Effect of Calcium Chloride Injection and *postmortem* Aging on Bovine *Biceps femoris* Muscle Tenderness

Gonzalez, Claudia; Salitto, Valeria; Carduza, Fernando & Lasta, Jorge.
Instituto Tecnología de Alimentos, Centro Agroindustria, CNIA, Instituto Nacional de Tecnología Agropecuaria (INTA), Casilla de Correo 77, (1708), Morón, Buenos Aires, Argentina.

Background

Meat tenderness is the most demanded palatability trait by consumers (Cross *et* al., 1986) and its variability is an area of major interest to the meat industry (Koohmaraie *et* al., 1998; Smith *et al.*, 1992; Morgan *et al.*, 1991). Studies carried out by Savell *et* al. (1992) reported a direct relationship between muscle tenderness and market price. Even more, Dransfield (1997) and Koohmaraie *et al.* (1998) concluded that consumers preferred to pay premium for high quality products. Consequently, it becomes clear that the application of *postmortem* processes to increase meat tenderness could be of benefit to the meat industry and consumers. Some of the potential solutions to this problem are the use of refrigerated storage in order to induce myofibrilar proteolysis or the incorporation of calcium chloride solution to enhance and accelerate the tenderization process. However, in our country, the procedure of aging meat during 7 days is highly expensive, consequently the present study was designed to reduce the storage time required to achieve a desired level of tenderness in the *Biceps femoris* muscle by using a combination of calcium chloride treatment and aging procedure.

Materials and methods

Twenty steers (36-42 months of age) were slaughtered following regular commercial procedures with Good Manufacture Practices. Muscles were removed after 20-22h postmortem, right and left sides were assigned randomly to the calcium treatment {10% (w/w) incorporation of 0.25 M calcium chloride solution (Gonzalez *et al.* 2000)} or kept as non-treated (without calcium but submitted to aging) samples. Assigned muscles, treated and non-treated, were then cut in 7 pieces, vacuum packaged and aged at 1 ± 1°C for 0, 1, 2, 3, 4, 5 and 7 days respectively. After each aging period the samples were submitted to the myofibrillar fragmentation measurement (MFI) as indicator of tenderness level (Parrish, Jr. *et al.* 1979; Olson *et al.* 1976).

Considering the results of tenderness scores a consumer panel evaluation was attained in order to prove if the combined treatment applied produced the same level of tenderness than the aging procedure alone, and if the consumers would be able to detect the increased tenderness.

Calcium-ion concentration.

Added calcium-ion concentration was determined in the tissue upon completion of the digestion process by atomic absorption spectrophotometry (Nakamura, 1973). Total protein concentration was determined by the macro Kjeldahl method (AOAC 1965).

Myofibril Fragmentation Index (MFI)

Muscular myofibrils were extracted following the procedure described by Olson *et* al., (1976) and protein concentration was determined by the Biuret method of Gornall *et al.* (1949).

Consumer panel evaluation

Two panel sessions were accomplished; each of them composed of 110 consumers of different ages, genders and professions, and paired comparison assays (IRAM Norm 20002, 1985) were utilized. The first panel (C1) was designed to compare control samples (without any treatment) to treated samples (calcium chloride injection and aging for 2 days) in order to know if the *postmortem* treatment produced a higher and evident tenderness level in the *Biceps femoris* muscle. The second comparison (C2) included the mentioned treated-samples and non-treated samples (aged during 7 days) and was designed to establish if the new combined treatment produced the same tenderness level than the traditional procedure of aging during 7 days.

All samples were cut in 3x3x0.8 cm cubes, breaded, fried at 180°C and served in special containers to keep them warm.

Statistical Analysis

MFI data was analyzed for significant differences by analysis of variance using the GLM procedure of SAS (1996). When significant ($p<0.05$) main effects were observed without interactions, mean separation was accomplished by the use of Tukey's means comparison test. Consumer panel data analysis was performed by the use of contingence tables (Roessler *et al.* 1956).

Results and discussion

Fig. 1 shows MFI values for non-treated samples (aged 0 to 7 days) and for treated samples (calcium injection + aging 0-7 days). MFI values of non-treated samples showed an increase on the second day of aging ($p<0.05$), this score was upheld during the rest of the aging period except on day 7 when a new increment was observed ($p<0.05$). Treated samples showed a different pattern, MFI values increased on day 2 ($p<0.05$) of aging and after this point no further increase was observed. Nevertheless MFI values of treated samples were higher than MFI values of non-treated samples during the complete aging period.

The percentage of MFI increment of each day compared to day 0 demonstrated that tenderness of non-treated samples achieved their maximum score on day 7 (38.2%). Contrasting, calcium chloride treatment achieved the highest % of increment of MFI score on day 2 of aging (45.1%).

Even though most of the authors utilized sensory and objective evaluations to scores tenderness (Wheeler *et al.*, 1997; Benito-Delgado *et al.*, 1994), MFI can be considered as a potential tool for the identification of tough and tender muscles since it was demonstrated that MFI values were close correlated with sensory and objective tenderness scores (Parrish *et al.* 1979; Culler *et al.* 1978). Consequently the MFI values obtained in this research made apparent the reduction of postmortem refrigeration storage time from the traditional 7 days to 2 days due to the enhancing influence of the calcium salt on this muscle tenderness.

The mode of action of calcium chloride-induced tenderization is nowadays a matter of discussion. The well-known theory which points out that the tenderization process occurs through proteolysis from activation of calpain proteinases (Koohmarie 1996, 1988) has a counterpart on "the calcium theory", which postulates that a calcium-ion concentration of 0.1 mM is responsible for the weakening of myofibrils structures and desmin-intermediate filaments (Takahashi, 1999). Bearing in mind any of both explanations, it is of great importance to know the percentage of incorporation, and the tissue distribution and concentration of the added calcium salt to merit the participation of this ion in the tenderization process.

Calcium determination demonstrated a homogeneous salt distribution through the muscle by the injection procedure utilized (data not shown). Besides, Table 1 shows the calcium salt incorporation and the average of calcium distribution through the *Biceps femoris* muscle. It can be seen that the percentage of incorporation was the desired one (11.7% in treated samples) and that this incorporation resulted in a calcium-ion concentration of approximately 4.25mg/ g protein, sufficient concentration to decrease meat toughness by any of the mentioned mechanisms.

Taking into account that calcium was present in the tissue in appropriate concentration, and that MFI values accounted for the tenderness enhancement of calcium treated samples, it was of great concern to establish if the consumers would be able to notice the tenderness increment due to the combined technique applied and if this technique produced the same level of tenderness than the procedure of aging meat for 7 days. For this purpose a two-consumer panel evaluation was completed. Fig. 2 shows the results obtained with comparison C1, it can be seen that panel members were able to detect tenderness increase, since they could differentiate between treated and control samples with a significance level of 5%. Seventy one percent of the panelists chose calcium-treated samples due to higher tenderness. The second comparison (C2) established that tenderness of samples calcium-treated and aged for 2 days was not significantly different from tenderness of samples aged for 7days (data not shown).

Conclusions

The combined technique of calcium injection and aging could be used in a tough muscle such us *Biceps femoris* muscle to improve tenderness (higher FMI values) in a shorter storage period under refrigeration . This combined technique has the advantage of the reduction of the aging period, which will result in a price benefit for the meat industry and for the consumers. Inasmuch us this treatment will produce meat which would be not only accepted but also preferred by the public due to the improvement in certain palatability traits. Besides calcium addition could be considered as an additional source of calcium fortification in special diets.

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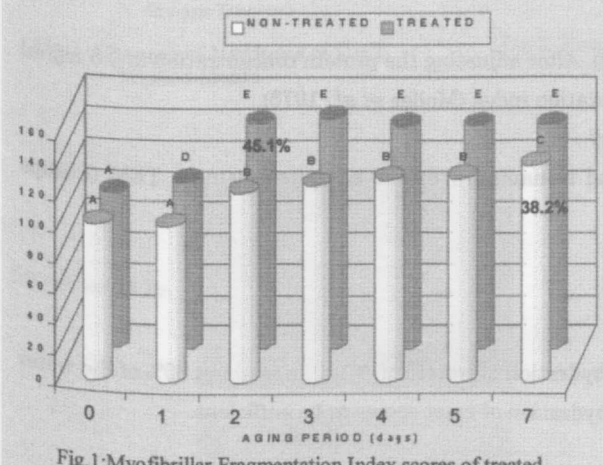

Fig.1:Myofibrillar Fragmentation Index scores of treated (0.25 mM calcium chloride injection+aging) and non-treated (aging) samples.

Table 1: Calcium salt incorporation (%), protein (g%) and calcium-ion (mg/g protein) concentration in control (non-treated), water-treated and calcium-treated samples of *Biceps femoris* muscle.

Biceps femoris	Incorporation (%)	Protein (g/100g tissue)	Ca (mg/g protein)
Control	-	*22.2*	*0.27*
Water-treated	*10.7*	*20.8*	*0.06*
Calcium-treated	*11.7*	*20.7*	*4.25*

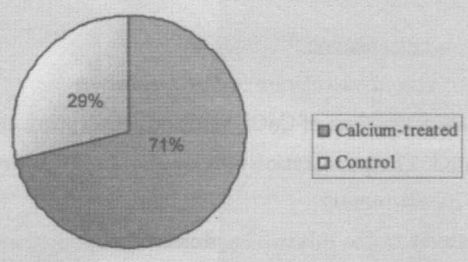

Fig. 2: Consumer Panel Evaluation. Comparison C1, calcium-treated (0.25 mM calcium chloride injection+aging for 2 days) *vs*. control (no treatment) samples