The Effect of Post Mortem Ageing and Muscle Type on Spent Hen Meat Quality

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ABSTRACT

Current investigation was carried out to study the changes in meat quality during ageing of thigh and breast muscles from spent hen meat at 4 °C. The pH of thigh muscle found to be higher (6.02) than breast muscle (5.78) throughout the ageing period. Water holding capacity increased ($P<0.05$) from 17.33% to 23.33 % and 33.33% to 38.33% in thigh and breast muscles, respectively. Variation ($P<0.05$) in protein extractability was observed throughout the ageing period. The % metmyoglobin increased ($P<0.05$) in thigh and breast muscles of spent hen meat. Myofibrillar fragmentation index was significantly ($P<0.05$) higher at 6 days of ageing in thigh and breast muscles compared to other ageing period. The metabolic activity as indicated by R Value increased progressively up to 6 days in both muscles. The SDS PAGE pattern of proteins at different ageing period as analyzed by Gel Analyzer showed appearance of few new bands on completion of ageing. The differentially expressed proteins were identified to be Vesicle associated membrane protein-7, Cartilage matrix protein, ProteinFAM210A and Phosphoglycerate mutase1, which are proposed to be generated during ageing. Present study indicates the quality changes and improvement in tenderness of thigh and breast muscles of spent hen meat during ageing.

Keywords: *2-DE, Muscle fiber diameter, Myofibrillar fragmentation index, Proteolysis, Protein extractability*

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INTRODUCTION

Skeletal muscle is a well organized and complex structure composed of muscular fibers, a cytoskeleton, an extracellular matrix and water that affects the molecular and mechanical characteristics of meat at various stages in the conversion of muscle to meat. Muscle is made up of different types of fibers that varies considerably in their biochemical, physiological, ultrastructural and chemical properties. Muscle fiber types of individual muscles can vary greatly between animals within a single species as well as across species. These variations in fiber type are due to adaptations of different activities by a muscle. In poultry carcasses, the thigh muscle is rich in red slow-twitch fibers and breast muscle is composed of a high proportion of white fast-twitch fibers. Red fiber has a high concentration of myoglobin and oxidative enzymes whereas, white fibers are rich in glycogen and glycolytic enzymes (Cassenas and Cooper, 1971). Meat tenderness is the primary quality attribute and the main reason for the consumer purchasing it, contributing to approximately 40% of the consumer's acceptance (Naveena *et al.,* 2013).

The physiological functions of the muscle tissue do not stop instantly after bleeding or death of the animal. Instead a large number of bio-physical and bio-chemical changes take place over a period of several hours and sometimes extend even to days. The post-mortem ageing of meat is a very important process having a significant effect on its microstructure and quality traits, especially texture, tenderness and water-holding capacity. Effect of ageing on sensorial, textural and proteolytic changes is well documented. In the post-genomic era, proteomic tools have been utilized to understand the biochemical mechanisms influencing conversion of muscle to meat tenderness in beef and pork (Laville *et al.,* 2009). Application of mass spectrometry and bioinformatics tool further increased the usage of the omic techniques to the meat science. However, there are limited publications demonstrating ageing changes in two different muscles of spent hen meat.

Hence, the objective of this study was to determine the extent to which muscle type contribute to variation in

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physicochemical and proteome characteristics. In this work variation in physicochemical and proteome characteristics in Thigh (*Iliotibialis*) and Breast (*Pectoralis major*) muscle stored at 4°C was studied during conditioning/ageing for 6 days. The muscles selected differ in composition of muscle fibres, content and composition of connective tissue.

MATERIALS AND METHODS

Slaughtering and ageing: Thigh (*Iliotibialis*) and Breast (*Pectoralis major*) muscle were procured from Broiler spent hen immediately after bleeding from local slaughter house, Bidar, Karnataka. The selected muscles were cut into uniform sized chunks and packed under atmospheric conditions using low-density polyethylene bags. The samples were randomly assigned to four different ageing periods (0, 2, 4 and 6 days), kept at 4 ± 1°C in a domestic refrigerator. Fresh meat samples were obtained in batches separately for each of the three replications (n = 3). At each designated ageing period, meat chunks were evaluated for pH, water holding capacity, R-Value, protein extractability, muscle fiber diameter, myofibrillar fragmentation index (MFI), Myoglobin and % metmyoglobin content.

The pH of the meat sample was determined by homogenizing 10 g of sample with 50 ml distilled water with the help of tissue homogenizer (Model: Z742486, Bench mark, D1000 Hand held homogenizer, Malaysia) for 1 minute.

Water holding capacity: Twenty g of minced meat sample was centrifuged with 30 ml 0.6 M NaCl and homogenate was mixed using a glass rod for 1 minute. The centrifuge tube was then kept at refrigeration temperature for 15 minutes, stirred again and centrifuged at 5000 rpm using refrigerated centrifuge (Model: Eppendorf Centrifuge 5804 R) for 15 minutes. The supernatant was estimated and the amount of water retained by the sample was estimated and WHC was expressed in percentage (Wardlaw *et al.,* 1973).

R Value: The R-value was determined as per the method reported by Honikel and Fischer (1977). Two g of muscle sample was homogenized in 10 ml of 1 M perchloric acid. Homogenate was filtered and 0.1 ml filtrate was diluted with 4.9 ml of 0.1 M phosphate buffer (pH-7.0). The absorbance at 250 nm and 260 nm were recorded using UV-VIS spectrophotometer (Model: Agilent Cary 60 UV-VIS, Germany) using phosphate buffer as a reference and the R-value was expressed as the ratio of absorbance at 250 nm and 260 nm.

Protein extractability: Protein extractability was determined according to procedure of Joo *et al.* (1999). Sarcoplasmic and total (sarcoplasmic+myofibrillar) protein were extracted separately by homogenizing two g muscle sample with 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2) and 20 ml of ice-cold 1.1M potassium iodide in 0.1 M phosphate buffer (pH 7.2), respectively. Homogenate was centrifuged and the protein concentration was estimated using biuret method. Myofibrillar protein extractability was obtained as difference between total and sarcoplasmic protein extractability.

Myofibrillar fragmentation index (MFI): About 10 g of minced muscle sample was homogenised with 50 ml of 0.24 M cold sucrose and 0.02 M Potassium chloride solution in a homogenization cup. After 5 minutes, each homogenate was blended for 40 seconds at high speed uniformly using tissue homogenizer. The resulting homogenate was filtered through a pre-weighed muslin cloth with constant stirring using a stirring rod to speed up filtration. The MFI was reported as the weight of the residue after draining out the excess water and expressed in gram per cent (Hawkins *et al.,* 1987).

Muscle fiber diameter: For Muscle fiber diameter estimation 1 cm core of muscle tissue was fixed in 10% formal saline for 24 hours. The fixed muscle tissue was homogenized and drop of the homogenate placed over a glass slide covered with cover slip and observed under a calibrated ocular micrometer. The diameter of minimum of 10 fibers was measured and the average muscle fiber diameter was expressed in microns (Tuma *et al.,* 1962).

Myoglobin content and % metmyoglobin: Myoglobin was extracted from muscle using a modified procedure of Warris (1979). Five g of muscle sample was homogenized with 25 ml of cold 0.04 M phosphate buffer at pH 6.8 for 10 seconds in a hand held homogenizer and centrifuged at 5000 rpm in refrigerated centrifuge for 30 minutes. The absorbance of the filtrate was recorded at 525, 572 and 700 nm using a UV-VIS spectrophotometer. The myoglobin concentration and % MetMb were calculated using formula as outlined by Trout (1989).

Proteome analysis: SDS PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis) was carried out by using the methods of Laemmli (1970) with mini-electrophoresis apparatus (Biorad, Mumbai, India). Protein extract was diluted with sample buffer and distilled water so as to obtain uniform protein concentration of 10 μg protein /10 μl of mixture. This reaction mixture was denatured by heating in water bath at 90°C for 5 minutes before loading into gel. Denatured proteins were separated using mini-electrophoresis apparatus at 100 V

with 100 mA/gel until tracking dye reached lower end of gel. The protein bands expressing significantly different expression were selected based on GelAnalyzer 2010 result and carefully removed for in-gel digestion and MALDI-TOF/TOF MS. In-gel digestion and MALDI-TOF/TOF MS of the proteins was performed as described by Shevchenko et al. (2006). Protein identification was performed by MS/MS by searching and comparing against existing data bases like Swiss port and NCBI using Mascot (Matrix Science, Boston; MA).

Statistical analysis: Statistical analysis was performed with the analysis of variance (ANOVA) using SPSS (SPSS version 13.0 for Windows; SPSS, Chicago, IL). The effect of ageing period (0, 2, 4 and 6 days) were analyzed using one way ANOVA considering thigh and breast muscle as separate variables and F test was calculated using Duncan Multiple Range tests and were considered significant at a 5% level.

RESULTS AND DISCUSSION

Results of variation in meat quality of thigh and breast muscles during ageing has been summarized in Table 1.

PH: The pH of thigh and breast were found to be 6.02 and 5.78 with significant ($P<0.05$) difference between them which decreased to 5.81 and 5.57 respectively on 6th day of ageing. The difference in ultimate pH between two muscles might be attributed to variation in proportion of white and red fibers. Similar pH values for breast and thigh muscle have been reported by many researchers (Choe *et al.*, 2010; Rathod *et al.*, 2017). The rate of pH decline are in agreement with Farouk and Lovatt (2000), who proposed that faster rate of pH decline in white fibers are due to high concentration of glycolytic enzymes.

Water holding capacity: The WHC of both muscles increased significantly ($P<0.05$) from day 0 to 6th day of ageing. Offer and Knight (1998) proposed that during the development of rigor mortis due to lateral shrinkage of the myofibrils, the cross-sectional area decreased about 9%. This shrinkage of myofibrils significantly influences water holding capacity. The degradation of the cytoskeletal proteins during ageing assists in minimising shrinkage and increasing WHC of meat by removing inter-myofibrillar and costameric connections in muscle (Melody *et al.*, 2004).

Table 1: Variation in biochemical, histological and textural qualities of thigh and breast muscles from spent hen meat during ageing (Mean ±SE)

Parameters	0 days	2 Days	4 days	6 days
		Thigh (*Iliotibialis*)		
pH	6.02±0.06^{c}	5.87±0.03^{bc}	5.83±0.09^{ab}	5.81±0.07^{a}
WHC (%)	17.33±1.20^{a}	23.00±1.53^{ab}	27.67±3.76^{ab}	33.33±1.76^{b}
R Value	1.17±0.01^{a}	1.31±0.05^{ab}	1.37±0.05^{b}	1.29±0.02^{ab}
SPE	83.10±3.09^{a}	92.31±3.93^{ab}	115.03±3.30^{c}	110.96±3.60^{c}
MFPE	151.00±2.73^{a}	180.38±8.82^{c}	170.28±3.80^{b}	177.49±2.63^{bc}
TPE	234.1±1.43^{a}	272.7±6.75^{b}	285.3±5.69^{bc}	288.5±2.77^{bc}
MFI	66.50±1.19^{a}	71.13±0.87^{bc}	74.67±0.87^{c}	74.30±0.95^{c}
MFD	62.583±0.73	61.08±1.59	59.58±1.92	60.08±1.59
Myoglobin	3.78±0.41^{b}	3.09±0.24^{b}	2.98±0.27^{a}	3.47±0.39^{b}
% MetMb	56.18±3.21^{a}	54.99±1.56^{a}	55.09±1.40^{a}	61.86±0.48^{b}
		Breast (*Pectoralis major*)		
pH	5.78±0.03^{b}	5.61±0.07^{a}	5.60±0.06^{a}	5.57±0.03^{a}
WHC (%)	23.33±1.67^{a}	24.67±5.78^{a}	30.00±2.89^{b}	38.33±4.06^{c}
R Value	1.16±0.01^{a}	1.38±0.05^{b}	1.47±0.05^{b}	1.35±0.02^{b}
SPE	79.92±1.38^{a}	89.87±3.96^{ab}	102.99±0.92^{bc}	111.66±3.89^{c}
MFPE	167.69±6.86^{a}	192.08±3.13^{b}	200.15±3.81^{b}	206.48±1.04^{b}
TPE	247.6±8.17^{a}	282.0±4.32^{b}	303.1±4.03^{c}	318.1±3.84^{d}
MFI	67.70±0.83^{a}	70.63±1.08^{ab}	73.07±0.66^{b}	73.40±0.44^{b}
MFD	62.08±1.62	62.41±0.60	60.92±1.69	59.98±0.82
Myoglobin	1.50±0.22^{a}	1.39±0.21^{a}	1.72±0.11^{b}	1.59±0.09^{a}
% MetMb	63.53±0.79^{a}	66.80±1.87^{b}	67.88±0.96^{b}	68.66±0.81^{b}

Means with different superscripts (between columns) differ significantly ($P<0.05$)

SPE: Sarcoplasmic protein extractability; MFPE: Myofibrillar protein extractability; TPE: Total protein extractability; MFI: Myofibrillar fragmentation index MFD; Muscle fibre diameter %MetMb: % Metmyoglobin

R Value: The R-Value increased from 1.17 and 1.16 to 1.29 and 1.35 on 6th day of ageing in thigh and breast respectively. There was no significant (P>0.05) difference between thigh and breast on zero day, but the rate of change in R Value within muscles was significant (P<0.05), indicating the variation in rate of ATP degradation among two muscles during ageing. Post Slaughter, oxygen and blood circulation stops and muscles switch to anaerobic metabolism and glycolysis to maintain the generation of ATP (Warner 2016). The increase in the R-Value might be due to decrease in the concentration of ATP in the muscle during completion of rigor mortis (Calkins *et al.*, 1982). The similar trend of R value change has been reported in chicken (Kiran *et al.*, 2013).

Protein extractability: Sarcoplasmic, myofibrillar and total protein extractability increased (P<0.05) with ageing in the thigh and breast samples. Myofibrillar protein extractability increased (P< 0.05) from 151.00 to 177.00 mg/g protein and 167.69 to 206.48 mg/g protein for thigh and breast muscle respectively. Similarly, total protein extractability increased (P<0.05) during ageing from 234.10 to 288.50 mg/g protein and 247.60 to 318.10 mg/g protein for thigh and breast muscle respectively. Proteolysis of myofibrillar proteins is suggested to be the reason for meat tenderization during post mortem ageing. The increased protein extractability is suggested to be due to proteolytic degradation of myofibrillar proteins which weakens myofibrils leading to tenderization (Koohmaraie *et*

2002). The correlation coefficients of WHC with sarcoplasmic protein solubility were higher than those of myofibrillar protein solubility (Joo *et al.*, 1999). The increased extractability along with ultimate pH during ageing contribute to development of tenderization in meat (Joo *et al.*, 1999).

Myofibrillar fragmentation index (MFI): There was signiûcant (P<0.05) increase in MFI from 66.50 to 74.30 and 67.70 to 73.40 during ageing in thigh and breast muscles respectively. MFI was proposed to be indicator of the extent of post-mortem myofibrillar structural protein degradation, indicating the degradation of key structural proteins of the myofibril and weakening of myofibril linkages (Taylor *et al.*, 1995). The values of MFI increased as ageing time extended, in agreement with some previous research (Naveena *et al.*, 2015).

Muscle fiber diameter: There was a signiûcant (P<0.05) decrease in the muscle ûber diameter of spent meat samples during ageing. The mean muscle ûber diameter observed in the current study decreased from 62.58 to 60.08 μ and 62.08 to 59.98μ on 6th day of ageing in thigh and breast samples respectively. The decrease in the muscle ûber diameter during ageing may be due to the modification of muscle ûber structure with ageing. The muscle fibre diameter has been positively correlated to shear force but negatively correlated to tenderness and sarcomere length of the muscle (Biswas *et al.*, 1989). The average muscle fiber diameters observed in the current study were similar to earlier reports (Anita *et al.*, 2018).

Myoglobin content and % Metmyoglobin: Myoglobin content was higher in thigh (3.78mg/g) in contrast to breast (1.50 mg/g) muscle which decreased significantly (P<0.05) during ageing. Oxidation of oxymyoglobin results in MetMb formation producing brown discoloration of meat (Naveena *et al.*, 2013). The %MetMb has increased (P<0.05) from 56.18 to 61.86 mg/g in the thigh samples, whereas in the breast samples, increase from 63.53 to 68.66 mg/g was observed during ageing. The rate of %MetMb was same in both thigh and breast muscles throughout ageing. These results indicate that color stability is matter of concern during ageing as meat becomes unsalable when proportion of %MetMb crosses 40% (Renerre and Labas, 1987).

Proteome analysis: During post mortem tenderization there are major changes in the myofibrillar structure predominantly due to the proteolysis of key myofibrillar and associated proteins. The post rigor cytoskeleton degradation and consequent tenderization (Rosenvold *et al.*, 2008) are known to improve WHC and tenderness of meat. Proteolysis leads to weakening of the myofibrils and hence tenderization of the meat. The SDS-PAGE photograph (Fig. 1) of total soluble proteins revealed the reduction in the protein band intensity with progress in ageing. The molecular weight, protein score, Gene ontology, biological function and molecular location of differentially expressed protein has been tabulated in Table 2. The differentially expressed proteins were identified to be Vesicle associated membrane protein-7, Cartilage matrix protein, and ProteinFAM210A and Phosphoglycerate mutase1. Previous researchers have also reported that degradation of high molecular weight proteins and an increase in the number of low-molecular-weight proteins as indication of proteolysis and degradation of myofibrillar proteins (Naveena *et al.*, 2015). The glycogen-phosphorylase has been reported as biomarker of meat quality in pig during ageing (Laville *et al.*, 2009).

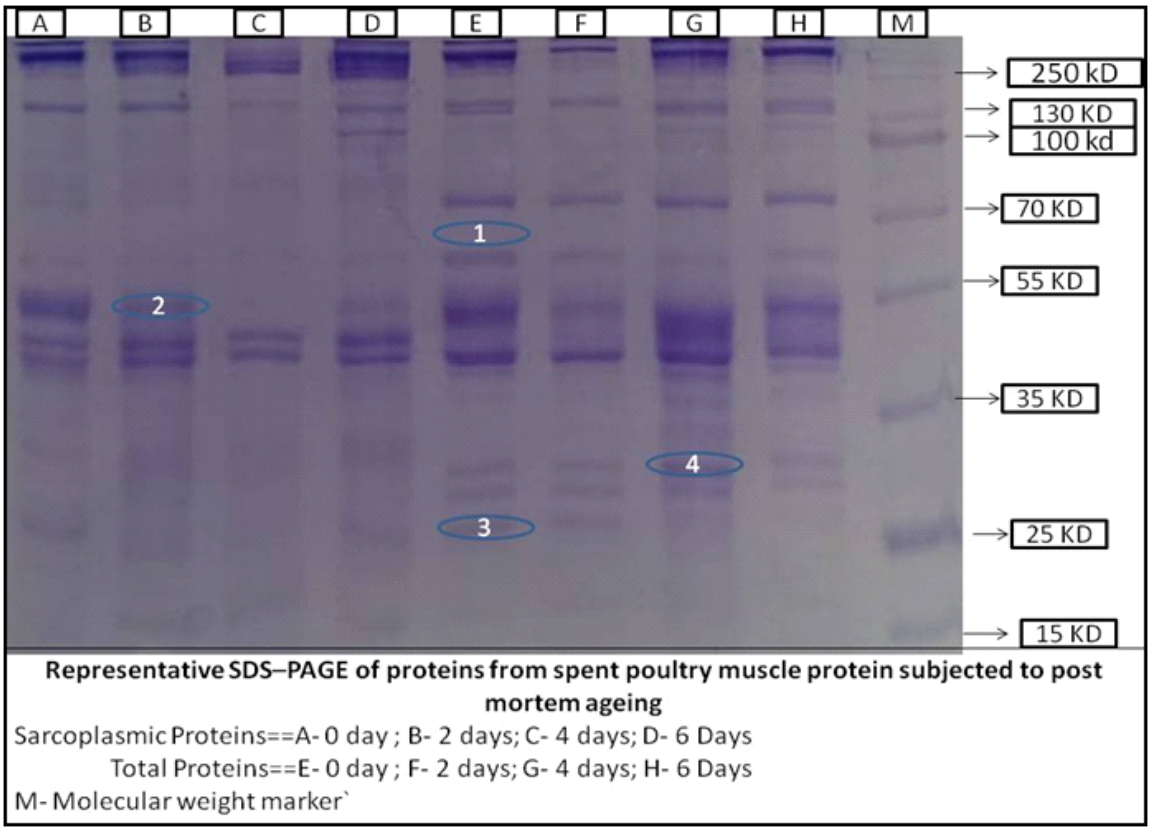

Fig. 1 Representative SDS PAGE photograph of spent hen meat subjected to various ageing period differentially expressed protein bands characterized by MALDI TOF MS/MS are indicated by number (1-4)

CONCLUSION

This study demonstrated that, tenderness an important meat quality characteristic of spent hen meat can be improved through post mortem ageing. However, the rate and extent of ageing vary considerably between meat from thigh and breast region. Appearance of the low molecular weight protein bands during ageing as evident from SDS-PAGE indicates the proteolysis and tenderization. Using proteomics approach, we have successfully identified few important proteins whose expression levels are associated with ageing of meat. The proteins identified in the present study includes: Vesicle associated membrane protein-7, Cartilage matrix protein, and ProteinFAM210A and Phosphoglycerate mutase1. These proteins may be useful as biomarkers for meat quality development during post mortem ageing in spent hen meat.

Table 2: Functional roles, molecular weight, accession number of differentially expressed muscle proteins during ageing.

#	Protein Name	Accession No	Gene ID	Protein Score	Molecular Function	Biological Process
1	Vesicle associated membrane protein 7	VAMP7_CHICK	ENSGALG00000007476	77	SNAP receptor activity SNARE binding	Calcium-ion regulated exocytosis endoplasmic reticulum to Golgi vesicle-mediated transport endosome to lysosome transport eosinophil degranulation exocytosis
2	Cartilage matrix protein	MATN1_CHICK		138	Calcium ion binding Extracellular matrix structural constituent	Growth plate cartilage Chondrocyte morphogenesis
3	Protein FAM210A	F210A_CHICK	ENSGALG00000013887	95	May play a role in the structure and strength of both muscle and bone.	
4	Phosphoglycerate mutase	PGAM1_CHICK	RCJMB04_5g20	116	Bisphosphoglycerate mutase activity Hydrolase activity Phosphoglycerate mutase activity	Gluconeogenesis Glycolytic process

Differentially expressed bands are numbered (1-4) in Figure 1

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ETHICS STATEMENT: Not applicable

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