BIOCHEMICAL AND STRUCTURAL MECHANISMS INVOLVED IN RABBIT MEAT AGEING AS ASSESSED BY THE USE OF PEPTIDASE (EC 3.4) INHIBITORS

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Background

The improvement of meat tenderness during the storage of animal carcasses at refrigeration temperatures (ageing) was firstly reported, in 1907, by Lehmann (quoted in [1]). However, the specific causes and the precise structural and biochemical mechanisms involved in the process remain controversial [2]. In addition, as tenderness is the most important sensory attribute of meat, for consumer selection and acceptability [3], an understanding of its structural and molecular basis is required for optimising meat storage and processing in the industry. This understanding is also required for rabbit meat since there is a scarcity of information relating to its ageing. In a previous report we suggested, based on *ante-mortem* injection of rabbits (*Oryctolagus cuniculus* L.) with peptidase (EC 3.4) inhibitors, that cysteine endopeptidases (EC 3.4.22), possibly calpains (EC 3.4.22.17), play a major role in rabbit meat ageing [4]. In addition, we also showed, based on the same approach, that myofibrillar structure weakening at or near N_2-line level might be the major structural change responsible for rabbit meat ageing [5].

Objectives

The aim of this work was to study further the biochemical and structural mechanisms involved in rabbit meat tenderisation at refrigeration temperatures, by relating the rate and extension of meat tenderness (shear force), myofibrillar proteins degradation (SDS-PAGE) and major structural changes in myofibrils (phase-contrast and electron microscopy), that occur during rabbit meat ageing (9 days at +4°C), for different muscle types (I, IIB and IID) and animal treatment groups (controls and *ante-mortem* injected with peptidase inhibitors).

Methods

New Zealand White male rabbits, with 12 ± 1 weeks of age and 2.8 ± 0.1 kg of b.w., were used in the experiments. The rabbits were injected *ante-mortem* (15 minutes before slaughter) with E-64, N-acetyl-Leu-Leu-norleucinal, Z-Phe-Ala-diazomethane, L-Leu-chloromethyl ketone, 0.9% NaCl (SSS control) or 20% DMSO (DMSO control). The muscles *semimembranosus proprius* (SP, type I), *semimembranosus accessorius* (SA, type IIB) and *psoas major* (PM, type IID) were removed from carcasses, at slaughter time (0 days) and after 3, 6 and 9 days of refrigeration at +4°C. Raw meat tenderness was assessed by shear force and the results expressed in N/cm^2, as described previously in [4]. The semi-quantification of meat myofibrillar proteins was performed by SDS-PAGE using BSA as an internal standard, as described previously in [4]. Myofibrillar structure weakening of meat was observed, after mechanical treatment, under phase-contrast microscopy and expressed as number of sarcomeres per myofibrillar fragment or myofibril (mns/mf), as previously described in [5]. Ultra-structural changes in meat myofibrils were observed and photographed under electron microscopy and the intensity and frequency of these changes classified (- to +++), as described in [5]. The parameters were analyzed by ANOVA at a significance level of 5% (H_0: $p<0.05$). Correlation coefficients were calculated using the Pearson method and their significance read in statistical tables.

Results and discussion

Of the peptidase inhibitors injected *ante-mortem* to the rabbits, only E-64 (cysteine peptidases inhibitor) and N-acetyl-Leu-Leu-norleucinal (calpain inhibitor) inhibited meat tenderisation (figure 1), myofibrillar proteins degradation and structural changes in myofibrils, during ageing. The other two peptidase inhibitors, Z-Phe-Ala-diazomethane (cathepsins B and L inhibitor) and L-Leu-chloromethyl ketone (cathepsin H inhibitor), did not modify the evolution of any of these parameters during meat ageing for the three muscles studied. A significant correlation (H_0: $p<0.05$) exists between myofibrillar fragmentation after mechanical treatment and meat tenderness (in all muscle types), for SSS control and E-64 injected rabbits, suggesting that both processes are closely related (figure 2). These results also indicate that myofibrillar fragmentation is a good ageing index for rabbit meat and is possibly directly involved in the tenderisation process. The data also show a clear relation between transversal disruption of sarcomeres at N_2-line level and meat tenderness (in all muscle types), for SSS control and E-64 injected rabbits, suggesting that both processes are closely related (table 1). The electron-dense N_2-line of sarcomere is due to the extensible N2A element of titin and is a site for intramyofibrillar calcium accumulation. The most susceptible to cleavage site of titin is located in its extensible segment at N_2-line region or very close to it [6]. By another way, *in vitro* degradation of nebulin, near C-terminal end, seems also to be related to a loss of the N_2-line in *post-mortem* muscle [7]. Moreover, calpains are activated and autolysed by calcium ions, diffuse from Z-line during storage at refrigeration temperatures, and its insertion sequence IS2 binds tightly to titin N_2-line region [8].

Conclusions

It is proposed that meat tenderisation during ageing depends mainly on the specific cleavage of titin molecules/filaments and nebulin molecules, at their susceptible sites located at or very close to the N_2-line region (extensible segment and near C-terminus, respectively), mediated by cysteine endopeptidases (possibly calpains).

Pertinent literature

[1] Koohmaraie M. (1994). *Meat Sci.*, 36, 93-104. **[2]** Martinaud A. *et al.* (1997). *J. Agric. Food Chem.*, 45, 2481-2487. **[3]** Warkup C. *et al.* (1995). *Expression of Tissue Proteinases and Regulation of Protein Degradation as Related to Meat Quality*. ECCEAMST, Utrecht, 225-237. **[4]** Prates J. *et al.* (2001). *Meat Sci.*, 57, 283-290. **[5]** Prates J. *et al.* (2002). *Meat Sci.*, 61, 103-113. **[6]** Kolmerer B. *et al.* (1999). *Rev. Phys. Biochem. Pharmac.*, 138, 19-55. **[7]** Tatsumi R. *et al.* (1993). *J. Biochem.*, 113, 797-804. **[8]** Sorimachi H. *et al.* (1997). *Biochem. J.*, 328, 721-732.

Acknowledgments

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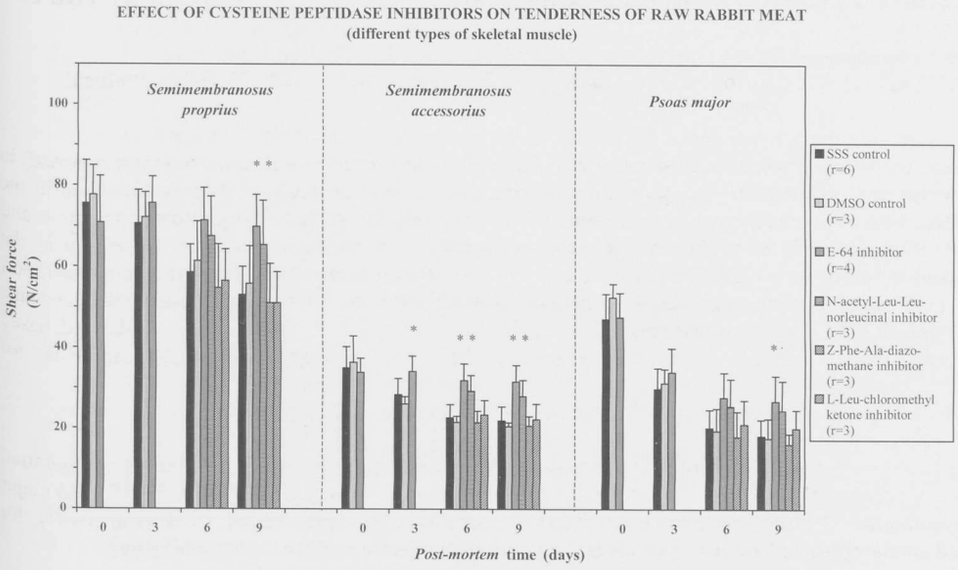

Figure 1. Effect of *ante-mortem* injection of peptidase inhibitors on the evolution of rabbit meat tenderness during ageing, for several muscles of different types. Vertical bars represent standard deviation of the mean of *r* replicates and means quoted with * are statistically significant (H_0: $p<0.05$).

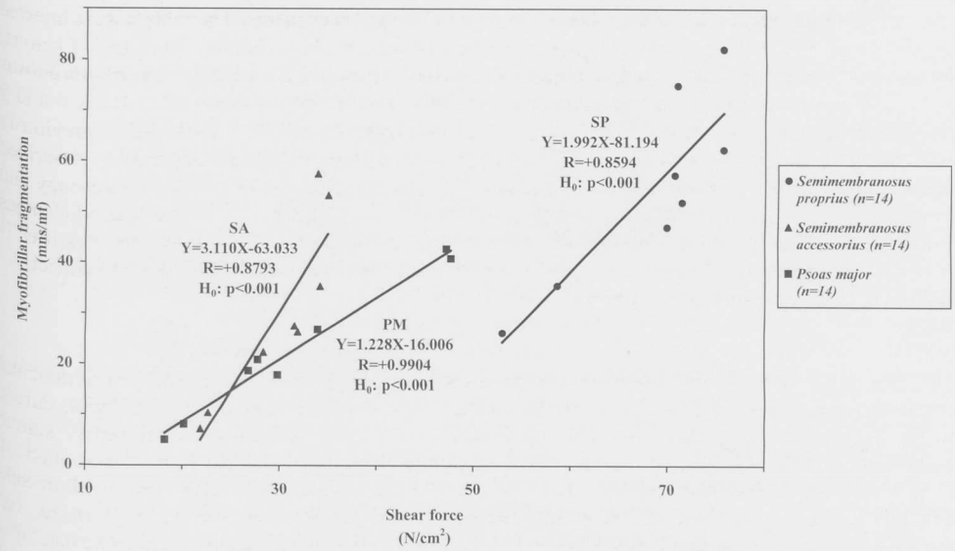

Figure 2. Correlation between meat tenderness and myofibrillar fragmentation during ageing, from SSS control and E-64 injected rabbits, for different muscles. The linear regression equation (Y=aX+b), correlation coefficient (R), degrees of freedom (*n*) and probability for H_0, are presented for each of the regression plots.

Table 1. Effect of *ante-mortem* administration of E-64 on major ultra-structural changes of rabbit meat during storage at +4°C, for different muscles. The following symbols mean: -, absent change; +, slight change; ++, moderate change; and +++, intense change.

ULTRA-STRUCTURAL CHANGES	MUSCLE	CLASSIFICATION (- to +++)					
		Post-mortem time / Treatment group					
		1 hour		3 days		6 days	
		SSS control	E-64	SSS control	E-64	SSS control	E-64
Z-Line degradation	SP	-	-	-	-	-	-
	SA	+	+	++	+	++	+
	PM	+	+	++	+	++	+
Loss of electron density of M-line	SP	-	-	++	+	++	+
	SA	-	-	++	+	++	+
	PM	-	-	++	+	++	+
Transversal disruption of sarcomeres at N_2-line level	SP	-	-	++	+	+++	+
	SA	+	+	++	+	+++	+
	PM	+	+	++	+	+++	+
Longitudinal fissure of myofibrils	SP	+	+	+++	+	+++	++
	SA	+	+	+++	+	+++	++
	PM	+	+	+++	+	+++	++
Loss of transversal alignments of Z- and M-lines	SP	+	+	+++	+	+++	++
	SA	+	+	+++	+	+++	++
	PM	+	+	+++	+	+++	++