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Proteomics and Meat Quality Aspects of Chilled Goat Meat

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ABSTRACT

Background: Chilling and ageing is an important step to enhance the quality attributes of meat. Improvement in meat quality is directly related to different muscle proteins expressed during ageing process. Identifying different protein markers is important in developing meat quality biomarkers and improve the profitability from goat meat marketing.

Methods: In this study, major changes in quality attributes and proteins of *semitendinosus* muscles of goat carcasses during chilling and ageing was investigated. Proteomic tools like LC-MS were used to identify the individual proteins expressed in chilled and aged goat muscle.

Result: Free water content and tenderness were significantly ($P<0.05$) higher in aged meat. Differential expression analysis of proteins revealed a total of 291 proteins of which 103 proteins were differentially expressed between aged and non-aged samples. Major up-regulated proteins in aged muscle samples were: myosin light chain/MLC-3 and MLC-1, myosin-I, myosin-II and myosin-III, troponin-C, histone, ATP synthase, cytochrome-C, annexin-A, creatine kinase and tropomyosin alpha chain.

Key words: Ageing, Goat meat, LC-MS/MS, Proteomics, Tenderness.

INTRODUCTION

Chevon (goat meat) is most preferred and widely consumed meat in the country. Generally, goat meat is marketed as fresh meat immediately after slaughter. Several times, consumers complain about the toughness of goat meat. This is mainly attributed to improper development of rigor mortis and hindering the process of conversion of muscle to meat. Hence, post-mortem chilling and ageing of carcasses are required to ensure food safety, maximize shelf-life, reduce shrinkage and improve the tenderness of meat. It is well established that meat tenderizes during post mortem storage. The biochemical and physiochemical mechanisms during the tenderization processes are reported (Lametsch *et al.* 2003). Several muscle proteins play important role in the meat tenderization process (Hopkins and Thompson 2002).

Previous studies on effect of chilling and ageing on expression individual skeletal muscles were focused on beef and lamb. In India, meat quality aspects in various breeds have been studied (Jayanthi *et al.* 2021; Raman *et al.* 2021; Prasad *et al.* 2022). However, no studies in goat meat were focussed on proteomics and ageing effects. With the objective to identify reliable proteins involved in ageing and tenderness, the present study was undertaken to compare meat quality characteristics and expression of different muscle proteins between aged and non-aged goat meat.

MATERIALS AND METHODS

This work was carried out in ICAR-Central Institute of Postharvest Engineering and Technology during the year 2014. Final data analysis was carried out in 2016 at ICAR-National Research Centre on Meat, Hyderabad. Adult goat carcasses (Beetel breed; 12 months age) from animals slaughtered in government approved local municipality abattoir were used. A total of 12 goat carcasses (6 for each treatment) were randomly assigned to two post mortem treatments: 1. Control/non-aged (4-6 h post mortem) and 2. Chilled and aged meat. Chilling was initiated at (16±1°C) within 2 h of post-mortem and continued for 6-8 h. For ageing fresh muscle samples of *semitendinosus* muscle obtained from chilled goat carcass were vacuum packaged in polyamide packaging films (Nylon-6, 100 µm) and stored in the chiller (6±2°C) for 72 h. Thus collected semitendinosus muscles were used to measure pH, colour, and shear force value, free and bound water, cooking loss, moisture and protein identification studies.

Carcass pH and temperature were measured using a portable pH-meter with a penetration pH electrode (Hanna Instruments, Woonsocket, RI, USA). Hunter L (lightness), a (redness) and b (yellowness) values were measured after the newly cut surface was exposed to ambient air (Hunter and Harold 1987). Hue (H*) and Chroma (C*) were calculated using the equations $H^* = \tan^{-1}(b/a)$ and chroma $C^* = (a^2+b^2)^{0.5}$. The cooking loss values were calculated based on the difference in weight before and after cooking at 80°C, for 25 min and the same samples were used for the determination of shear force with a Warner-Bratzler shear blade attached to TA-XTplus Texture analyser (Stable Micro

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Systems, Godalming, Surrey, UK). The crosshead speed was 5 mm/s. The water lost, termed "free water" or centrifuged free water, was the percentage difference in weight before and after centrifugation. (Kristensen and Purslow 2001).

The procedure described by Kang and Rice (1970) was used with slight modifications for extraction of salt soluble proteins (SSP) Proteomic service facilities of Centre for Cellular and Molecular Platforms (C-CAMP), Bangalore, India were utilized for the identifications of proteins using LC-MS/MS. Protein extracts were precipitated using ethanol and acetone (1:1), precipitated protein pellet was trypsin digested before subjected to MS analysis. Digested peptides were dried by speed vacuum and reconstituted in 100 µL of 2% acetonitrile with 0.1% formic acid and 1 µL of the same were injected on to the column. Digested peptides from in-sol samples were subjected to 70 LC run followed by acquisition of data on LTQ-Orbitrap-MS. Generated data was searched for the identity on MASCOT as search engine using Swiss-prot, TrEMBL and Capra Hircus databases. List of proteins and sequence generated through LC-MS/MS was used for the analysis. Spectra were processed and analysed by the Global Protein server Explorer 3.6 software (Applied Biosystems, Carlsbad USA). This software uses an internal MASCOT (Matrix Science, Marylebone, UK) program for matching MS and MS/MS data against database information. The data obtained were screened against mammalian database downloaded from Swiss-Prot/TrEMBL homepage (http://www.expasy.ch.sprot).

The meat quality data obtained from six goat carcasses were subjected to a one-way analysis Ageing/chilling treatment was considered as a major source of variation. When statistical differences were detected, differences were considered significant at the P=0.05 level.

Table 1: Physicochemical, shear-force values and colour characteristics of non-aged and chilled/aged *semitendinosus* muscle of goat.

Meat quality parameter	Non-aged meat	Chilled and aged meat
Initial temperature	32.85±1.16	34.86±2.71
Final temperature	26.69^{a}±2.92	6.38^{b}±0.96
Initial pH	7.18±0.18	6.93±0.14
Final pH	6.42^{a}±0.19	5.96^{b}±0.10
Cooking loss (%)	38.57±1.41	36.96±1.65
Free water (%)	12.59^{a}±0.97	22.61^{b}±1.24
Bound water (%)	65.81^{a}±1.17	56.56^{b}±0.97
Total moisture (%)	78.40±1.86	79.21±1.29
Warner-Bratzler shear force value (kg)	6.91^{a}±0.62	4.98^{b}±0.34
Hunter L value	38.79±1.27	37.28±0.66
Hunter a value	12.69±0.40	13.44±0.38
Hunter b value	10.48±0.31	9.10±0.32
Hue	39.69±1.64	34.11±1.03
Chroma	16.62±0.22	16.30±1.99

Mean values with in a row with different superscript are significantly different (P<0.05).

RESULTS AND DISCUSSION

Effect of chilling on quality characteristics of meat

The average carcasses temperature in chilled carcas dropped from 36.2±1°C to 14.4±1°C in 4 h. The pH of meat decreased from 6.65±0.2°C to 5.72±0.3°C during this period. Color values were not significantly different (Table 1). The chilled and aged meat samples showed higher release of moisture during cooking and thus higher cooking losses as compared to non-aged meat samples. Bendall (1978) suggested that carcasses should be chilled slowly so that the internal temperature does not drop to less than 10°C within first 10 h.

Shear force value

Chilled and aged meat had significantly (p<0.05) lower shear force value as compared to non-chilled fresh meat (Table 1). Difference in shear force values between aged and non-aged carcasses was nearly 30%. Several studies indicated that tenderization of meat during ageing can be attributed to various factors like sarcomere length, breakdown of proteins into smaller units, (Devine 1996). They further, supported that tenderization is dependent on the degree of sarcomere shortening and the activation of proteolytic enzymes and that these two factors act synergistically to give the tenderness.

Free and bound water and total moisture

The free water was significantly (P<0.05) higher in chilled/aged meat whereas bound water was significantly higher in non-aged/fresh meat (Table 1). Increase in free water content is also related to protein breakdown during ageing. Devine *et al.* (2004) observed that meat that entered rigor at 15°C and further aged was having higher tenderness and free water content.

Proteomic analysis of different proteins in un-aged and aged/chilled meat

LC-MS/MS identification and information related to the proteins are shown in Tables 2 and 3. Myofibrillar, sarcoplasmic, mitochondria and ribosomal proteins were dominant in both non-aged and aged meat. Four different isoforms of myosin were identified and myosin-1 was having highest scores and coverage and myosin-3 was having lowest score and coverage. Tropomyosin which provides physical integrity to muscle cells, was having higher score and coverage in aged than non-aged meat. Myosin light chains are distinct from the heavy chains and have their own properties. MLC found in aged meat had higher score and coverage compared to MLC observed in non-aged meat. Most of the sarcoplasmic proteins found are related to oxidative metabolism including citric acid cycle (*e.g.* creatine kinase), glycolysis (*e.g.* glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase) oxidative stress, transport, protein repair *etc*. Lactate dehydrogenase is generally released during tissue damage and was observed in aged and non-aged meat. Endoplasmic reticulum calcium ATPase- 1 was also observed in both the samples. Higher

Table 2: Major proteins from fresh/non-aged goat meat, identified by LC-MS (Total number of proteins identified = 163).

Accession	Protein name	Score	Sequence Coverage (%)	# Unique Peptides	MW (kDa)	calc. Pi
548508120	Myosin-1 (*Capra hircus*)	6837.59	38.78	12	223.8	5.69
548508115	Myosin-2 (*Capra hircus*)	5220.51	33.37	11	221.6	5.73
548508110	Myosin-4 (*Capra hircus*)	4327.07	27.24	1	218.1	5.77
548468331	:glyceraldehyde-3-phosphate dehydrogenase (*Capra hircus*)	3107.92	33.63	7	35.9	8.35
548466988	Myoglobin (*Capra hircus*)	2240.73	51.30	7	17.0	7.43
548505083	Creatine kinase M-type isoform X2 (*Capra hircus*)	2137.92	33.33	13	43.0	7.15
548466819	Myosin-binding protein C, slow-type isoform X7 (Capra hircus)	1471.60	29.96	0	126.4	6.24
548506055	Myosin-binding protein C, fast-type (*Capra hircus*)	1128.95	29.30	23	132.1	7.74
550822194	Lactate dehydrogenase (*Capra hircus*)	1122.81	34.94	10	36.5	8.21
548454736	Myosin light chain 1/3, isoform X3 (*Capra hircus*)	1114.81	48.95	0	20.8	5.02
548482979	Tropomyosin alpha-1 chain isoform X8 (*Capra hircus*)	1113.26	53.52	10	32.7	4.74
548524089	Myomesin-2 (*Capra hircus*)	985.11	23.78	26	161.1	6.23
548528199	Alpha-actinin-3 (*Capra hircus*)	687.10	25.90	15	101.4	5.59
548465514	Phosphofructokinase, muscle type (*Capra hircus*)	662.49	19.58	11	94.3	7.37
548528637	Troponin T, fast skeletal muscle (*Capra hircus*)	488.52	21.12	0	35.8	9.95
343432731	Heat shock protein 70.1 (*Capra hircus*)	379.39	21.53	0	70.2	5.82
548456586	Calsequestrin-1 (*Capra hircus*)	371.57	20.15	5	45.2	4.16
548475888	Creatine kinase S-type, mitochondrial (*Capra hircus*)	321.38	24.35	6	43.3	8.72
751372353	Cytochrome c (*Capra hircus*)	271.09	32.38	4	11.7	9.50
550821960	Troponin C type 1 (slow) (*Capra hircus*)	181.96	20.50	2	18.4	4.18
198385324	II alpha globin (*Capra hircus*)	169.01	45.77	4	15.2	8.68
548515867	Myosin light chain 3 (*Capra hircus*)	164.02	28.14	4	21.9	5.07
548485641	Calmodulin (*Capra hircus*)	162.26	20.13	0	16.8	4.22
389620461	HSP27 protein, partial (*Capra hircus*)	123.19	31.15	3	20.5	6.70
14245683	Slow skeletal troponin I isoform, partial (*Capra hircus*)	105.67	22.22	0	12.7	8.59
548485877	Ubiquitin-40S ribosomal protein S27a (*Capra hircus*)	86.95	16.03	0	18.0	9.64
548465403	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic (*Capra hircus*)	84.99	12.56	3	48.3	7.97

Major muscle proteins with a minimum coverage of 20% are listed above.

score and coverage for cytochrome c was observed in non-aged meat, but, the HSP 70 identified in aged meat showed higher protein score and coverage than HSP identified in non-aged meat. Calsequestrin, a calcium-binding protein of the reticulum; calmodulin, a small, highly conserved protein; cytochrome complex, or cyt *c,* a small heme protein were also identified. Other sarcoplasmic proteins identified were sarco/endoplasmic Calcium ATPase (SERCA). These identified metabolic enzymes are all associated with ATP-generating pathways, either the glycolytic pathway or energy metabolism.

During the conversion of muscle to meat, major changes in muscle protein architecture are primarily noticeable at the expression levels of major myofibrillar proteins like myosin, actin, titin, nebulin, troponin-T, desmin and filamin (Lonergan and Lonergan 2005). In a study by Jia *et al.* (2007) on protein changes in two different bovine muscles (*M. longissimus dorsi* and *semitendinosus*) after 24 h storage, five proteins (cofilin, lactoylglutathionelyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20) changed with a similar pattern in both muscles, while 15 proteins showed altered expression pattern specific for the two different muscle types. Stefania *et al.* (2010) observed changes in the insoluble protein fraction of bovine *longissimus thoracics* muscle from eight

Table 3: Major proteins from chilled and aged goat meat, identified by LC-MS (Total number identified =193).

Accession	Description	Score	Coverage	# Unique peptides	MW [kDa]	calc. pI
Q9BE40	Myosin-1 (*Bos taurus*)	4176.39	32.20	3	222.9	5.72
Q8MJV1	Myosin-2 OS (*Equus caballus*)	3883.19	30.56	1	222.6	5.80
Q9TV63	Myosin-2 (*Sus scrofa*)	3446.06	26.61	2	223.0	5.81
P10096	Glyceraldehyde-3-phosphate dehydrogenase (*Ovis aries*)	2733.98	33.63	0	35.8	8.35
Q9XSC6	Creatine kinase M-type (*Bos taurus*)	1659.31	22.83	0	43.0	7.12
Q5KR48	Tropomyosin beta chain (*Bos taurus*)	892.26	45.07	12	32.8	4.70
P19858	L-lactate dehydrogenase A chain (*Bos taurus*)	844.48	47.59	0	36.6	8.00
Q5KR49	Tropomyosin alpha-1chain (*Bos taurus*)	838.27	42.96	3	32.7	4.74
P02602	Myosin light chain 1/3, skeletal muscle isoform (*Oryctolagus cuniculus*)	644.21	40.10	8	20.9	5.03
P97457	Myosin regulatory light chain 2, skeletal muscle isoform (*Mus musculus*)	498.36	31.95	0	18.9	4.92
P04466	Myosin regulatory light chain 2, skeletal muscle isoform (*Rattus norvegicus*)	498.36	31.95	0	19.0	4.92
Q3ZC07	Actin, alpha cardiac muscle (*Bos taurus*)	478.02	31.56	0	42.0	5.39
P68138	Actin, alpha skeletal muscle (*Bos taurus*)	478.02	31.56	0	42.0	5.39
Q5KR47	Tropomyosin alpha-3 chain (*Bos taurus*)	460.73	41.55	0	32.8	4.72
Q3ZC09	Beta-enolase (*Bos taurus*)	338.85	28.57	6	47.1	7.72
P02190	Myoglobin (*Ovis aries*)	307.92	47.40	0	17.0	7.43
Q3T0P6	Phosphoglycerate kinase (*Bos taurus*)	303.41	20.14	0	44.5	8.27
Q32LG3	Malate dehydrogenase, mitochondrial (*Bos taurus*)	297.91	26.92	0	35.6	8.54
P13696	Phosphatidylethanolamine -binding protein (*Bos taurus*)	241.91	42.25	5	21.0	7.49
Q3SZX4	Carbonic anhydrase 3 (*Bos taurus*)	223.66	22.31	4	29.4	7.84
P00570	Adenylate kinase isoenzyme 1 (*Bos taurus*)	192.15	34.02	5	21.7	8.32
P63315	Troponin C, slow skeletal and cardiac muscles (*Bos taurus*)	182.42	20.50	0	18.4	4.18
P02077	Hemoglobin subunit beta -A (*Capra hircus*)	164.62	31.72	2	16.0	7.30
P62894	Cytochrome c (*Bos taurus*)	104.14	23.81	0	11.7	9.50
P23934	NADH dehydrogenase, mitochondrial (*Bos taurus*)	103.38	21.77	2	13.4	9.07
P0C0S9	Histone H2A type (*Bos taurus*)	92.33	21.54	0	14.1	10.90
P62146	Calmodulin-alpha	88.98	21.13	0	16.0	4.18
P00426	Cytochrome c oxidase subunit 5A, mitochondrial (*Bos taurus*)	77.19	25.66	0	16.7	6.92

Major muscle proteins with a minimum coverage of 20% are listed here.

Norwegian Red (NRF) dual-purpose young bulls during the first 48 h post-mortem. They found significant changes in a total of 35 proteins identified two metabolic enzymes (2, 3-bisphosphoglycerate mutase and NADH dehydrogenase) and one protein involved in the stress responses/apoptosis of the cell (Hsp70).

CONCLUSION

In comparison with non-aged meat, aged meat showed improved meat quality characteristics. In aged meat, higher expression myofibrillar proteins involved in tenderization was observed. Further, Myosin light chain/MLC-3 and MLC-1, myosin-I, myosin-II and myosin-III, troponin-C, are probable protein markers associated with tenderization during ageing of goat meat.

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Conflict of interest statement

The authors declare that there is no conflicts of interest.

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