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Benefits of prolonged ageing for the quality of Australian pork depends on cooking temperature and meat pH

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ABSTRACT

Context. Heating of meat leads to structural changes reflected in the juiciness and the tenderness of the cooked meat. **Aims.** This study aimed to characterise the effect of prolonged ageing and cooking on pork-quality traits. **Methods.** *Longissimus lumborum* samples from 12 carcasses were aged 3 days (conventional ageing) or 15 days (prolonged ageing) and pork cuboids were cooked at 50–80°C for 30 min. Cooking loss, total water content (TWC), Warner–Bratzler shear force (WBSF) and shrinkage (longitudinal, transverse and estimated volume) of the pork loin cuboids were measured. **Key results.** Prolonged ageing for 15 days reduced the WBSF of samples cooked at 50°C, and the cooking loss for samples cooked at 70°C and 80°C, relative to conventional ageing for 3 days. The WBSF of pork aged for 15 days was not different from that of pork aged for 3 days. Prolonged ageing reduced longitudinal shrinkage of cuboids, but TWC and transverse/volume shrinkage of cuboids were not affected by ageing. The diameter of cooked muscle fibre fragments was smaller in pork subjected to prolonged ageing. **Conclusions.** Prolonged ageing was favourable for minimising cooking loss at higher cooking temperatures but was only favourable for tenderness at the lowest cooking temperature. Low pH of the samples is likely to have caused the lack of tenderisation with ageing.

Keywords: ageing, cooking, cooking loss, heating, pH, pork, shrinkage, tenderness, WHC.

Introduction

Consumers prefer, and are more likely to pay a premium price, for tender and juicy pork (Bryhni *et al.* 2003; Aaslyng *et al.* 2007; Sanders *et al.* 2007). While ageing has been shown to improve the eating quality of beef and lamb, it has not always been considered an effective intervention strategy to improve pork quality, especially in Australia (Channon *et al.* 2016*a*). Conventionally, ageing of pork in Australia to reach consumer-acceptable tenderness is thought to require only 2–3 days (Channon *et al.* 2016*b*), in alignment with the concept that 50–80% of pork tenderisation, measured instrumentally as shear force, occurs in 2–5 days (Dransfield *et al.* 1981). In contrast, extended ageing of pork for 8 days has been shown to improve sensory tenderness and juiciness, compared with ageing for 4 days (Jonsäll 2000), 16 days compared with 2 and 9 days (Ellis *et al.* 1998) and 10 days in comparison to 1 day (Wood *et al.* 1996). There is conflicting evidence on the role of extended ageing on the cooking loss of pork. Li *et al.* (2009) related a reduced cooking loss in the first 8 days of ageing to improved water-holding capacity and an increased cooked loss between 8 and 16 days to cell destruction. Other authors have not found further improvement of cooking loss after 5 days (Dransfield *et al.* 1981). In addition, contradictory results have been reported for the effect of ageing on pork tenderness. Channon *et al.* (2014) reported improvement in sensory and instrumental tenderness after 2 or 3 days of ageing and Channon *et al.* (2016*a*) showed improvement after 7 days of ageing. Taylor *et al.* (1995) showed improvement in both instrumental and consumer tenderness by ageing for 7 and 12 days in comparison to 4 days in pork loins.

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Meat shrinks with heating and it has been proposed that cooking loss occurs due to shrinkage of the myofilament lattice (Offer and Trinick 1983). Shrinkage of meat is important not only for weight loss of the product, but also for impact on consumer perceptions of quality. Danish consumers associate lack of shrinkage with good pork quality, while Canadian consumers consider that shrinkage is a criteria for selecting pork (Diamant *et al.* 1976; Ngapo *et al.* 2004). Meat shrinkage occurs in two directions, namely, transverse, which occurs perpendicular to the muscle fibre direction and has been related to myosin denaturation (Kondjoyan *et al.* 2014), and longitudinal, which occurs parallel to the muscle fibre direction and has been associated with collagen (Kondjoyan *et al.* 2014) and actin denaturation (Purslow *et al.* 2016; for review, see Warner *et al.* 2017). Most of the published meat shrinkage studies discriminating between transverse and longitudinal shrinkage have been conducted in beef (Warner *et al.* 2017) and dimensional shrinkage is rarely quantified in pork (Becker *et al.* 2016). Additionally, it is not clear what effect conventional and prolonged ageing would have on the shrinkage process in both directions.

Mechanistically, ageing mainly leads to protein degradation, while cooking mainly leads to conformational changes in meat proteins (Vaskoska *et al.* 2020*a*, 2021). Conformation changes of proteins during cooking result in changes in meat tenderness and lead to loss of water. While an increase in cooking temperature can lead to tougher pork and higher cooking loss (Crawford *et al.* 2010), pork can also become more tender with heating to certain temperatures, particularly to ~60°C (Christensen *et al.* 2000). Channon *et al.* (2016*a*) investigated ageing periods of 7 days and 2 days and cooking temperatures of 70°C and 75°C, but did not find an interaction between the ageing period and the cooking temperature. The present study aims to investigate the combined effect of ageing (3 and 15 days) and a range of cooking temperatures (50°C, 60°C, 70°C and 80°C) on the texture, water-holding capacity and shrinkage of pork loins. Investigating shrinkage simultaneously with WBSF and cooking loss enables exploration of other potential benefits of prolonged ageing on pork quality. If an interaction emerges between prolonged ageing and cooking temperature regarding quality traits, combinations of ageing periods and cooking methods can be selected/recommended for industry to achieve optimal pork quality.

Materials and methods

Muscles and ageing treatments

Pork loins (*longissimus lumborum*) from 12 pig carcasses (hot carcass weight 81.66 ± 2.7 kg) were collected 1 day postmortem and randomly assigned to either 3 days ($N = 6$ carcasses) or 15 days of ageing ($N = 6$ carcasses). The muscles were placed in vacuum bags and vacuum packaged (C100 Chamber, Multivac Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) and then aged in a refrigerated unit at a controlled temperature of 4°C. When the ageing was complete, the pH was measured with a pH meter (WP 80, TPS Pty Ltd, Brisbane, Qld, Australia) with an electrode (IJ 44) and temperature compensation probe attachments (Ionode Pty Ltd, Brisbane, Qld, Australia).

Purge loss was determined by weighing samples on a scale (Scout SPX 1202, Ohaus Corporation, Parsippany, New Jersey, USA) before and after ageing. The purge loss was calculated as the weight after ageing (3, 15 days) relative to the weight before ageing (1 day postmortem).

Cooking

Five meat cuboids measuring 50 mm length (L) × 30 mm width (W) × 30 mm height (H) were cut from the muscles from each carcass and randomly allocated to a control (raw) and four cooking temperatures (50°C, 60°C, 70°C and 80°C). A pin was inserted at the top-left corner of each cuboid to enable measurement of the same dimensions before and after cooking. The weight of the cuboid was recorded and then the cuboid was placed in a plastic bag and immersed in a heated water bath (Julabo F38, John Morris Scientific, Melbourne, Victoria Australia). Internal temperature was measured with a T-type probe (Grant Instruments, Cambridge, United Kingdom) placed in the centre of the sample. The cuboids were cooked to the target temperature (accuracy was ±0.75°C) for 30 min, after which the bags were placed in iced water for 15 min to cool, and the cuboids were dried with a paper towel. The weight was measured again for estimating cooking loss after the cooling period. Subsamples for measurement of Warner–Bratzler shear force (WBSF) and fibre diameter, as described below, were collected from each cuboid (including raw) and processed after overnight storage in a plastic bag at 4°C.

Cooking loss, TWC and WBSF

Cooking loss (%) was calculated as the weight of the cuboids cooked to the specified temperature, relative to the weight of the raw cuboid (in %). TWC was measured by weighing a ~3 g sample, which was then dried in an oven (IPX5; Convotherm, Eglfing, Germany) with an air temperature of 104°C for 48 h (Oillic *et al.* 2011) and cooled in a desiccator before re-weighing. TWC was calculated as the weight after drying relative to the weight before drying (in %).

Warner–Bratzler shear force (WBSF) was measured on 1 cm^2 samples (length varied with cooking regime, but was 5 cm for raw samples), cut from the middle of the raw and cooked pork cuboids, with the fibres perpendicular to the blade. A texture analyser (Lloyd Instruments Ltd., Hampshire, UK), using a triangular blade, a speed of 300 mm/min and

×500N load cell was used. Toughness was expressed as peak WBSF (in Newtons).

Longitudinal, transverse and volume shrinkage

The length of each of the edges of the cuboids was measured before and after cooking with digital Vernier calipers (Kincrome, Melbourne, Victoria Australia), using the location of the pin for co-locating the same edges for calculation purposes. For estimating the shrinkage in each direction, the average dimensions for each direction of the raw and cooked cuboids were used and shrinkage was expressed relative to the raw values. Transverse shrinkage included both width and height shrinkage by combining them in a calculation of the cross-sectional area. The cross-sectional area (CSA) was calculated as the product of the average width and the average height for each cuboid. The volume was calculated as the product of the length, width and height on the side of the cuboid where the pin was placed. Transverse, longitudinal and volume shrinkage (in %) were calculated as the CSA, average length and volume of the cuboids respectively, cooked to each temperature relative to the same dimensions in the raw cuboids.

Transverse shrinkage on level of fibre fragments

Microscopy was performed to observe the dimensional changes in the muscle fibres. The samples were prepared according to the method of Purslow *et al.* (2016) with some modifications. For measurement of the diameter of the fibre fragments, 1 g of muscle (from each ageing and temperature combination treatment) was homogenised in 10 mL of cold mannitol buffer (380 mM mannitol, 5 mM potassium acetate, pH 5.6) for each sample with an Ultra Turrax T25, 10 mm head, (Janke & Kunkel Ika Labotechnik) at 4000*g* at 0–1°C, three times for 10 s, with pauses of 10 s in between, while holding it on ice. A drop of each suspension was transferred onto a separate glass slide (Sigma Aldrich), covered with a cover slip and observed with a compound microscope (Leica DM750). Measurements of the fibre diameter were performed on bright field images (Leica camera ICC50 W) taken using 200 times magnification (10× ocular and 20× objective). At least 25 muscle fibres were measured per ageing and temperature treatment. Only samples that appeared intact in width were measured for the diameter. Samples were counted continuously throughout the area of the slide and if there were fewer than 25 muscle fibres per slide, an additional slide was used. The fibre fragment diameter was measured with the Leica LAS software (Leica, Wetzlar, Germany).

Data analyses

ANOVA was performed in Genstat v16 (VSN International Ltd, Hemel Hempstead, UK), with temperature, ageing and their interaction as treatments and the standard error of difference (s.e.d.) was used for comparison between treatments. Principal-component analysis (PCA) was conducted in Minitab (ver. 19, University Park, Pennsylvania, USA) by selection of six components.

Results and discussion

The pH of the pork loin muscles was 5.45 ± 0.07 for 3 days of ageing and 5.36 ± 0.06 for 15 days of ageing, which is within the usual range encountered for Australian pork (Channon *et al.* 2003, 2014). Purge loss was 2.75% ± 0.53 (s.d.) for 3-days-aged pork and 7.22% ± 0.97 (s.d.) for 15-days-aged pork. While the purge amounts for the initial period of ageing (2–7 days) in the study of Juárez *et al.* (2009) were similar to those in our study (2.85%), the purge of 14-days-aged pork loins in their study (3.45%) was much lower than the purge found in our study for 15-days-aged pork. This could be a consequence of the lower pH of the pork used in our study than the pH of 5.6 of the pork in the study of Juárez *et al.* (2009), as lower pH is associated with an increased purge loss (Huff-Lonergan and Lonergan 2005). Furthermore, low pH is associated with the occurrence of PSE (pale, soft exudative) pork as well as reduced water-holding capacity (Warner *et al.* 1997) and failure to tenderise (Kim *et al.* 2014).

A significant ($P < 0.001$) interaction was found between ageing and the cooking temperature (Fig. 1*a*). Ageing for 15 days led to reduced cooking loss compared with ageing for 3 days when pork was cooked to 70°C and 80°C, but ageing period did not affect the cooking loss at 50°C and 60°C (Fig. 1*a*). The lower cooking loss at 70°C and 80°C for 15-days-aged samples in comparison to 3-days-aged pork was comparable to the results of Ellis *et al.* (1998) who found reduced cooking loss in prolonged (16 days) compared with conventional (2 days) ageing of pork. The reduced cooking loss with prolonged ageing could be a consequence of the greater purge loss as explained by the 'leaking out' hypothesis; water that is lost in drip cannot be lost again through cooking (Kim *et al.* 1993). Cooking loss increased continuously with each increase in temperature within each ageing period ($P < 0.001$; Fig. 1*a*), which has also been previously reported (Wood *et al.* 1995). For TWC, there was no change with ageing ($P > 0.05$; Fig. 1*b*), and it decreased with the increase in temperature (Fig. 1*b*; 50°C vs 80°C was 71.6% and 63.3% respectively; $P < 0.001$).

Ageing alone did not affect the WBSF of pork, but it had a significant interaction with the cooking temperature ($P = 0.017$) interaction with the cooking temperature (Fig. 1*c*). Specifically, ageing period affected the WBSF of aged pork only at 50°C, where ageing for 15 days resulted in a lower WBSF than did ageing for 3 days (Fig. 1*c*). It is unclear why there was a difference between ageing periods at 50°C. Meat texture at 50°C can be related to background toughness of

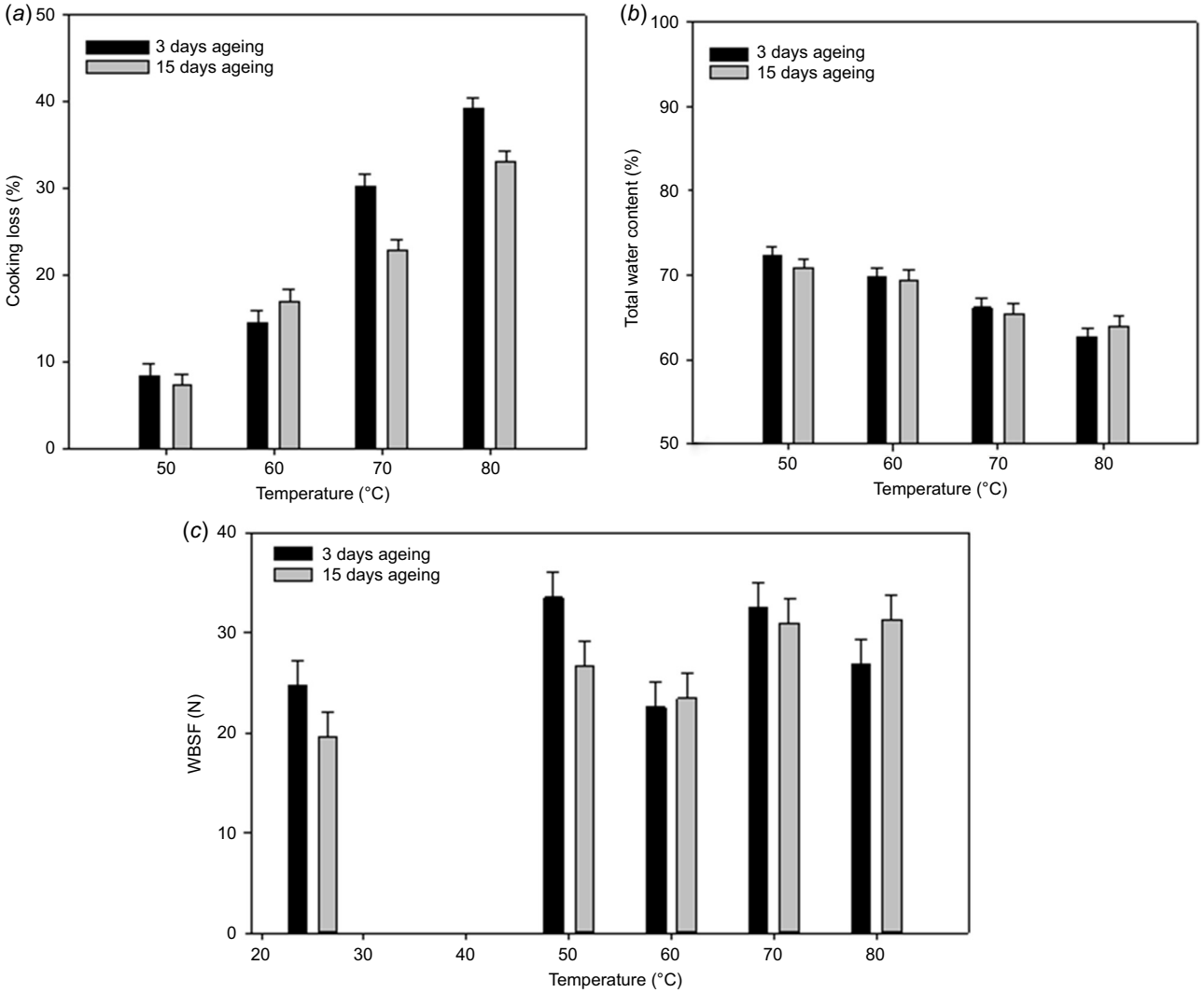

Fig. 1. Effect of cooking temperature (50°C, 60°C, 70°C and 80°C) and ageing (3 days and 15 days) on physical changes during cooking of cuboids of pork loins (*longissimus lumborum*). (*a*) Cooking loss (%); *P*-values are as follows: temperature $P < 0.001$, ageing $P < 0.001$, temperature × ageing $P < 0.001$. (*b*) Total water content (TWC, %); *P*-values were as follows: temperature $P < 0.001$, ageing $P > 0.05$, temperature × ageing $P > 0.05$. (*c*) Warner–Bratzler shear force (WBSF, *N*); *P*-values were as follows: temperature $P < 0.001$, ageing $P = 0.15$, temperature × ageing $P = 0.017$. The values are predicted means and vertical bars are the s.e.d. for the interaction.

the connective tissue or myosin denaturation (in beef: Martens *et al.* 1982) and several studies have shown that these two components might be sensitive to ageing-related proteolysis (Nishimura 2010; Anderson *et al.* 2012). Interestingly, Dransfield *et al.* (1981) found improvement in WBSF from 2 to 10 days of ageing in pork cooked to a higher temperature (80°C). Other studies on Australian pork have shown no change in consumer scores for tenderness for pork aged 3–7 days, or more than 7 days (Channon *et al.* 2017), and modelling has demonstrated that ultimate pH values of less than 5.5 will reduce consumer eating quality scores for pork tenderness (Channon *et al.* 2018). Hence, the low pH in the meat in our study is likely to explain the lack of tenderisation with ageing as low pH pork is well known to exhibit a lack of proteolysis and failure to tenderise (Channon *et al.* 2000, 2003; Kim *et al.* 2014). Cooking temperature of 60°C resulted in the lowest WBSF within both ageing periods, which was comparable to the WBSF of the raw meat. The reduced WBSF at 60°C in 3-days-aged pork in this study agrees with that in previous studies where WBSF for porcine *longissimus* was reduced by cooking between 53°C and 58°C (Christensen *et al.* 2011) and it can likely be explained by the solubilisation of collagen (Hamm 1966). The holding time of 30 min might have contributed to the solubilisation of collagen at 60°C, since Huang *et al.* (2011) did not find a reduction of WBSF in pork loin when cooking to end-point temperatures of 50°C and 60°C.

Ageing period and temperature did not demonstrate an interaction in relation to longitudinal, transverse or volume shrinkage (Table 1; $P > 0.05$) and, hence, the interactions were excluded from the analyses. Ageing affected the longitudinal shrinkage (Table 1; $P = 0.006$) but had no effect on the transverse or volume shrinkages ($P > 0.05$ for both). Pork aged for 15 days had a 2.6% reduction in longitudinal shrinkage compared with pork aged for 3 days (Table 1). Longitudinal shrinkage is associated with the denaturation of the myofibrillar and connective tissue proteins (Tornberg 2005) and its reduction with ageing indicates that a component of these mechanisms is affected by ageing. Although actin denaturation has commonly been implicated as a reason for longitudinal shrinkage (Purslow *et al.* 2016), it is not normally degraded during postmortem ageing. The muscle protein titin runs longitudinally, and its degradation could be a potential reason for the reduced longitudinal shrinkage during cooking in aged samples. Purslow *et al.* (2016) found that in beef *semitendinosus*, there were no differences in longitudinal shrinkage with ageing, which contradicts our results; however, this may be a consequence of differences between species. Transverse shrinkage remained the same in both ageing periods (Table 1), which is similar to the results of Straadt *et al.* (2007) who reported no changes between 4 and 14 days for the area of pork muscle fibres on microscopic transverse sections. Temperature had a significant ($P < 0.001$) effect on the levels of shrinkage in both cuboids and muscle fibre fragments. Transverse shrinkage of 10.7% was observed in pork loin cuboids cooked at 50°C, and this was doubled at 60°C (20.35%), with no further shrinkage occurring at >60°C. Significant longitudinal shrinkage occurred at temperatures >60°C (Table 1). We showed higher transverse shrinkage than that previously reported for pork loins aged for 5 days by Becker *et al.* (2016), whereas the longitudinal shrinkage at 60°C was comparable, and at 80°C it was lower. The completion of the transverse shrinkage at <60°C, and the onset of the longitudinal shrinkage at >60°C, correspond to previous findings in bovine muscles, (as reviewed in Warner *et al.* 2017). While the volume shrinkage was not affected by ageing alone or in interaction with temperature, it increased progressively with the increase in temperature and it reached up to 38% when the pork cuboids were cooked at 80°C. This agrees with the cooking loss of 39% and 33% for 3 days and 15 days respectively, confirming the relationship between volume shrinkage and cooking loss. However, at 50°C and 60°C, the shrinkage was greater than the cooking loss, indicating that the shrinkage at lower cooking temperatures is not always directly proportional to the loss of water. A similar trend was also seen in the study of Purslow *et al.* (2016) in bovine *semitendinosus*, but the differences in the extent of the shrinkage and the cooking loss in relation to temperature were smaller. The higher-volume shrinkage relative to the cooking loss at low temperatures, seen in the present study, may be attributed to the water being held strongly in the structure, and some of the water expelled by the shrinkage process is redistributed from the intracellular to the extracellular space, but does not escape the structure in the form of cooking loss.

Despite the absence of ageing effects on transverse shrinkage, the diameter of fibre fragments isolated from the cooked meat reduced with the extended ageing period (Table 1; $P = 0.004$). This was not related to specific temperature conditions, as there was no interaction between ageing and temperature ($P > 0.05$). This was in contrast to the absence of any effect of ageing on the transverse shrinkage in the present study, as well as in the study of Straadt *et al.* (2007), who measured diameters on transverse sections as described above. Discrepancies between studies is not surprising, since all three methods of assessing shrinkage are constrained by accuracy for cuboid measurements (Vaskoska *et al.* 2020*b*), sample preparation for sections and potential segmentation in the width for the fragments during homogenisation. The diameter of the fibres decreased with an increasing cooking temperature up to 70°C (Table 1;

Table 1. Effect of cooking temperature (50°C, 60°C, 70°C and 80°C) and ageing (3 days and 15 days) on longitudinal, transverse and volume shrinkage (%) of meat cuboids (50 mm × 30 mm × 30 mm) and on diameter of fibre fragments from raw and cooked pork loins (*longissimus lumborum*).

Dimensional change[A]	Ageing (days)		s.e.d. (ageing)	*P*-value (ageing)[B]	Cooking temperature (°C)					s.e.d. (temperature)	*P*-value (temperature)
	3	15			Raw	50	60	70	80		
Longitudinal shrinkage[C] (%)	9.85	7.24	0.9	0.006	NA[B]	1.86	3.19	10.3	18.83	1.27	<0.001
Transverse shrinkage[C] (%)	18.16	20.98	2.14	0.195	NA[B]	10.73	20.35	24.6	22.62	3.03	<0.001
Volume shrinkage (%)[C]	30.81	26.12	2.49	0.066	NA[B]	21.03	24.17	30.65	38.03	3.52	<0.001
Fibre fragment diameter (μm)	89.62	82.10	2.48	0.004	101.07	92.99	83.28	73.92	78.03	3.92	<0.001

The values are least squares means, and the standard error of difference (s.e.d.) and *P*-values for the main effects are shown. There were no significant ($P > 0.05$) two-way interactions.

[A]$N = 6$ for shrinkage (longitudinal, transverse, volume) and $N = 25$ for diameter.

[B]NA, not applicable, since shrinkage was calculated relative to raw.

[C]Longitudinal and transverse shrinkage are expressed relative to raw (~25°C).

$P < 0.001$), which confirms the findings of decreased fibre diameter with cooking in histology studies of pork sections (Straadt *et al.* 2007). Fibre dimensions are important for meat quality, since there is a general understanding that thinner fibres result in better tenderness (Hiner *et al.* 1953).

The PCA biplot (Fig. 2*a*) illustrates the overall relationships of the measured variables in cooked pork. As expected, scores for TWC and the cooking loss are opposite to each other, as well as diameter and transverse shrinkage, demonstrating their inverse relationship and, hence, negative correlation with each other. The positive relationship between cooking loss and volume shrinkage was supported by the study of Du and Sun (2005). Also apparent in the biplot is that cooking loss and WBSF of pork are more closely related to the longitudinal shrinkage than to transverse shrinkage (Fig. 2*a*). Du and Sun (2005) also found a high correlation ($r = 0.92$) between longitudinal shrinkage and hardness in pork ham. The PCA score plot (Fig. 2*b*) illustrates that cooking temperature separates the scores on the PC1 component, explaining 50.9% of the variability of the data, and that ageing period shows limited separation on the PC2 component (explaining 16% of the variance) at 50°C, but not consistently across temperatures (Fig. 2*b*). This confirms that the benefit of the prolonged ageing is only apparent under certain cooking conditions.

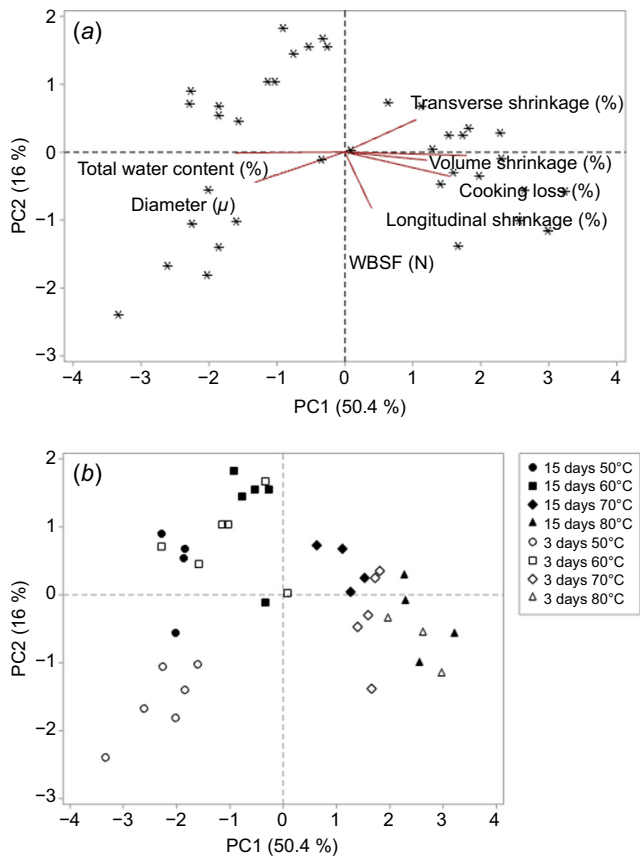

Fig. 2. (*a*) Biplot and (*b*) score plot from a principal-component analysis (PCA). PCA included the following variates: cooking loss, total water content (TWC), Warner-Bratzler shear force (WBSF), longitudinal shrinkage, transverse shrinkage, volume shrinkage and diameter, measured on cuboids from pork loins (*longissimus lumborum*) aged for 3 or 15 days and cooked to 50°C, 60°C, 70°C and 80°C. Line vectors on the Biplot *a* represent measured quality variate, dots on both plots (*a* and *b*) represent eigenvalue scores for each sample. Scores are grouped on the basis of temperature and ageing period in *b*).

Conclusions

Prolonged ageing for 15 days reduced cooking loss at 70°C and 80°C, reduced WBSF of pork at 50°C, as well as reduced overall longitudinal shrinkage and diameter of fibre fragments isolated from cooked meat, when compared with conventional ageing for 3 days. The lack of tenderisation with ageing for 12 days is likely due to the low pH of the samples in our study. TWC, as well as transverse and volume shrinkage, were not affected by the duration of ageing. Thus, it is evident that tenderness of pork, particularly if it is of low pH, will be improved by a combination of prolonged ageing and low cooking temperature, and the cooking loss of meat cooked to high temperatures might also benefit from prolonged ageing. Irrespective of cooking temperature, prolonged ageing will prevent some of the longitudinal shrinkage occurring during heating. Although the longitudinal shrinkage accounts for less in the overall volume shrinkage than does transverse shrinkage, it appears to have a higher contribution to WBSF and cooking loss.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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