J. Meat Sci. 2021, 16(1&2): 48-51

Research paper

Influence of Residual Blood on the Physico-chemical characteristics of Beef during Post-Mortem Refrigerated Storage

Vandana Sasidharan[a], V. N. Vasudevan, B. Sunil, T. Sathu, V. Ramnath, Arun Sankar K. J and Yazhinidevi R

Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, 680651 Kerala, India

ABSTRACT

Inefficient and improper bleeding causes more blood and subsequently more haemoglobin (Hb) to be retained in the muscles, which acts as powerful promoters of lipid oxidation and may decrease the shelf life of meat and meat products. A study was carried out at the Meat Technology Unit, Kerala Veterinary and Animal Sciences University, Mannuthy to examine the effect of bleeding efficiency on certain physico-chemical characters of muscle samples upon post-mortem refrigerated storage at 4±1 ˚C. Muscle samples were collected from imperfectly bled (IB), scientifically slaughtered (SS) and cold slaughtered (CS) carcasses and were packed in HDPE packages and the pH, lipid oxidation and total viable count of the muscle samples were evaluated during a six-day post-mortem refrigerated storage at 4±1 ˚C. The mean Hb concentration (mg/g) of IB and CS carcasses (0.07±0.003 and 0.09±0.008, respectively) were significantly ($p<0.01$) higher than that from SS carcasses (0.05±0.004). The pH values estimated for IB, SS and CS muscle samples on day 0 and day 6 were 6.35±012 and 5.66±0.035, 6.19±0.104 and 5.93±0.174 and 6.46±0.05 and 5.62±0.07, respectively. The thiobarbituric acid reacting substances value (TBARS, mg malonaldehyde/kg) increased significantly from 0.34±0.08 and 0.34±0.06 at day 0 to 1.02±0.18 and 0.75±0.05 for IB and CS muscles samples, respectively. The total viable count (TVC, log cfu/g) significantly increased from 5.29±0.07 and 5.24±0.02 at day 0 to 5.56±0.12 and 5.45±0.06, respectively for IB and CS muscle samples. No significant difference was found in the TVC and TBARS values for SS muscle samples. The results revealed that the level of bleeding had no significant influence on the muscle pH on storage at 4±1 ˚C, but had effect on the oxidative stability as well as the microbiological quality. The microbiological quality and oxidative stability of meat samples which underwent complete bleeding were superior to the other samples on all days of storage. Thus, it was concluded that bleeding levels do affect meat quality. Hence, it can be concluded that higher levels of residual blood can result in enhanced lipid oxidation and higher total viable count in meat upon storage at 4±1 ˚C. However, lipid oxidation status and total viable count of cold slaughtered meat were not significantly different for samples from IB carcasses.

Keywords: *Cold slaughter, Imperfect bleeding, Lipid oxidation, Shelf-life*

Received: 02/02/2022 Accepted: 22/04/2022

INTRODUCTION

Meat is a concentrated source of all essential nutrients and an important protein source for people across the world, particularly in developing countries. The sale of meat of dead animals commonly known as the cold slaughtered meat is an unethical practice frequently reported in the media raising concerns about the ethics, quality, and safety of such meat. Since the cold slaughtered meat is obtained from dead animals and not from healthy live animals, it does not undergo one of the vital steps of meat processing, which is sticking/bleeding/exsanguination. Alternatively, during the slaughter process, if an undue interval is allowed to elapse between stunning and bleeding, the carcass may be imperfectly bled which is of great significance from public health and religious perspectives.

Inefficient and improper bleeding during the slaughter process may cause more blood (haemoglobin) to be retained in the muscles which could cause increased oxidation resulting in rancidity and reduced shelf-life. Since blood is a perfect medium for the growth of microorganisms, if increased levels of residual blood are present or localised in a muscle, a corresponding susceptibility to microbial proliferation may exist (Gill and Newton, 1978). A limited number of studies are available comparing the quality attributes of meat from the dead and imperfectly bled beef carcasses. Therefore, the present study was conducted to determine the physico-chemical characteristics of beef samples harvested from imperfectly bled (IB), scientifically slaughtered ([1]SS) and cold slaughtered (CS) animals during refrigerated storage at 4±1 ˚C.

MATERIALS AND METHODS

Muscle sampling

Twelve female cross-bred cattle in the age group of 4-6 years from various farms under the Kerala Veterinary and Animal Sciences University were utilised in this study. Six animals were subjected to imperfect bleeding wherein bleeding was arrested by clamping either of the carotid arteries post-stunning using an artery forceps for 120 seconds to effect the arrest of bleeding followed by bilateral severance of carotid artery and jugular vein by throat incision. The carcasses were then subjected to the steps of routine slaughter procedures. The other group of six animals were slaughtered as per scientific procedures including mechanical stunning followed by bilateral severance of the carotid arteries for complete bleeding. The bleeding period continued for six minutes.

Muscle samples from six female cross-bred cattle in the age group of 4 to 6 years that have died due to natural causes and presented at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy for post-mortem examination were used for the collection of cold slaughtered meat. All the samples were collected within two hours of death.

Muscle samples representing four wholesale cuts viz. chuck, rib, loin, and round were immediately harvested from each carcass by hot deboning. Subcutaneous fat, fascia, and blood vessels were removed manually.

* Corresponding author E-mail : vandana.sasidharan474@gmail.com

DOI : 10.5958/2581-6616.2021.00010.4

Physico-chemical attributes of imperfectly bled, scientifically slaughtered, and cold slaughtered muscle samples during storage at 4±1˚C

The muscle samples for the storage study were packed in high-density polyethylene (HDPE) pouches and stored in a domestic refrigerator at 4±1˚C and the characteristics were evaluated on days 0, 2, 4, and 6. Fresh muscle samples immediately after collection were evaluated for pH, thiobarbituric acid value (TBA) value, and total viable count (TVC). The concentration of haemoglobin (Hb) was evaluated using a modified kinetic technique by Goyal and Basak (2009). The pH of the samples was evaluated using a digital pH meter (μ pH system- Systronics, India) as described by O'Halloran *et al*. (1997). The TBA values were determined by the extraction method of Witte *et al*. (1970). The TVC was estimated by the pour plate method, as described by APHA (2015) using Standard Plate Count Agar (Hi-Media, Mumbai) and incubated at 37°C for 24 hours, and the count was expressed as $\log_{10}$ cfu/g.

Data analysis

The data recorded were analysed statistically as per Snedecor and Cochran (1994) by repeated ANOVA measures using SPSS Software Version 24.0.

RESULTS AND DISCUSSION

Haemoglobin (Hb) estimation

The mean Hb concentration (mg/g) of imperfectly bled (IB) and cold slaughtered (CS) carcasses (0.07±0.003 and 0.09±0.008, respectively) were significantly ($p<0.01$) higher than that from scientifically slaughtered (SS) carcasses (0.05±0.004). The meat Hb content depended on the extent of carcass bleeding and the vascular bed in the muscles (Oellingrath *et al*., 1990). Thus, carcasses subjected to no or minimal bleeding retained more blood in muscles which was reflected in the mean Hb content values.

The pH value for the fresh samples (day 0) from IB, SS, and CS animal groups were 6.35 ± 0.120, 6.19 ± 0.104, and 6.46 ± 0.059, respectively. For all samples from all the three animal groups, the pH decreased significantly ($p<0.05$) from day 0 to day 2 and then increased subsequently. The pH values on day 0 were found to be significantly ($p<0.01$) higher compared with other days in all the animal groups. Hou *et al*. (2014) also reported that the pH values of beef samples reduced significantly after 24 hours (6.45 at 45 min to 5.5 at 24 h post-mortem) of the post-mortem period with no significant changes during the next 20 days of ageing.

There was a non-significant and gradual increase in the pH values from the second to sixth day among all groups. McKenna *et al.* (2005) also reported a minute increase of 0.1 units in bovine muscles from zero to five days of storage under refrigeration. D'Agata *et al.* (2009) reported that the pH increased from day 2 to day 6. A progressive increase in pH of muscle foods during refrigerated storage due to proteolysis and subsequent generation of nitrogenous compounds like ammonia and amines has been documented by Aksu *et al*. (2005). Between the animal groups, there was no significant difference in the mean pH values at all stages of storage. Alvarado *et al*. (2007) also reported no significant differences in pH 24 hours post-mortem of poultry meat samples subjected to varying levels of stunning and bleeding. The delay in post-mortem glycolysis among different treatment groups during storage might have resulted in the stable pH as reported by these authors.

Thiobarbituric acid reacting substances (TBARS) value

On day 0, there was no significant difference between the animal groups in the TBARS values. The TBARS value of IB samples increased significantly ($p<0.05$) from 0.34 ± 0.08 on day 0 to 1.02 ± 0.18 on day 6 while that of CS samples from 0.34 ± 0.06 on day 0 to 0.75 ± 0.05 on day 6. The values on the final day of the storage study for all the samples were well below the threshold value for detecting rancidity which has been reported to be 0.6 to 2.0 for consumer sensory panels, and 0.5 to 1.0 for trained sensory panels (Greene and Cumuze, 1982). For SS samples there was no significant difference in the TBARS values between the storage periods. McKenna *et al.* (2005) also reported that the TBARS value of bovine muscles increased with increasing days of refrigerated storage for five days and was well below the arbitrary threshold level. Addeen *et al.* (2014) reported that the impairment of the porphyrin ring during storage caused a breakdown of haeme molecules and subsequent release of iron which stimulated lipid oxidation of muscle.

Between the animal groups, a significant difference in the TBARS values was observed only on day 4 and day 6 of storage. On day 4, the TBARS value of IB samples (0.92 ± 0.24) was significantly ($p<0.05$) higher than SS samples (0.36 ± 0.09). On day 6, the TBARS values of IB and CS samples were not significantly different while that of SS samples were significantly ($p<0.01$) lower than the other two groups. At all stages of storage, SS samples revealed the lowest TBARS values. Sohaib *et al*. (2020) also reported a higher TBARS value for dead chicken meat compared with halal slaughtered samples. They explained the finding by pointing out that the ferric haeme pigments present in improperly bled samples initiated lipid oxidation and acted as pro-oxidants in living tissues.

Total viable count (TVC)

On day 0 of storage, the mean TVC of IB, SS, and animal groups were 5.29 ± 0.07, 4.73 ± 0.15, and 5.24 ± 0.02, respectively. For IB muscle samples there was no significant difference in the TVC till day 4 of storage, but the TVC increased significantly ($p<0.01$) on day 6. For SS samples there was no significant difference in the TVC between the storage periods. For CS samples also there was no significant difference in the TVC till day 4 of storage. However, the TVC of CS samples on day 6 were significantly ($p<0.05$) higher than that on day 0 and 2. On day 6, the TVC of IB, SS, and CS muscle samples were 5.56 ± 0.1, 5.12 ± 0.03, and 5.45 ± 0.06, respectively. Spoilage occurred between seven and 14 days in beef under refrigerated storage (Ercolini *et al*. 2006).

When the mean TVC was compared between groups, the TVC of SS samples was significantly ($p<0.01$) lower than that of CS and IB samples on all days of the storage study. The TVC values were numerically higher for IB samples compared to CS samples on the first and final days of the storage study. Gracey (1986) opined that the meat of dead animals in the absence of rigor mortis and due to the incomplete bleeding will be perishable, unlike the meat derived from the live slaughtered animals. Gill and Newton (1978) reported that blood was a perfect medium for the growth of microorganisms and if increased levels of residual blood were present or localised in a muscle, a corresponding susceptibility to microbial contamination existed.

Table 1: Physico-chemical attributes of imperfectly bled (IB), scientifically slaughtered (SS), and cold slaughtered (CS) muscle samples (Mean±S.E.)

Quality attribute	Storage days (days)			
	0	2	4	6
		pH		
IB	6.35 ± 0.120^{a}	5.53 ± 0.025^{b}	5.65 ± 0.044^{b}	5.66 ± 0.035^{b}
SS	6.19 ± 0.104^{a}	5.74 ± 0.174^{b}	5.79 ± 0.17^{b}	5.93 ± 0.174^{b}
CS	6.46 ± 0.059^{a}	5.53 ± 0.087^{b}	5.61 ± 0.091^{b}	5.62 ± 0.079^{b}
		TBARS		
IB	0.34 ± 0.08^{b}	0.58 ± 0.23^{b}	0.92 ± 0.24^{aA}	1.02 ± 0.18^{Aa}
SS	0.21 ± 0.02	0.24 ± 0.01	0.36 ± 0.09^{B}	0.34 ± 0.03^{B}
CS	0.34 ± 0.06^{b}	0.36 ± 0.03^{b}	0.52 ± 0.03^{bAB}	0.75 ± 0.05^{aA}
		TVC		
IB	5.29 ± 0.07^{Ba}	5.17 ± 0.04^{bA}	5.33 ± 0.09^{bA}	5.56 ± 0.12^{aA}
SS	4.73 ± 0.15^{B}	4.84 ± 0.13^{B}	5.06 ± 0.02^{B}	5.12 ± 0.03^{B}
CS	5.24 ± 0.02^{bA}	5.3 ± 0.04^{bA}	5.38 ± 0.06^{abA}	5.45 ± 0.06^{aA}

Means having a different lower case and upper case differ significantly

CONCLUSIONS

Based on the findings of the study it was concluded bleeding time during the slaughter has a significant effect on the quality of meat. The better the bleeding the better the quality of meat. The quality of meat from imperfectly bled and cold slaughtered animals showed significantly higher values of TBA and TVC when compared to scientifically slaughtered animals which proved that retention of blood has a deteriorative effect on the quality of meat. Hence, processors and retailers involved in the meat trade should resort to the scientific processing of meat animals thereby ensuring the efficient bleeding of animals.

ACKNOWLEDGMENTS

The authors acknowledge the technical support from the Meat Technology Unit (MTU), Mannuthy for the conduct of the research and the laboratory facilities at the Central Instruments Lab, Kerala Veterinary and Animal Sciences University.

COMPETING INTEREST

The authors have no known competing interests either financial or personal between themselves

ETHICS STATEMENT

The protocol and procedures employed were ethically reviewed and approved.

REFERENCES

Addeen A, Benjakul, Wattanachant S S, Maqsood S (2014) Effect of Islamic slaughtering on chemical compositions and post-mortem quality changes of broiler chicken meat. Int Food Res J 21 : 897- 905.

Aksu MI, Kaya M, Ockerman HW (2005) Effect of modified atmosphere packaging and temperature on the shelf-life of sliced pastirma produced from frozen/thawed meat. J Muscle Foods 16 : 192-206.

Ali SA, Abdalla HO, Mahgoub IM, Medani W (2011) Effect of slaughtering method on the keeping quality of broiler chickens' meat. Egypt Poult Sci 31 : 727-736.

Alvarado CZ, Richards M.P, O'Keefe SF, Wang H (2007) The effect of blood removal on oxidation and shelf life of broiler breast meat. Poult Sci 86 : 156-161.

Ercolini D, Russo F, Torrieri E, Masi, P, Villani F (2006) Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl Environ Microbiol 72 : 4663-4671.

Gill CO, Newton KG (1977) The development of aerobic spoilage flora on meat stored at chill temperatures. J Appl Bact 43 : 189-195.

Collins DS, Huey RJ (2014) Gracey's meat hygiene. John Wiley & Sons, United Kingdom.

Goyal MM, Basak A (2009) Estimation of plasma haemoglobin by a modified kinetic method using O-tolidine. Indian J Clinic Biochem 24 : 36-41.

Greene BE, Cumuze TH (1982) Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavour in cooked beef. J Food Sci 47 : 52-54.

Hou X, Liang R, Mao Y, Zhang Y, Niu L, Wang R, Liu C, Liu Y, Luo X (2014) Effect of suspension method and aging time on meat quality of Chinese fattened cattle M. Longissimus dorsi. Meat Sci 96 : 640-645.

Johns AM, Birkinshaw L, Ledward DA (1989) Catalysts of lipid oxidation in meat products. Meat Sci 25 : 209-220.

Lawrie RA (1979) Developments in meat science (Vol. 1), London.

McKenna DR, Mies PD, Baird BE, Pfeiffer KD, Ellebracht JW, Savell JW (2005) Biochemical and physical factors affecting discolouration characteristics of 19 bovine muscles. Meat Sci 70 : 665-682.

Morton RD (2001) Aerobic plate count. In: Downes, F.P. and Ito, K. (Ed.). Compendium of methods for the microbiological examination of foods, 4th edn, APHA, Washington, D.C.

Oellingrath IM, Iversen A, Skrede G (1990) Quantitative determination of myoglobin and haemoglobin in beef by high-performance liquid chromatography. Meat Sci 28 : 313-320.

O'Halloran GR, Troy DJ, Buckly DS (1997) The relationship between early post-mortem pH and tenderisation of beef muscles. Meat Sci 45 : 239-251.

Sabow AB, Sazili AQ, Aghwan ZA, Zulkifli I, Goh YM, Ab Kadir, MZA, Nakyinsige K, Kaka U, Adeyemi KD (2016) Changes of microbial spoilage, lipid-protein oxidation and physico-chemical properties during post mortem refrigerated storage of goat meat. Anim Sci J 87 : 816-826.

Snedecor GW, Cochran WG (1994) Statistical Methods, Iowa, USA.

Sohaib M, Zafar MS, Arshad MS, Nauman K, Malhi IY (2020) Evaluation of quality and safety attributes of slaughtered versus dead chicken birds Meat. Brazilian J Poult Sci 22 : 35-44.

Thiansilakul Y, Benjakul S, Park S, Richards MP (2012) Characteristics of myoglobin and haemoglobin-mediated lipid oxidation in washed mince from bighead carp (Hypophthalmichthys nobilis). Food Chem 132 : 892-900.

Witte VC, Krause JF, Bailey ME (1970) A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J Food Sci 35 : 582-585.

Zarasvand SA, Kadivar M, Aminlari M, Shekarforoush SS (2012) A comparative study of physico-chemical and functional properties, and ultrastructure of ostrich meat and beef during aging. Cyta J Food 10 : 201-209.