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Does Tenderness of Korean Native Pork is Related Fiber type?

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Introduction

The reddish feature of Korean native pork is a favorable characteristic to Korean consumers, and likely related to fiber composition[1]. Fiber type is a significant component affecting meat quality due to its relation to postmortem glycolytic rate, proteolytic rate and water-holding capacity. Current study was conducted to characterize fiber type of Korean native black pig (KNBP) and its relation to glycolysis, proteolysis, and objective meat quality with reference to landrace.

Materials and Methods

Animal, experimental design and treatment: Twenty market-weighted male pigs (10 landrace, 118 kg, and 10 KNBP, 72 kg) were sampled from the NLRI breeding program. The pigs were assigned to a 2 x 3 factorial which was composed of two chilling regimes (-3 and 5°C) and three ageing times (1, 7, and 14 d at 1°C). Pigs were conventionally slaughtered, and placed in a 1°C chiller until the following day.

pH, temperature and objective meat quality: pH, temperature, WB-shear force, and meat color were determined similar to those described by Hwang et al.[2].

Relative proportion of slow myosin heavy chain (MyHC-I): Relative composition of MyHC-I isoform in myofibril was determined by applying an indirect enzyme-linked immunosorbent assay (ELISA) following Picard et al.[3]. Longissimus muscle tissue was biopsied during bleeding, immediately frozen in liquid nitrogen, and stored at -70°C until analysis. Crude extracts were made by stirring 200 mg of tissue powder in 1.4 mL extraction buffer (0.3 M KCl, 0.1 M KH_2PO_4, 0.05 M K_2HPO_4, 0.04 M EDTA, pH 6.5, 1 mM DTT) for 15 min on ice. The homogenate was centrifuged at 10,000 x g for 20 min at 4°C, and the supernatant was diluted in two-fold with glycerol to a final concentration of 50%, and stored at -70°C until used[4]. Protein concentration of the extract was determined as described by Bradford[5], using BAS as a standard. Primary and secondary antibodies were human MyHC-I monoclonal antibody (F36.5B9, 2C8, isotype mouse IgG2a, Biocytex biotechnology) and rabbit anti-mouse IgG (conjugated with alkaline phosphatase, Bethyl, Lab. Inc)[6]. *p*-nitrophenyl phosphate solution (Sigma, SL, USA) was used for color development and absorbance was measured at 405 nm using a plate reader (MicroScreener LB 9260, EG & E BERTHOLD, Germany). Relative percentage of MyHC-I was calculated against a standard curve of *m. masseter* tissue[3].

Identification of proteolytic peptides: Postmortem proteolytic rate was quantified by a tricine-SDS-PAGE[7,8], and peptides were identified by a LC/MS/MS procedure described by Hwang et al.[9]. Longissimus muscle tissues were sampled during breeding (0 h), 1 d and 7 d postmortem. Samples for 1 and 7 d were taken from WB-shear force blocks, and all samples were prepared as for the ELISA procedure. 100 mg sample was homogenized in 2.5 mL of extraction solution (0.01M imidazole, pH 7.0, 2% SDS and 2% MCE), boiled for 10 min, and kept at room temperature overnight. 25 uL of the sample was separated using a Hoefer vertical slab gel unit SE600 (24 x 14 cm, 1.5 mm thick). Gel was composed of 1.5 cm stacking gel (T=4%, C=3%), 2.5cm spacer gel (T=10%, C=3%), and 17.5 cm resolving gel (T=14.5%, C=4%). Gels were stained for 48 h in 0.02% Coomassie brilliant blue R250 and 2% phosphoric acid. Destained gels were digitalized by an imaging system (Fluor-S MultiImager, Bio-Rad, USA) and quantified using a Quantity-One software (Bio-Rad, USA). For relative quantification, a horse myosin peptide cocktail (2.5 - 16.9 kDa, Amersham Biosciences) was run in triplicate, and changes in relative percentage of proteolytic products during ageing were calculated against 16.9 kDa peptide.

Results and Discussion

The current results are preliminary data obtained from a long-term co-project designed to identify the physical and biological characteristics of KNBP, with reference to other commercial breeds. Table 1 and Fig. 1 describe fiber composition, objective meat quality and rate of proteolysis during chiller ageing. The result demonstrated that KNBP longissimus muscle had a higher level in hunter a* value (red dimension), and that was related to a higher proportion of slow myosin heavy chain (MyHC-I). Given the result of an early study[10] which reported that MyHC-I was negatively related to carcass weight, we could not exclude that the lighter carcass weight of KNBP, with similar age, was a possible factor for the higher proportion of the slow fiber type. However, our previous study[11] found that old and heavy KNBP (ca. 100 kg and 13 month old) also showed a similar color characteristic. The result collectively implied that genetic components were involved in the distinct color feature of KNBP.

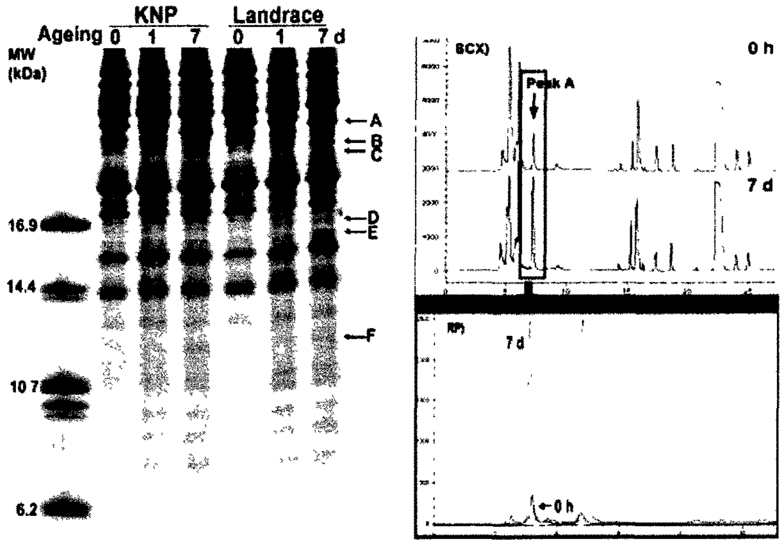
It has been well documented that carcass temperature of 10-15°C during rigor development could minimize muscle shortening and maximize proteolysis[12]. Based on longissimus temperature at pH 6.2, pH/temperature decline of KNBP during rigor development was greatly favorable for resulting in tender meat (Table 1). This could be a consequence of slower glycolytic rate due to the higher proportion of slow myosin heavy chain[13], and faster chilling rate for the small carcasses. As previous studies demonstrated, this was reflected on a fast appearance of proteolytic peptides (Fig. 1) and concomitantly tender meat (Table 1). With bearing a fact in mind that proteolytic rate is significantly faster in white type muscle, one might expect a faster proteolytic rate for landrace due to the higher frequency of white type fibers[13]. However, KNBP showed a significantly faster degradation rate for some proteins (ca. creatine kinase, GAPDH, myosin light chain, titin and troponin I). This raised a fundamental question whether pH/temperature window during rigor development is more important than muscle fiber type?

Table 1. Differences in objective meat qualities and postmortem proteolysis between landrace and Korean native black pig(KNBP)

	Breed		Av.se	F value		df^{d}
	Landrace	KNBP		Breed	Ageing	
pH at 3 h	6.3	6.5	0.06	6.12^{*}		1(1)/18
Temperature at 3 h	26.0	23.1	0.87	5.77^{*}		1(1)/18
Temperature at pH 6.2	21.5	11.4	2.52	7.93^{*}		
pH at 24 h	2.3	3.0	0.87	0.3		
Temperature at 24	5.5	5.6	0.05	2.58		
MyHC-I (%)	11.3	14.2	0.71	7.91^{*}		1(1)/18
WB-shear force (kg)	5.8	5.4	0.22	2.77	52.0^{***}	1(2)/56
Hunter a*	6.8	10.2	0.32	57.27^{***}	7.12^{**}	1(2)/56
Hunter L*	45.9	42.1	0.64	17.44^{***}	11.47^{***}	1(2)/56
Band A $(\%)^{f}$	157.7	133.4	9.17	3.5		1(1)/18
Band B $(\%)^{f}$	11.2	12.6	0.71	1.85	30.55^{***}	1(2)/56
Band C $(\%)^{f}$	8.2	10.2	0.51	7.37^{**}	26.64^{***}	1(2)/56
Band D $(\%)^{f}$	8.8	10.5	0.47	6.96^{*}	17.86^{***}	1(2)/56
Band E $(\%)^{f}$	9.4	11.4	0.74	3.87	13.88^{***}	1(2)/56
Band F $(\%)^{f}$	7.6	8.9	0.86	1.24	19.54^{***}	1(2)/56
Peak A	7.1	6.8	0.35	0.39	203.5^{***}	1(2)/17

	Ageing (d)				Av.se
	0^{e}	1	7	14	
WB-shear force (kg)		6.9^{a}	5.2^{b}	4.7^{b}	0.26
Hunter a*		7.4^{a}	8.7^{b}	9.4^{b}	0.39
Hunter L*		41.0^{a}	44.8^{b}	46.1^{b}	0.78
Band B $(\%)^{f}$	7.2^{a}	11.6^{b}	16.8^{c}		0.87
Band C $(\%)^{f}$	6.0^{a}	9.1^{b}	12.5^{c}		0.62
Band D $(\%)^{f}$	7.1^{a}	10.1^{b}	11.8^{c}		0.57
Band E $(\%)^{f}$	7.5^{a}	9.7^{a}	14.1^{b}		0.90
Band F $(\%)^{f}$	4.2^{a}	7.3^{b}	13.4^{c}		1.06
Peak A	3.4^{a}		10.5^{b}		0.35

[abc] Means bearing the same letter did not differ significantly (P>0.05).
*P<0.05, **P<0.01, ***P<0.001.
[d] Numerator/denominator degree of freedom for breed (ageing).
[e] Biopsied sample during bleeding.
[f] Relative percentage of 16.9 kDa horse myosin peptide.



	Consensus identity	gi number	*de novo* sequence
Band C	Creatine kinase	gi|13938619	LGSSEVEQVQLVVDGVK
			GTGGVDTAAVGSVFDVSNADR
	GAPDH	gi|40889050	AITIFQER
			AITIFQERDPANIK
			IVSNASTTTNCLAPLAK
	Myosin light	gi|127176	GADPEDVITGAFK
		gi|71708	GADPEETILNAFK
Band D	Titin	gi|34856454	LVISMTFADDAGEYTIVIR
	Superoxide dismutase 1	gi|15082144	DGVATVVYIEDSVIALSGDHSIIGR
	GAPDH	gi|65987	LISWYDNEFGYSNR
	Troponin I	gi|401209	SVMLQIAATELEK
Peak A	GAPDH	gi:3219753	VPTPNLPPVDL
	Myopodin protein	gi:5689736	SPPSFFAEPPSPVS
	Troponin T	gi:346622	DEEEVEHVEEEAGAEEVH

Fig. 1. Changes in small molecular proteins during chiller ageing for landrace and Korean native black pig(KNP), and 2DE-chromathgraphic profile (cation exchange, SCX, and reverse phase, RP) during ageing. Their identities are also tabulated.

Summary

More a reddish color of KNBP was related to higher frequency of slower fiber type. Tender meat with a faster ageing rate for KNBP was coincided with a faster

proteolytic rate, and likely a higher collagen solubility (data not shown). However, it is not confirmed whether the results were linked to the favorable pH/temperature window during rigor development, or fiber composition for tender meat.

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