Meat Science 82 (2009) 425–431

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Particle size analysis of lamb meat: Effect of homogenization speed, comparison with myofibrillar fragmentation index and its relationship with shear force

L.U. Karumendu [a,c], R. van de Ven [d], M.J. Kerr [a], M. Lanza [c], D.L. Hopkins [a,b,*]

[a] NSW Department of Primary Industries, Centre for Sheep Meat Development, P.O. Box 129, Cowra, NSW 2794, Australia
[b] Australian Sheep Industry Cooperative Research Centre, Armidale, NSW 2350, Australia
[c] Department DACPA-Animal Science section, University of Catania, via Valdisavoia, n. 5 95123 Catania, Italy
[d] NSW Department of Primary Industries, Orange Agricultural Institute, Forest Road, Orange, NSW 2800, Australia

ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form 12 February 2009
Accepted 18 February 2009

Keywords:
Lamb
Homogenization speed
Ageing
Particle size analysis
Myofibrillar fragmentation index
Shear force

ABSTRACT

The impact of homogenization speed on Particle Size (PS) results was examined using samples from the *M. longissimus thoracis et lumborum* (LL) of 40 lambs. One gram duplicate samples from meat aged for 1 and 5 days were homogenized at five different speeds; 11,000, 13,000, 16,000, 19,000 and 22,000 rpm. In addition to this LL samples from 30 different lamb carcases also aged for 1 and 5 days were used to study the comparison between PS and myofibrillar fragmentation index (MFI) values. In this case, 1 g duplicate samples ($n = 30$) were homogenized at 16,000 rpm and the other half (0.5 g samples) at 11,000 rpm ($n = 30$). The homogenates were then subjected to respective combinations of treatments which included either PS analysis or the determination of MFI, both with or without three cycles of centrifugation. All 140 samples of LL included 65 g blocks for subsequent shear force (SF) testing. Homogenization at 16,000 rpm provided the greatest ability to detect ageing differences for particle size between samples aged for 1 and 5 days. Particle size at the 25% quantile provided the best result for detecting differences due to ageing. It was observed that as ageing increased the mean PS decreased and was significantly ($P < 0.001$) less for 5 days aged samples compared to 1 day aged samples, while MFI values significantly increased ($P < 0.001$) as ageing period increased. When comparing the PS and MFI methods it became apparent that, as opposed to the MFI method, there was a greater coefficient of variation for the PS method which warranted a quality assurance system. Given this requirement and examination of the mean, standard deviation and the 25% quantile for PS data it was concluded that three cycles of centrifugation were not necessary and this also applied to the MFI method. There were significant correlations ($P < 0.001$) within the same lamb loin sample aged for a given period between mean MFI and mean PS (−0.53), mean MFI and mean SF (−0.38) and mean PS and mean SF (0.23). It was concluded that PS analysis offers significant potential for streamlining determination of myofibrillar degradation when samples are measured after homogenization at 16,000 rpm with no centrifugation.

1. Introduction

The inconsistency in meat tenderness at the consumer level has been identified as one of the major challenges facing the meat industry today (Koohmaraie, 1994; Monsón et al., 2004). An understanding of the causes of this inconsistency is imperative if appropriate and efficient methodologies are to be developed so as to respond to consumer demands for tender meat while at the same time reducing the inconsistency in meat tenderness.

A subjective measure of tenderness is the consumer appreciation of meat, with a high score being desirable (Hwang, Devine, & Hopkins, 2003) while it can also be measured objectively by means of shear force. Meat tenderization occurs as the structural proteins are degraded most notably during the process of ageing (Hopkins & Thompson, 2002). The extent of muscle myofibrillar protein degradation under *post-mortem* conditions can be tested by subjecting meat to homogenization since higher levels of degradation produce more disruption of myofibres. Several methods have been used, but the most widely used method involves the homogenization of muscle followed by the determination of the protein content and the measurement of the turbidity of samples adjusted to a common protein concentration (Olson, Parrish, &

* Corresponding author. Address: NSW Department of Primary Industries, Centre for Sheep Meat Development, P.O. Box 129, Cowra, NSW 2794, Australia.
E-mail address: David.Hopkins@dpi.nsw.gov.au (D.L. Hopkins).

0309-1740/$ - see front matter Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.02.012

Stromer, 1976), giving myofibrillar fragmentation index (MFI) values. Taylor, Geesink, Thompson, Koohmaraie, and Goll (1995) showed that this method reflected the degradation of key structural proteins in the I-band of the sarcomere. Olson et al. (1976) and Culler, Parrish, Smith, and Cross (1978) reported that MFI had a high correlation with measures of tenderness including sensory tenderness scores and MFI was reported to account for about 50% of the variation in tenderness (Olson & Parrish, 1977).

A number of variables are likely to impact on the results when determining fragmentation. In their paper on MFI using the turbidity method, Olson et al. (1976) showed that the length of homogenization time affected measures of MFI and using their method, samples needed at least 60 s of homogenization in order to provide reliable results for different ageing periods. In the published papers, a variety of different homogenizers have been used and in some cases the type of homogenizer has not been specified (Culler et al., 1978; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). Hopkins, Martin, and Gilmour (2004) undertook a study to establish whether a shaft homogenizer could be used as an alternative to an Omni-mixer which had been the standard in previous work (Hopkins, Littlefield, & Thompson, 2000). Hopkins et al. (2004) found that homogenizing at 11,000 rpm for two 30-s bursts with a shaft homogenizer produced a similar variance to the standard method using an Omni-mixer. These workers also found that homogenizing at 11,000 rpm was the best speed of a range of speeds tested. The method of Hopkins et al. (2004) was further refined by Martin, Hopkins, and Morgan (2004) and has been used in a number of studies (e.g. Hopkins, Stanley, Martin, Toohey, & Gilmour, 2007). Recently, a new approach to examine myofibrillar degradation was proposed by Lametsch, Knudsen, Ertbjerg, Oksbjerg, and Therkildsen (2007), using laser diffraction to measure particle size based on the principles of the Mie theory of light scattering. 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.

The purpose of this study was three-fold. Firstly, it was to examine the impact of homogenization speed on lamb loin particle size (PS) results without the use of centrifugation. Secondly, using the ideal homogenization speed, the purpose was also to compare PS values to MFI values after homogenization and centrifugation. Thirdly, it was to examine the relationship between PS and MFI values with respect to shear force using lamb loin samples.

Preliminarily results on the first phase were presented by Karumendu et al. (2008).

2. Material and methods

2.1. Design and muscle sampling – Phase 1

Samples of lamb loin (*M. longissimus thoracis et lumborum;* LL) aged for 1 and 5 days at 3–4 °C before they were frozen at −20 °C were used from 40 carcases. These samples of LL included 65 g blocks for subsequent shear force testing. Duplicate 1 g samples were homogenized at the following speeds; 11,000, 13,000, 16,000, 19,000 and 22,000 rpm using an Ystral homogensier (Series X10/25, Ystral, Germany) with a 10 mm shaft. The samples were homogenized twice for 30 s with a 30 s break on ice and then tested for PS. The testing, performed over 10 days, was designed with samples from the same lamb carcass (samples aged 1 and 5 days) tested on the same day using two of the five homogenization speeds assigned randomly. Each pair of homogenization speeds (e.g. 13,000 and 22,000 rpm) occurred together on four of the carcases.

2.2. Design and muscle sampling – Phase 2

Samples of lamb loin (*M. longissimus thoracis et lumborum*) aged for 1 and 5 days (one loin per lamb per age) at 3–4 °C before they were frozen at −20 °C were used from 30 carcases. These samples of LL included 65 g blocks for subsequent shear force testing in addition to samples for PS analysis and MFI determination. The following treatments were applied;

- 1 g sample (in duplicate), homogenization at 16,000 rpm and PS analysis with no centrifugation (Treatment 1).
- 0.5 g sample (in duplicate) homogenization at 11,000 rpm and determination of MFI with no centrifugation (Treatment 2).
- 1 g sample (in duplicate), homogenization at 16,000 rpm and PS analysis with three cycles of centrifugation (Treatment 3).
- 0.5 g sample (in duplicate), homogenization at 11,000 rpm and determination of MFI with three cycles of centrifugation (Treatment 4).

The design was balanced in that all treatments and aged samples were equally represented and so that each pair of treatments within each aged group occurred equally often within the same animal. This phase of the experiment was conducted over 18 days. On 12 of the 18 days, samples were randomly chosen from two lambs while for each of the remaining 6 days, samples from a single lamb were chosen.

2.3. MFI measurement

The method was based on that described originally by Culler et al. (1978), but modified as described by Hopkins et al. (2004) and Martin et al. (2004). Duplicate 0.5 g samples of muscle were taken from the samples to be tested on any particular day and sliced along the fibre direction avoiding any visible fat, or connective tissue. Muscle samples were homogenized in 50 ml falcon tubes containing 15 ml of ice-cold buffer and held on ice between homogenizations. The buffer was 0.1 M KCl, 1 mM EDTA (di-sodium), 25 mM potassium phosphate (K_2HPO_4 and KH_2PO_4) adjusted to a pH of 7 using K_2HPO_4 (base) or KH_2PO_4 (acid) at a temperature of 5 °C.

After homogenization, the myofibril suspensions were filtered into a 100 ml beaker using mesh strainers with 1 mm^2 holes to remove connective tissue. Washing the myofibrils with 5 ml of cold buffer facilitated filtration through the mesh. The filtrates for Treatment 2 were made up to 50 ml with buffer and those for Treatment 4 (20 ml) were centrifuged at 2 °C at 1000*g* for 10 min (Model CPR, Beckman Instruments, CA, USA) and the supernatant decanted. The pellets of myofibrils were re-suspended in 10 ml of buffer, shaken thoroughly and centrifuged again. This process was repeated and the pellet finally re-suspended in 40 ml of cold buffer. The protein concentration of the suspensions (Treatments 2 and 4) was determined in triplicate using the bicinchoninic acid (BCA) method (Pierce Chemical Company, Illinios, USA). Absorption was measured at 560 nm in a micro-plate reader (FLUOstar OPTIMA, BMG Labtechnologies, Victoria, Australia) in accordance with the specified protocol and a bovine serum albumin standard curve was used.

Aliquots of the suspensions were diluted in buffer to a final protein concentration of 0.5 mg/ml in triplicate. The diluted protein suspensions were poured into a cuvette, mixed and the absorbance measured immediately at 540 nm using a spectrophotometer

(Biochrom WPA Spectrawave S1000 Diode Array Spectrophotometer). The mean of the triplicate absorbance readings was multiplied by 150 to give index values for myofibrillar fragmentation termed, MFI.

2.4. Particle size analysis

Duplicate one gram samples of muscle were taken from the samples to be tested on any particular day and sliced along the fibre direction avoiding any visible fat, or connective tissue. Muscle samples were homogenized in 50 ml falcon tubes containing 15 ml of ice-cold buffer and held on ice between homogenizations as for the MFI method. After samples for Treatment 1 were homogenized, the particle size (PS) was measured using a laser diffraction particle size analyser (Beckman Coulter, Model LS 13 320, Miami, USA). The PS of samples from Treatment 3 was measured after the three cycles of centrifugation and the final pellet was suspended in 30 and 40 ml of buffer for muscles aged 1 and 5 days, respectively. The instrument measures the size distribution of particles suspended in a liquid (water in this case) by using the principle of light scattering (Beckman Coulter., 2003). The instrument was connected to a water unit and samples were added drop-wise to this unit. The data produced include the mean particle size, the standard deviation of the mean and the quantile distribution of the data (at 10%, 25%, 50%, etc.). The quantile is the fraction or a percentage of data points below a given value. For instance, the 0.25 (or 25%) quantile is the point at which 25% of the data fall below and 75% fall above that value.

2.5. Measurement of shear force

Frozen samples for shear force testing were cooked in plastic bags in batches of 20 for 35 min at 70 °C in a water bath. Cooking took place over a period of 5 days and samples were kept overnight in the fridge before they were subjected to shear testing the following day, using a Lloyd (Model LRX, Lloyd Instruments, Hampshire, UK) with a Warner–Bratzler shear blade fitted as described by Hopkins and Thompson (2001).

2.6. Statistical analysis

Three separate statistical analyses of the data were undertaken. These were analyses of the results of Phase 1, analyses of the results of Phase 2 and finally correlation analyses of shear force with the PS and MFI results from Phases 1 and 2.

2.6.1. Homogenization speed – Phase 1

Firstly, for Phase 1 the average particle size (MeanPS) for each sample was analysed using a linear mixed model (LMM) 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 (DevSpeed), 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 × ageing and at the lower strata Lamb × ageing × DevSpeed. This LMM, and subsequent LMM's, were fitted using the software package ASREML (Gilmour, Gogel, Cullis, & Thompson, 2006). The second analysis within Phase 1 analysed the standard deviation (SD) of the mean particle sizes within each test on the logarithm scale (logSD). The same LMM was used for logSD as for MeanPS. Thirdly, the maximum particle size for the quantiles (10%, 25%, 50%, 75% and 90%) for each sample were analysed, 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 sample.

2.6.2. Comparison between MFI and PS values – Phase 2

Here the data were analysed for differences between the means within methods (MFI and PS), the standard deviations (PS only) and the quantiles (PS only) across the relevant combinations of treatment (centrifugation vs. no centrifugation) and ageing (1 or 5 days) effects. The mean particle size for each sample was analysed using a LMM analysis. Fixed effects in the final model included effects for method, treatment, ageing and the interaction of these effects. Random effects included in the model were effects for method × date, method × lamb, method × lamb × ageing and random error with the random effects for the two methods (PS and MFI) having different variances and correlated within the same date and same lamb. For the analysis of logSD and log 25% quantiles (LT25), similar LMM analyses were undertaken, but without the inclusion of effects for method as these traits were only available for PS. The test for significance of the fixed effects was based on the *F*-tests with the denominator degrees determined using Kenward adjustments (Kenward & Roger, 1997).

2.6.3. Relationship between PS, MFI and shear force

All lamb loin × ageing combinations tested in Phases 1 and 2 were each tested for Warner–Bratzler shear force (unreplicated). The third stage of the analysis was correlation analyses of the results for the two phases separately and then each of these phases with these shear force (SF) values. Each response variable (mean, log(SD) and log(LT25)) from each of the two phases was separately analysed jointly with SF. For each correlation analysis, i.e. analysis of a given response for a given phase and SF, a LMM similar to the model for the given response/phase was used to model the data. The major differences in the model were inclusion of an effect for the SF test and the interaction of this effect with other effects in the model (age, lamb, ageing × lamb), inclusion of random effects for SF test results associated with cooking day, cooking batch, and inclusion of correlations across treatment effects at the lamb and the lamb × ageing level. Correlations across methods (MFI, PS and SF) were allowed to differ, but were assumed the same irrespective of treatment (e.g. speed and centrifugation).

3. Results

3.1. Homogenization speed (Phase 1)

In this study, the average mean particle sizes for samples aged 1 and 5 days differed significantly ($P < 0.001$) at a given speed and were described by the linear models average mean PS = 257.6 ± 0.94–7.69 ± 0.49 (speed/1000) and average mean PS = 215.1 ± 0.94–7.69 ± 0.49 (speed/1000), respectively. The differences in average mean particle size between the two ageing groups did not differ significantly across the range of speeds tested (see Fig. 1).

Analysis on the log scale of the standard deviation (SD) of PS indicated significant linear trends against the speed of homogenization for each of the two ages, with the linear trend significantly ($P < 0.001$) different for the two ages (Fig. 2; SD is predicted on the original scale). 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. The two models which describe the linear trends were average logSD = 5.92 ± 0.14–0.050 ± 0.008 (speed/1000) and average logSD = 6.47 ± 0.14–0.113 ± 0.008 (seed/1000) for samples aged for 1 and 5 days, respectively.

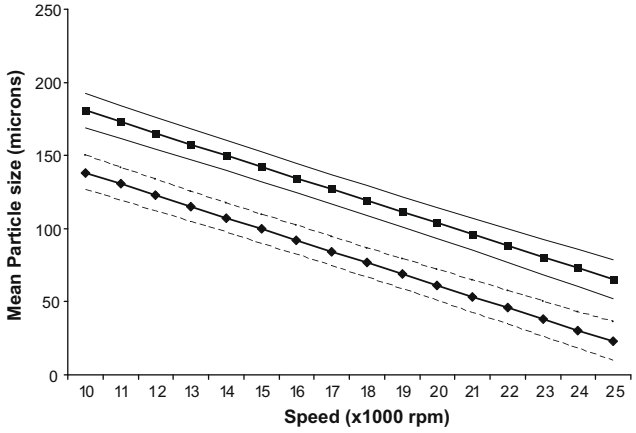

Fig. 1. Predicted mean particle size (μm) with the 95% confidence intervals indicated at various speeds of homogenization for meat samples aged 1 (■) and 5 days (♦).

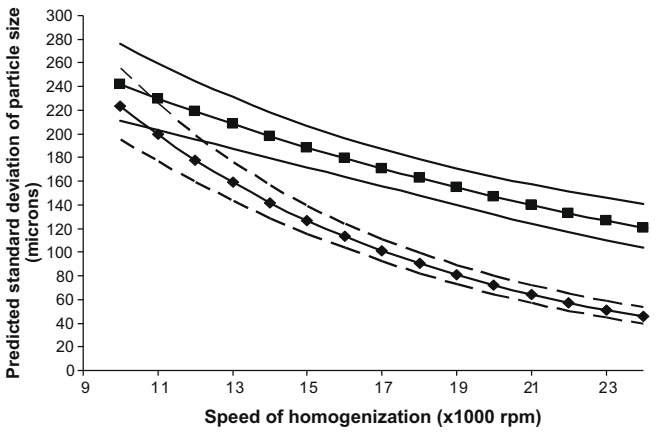

Fig. 2. Predicted standard deviation of mean particle size (μm) with the 95% confidence intervals indicated at various speeds of homogenization for meat samples aged 1 (■) and 5 days (♦).

Data analysis for the quantiles (10%, 25%, 50%, 75% and 90%) revealed that a speed of 16,000 rpm was the best for detecting differences between ageing treatments across the quantiles (Fig. 3) and the coefficient of variation for comparing ageing differences was uniformly minimized over the five quantiles at this speed (Fig. 4). Within these quantiles, the 25% level was the best for comparing the effect of ageing since it yielded the lowest coefficient of variation.

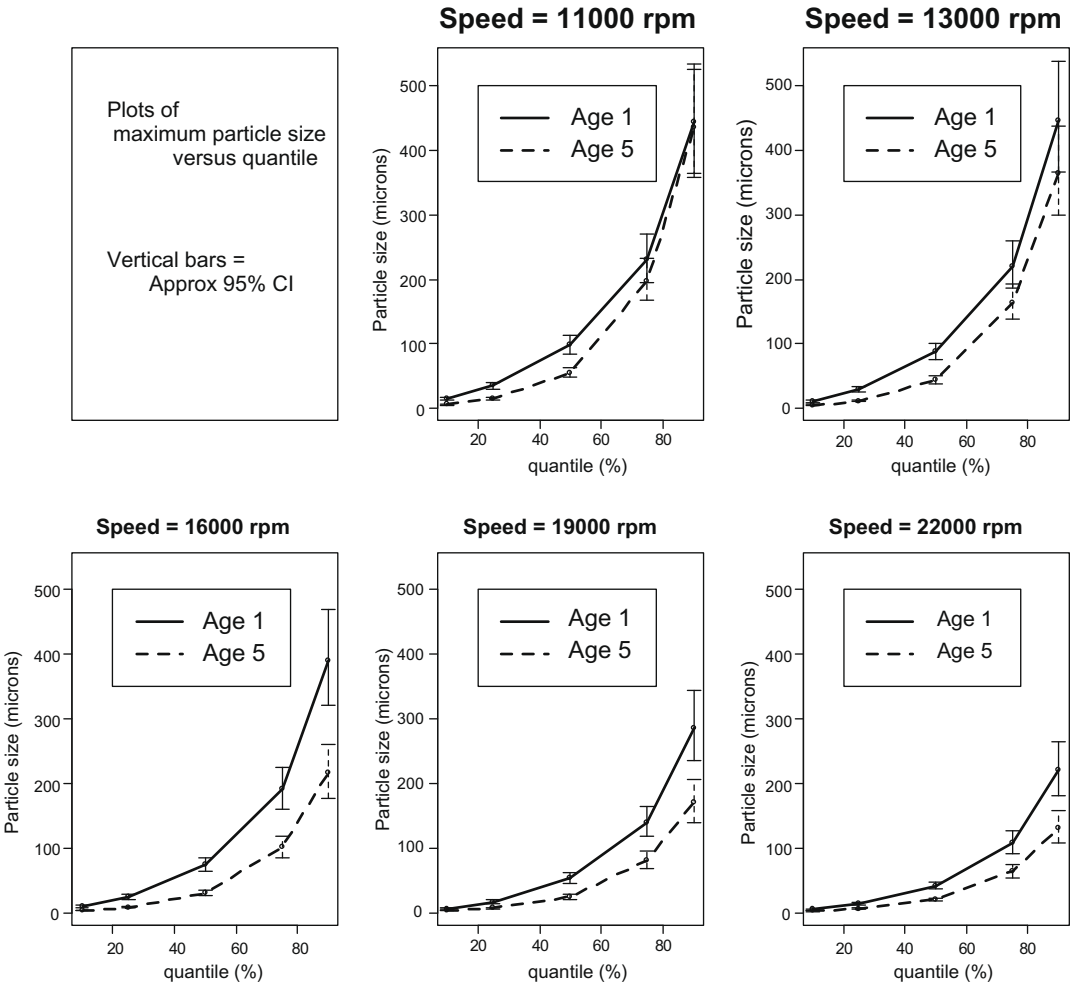

Fig. 3. The mean particle size (μm) with the 95% confidence interval against quantiles for meat samples aged 1 (♦) and 5 days (■) and homogenized at different speeds.

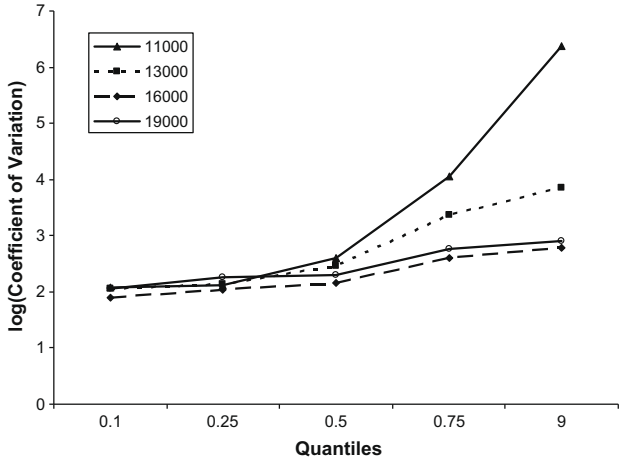

Fig. 4. The log(coefficient of variation) of particle size between lamb samples aged 1 and 5 days at different quantiles when homogenized at different speeds. Here the coefficient of variation, expressed as a percentage, is the magnitude of the standard error of the estimated difference relative to the mean estimated difference. Note the line for data at 22,000 rpm lies on the same line as that at 19,000 rpm.

3.2. Comparison between MFI and PS values (Phase 2)

3.2.1. Mean and standard deviation analysis

Fitting the model to the means for MFI and PS identified significant variation across testing dates with these effects for MFI and PS significantly correlated. The variation across lambs was significant for MFI, but was estimated as zero for PS. For the fixed effects, there were significant differences across treatments within method × ageing combinations. The predicted means and associated standard errors across the combinations of treatment × ageing, together with an LSD ranking of the predicted means within methods are given in Table 1. Also in Table 1 are given the estimated variance and coefficient of variation (with latter expressed as a percentage) for results from each treatment × ageing combination.

The analysis of SD on the log scale for particle size (logSD) indicated significant variation across dates, but with the variation due to lamb estimated as zero. Both treatment and ageing had significant effects with a significant interaction between treatment and ageing ($P < 0.05$). The predicted average SD's for the two methods at the different ageing times are shown in Table 2. The variation of results at the residual level of logSD was significantly different for the two ageing periods, with more variation for samples aged for 5 days.

3.2.2. Quantile analysis

For each quantile, analysed separately, there were significant random variations across dates while the variation across lambs was estimated as zero. The variation across samples (within lambs) did not differ significantly across combinations of treatment × ageing for the 10% and 25% quantiles. There was no significant difference ($P > 0.05$) between treatments at the 25% quantile (on log scale), but there was a significant ($P > 0.001$) ageing effect, with muscle aged for 1 day having a mean of 3.52 ± 0.07compared to muscle aged for 5 days with a mean of 2.19 ± 0.07. Both predicted means here were on the log scale.

3.2.3. Quality assurance

In conducting the comparison of the PS and MFI methods it became apparent that, for the means, the coefficient of variation for the PS method exceeded that of the MFI method, in particular for muscle aged for 5 days (see Table 1) and that a quality assurance system was required. The standard MFI method as developed by Martin et al. (2004) has an internal quality assurance system that detects when repeat testing is required. From this study it was possible to examine the repeatability of replicates and the associated variance for the PS method. Based on this if n results are obtained under repeatability condition and M and S are the mean and standard deviation of these results, then with a 5% false alarm rate further testing is required if;

$$S > \sigma\sqrt{\chi_v(0.95)/v}$$

where $v = n-1$, $\chi_v(0.95)$ is the 95 percentile of a χ^2 distribution on v degrees of freedom and σ^2 denoting the repeatability variance.

Based on a separate analysis of the results for Phase 2, we have that the repeatability standard deviation σ is estimated as $\sigma = 4.73 \times \exp(0.0058 \times \mu)$ where μ denotes the mean particle size for the sample, independent of ageing. In applying the above criteria to determine is further testing is required it is suggested that σ

Table 1
Predicted means and standard errors (s.e.) for mean particle size (PS; μm) and myofibrillar fragmentation index (MFI) at two ageing times according to centrifugation. The comparison for centrifugation is within methods.

Method	Centrifugation	Ageing (days)	Predicted mean	s.e.	LSD ranking	Variance	CV (%)
PS	No	1	172.3	9.31	b	1711.9	24.0
PS	No	5	80.6	9.24	c	1711.9	51.3
PS	Yes	1	195.0	9.31	a	1711.9	21.2
PS	Yes	5	64.0	9.12	c	1711.9	64.7
MFI	No	1	72.7	5.47	c	571.1	32.9
MFI	No	5	134.4	5.47	b	571.1	17.8
MFI	Yes	1	80.2	5.47	c	571.1	29.8
MFI	Yes	5	149.3	5.47	a	571.1	16.0

Table 2
Predicted means and standard errors for the standard deviation of the particle size (PS; μm) for centrifugation treatments at the two ageing times, (on log scale and on the original scale).

Centrifugation	Ageing (days)	Predicted mean (log scale)	Standard error (log scale)	Predicted mean SD	Standard error	LSD ranking
No	1	5.40	0.05	225.3	10.39	b
No	5	4.55	0.11	107.5	12.05	c
Yes	1	5.52	0.05	255.3	11.75	a
Yes	5	4.36	0.11	89.4	9.86	c

be replaced by $4.73 \times \exp(0.0058 \times M)$. This can then be applied to future results obtained for PS.

3.3. Relationship between PS, MFI and shear force

For Phase 1, there were no significant correlations between PS and SF within lamb samples for either, Mean PS, LT25 or logSD after adjusting for extraneous effects, e.g. ageing, speed and lamb (Table 3).

For Phase 2, after adjusting for extraneous effects, there were significant negative correlations between mean MFI and mean PS (−0.526) and mean MFI and mean SF (−0.382) while mean PS and mean SF were positively (0.233) correlated (Table 3). In this correlation analysis, cooking day was estimated to be a significant source of variation in SF values, contributing about 25% of the variation. This in turn contributed to reducing the correlation of mean MFI and mean PS with mean SF.

4. Discussion

4.1. Homogenization speed

As expected, the mean particle sizes for samples aged for 1 or 5 days differed significantly at a given speed. This is in agreement with the results of Lametsch et al. (2007) who found significant differences between samples aged 1 and 8 days and further supports the general notion that myofibrillar fragmentation increases with *post-mortem* storage of meat. It is also evident from previous work by Hopkins et al. (2004) that homogenization speed influences the size of myofibrillar fragments. Using the 10 mm shaft type homogenizer (Ystral), Hopkins et al. (2004) established that an ageing effect on MFI was best detected when homogenizing at 11,000 rpm. However, Lametsch et al. (2007) used a faster speed of 20,500 rpm with an 18 mm shaft type homogenizer (Ystral), which will have caused more destruction of myofibres compared to a slower speed and a smaller shaft. Apart from a different speed, Lametsch et al. (2007) also homogenized samples for only 30 s which was much shorter than used in the current study (60 s) and the 60 s proposed by Olson et al. (1976) as the optimum homogenization time. One important finding from this study was that among the various speeds tested, homogenizing at 16,000 rpm was best in terms of detecting an ageing effect for the new PS method since the coefficient of variation for the difference across ageing was uniformly minimized over the five quantiles. Furthermore, the standard deviation for particle size decreased with increasing speed and the ageing effect was best compared at the 25% quantile. These findings suggest that the results of Lametsch et al. (2007) may have been different if homogenization speed had been studied.

Table 3
Correlations between particle size (PS), myofibrillar fragmentation index (MFI) and shear force (SF) where LT25 is the PS data at the 25% quantile and SD is the standard deviation of the PS.

Traits	Correlation	Standard error
Phase 1		
Mean PS and mean SF	0.095	0.097
log(LT25) for PS and mean SF	0.081	0.089
log(SD) for PS and mean SF	0.290	0.113
Phase 2		
Mean MFI and mean PS	−0.526	0.031
Mean MFI and mean SF	−0.382	0.051
Mean PS and mean SF	0.233	0.028
log(LT25) for PS and mean SF	0.286	0.040
log(SD) for PS and mean SF	0.201	0.029

4.2. Comparison between PS and MFI

Lametsch et al. (2007) reported that the PS method was simpler and that the conventional MFI turbidity method was four times slower than the new PS method since the latter could be undertaken without centrifugation of the homogenate. They further proposed that measurements should be made on the homogenate in lieu of the partly purified myofibrils, hence further reducing sample preparation and analyzing time. In fact our results show that this also applies to the use of the MFI method.

From Table 1, it is apparent that there were significant differences ($P < 0.05$) across the two ageing groups for treatments within methods. For the PS method there was a significant difference, in the average mean particle size depending on whether centrifugation was used for samples aged for 1 day, with a higher average mean for samples subjected to centrifugation. Also for samples aged for 1 day, those subjected to centrifugation had a significantly higher standard deviation of the mean particle size (Table 2) than those not centrifuged. There were no significant differences between treatments (centrifugation vs. no centrifugation) in the averages for the mean and standard deviation for samples aged for 5 days. There was also no difference between treatments (centrifugation vs. no centrifugation) at the 25% quantile level, but an ageing effect was detected. Based on these results, we concur with Lametsch et al. (2007) that the PS method can be used without applying centrifugation, but as discussed below there is a need for a quality assurance system. Additionally based on the results for MFI when compared across treatments (centrifugation vs. no centrifugation), centrifugation is not required.

The coefficient of variation of mean particle sizes as measured by the PS method was higher compared to the MFI method because the MFI method as applied by Martin et al. (2004) was based on an internal quality assurance system while the PS method did not have any. Hence, in order to reduce the coefficient of variation in the mean particle size and to optimize the usefulness of the PS method, it was paramount to develop an internal quality assurance system for the PS method that could be used for any future PS work under the same repeatability conditions.

4.3. Comparison between shear force, PS and MFI

It was reported in a number of studies (eg. Culler et al., 1978; Olson et al., 1976; Takahashi, Fukazawa, & Yasui, 1967) that MFI had a high correlation with both Warner–Bratzler shear force and sensory tenderness scores. However, in this study, correlations between MFI and SF appeared generally low (−0.38), but similar to the −0.34 reported by Lametsch et al. (2007). Correlations between PS and shear force were low (0.23) and even lower than that reported by Lametsch et al. (2007). The coefficient of variation was greater in Phase 1 of this study compared to Phase 2. This outcome may be partly explained by the fact that various homogenization speeds were used since the primary aim was to establish the effect of speed on particle size. The coefficient of variation decreased significantly in the second phase where 16,000 rpm was used as the standard speed. Considering that various speeds were used in Phase 1, this reduced the amount of data analysed for a particular speed and to minimize the coefficient of variation associated with speed requires more data than we had for PS (Phase 1) and SF.

Another significant component of the variation across the means for the SF was variation across cooking days which contributed about 25% of the total SF variation. If this source of variation could be removed, correlations between the results could be increased. For instance the correlation between PS and SF was likely to increase to around 0.38 for log(LT25). Even though the cooking day effect was the main source of variation in this study, it was not considered in the work by Lametsch et al. (2007), nor did they give

any indication of the significance level of any other sources of variation so as allow a proper comparison and help explain the causes of variation.

5. Conclusions

The data from this study indicate that there is considerable scope to replace the conventional MFI method with a new method which is based on multi angle light scattering to measure particle size (PS). This was evidenced by the fact that the PS method could be used to detect differences in myofibrillar fragmentation due to ageing, without the requirement to centrifuge samples. However, to ensure the optimization of the proposed method, samples must be homogenized at 16,000 rpm and a quality assurance system applied. Correlations between PS and SF are low, but reducing the sources of variation offers considerable potential to improve these correlations.

Acknowledgments

The contribution of Tracy Lamb, David Stanley, Gordon Refshauge, Edwina Toohey and Sue Langfield (NSW DPI) to the collection of samples is gratefully acknowledged, as is the co-operation of Junee Abattoir (NSW). This work was funded by the NSW Department of Primary Industries and the Australian CRC for Sheep Industry Innovation. The senior author would like to thank the EU for funding his postgraduate scholarship under the Agris Mundus program.

References

Beckman Coulter. (2003). LS 13 320 laser diffraction particle size analyzer instrument manual. Beckman Coulter Inc., 11800 SW 147th Ave. Miami, FL 33196, USA.

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. *Journal of Food Science, 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 Science, 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 Science, 67*, 705–710.

Hopkins, D. L., Stanley, D. F., Martin, L. C., Toohey, E. S., & Gilmour, A. R. (2007). Genotype and age effects on sheep meat production. 3. Meat quality. *Australian Journal of Experimental Agriculture, 47*, 1155–1164.

Hopkins, D. L., & Thompson, J. M. (2001). The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. *Meat Science, 57*, 1–12.

Hopkins, D. L., & Thompson, J. M. (2002). Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact on tenderisation in beef and sheep meat. *Australian Journal of Agricultural Research, 53*, 149–166.

Hwang, I. H., Devine, C. E., & Hopkins, D. L. (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science, 65*, 677–691.

Karumendu, L., Hopkins, D. L., van den Ven, R., Kerr, M. J., Lamb, T. A., & Lanza, M. (2008). Particle size analysis: Effect of homogenization speed. In *Proceedings of the 54th International Congress of Meat Science and Technology, 8.7* (pp. 1–3). Cape Town, South Africa.

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics, 53*, 983–997.

Koohmaraie, M. (1994). Muscle proteinases and meat ageing. *Meat Science, 36*, 93–104.

Lametsch, R., Knudsen, J. C., Ertbjerg, P., Oksbjerg, N., & Therkildsen, M. (2007). Novel method for the determination of myofibril fragmentation post-mortem. *Meat Science, 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.

Monsón, F., Sañudo, C., & Sierra, I. (2004). Influence of cattle breed and ageing time on textural meat quality. *Meat Science, 68*, 595–602.

Olson, D. G., & Parrish, F. C. Jr., (1977). Relationship of myofibril fragmentation index to measures of beefsteak tenderness. *Journal of Food Science, 42*, 506–509.

Olson, D. G., Parrish, F. C., Jr., & Stromer, M. H. (1976). Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *Journal of Food Science, 41*, 1036–1041.

Takahashi, K., Fukazawa, T., & Yasui, T. (1967). Formation of myofibrillar fragments and reversible contraction of sarcomeres in chicken pectoral muscle. *Journal of Food Science, 32*, 409–413.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderisation? *Journal of Animal Science, 73*, 1351–1367.

Veiseth, E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2001). Technical note: Comparison of myofibril fragmentation index from fresh and frozen pork and lamb longissimus. *Journal of Animal Science, 79*, 904–906.