the LT muscle had lower cooking loss, which pertains to higher water-holding capacity of that muscle. All types of water loss in meat including drip, thawing, cooking and pressing loss are affected by the water-holding capacity of meat (38).

Shear force of the BF was significantly higher than that of the LT muscle ($p < 0.05$). Higher shear force values of the BF muscle may be partly due to the amount and structure of the connective tissue. Collagen and elastin contents of distinctive muscles have a several-fold difference. In general, the force muscles such as the BF have more collagen and are more cross-linked than the position muscles (23,31). Furthermore, according to the findings of Wheeler *et al.* (39), connective tissue content, sarcomere length and proteolysis of myofibrillar proteins account for most, if not all, of the explainable variations observed in tenderness, and their relative contribution is muscle dependent.

One of the most important indices to measure proteolysis degree is MFI, indicating both the I-band breaks and the myofibrillar loss (40). In the present study, the LT muscle had significantly higher MFI values than the BF. This can be due to increased proteolytic activity of calpains or decreased inhibitory activity of calpastatin in the LT muscle. The BF muscle had shorter sarcomere length than LT muscle. The reasons for this difference could be related to the variations in muscle fibre orientation and in the amount of tension placed on the BF and LT muscles in a hanging carcass during 24 h of chilling at slaughterhouse and *rigor mortis* development (41).

Effects of ageing on meat quality

All of the physicochemical and quality properties of the LT and BF muscles changed significantly ($p < 0.05$) over 14 days of storage in a refrigerator except for cooking loss (Table 1). The pH of muscles reached the ultimate values (pH_u) 72 h after slaughter (Table 1). In the camel's body, the content of glycolytic enzymes is lower than in cattle due to its hump, which may cause slower glycogen degradation and pH decline (42). In the current study, the pH increased slightly up to 7 days *post-mortem* (Fig. 1). The pH increase from day 7 to 14 was significant ($p < 0.05$), and the pH of the BF and LT at the end of storage was 5.93 and 5.75, respectively.

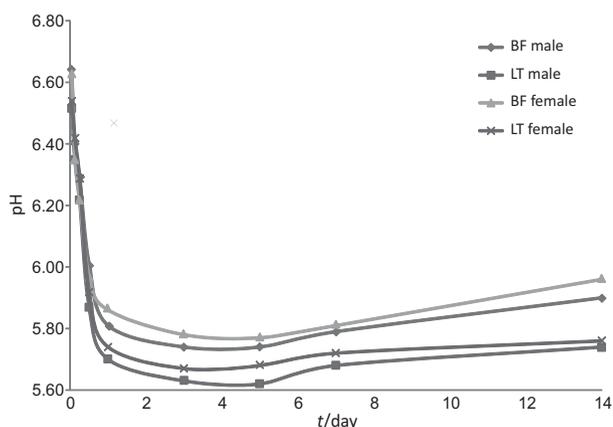


Fig. 1. Mean changes in the pH within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camel carcasses

Ageing of the LT and BF muscles influenced significantly ($p < 0.05$) the L^* , a^* and b^* values (Fig. 2). The L^* values of both muscles increased during storage because the *post-mortem* protein proteolysis intensifies light-scattering properties of meat and thereby increases the L^* value (43). Similar to L^* value, the b^* value showed an increasing trend during two weeks of storage. Increase of b^* value in meat was due to an increase in the relative concentrations of the oxygenated and oxidized forms of myoglobin (MbO_2 and $MetMb$) at the expense of the reduced form

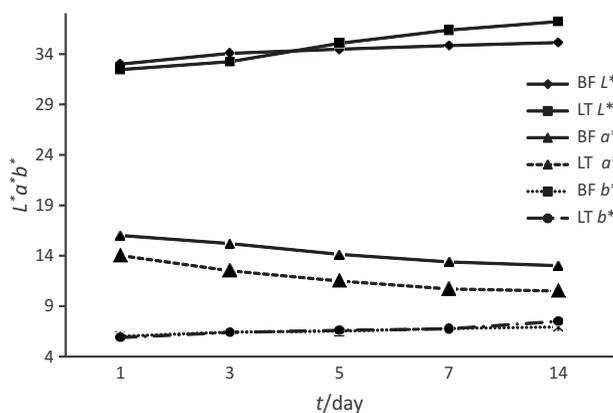


Fig. 2. Colour changes of the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

(Mb) (44). Ageing of meat resulted in an a^* value drop due to two reasons: (i) an increase in the relative concentration of MetMb on the surface of meat, which had a great impact on reducing the a^* value (44); and (ii) MetMb beneath the surface gradually thickened and moved towards the surface (45).

The mass fraction of expressed juice of both muscles decreased significantly ($p < 0.05$) during 14 days of storage (Fig. 3). Degradation of proteins, as well as an increase in net charge through absorption of potassium ions and release of calcium ions served to raise osmotic pressure and enhance both water-holding capacity and pH (after reaching a minimum pH in 72 h *post-mortem*) with ageing (46).

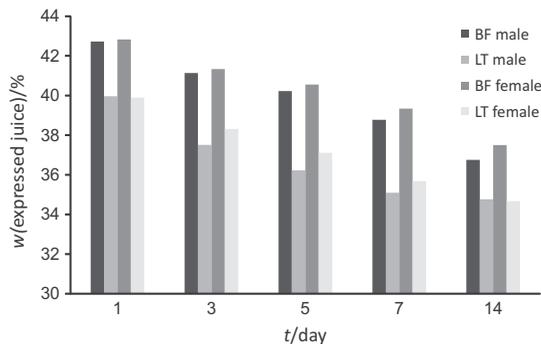


Fig. 3. Expressed juice changes within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

Significant increase ($p < 0.05$) in drip loss of the LT and BF muscles was observed as a result of ageing (Fig. 4). It seems that, in contrast with previous findings, desmin was not involved in drip loss, but other costameres were responsible for it. Similar results were reported by Melody *et al.* (47) for different porcine muscles.

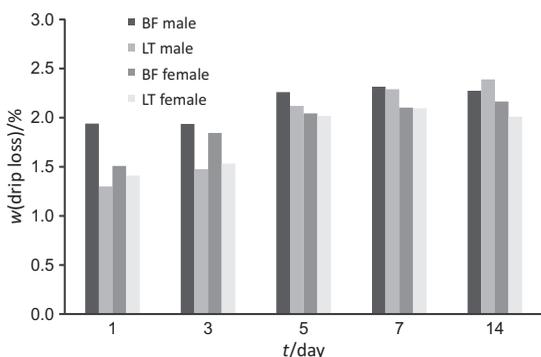


Fig. 4. Drip loss changes within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

Shear force of both muscles continuously decreased during 14 days *post-mortem* (Fig. 5). This observation was supported by the results obtained in other investigations on camel meat (1), beef (35), pork (47), *etc.* Proteolysis has an undeniable role in providing ultimate meat tenderness. Breakdown of titin, nebulin, desmin, and troponin-T proteins has been shown to be related to diminishing of shear force (47).

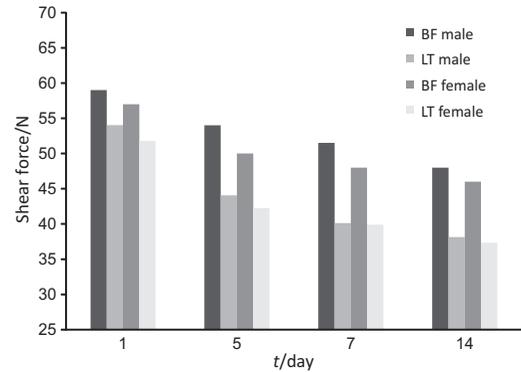


Fig. 5. Shear force changes within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

MFI is a useful indicator of the extent of myofibrillar protein degradation (28). Significant increase in MFI values of the LT and BF muscles occurred during *post-mortem* storage (Fig. 6). This is because myofibrils increasingly break into shorter fragments at or near the Z-band during *post-mortem* storage of the muscle (48).

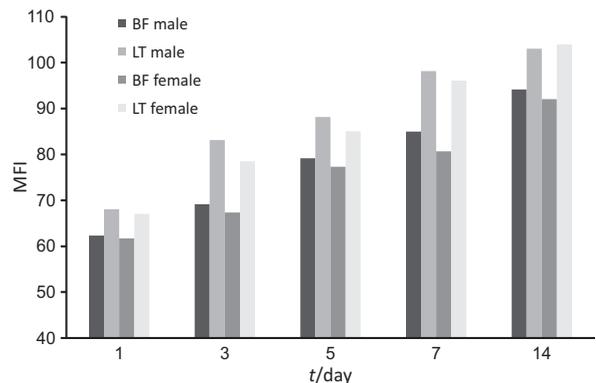


Fig. 6. Myofibrillar fragmentation index (MFI) changes within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

Length of sarcomeres rapidly decreased in the first 24 h after slaughter, followed by a slower rate of decline from days 1 to 14 (Fig. 7). Decrease in sarcomere length up to 3 days *post-mortem* (18 microns in the BF and 12 microns in the LT muscles) was due to increased actomyosin complex formation. As mentioned above, glycogen storage in camel meat, which provides required energy for sliding actomyosin, finished in 72 h *post-mortem*.

Proteomic analyses

Up to 300 spots per gel were detected. After removal of saturated or poorly reproducible zones, 100 spots were successfully matched across the whole set of images. Among them, the intensity of nineteen spots changed significantly ($p < 0.05$) with time. The reasons for these variations were degradation, post-translational changes and release from protein structures or complexes (5). Table 2 lists twelve identified spots whose intensity significantly differed between the two sampling times. Nine spots be-

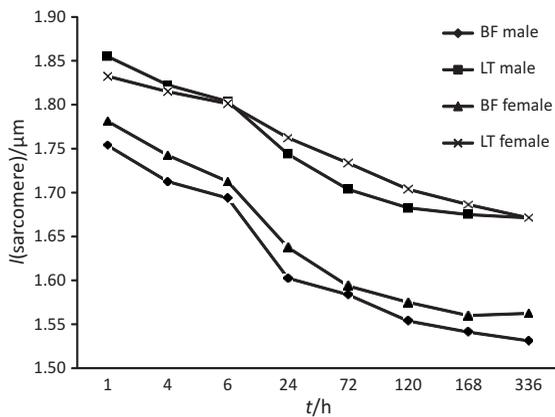


Fig. 7. Sarcomere length changes within the *longissimus thoracis* (LT) and *biceps femoris* (BF) muscles from male and female camels during 14 days of storage

long to myofibrillar proteins and three spots to sarcoplasmic proteins (NADH dehydrogenase and heat shock proteins (HSP)). Among myofibrillar proteins, four spots were identified as actin, one spot belonged to regulator proteins (capping protein), and four spots belonged to structural protein family (troponin T and desmin). Fig. 8 shows the image of a Coomassie Brilliant Blue-stained

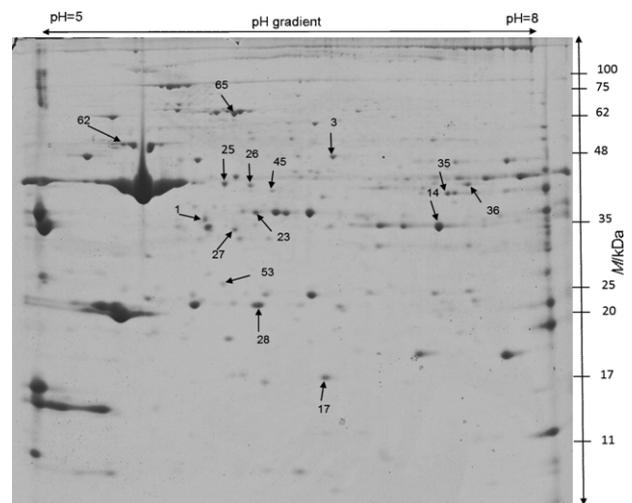


Fig. 8. Scanned 2-D gel electrophoresis image of BF muscle separated using an IPEG 5–8 strip in the first dimension and 12 % SDS gel in the second dimension. The protein loading was 1000 μ g and the gel was stained with colloidal Coomassie Brilliant Blue G-250. Numbered spots were identified and listed in Table 2

gel obtained from *biceps femoris* muscle myofibrillar proteins of camel.

Table 2. Identification and quantity of proteins and fragments significantly affected by the sampling time ($p < 0.05$)

Spot no.	Protein name	Accession no.	Organism	Location	Mascot score	AAS %	MP	Theoretical		Experimental		Fold change	
								M/kDa	pH	M/kDa	pH	3	7
1	actin, alpha cardiac muscle 1	Q3ZC07	<i>Bos taurus</i>	cytoplasm	315	12	3	42.3	5.23	39	5.66	-1.14	-1.54
25	actin, alpha cardiac muscle 1	Q3ZC07	<i>Bos taurus</i>	cytoplasm	263	12	3	42.3	5.23	41	5.79	-1.12	-1.29
26	actin, alpha cardiac muscle 1	Q3ZC07	<i>Bos taurus</i>	cytoplasm	264	12	3	42.3	5.23	41	5.94	-1.06	-1.44
36	actin, alpha cardiac muscle 1 (single peptide)	Q0PGG4	<i>Bos taurus</i>	cytoplasm	53	4	1	42.0	5.23	41	7.24	+2.04	+2.54
27	F-actin-capping protein subunit alpha-2	Q3T1K5	<i>Rattus norvegicus</i>	cytoplasm	92	13	3	33.1	5.57	32	5.86	+1.03	+2.27
14	troponin T, slow skeletal muscle	Q75ZZ6	<i>Sus scrofa</i>	cytoplasm	394	26	6	31.2	5.92	30	7.08	-1.06	-1.15
23	troponin T, slow skeletal muscle	Q7TNB2	<i>Rattus norvegicus</i>	cytoplasm	167	9	2	31.2	5.71	34	5.97	-1.71	-2.25
35	troponin T, fast skeletal muscle (single peptide)	Q8MKI3	<i>Bos taurus</i>	cytoplasm	80	5	1	32.1	5.99	37	7.11	+1.29	+1.37
17	heat shock protein beta-6 (single peptide) HSP20 (17 kDa)	Q148F8	<i>Bos taurus</i>	cytoplasm	67	6	1	17.5	5.95	17	6.38	+1.42	+1.78
28	heat shock protein beta-1 (single peptide) HSP27 (17 kDa)	Q3T149	<i>Bos taurus</i>	cytoplasm	122	7	1	22.4	5.98	22	5.98	-1.32	+1.15
53	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3	Q0MQG6	<i>Pongo pygmaeus</i>	mitochondria	133	9	2	30.4	6.54	26	5.78	-1.06	-1.39
62	desmin (single peptide)	Q5XFN2	<i>Canis familiaris</i>	cytoplasm	49	3	1	53.3	5.21	51	5.19	+1.07	+1.96
3	unidentified protein												
45	unidentified protein												

Protein names and accession numbers were derived from the UniProt database; AAS=coverage of the entire amino acid sequence, MP=number of matched peptides, theoretical=recorded in UniProt database and experimental=calculated from the spot position on the gel

Actin

Relatively poor resolution of one-dimensional SDS-PAGE might have led to limited identification of some major meat proteins such as actin in the last two decades, as *post-mortem* degradation of actin has not previously been reported. By using 2-D gel electrophoresis, it was found that actin degraded to small fragments in the few days of *post-mortem* (9,10). A relationship between *post-mortem* degradation of actin and meat tenderness was also found. In this study, three of the identified spots were found to be full-length actin and one was fragment of actin (Table 2), ranging from 39 to 41 kDa in molecular mass and from pH=5.66 to 7.24 in their isoelectric points. The spot intensities of full-length actins (spots 1, 25 and 26) decreased during *post-mortem* storage and spot intensity of actin fragment (spot 36) increased. *Post-mortem* breakdown of actin led to decrease in spot intensity of full-length actin, while accumulation of produced peptides resulted in an increase of spot intensity of actin fragment. Another explanation for the reduced abundance of full-length actins could be related to the disassociation of F-actin (filamentous) and release of G-actin (globular) during fractionation (14). Lametsch *et al.* (10) found that two fragments (40 and 32 kDa) in porcine muscle are derived from actin during 48 h after slaughter and that their spot intensity increased. Furthermore, actin degradation during 24, 48 or 72 h *post-mortem* was reported in many investigations on pork (5,7,9) and beef (3,14,15,17,18). In contrast, Jia *et al.* (15,17) stated that actin intensity did not change in bovine *longissimus dorsi* muscle during 24 h *post-mortem*, but it decreased in LT and *semitendinosus* muscles.

Significant correlations were found between a^* value, mass fraction of expressed juice, sarcomere length and MFI in one full-length actin (spot 1), and between b^* value and cooking loss ($p<0.05$) in another full-length actin (spot 25) (Table 3). Actin accounts for 20–25 % of myofibrillar proteins which *post-mortem* breakdown contributes to the most meat traits. This is in agreement with the results of Lametsch *et al.* (9) who observed a significant cor-

relation between the shear force in porcine muscle and three actin fragments at 72 h *post-mortem*. In addition, Laville *et al.* (19) stated that the 31-kDa fragment of actin can be used as an apoptosis marker. Results of a proteome analysis study showed that degradation of 9 actins and/or actin fragments were related to meat quality traits (L^* value and tenderness) during ageing (7). In the current study, there was no significant correlation between the actins and shear force, and a negative and significant correlation was observed between spot 1 and MFI. Also, a strong negative correlation between MFI and shear force ($p<0.01$) was found. It can be concluded that actin degradation and tenderness had very close correlation.

Troponin T

Troponin is a complex of three regulatory proteins (troponin C, troponin I and troponin T) and is involved in contraction of skeletal and cardiac muscles. In bovine muscle, two slow (sTnT) and six fast types of troponin T (fTnT) isoforms were identified (7). In the current study, two full-length sTnT (spots 14 and 23), and one fragment of fTnT were identified (spot 36; Table 3). *Post-mortem* proteolysis of troponin T by calpains was confirmed by coherent spot pattern changes of simultaneously decreasing full-length protein spots and an increasing fragment spot. The present study is in agreement with the previously reported proteolysis pattern of TnT in beef and pork (5,10,14). Bjarnadóttir *et al.* (3) reported that in bovine LT muscle sTnT isoform was more abundant 48 h *post-mortem*, while fTnT isoform was less abundant. This might be related to the changes in their solubility or degradation during *post-mortem* storage.

Pearson's correlation analysis showed that one of the sTnT isoforms had a positive relationship with a^* value and expressed juice, and a negative relationship with b^* value and cooking loss ($p<0.05$). Hwang *et al.* (7) found a significant correlation between three sTnT isoforms and L^* value and shear force and between one sTnT isoform and drip loss.

Table 3. Pearson's correlation coefficients between the proteins and physicochemical and quality traits of *biceps femoris* muscle during first week of storage

Spot no.	Protein name	pH	L^*	a^*	b^*	Expressed juice	Drip loss	Cooking loss	MFI	Shear force	Sarcomere length
1	actin	0.12	-0.96	0.99**	-0.99*	0.99**	-0.93	-0.99*	-0.99**	0.83	0.99**
25	actin	0.25	-0.99*	0.99*	-1.00**	0.99*	-0.87	-0.99**	-0.98	0.89	0.98
26	actin	-0.04	-0.90	0.98	-0.96	0.97	-0.98	-0.96	-0.99*	0.73	0.99*
36	actin (fragment)	-0.46	0.99*	-0.95	0.97	-0.95	0.74	0.96	0.91	-0.97	-0.91
17	HSP20 (fragment)	-0.32	0.99	-0.98	0.99*	0.98*	0.84	0.99*	0.96	-0.93	-0.96
28	HSP27 (fragment)	0.78	0.68**	-0.49	0.42	0.48	0.80	0.44	0.58	0.03	-0.57
27	capping protein	0.22	0.82	-0.93	0.89	0.92	0.99**	0.90	0.96	-0.60	-0.96
53	NADH dehydrogenase	-0.04	-0.91	0.98	-0.96	0.98	-0.97	-0.96	-0.99*	0.73	0.99*
62	desmin (fragment)	0.17	0.85	-0.94	0.91	-0.94	0.99**	0.92	0.97	-0.64**	-0.97
14	troponin T	0.19	-0.98	0.99**	-0.99**	1.00**	-0.90	-1.00**	-0.99*	0.87	0.99*
23	troponin T	0.53	-0.98	0.92	-0.95	0.92	-0.69	-0.94	0.87	0.98*	0.88
35	troponin T (fragment)	-0.56	0.98	-0.91	0.94	-0.91	0.66	0.93	0.86	-0.99*	-0.86

*significant at $p<0.1$, **significant at $p<0.05$; MFI=myofibrillar fragmentation index

Heat shock proteins

Heat shock proteins are members of the family of chaperones. Chaperone proteins protect native proteins against denaturation and subsequent aggregation, even after *post-mortem* (12). HSP27 may play a role in organising and protecting myofibrillar structures from proteolysis caused by stress conditions and is found in a low abundance in normal tissues (3,18). Hence, these stress proteins might be the potential markers of the change of myofibrillar structure in muscle *post-mortem* (16), and their increase is an indicator of myofibrillar protein change (15). HSP20 has roles in organising and protecting the myofibrillar structure and is localised on specific sarcomere structures such as the Z- and I-bands (17). In the present study, spots 17 and 28 belong to peptides of heat shock protein beta-6 (HSP20) and heat shock protein beta-1 (HSP27), respectively. The spot intensity of HSP27 decreased up to three days *post-mortem* and then increased, while the abundance of HSP20 increased about twofold up to day 7. HSP20 protects desmin, actin and titin, and HSP27 protects desmin against calpains. Decrease in the spot intensity of full-length actins and increase in the intensity of actin and desmin fragments indicated that protecting effect of HSPs diminished, which might be related to early postmortal degradation of HSPs. The reason for the decrease of HSP27 content on day 3 was probably due to solubility decrease. Results of previous studies showed that the HSP27 content increased at early times after slaughter, and that especially electrical stimulation intensified this increase, but *post-mortem* storage resulted in the decrease of HSP27 content (5,9,14–17). Similar trend was reported for HSP20 (15,17).

There was a significant positive correlation between the L^* value and HSP27 (Table 3). As abundance of HSP27 increased, the L^* value was enhanced too. This is consistent with previous observations of the relationship between HSP27 content and L^* value. Sayd *et al.* (12) found that HSP27 was overexpressed in darker beef meat. A negative correlation between HSP27 content and L^* value in pork (7) and between HSP27 content and L^* and a^* values in beef (18) have been reported. It can be concluded that proteolysis of HSP27 has a dramatic influence on meat colour and this protein might be a marker of colour variations in meat. Additionally, in the current study, HSP27 variations had no effect on meat texture. In contrast, researchers showed that degradation of HSP27 during meat ageing led to proteolysis increase of actin and myosin and consequently improved tenderness (7,15,18).

Desmin

In the present study, spot 62 was identified as a fragment of desmin (Table 3), which increased up to twofold during 7 days *post-mortem*. This was because desmin was mostly degraded by calpains during the first week of ageing. Similarly, Bjarnadóttir *et al.* (3) reported that in bovine *longissimus dorsi* abundance of desmin decreased 24 h after slaughter. Results of another proteomic study showed that the spot intensity of desmin reduced in half after 7 days of ageing (7).

There was a significant positive correlation between the fragment of desmin and drip loss. With the increase of desmin fragment, drip loss increased too (Table 3). As a

cytoskeletal protein, desmin contributes to the structural integrity and function of a muscle (13). Therefore, taking into account the functions of desmin in cytoskeleton, it was expected that higher degradation of desmin caused lower drip loss, however, inverse trend was observed. This could be because desmin is probably not, but other costameres are involved in the drip loss. This is in agreement with findings of Hwang *et al.* (7). On the contrary, comparison of porcine muscle proteome with high and low drip loss showed that the pork having low drip loss had more intact desmin and consequently, this protein was considered as a marker of drip loss (13).

Desmin was a good indicator of *post-mortem* proteolysis and meat tenderisation, as shown in earlier studies (35,40,47). In the present study, a negative correlation was observed between desmin proteolysis and shear force (Table 3). This is consistent with the findings of previous investigations by Zapata *et al.* (21) and Hwang *et al.* (7) in beef and pork, respectively. Based on the results of present and previous studies, it can be concluded that desmin is a potentially good biomarker for meat tenderness.

F-actin-capping protein subunit alpha-2

Capping proteins are key factors in maintaining a uniform thin filament length since they interact with both actin and additional components of the Z-band, and downregulation of capping proteins leads to myofibrillar disarray (14). Up to now, different isoforms of capping proteins have been identified. One of the most important isoforms is F-actin-capping protein subunit beta (CapZ). In this study, the spot intensity of capping protein (spot 27) did not change significantly up to 3 days *post-mortem*, but then increased more than twofold (Table 3). A decreasing trend of this protein during muscle ageing was expected, since capping proteins have a central role in actin stabilisation so with the beginning of actin degradation they are no longer needed (3). The reasons for the increase in abundance of the capping protein during storage of meat could be due to *post-mortem* modification, protein expression, or a slight degradation of the full-length proteins (9). Change in protein solubility during *post-mortem* storage could result in variation of protein extractability, and consequently fluctuation of spot intensity was found (3).

Lametsch *et al.* (9) reported that the spot intensity of CapZ increased 72 h after slaughter of pig. Also, Bjarnadóttir *et al.* (3) found that two capping proteins were less abundant at 48 h *post-mortem*, while one isoform (CapZ) increased threefold. Decrease of these capping isoforms was reported by Hwang *et al.* (7).

We found a positive correlation between capping protein and drip loss (Table 3). In a proteome study, there was a significant relationship between capping proteins and both tenderness and L^* characteristics of porcine muscle (7).

NADH dehydrogenase

NADH dehydrogenase is located in the inner mitochondrial membrane and involved in the respiratory chain and oxidative metabolism (3,12). This enzyme catalyses the transfer of electrons from NADH to coenzyme Q (3). In this study, NADH dehydrogenase, which is a sar-

coplasmic protein, was extracted within myofibrillar fraction *via* precipitation or aggregation, thereby changing from a TES buffer-soluble to an insoluble state (3). This enzyme decreased in abundance (1.4-fold) over 7 days of storage (Table 2). During the *post-mortem* period energy metabolism of the cell shifts from aerobic to anaerobic and as a result the need for NADH dehydrogenase decreases (3). This is in agreement with the finding of Bjarnadóttir *et al.* (3) in bovine LT 48 h after slaughter. In the present study, no significant relationship was established between NADH dehydrogenase and meat quality traits (Table 3). Sayd *et al.* (12) reported that NADH dehydrogenase was overexpressed in darker pork meat because of its more oxidative metabolism.

Conclusions

An investigation of the relationship between proteome changes and physicochemical and quality traits of *biceps femoris* muscle of Iranian one-humped camel was carried out. Changes in spot intensities of 19 proteins or peptides and objective meat quality traits occurred simultaneously during ageing. Twelve proteins were identified with MALDI-TOF/TOF including actin and its relevant peptide, troponin T, F-actin-capping protein subunit alpha-2, desmin, NADH dehydrogenase and small heat shock proteins (20 and 27 kDa). Among these proteins, actin correlated well with a^* value, b^* value, cooking loss, expressed juice content, sarcomere length and myofibrillar fragmentation index, troponin T correlated with b^* value and cooking loss, HSP27 with L^* value, desmin with shear force, and capping protein correlated with drip loss. The findings suggest that among the identified proteins, HSP27 and desmin may be beneficial as biomarkers for camel meat quality.

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