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The impact of homogeniser speed, dispersing aggregate size and centrifugation on particle size analyses of pork as a measure of myofibrillar fragmentation

T.M. Ngapo*, L. Vachon

Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 boul. Casavant Ouest, St-Hyacinthe, Québec J2S 8E3, Canada

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ABSTRACT

Particle size analysis has been proposed as a measure of myofibrillar fragmentation resulting from post-mortem proteolysis in meat. The aim of this study was to examine the effect of homogenisation speed, dispersing aggregate size and centrifugation on particle size characteristics of pork loin. Particle size characteristics were significantly ($P \leq 0.023$) greater for samples aged 2 than 8 d for all but the 80 and 90% quantiles. Differentiation with ageing was only achieved when homogenised at 11,000 rpm using the smaller dispersing aggregate (9 vs 13 mm rotor diameters). Centrifugation had no effect on particle size characteristics. Significant correlations with MFI ($r = -0.40$ to -0.81, $P < 0.001$) and shear force in meat aged at 3.7 °C ($r = 0.36$–0.47; $P < 0.001$) were observed. Weak or inconsistent correlations with shear force suggest adoption of particle size analyses as a method of tenderness classification unlikely. Rather, value lies in the detailed profiles of particle size distributions with meat ageing.

1. Introduction

The myofibrillar fragmentation index (MFI) is an indicator of the extent of post-mortem proteolysis in meat. This index has been estimated on homogenised muscle suspensions using techniques involving microscopy (Takahashi, Fukazawa, & Yasui, 1967), filtration (Davis, Dutson, Smith, & Carpenter, 1980) and turbidity (Davey & Gilbert, 1969; Olson, Parrish, & Stromer, 1976). The latter and most commonly used method has been optimised for type and speed of homogeniser (Hopkins, Littlefield, & Thompson, 2000; Hopkins, Martin, & Gilmour, 2004) with the particular aim of reducing sample preparation time. However, measurement of the protein concentration is a necessity for turbidity measures by spectrophotometry and the method is time consuming. Hence, particle size analyses which do not need prior information about the protein concentration of the sample have been proposed as an attractive alternative to the turbidity measures to estimate myofibrillar fragmentation (Karumendu, van de Ven, Kerr, Lanza, & Hopkins, 2009; Lametsch, Knudsen, Ertbjerg, Oksbjerg, & Therkildsen, 2007).

Lametsch et al. (2007) presented the use of multi-angle light scattering as a measurement of particle size in pork samples that had been homogenised with an 18 mm shaft type homogeniser at 20,500 rpm. Significant and strong correlations (r of -0.65 to -0.83; $P < 0.001$) were observed between particle size and turbidity measures. Furthermore, these authors found that the difference in surface mean diameter D[3,2] and the 10% quantile was more pronounced when measured on the homogenate than after two centrifugation steps and filtration. It was concluded that some of the smaller particles were lost during centrifugation and some of the larger fragments were removed during filtration and therefore particle size analyses should be made on the homogenate rather than the partly purified myofibrils.

While a relatively fast homogeniser speed was used by Lametsch et al. (2007) on pork samples, Karumendu et al. (2009) explored this new method of measurement of myofibril fragmentation by comparing five homogenisation speeds. Significant differences in mean particle size were observed and it was concluded that 16,000 rpm was the best homogeniser speed for detecting differences between lamb samples aged 1 and 5 d. Furthermore, the 25% quantile was the best particle size characteristic for comparing the ageing effect since it yielded the lowest coefficient of variation. These authors found a significant correlation between MFI and mean particle size reported as -0.526 (s.e. $= 0.031$). No significant differences in mean particle size were observed between centrifuged and non-centrifuged samples aged 5 d, but in samples aged 1 d a higher mean and standard deviation were observed in samples subject to centrifugation. It was concluded that particle size analyses can be undertaken without applying centrifugation, but that there is a

* Corresponding author.
E-mail address: tania.ngapo@agr.gc.ca (T.M. Ngapo).

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need for an internal quality assurance system to minimize the coefficient of variation.

The aim of the current study was to examine the impact of homogenisation speed, dispersing aggregate size and multiple centrifugation steps on particle size characteristics of pork loin aged 2 and 8 d with comparison to MFI. Furthermore, the study aims to validate the findings on a large number of samples and determine relationships with shear force.

2. Materials and methods

2.1. Pork, meat collection, ageing and portioning

The pork used in this project was described in an earlier study (Ngapo, Riendeau, Laberge, & Fortin, 2012). Briefly, pork loins were obtained from 108 Duroc × (Yorkshire × Landrace) pigs (54 each of gilts and barrows). The piglets were taken from about twenty different farms and raised, after weaning, in a conventional commercial environment at one location until slaughter. The animals were fed a diet of maize and soya meal base and were fasted for 16–20 h prior to slaughter at about 110 kg live weight. The pigs were electrically stunned and slaughtered by exsanguination in a commercial abattoir and the dressed carcasses were hung by Achilles tendon suspension in a chiller at 2 °C.

At 24 h post mortem (*p.m.*), boneless, long cut, skin-on loins were collected on-line from the left-side of the animal, at which time a slice (about 2.5 cm) at the level of the 3rd to 4th last ribs was removed. The remainder of the loin was vacuum-packaged, placed in boxes of two loins and transferred by refrigerated transport to the AAFC laboratory. The loins were randomly accorded an ageing period of 2, 4, 6, 8, 10 or 12 days *p.m.* at 3.7 ± 0.4 °C (18 loins per ageing period). At the completion of the assigned ageing period, skin and excess back fat were removed from the loins which were then portioned at 5 °C. The portions were individually vacuum-packaged and stored at − 40 °C until required. Portions were measured outwards from that removed at the level of the 3rd to 4th last ribs described above. The portions for MFI analyses were taken at 24–26 cm posterior to the 3rd to 4th last rib portion. None of the pork used in this study was classed as PSE (pH < 5.5 at 24 h *p.m.*, L* > 50, drip loss > 7.5%; Koćwin-Podsiadła, Krzęcio, & Przybylski, 2006), DFD (pH > 6.0 at 24 h *p.m.*, L* < 44, drip loss < 3%; Flores, Armero, Aristoy, & Toldrá, 1999) or cold shortened (sarcomere length measures were all ≥ 1.49 μm; Dransfield & Lockyer, 1985).

2.2. Chemicals, reagents and water

All chemicals and reagents used were at least analytical grade. Water was deionised except for that used in the particle sizer, which was distilled.

2.3. Homogeniser speed and dispersing aggregate size

Sample preparation was based on the methods described by Lametsch et al. (2007) and Karumendu et al. (2009). Duplicate samples of pork (2.0 g) were taken from the longissimus thoracis et lumborum (LTL) of the loin portion avoiding any visible fat and connective tissue and homogenised at 11,000, 13,000, 16,000 or 19,000 rpm using a Polytron (PT-MR 3100 with a PT 3012/2 T dispersing aggregate; Kinematica AG, Littau, Switzerland) or IKA (T25 DS1 Digital Ultra-turrax with S25N-18G dispersing tool, IKA®-Werke GmbH & Co. KG, Staufen, Germany) homogeniser. The Polytron PT 3012/2 T foam inhibiting dispersing aggregate was smaller (rotor and stator diameters of 9.0 and 12.0 mm, respectively, and referred to as 9/12 mm hereafter) than the IKA S25N-18G dispersing aggregate (rotor and stator diameters of 13.4 and 18.0 mm, respectively, and referred to as 13/18 mm hereafter). Samples were homogenised in 50 ml falcon tubes containing ice cold buffer (30 ml) twice for 30 s with 30 s held on ice between homogenisations. The buffer comprised 0.1 M KCl, 1 mM EDTA (di-sodium), 25 mM potassium phosphate (7 mM KH2PO4 and 18 mM K2HPO4, pH 7.0 at 4 °C), and 1 mM sodium azide. The myofibril suspensions were filtered (polyethylene 1.0 mm^2 mesh) to remove connective tissue and large particles, facilitated by washing with buffer (20 ml) and the filtrate was centrifuged (1000 *g*, 10 min, 2 °C). The supernatant was decanted and discarded and the pellets re-suspended in buffer (20 ml). Centrifugation and re-suspension steps were repeated two more times. Pellets were re-suspended in buffer (40 ml total) and the suspensions analysed for MFI and particle sizing.

The samples used for this comparison of homogeniser speed and dispersing aggregate size were from loins that had been aged to either 2 or 8 d *p.m.* (12 loins per ageing period, 24 loins total). Both dispersing aggregates were used on all loins. Two different homogeniser speeds were used on each loin. Therefore, each speed was tested on 12 loins (six each at 2 and 8 d ageing). The day of analysis of each speed × aggregate treatment was randomly selected. And, on a given day, two loins were tested for a speed × aggregate treatment.

2.4. Multiple centrifugation steps

Quadruplicate samples of pork (2.0 g) from eight loins were prepared as described in Section 2.3, but homogenising only at 11,000 rpm and using a Polytron (PT-MR 3100 with a PT 3012/2 T dispersing aggregate; Kinematica AG, Littau, Switzerland). The number of centrifugation/resuspension steps varied from 0 to 3. The samples were taken from loins that had been aged to either 2 or 8 d *p.m.* (4 loins per ageing period, 8 loins total). Every loin was tested for each of the four centrifugation step treatments.

2.5. Myofibrillar fragmentation measurement

2.5.1. MFI

The MFI method used was that of the classic turbidity method described by Olson et al. (1976) with modifications according to Hopkins et al. (2000). The protein concentration of the myofibril suspension was determined in duplicate using the biuret reaction (Gornall, Bardawill, & David, 1949) with a standard curve from 0 to 5 mg/ml BSA. Aliquots of the myofibril suspension were then diluted in buffer to a final protein concentration of 0.5 mg/ml in triplicate. The diluted protein was immediately poured into a disposable cuvette and the absorbance measured at 540 nm (Cary 50 Bio UV–Vis spectrophotometer, Varian Australia Pty Ltd., Australia). The mean of the triplicate absorbance readings was multiplied by 150 to give the MFI.

2.5.2. Particle size analyses

The particle size analyses were based on those of Karumendu et al. (2009) with modification. Particle size was analysed using a laser diffraction particle size analyser (Mastersizer 2000E, Malvern Instruments Ltd., Malvern, United Kingdom connected to a Hydro 2000MU large volume manual wet sample dispersion unit). The pump speed was set at 1500 rpm and myofibril suspension (6–15 ml) was added to stirring water (800 ml) in which the head of the dispersion unit was immersed, to achieve an obscuration of 10–11%. The suspension was allowed to stabilise 1 min after which data of particle size characteristics were obtained, including quantile distributions, average surface area moment mean (Sauter mean diameter or D[3,2]) and the average volume moment mean (De Broukere mean diameter or D[4,2]).

2.6. Validation

Comparisons of homogeniser speed, dispersing aggregate size and centrifugation steps were undertaken on a limited number of the samples described in Section 2.1. These samples were aged to either 2 or 8 d *p.m.* at 3.7 ± 0.4 °C. To validate the findings of these comparisons,