Effect of Breed (Yorkshir, Landrace and Korean Native Black Pig) on Extractable Level of Free Amino Acids

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Introduction

Structural and cytoplasmic proteins of meats are exposed to proteolytic actions of endogenous proteolysis (e.g., calpains) to result in polypeptides[1]. The degradation products consequently generate small peptide and free amino acids by subsequent actions of peptidases and aminopeptidases, respectively[2]. Free amino acids are of great importance in eating quality due to their specific tastes[3], and subsequent generation of volatile compounds [4,5]. On the other hand, the amino acids are also source of biogenic amines, which are taken place by microbial decarboxylases[6], leading to migraine, headache, and gastric and intestinal ulcers[7].

Korean native black pig (KNBP) is one of the most in demand meats for domestic consumers due to its unique characteristics of higher redness and palatability than other commercial breeds[8]. Cornet and Bousset[12] found that fiber type in porcine muscles significantly affected level of free amino acids, and postulated that difference in sensory characteristics between pork muscles possibly had a linage to free amino acids. In addition, other studies[9,10] indicated that activity of aminopeptidases were dependent on breeds. The previous results raised a possible scenario that discernible eating quality of KNBP meat was related to free amino acids. Thus, the current study was conducted to identify free amino acids by which Yorkshir, Landrace and KNBP meats can be classified during chiller ageing.

Materials and method

Animal, experimental design and treatment: A total of thirty market-weighted male pigs (10 Yorkshir, 10 Landrace and 10 Korean native black pig, KNBP) were sampled from the National Livestock Research Institute (NLRI) breeding program. Average live weight and age for Yorkshir, Landrace and KNBP were 118, 118 and 72 kg, and 194, 201 and 192 days, respectively. The pigs were conventionally transported to the NLRI abattoir, approximately 65 km away, with minimum transit stress. The pigs were assigned to a 2 × 3 factorial which were composed of two chilling regimes ($-3°$ C for five of each breed and $5°$ C for the rest five for each breed) and three ageing times (1, 3 and 7 days at $1°$ C). All pigs were

conventionally slaughtered over two consecutive days with an electronic stunner (230 volts for 2.5 sec), and placed in a 1°C chiller until the following day.

Free amino acids: Extractable levels of free amino acids were determined at 0 (biopsied tissue) and 7 d postmortem according to the method reported by Moya et al.[11], with minor modifications. Briefly, one gram of liquid nitrogen powdered sample was homogenized in 0.01N HCl with a polytron (3x15 sec) at 4°C, followed by centrifugation at 10,000×g for 20 min. 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 10,000xg for 15 min, and 16 primary amino acids were determined by online derivatization using o-phthalaldhyde (OPA). Amounts of free amino acids were analyzed by an Agilent 1100 HPLC system (Agilent Tech, Waldbronm, Germany) at diode array UV detector (338 nm, 10 nm band wide) using ZORBAX Eclipse-AAA C18 column (4.6× 150 mm, 5 um, Agilent Tech, Waldbronm, Germany). Separation for each sample was completed in 20 min at 40°C, using a gradient mobile phase between 40 mM Na_2HPO_4, pH 7.8 and acetonitril-methanol-water (45:45:10, v/v). Concentrations of amino acids were calculated against gradient linear external standards for each amino acid (0 to 250 pmol/uL), adjusted to the internal standard, and expressed as mg per 100 g wet tissue. Discriminant analysis was performed by a Systat package (version 10.2) using a backward stepwise method with alpha to enter = 0.15.

Results and Discussion

The current data are a part of a large join-project designed to characterize meat quality of KNBP, and identify its biological basis with reference to other commercial breeds. For the purpose, free amino acids were assessed to examine whether free amino acids profile differs from common commercial breeds (ie, Yorkshir and Landrace). As noted above, it was of particular interest as early study identified that KNBP was more palatable than Landrace[8]. It has been well documented that unique endo- and exogenous enzyme systems including aminopeptidase have been evolved not only for particular muscle, but also for each breed[9,10]. However, the current analysis failed to find significant difference in free amino acids between Yorkshir, Landrace and KNBP for biopsied muscle tissue when the data were analyzed using a general linear model containing fixed effects of breed, ageing and breed x ageing (data not shown). The discriminant analysis using 16 free amino acids of biopsied sample classified three breeds at ca. 53% (Table 1 and Fig. 1). It could mirror that the profile differed, but there was a large overlapping between breeds, and consequently the successful rate was relatively low.

On the other hand, when the same attempt was made after a 7-day ageing at 1°C, correction rate of classification for three breeds was noticeably improved, with an average of classification rate at 80%. Moya et al.[11] found that changes in free amino acids during ageing varied between PSE, RFN, RSE and DFD pork, and that was related to aminopeptidases[2]. Analysis of variance for 7-day samples indicated

that concentrations of Threonine and Alanine were significantly (P<0.05) higher and lower, respectively, than other breeds (data not shown). Taken together, the current result might be an indication that KNBP meat was subjected to different biological process during ageing, despite underlying mechanism(s) has not determined in terms of endo- and exogenous enzyme systems, biochemical environment of muscle tissue, and/or fiber type. These aspects are under examination. Nevertheless, the current preliminary data suggested that the divergence in free amino acids profile for aged KNBP meat could be one of the attributes associated with more palatable eating quality.

Table 1. Canonical discriminant functions of discriminant analysis, and successful rate of classifying each breed using extractable 16 free primary animo acids at 0 (biopsied sample) and 7 days postmortem. The analysis was performed by a backward stepwise method with alpha to enter=0.15. The model finally retained listed amino acids.

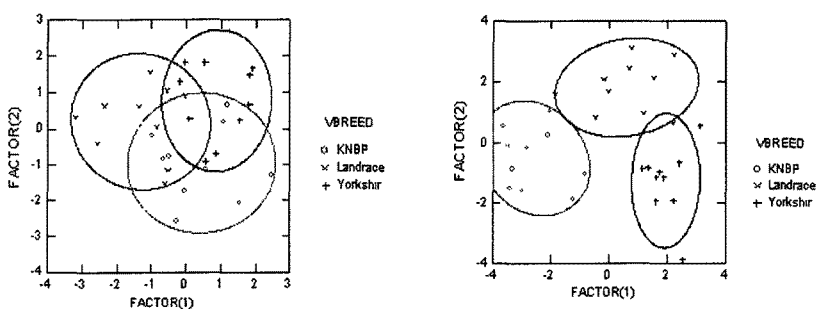|	Day 1		Day 7	
	Factor 1	Factor 2	Factor 1	Factor 2
Constant	3.425	4.824	−6.254	−13.814
GLY	−0.955	−0.287	0.895	−0.596
TYR	−0.822	0.52	−0.074	1.359
MET	−0.333	0.016	−0.807	0.196
ALA	−0.06	−0.452	.	.
GLU	0.432	0.123	−0.414	−0.198
LYS	2.248	0.522	−1.459	1.583
HIS	.	.	−0.317	0.273
ASP	.	.	0.309	0.167
PHE	.	.	0.659	−0.875
	Correction rate (%)			
KNBP	50		80	
Landrace	50		70	
Yorkshir	60		90	
Total	53		80	

Fig. 1. Score plot of discriminant alalysis presented at Table 1. Left panel: biopsied sample, right panel: day 7.

Summary

Sixteen primary free amino acids were determined for each 10 longissimus muscles of Yorkshir, Landrace and Korean native black pig. Eight amino acids at 7 d classified three breeds at ca. 80% confidence. The difference in free amino acids for aged meats could be one of factors responsible for more desirable palatability of Korean native black pig.

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