ANTIOXIDANT AND ANTI- INFLAMMATORY EFFECTS OF PORK MEAT EXTRACT

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Abstract – This study was conducted to evaluate the antioxidative and anti-inflammatory effects of the pork meat extracts in Landrace× Yorkshire×Duroc (LYD) and Korean Native Black Pig (KNBP). The HWEP in KNBP of ham had the highest the ABTS radical scavenging activity. Carnosine contents were higher in the HWEP from loin of KNBP. The anserine contents were significantly high in the shoulder ham of LYD and the loin of KNBP, respectively. The cytokines such as TNF-α, IL-6 were secreted by macrophage after LPS-activation. However those cytokines were significantly decreased after treatment with HWEP from the loin and ham at 200 to 500 μg/mL concentration. These results demonstrate that the HWEP of pork loin and ham shows antioxidative and anti-inflammatory effects.

Key Words – antioxidant, anti-inflammatory, Korean Native Black Pig, hot water extraction

I. INTRODUCTION

Histidine-di-peptides such as carnosine (β-alanyl-L-histidine) and anserine (N-β-alanyl-3-methyl-L-histidine) are one of functional peptide with antioxidative activity that widely distributed in skeletal muscles, the heart and the central nervous system at very high concentrations (up to 20 mM) [1]. These antioxidant di-peptide obtained from animal protein like beef, chicken, pork and fish. The acute phase of inflammatory in macrophages and monocytes usually play a important role in eliciting the response cascade [2]. After, they being stimulated make a number of chemokines and enzymes, for example cytokines, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)[3, 4]. Lipopolysaccharide (LPS) is a main component of the outer membrane of Gram-negative bacteria, is an endotoxin that induces septic shock syndrome by promoting the production of inflammatory mediators like tumor necrosis factor-a (TNF-a), interleukins[5]. Korean Native Black Pigs (KNBP) is highly appreciated by Korean consumers because it has favorable chewiness, redness, hard fat and better flavor compared with commercial pigs[6]. Therefore, this study was carried out to investigate antioxidant and anti-inflammatory effects of Boiled pork (BP), HWEP (hot water extraction in pig) of pork portions (loin, boston butt, shoulder ham and fresh ham) in LYD and KNBP.

II. MATERIALS AND METHODS

Materials

The different parts of four Landrace×Yorkshire×Duroc (LYD) and Korean Native Black Pigs (KNBP) were purchased from a local market in Chuncheon and a Kangwonsanwoori pig farms located in Hongcheon, Kangwon-do, respectively. All subcutaneous, intermuscular and visible connective fat were removed and then each raw meat added to five-fold distilled water. It is boiled for 1hrs and lyophilized.

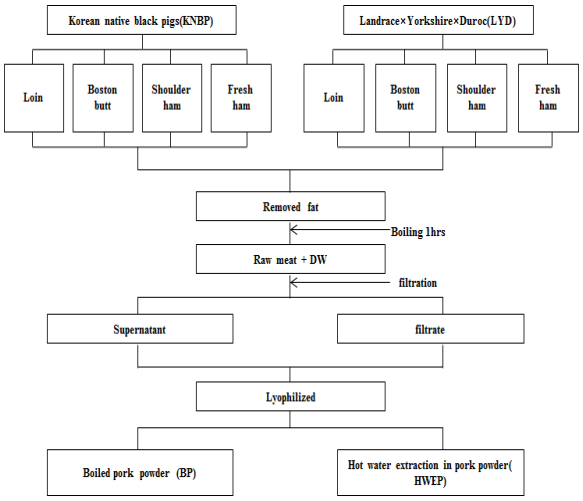

Figure 1. Preparation procedure of boiled pork and hot water extracts

ABTS+· radical scavenging activity

ABTS+· scavenging activity was carried out according to the procedure described by Re *et al*. (1999)**[7].** The reaction compound made up of 50 µL sample and 950 µL of the ABTS+· radical solution. The reaction mixture of absorbance measured (SpectraMax M2e, Molecular Devices, USA) at 30℃ after 30min. Trolox equivalents per gram of low molecular weight sample.

Di-peptide (carnosine and anserine) content
Carnosine and anserine content was analyzed in accordance with the methods described by Kim *et al*. (2013)[8]. The samples were conducted using the HPLC (Agilent Technologies 1260 Infinity, Santa Clara, CA, USA), zorbax eclipse XDB-C18 column (250×4.6 mm, 5 µm, Agilent, Palo Alto, CA, USA) and were analyzed at a flow rate of 1 mL/min for 20min, column temperature of 25℃ and UV absorbance at 210 nm. Sample was injected 20 µl. All sample and solvents ware filtered through 0.45 µm membrane filter. Standard carnosine and anserine were purchased from Sigma. (St. Louis, Missouri, USA)

Macrophage Cell culture
RAW 264.7, a mouse macrophage cell line, was obtained from American Type Culture Collection (ATCC ® TIB-71™, USA). RAW 264.7 cells were plated in 100-mm tissue culture dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Welgene) and 1% penicillin /streptomycin (Gibco, USA). RAW 264.7 cells were maintained at 37°C in a humidified incubator containing 5% CO_2.

IL-6 and TNF-α assays
Levels of IL-6 and TNF-α was analyzed by ELISA as per the manufacturer's instructions. The IL-6 and TNF-α were determined using mouse IL-6 BD OptEIA™ ELISA sets (BD PharMingen, San Diego, CA) and mouse TNF(Mono/Mono) BD OptEIA™ ELISA sets (BD PharMingen, San Diego, CA), respectively.

Statistical analysis
For the statistical analysis of all results data, an analysis of variance (ANOVA) of one way using General Linear Model (GLM) procedure SAS software (ver. 9. SAS Institute Inc., USA) was performed for all variables considered in the study. When treatment effects were significant ($P < 0.05$) mean values were compared with Duncan's multiple range test.

III. RESULTS AND DISCUSSION

ABTS indicated that the HWEP in KNBP of all pork cuts had the highest antioxidant. Especially, Fresh ham showed significantly high levels antioxidant effect.

Table 1 ABTS assay of fresh cuts in BP and HWEP of LYD and KNBP (uM TE)

Treatment[1)]		Loin	Boston butt	Shoulder ham	Ham
LYD	BP	34.6± 0.26^{Bd}	26.6± 0.47^{Cd}	39.3± 0.49^{Ac}	39.4± 0.33^{Ac}
	HW EP	39.2± 0.09^{Cb}	28.8± 0.34^{Dc}	40.6± 0.09^{Bb}	44.0± 0.08^{Ab}
KN BP	BP	37.5± 0.32^{Bc}	40.5± 0.10^{Ab}	36.0± 0.26^{Cd}	35.7± 0.20^{Cd}
	HW EP	45.1± 0.15^{Ba}	45.0± 0.28^{Ba}	45.5± 0.09^{Ba}	46.9± 0.40^{Aa}

$^{A-D}$ Means±SE within same row with different superscript letters differ significantly at p<0.05.
$^{a-d}$ Means±SE within same column with different superscript letters differ significantly at p<0.05.
[1)]LYD: Landrace×Yorkshire×Duroc, KNBP: Korean native black pig, BP: Boiled pork, HWEP: Hot water extraction in pork

The case of carnosine, all showed distinct highest carnosine contents in loin. Particularly, fresh ham in HWEP of KNBP had the highest significant difference. Anserine contents show significantly high levels of Shoulder ham and loin in LYD and KNBP, respectively.

Table 2 Carnosine (mg/ml) contents of fresh cuts in BP and HWEP of LYD and KNBP (mg/g dry basis)

Treatment[1)]		Loin	Boston butt	Shoulder ham	Ham
LYD	BP	106.7± 0.15^{Ab}	44.7± 0.03^{Dc}	68.3± 0.01^{Cb}	72.4± 0.04^{Bc}
	HW EP	117.8± 0.02^{Aa}	56.1± 0.02^{Da}	81.8± 0.03^{Ca}	82.8± 0.01^{Bb}
KN BP	BP	79.7± 0.09^{Ad}	39.7± 0.04^{Dd}	43.8± 0.06^{Cd}	66.5± 0.01^{Bb}
	HW EP	101.2± 0.02^{Ac}	54.0± 0.05^{Cb}	52.8± 0.43^{Dc}	83.9± 0.01^{Ba}

$^{A-D}$ Means±SE within same row with different superscript letters differ significantly at p<0.05.
$^{a-d}$ Means±SE within same column with different superscript letters differ significantly at p<0.05.

[1)]Refer to Table 1.

Table 3 Anserine (mg/ml) contents of fresh cuts in BP and HWEP of LYD and KNBP (mg/g dry basis)

Treatment[1)]		Loin	Boston butt	Shoulder ham	Ham
LYD	BP	3.7± 0.00^{Cd}	3.3± 0.02^{Dc}	5.3± 0.01^{Ab}	4.9± 0.01^{Bc}
	HWEP	4.3± 0.00^{Cc}	4.0± 0.01^{Da}	6.8± 0.00^{Aa}	4.7± 0.01^{Bb}
KNBP	BP	5.1± 0.01^{Ab}	3.0± 0.02^{Cd}	2.8± 0.01^{Dc}	3.7± 0.01^{Bd}
	HWEP	6.7± 0.01^{Aa}	3.8± 0.00^{Cb}	3.1± 0.28^{Dc}	4.8± 0.01^{Ba}

$^{A-D}$ Means±SE within same row with different superscript letters differ significantly at $p<0.05$.
$^{a-d}$ Means±SE within same column with different superscript letters differ significantly at $p<0.05$.
[1)] Refer to Table 1.

When antioxidant the studies were combined, the loin and fresh ham of all pork cut in KNBP mainly have high levels of antioxidant activity effect. To investigate that loin and fresh ham in HWEP of LYD and KNBP have anti-inflammatory effect in macrophage cells, RAW 264.7 cells were treated with LPS (1 μg/mL). In this study, Cytokines like TNF-α, IL-6 stimulated LPS-activated macrophage indicated concentration dependent decrease the loin and fresh ham in HWEP of LYD and KNBP. At a dosing rate of 200 to 500 μg/mL of the all treatment significantly showed reductions in expression of IL-6 and TNF-α sharply, respectively, as compared to the only LPS-stimulated group. Each TNF-α, IL-6 secreted as an inflammatory cytokine indicated the greater effects of inflammatory reduction that loin than fresh ham and loin in HWEP of KNBP.

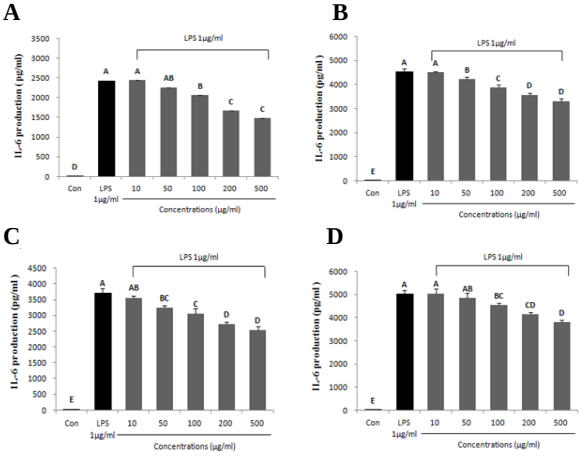

Figure 1. Effect of inhibition of LPS-activated IL-6 of loin and fresh ham in HWEP of LYD and KNBP. Values of bar with different letters differ significantly at $p<0.05$.
A, HWEP of loin in LYD; B, HWEP of ham in LYD; C, HWEP of loin in KNBP; D, HWEP of ham in KNBP

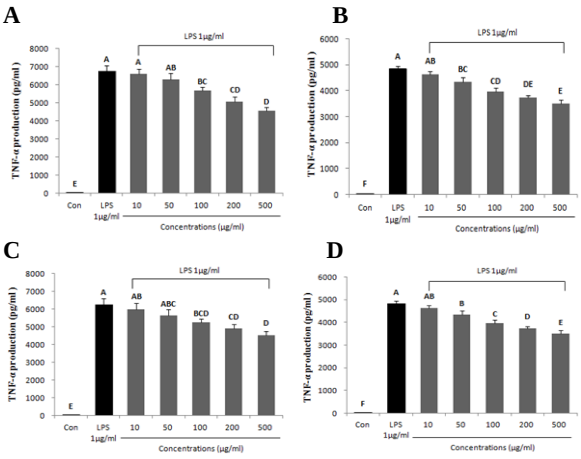

Figure 2. Effect of inhibition of LPS-activated TNF-α of loin and fresh ham in HWEP of LYD and KNBP. Values of bar with different letters differ significantly at $p<0.05$.
A, HWEP of loin in LYD; B, HWEP of ham in LYD; C, HWEP of loin in KNBP; D, HWEP of ham in KNBP

IV. CONCLUSION

The ABTS radical (ABTS+·) produced by the reaction ABTS and potasium persulfate is reduced to a colorless substance by cation decolorization [7]. Jung *et al*., (2010)[9] was investigated that effect of dietary mixture of gallic acid and linoleic acid (MGL) on the antioxidative potential of breast meat from broilers. The breast meat of the broiler fed 1.0% MGL had significantly higher ABTS + reducing activity. These results suggest that ABTS radical scavenging activity can have increased by internal meat are existed an antioxidant substance. Carnosine can act potently inhibitor of advanced lipoxidation end products (ALEs) and glycoxidation end-products (AGEs) formation through the mechanisms that inhibiting lipid oxidation and breakdown to RCS(reactive carbonyl species), detoxifying RCS(reactive carbonyl species) and reacting with carbonylated proteins (“carnosinylation”)[10]. Tinberge & Slump (1976)[11] reported 104-338, 7-16 carnosine and anserine concentrations (per 100 g of pork). Each carnosine and anserine of contents present 180-321 and 10-18 mg/100 g muscle in four pork muscles(masseter, trapezius, semimembranosus, longissimus dorsi) that reported by Aristoy and Toldrá (1998)[12], respectively. That compare to this result that lower than carnosine

and anserine of contents in raw pork. Higher fat content could decrease carnosine concentrations, because carnosine is mainly presence in the cytosol of skeletal muscle and the Higher adipose fat would decrease carnosine concentrations in skeletal muscle[13]**.** This is explain that carnosine and anserine concentrations of loin pork (low fat pork cut) used in this study indicated high contents and Boston butt had high fat pork cut maily showed low contents. The pro-inflammatory cytokines such as TNF-α, IL-6 are small secreted proteins, which mediate and regulate immunity and inflammation. The IL-6 is known pro-inflammatory cytokine, considered as an endogenous mediator of LPS-induced fever. TNF-a secreted by activated including macrophages, lymphocytes, neutrophils, and mast cells and monocytes was inflammatory cytokine[14]. Each TNF-α, IL-6 secreted as an inflammatory cytokine indicated the greater effects of inflammatory reduction that loin than fresh ham and loin in HWEP of KNBP. These findings concur with soy protein had rich protein that similar to the meat exerts potent inhibitory activity of TNF-α and IL-6 production by these LPS-stimulated RAW264.7 cells[15]. These results concluded that the HWEP of loin and fresh ham in pork has antioxidative and anti-inflammatory of effects.

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