

## **Effects of Calcium Chloride (CaCl<sub>2</sub>) Injection at Different Times post-mortem on Meat of Spent Layers**

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### **Abstract**

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The effect of post-mortem calcium chloride injection at different time was investigated on thigh meat of eighteen spent layers. The thirty-six thighs weighing between 81.16 and 86.21 g were randomly assigned to four treatments. The thigh muscles were injected with 0.1 M calcium chloride (10 % w/w) at 0, 1, 12 and 24 h post-mortem representing treatments 1, 2, 3 and 4 respectively. The samples were subsequently weighed and aged at -2 °C for 3 days.

Tenderness was evaluated by Warner Bratzler shear force (WBSF) machine and sensory panel evaluation. Drip loss and cooking losses were also investigated. WBSF values were not significantly different between samples injected at 12 and 24 h post-mortem while the least ( $P < 0.05$ ) WBSF values were obtained in samples injected 1 h post-mortem. Injection of samples with CaCl<sub>2</sub> solution improved tenderness as the non-injected samples had the highest WBSF value. CaCl<sub>2</sub> injection significantly ( $P < 0.05$ ) increased drip loss and as time postmortem increased the percent drip loss also increased. Cooking loss was not affected ( $P > 0.05$ ) by CaCl<sub>2</sub> injection.

The injection of CaCl<sub>2</sub> at 1 h post mortem improved significantly the organoleptic traits of the meat after ageing for 3 days while 24 h post mortem injection gave the least values for flavour, juiciness and ease of fragmentation. In terms of the overall acceptability, the panelist preferred samples injected at 1 and 12 h post mortem.

*Key words:* Calcium chloride, spent layers, post-mortem, injection and organoleptic traits

### **Introduction**

Consumers consider meat tenderness as the most important palatability trait of meat quality (Cross et al., 1986), while the other primary determinants of the eating

quality of meat are the flavour and juiciness (Breidenstein and Carpenter, 1983). Tenderness has also been identified as the most critical eating quality characteristic, which determines whether consumers are repeat buyers. Koohmaraie et al. (1998)

and Dransfield (1997) reported that consumers prefer to pay a premium for high quality product. Consequently, it is essential to improve and develop different post-mortem processes to increase meat tenderness particularly in those muscles that are traditionally tough and are usually punished with lower prices.

Several post-mortem methods of improving meat tenderness have been studied. Among the various methods are, temperature conditioning (Rees et al., 2002), injection of lactic acid (Berge et al., 2001). The use of calcium chloride has also gained prominence in reducing toughness in beef and lamb carcasses (Geesink, 1993, Morgan et al., 1991, and Wheeler et al., 1991). The tenderizing effect of calcium chloride has been attributed to the activation of calpains - the calcium ion dependent protease involved in the ageing of meat (Koochmaraie, 1994 and Geesink et al., 1994) and also to the increase in the intracellular ionic strength inducing protein solubilization (Takahashi, 1992). It is however reported that some of the properties such as colour and flavour can be altered by the use of calcium chloride and the alteration is concentration dependent (Lansdell et al., 1995 and Wheeler et al., 1993). In a similar way, Perez et al. (1998) found that the use of high concentration of calcium salts resulted into product with altered taste and bitter flavour while Gonzalez et al. (2001) found that marination with calcium chloride longer than 24 h resulted in bitter flavour, undesirable texture and colour changes.

Considering that the lack of tenderness is a common complaint against the meat of spent layers (chicken), the objective of this work was therefore to determine the effect of calcium chloride injection at different time post-mortem on tenderness,

drip loss, cooking loss, and other eating qualities of spent layer's thigh muscle.

## Materials and Methods

A total of eighteen spent layers which were at the end of lay (72 weeks) were slaughtered after starvation of feed for 16 hours but with adequate supply of water. Severing their jugular veins and carotid arteries with a sharp knife they were killed. Hanging on a railing for about 10 minutes the slaughtered birds were properly bled. Scalding, defeathering and evisceration were then carried out.

### *Sampling procedure*

After slaughtering under commercial conditions, the thigh was immediately removed from each carcass giving 36 thighs. The 36 thighs were randomly allotted into 4 treatment groups of no injection (control), calcium chloride injection at 1 h post-mortem (treatment 2), calcium chloride injection at 12 h post-mortem (treatment 3) and calcium chloride injection at 24 h post-mortem (treatment 4), giving a total of nine replications per treatment, in a completely randomized design.

### *Injection of calcium chloride*

From the time the thighs were removed until the time of injection, meat samples were held at 4 °C in the refrigerator. Each thigh was weighed and injected with 0.1 M calcium chloride 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 °C for 3 days.

### *Physical measurements*

Drip loss: This was measured by the method of Barton-Gade et al. (1993) with

some modifications. Each thigh was weighed immediately after ageing, hung in a laminate bag, closed tightly with string and allowed to thaw. After thawing for 24 hours, the meat samples were taken out, mopped and re-weighed and the drip loss calculated.

**Cooking loss:** The meat samples were broiled in a gas oven to an internal temperature of 75 °C. Each cooked sample was cooled to room temperature, blotted dry and weighed.

$$\text{Cooking loss} = \frac{\text{weight before cooking} - \text{weight after cooking} \times 100}{\text{Weight before cooking}}$$

**Shear force determination:** The objective evaluation of tenderness was performed using the modified Warner Bratzler shear force procedure (Bouton and Haris, 1978) by using the Instron Universal Testing Machine. Meat samples were wrapped in aluminum foil and cooked to an internal temperature of 75 °C as measured using Fluke type K temperature probe attached to Fluke 52 meters. About 0.5 cm<sup>2</sup> thick meats was cored from each cooked thigh parallel to the muscle fibre after the broiled meat has been allowed to cool to room temperature. Three cores (0.5 cm in diameter) were removed using an electrical coring machine. Each core was sheared at three locations parallel to the orientation of muscle fibre.

**Taste panel evaluation:** To determine if consumers could detect the improvement in meat tenderness or whether the injection of calcium chloride will affect other organoleptic properties of the meat, a panel session was carried out. A total of 16 trained individuals aged between 25 and 40 years (62.5 % male and 37.5 % fe-

male) were used to assess the cooked meat samples. The panelists were made to rate each of the 4 replicates of the meat samples. Equal bite size from each treatment were coded and served in an odorless plastic plate. Each sample was evaluated independent of the other. The panelists rated the samples on a 9-point hedonic scale for tenderness, ease of fragmentation, apparent adhesion, flavour juiciness and overall acceptability.

**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 software package (1999) was used for all statistical analysis.

## Results and Discussion

The result in Table 1 shows the mean weight, percentages and standard deviations of the experimental thigh meat samples at each processing step. The result shows an increase in the percent weight of samples from the initial weight in samples injected with calcium chloride since they were pumped with the solution to 110 % of the green weight.

There was a slight drop in percentage weight of injected samples after 3 days of ageing probably as a result of drip loss. However, these weights were heavier than the respective green weights except for the non-injected (control) samples. The non-injected samples lost 0.81 ± 0.41 percent of its initial green weight while the gain in weight after ageing for 3 days at -2 °C for the injected samples were 9.20 ± 0.31, 9.34 ± 0.37 and 6.61 ± 0.13 % for samples injected with CaCl<sub>2</sub> at 1 h, 12 h and 24 h post mortem respectively. Injec-

**Table 1**  
**Processing yield of spent layers' thigh muscle injected with calcium chloride at different time post mortem (mean  $\pm$  SD)**

Parameters	Treatments			
	Non-injected	Injected		
		0h	1h	12h
Initial weight, g	81.16 $\pm$ 2.10	86.21 $\pm$ 0.59	84.19 $\pm$ 0.43	82.48 $\pm$ 0.89
Initial weight, %	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Final injected, g	81.16 $\pm$ 2.10	94.83 $\pm$ 0.68	92.61 $\pm$ 4.70	90.76 $\pm$ 0.54
Final injected weight, %	100.00 $\pm$ 0.00 <sup>b</sup>	110.00 $\pm$ 0.07 <sup>a</sup>	110.00 $\pm$ 0.01 <sup>a</sup>	110.04 $\pm$ 0.46 <sup>a</sup>
Weight of CaCl <sub>2</sub> injected	0	8.62 $\pm$ 0.29	8.42 $\pm$ 0.36	8.25 $\pm$ 0.72
Aged weight, g	80.50 $\pm$ 0.98	94.14 $\pm$ 0.40	92.07 $\pm$ 0.44	87.93 $\pm$ 0.43
Aged weight, %	99.19 $\pm$ 0.41 <sup>b</sup>	109.20 $\pm$ 0.55 <sup>a</sup>	109.74 $\pm$ 0.34 <sup>a</sup>	106.89 $\pm$ 0.48 <sup>a</sup>
Loss/gain in wt. on ageing	* 0.66 $\pm$ 0.11 <sup>c</sup>	7.93 $\pm$ 0.52 <sup>a</sup>	7.88 $\pm$ 0.44 <sup>a</sup>	5.45 $\pm$ 0.61 <sup>b</sup>
Loss/gain in wt. on aging, %	* 0.81 $\pm$ 0.41 <sup>c</sup>	9.20 $\pm$ 0.31 <sup>a</sup>	9.34 $\pm$ 0.37 <sup>a</sup>	6.61 $\pm$ 0.43 <sup>b</sup>

\*Losses in grams and percentages.

Values denoted with different superscripts within the same row are significantly different ( $P < 0.05$ ).

tion of CaCl<sub>2</sub> solution at 12 h post mortem gave the highest increase in weight however, the difference in weight gain between the 12 h and 1 h post mortem injection were not significantly ( $P > 0.05$ ) different from each other.

**Drip loss:** It is generally accepted that the source of drip loss is intracellular water which, is lost from the muscle fibre post-mortem, driven by a pH and calcium induced shrinkage of myofibril during rigor development (Honikel et al., 1986 and Offer et al., 1989). The rate and quantity of drip formation in fresh meat is believed to be influenced by the extent of rigor shrinkage and the permeability of the cell membrane to water as well as other factors, such as the extent of protein denaturation (Table 2). The result of drip losses obtained in this present study gave values

of  $3.44 \pm 2.01$ ,  $5.28 \pm 1.60$ ,  $5.97 \pm 1.61$  and  $8.72 \pm 1.38$  percent for non-injected and injected meat at 1 h, 12 h and 24 h post mortem respectively. As time post mortem increased, the percent drip loss increased significantly ( $P < 0.05$ ) in the injected samples except for the values recorded in the 1 h and 12 h post mortem injection which were not different ( $P > 0.05$ ) from each other. The result of this study did not agree with that of Geesink et al. (1994) who reported that drip loss was much higher with pre-rigor rather than post rigor CaCl<sub>2</sub> injection as a result of drastic contraction and myofibrillar disorganization which were responsible for enormous drip loss. The difference in the two results could have emanated from the injection method. In this present study, single needle injection was used and this could

**Table 2**  
**Percent drip and cooking losses and shear force values of spent layers thigh muscle injected with calcium chloride at different time post mortem (mean ± SD)**

Parameters	Treatments			
	Non injected	Injected		
	0h	1h	12h	24h
Drip loss, %	3.44 ± 2.01 <sup>c</sup>	5.28 ± 1.60 <sup>b</sup>	5.97 ± 1.61 <sup>b</sup>	8.72 ± 1.38 <sup>a</sup>
Cooking loss, %	24.62 ± 2.79	21.47 ± 2.41	23.86 ± 2.99	26.19 ± 1.88
Shear force, kg/cm <sup>3</sup>	8.65 ± 0.51 <sup>a</sup>	6.44 ± 0.56 <sup>c</sup>	7.59 ± 0.91 <sup>b</sup>	7.89 ± 0.10 <sup>b</sup>

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

have led to difference in penetration and much less diffusion of CaCl<sub>2</sub> solution throughout the sample.

**Cooking loss:** Percentage cooking loss of the non-injected meat samples were not significantly different ( $P > 0.05$ ) from the injected samples irrespective of time of injection. The result obtained in this study agreed with the work of Morgan et al. (1991), who used beef samples. However, there was an increase in cooking loss as time post mortem increased. In a similar way, Wheeler et al. (1992) reported higher cooking loss values in beef injected with CaCl<sub>2</sub> 24 h post mortem compared with 0 h post mortem.

**Share force:** The result of this study demonstrated that calcium chloride injection at the pre rigor phase (1 h post - mortem) produced the highest tenderness level most probably because meat cooked in the pre-rigor phase is the most tender (Cia and Marsh, 1976). The data in Table 2 shows that the control (non-injected) samples required significantly ( $P < 0.05$ ) more shearing force (8.65 ± 0.51) than the other samples. However, the differences in the force required in samples injected at 12 h and 24 h post mortem were not

significantly different ( $P > 0.05$ ) from each other. The injection of calcium chloride irrespective of the time of injection greatly improved tenderness of the thigh meat. This was in agreement with earlier work of Koohmaraie et al. (1990) Koohmaraie and Shackelford (1991) and Wheeler et al. (1992) on different species of live-stock.

Three different mechanisms have been proposed as the means by which Calcium chloride improves tenderness, but its effect is probably due to a combination of this mechanism. Calcium chloride is thought to improve tenderness via increasing the activation of the calpains (Koohmaraie et al., 1988 and Koohmaraie et al., 1989), via causing extreme contraction of muscle fibbers resulting in disruption of the myofibrillar network (Morgan et al. (1991) or via altering the protein to protein interaction due to the elevated ionic strength (Wu and Smith 1987).

The change in ionic strength may also influence the stability of the lysosomal membranes, the release of the cathepsins and contribute to the improved tenderness seen in ion infused muscle. In a similar way, Takahashi (1992) linked the tender-

**Table 3**  
**Organoleptic characteristics of non-injected and injected thigh muscle of spent layer**

Parameters	Treatments			
	Non injected	Injected		
	0h	1h	12h	24h
Flavour	6.08 ± 0.75 <sup>a</sup>	6.25 ± 0.58 <sup>a</sup>	4.03 ± 0.46 <sup>b</sup>	3.75 ± 0.56 <sup>c</sup>
Juiciness	5.44 ± 1.35 <sup>a</sup>	6.50 ± 0.61 <sup>a</sup>	4.92 ± 1.19 <sup>b</sup>	3.38 ± 0.40 <sup>c</sup>
Tenderness	3.38 ± 0.48 <sup>c</sup>	6.50 ± 0.20 <sup>a</sup>	6.00 ± 0.85 <sup>a</sup>	4.88 ± 0.84 <sup>b</sup>
Ease of fragmentation	3.00 ± 0.74 <sup>c</sup>	6.13 ± 0.76 <sup>a</sup>	5.00 ± 0.17 <sup>b</sup>	3.88 ± 0.31 <sup>c</sup>
Overall acceptability	3.88 ± 0.54 <sup>c</sup>	6.50 ± 0.70 <sup>a</sup>	5.50 ± 0.65 <sup>a</sup>	4.61 ± 0.45 <sup>b</sup>

izing effect of CaCl<sub>2</sub> to the increase in the intracellular ionic strength inducing protein solubilization.

**Taste panel evaluation:** The results of the taste panel evaluation (Table 3) indicated that injection of calcium chloride at 0.1M (10 % w/w) concentration influence sensory score for flavour, juiciness, tenderness, ease of fragmentation and overall acceptability.

**Flavour:** For flavour, the intensity on meat treated with calcium chloride post rigor was lower significantly, ( $P > 0.05$ ) compared with those injected pre-rigor and non-injected muscles. The highest flavour perception of  $6.25 \pm 0.58$  was recorded in muscles injected with calcium 1 h post mortem while a value of  $6.08 \pm 0.75$  was obtained in the non-injected meat samples, these two results were however not significantly different ( $P > 0.05$ ). Typical meat flavour decreased with time post mortem while abnormal flavour increased. Flavour of meats is derived from the juice, which contains the important flavour components and plays an important role in the overall impression of palatability by consumers. The result of this study showed that there were significant differences ( $P < 0.05$ ) in flavour score between non-injected meat

and those of 12 h and 24 h post mortem injected meat samples. Wheeler et al. (1993) found that beef treated with calcium chloride were more tender than the control (non-injected) samples, but they appeared saltier, more bitter and with less flavour than the control samples. Eilers et al. (1994) also found some off-flavours in the calcium chloride treated samples but at concentrations greater than 0.2 m (5% w/w).

**Juiciness** is made up of two effects viz the impression of moisture released during chewing and also the salivation produced by flavour factor (Omojola et al., 2003).

The highest juiciness perception was given by the panelists to meat treated 1h post mortem with calcium chloride, followed by the non-injected samples. However, the two results were not significantly different ( $P > 0.05$ ) from each other. The value of  $5.44 \pm 1.35$ ,  $6.50 \pm 0.61$ ,  $4.92 \pm 1.19$  and  $3.38 \pm 0.40$  were obtained on a nine point hedonic scale for non-injected and injection of CaCl<sub>2</sub> at 1 h, 12 h and 24 h post mortem respectively. The juiciness rating decreased as time post mortem increased. The value obtained for the Calcium chloride injected thigh meat had a

direct relationship with the cooking loss (Table 2). The low value recorded for 24 h injected meat could be due to the high cooking loss compared to other treatments. The result of this work attest to the direct relationship between juiciness and tenderness: the more juicy the meat appears (Table 3), the more tender (Table 2) the meat is and the more quickly the juice is released upon chewing.

**Tenderness:** The result of the taste panel rating on a nine point hedonic scale for tenderness were  $3.38 \pm 0.48$ ,  $6.50 \pm 0.20$ ,  $6.00 \pm 0.85$  and  $4.88 \pm 0.84$  for non-injected samples and those injected at 1 h, 12 h and 24 h post mortem respectively. There were no significant differences ( $P > 0.05$ ) between 1 h and 12 h injected samples but there were significant differences ( $P < 0.05$ ) between non-injected and 24 h injected meat samples. The 1 h injected meat samples that were rated highest in tenderness by the taste panelist was also found the tenders by WBSF determination. The result obtained by the taste panel evaluation shows that the higher the drip loss in the injected samples the less tender the meat.

**Ease of fragmentation:** This explains how easily the fibers separate as chewing continued. The highest ( $P < 0.05$ ) ease of fragmentation was recorded in 1 h post mortem injected meat samples with a value of  $6.13 \pm 0.76$ . The ease of fragmentation decreased as the time of injection increased. The values of  $3.88 \pm 0.31$  and  $3.00 \pm 0.74$  were obtained for meat injected 24 h post mortem and non-injected samples while a value of  $5.00 \pm 0.17$ . The result above was consistent with the report of Koohmaraie et al. (1998) that Calcium chloride incubation significantly accelerates myofibril fragmentation of bovine meat. Samenjina and Wolfe (1976)

and Chou et al. (1994) also reported similar changes in chicken muscles. Previous reports (Fritz and Greaser, 1991 and Chou et al., 1994) have shown that titin and nebulin are quite susceptible to post mortem degradation.

**Overall acceptability:** The result obtained for the overall acceptability of meat from spent layers treated with calcium chloride indicated that the treatment increased quality of the product. However, as the injection time was delayed, the means for the samples gradually reduced (Table 3). The value of  $3.88 \pm 0.54$  obtained for the non-injected sample was significantly the least followed by  $4.61 \pm 0.45$  and  $5.50 \pm 0.65$  obtained for 24 h and 12 h injected samples respectively. The value of  $6.50 \pm 0.76$  obtained from samples injected 1 h post mortem were similar statistically to the value obtained from samples injected 12 h post-mortem.

## Conclusion

The result of this study shows that calcium chloride injection of spent layer is thigh meat was effective in increasing tenderness of the meat without adversely affecting the cooking loss. Although the greatest degree of tenderization was obtained in 1 h post mortem injected samples, injection at 12 and 24 h post mortem were also beneficial in lowering the shear force values. Also, increasing the ultimate yield is an added benefit because profit is affected by retail product yield.

The taste panel result showed that the 1 h injection of Calcium chloride improves all the organoleptic properties measured while the delay in injection till 24 h post mortem adversely affects the flavour, juiciness and the ease of fragmentation scores.

The parameters were evaluated on a

nine point hedonic scale with 9=most preferred and 1= least preferred.

Means in the same row with common superscripts are not significantly different ( $P>0.05$ ) from each other.

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