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Physico-chemical characteristics of commercially available and cold slaughtered broiler chicken meat#

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Abstract

In the current study, leg muscle samples of commercially available (CA) broiler chicken slaughtered traditionally by neck severance and cold slaughtered (CS) broiler chicken carcasses were compared for their physico-chemical characteristics. Sample harvesting was carried out at 0-2, 2-4, 4-6 and 6-8 h post mortem. The pH revealed significantly ($p<0.01$) lower values in CS leg samples compared to CA leg samples at all times of sample collection except at 6-8 h. Significantly lower pH values were recorded in samples collected at increasing post mortem time intervals with mean pH values shifting from 6.3±0.05 at 0-2 h to 5.78±0.05 at 6-8 h in CA samples. Water holding capacity (WHC) decreased significantly as time of sample collection post mortem increased with significantly lower WHC in CS group (48±0.894 % at 6-8 h), and thiobarbituric acid reactive substances (TBARS) values increased significantly as time of sample collection post mortem increased with mean values of 0.26±0.01 and 0.5±0.02, in CA and CS samples respectively at 6-8 h. The L values were higher in CA samples whereas a* and b*colour values were higher in CS samples. As time of sample collection post mortem increased, the mean a* values decreased from 3.9±0.33 at 0-2 h to 2.54±0.35 at 6-8 h in CA samples and from 11.17±0.76 at 0-2 h to 9.48±0.84 at 6-8 h in CS samples. In both CA and CS samples, L* values increased significantly as time of sample collection post mortem increased. Haem iron, haemoglobin and myoglobin contents of muscle samples were significantly higher in CS group, but as the time of sample collection post mortem increased the difference became less significant.*

Keywords: *Broiler chicken meat, cold slaughter, commercial, post mortem, meat pigments*

The poultry sector is a cornerstone of global food production, with chicken meat standing out as one of the most affordable, accessible, and widely consumed sources of animal protein. According to the Food and Agriculture Organization (FAO, 2024), poultry contributes to more than one-third of the world's total meat supply, a reflection of its efficient feed conversion, rapid production cycle, relatively low cost and broad cultural acceptance. India produced an estimated 10.25 million tonnes of meat in 2023–24, marking a 4.95 per cent increase from the previous year. Of this, the poultry industry contributed the largest share, making up about 48.96 per cent of national meat production

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(DAHD, 2024). In India, chicken is the most commonly consumed meat variety due to its low price and high-quality lean protein.With increasing population and people becoming more focussed on lean meat, the quality of meat is compromised at times to meet the demand. One serious issue is the illegal entry of carcasses from birds that died due to natural causes, illness or stress into the food chain. Carcasses from birds that die before proper slaughtering miss exsanguination leading to pooling of blood which results in meat that acts as breeding ground for microbial growth. In recent times, many such adulterations have been reported by various news channels all over India (Abhishek, 2012; Pawan, 2020 & Anon., 2023). Besides being unethical and unlawful, this can lead to food poisoning and spread of zoonotic diseases (Si et al*.,* 2021). Only limited studies have been done in this area for evaluating differences between cold slaughtered and commercially available chicken. Some researchers have tried to tell the difference between cold slaughtered meat using physical and chemical methods (Sohaib et al., 2020) while others used untargeted metabolomics, a mix of untargeted and pseudo-targeted metabolomics, metabolomic profiling (Sidwick et al., 2017;Abbas et al., 2020 & Cao et al., 2020) and isoelectric focusing for investigations (Saud et al., 2019). However, most of these studies were done on just a few slow-growing birds, and the observations were recorded only at early post mortem time periods with no consideration to post mortem progression and associated changes. Also, most of the studies were done with cold preserved samples and the real time scenario of retail markets have not been reflected in the results. Hence the current study was conducted to determine the physico-chemical characteristics and meat pigment characteristics of cold slaughtered and commercially available chicken leg meat samples taken at different post mortem time points.

Materials and methods

Sample collection

The study was conducted during the months of February-May, 2025 and the ambient temperature during this period ranged from 28-35º C. All the birds included in the study originated from a single commercial broiler farm and the birds were managed on identical conditions. Cold slaughtered broiler chicken meat samples were harvested from birds that were dead at the time of unloading at the local retail meat shops. The duration of transportation of birds from the farm was ascertained by enquiry with the suppliers of the birds. It was recorded that the birds arrived at the market within 2 h of leaving the farm. Hence, it was ensured that death of those birds which were dead at the time of unloading in the retail shops has occurred within the preceding 2 h. The carcasses of dead birds collected from retail shops were immediately transported to Meat Technology Unit (MTU), Mannuthy in plastic crates.

At, MTU, ten dead bird carcasses were deskinned and eviscerated. Postmortem examination of these carcasses and offals was conducted and those having indications of infectious diseases, fractures and trauma were eliminated from the study. Approximately, 200g portions of boneless leg muscles were collected separately and packed in low density polyethylene (LDPE) pouches. These portions of the sample were categorised as CS samples harvested 0-2 h post mortem.For collection of CS samples at 2-4, 4-6 and 6-8 h post mortem, the dead bird carcasses (ten each) were retained in the plastic crates for 2 h, 4 h and 6 h, respectively at ambient temperature. After the designated period, samples were collected as mentioned above.

Commercially available broiler chicken meat samples were collected from retail meat shops where the birds were slaughtered as per the traditional process of severing the blood vessels without preslaughter stunning. Before slaughter, it was ensured that birds were provided with 4 h of preslaughter rest after unloading at the retail shops. Only healthy birds (total forty birds) weighing around 2-2.2 kg were selected. A bleeding duration of 3-3.5 min was allowed for adequate removal of the blood. After the bleeding was over, the slaughtered birds were transferred to plastic crates and transported immediately to MTU, Mannuthy. At MTU, leg muscle samples at same time intervals as for CS samples were harvested after deskinning, evisceration and postmortem inspection. Diseased and injured bird carcasses were eliminated from the study.

Immediately after harvest, the samples were assessed for pH and L*, a* and b* colour values. Leg muscle samples were aerobically packed in LDPE pouches and stored for analyses of water holding capacity and thiobarbituric acid reactive substance (TBARS) value. A portion of the samples was stored at -80±1°C for haemoglobin, myoglobin and haem iron quantification.

Physicochemical characteristics

The pH of leg muscle samples of CA and CS groups was measured using a digital pH meter (µ pH System-Systronics, India) as described by O'Halloran et al*.* (1997).The instrumental colour was measured using a calibrated colour reader (Lovibond LC 100 Spectro colourimeter) with diffuse illumination. It was calibrated using black and white calibration tiles before starting of the measurement and the colourimeter score was recorded with L*of black equals zero and L* of white equals 100, a* of lower numbers denoting more green (less red), higher numbers denoting more red (less green) and b* of lower numbers indicating more blue (less yellow) and higher numbers denoting more yellow (less blue). The sensor of the colourimeter was kept in contact with the sample and readings taken. The reading was repeated three times at different points on the sample.

Water-holding capacity of CS and CA chicken leg samples was estimated by centrifugation method according to Devatkal et al. (2019). Thiobarbituric acid reactive substances (TBARS) values of CS and CA leg muscle samples were determined by the extraction method of Witte et al. (1970).

Haem iron content of chicken meat was determined as described by Addeen et al. (2014). Myoglobin content was determined according to the procedure given by Warris (1979) and the calculation was done according to Krzywicki (1979) as shown below.

Myoglobin (mg/g) = (A 525-A 700) x 2.303 × dilution factor

Haemoglobin from breast and leg muscle samples of commercially available and cold slaughtered broiler chicken was extracted following the method of O'Brien et al. (1992). Haemoglobin was evaluated using a modified kinetic technique of Goyal and Basak (2009) in which haemoglobin acted as a chemical catalyst to break down hydrogen peroxide into water and nascent oxygen. Nascent oxygen then oxidised o-tolidine to give an oxidised product of green-blue colour. The rate of colour development was directly proportional to haemoglobin concentration.

Statistical analysis

The physico-chemical attributes of CS and CA chicken leg samples collected at four different time intervals post mortem were analysed using univariate ANOVA by using SPSS software version 24.0.

Results and Discussion

The pH, WHC and TBARS values of CA and CS leg muscle samples are given in Table 1, the L*, a* and b* colour values in Table 2 and the haem iron, haemoglobin and myoglobin contents in Table 3.

pH

The mean pH of CA leg samples collected at 0-2 h post mortem was 6.3±0.05. Bhawana et al. (2023) reported pH values in the range of 6.2 to 6.3 for thigh muscle samples from broiler chicken carcasses collected from retail shops. The mean pH values of leg muscle samples from both CA and CS groups were consistently lower as the post mortem interval before sample collection increased. However, for CS samples, those collected at 6-8 h post mortem had significantly ($p<0.01$) higher pH values as compared to those collected at 4-6 h post mortem. Lawrie (1991) described that immediately after death the body shifted to an anaerobic glycolysis process and produced lactic acid in muscles which led to a gradual decline in the pH values. The combination of retained blood and higher ambient temperature could have caused rapid spoilage in meat that caused higher pH values in CS samples collected at 6-8 h postmortem. Lee and Shin (2019) stated that the alkalisation and rise in pH was due to release of products of protein denaturation and biogenic amines produced by spoilage bacteria.

The pH of samples differed significantly ($p<0.01$) between CA and CS groups at all the four times of sample collection post mortem with lower values seen in the CS group except at 6-8 h. This is in accordance with Sohaib et al. (2020) and Belore et al. (2023) who reported significantly ($p<0.01$) lower pH in CS samples than halal and scientifically slaughtered chicken meat samples. However, contrasting results had been reported by Ahmad et al. (2015) who observed significantly lower pH values for perfectly bled poultry meat samples in comparison to the imperfectly bled meat samples. The lower pH observed in CS samples at all times of sample collection in the present study could be attributed to the following factor. Till the CS samples were collected, the bird carcasses were left at ambient temperature in the undressed state to simulate the market conditions, whereas the CA carcasses were in the deskinned and eviscerated stage. This might have resulted in higher carcass temperatures and faster post mortem glycolysis in the CS carcasses.

Water Holding Capacity

The water holding capacity (WHC, per cent) of leg samples from CA group collected at 0-2 h post mortem was 77.4±1.26. For CS leg muscle samples collected at 0-2 h post mortem, the WHC values was 69.2±2.37. The samples collected at later times post mortem showed significantly ($p<0.01$) lower WHC values with the lowest values seen at 6-8 h in samples from CA and CS groups. Hamm (1961) attributed this to decrease in bound water during the onset of rigor mortis and further reduction upon cooking.

The WHC values were significantly ($p<0.01$) higher in CA group compared to CS group for samples at all the collection intervals. These observations concur with those of Belore et al. (2023) who reported significantly higher WHC in halal slaughtered chicken breast muscle samples in comparison with cold-slaughtered meat samples. Rahman et al. (2019) had also observed higher WHC in breast muscles from halal slaughtered chicken than samples from dead (unbled) birds.

Thiobarbituric Acid Reactive Substances

The mean thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde/kg) of leg muscle samples from CA group was 0.12±0.01 at 0-2 h post mortem. The mean TBARS values were significantly ($p<0.01$) higher in leg muscles of CA and CS birds collected at increasing intervals post mortem. Lipid peroxidation is a progressive reaction initiated by free radicles generated from iron rich

proteins. This is in accordance with Addeen et al. (2014) who found progressive increase in TBARS values during storage of meat samples from chicken killed by different methods.

The mean TBARS values of muscle samples from CA group were significantly lower than CS group. Sohaib et al. (2020) reported higher TBARS values in dead meat than meat from halal-slaughtered chickens. They attributed this to the presence of ferric heme pigments in inadequately bled tissues, which could promote lipid oxidation and act as pro-oxidants. Sasidharan et al. (2022) also reported higher TBARS values in unbled beef compared to incompletely bled and adequately bled beef samples.

L* value

The L* value of leg samples from both CA and CS groups were significantly higher ($p<0.05$) in samples collected at progressively later times post mortem. This correlates well with the lower pH values observed at these time periods. In the frequently cited work by Qiao et al. (2001), they had reported a negative correlation between broiler chicken breast muscle pH and L* values. These authors separated broiler chicken breast fillets into three groups according to meat lightness (L*) as follows: lighter than normal (light, L* > 53), normal (L* >48 and < 53), and darker than normal (dark, L* < 46). MacDougall (1972) attributed the variations in L* values to the changes that take place in the structure of meat during ageing, especially protein denaturation, which results in greater dispersion of light and thus, increased lightness.

The L* value of leg muscles samples differed significantly between CA and CS groups at all times of collection post mortem. These results are in accordance with Sohaib et al. (2020) who reported significantly higher L* values in breast muscle samples from halal slaughtered (without stunning) broiler chicken compared to meat from dead and decapitated birds. The significantly ($p<0.01$) lower L* values in CS leg muscles samples could be due to retention of haem pigments in muscles as these birds were not subjected to the process of bleeding. Boulianne and King (1998) showed a strong negative correlation between total pigment concentration, iron levels and L* values.

a* values

There was a non-significant decline in the a* values of leg muscle samples in CA muscle samples as the time of sample collection increased post mortem, whereas in CS samples the value decreased significantly ($p<0.05$). Petracci and Fletcher (2002) reported steady decline in a* in intact carcasses with time. Sujiwo et al. (2018) and Belore et al. (2023) also observed non-significant reduction in a* values on post slaughter storage.

The mean a* values were significantly ($p<0.01$) higher in leg muscle samples of CS group at all time periods of collection when compared to CA group. This result is expected as the birds in the CS group did not undergo the process of bleeding resulting in retention of larger proportion of hemoglobin. Alvarado et al. (2007) reported higher a* values in unbled birds killed by carbon dioxide slaughter and Sohaib et al. (2019) found higher a* in dead birds due to retention of blood in them. In normally bled broiler chicken carcasses, hemoglobin contributed about only 6–16% of the total pigment content in fresh meat (Crystal et al., 1981). Because of this, its impact on meat colour in normally bled chicken carcasses is relatively limited when compared with myoglobin.

b* Values

Non-significant increase in the b* values was

Table 1. pH, WHC (%) and TBARS (mg malonaldehyde/kg) values of leg muscle samples of commercially available (CA) and cold slaughtered (CS) broiler chicken at different times of collection post mortem

PARAMETER	GROUP	TIME OF COLLECTION (h)				F-value
		0-2	2-4	4-6	6-8	
pH	CA	6.3 ± 0.05^{aA}	6.22 ± 0.05^{abA}	6.15 ± 0.05^{bA}	5.78 ± 0.05^{cA}	20.382**
	CS	6.12 ± 0.05^{aB}	6.06 ± 0.05^{aB}	5.98 ± 0.05^{aB}	6.27 ± 0.05^{bB}	5.963**
F-value		6.702*	4.910*	5.599*	46.120**	
WHC	CA	77.4 ± 1.26^{aA}	75.8 ± 0.92^{aA}	72.8 ± 1.79^{aA}	64.2 ± 1.56^{bA}	11.997**
	CS	69.2 ± 2.37^{aB}	64.8 ± 1.08^{abB}	60.8 ± 2.72^{bB}	48 ± 0.894^{cB}	28.915**
F-value		11.649**	20.962**	24.947**	45.466**	
TBARS	CA	0.16 ± 0.01^{aA}	0.19 ± 0.01^{abA}	0.22 ± 0.01^{bA}	0.26 ± 0.01^{cA}	13.757**
	CS	0.23 ± 0.01^{aB}	0.24 ± 0.01^{abB}	0.3 ± 0.01^{bB}	0.5 ± 0.02^{cB}	115.415**
F-value		18.129**	12.242**	28.624**	213.888**	

** Significant at 0.01 level. * Significant at 0.05 level.

Means bearing different lower-case alphabets as superscript differ significantly within a row

Means bearing different upper-case alphabets as superscript differ significantly between CA and CS at each time of collection.

Table 2. L*, a* and b* values of leg muscle samples of commercially available (CA) and cold slaughtered (CS) broiler chicken at different times of collection post mortem

PARAMETER	GROUP	TIME OF COLLECTION(h)				F-value
		0-2	2-4	4-6	6-8	
L*	CA	42.27±1.35^{aA}	40.96±1.67^{aA}	43.87±1.30^{abA}	47.81±2.26^{bA}	3.160*
	CS	34.59±1.33^{aB}	36.35±2.71^{aAB}	37.04±1.67^{abB}	41.41±1.44^{bB}	3.013*
F-value		10.582*	3.801*	8.371**	7.328**	
a*	CA	3.9±0.33^{aA}	3.77±0.32^{aA}	2.82±0.37^{aA}	2.54±0.35^{aA}	1.348^{ns}
	CS	11.17±0.76^{aB}	10.85±0.69^{aB}	9.96±0.71^{aB}	9.48±0.84^{aB}	1.760^{ns}
F-value		76.696**	72.879**	74.190**	70.162**	
b*	CA	10.85±0.81^{aA}	12.36±0.82^{aA}	12.98±0.69^{aA}	13.03±0.59^{aA}	1.297^{ns}
	CS	11.02±0.71^{aA}	11.85±1.15^{abA}	12.71±1.36^{abA}	14.22±0.70^{bA}	2.350^{ns}
F-value		0.017^{ns}	0.166^{ns}	0.045^{ns}	0.890^{ns}	

** Significant at 0.01 level, * Significant at 0.05 level, ns non-significant
Means bearing different lower-case alphabets as superscript differ significantly within a row
Means bearing different upper-case alphabets as superscript differ significantly between CA and CS at each time of collection.

seen in leg samples of both CA and CS groups as the time of sample collection increased post mortem. Sujiwo et al. (2018) reported significant increase in b* values on storage over 12 days in chicken breast muscle samples. Contrasting results were reported by Sabikun et al. (2019) were the b* values decreased over time.

The b* values of leg muscle samples from CA and CS groups showed non-significant difference between them at all times of sample collection postmortem. Alvarado et al. (2007) also reported no significant differences in b* value among bled and unbled birds. Sohaib et al. (2020) on the contrary, reported that the chicken meat from halal slaughter had the lowest mean b* values, whereas dead chicken meat documented the highest mean b* values. They attributed higher residual blood contents and post-mortem changes for the difference.

Haem Iron Content

The mean haem iron contents of leg muscle samples from both CA and CS groups were significantly ($p<0.01$) lower in samples collected at 6-8 h post mortem than the 0-2 h samples. In CS leg muscle, the haem iron content was about 62 per cent less in 6-8 h samples than in the 0-2 h samples. Addeen et al (2014) also found that on storage, the haem iron and non-haem iron values increased and decreased in broiler breast muscle samples, respectively, with the unbled group experienced a substantial rise in non-haem values. The reduction in haem iron content could be attributed to the breakdown of porphyrin rings during storage, leading to the release of free iron from the haem structure (Benjakul & Bauer, 2001). Our results showing a time-dependent reduction in haem iron content aligned with these findings.

The mean haem iron content of CA group was significantly lower than CS group in meat samples collected 0-2 h postmortem. The haem group is made up of an iron atom bound within a heterocyclic ring structure called a porphyrin, where the iron is predominantly present in the ferrous (+2) state (Richards & Hutlin, 2002). The significantly higher haem iron content in CS group 0-2 h post-mortem was in accordance with findings of Belore et al. (2023). They found that mean haem iron content of 5.19±1.28 in cold slaughtered broiler breast meat samples were significantly higher than 1.98±0.45 seen in electrically stunned samples and 1.69±0.46 in halal slaughtered samples. They attributed these higher haem iron contents to the higher residual blood in CS meat samples. Higher haem iron contents in dead and unbled chicken samples had also been reported by Sohaib et al. (2019) and Addeen et al. (2014).

Myoglobin Content

The mean myoglobin content decreased with the advancement of post mortem interval before sample collection in leg muscle samples of CA and CS groups. Schreurs (2000) opined that the postmortem changes in chicken muscle, including the degradation of myofibrillar proteins and the development of rigor mortis contributed to the reduction in myoglobin concentrations over time. Sabikun et al. (2019) also reported that with increasing postmortem ageing, muscle underwent changes in protein oxidation, release of pro-oxidant enzymes and cellular compartmentalisation which contributed to myoglobin reduction with time.

The myoglobin concentrations of leg muscle samples from CA and CS groups differed significantly ($p<0.01$) at all times of sample collection with higher values in CS group. Belore et al. (2023) reported highest value of mean myoglobin in cold slaughtered broiler meat (2.33 ± 0.70), followed by meat from electrically stunned birds (1.96 ± 0.27) and the lowest in halal slaughtered chicken meat (1.84 ± 0.68).

Table 3. Haem iron content (mg/100g), myoglobin content(mg/g) and haemoglobin content(mg/g) of leg muscle samples of commercially available (CA) and cold slaughtered (CS) broiler chicken at different times of collection post mortem

PARAMETER	GROUP	TIME OF COLLECTION(h)				F-value
		0-2	2-4	4-6	6-8	
Haem iron	CA	5.12 ± 0.39^{aA}	3.52 ± 0.17^{bA}	3.32 ± 0.19^{bA}	2.88 ± 0.25^{bA}	4.466**
	CS	8.38 ± 1.11^{aB}	4.51 ± 0.26^{bA}	4.31 ± 0.08^{bcA}	3.18 ± 0.30^{cA}	24.080**
F-value		24.890**	2.290^{ns}	2.318^{ns}	0.202^{ns}	
Myoglobin	CA	1.67 ± 0.07^{aA}	1.23 ± 0.09^{bA}	1.07 ± 0.14^{bA}	0.96 ± 0.08^{bA}	5.606**
	CS	2.98 ± 0.18^{aB}	2.14 ± 0.19^{bB}	1.78 ± 0.13^{bB}	1.36 ± 0.13^{cB}	27.814**
F-value		50.459**	23.823**	14.564**	4.560*	
Haemoglobin	CA	0.07 ± 0.01^{aA}	0.05 ± 0.01^{abA}	0.04 ± 0.01^{bcA}	0.02 ± 0.01^{cA}	7.741**
	CS	0.15 ± 0.01^{aB}	0.1 ± 0.01^{bB}	0.07 ± 0.01^{bB}	0.04 ± 0.01^{cA}	42.423**
F-value		60.479**	21.276**	7.030**	2.944^{ns}	

**Significant at 0.01 level. * Significant at 0.05 level. ns non-significant

Means bearing different lower-case alphabets as superscript differ significantly within a row

Means bearing different upper-case alphabets as superscript differ significantly between CA and CS at each time of collection.

Haemoglobin Content

The haemoglobin contents of leg muscle samples from both CA and CS groups progressively decreased as post mortem time interval before sample collection increased. This might be due to biochemical changes such as protein denaturation, enzymatic breakdown and oxidative processes contributing to the reduction of haemoglobin in muscle tissue as reported by Sabikun et al. (2019).

Significantly lower mean haemoglobin concentration was observed in leg muscle samples from CA group. This was consistent with results of many researchers who compared haemoglobin content in birds slaughtered by different methods. Oellingrath et al. (1990) highlighted that the haemoglobin content of meat was influenced by how effectively the carcass was bled and the density of blood vessels within the muscles. Thus, higher haemoglobin levels in unbled or poorly bled carcasses was due to retention of blood in their tissues. Sasidharan et al. (2022) and Quawsar et al. (2024) had also reported that cold-slaughtered or unbled animals retained more blood in the muscles, leading to higher haemoglobin levels.

Conclusions

The results revealed a consistent decline in the pH of the samples as the time of sample collection increased post mortem. Contrastingly, higher pH value was seen in CS samples collected at 6-8 h post mortem. There was a consistent decline in the mean L* values and nonsignificant changes in a* and b* values of the samples of both CA and CS group as the time of sample collection increased post mortem.The WHC of leg muscles declined steadily from 0–2 h to 6–8 h post mortem while the TBARS values increased steadily from 0–2 h to 6–8 h post mortem in all groups with highest values in CS samples.Haem iron content, myoglobin content and haemoglobin content declined progressively post mortem, with significantly lower values at 6–8 h compared to 0–2 h. These findings suggest that physico-chemical and meat pigment attributes can potentially distinguish cold-slaughtered broiler chicken meat from meat of conventionally slaughtered broiler chicken in early time of post mortem. However, as the time after death elapses, the difference between CA and CS become non-significant in haem iron and haemoglobin contents.

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