Modelling postmortem evolution of pH in beef *M. biceps femoris* under two different cooling regimes

Kumsa D. Kuffi³ · Stefaan Lescouhier² · Bart M. Nicolai¹ · Stefaan De Smet² · Annemie Geeraerd¹ · Pieter Verboven¹

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Abstract A model based on enzyme kinetics was developed to predict differences in postmortem pH change in beef muscles as affected by cooling rate. For the calibration and validation of the model, pH and temperature measurements were conducted at different positions in *M. biceps femoris* following conventional carcass cooling or faster cooling of the muscle after hot boning. The glycogen conversion, and, hence, the pH fall, was observed to significantly vary with position and cooling regime but only during the initial hours of cooling. Comparison of the cooling regimes indicated that fast cooling following hot boning avoids heat shortening induced by the combined effect of high temperature and low pH.

Keywords Postmortem heat production · Modeling · Muscle type · Muscle pH · Glucose conversion rate

Abbreviations

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BF	<i>Biceps femoris</i>
BC	Buffering capacity ($\text{mol m}^{-3} \text{pH}_{\text{unit}}^{-1}$)
c_p	Specific heat capacity ($\text{J kg}^{-1} \text{K}^{-1}$)
Cr	Creatine
$c_{p,m}$	Specific heat capacity of meat ($\text{J kg}^{-1} \text{K}^{-1}$)

PCr	Phosphocreatine
E_a	Energy of activation (J mol^{-1})
F6P	Fructose-6-phosphate
F1,6P ₂	Fructose-1,6-bisphosphate
[C]	Concentration of component C (mol m^{-3})
HBIBF	Hot boned inner BF
HBOBF	Hot boned outer BF
ΔH_{ATP}	Enthalpy change during ATP hydrolysis ($\text{J per mol P}_i^{-1}$)
IIBF	Intact inner BF
IOBF	Intact outer BF
K_a	Acid dissociation constant
K_m	Michaelis–Menten constant (mol m^{-3})
L	One-half the thickness of the sample (m)
La^-	Lactate
m	Exponent (that indicates the number of hydrogen ions attachment site)
N	Number of measurements
P	Parameter value
p	Number of model parameters
$\text{pH}_{i,m}$	pH value of individual measurement
pH_p	Predicted pH
PFK	Phosphofructokinase
P_i	Inorganic phosphate
Δ	Difference operator
∇	Gradient operator
R	Universal gas constant ($\text{J mol}^{-1} \text{K}^{-1}$)
R^2	R-squared value
RMSE	Root mean square error
$S_{vG,P}$	Relative sensitivity of glucose conversion rate with respect to model parameter
T	Temperature (K)
t	Time (s)
T_i	Initial temperature (K)

✉ Pieter Verboven
 pieter.verboven@biw.kuleuven.be

¹ BIOSYST-MeBioS, University of Leuven, Willem de Croylaan 42, 3001 Heverlee, Belgium

² Laboratory of Animal Nutrition and Animal Product Quality, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

³ Institute of Technology, Ambo University, P.O. BOX :19, Ambo, Ethiopia

T_{ref}	Reference temperature (K)
T_s	Surface temperature (K)
v_{ATP}	ATP production rate ($\text{mol m}^{-3} \text{s}^{-1}$)
v_G	Rate of glucose conversion ($\text{mol m}^{-3} \text{s}^{-1}$)
$V_{max,ref}$	Maximum glycogen conversion rate at reference temperature ($\text{mol m}^{-3} \text{s}^{-1}$)
v_{La}	Lactate production rate ($\text{mol m}^{-3} \text{s}^{-1}$)
x	Sample depth at which measurement was conducted (m)

Greek symbols

α	Thermal diffusivity ($\text{m}^2 \text{s}^{-1}$)
δ	Fraction of inorganic phosphate existing as HPO_4^{2-}
λ_m	Thermal conductivity of meat ($\text{W m}^{-1} \text{s}^{-1}$)
ρ_m	Density of meat (kg m^{-3})
σ	Standard deviation

Introduction

Adenosine triphosphate (ATP) is an immediate energy source for the processes taking place in muscle fibers. The central outcome of the intracellular energy metabolism in muscles is the maintenance of the cellular concentration of the ATP (Hamm 1977; Vetharaniam et al. 2010). Postmortem, the production of ATP continues via glycogen conversion, phosphocreatine conversion and aerobic synthesis by residual oxygen in the muscle myoglobin (Greaser and Guo 2012). The production of ATP from myokinase reaction is small when compared to the other reactions (Gearhart and Mintz 1975; Korzeniewski 2006; Vetharaniam et al. 2010). A simplified schematic overview of the main pathways for ATP production is presented in Fig. 1a as to guide the model derivation below. The pathways are presented based on the more elaborate work of Greaser and Guo (2012). As reported by Pösö and Puolanne (2005), the amount of ATP produced by the creatine kinase reaction and via aerobic synthesis from residual oxygen are estimated to be 20 and 5%, respectively, of the amount that is produced through postmortem glycolysis. It can thus be assumed that the energy for postmortem energy supply comes mainly from glycogen conversion. The glycogen conversion process, that starts from glycogenolysis followed by glycolysis, is catalyzed by several enzymes acting on a series of separate reactions to finally produce ATP (Vinnakota et al. 2006). Postmortem, ATP is consumed for involuntary slow muscle contractions, maintenance of the membrane potential and especially to preserve the calcium ions within the sarcoplasmic reticulum (Pösö and Puolanne 2005). ATP provides energy for the myosin head to slide over the actin filament during the contraction process (James and James 2002). If ATP is

depleted, the myosin head will cross-bridge with the actin filament which causes the postmortem muscle to be inextensible at rigor mortis (Hamoen et al. 2013).

In the cell, as glycolysis proceeds and the level of glycogen declines, lactate starts to build up and hydrogen ions are either buffered, transported out of the cell or left accumulated (Hamm 1977; Savell et al. 2005; Scheffler et al. 2011). ATP hydrolysis is proposed to be the major source of proton load during anaerobic muscle metabolism (England et al. 2014; Gevers 1977). Apart from being the main source of hydrogen ions, the hydrolysis of ATP also produces heat. According to de Meis (2001) the hydrolysis of ATP releases an enthalpy $\Delta H_{ATP} = 134 \text{ kJ/mol Pi}$ which is the source of heat that can raise the temperature of beef by 2.1 to 2.7 °C above body temperature (Morley 1974).

The postmortem evolution of temperature and pH determines the extent of sarcomere shortening (Honikel et al. 1983). Thompson (2002) defined a pH–T diagram as a function of postmortem cooling time, in which combinations of pH and temperature may result in cold shortening or heat shortening. These combinations can to some extent be controlled by the rate of cooling. Sarcomere shortening can cause meat toughness (Hamoen et al. 2013; Jacob and Hopkins 2014). According to Hwang et al. (2004), minimum sarcomere shortening of beef *M. longissimus* and *M. semitendinosus* muscles occurred at a rigor temperature of 15 °C. At this temperature the sarcomere length was 1.76 μm for both muscles. The authors also reported that both muscles have a reduced sarcomere length of 1.52 and 1.44 μm at rigor temperatures of 5 and 36 °C, respectively. In addition to effects on tenderness, it is well established that the postmortem evolution of pH and temperature also strongly determines other meat quality traits, e.g. colour and colour stability (Pastsart et al. 2013).

Taking into account the cooling profile and the time evolution of pH during the postmortem cooling by a priori modeling allows to understand and design cooling processes better. Different modeling approaches have been presented in the literature. Vetharaniam et al. (2010) developed a model based on the energy metabolism in sheep muscle under anaerobic conditions at constant temperature. Hamoen et al. (2013) adapted this model for the decreasing temperature of veal carcass cooling in slaughterhouses. The original model of Vetharaniam et al. (2010) contains various sub models and parameters that were incorporated via heuristics and thus make the models complex.

The objective of this work was to develop a simple kinetic model of postmortem glycolysis in *M. biceps femoris* (BF) of Belgian Blue carcass under different chilling conditions. The kinetic model was used for prediction of pH and biochemical heat generation in muscles of beef carcasses during the postmortem period. The model was developed for engineering analysis and predictions of

Fig. 1 A simplified diagram of postmortem muscle energy metabolism used for kinetic modeling in this work, based on the work of Greaser and Guo (2012) (a); measured temperature (b) and pH (c) of the four sub-datasets according to muscle depth and cooling regime.

Values in **b** and **c** are averages of different samples, error bars are standard deviation ($n = 52, 26, 7$ and 7 for intact inner (IIBF), intact outer (IOBF), hot-boned inner (HBIBF) and hot-boned outer (HBOBF) *Biceps femoris*, respectively)

changes related to postmortem period. The *M. biceps femoris* was chosen for this study as this muscle extends from the carcass surface to the deepest parts of the hindquarter where cooling is slow. The model was validated and used to compare two cooling regimes and different positions in the carcass.

Methods

The model for the postmortem evolution of pH in the *M. biceps femoris* (BF) of beef was developed from a simplified glucose conversion model that is fitted to experimental data. To calibrate and validate the model, temperature and pH measurements were conducted on beef *M. biceps femoris* under two cooling regimes, i.e. 1/conventional cooling of the intact carcass side and 2/cooling of the muscles in a conventional cooler after hot boning at 1 h post-mortem. The aim of hot boning was to reduce the size of the muscles so that the cooling rate could be accelerated.

Model development

Model reactions of glycogen conversion

The process of conversion of glycogen to lactate involves numerous enzymatic reactions (Hamm 1977; Vinnakota et al. 2006). Here we assume that the process can be described by a rate limiting enzyme in the glycolytic pathway such that the reaction can be represented in the following form (Hui et al. 2005):

where P_i^{2-} and P_i^{1-} stand for HPO_4^{2-} and $\text{H}_2\text{PO}_4^{1-}$ respectively; δ represents the fraction of free phosphate existing as P_i^{2-} in equilibrium with P_i^{1-} , and $n\text{Glucose}$ represents glycogen.

Kinetic model

Studies by Rhoades et al. (2005) and England et al. (2014) showed that the critical enzyme controlling glycolysis is phosphofructokinase. This enzyme is responsible for catalyzing the rate limiting step of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate conversion. Since the primary source of F6P is glycogen, which is

expressed in glucose equivalents, we used the glucose concentration $[\text{G}]$ instead of $[\text{F6P}]$ for writing down the Michaelis–Menten kinetics. As is evident from Eq. (1), only one glucose residue in glycogen is converted to produce the metabolites at the right hand side of the equation. Hence, the conversion rate is expressed in terms of glucose conversion instead of glycogen conversion although the primary source of the conversion is glycogen.

The rate of enzyme catalyzed reactions increases with temperature up to a certain limit (Shuler and Kargi 2002). During the postmortem cooling of beef carcasses, we believe that the muscle temperature will not reach the maximum limit that inhibits the activity of enzymes. Hence, the rate of enzyme catalyzed reaction in post-mortem muscle decreases only as the temperature decreases. In this study, the impact of temperature on rate of enzyme catalyzed reaction is expressed by the Arrhenius law (Gwanpua et al. 2012).

In addition to temperature, the rate of enzyme catalyzed reactions is also affected by pH. Enzymes have ionic groups on their surface thus need an optimum pH for maximum activity. Below or above this optimum pH their activity decreases as the charges on ionic groups vary, according to their acid dissociation constant K_a , when pH of the environment changes. In practice, enzymes can have more than one active and/or allosteric site; hence, the hydrogen ion can be attached to more than one site. The suggested model for glucose conversion during the post-mortem period then reads

$$v_G = - \frac{V_{\max, \text{ref}} [\text{G}] \cdot \exp\left(\frac{E_a}{R} \left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right)}{(K_m + [\text{G}]) \left(1 + \left(\frac{[\text{H}^+]}{K_a}\right)^m\right)} \quad (3)$$

where v_G ($\text{mol m}^{-3} \text{ s}^{-1}$) is the rate of glucose conversion in anaerobic muscle, $V_{\max, \text{ref}}$ ($\text{mol m}^{-3} \text{ s}^{-1}$) is the maximum conversion rate of the reaction at reference temperature, T_{ref} (K), equal to 37 °C, K_m (mol m^{-3}) is the Michaelis–Menten constant, K_a (mol m^{-3}) the acid dissociation constant and m is the exponent that indicates the number of active or allosteric sites of the enzyme to which hydrogen ions can be attached.

The relation of pH fall to lactate (La) formation during postmortem is conventionally expressed as the buffering capacity (BC) which is the slope of the curve relating the two parameters (Bendall 1979; Hamm 1977; Hamoen et al. 2013). In most of the muscles of mammals BC is constant from about pH 7.0 to the final pH 5.4 (Bendall 1979). Thus in this region, BC , pH and $[\text{La}]$ are related as given in Eq. (4).

$$BC = - \frac{d[\text{La}]}{dpH} \quad (4)$$

From stoichiometry:

$$\frac{dpH}{dt} = \frac{-v_{La}}{BC} = \frac{2v_G}{BC} \quad (5)$$

and

$$v_{ATP} = -3v_G \quad (6)$$

where v_{La} ($\text{mol m}^{-3} \text{ s}^{-1}$), v_{ATP} ($\text{mol m}^{-3} \text{ s}^{-1}$) and BC ($\text{mol m}^{-3} \text{ pH}_{\text{unit}}^{-1}$) are rates of lactate and ATP production and buffering capacity, respectively.

The hydrogen ion concentration $[H^+]$ is expressed in terms of pH ($[H^+] = 10^{-\text{pH}}$) to use measured data for fitting during determination of the optimum parameters in Eq. (3). Similarly the parameter K_a is expressed in terms of pK_a ($K_a = 10^{-\text{pK}_a}$) to avoid using small numbers.

Experimental data and parameter estimation

The temperature and pH were measured in *M. biceps femoris* of 59 double-musled Belgian Blue young bull carcasses at four time points (1, 3, 5 and 48 h) starting from exsanguination. The animals originated from commercial farms in Flanders and were slaughtered in four different commercial abattoirs. The average age at slaughter was 20.9 (SD 2.2) months. The carcasses were all classified in the conformation class 'S' and fat class '2' according to the SEUROP beef carcass classification system. The carcass weight was approximately 500 kg. Animals were slaughtered and cooled according to common practices, i.e. stunned by captive bolt, shackled and bled immediately, dehided, eviscerated, split and sent to conventional chilling rooms after approximately 1 h. From the 59 carcasses, 7 carcass sides were used for hot boning of the BF. Hot boning was performed within 1 h postmortem to allow faster cooling. The hot-boned BF muscle was vacuum packed and stored in the dark at 12 °C during the first 5 h pm and thereafter at 4 °C until 48 h pm. Part of the group of animals that were used for measurements on the intact carcass side, were also used in the study of Pastsart et al. (2013) for meat quality analyses.

Measurements of pH and temperature were done at two muscle depths, i.e. in the inner part of BF at 10 cm depth (IBF) and in the outer part at 2 cm depth (OBF). It was avoided to measure in the same spot at the different time points. For analysis, we grouped the measurements according to position in the muscle and cooling regime into intact inner *biceps femoris* (IIBF), intact outer *biceps femoris* (IOBF), hot-boned inner *biceps femoris* (HBIBF) and hot-boned outer *biceps femoris* (HBOBF).

From the four time point measurements a complete cooling curve was reconstructed using the following curve fitting equation (inspired by the solution of the heat conduction equation):

$$\frac{T - T_f}{T_i - T_f} = \sum_{n=0}^{\infty} \frac{4(-1)^n}{(2n+1)\pi} \cos \frac{(2n+1)\pi x}{2L} \cdot \exp \left(-\alpha \left(\frac{(2n+1)\pi}{2L} \right)^2 t \right) \quad (7)$$

where x and L are fitted and n was set equal to 3. The fitted parameters could be interpreted as dimensional parameters of the carcass, but here they are used rather as empirical values to obtain a continuous and smooth cooling profile according to simplified heat conduction mechanisms. T , T_f and T_i (K) are the temperature at a given time, the final temperature and the initial temperature of the sample, respectively. n represents the number of terms in the solution of heat conduction equation. The initial temperature T_i (K), the temperature just after exsanguination, was set to 40 °C. The parameter α ($\text{m}^2 \text{ s}^{-1}$) was set equal to a value of $1.14 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ (which is an estimate for the thermal diffusivity of the meat).

OptiPa, a software tool to calibrate mathematical models (Hertog et al. 2007) was used to determine the optimum values of the parameters of the model Eqs. 3, 5 and 7 using the cooling profiles and measured pH data. 50% of the measured data from each class were used for model calibration. The remaining 50% of the measured data were used for model validation.

The empirical values (x and L) in Eq. (7) were estimated by fitting the curve for the four sub-datasets sorted according to muscle position and cooling. After obtaining the best fits of the cooling curves for each sub-dataset, common values of kinetic parameters in Eqs. (3) and (5) were determined with the same dataset selected for calibration. The value of m was initially set to 2, and the effect on the fits was assessed for values of 1, 3 and 4 as well. The initial values of the parameters were set to literature values as given in Table 1.

Parameter sensitivity analysis

A sensitivity analysis was performed to study how sensitive the glycogen conversion rate was with respect to small changes in model parameter values. Relative sensitivity is

Table 1 Initial parameter values of the glucose conversion model [Eqs. (3–5)]

Parameter	Initial guess	Reference
$V_{\text{max,ref}}$ ($\text{mol m}^{-3} \text{ s}^{-1}$)	9×10^{-4}	Rhoades et al. (2005)
pK_a (mol m^{-3})	6.9	Dobson et al. (1986)
K_m (mol m^{-3})	0.6	Rhoades et al. (2005)
BC ($\text{mol m}^{-3} \text{ pH}_{\text{unit}}^{-1}$)	50.7	Bendall (1979)
E_a (kJ mol^{-1})	40	Jeacocke (1977)

used to allow comparison between different parameters (Ho et al. 2013). In this study, the effect of changes in each model input parameter on glucose conversion rate was examined by varying one input parameter by 10% at a time and keeping the other parameter constant. Parameters were then ranked based on relative sensitivity so that more attention could be given to more sensitive parameters.

$$S_{v_G, P} = \left(\frac{\partial v_G}{\partial P} \right) \left(\frac{P}{v_G} \right) = \left(\frac{[v_{GP+\Delta P}] - [v_{GP-\Delta P}]}{2\Delta P} \right) \left(\frac{P}{v_G} \right) \quad (8)$$

Where $S_{v_G, P}$ is the relative sensitivity of glucose conversion rate with respect to the input parameter P .

Statistical analysis

Data in the text are presented as mean values $\pm$ standard deviation. A one-way ANOVA was used to investigate significance differences ($P < 0.001$) according to muscle position and cooling regime.

Results

Postmortem temperature and pH fall

The average experimental temperature course and pH fall in the BF muscle of the intact and hot-boned sides are presented in Fig. 1b, c. There was a significant difference in temperature decrease between IIBF and IOBF, due to the large diffusive resistance of the carcass side (Fig. 1b). Already after 1 h, the mean values differed significantly (39.0 ± 0.8 °C for inner versus 31.6 ± 3.4 °C for outer muscle). After 5 h, the temperatures were 36.1 ± 1.0 °C for the inner position in the muscle versus 21.4 ± 3.1 °C for the position closer to the surface. The temperature equalized after 48 h.

In the same figure, the mean temperature of the hot-boned muscles (HBIBF and HBOBF) is also presented. A significant difference between the two positions was only observed after 1 h with values of 39.1 ± 1.4 °C versus 31.1 ± 4.0 °C for the HBIBF and HBOBF, respectively. For the rest of the cooling, the two positions experienced very similar temperature trajectories. As expected, at the inner and outer measurement positions, hot-boned muscles cooled faster than the corresponding intact muscles.

As a result of the different cooling rates, significant differences in pH decline were observed between IIBF and IOBF (Fig. 1c). After 1 h, the mean pH values were 6.63 ± 0.16 at the inner position versus 6.92 ± 0.13 at the outer position. After 5 h of cooling, the difference became larger with values of 5.42 ± 0.08 and 6.58 ± 0.28 , respectively. Differences at time points 1, 3 and 5 h were

significant ($p < 0.001$). Contrary to the differences in pH in the intact carcasses, smaller and insignificant differences were observed between HBIBF and HBOBF (Fig. 1c). The mean pH values were 6.53 ± 0.31 for the inner position versus 6.87 ± 0.26 for the outer position after 1 h, and 6.10 ± 0.39 versus 6.29 ± 0.39 after 5 h. The pH in the inner part of the muscle was lower than in the superficial part, due to the effect that temperature has on enzyme activity. At higher temperature, the conversion rate of energy substrate is greater than at lower temperature, hence the production rate of hydrogen ions is greater in the inner part of the muscle than at the surface.

Parameter fitting and sensitivity analysis

The kinetic model of glycolysis was fitted to the observed pH profiles, using the temperature curves as inputs. Starting from an initial guess with literature values (Table 1), the model parameter values in Table 2 were obtained by fitting the model to 50% of the measured pH data from all muscle groups to obtain a single set of parameters. This 50% of the data includes measurements conducted on 46 sides at the four time points.

The actual value of m in Eq. (3) was determined by fitting the model to the measured pH values for $m = 1, 2, 3$

Table 2 Fitted values of model parameters, the corresponding standard deviation (σ) and R^2 -values of the fit for different values of exponent m in Eq. (3)

m	Parameter	Fitted value	σ	R^2
1	$V_{max,ref}$ (mol m ⁻³ s ⁻¹)	5.0×10^{-3}	2×10^{-4}	68.6
	pK_a (mol m ⁻³)	5.8	0.11	
	K_m (mol m ⁻³)	0.53	34.56	
	BC (mol m ⁻³ pH _{unit} ⁻¹)	93.2	2.7	
	E_a (kJ mol ⁻¹)	58.6	337.3	
2	$V_{max,ref}$ (mol m ⁻³ s ⁻¹)	4.4×10^{-3}	1×10^{-4}	69.4
	pK_a (mol m ⁻³)	5.79	0.13	
	K_m (mol m ⁻³)	0.57	117.94	
	BC (mol m ⁻³ pH _{unit} ⁻¹)	93.6	1.93	
	E_a (kJ mol ⁻¹)	58.4	291.7	
3	$V_{max,ref}$ (mol m ⁻³ s ⁻¹)	2×10^{-3}	5×10^{-4}	70.3
	pK_a (mol m ⁻³)	5.7	1.3×10^{-5}	
	K_m (mol m ⁻³)	0.63	18.9	
	BC (mol m ⁻³ pH _{unit} ⁻¹)	43.3	14.6	
	E_a (kJ mol ⁻¹)	57.6	501.9	
4	$V_{max,ref}$ (mol m ⁻³ s ⁻¹)	1.3×10^{-3}	1×10^{-4}	69.7
	pK_a (mol m ⁻³)	5.7	0.042	
	K_m (mol m ⁻³)	0.5	29.34	
	BC (mol m ⁻³ pH _{unit} ⁻¹)	27.7	2.93	
	E_a (kJ mol ⁻¹)	59.3	467.2	

and 4 and then the best fit was selected. As the value of m must be an integer greater or equal to one, this approach was preferred over fitting it together with the other model parameters. The best R^2 value was obtained at $m = 3$, as shown in Table 2.

Using the set of fitted parameter values from Table 2, a sensitivity analysis of the glucose conversion rate with respect to the individual parameters was performed. As shown in Fig. 2a, the glucose conversion rate is sensitive to all of the parameters considered except K_m . Moreover, as shown in Fig. 2b, the model is not sensitive to the initial glycogen concentration but very sensitive to the initial pH. With expected high glycogen levels and the low value of K_m the glucose conversion rate can be further simplified to:

$$v_G = V_{max,ref} \cdot \exp\left(\frac{E_a}{R} \left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \left(1 + \left(\frac{[H^+]}{K_a}\right)^3\right)^{-1} \quad (9)$$

During the fitting, we observed that the standard deviation for E_a tends to be high although the fitted values are almost similar in all cases. Hence we decided to keep the

value of E_a to 57 (kJ mol⁻¹) and continued further fitting with the three important parameters ($V_{max,ref}$, BC and pK_a). The new best fit values of these parameters are shown in Table 3. These new fitting values and value of $E_a = 57$ (kJ mol⁻¹) were used for the pH predictions of the muscle groups.

Predictions of postmortem pH

As shown in Fig. 2d, the prediction of the pH decline from the glucose conversion model is generally well showing the differences between the sub-datasets according to muscle position and cooling regime. From the figure, it is clear that the pH of IOBF, HBIBF and HBOBF significantly keeps decreasing at least up to 48 h after exsanguination which may indicate that low temperature does not inhibit the activities of the rate limiting enzyme. The temperature curves used for the pH predictions are presented in Fig. 2c.

The goodness of the pH predictions at the four time points for the four sub-datasets is computed via the Root Mean Square Error (RMSE), which is defined as:

Fig. 2 Relative sensitivity of glucose conversion rate with respect to small variations in different model parameters (a) and the initial values of glucose concentration and pH (b); comparison between experimental (asterisk) and predicted values (line) of temperature (c) and pH d for the validation dataset. The solid lines in c and d represent the predicted values while the symbols represent experimental values. The predictions were obtained with the parameter values in Table 3. The sub-datasets were intact inner (IIBF), intact outer (IOBF), hot boned inner (HBIBF) and hot boned outer (HBOBF) *Biceps femoris*

Table 3 Fitted values of model parameters, the corresponding standard deviation (σ) and R^2 -values of the fit using a value (57 kJ mol^{-1}) of E_a

m	Parameter	Value	σ	R^2
3	$V_{max,ref} (\text{mol m}^{-3} \text{ s}^{-1})$	2.6×10^{-3}	3.0×10^{-9}	70.4
	$pK_a (\text{mol m}^{-3})$	5.7	0.043	
	$BC (\text{mol m}^{-3} \text{ pH}_{unit}^{-1})$	55.1	1.95	

$$RMSE = \sqrt{\frac{\sum_i (pH_{i,m} - pH_p)^2}{N - p}} \quad (10)$$

where $pH_{i,m}$ and pH_p are the measured and predicted values respectively. N is the number of measurements and p is the number of fitted parameter ($p = 3$).

The RMSE values of the pH predictions for sub-dataset at each measurement time are calculated based on the dataset used for validation and are shown in Fig. 3a. The purpose of this figure is to compare the predictive errors of the model for each sub-dataset and to verify if the errors change with time of cooling. It is clear that the model predicted the ultimate pH (at 48 h) more accurately than the pH at 5 h for the outer position in the muscle. This difference is linked to the large variation among the measured data values at 5 h compared to those at 48 h.

In Fig. 3b, a $T(t)$ – $pH(t)$ diagram is presented based on Thompson (2002), in which for each time point the temperature and pH are plotted for each sub-dataset cooling profile using the model predictions. The predictions show that under conventional chiller conditions, IIBF has a risk for heat shortening ($pH < 6.0$ and $T > 35^\circ \text{C}$, according to Thompson (2002), which agrees with the previous work by Pastsart et al. (2013). The prediction for IOBF followed the ideal path where the occurrence of either heat or cold shortening is not expected. The predictions further show that HBIBF and HBOBF may be prone to cold shortening ($pH > 6.0$ and $T < 12^\circ \text{C}$, according to Thompson (2002). This was checked by measuring sarcomere length on HBIBF and HBOBF samples and cold-boned samples from the counterpart carcass sides. The average sarcomere length of the hot-boned BF samples at the outer position was $1.8 \pm 0.2 \mu\text{m}$ versus $1.9 \pm 0.2 \mu\text{m}$ for the cold-boned counterparts. The corresponding values at the inner position were $1.7 \pm 0.2 \mu\text{m}$ for the hot-boned samples versus $1.8 \pm 0.2 \mu\text{m}$ for the cold-boned counterparts. There was thus a slight but not significant sarcomere shortening that is not expected to induce differences in toughness.

Discussion

The model was shown to predict well the difference in pH decline of beef *M. biceps femoris* under different cooling regimes without having to go into every detail of

postmortem glycolysis. As observed from the sensitivity analysis, the glycogen conversion rate depends more strongly on the model parameters pK_a and BC but in general is not dependent on K_m . There are only slight differences between fitted values of these parameters and previously published values. These minor variations observed on the values in this study and those published in the previous studies can be linked to the difference in muscle types on which the experiments were conducted. Literature values of $V_{max,ref}$ were obtained from *M. beef sternocephalicus pars mandibularis* muscle (Rhoades et al. 2005) which could have different enzyme concentrations than *M. biceps femoris* muscle, while the pK_a value was obtained from an experiment conducted on rat muscle at 25°C (Dobson et al. 1986). All the other initial kinetic parameter values were obtained from experiments that were conducted on beef muscles other than *M. biceps femoris*.

The model uses as inputs the initial concentration of glycogen, the initial pH and the temperature as a function of time. The initial glycogen concentration depends on the conditions that the animals are exposed to during the pre-slaughter periods which includes, among other factors, the type of diet the animals have been fed (Ferguson et al. 2008). In bovine the mean initial glycogen concentration is around 88 mmol/kg (Immonen et al. 2000). With this high glycogen level, which is usually the case during normal slaughtering practice for beef, and low value of K_m the mathematical model for glucose conversion is simplified to Eq. (9), thus making the pH changes relatively insensitive to the exact glycogen concentration.

The initial pH value generally depends on the stress that the animal faces during the exsanguination. With the normal slaughtering practice in slaughterhouse, the initial pH value for beef carcass is around 7.2 which of course can vary among muscle types (Bendall 1979). The variation in initial pH of different muscles of the same species could be linked to the intensity of activity of the muscle type during death struggle. For this study we used an initial pH of 7.2 for all samples, but its high sensitivity points out the importance of proper stress control during slaughter.

We fitted the temperature profile in the BF muscle based on a limited number of measured data points in time. To overcome this limitation, heat transfer models are available to predict the temperature profile as a more continuous function of position and time in the carcass, as function of carcass properties and cooling conditions which is generally represented by Eq. (11) (Pham et al. 2009).

$$\rho_m c_{p,m} \frac{\partial T}{\partial t} = \nabla \cdot (\lambda_m \nabla T) + S \quad (11)$$

where $\rho_m (\text{kg m}^{-3})$, $c_{p,m} (\text{J kg}^{-1} \text{K}^{-1})$, $\lambda_m (\text{W m}^{-1} \text{K}^{-1})$, $S (\text{W m}^{-3})$ are the density, heat capacity, thermal

Fig. 3 Root Mean Square Error (RMSE) of the pH predictions at the different measurement time points for the validation dataset (a) and predicted pH values at the corresponding average temperature per sub-dataset as a function of cooling time (b). The predictions in b were done with parameter values in Table 3. The sub-datasets were intact inner (IIBF), intact outer (IOBF), hot boned inner (HBIBF) and hot boned outer (HBOBF) *Biceps femoris*

conductivity and heat source due to ATP hydrolysis, respectively. Until recently, the source term in Eq. (11) had been either ignored or included without explaining how it affects the temperature profile within the cooling carcasses. Also in this work, Eq. (7) ignores this term. Recently, Hamoen et al. (2013) modelled the source term as a function of ATP production rate for calves. The authors adapted the original mechanistic model of Vetharaniam et al. (2010) towards a model for varying temperatures in the postmortem cooling process and showed how the source term is linked to the temperature profile within the cooling carcasses. The dimensions of the calves were much smaller than those of the animals under study here; it can therefore be expected that the heat production may play an even larger role for animals with very muscular conformation.

The model assumptions made here are that ATP produced is totally hydrolyzed, which is associated with the muscle entering *rigor mortis* state, the rate of production of the final products of glycolysis are controlled by a rate limiting reaction step and also that the thermal properties of the muscle are uniform. In practice, the composition varies across a muscle (Trujillo and Pham 2003) and hence thermal properties can vary locally. Thus by using local thermal properties, instead of uniform properties, one can get relatively better accuracy. However, to use local thermal properties CT scans that show contrast for muscle, fat and bone have to be used.

During ATP hydrolysis about 1/3 of the energy is dissipated as heat. The rest of the energy is stored in electrochemical energy of the Ca^{+2} gradient over the sarcoplasmic reticulum membrane (de Meis 2001). Thus the rate of heat production per metric cube of muscle can be estimated as:

$$S = -1.25v_G\Delta H_{ATP} \quad (12)$$

It is clear from Eq. (3) that this heat production is significant during the initial hours of cooling of intact carcasses especially in the inner part of muscles where the rate of cooling is slow. For hot-boned muscles and near surface muscles of intact carcasses, the heat production would be negligible as they cool fast and soon after slaughter.

This work has demonstrated that hot boning of muscles leads to faster cooling compared to cooling the corresponding intact carcass side and hence reduces the rate of initial pH decline. This could be linked to improved conduction heat transfer in hot-boned muscles owing to the size reduction of the meat compared to the intact carcass sides. This result suggests hot boning as an alternative cooling method to maintain the desired meat quality traits compared to forced air cooling methods commonly employed in conventional carcass chillers. However, removal of the muscle from the carcass during the pre-rigor

phase can make the muscle contract more easily than the corresponding muscles remaining attached to the skeleton of the carcass side (Pinto Neto et al. 2013). Although the contraction process may thus be faster for hot-boned muscles, we assumed that both carcass cooled and hot-boned muscles have similar energy metabolism.

The kinetic model presented here, in combination with the above discussed heat transfer models, and could then be used to investigate the applicability of other cooling methods that have the potential to increase cooling rates. However, hot boning can drive the meat into conditions for cold shortening, hence, the increment in cooling rate should be controlled in the way that avoids the quality damage due to too fast cooling. These cooling methods may include blast/rapid chilling, cold pipes, perfusion chilling and CO_2 chilling, all of which are designed to rapidly reduce the carcass temperature as result of which weight loss and microbial growth can be minimized. Other quality issues like tenderness should be accounted for with this improved chilling rate as well. This can be achieved by optimizing the cooling rate and rate of metabolic conversions in such a way that the decline in pH should pass through the ideal window proposed by Meat Standards Australia (MSA).

Conclusion

The rate of cooling of beef muscles is affected by the chilling method employed for this purpose. Hot boning the *M. biceps femoris* accelerates cooling and slows down the metabolism resulting in a decreased rate of pH decline. The combination of cooling rate and pH decline determines the fate of the final meat quality such as tenderness which then indicates the importance of knowing the conversion rates of energy substrates that are linked to changes in pH and the temperature of the muscles to obtain a wholesome meat product. To this end, we developed models that predict pH decline and heat production rate so that chiller room designers/slaughterhouse operators can consider both the cooling and conversion rates simultaneously to achieve the desired quality meat product. The model was experimentally validated, and generally a good agreement was observed between predicted and measured values. Hence we recommend designers/operators of the chiller rooms to include models for conversion rates when striving for optimizing heat load to obtain meat with desired quality properties.

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Kumsa D. Kuffi, the first author of this manuscript, was a PhD student at KU Leuven, Belgium. The author is currently an assistant professor at Ambo University, Ethiopia.

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