

RELATIONSHIP BETWEEN PHYSICO-CHEMICAL MEASUREMENTS AND VEAL MEAT TENDERNESS DURING AGEING

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Introduction

In veal meat, despite the role of colour as an important factor determining the value of carcasses, tenderness, juiciness and cooking loss are also very important criteria of quality (Guignot *et al.*, 1992). Pearson (1994), refers to the following factors affecting meat tenderness: exercise, sex, breed, marbling, fat covering, conformation, type of muscle, onset of rigor mortis, cold-shortening, thaw-shortening, ageing and electrical stimulation. As referred by Smulders *et al.* (1991), connective tissue, fat and myofibrillar protein matrix are the main components to determine the tenderness of meat. The contribution of myofibrillar proteins to toughness is largely determined by rigor mortis and post-mortem ageing process (Smulders *et al.*, 1991). The mechanism of improvement of meat tenderness during post-mortem storage at refrigeration temperatures remains controversial. However, proteolysis of myofibrillar proteins may have an important role. In contrast to lysosomal cathepsins and the multicatalytic proteinase complex (MCP), substantial evidence suggests that calpains (mainly μ -calpain) are the primary system responsible for post-mortem proteolysis (Koochmariaie, 1996).

The aim of the present work was to determine the influence of storage of veal meat at refrigeration temperature in its quality and the relation between tenderness assessed objectively and subjectively and some variables that have been proposed as an influence of meat tenderness: sarcomere length, myofibrillar fragmentation, myofibrillar solubility, intramuscular fat and cooking loss.

Materials and Methods

In this study, males ($n=23$) of a portuguese autochthonous breed (Maronesa) with 8-11 months and carcasses weight of 100-164 kg were used. Carcasses were chilled 1 h at 0°C, 4 m/s and kept at 1°C until 24 h post-mortem. At 28 h post-mortem longissimus was excised (between 8th rib and 2nd lumbar vertebra) and cut in three parts (± 600 g). One part of the muscle was used for meat characterization at day 1 post-mortem and the two others were vacuum packed and aged at $2\pm 2^\circ\text{C}$ until 6 and 13 days post-mortem.

The pH was measured directly in the muscle using a combined glass electrode with a pH-Meter Crison 2002.

For determination of sarcomere length, about 200 mg of muscle were fixed in a solution of 2% glutardialdehyde and 0.2M sucrose in 0.2M phosphate buffer pH 7.1 (Honikel *et al.*, 1981). Bundles of 3-4 fibers were removed of the fixed muscle tissue and the length of 10 consecutive sarcomeres was measured (30 groups of 10 sarcomeres for each sample) on a optic microscope ($\times 1000$) using phase contrast (Jaime *et al.*, 1993).

Myofibrillar fragmentation index (MFI) was determined in frozen samples as described by Culler *et al.* (1978). After determination of protein concentration of the suspension by the biuret method (Gornall *et al.*, 1949), the suspension was diluted with 0.02 M potassium phosphate buffer (pH 7.0) to 1.0 mg/ml protein concentration. The exact protein concentration was determined using the micro-biuret method (Itzhaki and Gill, 1964 as cited by Clark, 1984) and the suspension of myofibrils diluted to 0.5 ± 0.05 mg/ml. MFI is the value of absorbance of myofibrillar suspension, measured at 540 nm multiplied by 200. Myofibrillar protein solubility (MPS) was determined in a high ionic strength buffer at pH 7.0 (0.4 M NaCl, 1 mM EDTA, 19 mM KH_2PO_4 , 31 mM Na_2HPO_4 and 1 mM NaN_3) for the determination of MPS pH7.0 or at pH 5.5 (0.4 M NaCl, 1 mM EDTA, 100 mM citric acid and 1 mM NaN_3) for determination of MPS pH5.5 according to Claeys *et al.* (1994). The protein concentration was determined in the supernatant by the biuret method (Gornall *et al.*, 1948) and results expressed as mg solubilized protein / g muscle.

Intramuscular fat was determined by extraction in a soxhlet apparatus using petroleum ether (ISO, 1973). The Cooking loss was determined in meat samples heated to an internal temperature of 70°C and was expressed as percentage of loss by heating based on the initial weight. After measurement of cooking loss the samples were used for determination of Warner-Bratzler shear force (WBSF) measured in 12 sub-samples with ± 1 cm² cross section and 4-5 length with fibres perpendicular to the direction of the blade attached to a Stevens QTS apparatus.

For sensory evaluation ± 1.5 cm thick steaks were covered with an aluminium foil and heated in a double side contact grill (230°C) to an internal temperature of 70°C. To a semi-trained panel of 6 members were asked to rank the meat on a 9-point scale: for tenderness (1 = extremely tough, 9 = extremely tender), juiciness (1 = extremely dry, 9 = extremely juicy) and flavour (1 = extremely undesirable, 9 = extremely desirable). Data analysis was performed with Systat 5.0. Tukey HSD was used to locate differences between means.

Results and Discussion

Table 1 shows the means and standard deviation of the parameters study in the three days post-mortem. The results show a significant increase of tenderness of meat from day 1 to day 6, evaluated by panel ($P < 0.05$) and by WBSF ($P < 0.001$). A period of ageing longer than 6 days did not increase the tenderness evaluated by both methods. It was also observed that the tenderness increase linearly with ultimate pH (measured at 28 h post-mortem) and significant ($P < 0.001$) correlations were found between the pH₂₈ and the values of WBSF and panel tenderness to the three days post-mortem (Table 2). The significant ($P < 0.001$) correlation of the cooking loss and juiciness with tenderness assessed objectively and subjectively, may partially explain the increase in tenderness with pH₂₈ (Guignot *et al.*, 1992). The strong correlation found between WBSF and panel tenderness (-0.852, -0.801 and -0.881 at 1, 6, and 13 days post-mortem) suggest that it is sufficiently correlated to justify the use either one or other for assessing veal meat tenderness.

Despite some authors have found an increase of toughness with decrease of sarcomere length (Bouton *et al.*, 1973), other works do not support this relation (Culler *et al.*, 1978; O'Halloran *et al.*, 1994). In this work, the sarcomere length was not significantly ($P > 0.05$) correlated with WBSF and sensorial tenderness, excluding curiously at day 6 and only between sarcomere length and WBSF (Table 2).

Various works showed that MFI increases with ageing of meat (Olsson *et al.*, 1976; Olsson and Parrish, 1977; Heinz *et al.*, 1994). This increase of MFI with ageing seems to be related to the phenomenon of myofibrils breaking into shorter segments at or near the Z-disk during post-mortem storage of meat (Olsson *et al.*, 1976). A high negative correlation between MFI and WBSF has been documented and, as referred by Culler *et al.* (1978) the MFI is a potential method for identifying tough and tender beef carcasses. These results show that MFI increases with ageing, although between day 6 and day 13 this increase was not statistically significantly ($P > 0.05$). At day 1 post-mortem the MFI is strongly correlated with WBSF and with panel tenderness, (-0.676 and 0.671 respectively) but, at day 6 and 13 post-mortem the relationship was lower. These results were partially according with Shackelford *et al.* (1991) who found correlation coefficients -0.91, -0.74, -0.63 and -0.40 between WBSF and MFI in beef longissimus dorsi at 1, 3, 7 and 14 days post-mortem respectively.

Myofibrillar protein salt solubility at pH 7.0 and pH 5.5 increased significantly ($P < 0.05$) from day 1 to day 6. Claeys *et al.* (1994), have found a significantly negative correlation ($P < 0.01$) between MPS at pH 7.0 and at pH 5.5 with WBSF in *longissimus thoracis* muscle three days *post-mortem*, which suggests that myofibrillar protein solubility is the result of proteolytic breakdown of key proteins that causes minor but important changes in the myofibrillar meat structure and promotes tenderization. In this study it was observed a significant ($P < 0.001$ at 1 and 6 days, $P < 0.01$ at 13 days *post-mortem*) correlation between MPS at pH 7.0 and the tenderness assessed by both methods. However, no significantly ($P > 0.05$) correlation was found for the MPS at pH 5.5. It is to remark that, in day 1, the MFI and MPS pH 7.0 were strongly

TABLE 1

Means and standard deviations (sd) of various parameters measured at 1, 6 and 13 days *post-mortem*.

	1	6	13
	Mean \pm sd	Mean \pm sd	Mean \pm sd
pH	6.08 ^a \pm 0.36	6.09 ^a \pm 0.36	6.01 ^a \pm 0.32
Sarcomere length (μ m)	1.66 ^a \pm 0.21	1.59 ^a \pm 0.17	1.57 ^a \pm 0.13
WBSF (Kg/cm ²)	12.39 ^a \pm 3.00	8.75 ^b \pm 3.27	8.67 ^b \pm 3.30
MFI	79.28 ^a \pm 16.51	118.73 ^b \pm 15.37	124.67 ^b \pm 12.31
MPS pH 7.0 (mg/g)	26.86 ^a \pm 10.51	32.41 ^b \pm 8.01	33.71 ^b \pm 8.55
MPS pH 5.5 (mg/g)	19.37 ^a \pm 3.71	21.90 ^b \pm 3.22	21.51 ^b \pm 2.90
Cooking loss (%)	14.36 ^a \pm 3.27	14.67 ^a \pm 4.47	15.74 ^a \pm 3.89
Panel tenderness	5.28 ^a \pm 1.30	6.21 ^b \pm 1.29	6.12 ^b \pm 1.25
Flavor	5.51 ^a \pm 0.94	5.81 ^a \pm 0.80	5.70 ^a \pm 0.78
Juiciness	5.04 ^a \pm 1.02	5.70 ^a \pm 0.78	5.13 ^a \pm 1.21

In the same row, means with different superscripts are significantly different ($P < 0.05$).

correlated ($P < 0.001$) with WBSF and with sensorial tenderness but, at day 6 the MPS pH 7.0 showed a better relationship with the WBSF (-0.825) and with the sensorial tenderness (0.760) than MFI (-0.521 and 0.354 for the WBSF and sensorial tenderness respectively). This suggests that at day 6 *post-mortem* MPS pH 7.0 is a better predictor of tenderness than MFI.

As it is shown (Table 2), intramuscular fat is not related with tenderness. Usually, higher intramuscular fat contents are associated with increased tenderness, possibly because: per volume unit of muscle, less tissue and fibrillar elements are present (Smulders *et al.*, 1991); fat promotes salivary flux and easily chewing and facilitate the separation of fibres bundles during chewing (Wood, 1993). The low mean content of intramuscular fat found (1.9%) was probably the reason of neglected relationship between intramuscular fat and tenderness.

The losses of moisture determined as cooking loss did not change with the time of storage as previously showed by Honikel (1987) in pig meat. Flavour and juiciness also showed no significant differences with storage of meat.

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TABLE 2

Simple correlations of various parameters with WBSF (above) and panel tenderness (below) at 1, 6 and 13 days *post-mortem*.

	1	6	13
pH ₂₈	-0.832*** 0.779***	-0.702*** 0.710***	-0.784*** 0.812***
Sarcomere length	-0.109 ^{ns} -0.004 ^{ns}	-0.551** 0.280 ^{ns}	-0.230 ^{ns} 0.203 ^{ns}
MFI	-0.676*** 0.671***	-0.521* 0.354 ^{ns}	-0.506* 0.435*
MPS pH 7.0	-0.655*** 0.701***	-0.825*** 0.760***	-0.595** 0.552**
MPS pH 5.5	-0.220 ^{ns} 0.474*	0.207 ^{ns} -0.036 ^{ns}	0.294 ^{ns} -0.185 ^{ns}
Intramuscular fat	0.055 ^{ns} 0.033 ^{ns}	0.097 ^{ns} -0.208 ^{ns}	0.130 ^{ns} -0.226 ^{ns}
Cooking loss	0.791*** -0.844***	0.665*** -0.642***	0.741*** -0.764***
Panel tenderness	-0.852*** -0.566**	-0.801*** -0.606**	-0.881*** -0.412 ^{ns}
Flavor	0.741*** -0.784***	0.684*** -0.742***	0.609*** -0.749***
Juiciness	0.876*** 0.876***	0.803*** 0.803***	0.868*** 0.868***

ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$