Full Length Research Paper

Post-mortem tenderization of spent layer's breast meat with calcium chloride

Okubanjo A. O., Omojola A. B*, Olusola, O. O and Oladepo, O. O

Meat Science Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Accepted 23 December, 2010

The effect of calcium chloride (CaCl₂) injection at different time post-mortem (PM) on breast meat of spent layer was investigated. Each breast was dissected into two halves with each half weighing between 151.75 and 168.22 g. A total of 36 half breast were randomly assigned to four treatment groups in a completely randomised design. The breast meat were injected with 0.1 M CaCl₂ (10% W/W) at 1, 12 and 24 h PM representing Treatments 2, 3 and 4 respectively while treatment one represents non injection at 0 h PM. The injected and non injected samples were aged at 2° C for 24 h. Tenderness was measured with Warner Bratzler shear force machine, drip and cooking losses were determined, proximate composition and eating qualities were also assessed. Injection of samples with CaCl₂ inperved tenderness by 11.85 to 39.12%. Cooking loss was not affected (P>0.05) by CaCl₂ injection while drip loss increased (P<0.05) as time PM injection with CaCl₂ increased. Crude protein, moisture and ether extracts were not affected by CaCl₂ injection while the ash content of treated samples increased with time PM. The injection of CaCl₂ improved the panellist rating (P<0.05) for juiciness, tenderness, ease of fragmentation and overall acceptability however, the flavour score decreased with CaCl₂ injection.

Key words: Breast, injection, spent layer, calcium chloride, post-mortem.

INTRODUCTION

The use of spent layer has been limited in scope and it has been a problem to the food industry because of its unacceptable toughness. The toughness associated with spent layer's meat is primarily due to the increased crosslinking in the connective tissue of older animals (Bailey and Light, 1989). Presently, there is no commercially feasible way to increase tenderness by decreasing collagen cross-linking in meat spent layer. Consequently, it is essential to improve and develop different postmortem processes to increase the tenderness of spent layer's meat that are traditionally tough and are usually punished with low price. Meat tenderness is considered as the most important palatability attribute of meat (Cross et al., 1986) and it is the most critical eating quality which determines whether consumers are repeat buyers (Koohmaraie et al., 1989).

Several post-mortem methods of improving meat

tenderness have been studied. Among the various methods are; injection of lactic acid (Berge et al., 2001) and temperature conditioning (Rees et al., 2002). The involvement of ionic strength as tenderizer is another possibility (Wu and Smith, 1987). The use of calcium chloride has also gained prominence in reducing toughness in beef and lamb carcasses (Geesink, 1993; Wheeler et al., 1991; Morgan et al., 1991).

The tenderizing effect of calcium chloride has been attributed to the activation of calpain (the calcium ion dependent protease involved in ageing of meat (Koohmaraie, 1994) and also to the increase in the intracellular ionic strength including protein solubilization (Takahashi, 1992). Wu and Smith (1987) also suggested that both ionic strength and enzymatic proteolysis synergistically contributed to post-mortem myofibrillar protein solubilization.

It was however reported that some of the organoleptic properties such as colour and flavour can be altered by the use of calcium chloride and that such alteration is concentration dependent (Wheeler et al., 1993; Landsdell et al., 1995; Perez et al., 1998) while meat marinating

^{*}Correspondence author. E-mail: omojolababs@yahoo.com.

with calcium chloride longer than 24 h was found to result in bitter flavour, undesirable texture and colour changes (Gonzalez et al., 2001). It was therefore the objective of this study to evaluate the effect of calcium chloride injection at different time post-mortem on tenderness, physico-chemical and eating qualities of spent layer's breast meat.

MATERIALS AND METHODS

Sample preparation

A total of 18 Isa-Brown spent layers (2 years of age) were obtained from a local commercial layer farm. Prior to slaughtering, the birds were starved of feed for 12 h but were allowed access to fresh cool water. After slaughtering under commercial conditions, the birds were properly bled and scalded in hot water (85 °C), they were defeathered and eviscerated. The breast were immediately removed from each carcass and dissected into two halves (n = 36). Each half breast was randomly allotted to the four treatment groups of no injection at 0 h post-mortem (Treatment 1), calcium chloride injection at 1, 12 and 24 h post-mortem representing Treatments 2, 3 and 4 respectively. Each treatment was replicated 9 times in a completely randomized design.

Injection of calcium chloride

Each breast was weighed and injected with 0.1 M CaCl₂ solution at 10% (W/W) using a hand held single needle syringe. After injection and packaging in laminate bags, each sample was weighed and aged at 2° for 24 h.

Measurements

Measurements were made after 24 h of ageing.

Cooking loss

Control and CaCl₂ treated breast muscles were cut into steaks (25.18 \pm 1.14 g). Two steaks from each treatment were weighed and cooked on a Teval super barbeque electric broiler (Teval, France) to an internal temperature of 70 °C. Each sample was turned over after reaching 40 °C. Internal temperatures were monitored with a Fisher alarm thermometer (Model No 15-077-8B, Fisher Scientific, Pittsburgh, P.A). After cooking, the meat samples were allowed to equilibrate to room temperature (25 °C), weighed and percent cooking loss calculated according to the formula.

Cooking loss = <u>Weight of sample before cooking - weight after cooking</u> X 100 Weight of sample before cooking

Shear force determination

The objective evaluation of tenderness was performed using the modified Warner Bratzler shear force procedure (Bouton and Haris, 1978). Meat samples were wrapped in aluminum foil and cooked to an internal temperature of 75 ℃ as measured using Fluke type K temperature probe attached to Fluke 52 m. Three cores of 1.0 cm² were removed from each sample using an electrical coring machine. Each core was shared at three locations parallel to the orientation of muscle fibre, using a Warner Bratzler shear force instrument.

Drip loss

This was measured by the method of Barton-Gade et al. (1993) with some modifications. Each breast was weighed immediately after ageing, hung in a laminate bag, closed loosely with string and allowed to thaw. After thawing for 24 h at 4° C, the meat samples were taken out, mopped and re-weighed and the drip loss calculated.

Taste panel evaluation



A total of 20 trained individuals, aged between 25 and 35 years (60.0% male and 40.0% female) were used to determine whether consumers could detect improvement in meat tenderness or whether the injection of $CaCl_2$ will affect other organoleptic properties of the meat or not. The panellists were made to rate each of the four replicates of the cooked breast meat on a nine-point hedonic scale for tenderness, ease of fragmentation, juiciness, flavour and overall-acceptability. Equal bite size (12 g) from each treatment replicate was coded in a way to prevent bias by the panellists. The samples were served in odourless plastic plates. Each sample was evaluated independent of the other.

Chemical composition

Moisture, crude protein, ash and ether extracts were determined on broiled breast muscle according to the methods described by AOAC (1990).

Statistical analysis

All data obtained were subjected to analysis of variance and where statistical significance were observed, the means were compared using the Duncan's multiple range test (Duncan, 1955). The SAS computer soft ware package was used for all statistical analysis (SAS, 1999).

RESULTS AND DISCUSSION

The result of the processing procedure (Tables 1 - 4) indicated an increase in weight of samples injected with $CaCl_2$. The increase in weight was commensurate with the weight of $CaCl_2$ pumped into the green samples. There was a drop in the weight upon ageing, the loss in weight was more (P<0.05) in samples injected with $CaCl_2$ however, samples injected with $CaCl_2$ at 12 h postmortem had a mean value that was statistically different from samples injected at 1 h and 24 h post-mortem respectively.

Shear force

The result of the present study showed that the noninjected samples required significantly more shearing force (P<0.05) than the injected samples. Samples injected with $CaCl_2$ at 1 h post-mortem produced the most tender meat probably because meat cooked at the pre-rigor phase is the most tender (Cia and Marsh, 1976).

	Treatment				
Parameters	Non-injected	Injected			
	0 h	1 h	12 h	24 h	
Initial wt (g)	151.75 ± 1.15	152.92 ± 0.54	151.49 ± 2.01	152.06 ± 0.15	
Final wt (g)	151.75 ± 1.15	168.22 ± 0.60	166.67 ± 2.19	167.27 ± 0.60	
Final injected wt (%)	100.00	110.00 ± 0.01	110.02 ± 0.04	110.00 ± <mark>0</mark> .06	
Weight of CaCl ₂ (g)	0.00	15.80 ± 0.16	15.18±0.21	15.21 ± 0.30	
Weight of CaCl ₂ (%)	0.00	10.00	10.00	10.00	
Aged wt (g)	151.01 ± 0.96 ^b	167.24 ± 0.40 ^a	161.44 ± 0.48 ^a	165.95 ± 0.53 ^a	
Aged wt (%)	$99.53 \pm 0.09^{\circ}$	109.36 ± 0.32 ^a	106.57 ± 0.52 ^b	109.13 ± 0.91 ^a	

Table 1. Yield of spent layers breast muscle injected with calcium chloride at different time post -mortem.

Means with different superscripts within the same row are significantly different from each other (P < 0.05).

Table 2. Physical properties of spent layers breast muscle injected with calcium chloride at different time post-mortem.

		٦	Freatments	
Parameters	Non-injected		Injecte	d
	0 h	1 h	12 h	24 h
Shear force (kg/cm ³)	5.65 ± 0.51 ^a	3.44 ± 0.54°	4.69 ± 0.91 ^b	4.98 ± 0.10 ^b
Change in shear force(%)	0.00	39.12	16.99	11.85
Cooking loss (%)	25.18 ± 1.50	22.93 ± 2.69	24.41 ± 3.70	25.62 ± 2.09
Drip loss (%)	1.47 ± 0.49 ^d	3.0 <mark>2±0.49°</mark>	4.01 ± 0.78^{b}	7.55 ± 0.49 ^a

Means with similar superscripts along the same row are not significantly different (P>0.05).

Table 3. Proximate composition of injected an	d non-injected breast muscle	e of spent layers after broiling (g/100g).
---	------------------------------	--

_		Treatme	ents	
Parameters	Non-injected	Injected		
	0 h	1 h	12h	24h
Ether extract	4.7 <mark>5 ± 0.3</mark> 2	3.98 ± 0.57	3.92 ± 0.52	4.05 ± 0.23
Ash	1.87 ± 0.04 [°]	2.37 ± 0.04 ^b	2.48 ± 0.02^{ab}	2.79 ± 0.10 ^a
Moisture	62.84 ± 1.24	65.48 ± 1.45	64.89 ± 1.55	63.30 ± 1.20
Crude protein	30.98±0.01	28.15±4.65	29.65±0.75	30,84±2.66

Means with similar superscripts along the same row are not significantly different (p>0.05).

Table 4. Organoleptic characteristics of injected and non-injected breast muscle of spent layer.

	Treatments			
Parameters	Non-injected	Non-injected Injected		
	0 h	1 h	12 h	24 h
Flavour	6.75 ± 0.64 ^a	5.71 ± 1.70 ^b	4.08 ± 1.55 ^{cd}	3.42 ± 0.51 ^d
Juiciness	6.38 ± 0.61^{ab}	7.64 ± 0.54 ^a	6.02 ± 0.80^{b}	$4.06 \pm 0.38^{\circ}$
Tenderness	3.56 ± 0.48^{d}	7.35 ± 0.42^{a}	6.33 ± 0.56 ^b	$4.92 \pm 0.84^{\circ}$
Ease of fragmentation	3.55 ± 0.69 ^d	7.21 ± 0.46 ^a	6.05 ± 1.53 ^b	4.62 ± 0.98 ^c
Overall acceptability	$4.36 \pm 0.73^{\circ}$	7.01 ± 0.29 ^a	6.85 ± 0.45 ^a	5.43 ± 1.02 ^b

Means in the same row with similar superscripts are not significantly different (P>0.05).

However, there was no significant difference (P>0.05) in the shear force values of samples injected with CaCl₂ at 12 and 24 h. The percentage increase in tenderness ranged between 11.85 and 39.12% over the non-injected samples. Injection of CaCl₂ irrespective of the time postmortem improved tenderness of breast meat, this finding was in agreement with the report of Koohmaraie and Shackelford (1991) and Wheeler et al., (1992) on different species of livestock.

The effect of $CaCl_2$ on meat tenderness was probably due to its effect on activation of calpains (Koohmaraie et al., 1987) or through the alteration of protein to protein interaction as a result of the elevation of ionic strength (Wu and Smith, 1987).

Drip loss

The rate and quantity of drip formation in fresh meat is influenced by the extent of rigor shrinkage and permeability of the cell membrane to water as well as other factors such as the extent of protein denaturation. It is generally accepted that the source of drip loss is intracellular water, which is lost from the muscle postmortem, driven by a pH and calcium induced shrinkage of myofibrils during rigor development (Offer et al., 1989). The result obtained in this study gave values of 1.47 ± 0.99% for non-injected at 0 h post-mortem and 3.02 ± 0.49, 4.01 ± 0.78 and 7.55 ± 0.49% for injected breast muscles at 1, 12, and 24 h post mortem respectively. It was observed that as time post-mortem increased, the percent drip loss increased (P<0.05) in the injected samples. The result obtained in this study differed from that of Geesink et al. (1994) that reported higher drip loss in pre-rigor than in post rigor CaCl₂ injected meat samples.

The difference in the two results could probably be due to the injection method employed. In the present study, single needle injection was used and this could have led to the differences in penetration and diffusion rate of CaCl₂ solution through the sample.

Cooking loss

The result of the present study showed that the percent cooking losses were similar (P>0.05) irrespective of the injection time or whether samples were injected or not. This was in agreement with the result obtained by Morgan et al. (1991) who used beef samples.

Proximate composition

The crude protein, ether extract and moisture content of the injected samples irrespective of the injection time and the non-injected were not significantly (P>0.05) different

however, the ash content increased as time post-mortem increased. The lowest ash content was obtained from the non-injected sample most probably because the CaCl₂ injected into the breast muscle increased the mineral profile of such samples.

Organoleptic characteristics

The highest flavour perception (P<0.05) was recorded in the non-injected samples. The flavour rating decreased as the time post-mortem increased however, there was no significant difference (P>0.05) in samples injected with CaCl₂ at 12 and 24 h post mortem. The fact that typical meat flavour decreased with increase in abnormal flavour as time post mortem increased could be responsible for the trend observed in the result of this work. The result obtained in this study was in agreement with that of Eilers et al., (1994) who reported off flavour in the CaCl₂ treated samples. The findings of Wheeler et al. (1993), Landsdell et al. (1995) and Perez et al. (1998) showed that some organoleptic properties of meat such as colour and flavour can be altered by the use of calcium chloride and that such alteration is concentration dependent while meat marinating with calcium chloride longer than 24 h was found to result in bitter flavour, undesirable texture and colour changes (Gonzalez et al., 2001).

Juiciness is made up of two effects namely; the impression of moisture released during chewing and also the salivation produced by flavour factor (Omojola et al., 2003). The juiciness rating decreased as the time postmortem increased in the injected samples. The panellists rated meat samples at 1 h post-mortem higher than those of 12 and 24 h while there was no noticeable difference (P>0.05) between the non-injected and those injected at 1 h post-mortem. Ease of fragmentation explains how easily the muscle fibre separates upon chewing. The highest value was obtained in 1 h post-mortem injected samples followed by samples injected at 12 and 24 h respectively while the list value was obtained from the non-injected samples most probably because the injection of CaCl₂ significantly accelerates myofibril fragmentation (Koohmaraie et al., 1998). Chou et al. (1994) also reported similar changes in chicken muscles.

Consumers consider meat tenderness as the most important palatability trait of meat quality (Cross et al., 1986) and it has been identified as the most critical eating characteristics which determine whether consumers are repeat buyers. The tenderness score followed a similar trend as observed in the ease of fragmentation with the highest value from samples injected with CaCl₂ 1 h postmortem and the least score from the non-injected samples. The result of the present study showed that injection of breast muscle of spent layers with CaCl₂ improves tenderness rating while the rating decreased as time post-mortem injection increased.

The result obtained for overall acceptability showed

that CaCl₂ injection improved quality of the product. However, as the injection time was delayed the mean rating for overall acceptability gradually decreased.

Conclusion

Injection of $CaCl_2$ 1 h post-mortem produced the highest tenderisation score and in a similar manner the taste panellist rated samples injected at 1 h post-mortem highest for tenderness, ease of fragmentation, juiciness and overall acceptability. $CaCl_2$ injection however decreased the flavour perception of spent layer's breast meat most probably because of the concentration used.

REFERENCES

- AOAC (1990). Official Methods of Analysis (12thed.) Association of Official Analytical Chemists, Washington, D.C.
- Bailey AJ, Light NDD (1989). The connective tissue of meat and meat products. Elsevier Applied Science, London.
- Barton-Gade PA, Demeyer D, Honikel KO, Joseph RL, Poulanne E, Severini M Smuldders FJM, Tomberg E (1993). Reference method for water holding capacity in meat and meat products. Procedures recommended by an OECD working group.39th International Congress of Meat Science and Technology. August 1-6 (1993), Calgary, Alberta. Canada.
- Berge P, Per E, Lone ML, Therry A, Xavier V, Anders JM (2001). Tenderization of beef by lactic acid injected at different times post mortem. Meat Sci., 57: 347-357.
- Bouton PE, Harris PV (1978). Factors affecting tensile and Warner-Bratzler shear values of raw and cooked meat. J. Texture Stud., 9: 395-413.
- Chou RGR, Tseng TF, Lin KJ, Yang JH (1994). Postmortem changes in myofibrillar proteins of breast and leg meat from broilers, spent hens and Taiwanese country chickens. J. Food Sci. Agric., 65: 297-302.
- Cia G, Marsh BB (1976). Properties of beef cooked before rigor onset. J. Food Sci., 41: 1259-1262.
- Cross HR, Savell JW, Francis JJ (1986). National consumer retail beef study.Proc. 38th Annu. Reciprocal Meat Conf., 39: 112-114.
- Duncan PB (1955). New Multiple Range and Multiple F-tests. Biometrics, 11: 1-42.
- Eilers JD, Morgan JB, Martin AM, Miller RK, Hale JD, Acuff GR, Savell JW (1994). Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. Meat Sci., 38: 443-451.
- Geesink GH (1993). Post mortem muscle proteolysis and beef tenderness with special reference to the action of the calpain calpastatin system. Ph. D thesis, Netherlands.
- Geesink GH, Smulders FJ, van Laack RLJM (1994). The effect of Calcium, Sodium and Zinc Chlorides treatment on quality of beef. Food Sci., 14: 485-502.
- Gonzalez CB, Valeria AS, Fernado JC, Adriana AP, Jorge A. L (2001). Effect of Calcium chloride marination on bovine *Cutaneus trunci* muscle. Meat Sci., 57: 251-256.

- Koohmaraie M, Seldeman SC, Schollmeyer JE, Dutson TR, Crouse JD. (1987). Effect of post-mortem storage on Ca⁺⁺ dependent protease, their inhibitor and myofibril fragmentation. Meat Sci., 9: 187-196.
- Koohmaraie, M, Crouse JD, Mersman HJ (1989). Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride. Effect of concentration and ionic strength. J. Anim. Sci., 67: 934-942.
- Koohmaraie M (1994). Muscle proteinases and meat ageing. Meat Sci., 38: 93-104.
- Koohmaraie M, Wheeler TL, Shackelford SD (1998). Beef tenderness regulation and prediction. In Proceedings; International Livestock Congress February 1998, Houston, Texas, pp. 25-27.
- Koohmaraie M, Shackelford SD (1991). Effect of calcium chloride infusion on the tenderness of lambs fed a β- adrenergic agonist. J. Anim. Sci., 69: 2463-2471.
- Lansdell JL, Miller MF, Wheeler TL, Koohmaraie M, Ramsey CB (1995). Post mortem injection of calcium chloride effect on beef quality traits. J. Anim. Sci., 73: 1735-1740.
- Morgan JB, Savell JW, Hale DS, Miller RK, Griffin DB, Cross HR, Shackelford SD (1991). National Beef Tenderness Survey. J. Anim. Sci., 69: 3274–3283.
- Offer GP, Knight R, Jeacocke R, Almond T, Cousins J, Elsey N, Parsons A, Sharp RS, Purslow (1989). The structural basis of the water-holding, appearance and toughness of meat and meat products. Food Microstruct., 8: 151-170.
- Omojola AB, Isah OA, Adewunmi MK, Ogunsola OO, Attah S (2003). Evaluation of the effect of various additives on the acceptability of Kilishi. Trop. J. Anim.Sci., 6(2): 97-101.
- Perez ML, Escalona H, Guerro I (1998). Effect of calcium chloride marination on Calpain and quality characteristics of meat from chicken, horse, cattle and rabbit. Meat Sci., 48: 125-134.
- Rees MP, Graham R, Robyn DW (2002). Effect of calcium infusion on tenderness and ageing rate of pork *M. Longissimus thoracis et lumborum* after accelerated boning. Meat Sci., 61: 169-179.
- SAS (1999). Statistical analysis system institutes. User's guide SAS institute Inc. Cary, NC.
- Samenjina K, Wolfe FH (1976). Degradation of myofibrillar protein components during postmortem ageing of chicken meat. J. Food Sci., 41: 250-254.
- Takahashi K (1992). Non–enzymatic weakening of meat; Calcium ion at 0.1 M and their effects on meat tenderness. Biochem., 74: 247-250.
- Wheeler TL, Crouse JD, Koohmaraie M (1992). The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. J. Anim. Sci., 70: 3451-3457.
- Wheeler TL, Koohmaraie M, Lansdell JL, Siragusa GR, Miller MF (1993). The effects of post mortem injection time, injection level and concentration of calcium chloride on beef quality trait. J. Anim. Sci., 71: 2965 –2974.
- Wheeler TL, Koohmaraie M, Crouse JD (1991). Effect of calcium chloride and hot boning on the tenderness of beef round muscle. J. Anim. Sci., 69: 4671-4678.
- Wu FY, Smith SB (1987). Ionic strength and myofibrillar protein solubilization J. Anim. Sci., 65: 597-608.