BEEF MYOFIBRILLAR PROTEIN SALT SOLUBILITY IN RELATION TO TENDERNESS AND PROTEOLYSIS

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SUMMARY

Myofibrillar protein salt solubility (MPS) (20 °C, pH 7.0 and 5.5, 0.4 M NaCl) was determined in 24 beef longissimus thoracis samples (7th thoracic rib, 3 days post mortem). A significant higher ($p<0.05$) MPS at pH 7 could be explained by higher concentrations of the high molecular weight proteins titin, nebulin, filamin and myosin heavy chain, as determined by semi-quantitative SDS-PAGE. Significant ($p<0.01$) correlations of MPS at both pH 5.5 and 7 with shear force and with the amount of TCA soluble tryptophan suggest beef MPS to be a reflection of post mortem proteolysis, which is in contrast with pork MPS, influenced mainly by protein denaturation. (DE SMET et al, 1993).

Introduction

Within identical muscles of homogenous animal groups, a large variability in beef tenderness is observed (Buts et al, 1984; Ouali, 1990; Morgan et al, 1991). Tenderness development greatly occurs during post mortem ageing and is mainly due to endogenous proteinases (Davey and Winger, 1988; Uytterhaegen et al, 1994). Most of the tenderizing related to ageing happens in the myofibrillar structure (Tarrant, 1987; Goll et al, 1983; Davey and Winger, 1988). The considerable improvement in tenderness during ageing is in contrast with the small changes in myofibrillar composition as observed by SDS-PAGE (Wu and Smith, 1987) and it is possible that minor changes are of critical importance or that the important changes are not apparent in SDS-PAGE-patterns. We tried to elucidate this potential changes by investigating beef myofibrillar salt solubility and related the results to tenderness and proteolysis.

Material and methods

Animals, treatment of carcasses and sampling

Twenty four Belgian Blue White bulls, 13 months old, were slaughtered at the slaughterhouse DERWA of Liege (Belgium). Carcasses were cooled rapidly at -3 °C during 2 hours and further kept at 0 °C for 3 days. Then, a longissimus thoracis steak adjacent to the 7th thoracic rib was removed and frozen at -18 °C until analysis. Three months later, samples were thawed overnight at 10 °C. To obtain a homogenous subsample, a slice (ca. 100g) covering the whole cutting surface of the sample was removed, passed through a grinder, vacuum packed in polyamide laminated polyethylene bags and again frozen at -18 °C for determinations. The remaining cuts were used to determine Warner Bratzler shear force.

Warner Bratzler shear force (WBS)

WBS was determined using a Warner Bratzler shear mounted on an Instron 1140 Food Tester, Instron Ltd, High Wycombe. Samples were heated in open plastic bags by immersion in a water bath at 75 °C for 1 hour. After cooling under running tap water, 1.27 cm cork bore samples were taken parallel to the fiber direction of the steaks. WBS was determined perpendicular to the fiber direction using 15 - 30 bore samples per steak.

Myofibrillar protein solubility (MPS)

To assay myofibrillar salt solubility at pH 5.5 and pH 7, the following empirical technique was developed: ground muscle (3.00 g) was homogenized with 30 ml of low ionic strength buffer at pH 7 (100 mM KCl, 1 mM EDTA, 8 mM KH_2PO_4, 12 mM K_2HPO_4 and 1 mM $MgCl_2$) using an Ultra Turrax (Janke and Kunkel, Staufen, Germany) and centrifuged at 1500 g for 10 minutes. The supernatant was decanted and the pellet resuspended

with a spatula in 30 ml buffer and centrifuged. To the pellet of isolated myofibrils, 45.0 ml of high ionic strength buffer at pH 7.0 (31 mM Na_2HPO_4, 19 mM KH_2PO_4, 1 mM EDTA, 0.4 M NaCl and 1 mM NaN_3) or at pH 5.5 (100 mM citric acid, 1 mM EDTA, 0.4 M NaCl and 1 mM NaN_3) was added and myofibrils suspended using the Ultra Turrax homogenizer at low speed for a few seconds. Tubes were placed in a water bath at 20 °C for 4 hours and mixed manually every 30 minutes. The solubilized proteins were isolated by centrifugation (3000 g, 30 minutes) and the supernatant decanted carefully. The protein content was determined using the biuret method and results expressed as mg solubilized protein/g muscle. The obtained values are further referred to as myofibrillar protein solubility (MPS).

Sodium Dodecylsulphate Polyacryamide Gel Electrophoresis (SDS-PAGE)
Total muscle myofibrillar protein concentrations were determined in a semi-quantitative manner using bovine serum albumin (BSA) as internal standard (Claeys et al, 1994). Results were expressed as mg BSA-equivalents/g muscle. Protein solutions for determination of MPS were diluted 2 times with a buffer containing 2% SDS, 2% 2-mercaptoethanol and 0.1333 mg/ml BSA for separation of solubilized proteins (both at pH 5.5 and 7) by SDS-PAGE, loading 15 µl on both 8% and 4.6% polyacrylamide gels (Claeys et al, 1994). Concentrations of soluble proteins were expressed as mg BSA-equivalents per g muscle.

TCA-soluble tryptophan
To obtain a measure of total peptides and amino acids in the meat samples, tryptophan (Try) was determined on available suspensions of 2.00 g of ground muscle in 2.0 ml 0.9% NaCl. After addition of 1 ml of 10% trichloric acetic acid (TCA) and vigorous mixing, suspensions were centrifuged (3000 g, 10 min) and the supernatant filtered (Schleicher & Schuell 597½). Total (bound as well as free) Try was determined on the filtrate using the method discribed by Messineo and Musarra (1972). Results were expressed as µg TCA-soluble Try/g muscle. This determination is considered to give a measure of the overall proteolytic activity that took place between the moment of slaughter and the sampling time at 3 days post mortem.

Results and Discussion

Table 1 shows the mean values for MPS, shear force and TCA-soluble Try for the 24 longissimus thoracis samples. Myofibrillar protein solubility at pH 7 is significantly ($p<0.05$, paired t-test) higher than at pH 5.5. This is in agreement with the results found by Wu and Smith (1987). Based on muscle protein tryptophan content (1 à 1.5%; Lawrie, 1974), the data for TCA soluble tryptophan suggest that less than 1% of myofibrillar protein was subjected to proteolysis.
From table 2 it is clear that the higher MPS at pH 7 can almost completely be explained by a higher solubility of the high molecular weight proteins titin, nebulin, filamin and myosin heavy chain. This is in line with the findings of Wu and Smith (1987). Based on visual comparison of different lanes on a SDS-PAGE pattern, Wu and Smith (1987) found more actin and M-protein solubilized at pH 7, but our results do not confirm this finding. Most of the proteins with a molecular weight (MW) lower than myosin heavy chain dissolve better at pH 5.5, again stressing the importance of the high molecular weight protein fraction dissolved at pH 7.
Of the lower MW proteins, only creatine phospho kinase (CPK, MW ca. 43 kDalton) dissolves better at pH 7. The presence of this protein in the myofibrillar fraction is probably a result of denaturation upon post mortem storage (Offer and Knight, 1988) and it is therefore not surprising that its solubility is higher at pH 7. Taylor and Etherington (1991) found that C-protein was the major protein band in SDS-PAGE patterns of protein solutions obtained after extraction of myofibrils with 30 mM $CaCl_2$. The results in table 2 agree well with this finding, showing that C-protein is about completely dissolved at both pH values, although NaCl was used in the present study.
Table 3 shows correlations between shear force, MPS at pH 5.5 and 7, the total muscle concentrations of some myofibrillar proteins and TCA soluble tryptophan (Try). Shear force (WBS) is significantly ($p<0.01$) correlated with MPS at both pH 5.5 and 7. Significant ($p<0.01$) correlations between WBS and concentrations of titin, troponin T and the 30 kDalton protein confirm earlier results (Buts et al, 1986; Buts et al, 1989) and explain post mortem tenderness development as a result of proteolysis. This is also confirmed by a significant ($p<0.01$) negative relation between WBS and TCA soluble tryptophan. Significant ($p<0.01$) correlations of MPS at both pH 5.5 and 7, with the total concentration of the same proteins and with TCA soluble tryptophan suggest that beef myofibrillar protein solubility is the result of proteolytic activity. Recently, this conclusion was quite convincingly confirmed by the finding that injection of longissimus thoracis samples with a cysteinproteinase inhibitor at 24 h post mortem (p.m.) completely blocked p.m. proteolysis, tenderization and MPS increase

(Uytterhaegen et al, 1994). The lack of significant ($p<0.05$) correlations between MPS at both pH 5.5 and 7 with total CPK and 34 kDalton concentrations, both known to be present in the myofibrillar fraction because of denaturation of sarcoplasmic proteins (Offer and Knight, 1988; Claeys et al, 1994), suggest that MPS is not determined by protein denaturation. This in contrast with earlier work carried out on pork Longissimus thoracis where MPS was mainly influenced by protein denaturation (De Smet et al., 1993). The correlations given in table 4 show that at pH 5.5 the variations in MPS are best explained by differences in soluble α-actinin, titin and myosin light chain 2. The correlations between concentrations of solubilized proteins at pH 5.5 with WBS are highest for titin, 30 kDalton, α-actinin and troponin T. The significant correlations between WBS with troponin T and 30 kDalton may be a reflection of the existing relation between WBS and the total amounts of these two proteins (table 3).
At pH 7, most of the concentrations of solubilized proteins show significant correlations ($p<0.05$) with MPS. This indicates that the solubility of the individual proteins in the first place reflects the total amount of solubilized proteins. Significant correlations ($p<0.01$) between WBS and soluble titin and α-actinin at pH 7 are found. The relation with soluble 30 kDalton found at pH 5.5 still exists at pH 7, but the relation with troponin T is lost at pH 7. This is probably because a much smaller part of the total amount of troponin T was dissolved at pH 7 (table 2). Concentrations of soluble myosin and actin at pH 7 are also significantly ($p<0.01$) correlated with WBS. This was not found at pH 5.5 (table 4). The significant correlation ($p<0.01$) between WBS and the solubility of α-actinin at both pH 5.5 and 7 indicate a possible relationship between the integrity of the Z-line and meat tenderness, and the highly significant ($p<0.001$) correlations between the concentrations of soluble α-actinin and TCA soluble tryptophan at both pH-values strongly suggest that this is also a result of proteolysis.

Conclusion

The overall conclusion of this experiment is that beef myofibrillar protein salt solubility (MPS) is a reflection of post mortem proteolytic activity. Significant correlations between MPS and shear force values are a consequence of the relation proteolysis - tenderness development. We assume that proteolytic breakdown of key proteins causes minor but important changes in the myofibrillar meat structure so that tenderization occurs and salt can enter the myofibrillar structure easier, increasing the protein solubility. The nature of these key proteins remains to be elucidated.

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