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A comprehensive study on effect of electrical stimulation on biophysical and biochemical parameters of spent hen meat using discriminant function analysis

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Abstract

The objective of this study was to assess the effect of electrical stimulation (ES) on breast and thigh muscles in spent hen. A total of 24 spent hens (White Leghorn) were slaughtered and split into two halves, each half was either treated with postmortem ES (150V) or was used as a non-stimulated (NS) control. Breast and thigh muscles were excised and analysed for biophysical, colour and sensory qualities after ageing for 24 h at 4 ± 1 ˚C. Biochemical parameters were recorded immediately (0h) and after 24h of ES. Biophysical parameters like water holding capacity (WHC), protein extractability, shear force value (SFV) and myofibre fragmentation index (MFI) of both types of muscles varied significantly with ES. Electrical stimulation significantly reduced the pH and glycogen content in both muscles and the thigh muscle showed a significantly higher pH and lower glycogen content than the breast muscle. No significance difference in metabolic rate (R-value) was observed between ES and NS thigh at 24 h. ES had no significant effect on colour and ES significantly improved the sensory value of both breast and thigh muscle. Canonical

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discriminant and Mahalanobis distance (D^2) analysis of biophysical and biochemical parameters demonstrated that the effect of electrical stimulation was comparatively more in thigh muscle than breast with an ageing period of 24 h.

Key words: *Electrical stimulation, spent hen, discriminant analysis, physicochemical properties*

Indian poultry industry has transformed from a mere backyard activity into a major commercial activity. The rapid growth of the poultry industry during the last four decades had propels India into becoming the third largest producer of table eggs in the world, with production of 95.5 million eggs per annum (DAHDF, 2019). Excessive expansion of the egg industry has resulted in an increased availability of culled or spent hens. In India, around 375 million laying hens were housed in an year (2015-16) and this is projected to reach 550 million by 2022 (DAHDF, 2017), considering the number of pullets introduced to replace old (spent) hens, and the fact that a similar number of spent hens would be being culled during the same period. The sale of spent hens at reasonable prices has become more difficult with the increase in broiler production (Navid *et al.,* 2011; Indumathi *et al.,* 2019). In addition, most important attributes of consumer palatability like tenderness, flavour and juiciness tend to decrease with increase in age of birds (Lawrie, 1991). The toughness is due to the increase in cross-linking in the connective tissue (Archile-Contreras *et al*., 2011) and structural integrity of myofibrils (Calkins and Sullivan, 2011). Even colour of meat is also one of the major sensory qualities, which varies among individuals. Some prefer dark coloured meat and others light coloured meat depending on the region and previous experience (Navid *et al.,* 2011). Although, spent hens yield comparatively lesser meat than broilers, satisfactory amounts of meat are available in the breast and thighs for processing. It has health promoting benefits due to enriched omega-3 fatty acids and low cholesterol and is thus a good protein source with high myofibrillar protein content (Lee *et al*., 2002). So, it is essential and economically viable to improve the meat quality of these spent hens at the end of laying cycle as this could provide economic benefits for the Indian poultry industry.

To improve the tenderness of meat from spent animals and birds, various technique have been developed like physical, chemical, thermal, enzymatic and other processing methods. (Naveena *et al*., 2011; Barekat and Soltanizadeh, 2017; Alves *et al*., 2018). Among various techniques, electrical stimulation is a simple, low cost technology and does not involve incorporation of chemicals or additives to the carcasses. Initial investment and maintenance are also inexpensive. In addition, it can be used in large slaughter houses also, with no interruption to the carcass flow as it requires very less time and does not affect the carcass appearance (Janz *et al.,* 2001). Electrical stimulation (ES) is widely used for improving the tenderness of mutton and beef. Electrical stimulation accelerates the development of rigors mortis in lesser time due to forceful contraction of muscle and thus reduce the costs of processing, cooling, storing and the power spent in the factory and even reduces the microbial load of chicken carcasses (Warriss, 2010; Adeyemi and Sazili, 2014).

Meat quality depends on various factors like pre-slaughter factors (genetic, nutritional and managemental), slaughter, and chilling and processing conditions, to mention some. Along with the pre- and post-slaughter factors, there are differences among the muscles within regard to individual carcass like biophysical, biochemical and nutritional properties (Naveena *et al*., 2011; Suriani *et al.,* 2014). It is absolutely clear that biophysical, histological and biochemical characteristics of muscle fibres play a key role in meat quality (Tůmová *et al*., 2009) which includes the muscle fibre number and type and fibre size. The skeletal muscles are composed of differing fiber types, these fibre type variations differ according to their molecular, metabolic, structural, and contractile properties (Choi and Kim, 2008). In chicken, the pectoralis muscle (breast) is composed of only type IIB muscle fibre and the biceps femoris (thigh) is composed of Type I, IIA and IIB (Roy *et al*., 2006; Papinaho *et al*., 1996). All these characteristics are involved in rigor mortis, which influences meat quality. With this view, the work conducted to

study the effect of electrical stimulation on biophysical, biochemical, colour and sensory parameters of two different muscles of spent hen was undertaken.

Materials and methods

Sampling and experimental design

In the present study, a total of twenty-four spent hens (White Leghorn) of approximate age of 72 weeks, weighing 1.2-1.3 kg, which were fed and handled under the same management conditions were purchased from local market of Chennai, Tamil Nadu. Feed was withdrawn from birds for 12h and these were slaughtered manually in different batches at the slaughter hall of Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Chennai. In brief, birds were restrained and bled by bilateral neck cut for 180 s, followed by hard-scalding at 61^{0}C for 40 s, picked in a rotary drum for 40 s and eviscerated manually. After dressing, carcasses were split into two halves using electric carcass splitting saw by leaving neck at the left side and without affecting the muscle integrity. Left half of the carcasses were electrically stimulated (ES) (alternative current of 150V; frequency 50Hz; period-20ms; 5s on/off duration of 90 s) using electrical stimulator (custom designed by Joe scientific equipment, Chennai, Tamil Nadu) within 20 min of exsanguination and right half was used without stimulation (NS) as a control.

Immediately after electrical stimulation (0 h) about 5 grams meat from each breast (*Pectoral major*) and thigh (*Biceps femoris*) was excised and frozen in liquid nitrogen for analysis of biochemical parameters and remaining carcasses were aged in the chiller (4 ±1 ˚C) for 24 h. After ageing, the pectoral and thigh muscles were separated and immediately analysed for biophysical, biochemical, colour and sensory parameters.

Biophysical parameters

Water-holding capacity (WHC) was measured by the filter paper press method as per Grau and Hamm (1953). Area were measured using Placom KP–90N planimeter (Koizumi Sokki Mfg. Co. Ltd., Japan) and WHC was calculated as the ratio of meat area to fluid area on the filter-paper as per George *et al*.(1979). Total protein extractability (PE) was determined according to procedure of Joo *et al*.(1999) and results were expressed as mg/g. Myofibrillar fragmentation index (MFI) values were determined by the procedure of Davis *et al*. (1980) and results were reported as the weight of the residue in grams times one hundred. Cooking loss (CL) was calculated as the difference in sample weight before and after cooking, and expressed as a percentage of the initial sample weight (Jama *et al*., 2008).

The shear force values (SFV) were assessed following the standardized protocol of Wheeler *et al*. (1997).Where,both pectoral and thigh samples were trimmed, touniform size (30mm x 10mmx 8mm)with length parallel to the muscle fibre and sheared perpendicular to the muscle fiber using Warner Bratzler Shear (G.R. Electric Manufacturing Company, Manhattan, USA),the average of the five readings were recorded and expressed as kgf.

Biochemical parameters

Biochemical parameters of control (NS) and electrical stimulated (ES)breast and thigh muscles were evaluated at zero as well as 24hr of treatment. The pH of the chicken meat samples was measured using a pre calibrated digital pH meter (Cypberscan 510, Eutech Inst., Singapore) according to Troutt *et al*. (1992). The R-value (RV) was estimated as per the method described by Honikel and Fischer (1977) and expressed as the ratio of IMP:ATP. Glycogen (GLY) was estimated according to Mendel *et al*. (1954) and expressed in milligram per gram. The lactic acid (LA) was estimated as per Barker and Summerson (1941) and calculation were done using lithium lactate as standard and results were expressed as milligram per gram. All spectrometric reading was taken using UV-VIS spectrophotometer (Model: Cary 60, AGILENT, US).

Colour and sensory evaluation

Meat colour was measured with Spectro-colourimeter (Hunter colour lab Mini scan XE plus, Model No. 45/O-L, Reston, Virginia, USA), calibrated prior to each session

for the CIE colour space system (Commission Internationale de l'Eclairage) using a white tile (L*: 94; a*: 1.10; b*:0.6) with geometry of diffuse/80 (sphere - 8mm view) and an illuminant of D65/10 deg (Hunt *et al*., 1991).

The numerical total colour difference (ΔE) among NS and ES samples of breast or thigh meat was calculated by the formula given by Mancini *et al.* (2015)

$$\Delta E = \sqrt{(\Delta L)^2+(\Delta a^*)^2+(\Delta b^*)^2}$$

where"Δ" means the difference between colours of muscles (thigh vs breast) or treatment groups (NS vs ES). A variation in colour (ΔE) equal to 2.3 units corresponds to a just-noticeable difference (JND) for the human eye; higher variation is considered discernable (Sharma, 2003).

For sensory evaluation, meat samples were wrapped in aluminum foil and cooked in water bath at 100°C for 30 minutes(until internal temperature reaches 75°C). The cooked meat chunks of uniform size were served to semi-trained panelists and evaluated for flavour, juiciness, tenderness and overall acceptability using a 9-point descriptive scale, where; 9=extremely desirable, 1= extremely undesirable.

Statistical analysis

Experimental results were expressed as mean±standard deviation of multiple determinations. The data were analysed by independent sample *T*- test (p = 0.05) or one-way analysis of variance. Tests of significant differences were determined by Duncan's multiple range tests at p = 0.05.

Discriminant function analysis (DFA) was carried out with the biophysical parameters and biochemical parameter (0 hr or 24 hr) separately using the SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) software package for Windows.

The experimental treatments consisted of combinations of two treatments (non-stimulated-NS and electrical stimulated-ES) and two muscles (breast-B and thigh-T) in eachwith two muscles in each treatment), in a total of four treatments. The null hypothesis for the equality of the mean vectors for the five parameters in the four tested treatments is H_0 :NSB=ESB=NST=EST. The alternative hypothesis H_a that at least one of these mean vectors is different from the others. The resulting subset of SDA was used in the discriminant analysis to describe differences among groups and observations to groups were allocated.

Mahalanobis distance between the two groups in two groups were manually calculated using pairwise F-ratios results in below equation (IBM, 2018)

$$D_{ab}^2 = [q(N-g)(Na+Nb)]/[(N-q-g+1)NaNb] * Fab$$

Where, a and b - different groups,

q - number of predictors,

N - total number of cases used across all groups,

g - number of groups,

Na - number of cases in group a,

Nb - number of cases in group b, and

Fab - F-statistic (pairwise distances) for comparing groups a and b

Results and discussion

Biophysical parameters

Results of electrical stimulation on biophysical parametersof breast and thigh meat are given in the table1. WHC, PE, MFI, CL and SFV are important attributes of meat which decides the quality and yield of the processed meat and meat products. In present study, WHC of the breast meat samples was found to be significantly (P<0.01) lower than that of the thigh meat samples. There was a significant (P<0.01) decrease in the WHC of ES breast and thigh meat samples than NS samples. Protein damage and decrease in pH of the meat samples by the ES may be responsible for reduction in WHC (Sams, 1999; Kim *et al*., 2014). According to Bowker and Zhuang (2015), denaturation of sarcoplasmic proteins has more influence on WHC than myofibrillar protein denaturation. Kadıoğlu *et al.* (2019)also reported that WHC of breast meat was lower than thigh meat. However, meat quality of both breast and thigh samples were noticeably good as WHC values are more than 1, according to George

et al.(1979).WHC value 1 or above indicate high WHC and values below 0.7 increasingly unacceptable WHC. There was a significant (P<0.01) increase in total protein extractability between NS and ES samples of breast and thigh meat. Between the breast meat and thigh meat, former showed higher (P<0.01) protein extractability. Increase inprotein extractability might be due to early activation of calpain enzyme leading to proteolysis and enhanced protein extractability (Rodas-González*et al.,* 2012). According to Lan *et al.*(1993)muscle fiber type and the extraction condition has a large influence on amount and composition of proteins extracted from muscles. MFI results are also significantly(P<0.01) varied between the muscles as well as corresponding treatment groups (Table 1), lower the MFI value indicates the higher fragmentation of muscle. In low volt ES a better balance between a reduction in muscle shortening and sufficient intrinsic enzymatic action (metabolic acceleration) which yields higher myofibrillar fragmentation (Walker *et al*., 1995).The apparent difference in fragmentation between the muscle types may have contributed to the difference in treatment effect between muscle types (Walker *et al*., 1996). Sams *et al*.(1992) and Birkhold and Sams (1993) also reported reductions in myofibrillar fragmentation with ES treatments. MFI is also positively correlated with sensory and Warner-Bratzler measures of tenderness and related to the degradation of the myofibrillar proteins (Olson and Stromer, 1976).

The CL is one of the important concerns in meat industries, which impacts not only on the final yield of product but also affects the eating quality of the meat (Dhital and Vangnai, 2019).In present study cooking loss of thigh meat was significantly (P<0.01) lower than the breast meat and no significant difference observed between NS and ES samples of breast or thigh meat. These results are in concurrence with the findings other worker(Alvarado and Sams, 2000; Dickens *et al*., 2002; Kahraman *et al*., 2011). However, these results are inconsistent with other studies including Young *et al.* (2005) in poultry and Agbeniga and Webb (2014)in beef. Kadıoğlu*et al.* (2019) reported the converse result for cooking losses in thigh and breast meat, which may be due to difference in sampling.

The shear force values were mainly used to assess the tenderness of cooked meat products. In present study the mean shear force values were lower in stimulated samples than control, ES has significantly (P<0.01) reduced

Table 1: Effect of electrical stimulation on biophysical parameters of spent hen breast and thigh muscle.

Parameters		Breast	Thigh	t-value
WHC	NS	1.35±0.07	1.66±0.14	6.742**
	ES	1.19±0.06	1.52±0.08	10.291**
	t-value	5.841**	2.891**	
Protein Extractability(mg/g)	NS	169.18±4.66	124.28±9.54	14.637**
	ES	184.35±10.32	151.11±8.77	10.991**
	t-value	5.194**	8.723**	
Cooking loss (%)	NS	25.99±0.941	21.48±1.72	7.942**
	ES	26.27±1.23	20.63±1.67	9.213**
	t-value	0.639^{n}	1.207^{n}	
Shear force value(kg)	NS	3.46±0.15	4.8±0.12	23.186**
	ES	2.62±0.25	3.5±0.15	10.15**
	t-value	23.186**	20.765**	
MFI	NS	749.35±19.37	848.88±39.36	7.857**
	ES	659.09±20.70	778.17±35.10	4.310**
	t-value	17.699**	2.477**	

Data are presented as mean±standard deviation (n = 12), '*' indicates the significant difference between the muscles (Breast and thigh) or treatments (NS and ES) for respective parameter and 'n' indicates non-significant difference.

SFV in both breast and thigh samples. Breast muscle has showed significantly (P<0.01) lower SFV in both treatment groups than thigh muscle and similar observations were reported by Wattanachant *et al.* (2004) and Jaturasitha *et al.* (2008)It is reported that, shear force values were significantly positively correlated with collagen content, yield of muscle and the diameter and area of myofibre and negatively correlated with myofiber density of the muscle Collagen content of the thigh meat is higher than the breast meat (Jaturasitha *et al.,* 2008; Jeon *et al*., 2010), which may be the reason for difference in SFV between the breast and thigh samples. In addition, many workers (Lowe *et al*., 2004; Li *et al*., 2006) correlated SFV to ultimate pH and reported that the differences in pH might be the cause of the lower SF values in the ES samples.

Biochemical parameters

After slaughter, biochemical changes, causing the conversion of muscle to meat, will determine final meat quality. Even after death due to asphyxia resulting from bleeding, muscle cells utilize available glycogen to produce ATP as long as pH conditions are optimal (Mir *et al*., 2017). According to Greaser (1986), this anaerobic metabolism results in the depletion of glycogen and accumulation of lactic acid and leads to decline in pH. Results of pH, R-value, glycogen and lactic acid of NS and ES samples are given in table 2. The results indicated that the mean pH of ES sample at both 0h and 24h was significantly (P<0.01) lower than the mean pH of NS samples. Similar results were reported by Šulcerová *et al*. (2014) and Karakayain and Ünal (2015). Breast meat showed lower pH values than thigh meat at 0 h and 24 h in both NS and ES groups. According to Yu *et al.* (2005) breast muscle, which contains a greater proportion of white fibrils, pH declined more rapidly than that of thigh, which contains a greater proportion of red fibrils.ES accelerates the catabolism of glycogen and conversion of adenosine to inosine nucleotides (R-value),causes rapid decrease in pH 50, which is also evident in the results of present study. The R-value was higher (P<0.01) in both ES (breast and thigh) compared to corresponding NS meat at 0 h. However, significantly (P<0.05) higher R-values were observed at 24 h of ageing than 0h.But no significance difference was observed between NS and ES thigh muscle at 24 hr. These results are consistent with Walker *et al.* (1996), in which a combined ES and muscle tensioning were shown to accelerate R-value in the red-fibered muscles compared with the white-fibered muscles of broilers. These differences in post mortem metabolism can be explained by white fibre, as opposed to red fibre, continuing to respire in the anoxic conditions of rigor development (Alvarado and Sams, 2000).

Table 2: Effect of electrical stimulation on biochemical properties of spent hen breast and thigh muscle**.**

Parameters	Treatment	Breast		Thigh		F-value
		0 hr	24 hr	0 hr	24 hr	
pH	NS	6.27±0.03^{c}	5.77±0.01^{a}	6.31±0.07^{d}	5.93±0.01^{b}	417.25**
	ES	5.99±0.01^{c}	5.67±0.03^{a}	6.06±0.01^{d}	5.8±0.03^{b}	570.32**
	t-value	22.02**	9.826**	11.04**	12.14**	
R-value	NS	0.89±0.03^{a}	1.32±0.03^{c}	0.94±0.03^{b}	1.39±0.03^{d}	669.91**
	ES	1.17±0.02^{b}	1.36±0.02^{a}	1.12±0.01^{c}	1.36±0.03^{c}	261.94**
	t-value	25.713**	3.414**	17.024**	1.28	
Glycogen (mg/g)	NS	7.09±0.23^{d}	1.22±0.15^{a}	6.06±0.25^{c}	1.98±0.18^{b}	2288.33**
	ES	3.74±0.37^{d}	1.00±0.12^{a}	3.00±0.39^{c}	1.5±0.11^{b}	239.29**
	t-value	25.98**	3.79**	22.6**	7.39**	
Lactic acid (mg/g)	NS	0.44±0.08^{b}	3.98±0.15^{d}	0.28±0.06^{a}	3.27±0.56^{c}	487.12**
	ES	1.83±0.35^{b}	4.55±0.17^{d}	1.51±0.27^{a}	4.28±0.20^{c}	436.44**
	t-value	14.935**	8.488**	14.959**	5.783**	

Data are presented as mean±standard deviation (n = 12). a, b, c and d indicates significance (p<0.05) different between muscles at corresponding time interval (row-wise) and '*' indicates the significant difference between the control and treatment (coloumn-wise)and 'n' indicates non-significant difference.

Electrical stimulation significantly increased the glycolysis in both the types of muscle, which is apparent in present study. Glycogen content of ES breast or thigh samples were significantly ($p<0.05$) lesser than corresponding NS samples and the glycogen content was relatively greater in breast muscle than in thigh muscle at 0h ($p<0.05$). But, lesser glycogen content was observed ($p<0.05$) in breast muscle than in thigh muscle at 24 h of postmortem. This finding was similar to that of a previous studies conducted by Mckee and Sams (1998) and Yu *et al.* (2005)in turkey (*Pectoralis*)and chicken (breast and leg), respectively. As discussed earlier, the reason for differences may be the type of the muscle fibres. Fernandez *et al.*(1995) observed that fast-twitch glycolytic fibres of the white fibres (fast-twitch) had a significantly greater glycogen content than red (fast- and slow-twitch) fibres and increased expression of glycolytic and fast isoforms of contractile proteins and decreased expression of oxidative enzymes in white compared with red skeletal muscle has been well-characterized previously (Glancy and Balaban, 2011). Likewise, results of lactic acid showed significant ($p<0.05$) difference between ES and NS samples of both muscles (breast and thigh) at both intervals i.e., 0hr and 24 hr. The results from our study supports the finding of Ryu *et al.* (2005),who described muscle pH negatively correlated with lactic acid content and positively correlated with glycogen content at postmortem. It was also reported that, the R-value positively correlated with lactic acid content and negatively correlated with glycogen content at 45 min postmortem. In this study, muscles with higher lactate levels at early postmortem showed faster glycolytic rates than muscles with lower lactate levels, similar results reported by Choe *et al.* (2008). Muscles with higher glycogen (breast) content at early postmortem also showed faster glycolytic rates than muscles with lower glycogen content, but these results were contrary to the results reported by Choe *et al.* (2008). The differences in the results of biochemical parameters between the muscles and response of each muscle to ES could be due to the fact that they differ in fiber type composition. Another possible factor is the muscle location which should be important in this study because both muscles are located at different parts of the carcass and function is also differs.

Colour and sensory evaluation

The CIELAB results were presented in table 3, indicates that breast meat from spent hens was lighter ($p<0.05$) in colour than thigh meat. No significant ($p<0.05$) difference observed for L*, a* and b* values between ES and NS of breast or thigh meat. The total colour differences (ΔE) between ES and NS breast, ES and NS thigh, NS breast and thigh, ES breast and thigh, were 1.84, 2.15 9.42 and 8.15 respectively. The difference between ES breast and thigh meat, NS breast and thigh meat were over the JND threshold (2.3 units). But results of no effect of electrical stimulation were observed among the treated and untreated muscles. Similarly, Owens and Sams (1998) found no differences between ES and NS. In contrast, Sams and Dzuik (1999) found that stimulated samples were darker and redder than control samples and Young *et al.* (2005) indicated that rapid muscle acidification by protein denaturation caused greater light reflectance on the meat surface.

Results of sensory attributes of NS and ES cooked breast and thigh chunks were presented in figure 1. No significance difference was observed for flavour between ES and NS samples of breast muscle. However, juiciness, tenderness and overall acceptability were found significantly ($P<0.01$) high in ES samples. No significance difference was observed for flavor and juiciness between ES and NS samples of thigh meat. However, significantly higher values were found for tenderness ($P<0.05$) and overall acceptability ($P<0.01$) for ES samples of thigh meat. Although ES breast showed higher overall acceptability, the difference in the scores for overall acceptability of ES thigh and NS thigh is more than breast.

Discriminant function analysis

Multivariate analyses have been used in different studies related to meat qualities. Like, effect of ultrasound treatment on beef quality (Alves *et al*., 2018), difference in yield and meat quality by gender in the Korean native

Table 3: Effect of electrical stimulation on colour of spent hen muscle

Parameters/treatment		Breast	Thigh	t-value
L	NS	56.03±2.14	47.14±2.62	9.09**
	ES	54.75±1.05	44.58±1.60	13.77**
	t-value	1.858	1.33	
a	NS	4.2±1.19	7.08±1.59	5.00**
	ES	4.53±1.32	8.36±1.00	8.254
	t-value	0.352	1.354	
b	NS	12.00±3.09	11.8±2.33	2.47*
	ES	13.32±3.67	11.5±2.89	2.08*
	t-value	0.952	0.782	

Data are presented as mean±standard deviation (n = 12) and '*' indicates the significant difference between the muscles (Breast and thigh) or treatments (NS and ES) for respective parameter

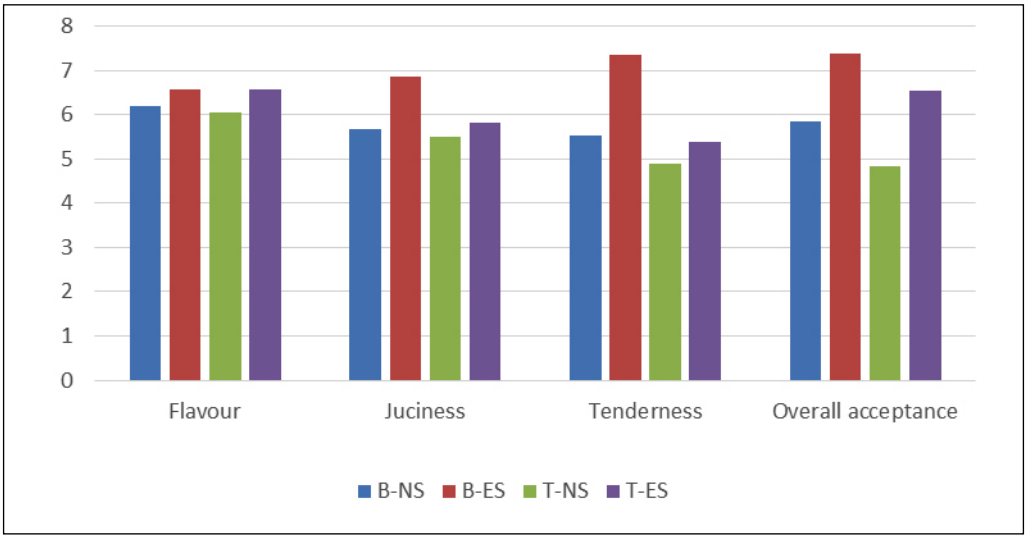

Fig. 1. Results of sensory evaluation

cattle, the use of illicit growth promoters in Charolais bulls (Choi and Kim, 2008; Roy *et al*., 2006), broiler chicken performance (Rosario *et al*., 2008). Principal components analysis was applied to studied the physical, colour and sensory characteristics of chicken breasts deboned at different time interval by Liu *et al.* (2004). In present study, canonical discriminant analysis was used to evaluate the effect of electrical stimulation on breast and thigh muscles of spent hen. The application of SDA to five biophysical parameters, four biochemical parameters at 0h and four biochemical parameters at 24h resulted in the selection of three (PE, SFV, CL), four (GLY, LA, RV, pH) and three (GLY, LA, RV) parameters, respectively. The discriminant function coefficient of all three sets of data is given in the table 4. Three discriminant functions have been developed for each sets of data, and contributed about 99.9, 99.6 and 97.4 per cent to the total variance in biophysical, biochemical-0hr and biochemical-24hr parameters, respectively. CDA was able to differentiate (100 %) all four groups (Fig. 2a) using biophysical parameters. Furthermore, 93.8 per cent and 95.8 per cent of original grouped cases correctly classified based on biochemical parameters at 0hr (Fig. 2b) and 24hr (Fig. 2c), respectively. The calculated results of Mahalanobis distances (D^2) are showed in Fig.

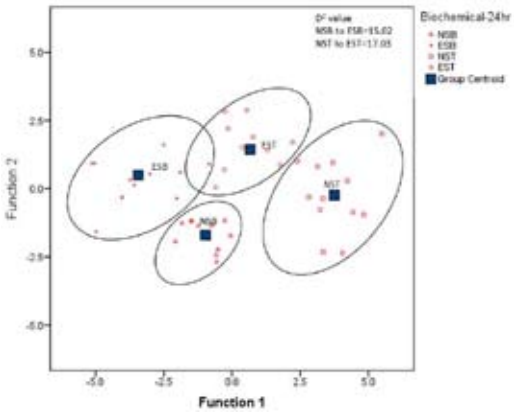

Fig. 2a

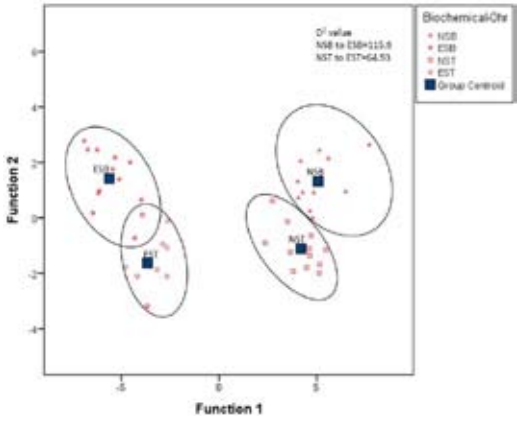

Fig. 2b

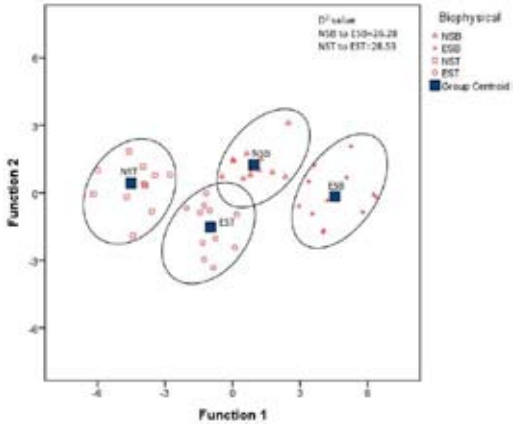

Fig. 2c

Figure 2: Canonical discriminant analysis of the three different sets of measured parameters described by the first and second canonical variables which are based on biophysical quality parameters (a), biochemical parameters at 0hr (b) and biochemical parameters at 24hr (c) selected by the stepwise discriminant analysis procedure. Mahalanobis distance (D^2) between the two groups are mention in the chart.

2**.** TheD^2 value between NSB and ESB is lesser than NST and EST for biophysical parameters (Fig.2a) and biochemical parameters (Fig.2c), which indicates the electrical stimulation cum 24 h of ageing has comparatively more effect on thigh muscle than breast muscle. Similarly, TheD^2 value between NSB and ESB showed higher than NST and EST for biochemical parameters at 0hr (Fig.2b), these differences may due to the immediate response of the muscle (fibre) types to electrical stimulation. However, biophysical and biochemical parameters of electrical stimulated breast and thigh muscle showed significant difference than non-stimulated.

Table 4: Discriminant functions' coefficients

Parameters	Function		
	1	2	3
Biophysical			
Protein Extractability	.762	.054	.655
Cooking Loss	.111	.955	-.385
Shear Force Value	-.598	.579	.606
Biochemical-0			
pH	.697	-.338	-.079
R-Value	-.601	.219	.752
Glycogen	.643	.783	.231
Lactic acid	-.534	.308	-.666
Biochemical-24			
R-Value	.349	.735	.740
Glycogen	.936	.526	-.282
Lactic acid	-.381	.831	-.450

Conclusion

Use of electrical stimulation significantly improves the biophysical, biochemical and sensory quality of both breast as well as thigh muscles. Colour of both breast and thigh were not influenced by electrical stimulation. In addition, significant difference was observed for all parameters of NS and ES groups of breast and thigh muscles, which may be due to the difference in muscle fibre types. Although, electrical stimulation has significant effect on both breast and thigh muscle, multivariate analysis based on the canonical discriminant and Mahalanobis distance (D^2) analysis demonstrated that the effect of electrical stimulation is comparatively more in thigh muscle than breast with ageing period of 24 h.

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Conflict of interest

Authors do not have any conflict of interest for this manuscript.

References

Adeyemi, K.D. and Sazili, A.Q. 2014. Efficacy of carcass electrical stimulation in meat quality enhancement: A review. *Asian-Australas J Anim. Sci.* **27**(3): 447.

Agbeniga, B. and Webb, E.C. 2014. Influence of electrical stimulation on carcass and meat quality of Kosher and conventionally slaughtered cattle. *S. Afr. J. Anim.* **44**(5):58-63.

Alvarado, C.Z. and Sams, A.R. 2000.The influence of postmortem electrical stimulation on rigor mortis development, calpastatin activity, and tenderness in broiler and duck pectoralis. *Poult. Sci.* **79**(9):1364-1368.

Alves, L.L., Rampelotto, C., Silva, M.S., De Moura, H.C., Durante, E.C., Mello, R.O., Menezes, C.R., Barin, J.S., Campagnol, P.C.B. and Cichoski, A.J. 2018. The effect of cold storage on physicochemical and microbiological properties of beef Semitendinosus muscle subjected to ultrasonic treatment in different systems (bath or probe). *Int. Food Res. J*. **25**(2):504-514.

Archile-Contreras, A.C., Cha, M.C., Mandell, I.B., Miller, S.P. and Purslow, P. P. 2011. Vitamins E and C may increase collagen turnover by intramuscular fibroblasts. Potential for improved meat quality. *J. Agric. Food Chem*. **59**(2):608-614.

Barekat, S. and Soltanizadeh, N. 2017. Improvement of meat tenderness by simultaneous alication of high-intensity ultrasonic radiation and papain treatment.*Innov.Food Sci. Emerg. Technol.* **39**:223-229.

Barker, S.B. and Summerson, W.H. 1941. The colourimetric determination of lactic acid in biological material.*J. Biol. Chem.***138:**535-554.

Birkhold, S.G. and Sams, A.R. 1993. Fragmentation, tenderness, and post-mortem metabolism of early-harvested broiler breast fillets from carcasses treated with electrical stimulation and muscle tensioning. *Poult. Sci.* **72**(3):577-582.

Bowker, B. and Zhuang, H. 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. *Poult. Sci.* **94**(7):1657-1664.

Calkins, C.R. and Sullivan, G. 2007. Adding enzymes to improve beef tenderness. Beef facts product enhancement, National cattleman's beef association. Centennial Colourado: Cattlemen's Beef Board.

Choe, J.H., Choi, Y.M., Lee, S.H., Shin, H.G., Ryu, Y.C., Hong, K.C. and Kim, B.C. 2008. The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. *Meat Sci.* **80**(2): 355-362.

Choi, Y.M. and Kim, B.C. 2009. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Sci.***122**(2-3):105-118.

DAHDF [Department of Animal Husbandry, Dairying, and Fisheries]. 2017. National Action Plans focusing on various sub-sectors of India's dynamic livestock industry, Ministry of Agriculture and Farmers' Welfare, Government of India. 34p

DAHDF [Department of Animal Husbandry, Dairying and Fisherie]s . 2019. Ministry of Agriculture & Farmers, Welfare, Government of India.47p.

Davis, G.W., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. 1980. Fragmentation procedure for bovine longissimus muscle as an index of cooked steak tenderness.*J. Food Sci.* **45**(4):880-884.

Dhital, S. and Vangnai, K. 2019. Meat tenderisationeffect of protease from mango peel crude extract. *Int. Food Res. J.* **26**(3):991-998.

Dickens, J.A., Lyon, C.E. and Buhr, R.J. 2002. The effects of electrical stimulation during bleeding on shear values and cook loss of breast fillets from mature chickens deboned at two or twenty-four hours post-evisceration. *J. Appl. Poult. Res.* **11**(1):111-116.

Fernandez, X., Lefaucheur, L. and Čandek, M. 1995. Comparative study of two classifications of muscle fibres: consequences for the photometric determination of glycogen according to fibre type in red and white muscle of the pig. *Meat Sci.***41**(2):225-235.

George, A.R., Bendall, J.R. and Jones, R.C.D. 1980. The tenderising effect of electrical stimulation of beef carcasses. *Meat Sci*. **4**(1):51-68.

Glancy, B. and Balaban, R.S., 2011.Protein composition and function of red and white skeletal muscle mitochondria. American Journal of Physiology-Cell Physiology, 300(6);1280-1290.

Grau, R. and Hamm, R. 1953. Eineeinfachemethodezurbestimmung der wasserbindungimmuskel. *Naturwissenschaften*, **40**(1):29-30.

Greaser, M. L. 1986. Conversion of muscle to meat. In: Bechtel, P.J.(ed). *Muscle as food.* Academic press, Orlando, USA, pp. 37-102.

Honikel, K.O. and Fischer, C. 1977. A rapid method for the detection of PSE and DFD porcine muscles. *J. Food Sci.* **42(**6):1633-1636.

Hunt, M.C., Acton, J.C., Benedict, R.C., Calkins, C.R., Cornforth, D.P., Jeremiah, L.E., Olson, D.G., Salm, C.P., Savell, J.W. and Shivas, S.D. 1991. Guidelines for meat colour evaluation. In 44th Annual Reciprocal Meat Conference (9-12). National Livestock and Meat Board Chicago, Ill.

Indumathi, J., Shashikumar, M., Reddy, G.V.B., Babu, A.J. and Prakash, M.G. 2019. Utilization of spent broiler breeder hen meat to develop value added sausages. *Int. J. Curr. Microbiol. Al. Sci.* **8**(12):754-765.

Jama, N., Muchenje, V., Chimonyo, M., Strydom, P.E., Dzama, K. and Raats, J.G. 2008. Cooking loss components of beef from Nguni, Bonsmara and Angus steers. *Afr. J. Agric. Res*. **3**(6):416-420.

Janz, J.A.M., Aalhus, J.L. and Price, M.A. 2001. Blast chilling and low voltage electrical stimulation influences on bison (Bison bison bison) meat quality. *Meat Sci.* **57**(4):403-411.

Jaturasitha, S., Srikanchai, T., Kreuzer, M. and Wicke, M. 2008.Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red).*Poult. Sci.* **87**(1):160-169.

Jeon, H.J., Choe, J.H., Jung, Y.K., Kruk, Z.A., Lim, D.G. and Jo, C.R. 2010. Comparison of the chemical composition, textural characteristics, and sensory properties of North and South Korean native chickens and commercial broilers. *Food Sci. Anim. Resour.* **30**(2):171-178.

Joo, S.T., Kauffman, R.G., Kim, B.C. and Park, G.B. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Sci*. **52**(3):291-297.

Kadıoğlu, P., Karakaya, M., Unal, K. and Babaoğlu, A.S. 2019.Technological and textural properties of spent chicken breast, drumstick and thigh meats as affected by marinating with pineale fruit juice.*BritishPoult. Sci.* **60**(4):381-387.

Kahraman, T., Bayraktaroglu, A.G., Vural, A., Issa, G. and Ergun, E.M.E.L. 2011. Electron microscopy of contractile bands and quality characteristics in high-voltage electrical stimulation broiler breast meat.*Poult. Sci.* **90**(2):486-490.

Karakayain, M. and Ünal, M.K. 2015.Effect of sonication time on technological and emulsification properties of spent chicken breast and thigh meat.Fleischwirtschaft international: *J Meat Prod.Meat Proces.* **1**:86-90.

Kim, Y.H.B., Warner, R.D. and Rosenvold, K. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. *Anim. Prod. Sci.,* **54**(4):375-395.

Lan, Y.H., Novakofski, J., Carr, T.R. and McKeith, F.K. 1993. Assay and storage conditions affect yield of salt soluble protein from muscle. *J. Food Sci.* 58(5), .963-967.

Lawrie, R.A. 1991. *Meat Science* (5th Ed). Pergamon Press, New York, 60p

Lee, S.O., Min, J.S., Kim, I.S. and Lee, M. 2003. Physical evaluation of poed cereal snacks with spent hen meat. *Meat Sci.***64**(4):383-390.

Li, C.B., Chen, Y.J., Xu, X.L., Huang, M., Hu, T.J. and Zhou, G.H. 2006. Effects of low-voltage electrical stimulation and rapid chilling on meat quality characteristics of Chinese Yellow crossbred bulls.*Meat Sci.***72**(1):9-17.

Liu, Y.O.N.G.L.I.A.N.G., Lyon, B.G., Windham, W.R., Lyon, C.E. and Savage, E.M. 2004. Principal component analysis of physical, colour, and sensory characteristics of chicken breasts deboned at two, four, six, and twenty-four hours postmortem. *Poult. Sci.* **83**(1):101-108.

Lowe, T.E., Devine, C.E., Wells, R.W. and Lynch, L.L. 2004. The relationship between postmortem urinary catecholamines, meat ultimate pH, and shear force in bulls and cows.*Meat Sci.* **67**(2):251-260.

Mancini, S., Preziuso, G., Dal Bosco, A., Roscini, V., Szendrő, Z., Fratini, F. and Paci, G. 2015.Effect of turmeric powder (Curcuma longa L.) and ascorbic acid on physical characteristics and oxidative status of fresh and stored rabbit burgers. *Meat Sci.* **110**:93-100.

McKee, S.R. and Sams, A.R. 1998. Rigor mortis development at elevated temperatures induces pale exudative turkey meat characteristics. *Poult. Sci.* **77**(1):169-174.

Mendel, B., Kemp, A. and Van Heijningen, A.J.K. 1954. A colourimetric micro-method for the determination of glycogen in tissues. *Biochem. J.* **56**(4):646.

Mir, N.A., Rafiq, A., Kumar, F., Singh, V. and

Shukla, V. 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. *J. Food Sci. Technol*. **54**:2997-3009.

Naveena, B.M., Kiran, M., Reddy, K.S., Ramakrishna, C., Vaithiyanathan, S. and Devatkal, S.K. 2011. Effect of ammonium hydroxide on ultrastructure and tenderness of buffalo meat.*Meat Sci.* **88**(4):727-732.

Navid, S., Sheikhlar, A. and Kaveh, K. 2011. Influence of the combination of vitamin D3 and papaya leaf on meat quality of spent layer hen. *Agric. J* .**6**:197-200.

Olson, D.G. and Stromer, M.H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* **41**(5):1036-1041.

Owens, C.M. and Sams, A.R.. 1998. Meat quality of broiler breast meat following post-mortem electrical stimulation at the neck.*Poult. Sci.* **77**(9):1451-1454.

Papinaho, P.A., Ruusunen, M.H., Suuronen, T. and Fletcher, D.L. 1996.Relationship between muscle biochemical and meat quality properties of early deboned broiler breasts.*J. AliedPoult. Res.* **5**(2):126-133.

Rodas-González, A., Pflanzer, S.B., Garmyn, A.J., Martin, J.N., Brooks, J.C., Knobel, S.M., Johnson, B.J., Starkey, J.D., Rathmann, R.J., De Felicio, P.E. and Streeter, M.N. 2012. Effects of postmortem calcium chloride injection on meat palatability traits of strip loin steaks from cattle sulemented with or without zilpaterol hydrochloride.*J. Anim. Sci*. **90**(10):3584-3595.

Rosario, M.F., Silva, M.A.N., Coelho, A.A.D., Savino, V.J.M. and Dias C.T. 2008. Canonical discriminant analysis alied to broiler chicken performance.*Animal*, **2**(3):419-424.

Roy, B.C., Oshima, I., Miyachi, H., Shiba, N., Nishimura, S., Tabata, S. and Iwamoto, H. 2006. Effects of nutritional level on muscle development, histochemical properties of myofibre and collagen architecture in the pectoralis muscle of male broilers. *British Poult. Sci.* **47**(4):433-442.

Ryu, Y.C., Choi, Y.M. and Kim, B.C. 2005. Variations in metabolite contents and protein denaturation of the longissimusdorsi muscle in various porcine quality classifications and metabolic rates. *Meat Sci.* **71**(3):522-529.

Sams, A.L.A.N. 1999. Commercial implementation of postmortem electrical stimulation.*Poult. Sci.* **78**(2):290-294.

Sams, A.R. and Dzuik, C.S. 1999. Meat quality and rigor mortis development in broiler chickens with gas-induced anoxia and postmortem electrical stimulation.*Poult. Sci.* **78**(10):1472-1476.

Sams, A.R., Janky, D.M. and Dukes, M.G. 1992. Research note: anatomical location of alication influences the tenderizing effectiveness of electrical stimulation of broiler carcasses. *Poult. Sci.* **71**(9):1564-1567.

Sharma, G. 2017. Colour fundamentals for digital imaging.In Digital colour imaging handbook. CRC press 114p.

Šulcerová, H., Mihok, M., Jůzl, M. and Haščík, P. 2011.Effect of addition of pollen and propolis to feeding mixtures during the production of broiler chickens ROSS 308 to the colour of thigh and breast muscle and pH determination. *Acta Univ. Agric. et Silvic,* **59**(6):359-366.

Suriani, N.W., Purnomo, H., Estiasih, T. and Suwetja, I.K. 2014. Physicochemical properties, fatty acids profile and cholesterol content of indigenous manado chicken, broiler and spent hen meat. *Int. J. Chemtech Res.* **6**(7):3896-3902.

Troutt, E.S., Hunt, M.C., Johnson, D.E., Claus,

J.R., Kastner, C.L., Kropf, D.H. and Stroda, S. 1992. Chemical, physical, and sensory characterization of ground beef containing 5 to 30 per cent fat. *J. Food Sci.* **57**(1):25-29.

Tůmová, E. and Teimouri, A. 2009. Chicken muscle fibres characteristics and meat quality: a review. *Sci. Agric. Bohem,* **40**(4):253-258.

Walker, L.T., Birkhold, S.G., Kang, I.S., Hirschler, E.M. and Sams, A.R. 1996. The effects of post-mortem electrical stimulation and muscle tensioning in two broiler muscles.*Poult. Sci.,* **75**(9):1118-1120.

Walker, L.T., Shackelford, S.D., Birkhold, S.G. and Sams, A.R. 1995.Biochemical and structural effects of rigor mortis-accelerating treatments in broiler Pectoralis. *Poult. Sci.,* **74**(1):176-186.

Warriss P.D. 2010. *Meat Science* (2nd Ed) Cambridge, UK,pp. 1-12

Wattanachant, S., Benjakul, S. and Ledward, D.A. 2004. Composition, colour, and texture of Thai indigenous and broiler chicken muscles.*Poult. Sci.* **83**(1):123-128.

Wheeler, T.L., Shackelford, S.D., Johnson, L.P., Miller, M.F., Miller, R.K. and Koohmaraie, M. 1997. A comparison of Warner-Bratzler shear force assessment within and among institutions. *J. Anim. Sci.* **75**(9):2423-2432.

Young, L.L., Cason, J.A., Smith, D.P., Lyon, C.E., Dickens, J.A. and Walker, J.M. 2005. Effects of electrical stimulation and simulated conventional and extended chilling method on cooked chicken breast meat texture and yield. *Int. J. Poult. Sci*. **4**(2):60-63.

Yu, L.H., Lee, E.S., Jeong, J.Y., Paik, H.D., Choi, J.H. and Kim, C.J. 2005. Effects of thawing temperature on the physicochemical properties of pre-rigor frozen chicken breast and leg muscles. *Meat Sci.***71**(2):375-382. ■