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Effect of rigor temperature, ageing and display time on the meat quality and lipid oxidative stability of hot boned beef *Semimembranosus* muscle

Tanyaradzwa E. Mungure [a,*], Alaa El-Din A. Bekhit [a], E. John Birch [a], Ian Stewart [b]

[a] Department of Food Science, University of Otago, PO Box 56, Dunedin, New Zealand
[b] Department of Chemistry, University of Otago, PO Box 56, Dunedin, New Zealand

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ABSTRACT

The effects of rigor temperature (5, 15, 20 and 25 °C), ageing (3, 7, 14, and 21 days) and display time on meat quality and lipid oxidative stability of hot boned beef M. *Semimembranosus* (SM) muscle were investigated. Ultimate pH (pH_u) was rapidly attained at higher rigor temperatures. Electrical conductivity increased with rigor temperature ($p < 0.001$). Tenderness, purge and cooking losses were not affected by rigor temperature; however purge loss and tenderness increased with ageing ($p < 0.01$). Lightness (L*) and redness (a*) of the SM increased as rigor temperature increased ($p < 0.01$). Lipid oxidation was assessed using ^{1}H NMR where changes in aliphatic to olefinic (R_{ao}) and diallylmethylene (R_{ad}) proton ratios can be rapidly monitored. R_{ad}, R_{ao}, PUFA and TBARS were not affected by rigor temperature, however ageing and display increased lipid oxidation ($p < 0.05$). This study shows that rigor temperature manipulation of hot boned beef SM muscle does not have adverse effects on lipid oxidation.

1. Introduction

Several studies reported significant effects of rigor temperature on meat quality attributes (Bekhit, Farouk, Cassidy, & Gilbert, 2007; Farouk & Swan, 1998; Geesink, Bekhit, & Bickerstaffe, 2000). As rigor temperature increases, the rate of glycolysis in muscle also increases (Hertzman, Olsson, & Tornberg, 1993). This leads to a rapid decline in pH while the muscle has relatively high temperature providing protein denaturing conditions. This process results in pale, soft, and exudative (PSE)-like meat characteristics (Thompson et al., 2005; Warner, Dunshea, Gutzke, Lau & Kearney, 2014).

High rigor temperatures (>25 °C) have been reported to have an adverse impact on the most important quality attribute, meat tenderness, due to heat shortening (Hertzman et al., 1993; Rees, Trout, & Warner, 2002). While the pH and μ-calpain activity rapidly decline with increasing rigor temperature (Geesink et al., 2000), lightness (L*) of meat have been reported to increase with increased rigor temperatures (Farouk & Swan, 1998; Warner, Kerr, Kim & Geesink, 2014). Most of the reported studies have examined the effects of rigor temperature on *longissimus* et *lumborum* muscle as it is the most important commercial muscle and little attention was given to metabolically different muscles.

Furthermore, the effects of rigor temperature, ageing and display time on lipid oxidative stability of fresh meat are rarely reported. Lipid oxidation is a major mechanism of meat quality deterioration manifesting changes in flavour and colour. Lipid oxidation can also cause formation of toxic compounds such as aldehydes and ketones; this leads to reduced shelf life (Gray, Gomaa, & Buckley, 1996). The present study investigated the effect of rigor temperature on the quality of hot boned beef *semimembranosus* (SM) muscle at various ageing and display times. Lipid oxidation was also assessed using thiobarbituric acid reactive substances (TBARS) method. ^{1}H NMR was used as a novel technique to monitor the change in oxidation rate by analysis of the relative change in aliphatic to olefinic proton ratio (R_{ao}) and the aliphatic to diallylmethylene proton ratio (R_{ad}). Lipid oxidation was also analysed using changes in polyunsaturated fatty acids (PUFAs) as assessed by gas chromatography flame ionisation detection (GC-FID).

2. Materials and methods

2.1. Meat

Topside (SM) muscles were obtained from 6 heifers ($n = 6$) raised on pasture with an average hot-carcass weight of 278.9 ± 28.4 kg. The animals were slaughtered in an export licensed meat-processing facility (Alliance Group, Pukeuri plant, Oamaru, New Zealand), electrically stimulated [Square mono wave, 80 V, 25 s0], hot boned at 45 min post-mortem and processed within 2 h. Muscles from right and left top sides of each animal were divided into 4 samples from each animal. Samples were randomised to 5, 15, 20 and 25 °C rigor temperature treatment groups. Samples measured for their pH decline every hour and then removed from the treatment incubator once the samples reached their ultimate pH thus attaining rigor. They were vacuum

* Corresponding author.
E-mail address: tanyaradzwa.mungure@otago.ac.nz (T.E. Mungure).

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packed and aged for 3, 7, 14, and 21 days at 4 °C. At the designated ageing time, the pH, purge loss and conductivity of samples were measured then subsampled for colour, tenderness and lipid oxidation. Samples for colour were processed as described below and samples for tenderness measurements and lipid analysis were stored at −80 °C until analysis.

2.2. pH analysis

The pH of the meat samples was measured using a puncture pH electrode (InLab 427, Mettler-Toledo Process Analytical Inc., Wilmington, MA) attached to a pH metre Hanna HI 9025 (Hanna Instruments, Woonsocket, RI) every hour during the incubation time until pH_u was reached. The pH was also measured directly after the ageing period.

2.3. Electrical conductivity

The electrical conductivity ($mS \cdot cm^{-1}$) of the samples was measured after the ageing period using a hand held electrical conductivity metre (LF-Star, Matthäus, Pöttmes, Germany).

2.4. Purge loss

Sample weight was determined prior to and following the ageing process. Following ageing, the samples were blotted dry with paper towels before reweighing. Purge loss was calculated as a percentage using the formula below:

$$\text{Purge loss } (\%) = \left(\frac{(\textit{weight before storage} - \textit{weight after storage time})}{\textit{weight before storage time}}\right) \text{X } 100$$

2.5. Cooking loss

The samples were thawed at 2 °C overnight and cooked individually in plastic bags immersed in a water bath at 80 °C until the internal temperature reached 75 °C as measured by temperature probes. The cooked meat was cooled on ice, blotted dry with paper towels and weighed. The cooking loss was expressed as a percentage and calculated by the formula below:

$$\text{Cooking loss } (\%) = \left(\frac{(\textit{weight before cooking} - \textit{weight after cooking})}{\textit{weight before cooking}}\right) \text{X } 100$$

2.6. Shear force

Shear force was determined using a MIRINZ tenderometer as described by Chrystall, Devine, Graafhuis, and Muir (1993). The cooked samples were sliced parallel to the muscle fibre axis to produce 6 subsamples with a 10 mm × 10 mm cross section. The subsamples were each sheared on a MIRINZ tenderometer with a wedged shaped tooth and the peak shear force was recorded. The values were converted to Newtons using the following formula;

$$\text{Shear force (N)} = [(0.2035 \times \text{shear force/kPA}) - 2.2945] \times 9.8$$

2.7. Colour analysis

Objective colour measurements were obtained as described by Bekhit et al. (2007). After ageing, the beef SM muscle samples were placed in polystyrene trays and wrapped with oxygen permeable polyvinylchloride film (O_2 permeability >2000 $mL \cdot m^{-2} \cdot atm^{-1} \cdot 24\ h^{-1}$ at 25 °C) (AEP FilmPac Ltd., Auckland, New Zealand). Samples were displayed under cool fluorescent light (1076 lux) at 4 °C. Colour measurements were taken at 1, 3, 5 and 7 days of display time using a HunterLab colorimeter (model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA). The colorimeter was calibrated with a black and a white standard tile C2-36852. CIE L*, a*, b* values were taken using illuminant *C* and a 10° observer with an aperture size 2.5 cm and the reflectance values in the wavelength range of 400 to 700 nm. The Chroma ($C = [a^{*2} + b^{*2}]^{[1/2]}$) and hue angle ($HA = \tan^{-1} b^*/a^*$) were calculated. The browning index parameter 630 nm/580 nm ratio was determined.

2.8. Lipid oxidation analysis using thiobarbituric acid reactive substance (TBARS) Assay

TBARS assay was used to determine lipid oxidation as described by Du, Nam, and Ahn (2001). TBARS were measured at each ageing time and after 7 days of display time. Meat samples from the surface (3 g) were placed in 50 mL tubes and homogenised with 9 mL of deionised distilled water (DDH_2O) using a Polytron homogeniser at 14,000 rpm (PT-MR 2100, Kinematica AG, Switzerland) for 30 s. Meat homogenate (1 mL) was transferred to a 10 mL Falcon tube and 50 μL butylated hydroxytoluene (BHT, 7.2% w/v in ethanol) and 2 mL thiobarbituric acid (TBA)/trichloroacetic acid (TCA) (20 mM TBA in 15% TCA, (w/v)) solution were then added and the mixture vortexed for 30 s. The samples were incubated in a water bath at 90 °C for 30 min for colour development, and then cooled in cold slurry of ice water for 10 min. The samples were vortexed (30 s) and centrifuged at 2520 ×*g* (Beckman Coulter, Inc. *California*, USA) for 15 min at 5 °C. The absorbance of the resulting supernatant was read at 531 nm against a blank prepared with 1 mL DDH_2O and 2 mL TBA/TCA solution. The amount of malondialdehyde ($mg \cdot kg^{-1}$) in the sample was determined by using the following equation;

$$\textit{Mg MDA/kg meat} = A@531 \div E \times 72.063/100\ \textit{x dilution factor}$$
$$\textit{Where E is the molar extinction coefficient of MDA} = 156,000 M^{-1} cm^{-1}$$
$$\textit{Molar mass of malondialdehyde} = 72.063 g mol^{-1}$$

2.9. Lipid extraction for ^{1}H NMR lipid oxidation analysis and GC-FID

Lipid extraction was performed as described by Folch, Lees, and Sloane-Stanley (1957). Meat samples (100 g), from rigor temperatures 5 and 25 °C only due to financial constraints, aged for 7 and 14 days, and subsequent post display time were cut into small pieces and extracted in an Omnimixer with 200 mL chloroform/methanol (2:1 *v/v*) in a 1.9 L Mason jar. After dispersion, the whole mixture was agitated for 20 min in an orbital shaker at room temperature. The homogenate was then filtered through a funnel with Celite 545 with a folded filter paper. The solvent was washed with a 0.9% NaCl solution. The samples were vortexed for 30 s and the mixture was centrifuged at 2000 rpm for 5 min to separate the two phases. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was evaporated under vacuum in a rotary evaporator and under nitrogen gas.

2.9.1. ^{1}H NMR analysis

^{1}H NMR spectra were acquired using a Bruker Avance 400 spectrometer operating at 400 MHz. Each lipid sample (30 mg) was mixed with 650 μL of deuterated chloroform (purity 99.8%) and 6.5 μL 1, 4-dioxane as an internal reference in a 5 mm diameter tube. The acquisition parameters were: spectral width 5000 Hz, relaxation time 3 s. Spectra were recorded at room temperature with 65,536 data points where the number of scans was 128, acquisition time 1.6 s and pulse width 80° with a total acquisition time of 21 min 27 s. Exponential line broadening 0.30 Hz, automatic phase correction and baseline correction were applied to each spectrum. The assignment of the signals integrated and used for lipid oxidation analysis was made as described in previous studies (Guillén & Ruiz, 2004) and is given in Table 1. The ratios for