STREAMLINING THE DETERMINATION OF MYOFIBRILLAR FRAGMENTATION INDEX

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It has recently been reported by Hopkins et al. (2004) that a Ystral shaft-type homogeniser could be used for the determination of myofibrillar fragmentation index (MFI) as an alternative to an Omni blade-type homogeniser. This followed an earlier study (Hopkins et al. 2000) that developed a standard method based on a blade homogeniser operated at 15,000 rpm at 2 passes of 30 seconds duration. It was shown by Hopkins et al. (2004) that homogenising at 11,000 rpm for two 30-second bursts with the Ystral produced a similar variance to the standard method using the Omni. A shaft-type homogeniser is advantageous for several reasons: 1) it can be used for other applications, 2) the capital cost is lower, and 3) it reduces the time between successive homogenisations. It was contended that the repeatability between duplicates using the Ystral homogeniser could be improved by homogenising samples in a falcon tube using a 10 mm shaft, instead of in a beaker using an 18 mm shaft as reported by Hopkins et al. (2004). A study was conducted to test this contention.

Myofibrillar fragmentation index values were determined by the turbidity method on 10 samples of ovine Longissimus muscle, aged for 1 and 5 days, and processed from a frozen state. The samples were homogenised at 11,000 rpm for 2 x 30 second periods using a Ystral shaft homogeniser with a 10 mm blade, and held on ice for 30 seconds between homogenisations. Homogenisation was in 50 mL falcon tubes. Several other changes were made to the method as reported by Hopkins et al. (2004): 1) the final re-suspension of the pellets was in 40 mL of cold buffer instead of 10 mL, 2) a micro-plate reader (FLUOstar OPTIMA, BMG Labtechnologies, Victoria) operating at 560 nm was used for protein determination, and 3) turbidity was measured at 540 nm using a different spectrophotometer (Biochrom WPA Spectrawave S1000 Diode Array Spectrophotometer).

The mean MFI values for 1 and 5 day samples were 65 and 133, respectively, whereas in the report of Hopkins et al. (2004), the mean values were 37 and 103, respectively. Whilst the values with the smaller shaft were higher, the difference between day 1 and 5 samples (68) was comparable with the 66 recorded by Hopkins et al. (2004). Further, these differences were much greater than when using the Omni, but this could be an advantage in other experiments where a smaller effect on MFI values is being tested. The samples were also examined under a light microscope and a qualitative visual difference between 1 and 5 day aged samples was observed. Greater fragmentation of the myofibres was seen in the 5 day aged samples, consistent with Hopkins et al. (2004).

The most important finding from this work was that there was a high degree of repeatability (r = 0.95) between the duplicates within samples, much higher than the r = 0.55 reported by Hopkins et al. (2004).

In conclusion, it appears that a shaft type homogeniser can be used for the determination of MFI instead of a blade homogeniser. Refining the method by using a 10 mm shaft and homogenising in a falcon tube instead of in a beaker reduced the time between successive homogenisations, streamlined the method and, most importantly, it has increased the repeatability between duplicates.

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