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Impact of cooking temperature on pork *longissimus*, and muscle fibre type, on quality traits and protein denaturation of four pork muscles

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ABSTRACT

Variations in pork quality impact consumer acceptance, and fibre type differences between muscles contribute to this variation. The aim was to investigate the influence of variations in muscle fibre types and protein denaturation peaks across four pork muscles and the influence of ageing and cooking temperature on *longissimus* quality traits. The *longissimus*, *masseter, cutaneous trunci,* and *psoas major* from 13 carcases were removed 1-day *postmortem* and subjected to 0- or 14-days ageing (d0, d14). Quality traits, protein denaturation peak temperature (DSC), fibre diameter and fibre type proportions were measured. Cook loss for *longissimus* was similar on d0 and d14, but was higher on d14 for *masseter*, *cutaneous trunci,* and *psoas major*. Warner-Bratzler shear force was highest, and ultimate pH was lowest, for *longissimus*, and similar among *cutaneous trunci*, *masseter*, and *psoas major*. *Masseter* had lowest L* and highest a* and *longissimus* and *cutaneous trunci* had highest L* and lowest a*. The DSC temperature peaks for *longissimus* occurred at lower temperatures relative to the other muscles. Fibre diameter was largest for type-IIb fibres relative to type-IIa and type-I. *Longissimus* and *cutaneous trunci* had predominantly type-IIb glycolytic (71%, 51% respectively), *masseter* had predominantly type-IIa intermediate (50%) and *psoas major* had predominantly type-I oxidative (48%) fibres. The glycolytic l*ongissimus* had the lowest DSC temperature peaks and the lowest quality meat. *Masseter* had the highest proportion of type-I fibres but was generally similar in quality traits to *psoas major*, and also similar to *cutaneous trunci* which had more glycolytic fibres than *masseter*.

1. Introduction

The pork industry has anecdotal evidence of a decline in pork quality, expressed as a lack of tenderization *postmortem* resulting in increased toughness (Channon, Taverner, D'Souza, & Warner, 2014; Han et al., 2012). Pork quality, including tenderness and water-holding capacity (WHC), can be influenced by many factors, including pH, protease activity (Koohmaraie, 1992), collagen content and cross-linking (Hanan & Shaklai, 1995), sarcomere length (Pearson & Dutson, 1985), and protein denaturation *postmortem* and during cooking (Xiong, Brekke, & Leung, 1987). Muscle ultimate pH impacts quality in many ways, including colour, WHC, Warner-Bratzler peak shear force (WBPSF) (Bee, Anderson, Lonergan, & Huff-Lonergan, 2007; Lindahl, Henckel, Karlsson, & Andersen, 2006), consumer flavour, juiciness and tenderness, as well as shelf-life and microbial growth (Aberle, Forrest, Gerrard, & Mills, 2012; Kerth, 2013). The frequency and size of muscle fibres can also impact quality by playing a role in pH decline, ultimate pH, colour, and protein denaturation during cooking, which in turn influences, WHC and tenderness (Bhat, Morton, Mason, & Bekhit, 2018; Brewer & McKeith, 1999; Christensen, Kok, & Ertbjerg, 2006) although the underlying mechanisms and causes are unclear.

The various fibre types have different metabolism and function in the muscle. Type IIb fast twitch, glycolytic fibres predominantly utilize anaerobic glycolysis for energy and muscles with a predominance of type IIb fibres are characterised by faster rates of pH fall and lower ultimate pH *postmortem* (Schiaffino & Reggiani, 1994). Glycolytic fibres (Fernandez, Forslid, & Tornberg, 1994) have higher myofibrillar ATPase activity, glycogen content, and contraction speed coupled with a low

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concentration of myoglobin which results in lighter, paler meat. Type I slow twitch, oxidative fibres use aerobic metabolism, which results in higher *postmortem* muscle pH in muscles rich in type I muscle fibres. Oxidative fibres have low myofibrillar ATPase activity, glycogen content, and slower contraction speed, as well as increased concentrations of myoglobin, resulting in increased redness in meat (Fernandez et al., 1994; Klont, Brocks, & Eikelenboom, 1998; Schiaffino et al., 1989). Type IIa muscle fibres have intermediate myofibrillar ATPase activity relative to type I and IIb (Christensen, Henckel, & Purslow, 2004; Klont, Talmant, & Monin, 1994). Ashmore and Doerr (1971) noted that type IIa muscle fibres have oxido-glycolytic metabolism, and these intermediate muscle fibres can differentiate into either glycolytic type IIb fibres or oxidative type I fibres. Other fibre types have been identified including fibres which are transitional between the main fibre types and these are type I-IIa, IIa-IIb and I-IIb (Christensen et al., 2004).

Protein denaturation during cooking impacts the eating quality of pork as well as the physicochemical traits of WHC and WBSPF. As cooking proceeds and the temperature increases, specific proteins denature, and myofibrils, and consequentially muscle fibres, shrink transversely, then longitudinally. Both transverse and longitudinal shrinkage result in increased water expelled from the muscle, which negatively impacts tenderness (Purslow, Oiseth, Hughes, & Warner, 2016). We hypothesised that pork muscles with predominantly glycolytic fibres will have increased cook loss, drip loss, and WBPSF values compared to muscles with more oxidative fibres. The muscles *longissimus*, *masseter*, *cutaneous trunci*, and *psoas major* were analysed as they were expected to have different fibre type proportions. We expected the *longissimus* and *psoas major* to contain a mixture of fibre types, the *cutaneous trunci* to be predominantly type IIb glycolytic and the *masseter* to be predominantly type I oxidative based on previous studies in beef (Choi & Kim, 2009; Kang, Lee, & Hong, & Kim., 2011; Vaskoska et al., 2021) and pork (Lefaucheur, Edom, Ecolan, & Butler-Browne, 1995). The aim of this research was therefore to investigate the influence of muscle fibre proportion and diameter on pork quality and a further investigation was conducted onthe effect of cooking temperature of pork *longissimus* on WHC and WBPSF.

2. Materials and methods

2.1. Muscle collection, treatments, and sample collection

Ten *longissimus thoracis et lumborum* (hereafter called *longissimus*) and eight *masseter*, *cutaneous trunci*, and *psoas major,* were obtained from one side of 13 female pork carcasses at one day *postmortem* providing a total of 34 muscles. Note that we were not able to collect all muscles from all carcases hence the discrepancy between carcase and muscle numbers. Samples were trimmed of excess skin and fat. The *masseter*, *cutaneous trunci* and *psoas major* were cut in half and one half was randomly allocated to immediate processing (0 days ageing) and the other half was allocated to 14 days ageing. All samples destined for 14 days ageing were weighed, vacuum packed and stored at 1 °C for 14 days. On day 0, for the *longissimus* only, each *longissimus* was cut into 18 x 25 cm segments and each segment was randomly allocated to 0 or 14 days ageing. On day 0 for all muscles, colour was measured as described below in Section 2.5, approximately 5 g of tissue was cut from each muscle and used for histochemistry, approximately 1 g was cut for differential scanning calorimetry, and approximately 1 g was cut from each muscle for determination of pH (see Section 2.4 below). For both 0 and 14 days ageing, samples from each *longissimus* were randomly allocated to end point cooking temperatures of 50 to 85 °C as described in Section 2.6 below, for measurement of cook loss and WBPSF. *Masseter*, *cutaneous trunci*, and *psoas major* were cooked to the end point temperature of 70 °C for measurement of cook loss and WBPSF on day 0 and day 14, also as described in Section 2.6 below.

2.2. Purge loss

Samples stored for 14 days were weighed prior to vacuum packaging and storage. On day 14, samples were removed from the bag and weighed to determine purge loss. Purge loss was calculated using the final weight of samples after the ageing period, subtracted from the intial weight, and expressed as a percent of the initial weight prior to ageing.

2.3. Differential scanning calorimetry (DSC)

For each muscle, 1 g of tissue was used for DSC analysis; samples from $n = 10$ muscles were used for *longissimus*, samples from $n = 8$ muscles were used for *masseter*, *cutaneous trunci*, and *psoas major*. The samples were kept at 1 °C and prepared similar to previous studies (Vaskoska et al., 2021); briefly, 20 ± 1 mg was dissected from the 1 g sample, placed in a hermetically sealed aluminum pan and then placed in a furnace for DSC (Model 8000, Perkin Elmer, Waltham, Massachusetts, USA). The reference was a blank hermetically sealed aluminum pan. The heating for the DSC was conducted with a 1-min isothermal step to equilibrate the sample to 25 °C followed by a heating step of 5 °C/min from 25 °C to 90 °C. Once 90 °C was reached, an isothermal equilibration step occurred for 1 min at 90 °C followed by a cooling step with a change in sample temperature of 30 °C/min from 90 °C to 25 °C. The Pyris software (Perkin Elmer, Waltham, Massachusetts, USA) was used to calculate the maximum heat input for denaturation (peak temperature, Tmax, °C) and enthalpy (the heat of transition, ΔH, J/g). The enthalpy of each peak was calculated by integrating peak area determined by a baseline drawn for each peak, normalized for sample weight.

2.4. Muscle pH

Muscle pH was determined in duplicate for each sample using previously described methods (Bendall, 1973). The pH probe was calibrated at 4, 7, and 10 each day, at room temperature. A 100 ± 2 mg sample was powdered in liquid nitrogen, then homogenized in a buffer [5 mM iodoacetic acid, 150 mM HCl (pH 7.0)] at a 1:8 ratio (wt/vol). Samples were centrifuged at 10,000 RPM for 5 min, left to equilibrate to 25 °C, and then measured using a semi-micro pH glass electrode (HI 1093) and pH meter (HI 5221) (Hanna instruments, Woonsocket, RI, USA).

2.5. Colour measurement

Samples were allowed to bloom after cutting for 30 min at 1 °C, on day 0 and 14 prior to measurement. Following blooming, the samples were measured in triplicate in different places on the same surface of the muscle for CIE-$L^*a^*b^*$ [lightness (L^*), redness (a^*), and yellowness (b^*)] using a CR400 Chroma Meter (Konica Minolta, Japan) with an 8 mm aperture, light source D65, and 0° viewing angle (Frank et al., 2017).

2.6. Cooking procedure, cook loss and Warner-Bratzler peak shear force (WBPSF)

For 0 and 14 days ageing, samples from each *longissimus* were randomly allocated to control (for continual temperature monitoring), or end point cooking temperatures of 50, 55, 60, 65, 70, 75, 80, or 85 °C, for measurement of cook loss and WBPSF. *Masseter*, *cutaneous trunci*, and *psoas major* were cooked to the end point temperature of 70 °C after 0 and 14 days ageing. Samples were weighed prior to cooking to calculate cook loss (%) similar to previous studies (Vaskoska et al., 2021). A 1.2 mm gauge injection thermometer (9FX1150, FusionChef Core Temperature Sensor (PT100) for Diamond, Julabo) was placed in the geometric centre of the control samples and was used as an indicator for when samples reached the target temperature. Each sample was placed at the bottom of a polyethylene bag with 4 marbles added to each bag to prevent bags from floating in the water bath. Each bag was fixed

on a metal rack with clips which allowed for easy removal of the samples when the desired temperature was reached. Samples were placed in a preheated water bath (Julabo F38 water bath; John Morris Scientific, Melbourne, VIC, Australia) set to 25 °C. When the internal temperature of the control sample reached 25 °C, the water bath was set to 90 °C to achieve a heating rate of 5 °C/min. At the specified endpoint temperature, the samples were removed from the water bath and placed in iced water for 30 min and then transferred to a 1 °C chiller overnight until processing for WBPSF. After cooking and cooling, samples were weighed to determine cook loss (%) using cooked weight relative to raw weight, expressed as a percent of raw weight. At 24 h post-cooking, samples were cut into six blocks (*longissimus*, *cutaneous trunci*, *psoas major*) or three blocks (*masseter*) (approximately 50 mm × 10 mm × 10 mm) for each cooked sample ensuring the longest dimension was parallel to the muscle fibres. WBPSF was measured using a Lloyd texture analyser (LS5; Ametk, Berwyn, PA, USA) equipped with a Warner-Bratzler blade (V-shaped blade with a 60°angle, BesTech, Dingley, VIC, AUS) using a cross-head speed of 300 mm/min, and a load cell of 500 N. Each 50 mm × 10 mm × 10 mm section was cut with the WBPSF blade perpendicular to the muscle fibres. The data was obtained from the Nexygen software (Version 3; Bestech, Dingley, VIC, Australia) and the peak shear force (N) was recorded.

2.7. Histological analysis

Histological characterization of muscle fibres was conducted on the *longissimus*, *masseter*, *cutaneous trunci*, and *psoas major* from six randomly chosen pork carcasses ($n = 6$ for each muscle). Samples were cut from these muscles in 10 mm × 10 mm × 10 mm (H) cubes. These cubes were attached to a wooden cork board using Optimal Cutting Temperature compound (OCT, Leica, Wetzlar, Germany) and immersed in a pre-chilled solution of isopentane in liquid nitrogen. The cubes were stored at −80 °C until further analyses. While samples were still frozen, the cubes were sectioned into 10 μm slices using a cryostat (Model 1860, Leica, Wetzler, Germany) at −20 °C. Once cut, the 10 μm sections were collected on Superfrost glass slides (8 slides for each muscle) (Thermo Fischer Scientific, Waltham, Massachusetts, USA). The sections were then stored at −80 °C until further analysis. The sections were processed for histological determinations of fibre type frequency (%) and fibre diameter (μm) as described below.

Two staining methods were used to determine the diameter and proportion of fibre type I, IIa, and IIb; Nicotinamide Adenine Dinucleotide tetrazolium reductase (NADHTR) staining and acidic myosin Adenosine Triphosphatase (mATPase) staining. For NADHTR staining, muscle sections on the slides were thawed by incubation of the muscle section at 37 °C with NitroBlue Tetrazolium (mass concentration 1.25%, Merck, Kenilworth, New Jersey, USA) and β-Nicotinamide Adenine Dinucleotide (mass concentration 1%, Merck, USA) in Tris Buffer (0.2 M, pH 7.4) for 30 min similar to previous studies (Vaskoska et al., 2021). After incubation, samples were processed through a series of acetone: water solutions (30:70, 60:40, 90:10) and then rinsed in distilled water. Pale blue staining indicated glycolytic IIb fibres, dark blue staining indicated oxidative I fibres, and an intermediate light blue represented an intermediate IIa fibres (Vaskoska et al., 2021). ATPase staining was based on procedures using an acid preincubation step where the pH of the preincubation solution was determined specifically for each muscle using previous methods (Brooke & Kaiser, 1970; Guth & Samaha, 1969). Specifically, the pH of the preincubation solutions were as follows; *longissimus*, pH 4.35; *cutaneous trunci*, pH 4.40; *masseter*, pH 4.30; *psoas major*, pH 4.30–4.40. Black stained fibres are classified as type I, white stained muscle fibres are classified as type IIb/IIx and intermediate grey fibres are classified as type IIa. The proportion (%) and fibre diameter (μm) for mATPase stained samples were calculated at 5 random locations across each slide using ImageJ (Version 1.53, Java 1.8.0_172, 64-bit, https://imagej.nih.gov/ij/download.html National Institute of Health, USA). The NADHTR images were used to verify the muscle fibres identified using mATPase staining from each serial image. Briefly, fibre proportion was calculated as the number of muscle fibres counted for each muscle section and calculated as a percentage of the total muscle fibre number counted. The feret diameter (μm) of muscle fibres, which is calculated as the minimum distance of parallel tangents at opposing borders of the muscle fibre, was measured using a hemocytometer in ImageJ. The method used was similar to that of Briguet, Courdier-Fruh, Foster, Meier, and Magyar (2004) who reported that the diameter, along with proportion, of fibre types, allows for detection of subtle differences between muscle samples. Feret diameter is hereafter called diameter. The allocation of a fibre to type I, type IIa or type IIb/IIx was based on the mATPase staining after confirming the classification to muscle fibres based on the NADHTR staining. These staining procedures do not distinguish between muscle fibres IIx and IIb. In this study, where we use the term 'type IIb', these fibres are actually 'type IIb/IIx' fast glycolytic muscle fibres.

2.8. Statistical analysis

Data analysis was conducted using restricted maximum likelihood (REML) in GenStat (edition 16). Due to the differences in sample collection day and carcase, sampling collection day (Sampling_day) and carcase (Carcase) were added to the random model to account for any variation between sampling days and carcases. For the raw muscle colour and pH and cooked muscle characteristics of cook loss and WBPSF across all muscles, the fixed model was Muscle+Ageing+Muscle.Ageing and the random model included Sampling_day/Carcase/Muscle where the effect of muscle and ageing were analysed as well as the interaction (expressed as Muscle.Ageing). For the cooked parameters for *longissimus* at different temperatures, a REML model was used that included a fixed model of Ageing+Cooking_Temperature + Ageing.Cooking Temperature and a random model of Carcase/Sampling_day. Muscle fibre proportion and diameter data were analysed using REML in GenStat (edition 16) where the fixed model was Muscle+Fibre type-+Muscle.Fibre type and the random model included Sampling_day/Carcase where muscle and muscle fibre type were analysed as well as the interaction between muscle and muscle fibre type (expressed as Muscle.Fibre_Type). For the DSC data, the mean, minimum, maximum, and standard deviation for the Tmax, and mean and standard deviation for enthalpy, for each peak for each muscle were generated using Microsoft Excel (version 16).

3. Results

3.1. Effect of muscle and ageing

There was a difference in pH between muscles ($P < 0.001$) where the *longissimus* had a lower pH compared to the *cutaneous trunci*, *masseter*, and *psoas major* (Table 1). There was no effect of muscle on the purge loss during 14 days in a vacuum bag ($P > 0.05$). There was a difference in lightness (L^*), redness (a^*) and yellowness (b*), between muscles ($P < 0.001$ for all) where the *masseter* had the lowest L^*, highest a^* and highest b^* and the *cutaneous trunci* had the highest L^* and lowest a^* and the *longissimus* had the lowest b^*, and the other muscles were intermediate. The b^* values increased with ageing ($P < 0.05$). For cook loss, there was an interaction between muscle and ageing day ($P = 0.025$) where *masseter*, *cutaneous trunci*, and *psoas major* had much higher cook loss after 14 days ageing relative to 0 day ageing whereas for the *longissimus*, the cook loss was similar at day 0 and 14 (Table 1). For WBPSF, there was an effect of muscle ($P < 0.001$) where the *longissimus* had the highest WBPSF compared to *masseter*, *cutaneous trunci*, and *psoas major*.

3.2. Effect of cooking temperature and ageing for the longissimus

This study also investigated the effect of cooking temperatures and ageing for the *longissimus* (Fig. 1). There was a significant effect of

Table 1
Effect of muscle (M; *longissimus thoracis et lumborum*, LTL; *masseter,* Ma; *cutaneous trunci,* CutT; *psoas major,* PsoasM) and ageing (A; 0, 14 days) on meat quality traits. Least squares means are shown as well as the standard error of the difference (SED) and *P*-values. For the variables with ageing as a treatment, the SED is for the interaction.

							P-values		
	Day	LTL	Ma	CutT	PsoasM	SED	M	A	M × A
pH	0	5.78	6.06	6.16	6.01	0.091	< 0.001		
Purge (%)	14	5.92	3.61	3.64	4.67	1.488	0.41		
*L**	0	56.9	48.3	60.1	50.9	1.60	< 0.001	0.51	0.85
	14	57.6	47.9	60.9	51.1				
*a**	0	7.3	19.9	8.4	14.2	1.22	< 0.001	0.39	0.26
	14	6.2	21.0	10.7	14.7				
*b**	0	6.7	9.4	9.6	8.9	0.85	<0.001	0.011	0.12
	14	8.6	10.9	9.7	9.3				
Cook loss (%)	0	20.3	10.7	9.40	13.3	3.671	0.36	< 0.001	0.025
	14	22.8	21.8	27.7	23.1				
WBPSF[1] (N)	0	36.5	27.8	25.0	21.4	4.364	< 0.001	0.28	0.47
	14	36.3	19.8	25.5	19.4				

[1] WBPSF = Warner-Bratzler peak shear force.

temperature ($P < 0.001$) and ageing ($P = 0.028$) on *longissimus* WBPSF. As temperature increased, three stages of change in WBPSF are evident; WBPSF increased from 55 °C to 60 °C, showed no change from 60 °C to 70 °C, and increased from 70 °C to 80 °C. On day 14, the WBPSF values were lower than day 0 samples. There was no interaction between temperature and ageing for WBPSF ($P = 0.26$). However, there was an interaction between temperature and ageing for cook loss ($P = 0.002$) where cook loss of samples was higher at 14 days, relative to day 0 samples, over 60–65 °C but was cook loss was lower for 14 days samples at 80 °C.

3.3. Muscle fibre histological analysis

There was a mix of muscle fibres in each muscle and Fig. 2 shows the effects of muscle fibre type and muscle on the proportion of each fibre type and on the muscle fibre diameter. The *longissimus* had a higher proportion of type IIb fibres (71%) compared to type IIa (15.6%) and type I (12.9%). The *cutaneous trunci* had a high proportion of type IIb muscle fibre (68.4%), followed by type IIa (19.6%) and type I (12.0%). *Masseter* was composed of similar proportions of type IIa (49.7%) and type I (42.4%) and a low proportion of type IIb (7.9%). The *psoas major* was predominantly type I (41.9%) and had a lower percentage of type IIb (32.0%) and type IIa (26.1%), There was an interaction between muscle and muscle fibre ($P < 0.001$) where *longissimus* and *cutaneous trunci* had much higher proportions of type IIb glycolytic fibres while *masseter* and *psoas major* had higher proportions of intermediate and oxidative fibres with fewer glycolytic fibres. Muscle fibre diameter was different between muscle fibre types ($P < 0.001$) and between muscles ($P < 0.001$) where type IIb fibres had a greater diameter compared to intermediate IIa fibres which were generally larger than type I fibres, except for *psoas major,* where type I and IIa had similar diameter. There was an interaction between muscle and fibre type ($P < 0.001$) where the diameter of type IIa was generally higher then type I except in *psoas major* and the *longissimus* had much higher diameter for type IIb fibres.

3.4. Differential scanning calorimetry

The specific denaturation peaks for each muscle are illustrated in Fig. 3 and the results are shown in Table 2. Table 2 shows that four distinguishable denaturation peaks were present for the *longissimus* and *psoas major*, three denaturation peaks for *cutaneous trunci*, and two denaturation peaks for *masseter*.

4. Discussion

It is well-known that muscle pH directly impacts meat quality characteristics, and in this study, the muscles varied in pH. The *longissimus* pH had the lowest pH, with a pH of 5.80 and the pH of the other muscles were 6.0–6.2. The *longissimus* pH was higher than found in some other studies in Australia where pH values in the *longissimus* were between 5.45 and 5.70 (Channon et al., 2014; Channon, D'Souza, Hamilton, & Dunshea, 2013; Channon, Payne, & Warner, 2000; Jose, Trezona, Mullan, McNaughton, & D'souza, 2013). The high pH across all muscles, especially in the *cutaneous trunci*, is indicative of stress, physical activity and/or feed withdrawal prior to slaughter. Unfortunately, no data was provided on the pre-slaughter conditions for the pig carcases we sampled. A recent survey of almost 15,000 carcases across all the major supply chains in Australia showed that the average pH of the *longissimus* was 5.70 where the lower and upper quartile cut-offs of *longissimus* pH were 5.59 and 5.78 (Dunshea, Lealiifano, Trezona, Gole, & Hewitt, 2022). Thus, in our study, the *longissimus* pH was only 0.1 units higher than the industry average, hence typical for the Australian pig population. Muscles composed of a greater proportion of intermediate and oxidative fibres, generally have higher ultimate muscle pH (England et al., 2016). Oxidative muscles, such as the *masseter* and *psoas major*, tend to have higher muscle pH because muscle glycolysis and pH decline terminate prematurely (England, Matarneh, Scheffler, Wachet, & Gerrard, 2014) hence causing darker colour (Kim, Yang, & Jeong, 2017). Shen et al. (2015) compared eight pork muscles and found that ultimate muscle pH values decreased in order of oxidative fibres > intermediate oxidative fibres > glycolytic fibres where the *masseter* was considered oxidative, the *psoas major* was considered intermediate, and the fast twitch glycolytic muscles included the *longissimus*. The high pH in the glycolytic *cutaneous trunci* muscle was not expected as others have reported that the beef *cutaneous trunci* has a low pH of 5.60 (Vaskoska et al., 2021).

The proportion of each muscle fibre type was different between muscles, as indicated by the interaction, and each fibre type was different in diameter. As expected, and across all muscles, type IIb fibres had the biggest diameter, type I fibres had the smallest, and type IIa fibres were generally a little bigger than type I, except the *psoas major* where type I and IIa had similar diameter. Across the *longissimus* and *semimembranosus*, others have measured cross-sectional area (CSA) and have shown that for muscles in the pork carcass, type IIb fibres are always a lot bigger, and type IIa and type I fibres are generally similar in CSA, with some authors showing that type IIa were bigger then type I, and other authors showing type I were bigger then type IIa (Christensen et al., 2006; Gil et al., 2008; Henckel, Erlandsen, & Bejerholm., 1997; Kim, Jung, Yang, Lim, & Joo, 2013; Lee et al., 2012; Maltin, Matthews, Porteig, & Delday, 1997). In our study, the *longissimus* had the highest frequency of type IIb fibres (71%) and thus was defined as glycolytic, similar to other studies (Essén-Gustavsson & Fjelkner-Modig, 1985; Kim, Jung, et al., 2013; Ryu & Kim, 2005a; Wheeler, Shackelford, & Koohmaraie, 2000) although Gil et al. (2003) showed lower (53%) levels of

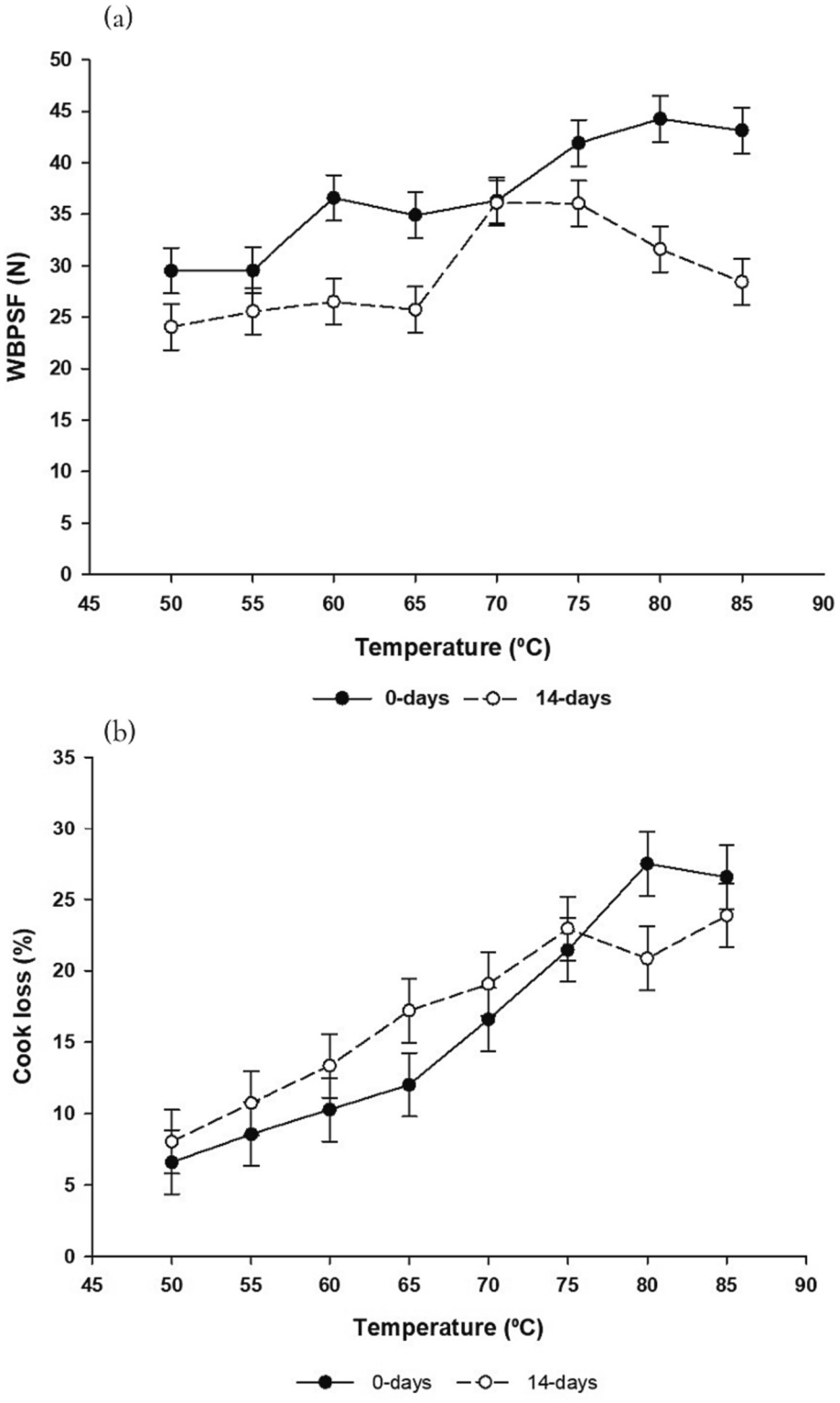

Fig. 1. Effect of cooking temperature (T; 50, 55, 60, 65, 70, 75, 80, and 85 °C) and ageing (A; 0, 14 days) on (a) Warner-Bratzler peak shear force (WBPSF, N) and (b) cook loss (%), for the *longissimus thoracis et lumborum* (LTL) Least squares means are shown and the vertical line is the least significant difference for the interaction between Cooking Temperature and Ageing (T × A). *P*-Values for WBPSF: T, $P < 0.001$; A, $P = 0.028$; T × A, $P = 0.254$. *P*-values for cook loss: T, $P < 0.001$; A, $P = 0.408$; T × A, $P = 0.002$.

IIb fibres in pork *longissimus* About 70% of the muscle fibres for the *cutaneous trunci* were type IIb, hence this muscle would also be defined as glycolytic, which is different to Gunawan et al. (2007) who found pork *cutaneous trunci* had ~45% type IIB and ~ 53% type IIA/IIX. *Psoas major* had a slightly higher proportion of type I fibres and an equivalent proportion of type IIa and IIb fibres and hence would be defined as oxido-glycolytic or mixed fibre type muscle, whereas Cheng, Song, and Kim (2021) defined pork *psoas major* as oxidative. The *masseter* was the only muscle with a high proportion of type I and IIa, with a very low proportion of type IIb, hence would be defined as oxidative, which is

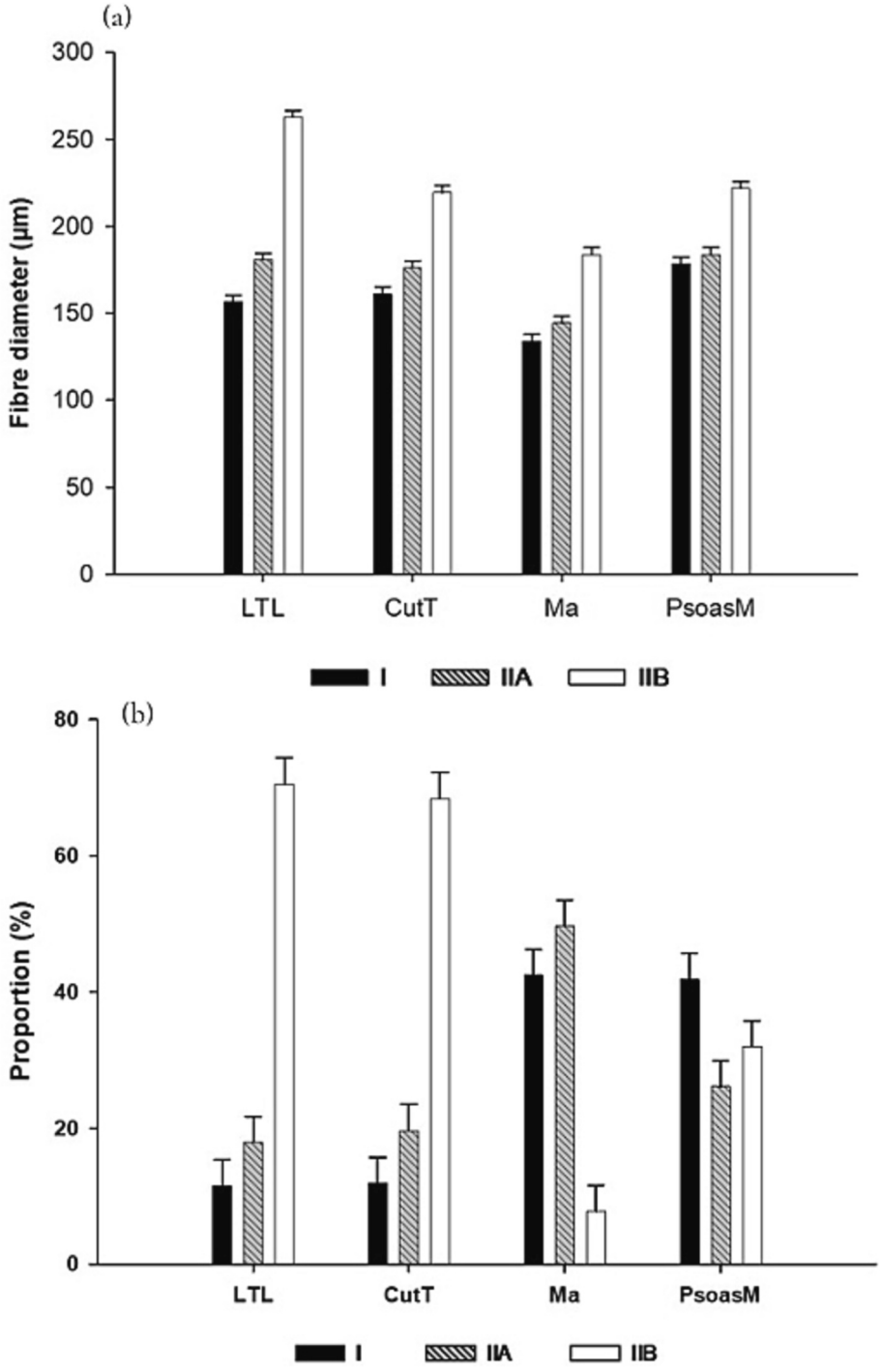

Fig. 2. The effect of muscle (M; *longissimus thoracis et lumborum* LTL, *masset*er Ma, *cutaneous trunci* CutT, and *psoas major* PsoasM) and fibre type (FT; I,IIa, IIb) on (a) the fibre diameter (µm) and (b) proportion (%) of fibre types. The means are least squares means and the SED is shown as a bar above the mean. *P*-values for fibre type proportion: M, $P = 1.0$; FT, $P < 0.001$; M × FT, $P < 0.001$. *P*-values for diameter: M, $P < 0.001$; FT, $P < 0.001$; M × FT, $P < 0.001$.

similar to others definition of pork *masseter* as oxidative (Gunawan et al., 2007; Shen et al., 2015) although Realini et al. (2013) reported that pork *masseter* was oxido-glycolytic as it had twice the number of type IIa fibres relative to type I. In beef, the *masseter* is composed of predominantly type I oxidative fibres (Vaskoska et al., 2021), hence although the pigs anatomy and physiology is very different to cattle, our study indicates that the oxidative nature of the *masseter* is similar between pork and beef.

The variation in muscle fibre proportion and fibre diameter between muscles in our study can be compared to the observed variation in quality traits. Our study found that the *longissimus* had lighter, less red surface colour, higher cooking loss and higher WBPSF which confirms other studies which showed that muscles composed of predominantly glycolytic fibres exhibit increased lightness, drip loss, cook loss, and toughness, and these studies attributed the quality differences to increased *postmortem* glycolytic metabolism and muscle fibre size (Kim, Ryu, Jeong, Yang, & Joo, 2013; Lee et al., 2012; Lee, Joo, & Ryu, 2010) rather than muscle proportion. In our study, the glycolytic *cutaneous trunci* did not exhibit the typical quality traits of low WHC and toughness likely due to the higher ultimate pH. Increased muscle fibre diameter is

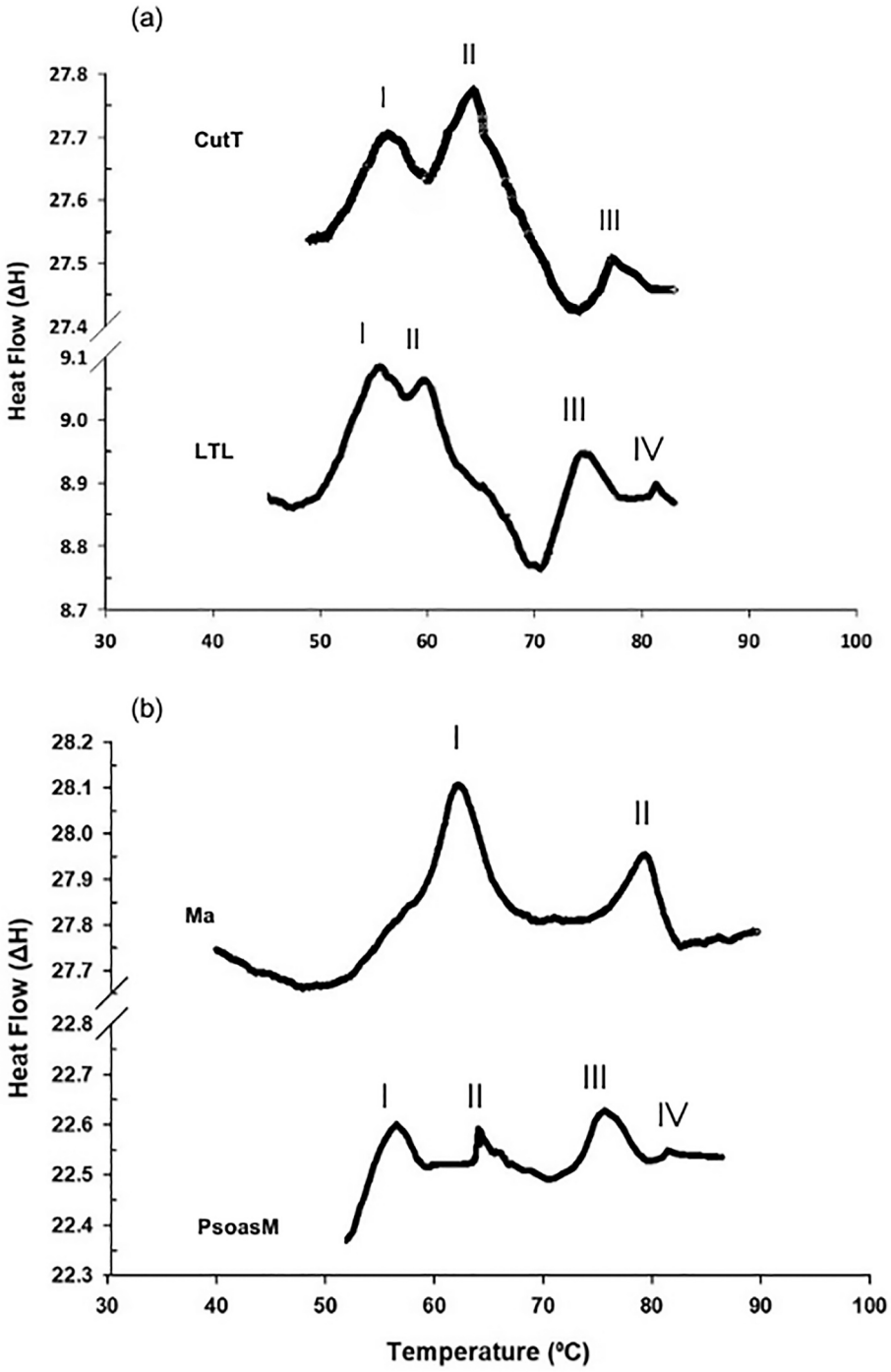

Fig. 3. Representative endothermic heat flow (thermogram) for (a) *longissimus thoracis et lumborum* (LTL) and *cutaneous trunci* (CutT), and (b) *masseter* (Ma) and *psoas major* (PsoasM) measured by differential scanning calorimetry (DSC) at a heating rate of 5 °C /min. The endothermic peaks for each muscle are marked as I, II, III and IV.

generally associated with increased toughness (Kim, Ryu, et al., 2013) and this was evident in the tougher meat in the *longissimus*, which had larger fibre diameter glycolytic fibres, but was not evident in the *cutaneous trunci*. Ryu and Kim (2005b) found a positive correlation between proportion of type I fibres and WBPSF ($r = 0.23$) in the pork *longissimus* and a negative association with drip loss ($r = -0.06$) and L^* ($r = -0.18$). They also found a positive relationship between type IIb fibres and L^* ($r = 0.34$) and drip loss ($r = 0.36$) indicating that increased proportions of type I fibres is associated with lower drip loss and results in darker products compared to muscles with more glycolytic fibres. Ryu and Kim (2005b) also found a negative correlation between the WBPSF in pork *longissimus* and CSA of type I ($r = -0.16$), type IIa ($r = -0.24$) and IIb ($r = -0.18$) fibres. Nam et al. (2009) found a negative correlation between CSA of fibres and sensory tenderness ($r = -0.15$) in pork *longissimus*, where sensory tenderness decreased as fibre area increased, indicating a tougher product as perceived by consumers. Therefore, both the diameter and the proportion of muscle fibres are important due to their impact on tenderness.

Tenderness of pork products is an important quality trait and varies between muscles and during ageing. In our study, the *longissimus* had higher WBPSF values compared to the other muscles, similar to findings by Channon, D'Souza, and Dunshea (2016) and Rees, Trout, and Warner

Table 2
Summary data for the transition temperature peaks (Tmax) and denaturation enthalpy ΔH of the major DSC peaks on the thermograms for *longissimus thoracis et lumborum* (LTL), *masseter* (Ma), *cutaneous trunci* (CutT), and *psoas major* (PsoasM) using a heating rate of 5 °C/min. SD = standard deviation.

Muscle	Peak	Tmax (°C)				Enthalpy ΔH (J/g)	
		Mean	SD	Minimum	Maximum	Mean	SD
LTL	I	52.4	3.75	42.5	60.4	0.21	0.165
	II	60.5	7.35	52.2	71.5	0.30	0.258
	III	74.5	5.40	61.9	78.6	0.13	0.285
	IV	82.5	3.41	77.1	87.9	0.13	0.192
Ma	I	–	–	–	–	–	–
	II	62.0	0.85	60.0	63.3	0.45	0.222
	III	78.9	0.60	78.0	80.1	0.33	0.151
	IV	–	–	–	–	–	–
CutT	I	57.2	4.75	46.0	62.5	0.59	0.955
	II	65.5	5.65	52.1	74.4	0.57	1.032
	III	77.0	4.96	61.1	79.9	0.28	0.123
	IV	–	–	–	–	–	–
PsoasM	I	56.8	4.96	45.1	62.5	0.19	0.228
	II	65.0	4.14	61.1	73.3	0.23	0.275
	III	75.0	4.58	67.0	79.4	0.22	0.175
	IV	82.2	2.60	78.8	85.7	0.15	0.181

(2002). The WBPSF values for *masseter*, *cutaneous trunci*, and *psoas major* were similar and overall, the muscles had lower WBPSF values after 14 days ageing although the difference was very small. The limited tenderization in the pork muscles in this study is similar to Channon et al. (2016) who also found no effect of ageing on sensory tenderness of *longissimus* and *biceps femoris*.

Protein denaturation impacts cooked pork quality, and the denaturation peaks for specific proteins can vary with species and muscle fibre type as well as the pH of the muscle (Amako & Xiong, 2001). In our study, the *longissimus* had the lowest peak denaturation temperatures for peaks I and II, which are generally assigned to myosin and collagen/sarcoplasmic proteins respectively (Purslow et al., 2016). The *masseter* only had two protein denaturation peaks at 62 and 79 °C which can be assigned to myosin/collagen/sarcoplasmic proteins and actin respectively (Vaskoska et al., 2021). The *cutaneous trunci* which is similar in fibre type proportions and glycolytic classification to the *longissimus*, had higher peak temperatures then the *longissimus* for peaks I and II, which was not expected. Our results for the denaturation peaks for each muscle are similar to other data in the pork *longissimus* (Bertram, Wu, van den Berg, & Andersen, 2006), beef *cutaneous trunci* and *masseter* (Vaskoska et al., 2020), and pork *psoas major* (Zielbauer, Franz, Viezens, & Vilgis, 2016). The *psoas major* and *masseter* likely have increased peak denaturation temperatures for peaks I and II due to more oxidative fibres compared to the *cutaneous trunci* and *longissimus* as previously described by Vaskoska et al. (2021), as the myosin heavy chain in type I oxidative fibres has increased thermal stability compared to myosin heavy chain of type IIb (Egelandsdal, Martinsen, Fretheim, Pettersen, & Harbitz, 1994). In this study, the denaturation peaks occurred at lower temperatures for pork *longissimus* muscles compared to beef studies (Pospiech, Greaser, Mikolajczak, Chiang, & Krzywdzinska, 2002; Vaskoska et al., 2021; Xiong et al., 1987). This supports the findings of Xiong et al. (1987) who analysed beef *longissimus,* pork *longissimus,* and chicken *pectoralis major* and *pullum femur* and demonstrated that beef had higher protein denaturation peaks then pork and chicken due to higher proportion of oxidative fibres.

Denaturation results in macromolecular structural change in proteins and can result in muscle fibre shrinkage, impacting cook loss and tenderness. In our study, the unaged and 14 days aged *longissimus* showed a linear increase in cooking loss with temperatures up to 75 °C. These increases in cook loss with temperature are similar to those for beef (Xiong, 1994; Xiong et al., 1987) and can be explained by our pork *longissimus* DSC thermograms for protein denaturation associated with myofibril shrinkage. Bendall and Restall (1983) reported that, longitudinal shrinkage of individual beef myofibrils generally begins to occur at 64 °C which could be associated with the increased cook loss observed in our study that occurred between 60 and 70 °C. Channon et al. (2016) found an increase in cook loss between 70 °C and 75 °C in pork *longissimus* but found there was no significant difference in cook loss for pork *biceps femoris* cooked to the same temperature which could be a result of a greater proportion of type I fibres in *bicep femoris* (King et al., 2018) compared to the *longissimus* (Ryu, Rhee, & Kim, 2004). During cooking, myofibrils first shrink transversely between 50 °C and 65 °C (Bendall & Restall, 1983; Purslow et al., 2016; Tornberg, 2005) while longitudinal shrinkage begins around 70 °C and predominantly occurs between 70 °C and 75 °C but can continue to occur up to 80 °C (Purslow et al., 2016). Similar to our results, others have demonstrated a linear increase in cook loss with cooking temperature in muscles and muscle blocks and attribute this to water expulsion from the muscle structure (Vaskoska et al., 2021; Vaskoska, Ha, Naqvi, White, & Warner, 2020). Water expulsion results from the transverse and longitudinal shrinkage but although it would be logical to consider this shrinkage is co-operative, it appears that longitudinal shrinkage is most closely associated with cooking loss, rather than transverse shrinkage (Vaskoska et al., 2021; Vaskoska, Ha, White, & Warner, 2023).

Cook loss and WBPSF are strongly influenced by the cooking temperature and in our study, we measured changes in these quality traits in the *longissimus* at cooking temperatures 50–85 °C. Cooking temperatures between 70 and 75 °C (Purslow et al., 2016) are associated with actin denaturation (Andersson, Andersson, & Tornberg, 2000; Pospiech et al., 2002; Xiong et al., 1987) and longitudinal shrinkage which, initiates at 70 °C and accelerates to 75 °C and, in some cases, can continue up to 80 °C (Purslow et al., 2016), causing increased cook loss. Importantly, on day 0, WBPSF increased between cooking temperatures 70 and 75 °C, indicating a tougher product, which corresponds to the increase in toughness in beef *sternomandibularis* seen by Davey and Gilbert (1974). This similarity in peak toughness between pork *longissimus* and beef *sternomandibularis* supports the concept that actin denaturation, not collagen, is the driving force for shrinkage and toughness, as the thermal sensitivity of actin is reasonably conserved across species and muscles (Xiong et al., 1987). Higher WBPSF values in the *longissimus* occurred at all end-point cooking temperatures at or above 70 °C, relative to below 70 °C, in the current study. This indicates that the transverse and longitudinal shrinkage in pork *longissimus* occurs at lower temperatures during the cooking process compared to more oxidative pork muscles, such as *masseter* and *psoas major* and potentially muscles from species that have a greater proportion of oxidative fibres, such as beef. In this study, the WBPSF toughness of fresh (0 days aged) pork *longissimus* increased when cooked to temperatures $\geq$70 °C whereas the pork *longissimus* aged for 14 days was more tender at temperatures $\geq$75 °C. This suggests that if pork *longissimus* is cooked to temperatures <65 °C), water loss and WBPSF values would be reduced, as the lower temperature would limit longitudinal shrinkage within the *longissimus* during cooking. However, there needs to be further investigation to ensure food safety (Purslow et al., 2016). Adjusting the recommended cooking temperature for specific muscles could help avoid unnecessary shrinkage, reduce cook loss, and improve tenderness.

Water-holding capacity of raw and cooked meat is related to transverse and longitudinal shrinkage, which are related to muscle ultimate pH and rate of *postmortem* pH decline (Purslow et al., 2016). In our study, the three muscles with a higher pH were similar in purge loss to the *longissimus*, which had a lower pH than the other muscles. The lower muscle pH for the *longissimus* is similar to Warner, Kauffman, and Russel (1993) who compared ten muscles, including *longissimus* and *psoas major*, and the low pH in the *longissimus* in our study likely caused the higher cook loss and WBPSF compared to the other muscles. For all muscles in our study, cook loss was higher on day 14 compared to day 0, which was similar to the higher cook loss with ageing for 8 days in sheep *longissimus* (Warner, Kerr, Kim, & Geesink, 2014) and with ageing for 14 days in beef *psoas major*, *semitendinosus* and *biceps femoris* (Vaskoska, Ha,

Naqvi, et al., 2020). The *longissimus* had higher cook loss on day 0, compared to the *masseter*, *cutaneous trunci*, and *psoas major*, which as discussed above, is likely due to the lower pH in pork *longissimus* in conjunction with a greater proportion of glycolytic type IIb fibres. Bidner et al. (2004) demonstrated that as the pork longissimus pH increases from 5.3 to 6.8, the cook loss decreases from 27% to as low as 15%.

Meat ultimate pH and muscle fibre proportion also influence meat colour. In our study, the glycolytic muscles *longissimus* and *cutaneous trunci* were the lightest and least red and the oxidative muscles *psoas major* and *masseter* were the darkest (lowest L^*) and most red (highest a^*), similar to the results of Warner et al. (1993), Phillips et al. (2001) and Sánchez Del Pulgar, Gázquez, and Ruiz-Carrascal (2011). This was expected as muscles composed of predominantly glycolytic fibres have lower concentrations of myoglobin and are expected to be a lighter, less red colour compared to muscles composed of predominantly oxidative fibres (Joo, Kauffman, Kim, & Park, 1999; Karlsson, Klont, & Fernandez, 1999; Klont et al., 1998). Muscles with lower pH generally have increased light reflectance and scattering which causes a paler colour, and elevated pH is associated with a darker, less red product (Adzitey & Nurul, 2011; Hughes, Clarke, Purslow, & Warner, 2017; Hughes, Purslow, & Warner, 2014; Warriss, 2010). This was not evident in our study as the *cutaneous trunci* which had a high ultimate pH, had the lightest, and least red surface. Hence in our study, the variations in colour between the muscles were driven by differences in fibre types between muscles, not by differences in ultimate pH. Our $L^*a^*b^*$ values cannot be directly compared to other studies as it is well-known that chromameters vary widely in their reported $L^*a^*b^*$ values (Holman, Diffey, Logan, Mortimer, & Hopkins, 2021; Holman & Hopkins, 2019). We found no change in colour traits L^* and a^* with 14 days ageing whereas others report that pork *longissimus* L^* increases, and a^* decreases with ageing time (Hwang, Lee, Lee, & Joo, 2020; Li et al., 2009).

5. Conclusion

There was a significant difference in muscle fibre type between glycolytic muscles, *longissimus* and *cutaneous trunci*, and oxidative muscles, *masseter* and *psoas major*, in this study. However, all muscles analysed were composed of mixed muscle fibres. The *longissimus* had the highest cook loss over the ageing period and, cook loss in *longissimus* increased as cooking temperatures increased. Interestingly, the *longissimus* muscle had no change in WBPSF between day 0 and day 14 when cooked to 70 °C. However, WBPSF was lower for day 14 ageing when cooked at 60–65 °C. Importantly, we found that the first protein denaturation peak for *longissimus* identified during DSC, and attributed to myosin, occurs at a lower temperature compared to *masseter*, *cutaneous trunci* and *psoas major*, which indicates transverse shrinkage occurs at a lower cooking temperature. Future studies should include microscopic analysis of transverse and longitudinal shrinkage of pork muscle fibres across a range of heating temperatures, compared to other species as well as evaluating food safety when pork *longissimus* is cooked to lower temperatures. These results indicate that for optimum eating quality, pork should be cooked to a final internal temperature of 65 °C rather than 70 °C due to increased shrinkage, cook loss, and toughness when pork is cooked to 70 °C and above.

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CRediT authorship contribution statement

Michelle N. LeMaster: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Minh Ha:** Methodology, Validation, Writing - review and editing. **Frank R. Dunshea:** Conceptualization, Writing – review & editing, Supervision. **Surinder Chauhan:** Writing – review & editing, Supervision. **Darryl D'Souza:** Conceptualization, Writing – review & editing, Supervision. **Robyn D. Warner:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability statement

Data is available on request.

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References

Aberle, E., Forrest, J., Gerrard, D., & Mills, E. (2012). Meat as a food - properties of fresh meat. In R. Wood (Ed.), *Principles of meat science* (5th ed., pp. 1–140). Dubuque, IA: Kendall Hunt.

Adzitey, F., & Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences – A mini review. *International Food Research Journal, 18*, 11–20.

Amako, D. E. N., & Xiong, Y. L. (2001). Effects of carrageenan on thermal stability of proteins from chicken thigh and breast muscles. *Food Research International, 34*(2), 247–253. https://doi.org/10.1016/S0963-9969(00)00161-7

Andersson, A., Andersson, K., & Tornberg, E. (2000). A comparison of fat-holding between beefburgers and emulsion sausages. *Journal of the Science of Food and Agriculture, 80*(5), 555–560. https://doi.org/10.1002/(Sici)1097-0010(200004)80:5<555::Aid-Jsfa573>3.0.Co;2-1

Ashmore, C., & Doerr, L. (1971). Comparative aspects of muscle fiber types in different species. *Experimental Neurology, 31*(3), 408–418.

Bee, G., Anderson, A. L., Lonergan, S. M., & Huff-Lonergan, E. (2007). Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. *Meat Science, 76*(2), 359–365. https://doi.org/10.1016/j.meatsci.2006.12.004

Bendall, J., & Restall, D. (1983). The cooking of single myofibres, small myofibre bundles and muscle strips from beef *M. psoas* and *M. sternomandibularis* muscles at varying heating rates and temperatures. *Meat Science, 8*(2), 93–117.

Bendall, J. R. (1973). Postmortem changes in muscles. In G. H. Bourne (Ed.) (2nd ed.,, *vol. 2. The structure and function of muscle* (pp. 243–309). New York, New York: Academic Press.

Bertram, H. C., Wu, Z., van den Berg, F., & Andersen, H. J. (2006). NMR relaxometry and differential scanning calorimetry during meat cooking. *Meat Science, 74*(4), 684–689. https://doi.org/10.1016/j.meatsci.2006.05.020

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. A. (2018). Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness, 7*(3), 196–204. https://doi.org/10.1016/j.fshw.2018.08.002

Bidner, B. S., Ellis, M., Brewer, M. S., Campion, D., Wilson, E. R., & McKeith, F. K. (2004). Effect of ultimate pH on the quality characteristics of pork. *Journal of Muscle Foods, 15*(2), 139–154. https://doi.org/10.1111/j.1745-4573.2004.tb00717.x

Brewer, M. S., & McKeith, F. K. (1999). Consumer-rated quality characteristics as related to purchase intent of fresh pork. *Journal of Food Science, 64*(1), 171–174. https://doi.org/10.1111/j.1365-2621.1999.tb09885.x

Briguet, A., Courdier-Fruh, I., Foster, M., Meier, T., & Magyar, J. P. (2004). Histological parameters for the quantitative assessment of muscular dystrophy in the mdx-mouse. *Neuromuscular Disorders, 14*(10), 675–682. https://doi.org/10.1016/j.nmd.2004.06.008

Brooke, M. H., & Kaiser, K. K. (1970). Muscle fiber types: How many and what kind? *Archives of Neurology, 23*(4), 369–379. https://doi.org/10.1001/archneur.1970.00480280083010

Channon, H., D'Souza, D., Hamilton, A., & Dunshea, F. (2013). Eating quality of pork shoulder roast and stir fry outperforms cuts from the loin and silverside in male pigs. In J. R. Pluske, & J. Pluske (Eds.), *vol. XIV'. Manipulating pig production* (p. 227).

Channon, H., Payne, A., & Warner, R. (2000). Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. *Meat Science, 56*(3), 291–299.

Channon, H. A., D'Souza, D. N., & Dunshea, F. R. (2016). Developing a cuts-based system to improve consumer acceptability of pork: Impact of gender, ageing period, endpoint temperature and cooking method. *Meat Science, 121*, 216–227. https://doi.org/10.1016/j.meatsci.2016.06.011

Channon, H. A., Taverner, M. R., D'Souza, D. N., & Warner, R. D. (2014). Aitchbone hanging and ageing period are additive factors influencing pork eating quality. *Meat Science, 96*(1), 581–590. https://doi.org/10.1016/j.meatsci.2013.08.016

Cheng, H., Song, S., & Kim, G. D. (2021). Frozen/thawed meat quality associated with muscle fiber characteristics of porcine longissimus thoracis et lumborum, psoas major, semimembranosus, and semitendinosus muscles. *Scientific Reports, 11*(1), 13354. https://doi.org/10.1038/s41598-021-92908-3

Choi, Y. M., & Kim, B. C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Science, 122*(2–3), 105–118. https://doi.org/10.1016/j.livsci.2008.08.015

Christensen, M., Henckel, P., & Purslow, P. P. (2004). Effect of muscle type on the rate of post-mortem proteolysis in pigs. *Meat Science, 66*(3), 595–601. https://doi.org/10.1016/S0309-1740(03)00175-X

Christensen, M., Kok, C., & Ertbjerg, P. (2006). Mechanical properties of type I and type IIB single porcine muscle fibres. *Meat Science, 73*(3), 422–425. https://doi.org/10.1016/j.meatsci.2005.12.010

Davey, C. L., & Gilbert, K. V. (1974). Temperature-dependent cooking toughness in beef. *Journal of the Science of Food and Agriculture, 25*(8), 931–938. https://doi.org/10.1002/jsfa.2740250808

Dunshea, F. R., Lealiifano, A., Trezona, M., Gole, V., & Hewitt, R. J. E. (2022). National audit of pork pH in Australia. In *Paper presented at the 73rd meeting of the European Federation of Animal Science Porto, Portugal*.

Egelandsdal, B., Martinsen, B. K., Fretheim, K., Pettersen, M., & Harbitz, O. (1994). Myosins from red and white bovine muscles: Differences measured by turbidimetric, calorimetric and rheological techniques. *Journal of the Science of Food and Agriculture, 64*(1), 75–85. https://doi.org/10.1002/jsfa.2740640112

England, E., Matarneh, S., Oliver, E., Apaoblaza, A., Scheffler, T., Shi, H., & Gerrard, D. (2016). Excess glycogen does not resolve high ultimate pH of oxidative muscle. *Meat Science, 114*, 95–102. https://doi.org/10.1016/j.meatsci.2015.10.010

England, E. M., Matarneh, S. K., Scheffler, T. L., Wachet, C., & Gerrard, D. E. (2014). pH inactivation of phosphofructokinase arrests postmortem glycolysis. *Meat Science, 98*(4), 850–857. https://doi.org/10.1016/j.meatsci.2014.07.019

Essén-Gustavsson, B., & Fjelkner-Modig, S. (1985). Skeletal muscle characteristics in different breeds of pigs in relation to sensory properties of meat. *Meat Science, 13*(1), 33–47. https://doi.org/10.1016/S0309-1740(85)80003-6

Fernandez, X., Forslid, A., & Tornberg, E. (1994). The effect of high post-mortem temperature on the development of pale, soft and exudative pork: Interaction with ultimate pH. *Meat Science, 37*(1), 133–147. https://doi.org/10.1016/0309-1740(94)90150-3

Frank, D. C., Geesink, G., Alvarenga, T. I. R. C., Polkinghorne, R., Stark, J., Lee, M., & Warner, R. (2017). Impact of high oxygen and vacuum retail ready packaging formats on lamb loin and topside eating quality. *Meat Science, 123*, 126–133. https://doi.org/10.1016/j.meatsci.2016.09.010

Gil, M., Delday, M. I., Gispert, M., Furnols, M. F., Maltin, C. M., Plastow, G. S., … Carrión, D. (2008). Relationships between biochemical characteristics and meat quality of Longissimus thoracis and Semimembranosus muscles in five porcine lines. *Meat Science, 80*(3), 927–933. https://doi.org/10.1016/j.meatsci.2008.04.016

Gil, M., Oliver, M. A., Gispert, M., Diestre, A., Sosnicki, A. A., Lacoste, A., & Carrion, D. (2003). The relationship between pig genetics, myosin heavy chain I, biochemical traits and quality of M. longissimus thoracis. *Meat Science, 65*(3), 1063–1070. https://doi.org/10.1016/S0309-1740(02)00324-8

Gunawan, A. M., Park, S. K., Pleitner, J. M., Feliciano, L., Grant, A. L., & Gerrard, D. E. (2007). Contractile protein content reflects myosin heavy-chain isoform gene expression. *Journal of Animal Science, 85*(5), 1247–1256. https://doi.org/10.2527/jas.2006-511

Guth, L., & Samaha, F. J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Experimental Neurology, 25*(1), 138–152. https://doi.org/10.1016/0014-4886(69)90077-6

Han, X., Jiang, T., Yang, H., Zhang, Q., Wang, W., Fan, B., & Liu, B. (2012). Investigation of four porcine candidate genes (H-FABP, MYOD1, UCP3 and MASTR) for meat quality traits in large White pigs. *Molecular Biology, 39*(6), 6599–6605. https://doi.org/10.1007/s11033-012-1490-6

Hanan, T., & Shaklai, N. (1995). The role of H2O2-generated myoglobin radical in crosslinking of myosin. *Free Radical Research, 22*(3), 215–227. https://doi.org/10.3109/10715769509147541

Henckel, O., Erlandsen, B.-G., & Bejerholm.. (1997). Histo- and biochemical characteristics of the longissimus dorsi muscle in pigs and their relationships to performance and meat quality. *Meat Science, 47*(3–4), 311–321. https://doi.org/10.1016/S0309-1740(97)00063-6

Holman, B. W. B., Diffey, S. M., Logan, B. G., Mortimer, S. I., & Hopkins, D. L. (2021). Nix pro color sensor comparison to HunterLab MiniScan for measuring lamb meat colour and investigation of repeat measures, illuminant and standard observer effects. *Food Analytical Methods, 14*(4), 697–705. https://doi.org/10.1007/s12161-020-01914-0

Holman, B. W. B., & Hopkins, D. L. (2019). A comparison of the Nix Colour Sensor ProTM and HunterLab MiniScanTM colorimetric instruments when assessing aged beef colour stability over 72 h display. *Meat Science, 147*, 162–165. https://doi.org/10.1016/j.meatsci.2018.09.009

Hughes, J., Clarke, F., Purslow, P., & Warner, R. (2017). High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Research International, 101*, 228–238. https://doi.org/10.1016/j.foodres.2017.09.003

Hughes, O., Purslow, & Warner. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science, 98*(3), 520–532. https://doi.org/10.1016/j.meatsci.2014.05.022

Hwang, Y.-H., Lee, S.-J., Lee, E.-Y., & Joo, S.-T. (2020). Effects of carcass weight increase on meat quality and sensory properties of pork loin. *Journal of Animal Science and Technology, 62*(5), 753.

Joo, S. T., Kauffman, R. G., Kim, B. C., & Park, G. B. (1999). The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Science, 52*(3), 291–297. https://doi.org/10.1016/s0309-1740(99)00005-4

Jose, C. G., Trezona, M., Mullan, B., McNaughton, K., & D'souza, D. N. (2013). *Determining the variability in eating quality of Australian fresh pork*. Co-operative Research Centre for High Integrity Australian Pork.

Kang, C., Lee, C., & Hong, & Kim.. (2011). Effects of myosin heavy chain isoforms on meat quality, fatty acid composition, and sensory evaluation in Berkshire pigs. *Meat Science, 89*(4), 384–389. https://doi.org/10.1016/j.meatsci.2011.04.019

Karlsson, A. H., Klont, R. E., & Fernandez, X. (1999). Skeletal muscle fibres as factors for pork quality. *Livestock Production Science, 60*(1–2), 255–269.

Kerth, C. R. (2013). *Preventing foodborne illness the science of meat quality* (pp. 249–282). Blackwell Publishing.

Kim, G. D., Ryu, Y. C., Jeong, J. Y., Yang, H. S., & Joo, S. T. (2013). Relationship between pork quality and characteristics of muscle fibers classified by the distribution of myosin heavy chain isoforms. *Journal of Animal Science, 91*(11), 5525–5534. https://doi.org/10.2527/jas.2013-6614

Kim, G.-D., Yang, H.-S., & Jeong, J.-Y. (2017). *Intramuscular variations of proteome and muscle fiber type distribution in semimembranosus and semitendinosus muscles associated with pork quality*. https://doi.org/10.1016/j.foodchem.2017.10.046

Kim, J., Jung, E. Y., Yang, H. S., Lim, H. T., & Joo, S. T. (2013). The influence of fiber size distribution of type IIB on carcass traits and meat quality in pigs. *Meat Science, 94*(2), 267–273. https://doi.org/10.1016/j.meatsci.2013.02.001

King, D. A., Shackelford, S. D., Schnell, T., Pierce, L., Wheeler, T. L., Shackelford, S. D., & Wheeler, T. L. (2018). Characterizing the ham halo condition: A color defect in fresh pork biceps femoris muscle. *Meat and Muscle Biology, 2*(1).

Klont, R. E., Brocks, L., & Eikelenboom, G. (1998). Muscle fibre type and meat quality. *Meat Science, 49*(1), 219–229. https://doi.org/10.1016/S0309-1740(98)90050-X

Klont, R. E., Talmant, A., & Monin, G. (1994). Effect of temperature on porcine-muscle metabolism studied in isolated muscle-fibre strips. *Meat Science, 38*(2), 179–191.

Koohmaraie, M. (1992). The role of Ca2+−dependent proteases (calpains) in post mortem proteolysis and meat tenderness. *Biochimie, 74*(3), 239–245. https://doi.org/10.1016/0300-9084(92)90122-U

Lee, S. H., Choe, J. H., Choi, Y. M., Jung, K. C., Rhee, M. S., Hong, K. C., … Kim, B. C. (2012). The influence of pork quality traits and muscle fiber characteristics on the eating quality of pork from various breeds. *Meat Science, 90*(2), 284–291. https://doi.org/10.1016/j.meatsci.2011.07.012

Lee, S. H., Joo, S. T., & Ryu, Y. C. (2010). Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. *Meat Science, 86*(1), 166–170. https://doi.org/10.1016/j.meatsci.2010.04.040

Lefaucheur, L., Edom, F., Ecolan, P., & Butler-Browne, G. S. (1995). Pattern of muscle fiber type formation in the pig. *Developmental Dynamics, 203*(1), 27–41. https://doi.org/10.1002/aja.1002030104

Li, C., Wu, J., Zhang, N., Zhang, S., Liu, J., Li, J., … Zhu, Z. (2009). Effects of boning method and postmortem aging on meat quality characteristics of pork loin. *Animal Science, 80*(5), 591–596.

Lindahl, G., Henckel, P., Karlsson, A. H., & Andersen, H. J. (2006). Significance of early postmortem temperature and pH decline on colour characteristics of pork loin from different crossbreeds. *Meat Science, 72*(4), 613–623. https://doi.org/10.1016/j.meatsci.2005.09.014

Maltin, W., Matthews, G., Porteig, & Delday. (1997). *Pig muscle fibre characteristics as a source of variation in eating quality meat science* (vol. 41, pp. 231–248). ELSEVIER.

Nam, Y. J., Choi, Y. M., Lee, S. H., Choe, J. H., Jeong, D. W., Kim, Y. Y., & Kim, B. C. (2009). Sensory evaluations of porcine longissimus dorsi muscle: Relationships with postmortem meat quality traits and muscle fiber characteristics. *Meat Science, 83*(4), 731–736. https://doi.org/10.1016/j.meatsci.2009.08.015

Pearson, A. M., & Dutson, T. R. (1985). Cold-induced toughness of meat. In R. H. Locker (Ed.) (1st ed.,, *vol. 1. Advances in meat research: Volume 1 electrical stimulation* (pp. 1–44). Westport, Connecticut: Springer Science & Business Media.

Phillips, A., Faustman, C., Lynch, M., Govoni, K., Hoagland, T., & Zinn, S. (2001). Effect of dietary α-tocopherol supplementation on color and lipid stability in pork. *Meat Science, 58*(4), 389–393.

Pospiech, E., Greaser, M. L., Mikolajczak, B., Chiang, W., & Krzywdzinska, M. (2002). Thermal properties of titin from porcine and bovine muscles. *Meat Science, 62*(2), 187–192. https://doi.org/10.1016/s0309-1740(01)00245-5

Purslow, P. P., Oiseth, S., Hughes, J., & Warner, R. D. (2016). The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Research International, 89*(Pt 1), 739–748. https://doi.org/10.1016/j.foodres.2016.09.010

Realini, C. E., Venien, A., Gou, P., Gatellier, P., Perez-Juan, M., Danon, J., & Astruc, T. (2013). Characterization of Longissimus thoracis, Semitendinosus and Masseter muscles and relationships with technological quality in pigs. 1. Microscopic analysis of muscles. *Meat Science, 94*(3), 408–416. https://doi.org/10.1016/j.meatsci.2013.03.009

Rees, M. P., Trout, G. R., & Warner, R. D. (2002). Tenderness, ageing rate and meat quality of pork *M. longissimus* thoracis et lumborum after accelerated boning. *Meat Science, 60*(2), 113–124. https://doi.org/10.1016/S0309-1740(01)00085-7

Ryu, Y. C., & Kim, B. C. (2005a). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. *Meat Science, 71*(2), 351–357. https://doi.org/10.1016/J.MEATSCI.2005.04.015

Ryu, Y. C., & Kim, B. C. (2005b). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. *Meat Science, 71*(2), 351–357. https://doi.org/10.1016/j.meatsci.2005.04.015

Ryu, Y. C., Rhee, M. S., & Kim, B. C. (2004). Estimation of correlation coefficients between histological parameters and carcass traits of pig longissimus Dorsi muscle. *Asian-Australasian Journal of Animal Sciences, 17*(3), 428–433. https://doi.org/10.5713/ajas.2004.428

Sánchez Del Pulgar, J., Gázquez, A., & Ruiz-Carrascal, J. (2011). Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science, 90*(3), 828–835. https://doi.org/10.1016/j.meatsci.2011.11.024

Schiaffino, S., Gorza, L., Sartore, S., Leopoldo, S., Ausoni, S., Vianello, M., … Lomo, T. (1989). Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *Journal of Muscle Research and Cell Motility, 10*, 197–205. https://doi.org/10.1007/BF01739810

Schiaffino, S., & Reggiani, C. (1994). Myosin isoforms in mammalian skeletal muscle. *Journal of Applied Physiology, 77*(2), 493–501.

Shen, L., Luo, J., Lei, H., Jiang, Y., Bai, L., Li, M., … Zhu, L. (2015). Effects of muscle fiber type on glycolytic potential and meat quality traits in different Tibetan pig muscles and their association with glycolysis-related gene expression. *Genetics and Molecular Research, 14*(4), 14366–14378.

Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science, 70*(3), 493–508. https://doi.org/10.1016/j.meatsci.2004.11.021

Vaskoska, R., Ha, M., Naqvi, Z. B., White, J. D., & Warner, R. D. (2020). Muscle, ageing and temperature influence the changes in texture, cooking loss and shrinkage of cooked beef. *Foods, 9*(9). https://doi.org/10.3390/foods9091289

Vaskoska, R., Ha, M., Ong, L., Chen, G., White, J., Gras, S., & Warner, R. (2021). Myosin sensitivity to thermal denaturation explains differences in water loss and shrinkage during cooking in muscles of distinct fibre types. *Meat Science, 179*, Article 108521. https://doi.org/10.1016/j.meatsci.2021.108521

Vaskoska, R., Ha, M., Tran, H. T. T., Khoshelham, K., White, J. D., & Warner, R. D. (2020). Evaluation of 3D laser scanning for estimation of heating-induced volume shrinkage and prediction of cooking loss of pork cuboids compared to manual measurements. *Food and Bioprocess Technology, 13*(6), 938–947. https://doi.org/10.1007/s11947-020-02421-0

Vaskoska, R., Ha, M., White, J. D., & Warner, R. D. (2023). Benefits of prolonged ageing for the quality of Australian pork depends on cooking temperature and meat pH. *Animal Production Science, 63*(8), 816–823. https://doi.org/10.1071/AN22389

Warner, R. D., Kauffman, R. G., & Russel, R. L. (1993). Quality attributes of major porcine muscles: A comparison with the longissimus Lumborum. *Meat Science, 33*(3), 359–372. https://doi.org/10.1016/0309-1740(93)90007-5

Warner, R. D., Kerr, M., Kim, Y. H. B., & Geesink, G. (2014). Pre-rigor carcass stretching counteracts the negative effects of high rigor temperature on tenderness and water-holding capacity – using lamb muscles as a model. *Animal Production Science, 54*(4), 494–503. https://doi.org/10.1071/An13062

Warriss, P. D. (2010). *Measuring the composition and physical characteristics of meat meat science: An Introductory Text* (2nd ed., pp. 229–251). Oxfordshire, United Kingdom: CABI.

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *Journal of Animal Science, 78*(4), 958–965. https://doi.org/10.2527/2000.784958x

Xiong, Y. L. (1994). Myofibrillar protein from different muscle Fiber types: Implications of biochemical and functional properties in meat processing. *Critical Reviews in Food Science and Nutrition, 34*(3), 293–320. https://doi.org/10.1080/10408399409527665

Xiong, Y. L., Brekke, C. J., & Leung, H. K. (1987). Thermal-denaturation of muscle proteins from different species and muscle types as studied by differential scanning calorimetry. *Canadian Institute of Food Science and Technology Journal-Journal De L Institut Canadien De Science Et Technologie Alimentaires, 20*(5), 357–362. https://doi.org/10.1016/S0315-5463(87)71331-5

Zielbauer, B. I., Franz, J., Viezens, B., & Vilgis, T. A. (2016). Physical aspects of meat cooking: Time dependent thermal protein denaturation and water loss. *Food Biophysics, 11*(1), 34–42. https://doi.org/10.1007/s11483-015-9410-7