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Full Length Research Paper

Determination of free amino acids of porcine serum responsible for the meat quality by ^{1}H NMR and HPLC analyses

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The objective of this study was to determine alternative meat-quality factors in porcine sera. We investigated serum metabolites from high pH group (HpHG) and low pH group (LpHG) on the basis of pH 24 h of post-mortem muscle (pH_{24h}). The pH_{24h} correlated well with the water holding capacity (WHC) of porcine meat, whereas a strongly negative correlation was observed between pH_{24h} and serum sodium level. For serum metabolites obtained by ^{1}H NMR spectra and PicoTag™ based HPLC, principal components analysis showed clear differences between the HpHG and the LpHG. The ^{1}H NMR spectra of serum metabolites at 600 MHz showed that free amino acids such as alanine, leucine, phenylalanine, and valine were qualitatively higher in the HpHG than in the LpHG. The relative abundance of three amino acids was quantitatively verified by HPLC: Phenylalanine and valine ($P<0.01$) and leucine ($P<0.05$). These free amino acids in porcine serum are considered as suitable indicators of meat quality in Berskshire pigs.

Key words: Porcine meat quality, muscle pH, principal components analysis, serum metabolites, free amino acids.

INTRODUCTION

Porcine meat quality can be influenced by various intrinsic factors such as meat colour, firmness, wetness, and intramuscular fat contents (Rosenvold and Andersen, 2003; Wood et al., 2008). In general, the extent of meat colour, firmness, and wetness can be primarily determined by the post-mortem biochemical process (Puolanne et al., 2002). The post-mortem metabolism in muscles critically relies on anaerobic glycolysis, which results in a decrease in muscular pH as a result of the production of lactate and hydrogen ions. In detail, the circulatory failure caused by exsanguination after slaughter, results in a lack of the oxygen that is required for aerobic glycolysis, leading to an adenosine triphosphate (ATP) homeostatic imbalance in muscle tissues.

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Abbreviations: HpHG, high pH group; **LpHG,** low pH group; **pH_{24h},** pH value after 24 h slaughter.

To maintain the cellular ATP concentration under anoxic conditions, muscle glycogen is metabolized via anaerobic glycolysis, which is less efficient at generatingATP than aerobic glyco-lysis.Consequently, levels of glycogen and ATP decrease, and the lactic acid, a waste product of the anaerobic glycolysis, accumulates, lowering muscle pH (Briskey and Wismer-Pedersen, 1961; Kastenschmidt et al., 1968). Especially, the rapid initial post-mortem decline in pH stimulates the denaturation of muscle proteins, affecting meat quality (Bowker et al., 2000) and thus, the post-mortem pH can be used as a common indicator for the evaluation of pork quality. In particular, the ultimate pH of post-mortem muscle (that is, the pH value after 24 h slaughter; pH_{24h}) has been considered as a critical factor that determines a meat-quality trait that is highly correlated with water-holding capacity (WHC) (Warner et al., 1997). Noticeably, few studies have investigated the direct relationship between metabolites and meat quality. For example, free amino acids from three types of muscles were quantitatively compared, and the free amino acid profiles of porcine sera reflected the decline in muscular pH (Cornet and Bousset, 1999). Recently, profiling of serum metabolites in crossbred pigs, experiencing different lairage time, implied the significant correlationship of the serum metabolite with meat quality. Accordingly, it is suggested that specific serum metabolites may be used as suitable indicators of meat quality traits.

In this study, we investigated them from high pH group (HpHG) and low pH group (LpHG) on the basis of pH 24 h of post-mortem muscle (pH_{24h}), to determine whether porcine serum metabolites reflect meat-quality traits. Various metabolic approaches revealed the clear differences between serum metabolic profiles of the HpHG and LpHG. Especially, the free amino acids such as alanine, leucine, phenylalanine, and valine were qualitatively higher in the HpHG than in the LpHG. Taken collectively, our results provide insight into the metabolic relationship between pH_{24h} and serum metabolites, and suggest that the free amino acids in porcine serum can be considered as suitable indicators of pork meat quality traits.

MATERIALS AND METHODS

Experimental procedure

Meat samples from a total of 155 Berkshire pigs were divided into two groups: high pH group (HpHG; upper 10% pH_{24h}) and low pH group (LpHG; lower 10% pH_{24h}). Whether these meat-quality traits from *m. longissimus dorsi* are correlated with pH_{24h} was investigated. Conversely, chemical components in porcine serum were analyzed from both meat-quality groups and their correlation with pH_{24h} was investigated. We also attempted to determine whether free amino acids of porcine sera reflect meat-quality traits. A chemometric approach using principal components analysis (PCA) was used to valuate serum free amino acids from both meat-quality groups. In addition, serum metabolites, including free amino acids, were qualitatively and quantitatively measured by analyzing ^{1}H NMR spectra (600 MHz) and using a Pico-tag method–based reversed-phase HPLC.

Animals and porcine sera preparation

A total of 155 healthy Berkshire pigs were bred under same condition (Da-San-Jong-Don Co. Ltd., Namwon-city, Korea) and then slaughtered according to standard slaughtering procedures, when their body weight reached 110 kg. Porcine blood samples from the jugular vein were directly collected and centrifuged at 3,000 rpm at room temperature for 20 min. The supernatants from serum samples were stored at −80°C in a deep freezer prior to biochemical analysis. Tissue samples from the Berkshire pigs were divided into two groups on the basis of post-mortem pH_{24h} values of *m. longissimus dorsi*: upper 10% pH_{24h} (n=17) and lower pH_{24h} (n=13). Albumin, globulin, glucose, cholesterol, creatinine, and electrolyte (example, sodium, potassium, and chloride) levels were measured with an automated chemical analyzer (AU400; Olympus, Tokyo, Japan) using a kit for each item.

Physical measurement of meat quality

The ultimate pH_{24h} of the muscles was determined in triplicate and monitored using a portable pH meter (pH-K21; NWKbinar GmbH, Landsberg, Germany). The pH of *m. longissimus dorsi* was measured at the 5th thoracic vertebrate for 24 h post-mortem. Objective meat color was determined using a Minolta Chromameter (CR400; Osaka, Japan) on a freshly cut surface after a 30-min blooming at 1°C. Water-holding capacity (WHC) was determined using a centrifugation method (Kristensen and Purslow, 2001) with minor modifications. In detail, 1 g of homogenized tissue was placed in a 2-ml centricon tube (VIDAS, France). The sample tube was then placed in a 50-ml centrifugation tube, heated in a 70°C water bath for 30 min, and centrifuged at 100 × *g* (SCR20BA; Hitachi, Japan) for 10 min at 18°C. WHC was expressed as a percentage of tissue sample weight loss during centrifugation. Water content (%) was determined as the weight loss of 5 g of muscle tissue at 102°C for 24 h. The intramuscular fat content (%) was measured by using a Foodscan (Type 78810 Food scan^{TM} Lab; Foss Co., Denmark) (Anderson et al., 2007). For the measurement of shear force, each sample (3-cm thickness, 120 g) was prepared and cooked individually in a plastic bag immersed in a water bath at 75°C for 30 min. The cooked sample was cooled at room temperature for 30 min and sampled by using a 12.7-mm circular core to determine shear force.

The sample cores from each sample were sheared across the length of the core with a Warner-Bratzler shear attachment on the texture analyzer (Model 5543; Instron Universal Testing Machine, Pittsfield, MA). Texture expert for the WINDOWS operating system was used to analyze data with the following operating parameters: a 50-kg load cell and a cross-head speed of 150 mm/min. The sheer force value was the mean of the maximum forces required to shear each set of core samples. Drip loss was obtained by measuring the weight loss during suspension of a standard 200-g sample at 2°C. The meat samples were weighed after 20 h of storage at 2°C. Cooking loss was determined by calculating the percentage weight loss of a 120-g meat sample during 30 min of cooking at a 75°C water bath.

^{1}H NMR analysis

Frozen serum sample was thawed, vortex mixed, and allowed to stand for 10 min before mixing an aliquot (250 µl) with saline containing 10% D_2O (500 µl). Subsequently, the mixture was centrifuged at 12,000 rpm for 10 min to remove solids during

Table 1. Characteristics of physical meat quality traits of Berkshires.

Component		LpHG	HpHG
Sample number		13	17
pH_{24h}		5.44±0.06	5.84±0.14[b]
CIE	*L*	50.07±2.20	49.09±3.38[b]
	a	6.09±0.91	5.26±1.08[a]
	b	2.58±0.77	1.54±0.83[b]
Water Holding Capacity (%)		54.81±1.70	56.29±1.13[b]
Intramuscular fat content (%)		2.73±1.50	2.22±0.74
Intramuscular collagen (%)		0.91±0.12	0.94±0.11
Warner-Bratzler shear force (kg/in^2)		3.37±0.45	3.34±0.55
Drip loss (%)		6.78±1.42	4.33±1.86[b]
Cooking loss (%)		26.50±3.49	24.54±4.29

[a]P<0.05, [b]P<0.01.

collection. A 600 µl supernatant was transferred into an NMR tube. NMR spectra were acquired with a Varian VNMRS-600 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) operating at a 599.84-MHz ^1H frequency and a temperature of 298 K using a triple resonance 5-mm HCN salt-tolerant cold probe. Chemical shifts were referenced to those of α-$_D$-glucose (^1H, δ5.23), and D_2O was provided by a field-frequency lock. The Carr-Purcell-Meiboom-Gill spin-echo pulse sequence was applied to acquire ^1H NMR spectra for all serum samples. For each serum sample, 128 transients were collected into 32-K data points, using a spectral width of 6720 Hz with a relaxation delay of 2 s and an acquisition time of 2.38 s.

All NMR spectra were phased and baseline-corrected by using Chenomx NMR suite 4.6 software (Chenomx Inc., Canada). The spectra were then normalized to the total spectral area and converted to ASCII format. The ASCII format files were imported into matlab (R2006a; Mathworks Inc., 2006), and all spectra were aligned using a correlation-optimized warping method (Christin et al., 2008). Spectral resonances of metabolites were assigned in accordance with those in the literature (Tang et al., 2004) and with the 600-MHz library from Chenomx NMR suite 5.1.

Free amino acid analysis

Porcine serum free amino acids were measured by reversed-phase HPLC using Pico-tag analysis (Razal et al., 1994) with a minor modification. In brief, porcine serum samples were filtered using a 0.45-µm PVDF membrane. The filtrate of serum (100 µl), supplemented with 2.5 mM norleucine (10 µl) as an internal standard amino acid, was dried in a speed vacuum (Hanil Co., Seoul, Korea) and then derivatized with 20 µl of labeling mixture (methanol : water : triethylamine : phenylisothiocyanate; 7:1:1:1, v/v/v/v) at room temperature for 15 min.

After the filtrate was completely dried in a speed vacuum, the resultant, phenylthiocarbamyl (PTC)-amino acids were dissolved in 400 µl of solvent A (140 mM sodium acetate trihydrate, 0.05% [v/v] triethylamine; pH 5.9). The mixture was centrifuged at 10,000 rpm at room temperature for 10 min, and then a 10 µl supernatant was injected in a Pico-tag^{TM} C_{18} reversed-phase column (3.9 × 300 mm; Waters, Milford, MA, USA). The PTC–amino acids were eluted by the binary gradient with solvents A and B (acetonitrile:water = 60:40 [v/v] containing 0.02% [w/v] EDTA) at a flow rate of 1 ml/min. Free amino acids in porcine serum were calibrated with norleucine and quantified with the external physiological PITC–amino acid standards.

Statistical analysis

All data were analyzed using a paired Student's *t*-test, and differences with a *P* value <0.05 were considered significant. The pattern recognition by PCA was performed using DAnTE version1.2 software based on R statistical software (Polpitiya et al., 2008).

RESULTS AND DISCUSSION

Relation between meat-quality traits and post-mortem muscle pH_{24h}

We chose individual serum samples separately corresponding to the upper 10% (n=17) and lower 10% pH_{24h} groups (n=13). As shown in Table 1, the average pH_{24h} values for the HpHG and LpHG were 5.84 □ 0.14 and 5.44 □ 0.06, respectively (P<0.01). Meat colours consisting of lightness, redness, and yellowness were significantly lower in the HpHG than in the LpHG. WHC is one of the most important traits influencing cooking and taste by consumers (Huff-Lonergan and Lonergan, 2005). WHC as a percentage was higher in the HpHG than that of the LpHG (P<0.01), whereas drip loss (%) (P<0.01) and cooking loss (%) were lower in the HpHG than that of the LpHG.

The extent of the decline in pH after slaughter was considered the most important factor affecting meat quality, which is strongly correlated with drip loss (Fischer, 2007). The WHC of pork muscle samples was previously shown to be influenced by the ultimate muscle

Table 2. Biochemical properties of Berkshire sera.

Component	LpHG	HpHG
Sample number	13	17
Albumin-to-globulin ratio	2.75±1.04	2.33±0.66
Glucose (mg/dl)	110.18±61.25	81.23±37.30
Cholesterol (mg/dl)	103.64±10.60	95.54±10.86
Creatinine (mg/dl)	1.81±0.32	1.57±0.26
Sodium (mEq/L)	159.10±11.07	149.30±11.19[a]
Potassium (mEq/L)	23.82±3.22	17.77±7.07
Chloride (mEq/L)	104.09±7.09	98.72±6.83
Sodium-to-potassium ratio	6.75±0.79	11.02±7.42

[a]$P<0.05$.

Table 3. Pearson correlation coefficients among Berkshire meat quality and serum factors.

Factor x Factor	pH_{24h}	WHC	$[Glucose]_{serum}$	$[Na^+]_{serum}$	$[K^+]_{serum}$
pH_{24h}	1.000	0.410[a]	-0.361[a]	-0.561[b]	-0.416[a]
WHC		1.000	-0.014	-0.029	0.030
$[Glucose]_{serum}$			1.000	0.472[a]	0.074
$[Na^+]_{serum}$				1.000	0.347[a]
$[K^+]_{serum}$					1.000

[a]considerable, [b]strong degreee of Pearson correlation. WHC, Water holding capacity.

pH, protein denaturation, and sarcomere length (Offer and Knight, 1988). A high pH_{24h} in post-mortem muscle is primarily due to an improvement in WHC (Rao et al., 1989). In addition, a high WHC is advantageous when preserving meat for several months by rapid freezing, which caused by inhibited glycolysis (Fischer et al., 1980). It is suggested that mild metabolic acidosis and elevated plasma protein levels may be the result of a decrease in body water (Parker et al., 2003).

Correlations between muscle-quality characteristics and serum metabolites

In order to determine which serum metabolites correlate with porcine meat-quality traits, serum samples were collected from both meat-quality groups and the levels of biochemical components were compared. As shown in Table 2, the albumin-to-globulin ratio and levels of glucose, cholesterol, creatinine, and electrolytes (sodium, potassium, and chloride) were lower in the HpHG than in the LpHG. Thus, a low muscular pH reflects a compensatory reaction to a decrease in blood pH, which results in a blood acid-base imbalance that leads to anionic ion like chloride into blood serum (Las et al., 2007). An elevated glucose level in serum, promotes the secretion of insulin and deposition of fat tissue (Jenny et al., 1974). Likewise, the high glucose level in the LpHG reflects a high content of intramuscular fat (Table 1). Serum potassium level can be influenced by the physical exercise condition (Fosha-Dolezal and Fedde, 1988; Schaefer et al., 1997).

The significantly lower sodium levels were observed in the HpHG than in the LpHG ($P<0.05$), which may be attributed to differences in buffering capacity between the two groups. A strong correlation between ultimate muscle pH_{24h} and WHC was observed (Table 3). This result is in good agreement with that of a previous study (Van Laack and Kauffman, 1999). A considerable positive correlation was observed between pH_{24h} and WHC or serum Na^+ and K^+ values, whereas a considerable negative correlation was observed between pH_{24h} and serum glucose or between pH_{24h} and K^+ values. A strong negative correlation was observed between pH_{24h} and serum Na^+ values. These data suggest that quantitative differences in serum metabolites indirectly reflect meat quality.

Diffrences in serum metabolites between the HpHG and LpHG

Metabolic profile of serum constituents provides useful information for determining the strain, age, sex, and health status of animals (Bollard et al., 2005). In particular, metabolite analysis of urine, sweat, and blood serum by ^1H NMR spectroscopy, enables the monitoring

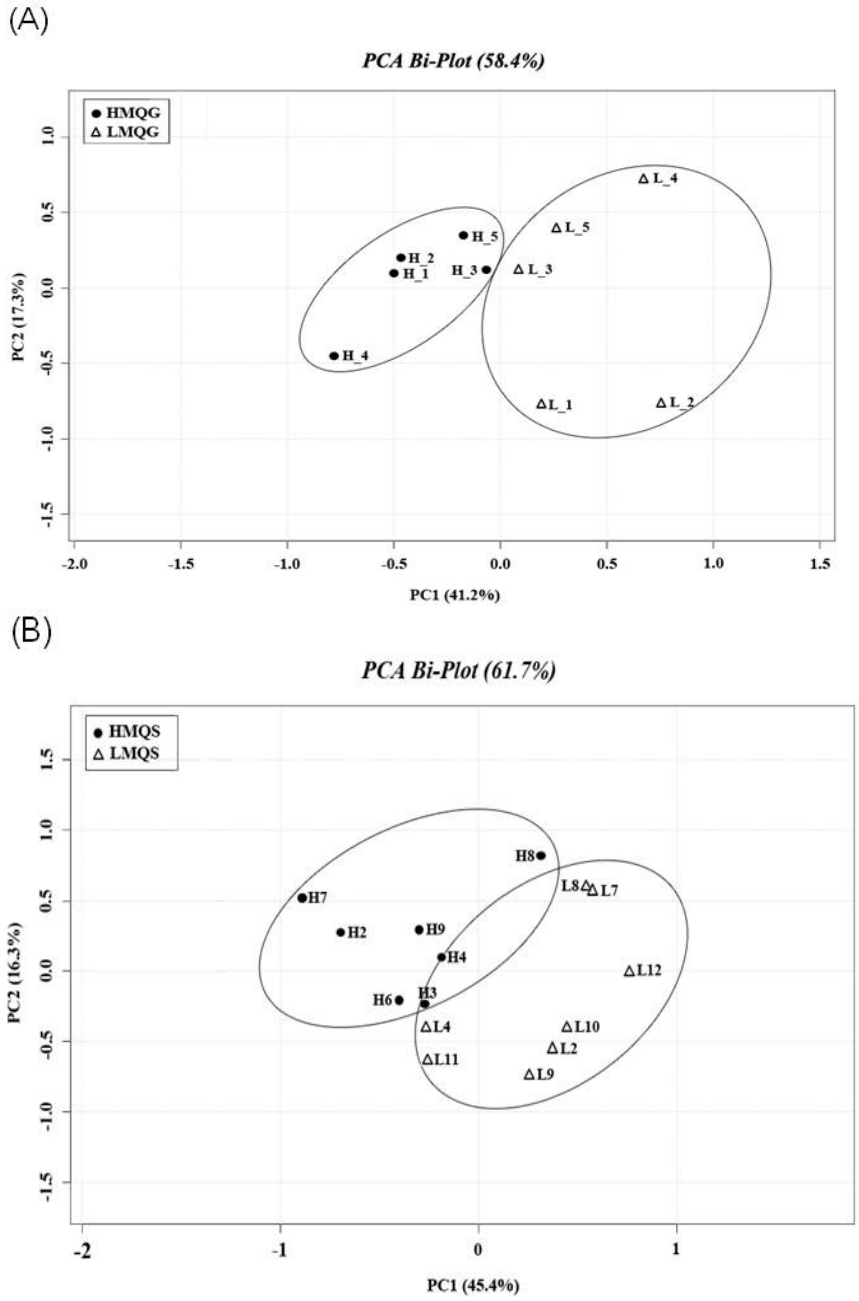

Figure 1. Score scatter plots of PCA of serum metabolites from (A) twenty free amino acids obtained by PicoTag^{TM} HPLC (n=5 for each group) and from (B) twelve metabolites by ^{1}H-NMR spectra (n=7 for HpHG, n=8 for LpHG).

of physiological conditions via pattern recognition of biofluid metabolites. The perturbation of biofluid metabolites is affected by intrinsic and extrinsic factors. Thus, we conducted an unbiased comparison of serum metabolites from the HpHG and LpHG to determine whether an intrinsic factor such as the innate meat quality of Berkshire pigs is associated with pH_{24h}, as defined earlier. As shown in Figure 1, the PCA data showed a clear distinction between the sera of the HpHG and LpHG, suggesting that the pattern of serum metabolites is an indicator of meat quality.

Qualitative and quantitative analysis of free serum amino acids

Blood serum or plasma can provide information regarding the effects of a variety of intrinsic and extrinsic factors

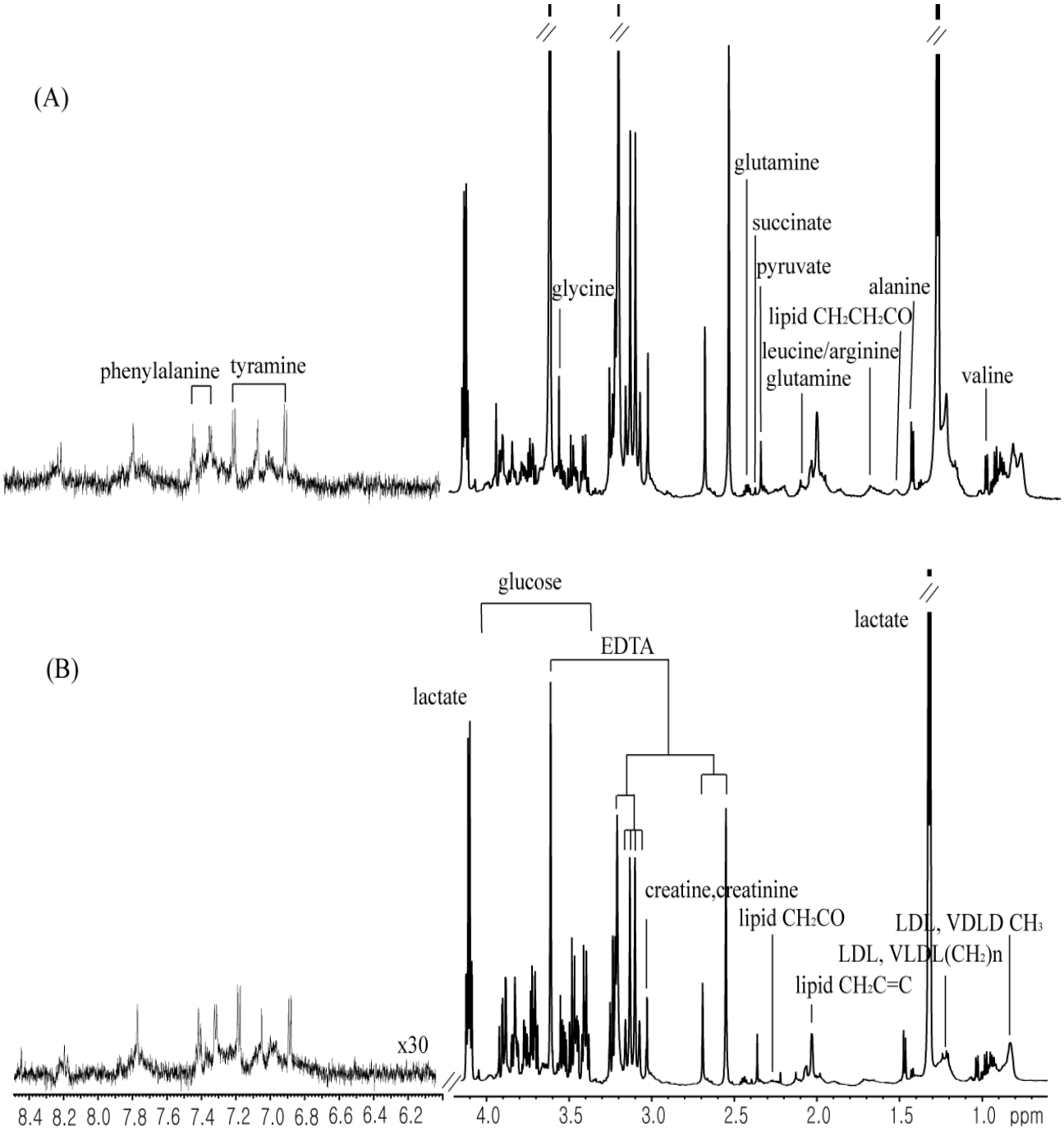

Figure 2. Representative ^{1}H NMR spectra of Berkshire pigs serum from (A) HpHG and (B) LpHG..

(example, the state of disease, infection, and metabolic disorders) (Wevers et al., 1994). In addition, free amino acids in tissue or blood are associated with the flavor and scent of pork meat through their reaction with sugars and oxidized lipids during cooking (Penet et al., 1983). Thus, we used ^{1}H NMR and HPLC to identify and quantify serum metabolites from both meat-quality groups. As shown in Figure 2, 600-MHz ^{1}H NMR spectra of representative serum samples were obtained from the LpHG and HpHG. The metabolites measured in most of the serum samples included glucose, lactate, pyruvate, succinate, creatine, creatinine, and lipids. In addition, amino acids were detected in the ^{1}H NMR spectra of porcine serum. On the basis of the ^{1}H NMR chemical shift assignment of known metabolites, levels of 8 metabolites were slightly higher in the HpHG than in the LpHG (Table 4), one of which was creatine. Supplementation of creatine monohydrate before slaughter has been found to

Table 4. ^{1}H NMR chemical shift assignment of metabolites observed in Berkshire sera.

Metabolite	Chemical shift (ppm) [a]	Fold change[b] (HpHG/LpHG)	P-value
Alanine	1.47(d), 3.76(d)	1.18	0.211
Leucine	0.95(d), 1.70(m), 3.73(q)	1.18	0.050
Phenylalanine	3.12(q), 3.27(q), 3.99(q), 7.31(t), 7.4(t)	1.22	0.026
Creatine	3.026(s), 3.92(s)	1.31	0.031
Valine	1.03(d), 2.26(m), 3.6(d)	1.32	0.033
Glucose	3.23(t), 3.40(t), 3.70(t), 3.84(m), 4.64(d), 5.22(d)	0.93	0.626
LDL, VLDL$(CH_2)_n$	1.25(m)	1.68	0.398
Lipid CH_2 CH_2 CO	1.60(m)	0.89	0.546
Lactate	1.31(d), 4.10(q)	0.95	0.654
Pyruvate	2.36(s)	1.02	0.804
Succinate	2.39(s)	0.86	0.080
Tyramine	2.91(t), 3.23(t), 6.88(d), 7.18(d)	1.53	0.027

[a]Keys: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet. [b]Data size: HpHG (n=9), LpHG (n=12).

Table 5. Free amino acid composition of Berkshire sera.

Amino acid	LpHG[a]	HpHG[a]	Fold change (HpHG/LpHG)	Statistical significance
Aspartic acid	0.62±0.26	0.32±0.05	0.52	P<0.05
Glutamic acid	2.22±0.58	2.67±0.38	1.2	NS[b]
Asparagine	0.34±0.08	0.46±0.04	1.35	P<0.05
Serine	0.54±0.09	0.59±0.14	1.09	NS
Glutamine	2.28±0.67	2.48±0.43	1.09	NS
Glycine	1.53±0.41	1.78±0.33	1.16	NS
Histidine	0.70±0.08	0.76±0.09	1.09	NS
Arginine	1.52±0.45	1.63±0.36	1.07	NS
Threonine	0.99±0.09	1.10±0.08	1.11	NS
Alanine	2.95±1.32	3.17±0.82	1.07	NS
Proline	0.67±0.12	0.53±0.24	0.79	NS
Tyrosine	1.43±0.22	1.46±0.13	1.02	NS
Valine	2.13±0.21	3.32±0.60	1.56	P<0.01
Methionine	0.52±0.08	0.94±0.32	1.81	P<0.05
Cysteine	0.06±0.04	0.03±0.01	0.5	NS
Isoleucine	1.29±0.17	1.85±0.37	1.43	P<0.05
Leucine	1.38±0.17	1.94±0.39	1.41	P<0.05
Phenylalanine	0.88±0.14	1.33±0.20	1.51	P<0.01
Tryptophan	0.56±0.13	0.79±0.14	1.41	P<0.05
Lysine	0.66±0.20	0.84±0.18	1.27	NS
Total free amino acid	23.27±5.50	27.98±5.30	1.2	P<0.05

[a]Data were presented as mean ± standard deviation (n=5). [b]NS means no significance. LpHG, Low pH group; HpHG, high pH group.

delay the muscle pH decline post-mortem in pigs (Berg and Allee, 2001; Young et al., 2005).

Similarly, it was previously shown that an elevated level of creatine in chicken muscle delayed lactic acid formation, resulting in a delay in pH decline (Nissen and Young, 2006). The content of total free amino acids was up to 20% higher in the HpHG than in the LpHG (Table 5). In particular, levels of leucine, phenylalanine, and valine were significantly higher in the HpHG than in the LpHG by ^{1}H NMR analysis. These data are consistent with the levels of free amino acids determined with Pico-tag–based reversed-phase HPLC. The relative abundance of three amino acids was quantitatively verified by HPLC analysis: phenylalanine and valine (P<0.01) and leucine (P<0.05). It is not clear why these amino acids were up- regulated in the HpHG.

However, it is postulated that some amino acids in blood may be indicators of internal or external signals (Tom and Nair, 2006). Interestingly, it was reported that levels of several free amino acids, including leucine, phenylalanine, and valine, were significantly higher under water-immersion stress in rats (Kitajima et al., 2002). Likewise, the significantly higher level of three amino acids in the HpHG observed in the present study may reflect the meat quality of Berkshire pigs.

Conclusions

Considerable variation was observed between muscular pH_{24h} and WHC, whereas a strongly negative correlation was observed between pH_{24h} and sodium content. This finding suggests that the maintenance of a high pH_{24h} value after slaughter retards anaerobic glycolytic degradation in muscle, which results in high meat quality. The serum metabolites from both meat-quality groups were clearly distinguished by pattern recognition analysis. The qualitative and quantitative analyses with ^{1}H NMR and HPLC showed that the free forms of leucine, phenylalanine, and valine in serum were significantly higher in the HpHG than in the LpHG. Thus, these specific amino acids in porcine sera could be adopted as indicators (biomarkers) of meat quality. Taken collectively, our data indicate that some free amino acids from Berkshire pig serum are determinants of meat quality based on pH_{24h} and that ^{1}H NMR analysis combined with the chemical analysis of porcine sera is a useful non-invasive technique for selecting high-quality meat.

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REFERENCES

Anderson S, Aldana S, Beggs M, Birkey J, Conquest A, Conway R, Hemminger T, Herrick J, Hurley C, Ionita C, Longbind J, McMaignal S, Milu A, Mitchell T, Nanke K, Perez A, Phelps M, Ritz J, Salazar A, Shinkle T, Strampe M, van Horn K, Williams J, Wipperfurth C, Zelten S, Zerr S (2007). Determination of Fat, Moisture, and Protein in Meat and Meat Products by Using the FOSS Food Scan TM Near-Infrared Spectrophotometer with FOSS Artificial Neural Network. J. AOAC Int. 90: 1073-1083.

Bollard M, Stanley E, Lindon J, Nicholson J, Holmes E (2005). NMR based metabonomic approaches for evaluating physiological influences on biofluid composition. NMR Biomed. 18: 143-162.

Bowker B, Grant A, Forrest J, Gerrard D (2000). Muscle metabolism and PSE pork. J. Anim. Sci. 79: 1-8.

Briskey E, Wismer-Pedersen J (1961). Biochemistry of Pork Muscle Structure. Rate of Anaerobic Glycolysis and Temperature Change versus the Apparent Structure of Muscle Tissuea. J. Food Sci. 1(26): 297-305.

Christin C, Smilde A, Hoefsloot H, Suits F, Bischoff R, Horvatovich P (2008). Optimized Time Alignment Algorithm for LC- MS Data: Correlation Optimized Warping Using Component Detection Algorithm Selected Mass Chromatograms. Anal. Chem. 80: 7012-7021.

Cornet M, Bousset J (1999). Free amino acids and dipeptides in porcine muscles differences between red and white muscles. Meat Sci. 51: 215-219.

Fischer C, Honikel K, Hamm R (1980). Influence of below freezing temperatures on the rate of post mortem metabolism and the water holding capacity in prerigor frozen beef muscles (author's transl). Zeitschrift fur Lebensmittel Untersuchung und-Forschung, 171: 200-205.

Fischer K (2007). Drip loss in pork: influencing factors and relation to further meat quality traits. J. Anim. Breed. Genet. 124: 12-18.

Fosha-Dolezal S, Fedde M (1988). Serum potassium during exercise in Hereford calves influence of physical conditioning. J. Appl. Physiol. 65: 1360-1366.

Huff-Lonergan E, Lonergan SM (2005). Mechanisms of water holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71: 194-204.

Jenny B, Polan C, Thye F (1974). Effects of high grain feeding and stage of lactation on serum insulin, glucose and milk fat percentage in lactating cows. J. Nutr. 104: 379-385.

Kastenschmidt L, Hoekstra W, Briskey E (1968). Glycolytic intermediates and cofactors in “fast” and “slow glycolyzing” muscles of the pig. J. Food Sci. 33: 151-158.

Kitajima H, Shiomoto H, Osada K, Yokogoshi H (2002). Effect of dietary amino acids on behavior and serum levels of amino acids in stress loaded rats. J. Nutr. Sci. Vita. 48: 194-200.

Kristensen L, Purslow P (2001). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci. 58: 17-23.

Las J, Odongo N, Lindinger M, AlZahal O, Shoveller A, Matthews J, McBride B (2007). Effects of dietary strong acid anion challenge on regulation of acid-base balance in sheep. J. Anim. Sci. 85: 2222-2229.

Nissen P, Young J (2006). Creatine monohydrate and glucose supplementation to slow and fast growing chickens changes the postmortem pH in pectoralis major. Poult. Sci. 85: 1038-1044.

Offer G, Knight P (1988). The structural basis of water holding in meat. Drip losses. Dev. Meat Sci. 2(4): 173-243.

Parker A, Hamlin G, Coleman C, Fitzpatrick L (2003). Quantitative analysis of acid base balance in Bos indicus steers subjected to transportation of long duration. J. Anim. Sci. 81: 1434-1439.

Penet C, Worthington R, Phillips R, Moon N (1983). Free amino acids of raw and cooked ground beef and pork. J. Food Sci. 48: 298-299.

Polpitiya AD, Qian WJ, Jaitly N, Petyuk VA, Adkins JN, Camp DGI, Anderson GA, Smith RD (2008). DAnTE: a statistical tool for quantitative analysis of omics data. Bioinformatics, 24: 1556-1558.

Puolanne EJ, Ps AR, Ruusunen MH, Sepponen KV, Kylä-Puhju MS (2002). Lactic acid in muscle and its effects on meat quality. Proceedings of 55th Reciprocal Meat Conference, East Lansing, MI. pp. 57-62.

Rao M, Gault N, Kennedy S (1989). Variations in water holding capacity due to changes in the fibre diameter, sarcomere length and connective tissue morphology of some beef muscles under acidic conditions below the ultimate pH. Meat Sci. 26: 19-37.

Razal R, Lewis N, Towers G (1994). Pico tag analysis of arogenic acid and related free amino acids from plant and fungal extracts. Phytochem. Anal. 5: 98-104.

Rosenvold K, Andersen HJ (2003). Factors of significance for pork quality a Rev. Meat Sci. 64: 219-237.

Schaefer A, Jones S, Stanley R (1997). The use of electrolyte solutions

for reducing transport stress. J. Anim. Sci. 75: 258-265.
Tang H, Wang Y, Nicholson J, Lindon J (2004). Use of relaxation edited one dimensional and two dimensional nuclear magnetic resonance spectroscopy to improve detection of small metabolites in blood plasma. Anal. Biochem. 325: 260-272.
Tom A, Nair KS (2006). Assessment of branched chain amino acid status and potential for biomarkers. J. Nutr. 136: 324S-330S.
Van Laack R, Kauffman R (1999). Glycolytic potential of red, soft, exudative pork longissimus muscle. J. Anim. Sci. 77: 2971-2973.
Warner R, Kauffman R, Greaser M (1997). Muscle protein changes post mortem in relation to pork quality traits. Meat Sci. 45: 339-352.
Wevers R, Engelke U, Heerschap A (1994). High resolution ^{1}H-NMR spectroscopy of blood plasma for metabolic studies. Clin. Chem. 40: 1245-1250.
Wood J, Enser M, Fisher A, Nute G, Sheard P, Richardson R, Hughes S, Whittington F (2008). Fat deposition, fatty acid composition and meat quality: Rev. Meat Sci. 78: 343-358.