CALCIUM CHLORIDE CONCENTRATION AND MARINATION TIME ON CALPAIN ACTIVATION AND THE EFFECT ON RABBIT MEAT TENDERNESS.

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Background

Meat maduration is attributed to the endogenous enzymes action, with two main enzymatic systems: calpain and cathepsin (Jaarseveld et al., 1997). These two systems are responsible of the myofibrillar breakdown, resulting in the meat tenderisation (Masayuki et al., 1987). Nonetheless, catepsins are acid enzymes and their effect is more retarded than the calpains activity. Calpains are proteases with two subunits (80 and 30 kDa) (Koohmaraie, 1992). There are two kinds of calpains: calpain I requires 50-70 mM of calcium to be activated, and calpain II requires only 1-5 mM of calcium for their activation (Thompson et al., 1996). Calpains activation can be achieved by two ways: injection of 0.3 M $CaCl_2$ solution or by marination immersion in a 150 mM $CaCl_2$ solution 48 h at 4°C (Uytterhaegen y col., 1994). Studies in regulatory proteins degradation had demonstrated that calpains act on troponine T, troponine I, tropomyosin, α-actinine, titine and nebuline (Zeece et al., 1992). But there are no effect of calpains on myosin and actin (Whipple y Koohmaraie, 1991, Greaser y Fritz, 1995). Meat texture is determined by myofibrillar properties and intramuscular connective tissue (Nishimura et al., 1998). Is one of the main meat characteristic and can be determined by chemical methods, force evaluation during compression, sensory test and microscopically (Taylor y Goll, 1995).

Objectives

Determine the marination time and calcium chloride concentration to activate calpains in rabbit meat.

Methods

Rabbit meat sample were obtained from the animal house at the University. After slaughter and evisceration, samples of leg muscles were taken from the right side of carcasses. Half of the muscle was treated with 75, 150 and 250 mM $CaCl_2$ during 24 and 48 h at 4°C, and the other half, stored at 4°C, was used a control. All the experiments were done by triplicate.
Enzymatic activity was determined by the technique reported by Etherington et al. (1987), using pepstatine and leupeptine as inhibitors, expressing the results as micrograms of solubilisated casein per minute per gram of muscle. Meat hardness was determined in an Instron Universal Testing Machine, with a 10 N load cell. The samples (1 cm^3) were compressed 50% and the force required reported.

Results and discussion

Meat tenderness increase gradually with postmortem storage. This process could be accelerated with calcium chloride marination reaching a compete tenderization after rigor mortis ends (Morgan et al., 1991). The results shown that the maximum enzymatic activity was found at 24h and a 250 mM $CaCl_2$ concentration (Table 1), but with no difference in meat texture during the experimental period (Table 2). This results are not in agree with the reported by Wheeler et al. (1992), where calcium treatment provoked a reduction in beef hardness. However, Morgan et al. (1991) reported that in *Semimebranous* muscle the calpain tenderization is not so marked due to the connective tissue, being this one of the main factors that affects meat tenderness, besides to collagen tyoe (Seideman y Koohmaraie, 1987). Rabbit meat had a great collagen amount, so the calpain activity could be minimized due to this reason. In other hand, Shackelford et al. (1995) found no differences in bovine treated with calcium chloride using compression test. Another techniques (v.g., Warner-Bratzler device) could be more effective to detect changes in meat tenderization.

Conclusions

Maximum calpain activity in rabbit meat can be achieved with 250 mM of $CaCl_2$ marination during 24h, but with no effect on hardness reduction due to collagen content. The use of rabbit meat in restructured products could be an alternative to reduce the hardness of this specie.

Pertinent literature

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Table 1. Enzimatic activity for marinated rabbit meat*

$CaCl_2$	24h	48h
75 mM	447.428	437.714
150 mM	531.142	480.571
250 mM	569.428	508.571

*Casein micrograms solubilisated/ min/g of muscle.

Table 2. Displacement and maximum load required to compress rabbit meat samples

$CaCl_2$	Displacement (mm)	Maximum load (N)
Reference	4.971	0.866
75 mM	1.665	0.736
150 mM	1.700	0.577
250 mM	1.680	0.511