THE INFLUENCE OF AGEING PERIOD AND BLADE SPEED ON THE DETERMINATION OF MYOFIBRILLAR FRAGMENTATION INDEX OF BEEF MM. LONGISSIMUS THORACIS ET LUMBORUM

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SUMMARY

Ten Bonsmara bull carcases were electrically stimulated (500 V, 60 sec) within 10 minutes of exsanguination. Carcases were chilled overnight at 0 °C. Samples from the *Mm. longissimus thoracis et lumborum* were vacuum packaged and aged at 0 °C until 1, 4, 7, 14 and 21 days *post mortem*. The myofibrillar fragmentation indices (MFI) were determined with homogenisation blade speeds of 5000, 10000, 20000 and 30000 rpm. Fragmented myofibre fibre lengths were also measured using Video Image Analysis. Ageing period and blade speed both influenced the MFI value and fibre length ($P<0.0001$). The higher the blade speed, the higher the MFI value and shorter the fibre length. MFI value increased and fibre length decreased from 1 to 14 days, reaching a plateau after 14 days *post mortem*. This method of MFI determination adequately reflected the changes in myofibrillar fibre lengths. Between 14 and 21 days of ageing no significant changes in myofibril length of electrically stimulated beef carcases occurred. A homogenisation speed of between 10000 and 20000 rpm is recommended for MFI determination.

Introduction

As the tenderness is one of the most important quality characteristics of meat, it has attracted much research. Ageing of meat is one of the oldest methods for *post mortem* improvement of tenderness. This *post mortem* ageing is usually restricted to the myofibrillar proteins (Olson *et al.*, 1976) with no apparent influence on the connective proteins (Sharp, 1963), at least not detectable in the normal period of ageing of 7 to 21 days as practised by the meat trade. The Myofibrillar Fragmentation Index (MFI) assesses the measure of fragmentation of the myofibrils which is caused by proteolysis (Olson and Parrish, 1977). Many studies in which the improvement in the tenderness as a result of myofibrillar degradation has been studied using MFI values (Olson *et al.*, 1976; Culler *et al.*, 1978). The method suggested by Culler *et al.* (1978) has been extensively used by certain research laboratories in the USA (Koohmaraie *et al.*, 1987; Koohmaraie *et al.*, 1988; Crouse *et al.*, 1991). It has rarely been used outside the USA. As the method described by Culler *et al.* (1978) seemed practical, it was decided to investigate the method and use it to measure the influence of ageing of muscle from electrically stimulated carcases on this MFI value. The influence of homogenisation speed was also included in this investigation.

Materials and methods

Ten Bonsmara bulls (273.0 ± 13.9 kg) were available for the study. The animals were slaughtered after captive bolt stunning. Within 10 minutes after exsanguination the carcases were electrically stimulated (500 V, 120 sec), and thereafter eviscerated and dressed according to normal slaughter procedures. The carcases were chilled overnight at 0 °C. One day *post mortem* about 1 kg of the *Mm. longissimus thoracis et lumborum* was removed and divided into 5 equal pieces. The pieces were vacuum packaged and aged until 1, 4, 7, 14 and 21 days *post mortem*, after which they were frozen until determination of the MFI value and myofibre length.

MFI values were determined according to a modified method of Culler *et al.* (1978). The frozen samples were thinly sliced with a knife. Any visible fat and connective tissue were removed and thereafter scissor minced. About 3 g of the minced sample was homogenised for 30 sec. in 50 ml 0.02 M potassium phosphate buffer (pH 7.0) containing 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA and 1 mM NaN_3. The blade of the Bühler HO 4 homogeniser was turned around in order to fragment the myofibrils with the blunt edge

rather than to cut them with the sharp edge. To determine the influence of blade speed on the MFI value, blade speeds of 5000, 10000, 20000 and 30000 rpm were used. The homogenate was centrifuged (4 °C, 1000 x g, 15 min). After discarding the supernatant the pellet was resuspended in 50 ml buffer, and re-centrifuged. The second supernatant was discarded and the pellet resuspended in 10 ml buffer before filtration through a 1000 µm polyethylene strainer under a light vacuum. An additional 5 ml buffer was added to facilitate the passage of myofibrils through the strainer before subsequent filtration through a 250 µm polyethylene strainer under a light vacuum. The protein concentration was determined using the Biuret method (Gornall *et al.*, 1949 as cited by Bailey, 1967). The filtrate was diluted to roughly 1.5 g/l protein and the exact protein concentration determined using the micro-biuret method (Bailey, 1967). Thereafter the sample was diluted to 0.5 g/l protein, and the protein concentration of this dilution determined using the micro-biuret method (Bailey, 1967). Only if the concentration was 0.5 ± 0.05 was the absorbance of the sample suspension determinded at 540 nm. The MFI-value was this absorbance multiplied by 200.

The myofibre lengths were measured on the sample from the final dilution for the MFI determination using a video image analysis system (Kontron). A few drops were taken and put on a microscope slide and covered with a cover slip. A microscope objective magnification of 25x was used. The lengths of the first 50 myofibrils with a sarcomere count of more than 5 were measured to ensure that it is a myofibril and not debris. The average over the 50 measurements was taken for statistical analyses.

The statistical analyses were done using the programme package Statgraphics V5.0.

Results and discussion

Both the ageing period and the homogenisation blade speed influenced the MFI value and myofibre length significantly ($P<0.0001$). As the ageing period increased from 1 to 14 days *post mortem*, the MFI value increased, but remained 21 days *post mortem* at about the level of the 14 days *post mortem* value. This would indicate that at 14 days *post mortem* the maximum ageing for this muscle under these conditions has been reached, or alternatively, that this method to determine the MFI value reached a stage where it became insensitive to any additional changes in myofibrillar degradation. Myofibre length also showed that the general trend is towards a shorter length with an increase in the ageing period from 1 to 21 days, although the values for 7 and 14 days *post mortem* did differ significantly.

The greatest increase in MFI value took place over the first 7 days *post mortem*. Koohmaraie *et al.* (1987) also showed that the MFI value (*Mm. longissimus thoracis et lumborum*) increase at more or less a steady rate from 1 to 6 days *post mortem*, after which a decrease in the rate of change to 14 days *post mortem* was found, very similar to the results of this study. This is in contrast to the work of Olson *et al.* (1976) who found that in the *M. semitendinosus* the biggest increase took place between 1 and 3 days *post mortem*. Nevertheless, they also encountered that the MFI of the *Mm. longissimus thoracis et lumborum* increased with ageing period from 1 to 6 days *post mortem*.

Sonaiya *et al.* (1982) have established that the MFI values of muscle from electrically stimulated carcases tend to be higher than those of non-stimulated carcases. This was confirmed for one day post mortem by Geesink (1993) only if the stimulation time was 64 sec. using relatively low voltage stunning (85 V). Seven and 14 days *post mortem* these values were not significantly different from those of the non-stimulated carcases, but tended to be lower than those of the non-stimulated carcases. As was encountered in this study, Geesink (1993) also found that the mean MFI value decreased from 14 to 21 days *post mortem*. The results of this study further indicates that even after electrical stimulation, ageing still takes place *post mortem*, and is still measurable by MFI value (14 days *post mortem*) and myofibre length measurement (21 days *post mortem*).

Regarding the influence of blade speed, the results were as expected. The higher the blade speed, the higher the MFI values were. The values all differed significantly between the various blade speeds. Although no significant difference was found between the myofibre lengths between 5000 and 10000 rpm, the 20000 and 30000 rpm lengths differed significantly from each other, and from that of the 10000 rpm value. With increasing blade speed the myofibre length was reduced. These results indicate that a blade speed of between 10000 and 20000 rpm is sufficient for the determination of MFI values.

Conclusion

The measurement of the MFI value by the method described in this study gives an adequate estimation of the proteolytic degradation which takes place post mortem during ageing. The results indicate that even after electrical stimulation ageing as measured by MFI value and myofibre length still continues up to 14 to 21 days

post mortem, measured by MFI and myofibre length respectively. A blade speed of between 10000 and 20000 rpm is sufficient for MFI determinations.

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