EFFECTS OF POSTMORTEM PH/TEMPERATURE DECLINE ON CHANGES IN FREE AMINO ACIDS DURING AGEING IN PIG LONGISSIMUS MUSCLE

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Background

Structural and cytoplasmic proteins of meats are exposed to proteolytic actions of endogenous proteolysis during ageing and result in polypeptides. The degradation of products consequently generate small peptide and free amino acids by subsequent actions of peptidases and aminopeptidases, respectively (Toldra et al., 2000). It has been well documented that interaction between pH and temperature during rigor development directly affects the incidence of PSE meats (Henckel et al, 2000). Moya et al. (2001) showed that changes in free amino acids during ageing varied between PSE, RFN, RSE, and DFD in pork. These changes are related to aminopeptidases activity under a particular class of pork (Toldra and Flores, 2000). The results mirrored that pH/temperature window during the onset of rigor can have an effect on changes in free amino acids during ageing. Lawrie (1991) noted that the accumulation of some free amino acids could result in an undesirable flavor and decrease in the water-holding capacity. However, free amino acids are of great importance in eating quality due to their specific tastes (Nishimura and Kato, 1988). Their subsequent degradation generates volatile compounds (Toldra, 1998; Hernandez-Jover et al., 1996).

Objective

To determine the effects of pH/temperature profile during rigor development on changes in the concentration of free amino acids for 7 days at 1°C.

Materials and methods

Animals, experimental design, and treatment. A total of 20 male pigs weighing an average of 118 kg (10 head of 194-day-old Yorkshire and 10 head of 201-day-old Landrace) were sampled from the National Livestock Research Institute (NLRI) feeding program. The pigs were conventionally transported to the NLRI abattoir, approximately 65 km away, with minimum transit stress, and kept off feed but with access to water, a day before they were slaughtered. To generate a large range of declines in pH and temperature, researchers placed the pigs in a -3°C chiller (five of each breed), or in a 5°C chiller (for the rest of the five of each breed) until the following day. All pigs were conventionally slaughtered over two consecutive days with an electronic stunner (230 volts for 2.5 sec).

pH, temperature, sampling, and objective quality measurements. Muscle temperature was logged at a 5-min interval from approximately 30 min after stunning for 24 hours (Thermo Recorder, TR-50C, Japan) by using thermocouples inserted into the geometrical center of the muscle between the 3rd and 4th lumbar vertebrae. The pH was measured by using a portable needle-tipped combination electrode (NWKbinar pH-K21, Germany) inserted into the center of the muscle between the 3rd and 4th lumbar vertebrae at a 15-min interval from approximately 30 min postmortem, until the muscle was judged to have reached the ultimate pH. Another measurement was made the following day, approximately 24 h postmortem.

The day after slaughter, *m. longissimus* muscles (from the 7th thoracic vertebrae to the last lumbar vertebrae) were removed, cut into three portions, vacuum-packed, and randomly assigned to one of three ageing periods (1, 3, and 7 d). These were used to get the objective measurements of WB-shear force, meat color, drip loss, and cooking loss. The samples were held at 1°C for the relevant ageing period.

For amino acid analysis, approximately 2 g of muscle tissue were taken at the end of the lumbar vertebrae by using a home-made biopsy sampler during breeding (i.e., 0 h), 4, 12, and 24 h postmortem. Similar amounts of muscle tissues were also sampled at 3 and 7 days postmortem from the WB-shear force block. Muscle tissue was frozen in liquid nitrogen immediately after sampling, powdered in liquid nitrogen by using a mortar-based homogenizer (Warning, Dynamics Corp., USA), and stored at -70°C until analysis. WB-shear force, meat color, and cooking loss were determined according to the procedures of Hwang et al. (2004).

Free amino acids. Level of free amino acids was determined at 1 and 7 d postmortem, largely according to the method reported by Moya et al. (2001), but with minor modification. Briefly, one (1) gram of liquid

nitrogen powdered sample was homogenized in 0.01N HCl with a polytron (3x15 sec) at 4C and centrifuged at 10000g for 20 min. About 300 uL of supernatant was filtered and deproteinised for 30 min at room temperature after mixing with 690 uL of acetonitrile and 10 uL of internal standard (L-Citrulline, 250 pmol/uL). Samples were centrifuged at 10000g for 15 min and 16 primary amino acids were determined by online derivatization using o-phthalaldhyde (OPA). Amount of free amino acids were analyzed by using the Agilent 1100 HPLC system (Agilent Tech, Waldbronm, Germany) at diode array UV detector (338 nm, 10 nm band wide) with ZORBAX Eclipse-AAA C18 column (4.6x150 mm, 5 um, Agilent Tech, Waldbronm, Germany). Separation for each sample was completed in 20 min at 40C. A gradient mobile phase between 40 mM Na_2HPO_4, pH 7.8 and acetonitril-methanol-water (45:45:10, v/v) was used and expressed as pmol. The effects of breed and ageing time on objective meat quality and free amino acid contentrations were examined by applying a general linear model (SAS, 1997), where the effects were tested against residual error.

Results and discussion

The temperature treatments (ie., -3°C and 5°C) did not affect pH/temperature profile in longissimus muscle during rigor development, but the experiment design resulted in wide ranges in pH (6.0-6.9 at 3 h pm) and temperature (20-29°C, data not shown). An average sarcomere length of 1.74 µm without any treatment effect indicated that there was no muscle shortening despite the temperature of 0.6 to 36°C with a pH of 6.2 (Table 1). The pH/temperature profile did not significantly differ between breeds, but slow rate in pH decline was noticeable in this study (ie. 6.2 at 4 h pm), with a normal ultimate pH of approximately 5.4 (data not shown). The result was not clearly understood as our previous data showed a pH of ca. 6.1 at 3 h by applying the same procedure (Hwang et al., 2004). But it might be in part related to a delayed transit time with extreme traffic jam. Landrace showed significantly ($P<0.05$) lower WB-shear force and higher hunter L* value than Yorkshire, but there was no correlation between breed and ageing time.

It has been well documented that proteolysis takes place in structural and cytoplasmic protein during ageing, and this significantly is affected by pH/temperature decline during rigor development (Dransfield, 1994; Hwang and Thompson, 2001). The latter study showed a significantly faster reduction in u-calpain activity when muscle had a rapid pH decline with slow pH decline, resulting in early exhaustion of the calpain and slow ageing rate. Despite activities of exopeptidases (ie, dipeptidylpeptidases and aminopeptidases) significantly varying between pig breeds (Armero et al., 1999) and muscles (Cronet and Bousset, 1999), the result of Toldra and Flores (2000) implied that pH/temperature profile at early postmortem might affect the rate and extent of aminopeptidase activities. These consequently affect changes in free amino acids during ageing.

In the current study, two amino acids (Gly and Cyc) out of 16 primary amino acids were significantly ($P<0.05$) affected by breed, where landrace had higher concentrations (Table 1). While concentration of all examined amino acids (except Met) increased over 7 days, the interaction between breed and ageing was not detectable. To examine the effects of pH/temperature profile during rigor development on changes in free amino acids for 7 d, the amino acid concentrations at 0 day (sampled during bleeding) were subtracted from those at the 7th day, and estimated the levels as a function of muscle temperature at pH 6.2 (Tehmph62). Tehmph62 has been used as an important threshold, because it could be an indirect indication of cold and heat shortening (Pearson & Young, 1989), denaturation of myofibril and sarcoplasmic proteins (Offer & Cousins, 1992), and proteolytic and/or autolytic activity of µ-calpain (Dransfield, 1994).

Tempph62 had simple correlation coefficients (r) of -0.9 and –0.93 with pH at 2 and 4 h; respectively, and of 0.4 and 0.5 with temperature at 2 and 4 h , respectively. Tempph62 did not affect changes in extractable concentrations for His, Tyr, Cyc, Gly, Iso, Ala. On the other hand, Tempph62 had significant curvelinear effects on eight amino acids (Asp, Glu, Leu, Lys, Val, Thr, Ser, Arg), varying in magnitude, while it showed only a linear effect on Met and Phe (Figure 1). Although the curvelinear effect was relatively weak for most amino acids, as seen in figure 1, there was a clear tendency of free amino acids to have higher concentration when muscle temperature at pH 6.2 (say during rigor development) was low. Aminopeptidase activities were not determined. However, an early study (Toldra and Flores, 2000) reported that Alanyl had a significantly lower activity in PSE meat than in DFD meat at 2 h pm. Alanyl, arginyl, and leucyl in PSE meat had significantly lower activities than those in DFD meat. Although, in our data set , changes in Ala were not affected by Tempph62, the results implied that pH/temperature interaction during rigor development not only affected the rate of proteolysis (Hwang et al., 2004), but also affected subsequent release of free amino acids.

Conclusion

Prelimarily data of current study show that pH/temperature significantly affect the release of free amino acids during ageing, with higher levels at a lower temperature and pH of 6.2. However, some amino acids are not affected by declines in pH and temperature. This may have a relation to taste development during ageing and cooking.

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Table 1. Least square mean and significance level of carcass traits, objective meat quality and changes in concentration of free amino acids from death to 7 d postmortem as a function of breed and ageing time.

	Breed		Ageing			Model terms	
	Landrace	Yorkshire	1	7	Av. Se	breed	ageing
Carcarss traits and objective meat quality							
pH at 4 h pm	6.2	6.2	na	na	0.07	ns	
Temperature at 4 h pm (°C)	22.1	21.1	na	na	0.09	ns	
Temperature at pH 6.2 (°C)	21.5	20.4	na	na	3.05	ns	
Sarcomere length (um)	1.74	1.74	na	na	0.02	ns	
WB-Shear force (kg)	6.25	7.50	8.04	5.71	0.26	***	***
Hunter L*	45.02	42.34	41.95	45.41	0.91	*	*
Concentration free amino acids (mg/ 100g wet tissue)							
ASP	0.9	0.8	0.0	1.7	0.20	ns	***
GLU	7.1	7.7	5.5	9.3	1.03	ns	*
SER	3.3	2.9	1.2	5.1	0.32	ns	***
HIS	2.4	2.7	0.0	5.1	0.35	ns	***
GLY	6.3	5.4	4.2	7.5	0.29	*	**
THR	2.5	2.3	1.9	2.9	0.28	ns	*
ARG	3.7	3.8	1.9	5.6	0.39	ns	***
ALA	65.8	66.0	14.0	117.8	5.68	ns	***
TYR	7.2	6.4	3.5	10.1	0.64	ns	***
CYC	0.5	0.2	0.0	0.8	0.09	*	***
VAL	3.5	3.1	2.1	4.6	0.30	ns	***
MET	3.3	3.1	ns	ns	0.70	ns	ns
PHE	4.0	3.9	1.8	6.0	0.22	ns	***
ISO	2.9	3.0	1.1	4.8	0.21	ns	***
LEU	3.5	3.4	1.2	5.7	0.34	ns	***
LYS	4.6	4.3	1.3	7.6	0.49	ns	***
df[a]						1/18(1/37)	1/18(1/37_

na-Not applicable, ns- P > 0.05, * P < 0.05, **P<0.01, ***P<0.001.
a -Numerator/denominator degree of freedom (where ageing term was applicable).

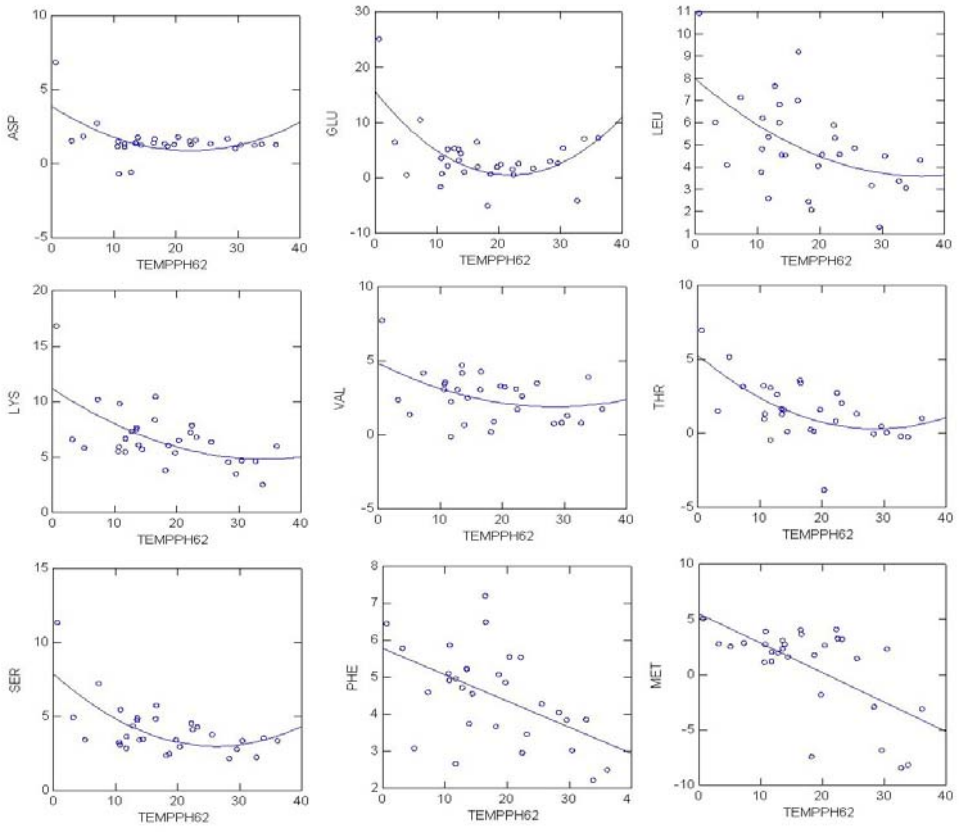

Figure 1. Changes in free amino acids from death to 7 d postmortem as a function of temperature at pH 6.2 (TEMPPH62).