Particle size analysis of lamb meat: Effect of homogenization speed

L. Karumendu[2, 4], D.L. Hopkins[1,2*], R. van den Ven[3], M.J. Kerr[1, 2], T.A. Lamb[1, 2] & M. Lanza[4]

[1]CRC for Sheep Industry Innovation, Armidale, N.S.W., 2350, Australia.

[2]NSW Department of Primary Industries, Centre for Sheep Meat Development, PO Box 129, Cowra N.S.W., 2794, Australia.

[3]NSW Department of Primary Industries, Orange Agricultural Institute, Forest Road, Orange, N.S.W., 2800, Australia.

[4]Department DACPA-Animal Science section, University of Catania, via Valdisavoia, n. 5 95123 Catania, Italy.

*E-mail: David.Hopkins@dpi.nsw.gov.au.

Abstract

The degradation of muscle myofibrillar proteins under post-mortem conditions can be assessed by determining the extent of the fragmentation of myofibrils when subjected to homogenization. A myofibrillar fragmentation index (MFI) can be derived by determination of protein content and measurement of the turbidity of samples adjusted to a common protein concentration. Although effective, this method is time consuming. An alternative method using particle size analysis (PSA) determined by laser diffraction has the potential to replace the MFI method and allow faster processing of samples, but a number of methodological questions need to be resolved. The speed of homogenization affects MFI results, hence the impact of this variable on PSA results was examined using 40 lamb loin samples. One gram duplicate samples from meat aged for 1 and 5 days were homogenized at different speeds; 11,000, 13,000, 16,000, 19,000 and 22,000 rpm. Homogenization at 16,000 rpm provided the greatest ability to detect ageing differences for particle sizes between samples aged for 1 and 5 days. The 25% quantile particle size provided the best result for detecting differences due to ageing. The relationship between PSA and MFI and PSA and shear force needs to be established for lamb, but PSA offers significant potential for streamling determination of myofibrillar degradation.

Introduction

A most widely-used method to determine the extent of myofibrillar fragmentation involves homogenization of muscles, followed by the determination of protein content and the measurement of the turbidity of samples adjusted to a common protein concentration. Taylor *et al.* (1995) showed that this reflected the degradation of key structural proteins in the I-band of the sarcomere and Culler *et al.* (1978) reported that MFI had a high correlation with measures of tenderness. A number of variables are likely to impact on the results when determining fragmentation. Using an Omni-mixer at 15,000 rpm as the standard from previous work (Hopkins *et al.*, 2000), a study was conducted by Hopkins *et al.* (2004) to establish whether a shaft homogenizer could be used as an alternative. This study found that homogenizing at 11,000 rpm for two 30-second bursts with a shaft homogenizer produced a similar variance to the standard method using an Omni-mixer. This method was further refined by Martin *et al.* (2004) and has been used in a number of studies. Recently, a new approach to examine myofibrillar degradation was proposed by Lametsch *et al.* (2007) using laser diffraction to measure particle size. This new approach has the potential to reduce sample preparation times compared to the method developed by Martin *et al.* (2004), but has not been tested on lamb meat. Further to this, Lametsch *et al.* (2007) homogenized their samples with an 18 mm shaft type homogenizer at 20,500 rpm which is much faster than the 11,000 rpm used by Martin *et al.* (2004) with a 10 mm shaft. Lametsch *et al.* (2007) also proposed that no centrifugation was required after homogenization, potentially increasing the number of samples that could be measured in a set time.

Using loin samples aged for 1 and 5 days, the objective of this study was to examine the impact of homogenization speed on particle size (PS) results without the use of centrifugation.

Materials and methods

One gram duplicate samples of lamb loin (m. *longissimus thoracis et lumborum*) aged for 1 and 5 days (40 of each) were homogenized at 5 different speeds, 11,000, 13,000, 16,000, 19,000 and 22,000 rpm. The testing, performed over 10 days, was designed with samples from the same lamb loin (samples aged 1 and 5 days) tested on the same day using two of the five homogenization speeds. Each pair of homogenization speeds (e.g. 13,000 and 22,000 rpm) occurred together on four of the loins. After samples were homogenized the PS was measured using a laser diffraction particle size analyzer (Beckman Coulter, Model LS 13 320). Three separate statistical analyses of the data were undertaken, using ASReml (Gilmour *et al.*, 2006). Firstly

the average particle size (mean) for each sample was analyzed using a linear mixed model analysis. Fixed effects in the model were effects for the two ageing classes, a linear speed effect, deviations from linearity in speed over the five speed settings (Dev), and interactions between ageing and linear speed and ageing and the five speed settings. Random effects in the model incorporated effects associated with the nested design structure. These were effects for day (at 10 levels), lamb (at 40 levels with samples from four lambs tested each day), lamb x ageing and at the lower strata lamb x ageing x dev. The second analysis analyzed the logarithm of the standard deviation (SD) of the mean particle sizes within each test (logSD). Thirdly the maximum particle size for the quantiles (10%, 25%, 50%, 75% and 90%) for each sample were analyzed, on the log scale, jointly using a linear mixed model similar to the above mentioned models, but also including trends with quantile and allowing for correlations between residuals for the same animal.

Results and discussion

In previous work, Lametsch *et al.* (2007) proposed PS as a novel method for determining myofibrillar fragmentation using a homogenization speed of 20,500 rpm with a shaft type homogenizer (Ystral). Using the same type of homogenizer, Hopkins *et al.* (2004) found that speed influences the size of myofibril fragments. They established that an ageing effect on MFI was best detected when homogenizing at 15,000 rpm. Hence, one is tempted to believe that the results of Lametsch *et al.* (2007) would have been optimized if appropriate homogenization speed was taken into account. In this study, the mean particle sizes for samples aged 1 and 5 days differed significantly at a given speed and were described by the linear models; mean = 257.6 ± 0.94 – 7.69 ± 0.49 (Speed/1000) + error and mean = 215.1 ± 0.94 – 7.69 ± 0.49 (Speed/1000) + error respectively. The differences in mean particle size between the two ageing groups did not differ significantly across the range of speeds tested.

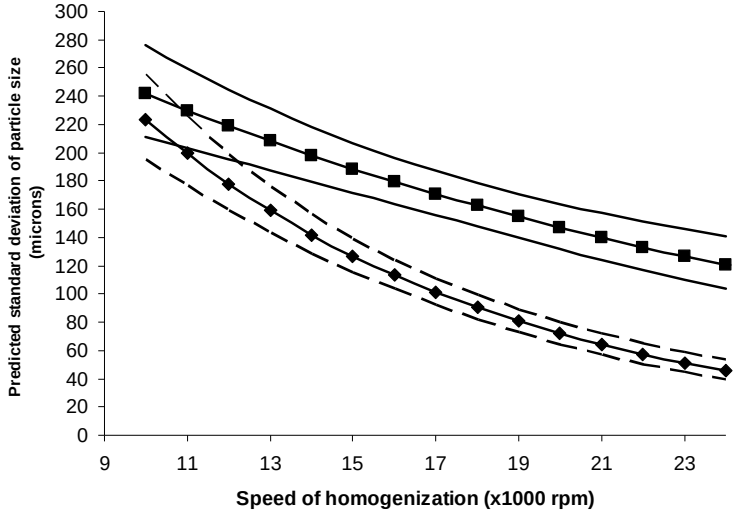

Figure 1. Predicted standard deviation of particle size (μm; 95% confidence intervals indicated) at various speeds of homogenization for meat samples aged 1 (■) and 5 day (♦).

However, analysis on the log scale of the standard deviation (SD) of PS indicated a significant ($P < 0.001$) linear trend against speed of homogenization (Figure 1; SD is predicted on the original scale non transformed). Differences in the standard deviation of PS between the two ageing groups at a common speed were maximized at approximately 22,000 rpm and this difference was estimated to decrease above this speed.

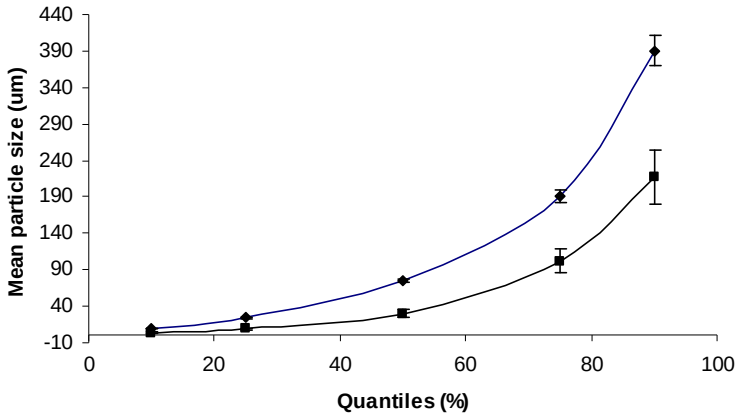

Figure 2. The mean particle size (μm) against quantiles for meat samples aged 1 (■) and 5 days (♦) and homogenized at 16,000 rpm.

A speed of 16,000 rpm was the best for detecting differences between ageing treatments across the quantiles (Figure 2) and the coefficient of variation was uniformly minimized over the five quantiles for each ageing period at this speed.

Conclusions

Homogenization speed influences particle size results and thus there is scope to optimize the use of the new laser diffraction method for determining myofibrillar fragmentation as proposed by Lametsch *et al.* (2007). Further work is currently under way to compare results obtained from PSA using the newly proposed homogenization speed (16,000 rpm) to results obtained using the conventional MFI method at 11,000 rpm as published by Martin *et al.* (2004). This work will also examine the relationship between PS, MFI and toughness measured by shear force.

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References

Culler, R. D. Parrish, F. C. Smith, G. C. & Cross, H. R., 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. of Food Sci. 43, 1177-1180.

Gilmour, A.R. Gogel, B.J. Cullis, B.R. & Thompson, R., 2006. *ASReml User Guide Release 2.0*. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Hopkins, D.L. Littlefield, P.J. & Thompson, J.M., 2000. A research note on factors affecting the determination of myofibrillar fragmentation. Meat Sci. 56, 19-22.

Hopkins, D.L. Martin, L. & Gilmour, A.R., 2004. The impact of homogenizer type and speed on the determination of myofibrillar fragmentation. Meat Sci. 67, 705-710.

Lametsch, R., Knudsen, J.C. Ertbjerg, P. Oksbjerg, N. & Therkildsen, M., 2007. Novel method for the determination of myofibril fragmentation post-mortem. Meat Sci. 75, 719-724.

Martin, L.C. Hopkins, D.L. & Morgan, J.E., 2004. Streamlining the determination of myofibrillar fragmentation index. Animal Production Australia 25, 279.

Taylor, R. G. Geesink, G. H. Thompson, V. F. Koohmaraie, M. & Goll, D. E., 1995. Is Z-disk degradation responsible for postmortem tenderisation? J. of Anim. Sci. 73, 1351-1367.