FREE CALCIUM ION CONCENTRATION FOLLOWING CALCIUM ADDITION TO MEAT

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Background

Calcium ions play a critical role in many cellular reactions. The content of calcium in skeletal muscle is similar in pork, beef and poultry and is about 1.5 mmoles Ca/kg fresh muscle (Zarkadas *et al.*, 1987) but most of the calcium is 'bound'. The 'free' calcium content in muscle rises during rigor development from about 0.2 µmoles Ca/kg to between 100 and 130 µmolesCa/kg (Dransfield, 1992; Jeacocke, 1993) at full rigor. Free calcium ions are implicated in meat tenderness mainly by stimulating proteolysis by activating calpains (Dransfield, 1992) but may also have a direct tenderising action on the myofibrillar proteins (Takahashi, 1999). The level of free calcium in post-rigor meat is therefore sufficient to activate µ-calpain but not calpain II which requires about 500 µM Ca for half maximal activity *in vitro*. Additional tenderness can therefore be obtained by activating calpain II by the addition of calcium chloride to meat. However for maximum tenderisation, calcium ions needs to be added at a final concentration of between 20 and 40 mM (Alarcon-Rojo and Dransfield, 1989) which is well above the level needed to fully activate calpain II. The reason why such a high concentration is required has not been studied.

In practice, the addition of calcium chloride solutions (to a final concentration of between 10 and 40 mM) to pre-rigor or post-rigor muscle usually increases tenderness but pre-rigor additions can give toughening. Injection of calcium chloride (30 mM final concentration) into pre-rigor muscle can also cause a bitter taste (Morgan *et al.*, 1991) and bitterness tends to increase with increasing chilled storage time of calcium-injected meat (Morgan *et al.*, 1991; Rousset-Akrim *et al.*, 1996). The origin of the bitterness is unknown.

Objectives

The aim of this work was to determine the available (free) calcium ions following addition of calcium chloride to beef and to estimate if their concentration could activate calpain II and be responsible for the bitter taste sometimes observed in calcium-injected beef.

Methods

M. Sternomandibularis muscles were removed at 1 hour (pre-rigor) or at 24 hours (post-rigor) post-mortem, minced and solutions of calcium chloride (up to 20 ml/kg of 2 M $CaCl_2$) added to give final concentrations up to 50 mmoles added calcium chloride/kg of muscle. The samples were stored at 4°C and the samples, together with the drip, centrifuged at 90 000g for 25 min. and the free calcium ion concentration in the supernatant determined by ion-specific electrode (Orion, ion plus) used according to the manufacturer's recommendations. For determinations in cooked meat, the meat was heated at 70°C for 25 min. and the cooking loss was recorded. The cooking loss fluid was discarded and the remaining cooked meat was centrifuged and the free calcium content of the supernatant measured as done for the raw samples. Measurements of free calcium ion concentrations were done on duplicate samples of meat.

Results and Discussion

The concentration of calcium by selective ion probe in the supernatant of post-rigor beef was 128 ± 60 µM (Table 1). This is in agreement with estimates (Dransfield, 1992) and measurements using specific chromophores (Jeacocke, 1992) and indicates that only calpain I would become active during the development of rigor mortis in muscle. Early studies (Parrish *et al.*, 1981) indicated that the level of calcium in a TCA supernatant from beef may rise to 1000 µM during chilled storage for 10 days which could be expected to be sufficient to activate calpain II. However, it is likely that this over-estimates the free calcium ion concentration and the amount of free calcium ions determined in this work did not change during chilled storage for up to 15 days (Table 1). This suggests that there would not be sufficient free calcium ions, even in aged meat, to activate calpain II which is consistent with the stable content of calpain II during storage (Dransfield, 1992). The total concentration of magnesium ions (8 to 10 mmoles/kg fresh muscle) was similar to findings in other meats (Feidt and Brun-Bellut, 1999) and did not increase during chilled storage for up to 2 weeks (results not reported). Similar to our findings on free calcium ions, the content of free Mg (4 mmole/kg) and K (70 mmole/kg) ions in goat muscle was found to be stable from 1 to 2 days chilled storage (Feidt and Brun-Bellut, 1999). Proteolysis during storage would therefore not appear to change the calcium ion binding.

The amount of free calcium ions was directly proportional to the amount of added calcium ions (Figure 1). Following post-rigor addition, about 25% of the added calcium was free in raw muscle and about 20% in cooked muscle (Figure 1a). Free calcium ion concentration following calcium addition to pre-rigor muscle was 18% in raw muscle and 12% in cooked muscle (Figure 1b). So, in both raw and cooked muscles, additions of calcium chloride to pre-rigor muscle (Figure 1b) tended to give lower free calcium concentrations than did calcium additions to post-rigor muscle (Figure 1a). This is inconsistent with the observation that pre-rigor injections of calcium chloride into pre-rigor meat tends to give more bitter flavour that when injected in to post rigor meat (Rousset-Akrim *et al.*, 1996).

Morgan *et al.* (1991) used mature cow carcasses and injected pre-rigor muscles to a calcium concentration of 30 mM which gave more tender meat than the non-injected cold-boned meat. The Ca-injected meat had higher flavour scores described as metallic, bitter and livery which increased with chilled storage. They suggested further studies should be done on the flavour of Ca-injected meats. Injections equivalent to 30 mmoles Ca/kg produced metallic off-flavours (Morgan *et al.*, 1991) however, at 10 mmoles Ca/kg there was no off-flavour detected (Wheeler *et al.*, 1993; Hoover *et al.*, 1996). In a later study, Rousset-Akrim *et al.* (1996) used 10 mM $CaCl_2$ injected at 1 hour and 24 hours post-slaughter. In pre-rigor injected muscle, they found no tenderising effect, an increased bitterness and saltiness which increased further during chilled storage. When injected post-rigor, the meat was more tender and, again bitterness scores tended to increase during chilled storage. Wheeler *et al.* (1993) and Eilers *et al.* (1994) also observed sour, bitter and livery flavours in injected meat, especially when the concentration of $CaCl_2$ was more than 10 mM.

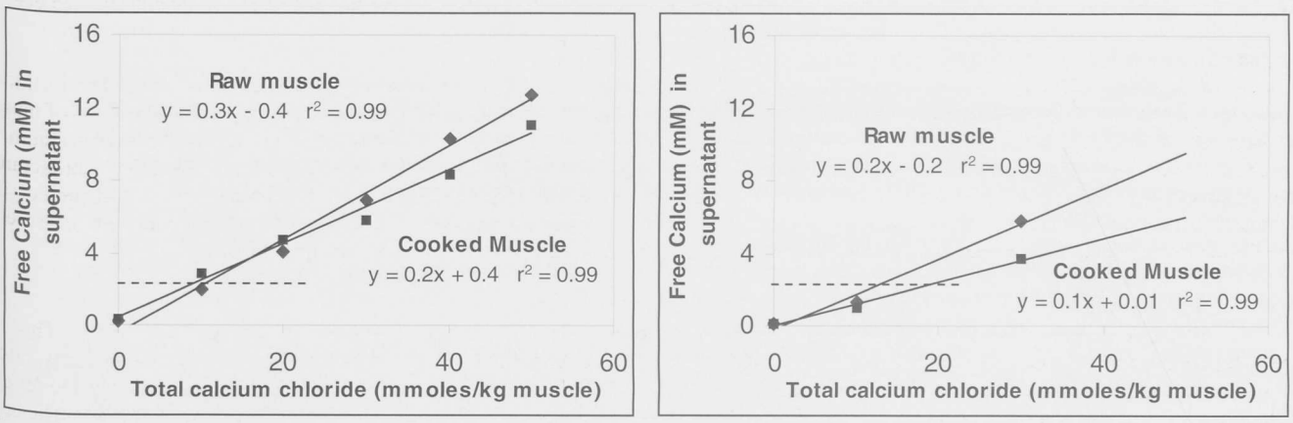
Calcium chloride in water has a bitter or salty taste with a threshold between 2 and 30 mM and a median threshold of 10 mM (Amerine *et al.*, 1965). It seems likely that, in meats, the taste of salts is related to the concentration of free ions. With calcium addition, 2 mM free calcium ion concentration is achieved with post-rigor addition of $CaCl_2$ to 10 mM in both raw and cooked muscle (Figure 1a). More calcium chloride (to 15 and 20 mM respectively) would need to be added to pre-rigor muscle to give a 2 mM free calcium ion concentration (Figure 1b). It seems likely then that a bitter taste would be detected by some consumers at additions as low as 10 mM.

Calcium injection is usually carried with concentrations from 10 to 30 mM $CaCl_2$, concentrations sufficiently high to give the bitter taste observed in some trials. However, the low free calcium ion concentrations following addition to pre-rigor muscle cannot explain the apparent bitterness observed following pre-rigor injections. Also, as no measured changes were found in free calcium ion concentrations in

the supernatants from raw or cooked meat during storage of raw meat at 4°C for up to 2 weeks, no explanation can be offered for the observed increase in bitterness during storage of calcium injected muscle.

Table 1. Free calcium (mM; means and standard deviations) following calcium additions and chilled storage

		Added $CaCl_2$ (mmoles/kg)	Chilled storage (days post-slaughter)			
			1	3	8	15
RAW MUSCLE	Pre-rigor injection	0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
		10	1.1 ± 0.0	1.3 ± 0.1	1.3 ± 0.0	1.5 ± 0.0
		30	6.2 ± 0.2	5.8 ± 0.5	6.3 ± 0.4	4.9 ± 0.0
	Post-rigor injection	0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
		10	1.8 ± 0.6	1.9 ± 0.5	2.0 ± 0.6	2.0 ± 0.6
		30	6.6 ± 1.1	7.0 ± 1.5	7.0 ± 1.6	6.8 ± 1.9
COOKED MUSCLE	Pre-rigor injection	0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
		10	1.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
		30	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	2.9 ± 0.1
	Post-rigor injection	0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
		10	2.8 ± 0.0	2.8 ± 0.0	2.9 ± 0.0	3.0 ± 0.2
		30	5.8 ± 0.2	5.7 ± 0.1	5.5 ± 0.1	6.4 ± 0.2



a. Addition of calcium to post-rigor muscle

b. Addition of calcium to pre-rigor muscle

Figure 1. Relationships between free and total calcium ions added to minced beef Sternomandibularis
Horizontal dotted lines indicate the minimum (2 mM) bitterness threshold concentrations of calcium chloride in water.

Pertinent Literature

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