RELATIONSHIP BETWEEN HEATING TEMPERATURES, HCAs FORMATION AND AMINO ACIDS COMPOSITION OF PAN-ROASTED PORK PATTIES

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I. INTRODUCTION

Meat is mainly consumed after heating. Heating improves the taste and flavor as well as microbial stability. During heating, the meat's free amino acids, creatine, creatinine, and sugar react, resulting in the Maillard reaction [1]. The Maillard reaction produces several substances that affect the flavour and colour of meat, and harmful substances such as Heterocyclic amines (HCAs) are also produced [2]. HCAs are powerful mutants of cancer at the ng/g level of cooked food and significantly impact its incidence in humans. The main precursors of HCAs are creatine, creatinine, amino acid, and sugar. The content or type of HCAs produced varies depending on the meat species, heating temperature, and time [3]. Therefore, this study will determination the amino acid composition, type, and content of HCAs in heated pork patties at different heating temperatures to confirm their relationship.

II. MATERIALS AND METHODS

The lean pork meat was purchased from the local supermarket of Jinju-si, Korea. Excess connective tissues and intermuscular fat were removed and ground with a 3 mm plate meat grinder. After that, the ground meat was kneaded to form a patty. The weight of each patty was 100±2 g, and the size of each one was approximately 100 mm in diameter and 15 mm in thickness. The prepared pork patties were cooked until to a central temperature of 75°C for a total of 10 min, 5 min per side, in a frying pan preheated to 220±5°C (Low-temperature heating, LTH) and 320±5°C (High-temperature heating, HTH). Subsequently, the amino acid composition and HCAs production of non-heated pork patty (NH), LTH, and HTH were measured using high-performance liquid chromatography (HPLC) and external standard methods. All data from three independent experiments were subject to statistical analyses using SAS software 9.4 (SAS Institute Inc., Cary, USA). Data obtained from amino acid composition and HCAs identification were determined with a one-way analysis of variance (ANOVA) using the cooking temperature as an independent factor. Duncan's multiple range test ($P<0.05$) determined differences between treatment means**.** The results of all analyses were stated as mean ± standard deviation.

III. RESULTS AND DISCUSSION

The content of total HCAs, including non-polar HCAs and polar HCAs in HTH was detected as higher than that of NH and LTH (Table 1). In particular, the increase in production of IQ, IQx, MeIQx, 4,8-DiMeIQx, PhIP, Harman, Aαc, and MeAαc were significant ($P<0.05$). It may result from the difference in heating temperature. First, HTH promotes the Maillard reaction [1], creating an ideal environment for producing HCAs. In addition, HTH promotes the conversion of creatine, a non-protein amino acid, to creatinine, a physiologically inactive substance [3]. The converted creatinine can serve as a precursor for forming imidazoquinoline or imidazoquinoxaline by aldol reaction with pyridine or pyrazine derivatives to form HCAs [2]. Finally, more moisture loss occurs under high-temperature conditions than at low temperatures. Increased moisture loss causes more leakage of water-soluble precursors (creatine, creatinine, and sugar) present in meat, thereby promoting HCAs formation. For this reason, HTH has more HCAs content than LTH. The content of amino acids in HTH increased compared to NH and LTH (Table 2). The contents of histidine, valine, lysine, isoleucine, leucine, phenylalanine, glycine, glutamic acid, proline, tyrosine, arginine, and alanine were significantly increased ($P<0.05$). Lopes *et al.* [4] explained that this could be due to moisture loss and protein deformation during heating. Increased amino acids can be used as precursors for the formation of HCAs. Phenylalanine, which showed a significant increase at HTH, is converted to phenylacetaldehyde during heating, which reacts with creatinine to form PhIP. Other amino acids (glycine, alanine, lysine, tyrosine, and proline) increased in HTH also form pyrazine, pyridine, and aldehyde by heating and become precursors for the formation of IQ-type HCAs [2]. Therefore, it is believed

that the increase in amino acids occurring during heating significantly affected the increase in HCA production.

Table 1. Effect of different heating temperatures on heterocyclic amines (HACs) formation in pan-roasted pork patties

Parameters (ng/g)	NH	LTH	HTH	SEM	P-value
IQ	N.d.	2.44^{b}	8.91^{a}	0.588	< 0.0001
IQx	N.d.	3.07^{b}	7.24^{a}	1.919	0.0104
MeIQx	N.d.	44.4^{b}	77.8^{a}	8.909	0.0001
4,8-DiMeIQx	N.d.	5.66^{b}	14.6^{a}	5.145	0.0356
7,8-DiMeIQx	N.d.	3.16	4.44	3.164	0.2833
PhIP	N.d.	4.42^{b}	8.01^{a}	2.311	0.0154
Harman	N.d.	1.60^{b}	28.1^{a}	2.701	<0.0001
Norharman	N.d.	3.69	16.4	7.382	0.0771
Aαc	0.31^{b}	1.84^{ab}	8.37^{a}	2.905	0.0314
MeAαc	N.d.	2.97^{b}	4.85^{a}	0.680	0.0004
Total HCAS	0.31^{b}	73.2^{b}	180^{a}	11.13	<0.0001
Polar HCAs	N.d.	63.2^{b}	121^{a}	10.81	0.0012
Non-polar HCAs	0.31^{b}	9.99^{b}	58.8^{a}	17.55	<0.0001

NH: uncooked pork patty; LTH: pork patty cooked at 220±5°C; HTH: pork patty cooked at 320±5°C. $^{a-b}$ Mean with a different letter within a different column are significantly different (P<0.05). SEM: Standard error of the mean. N.d.: Not detected.

Table 2. Effect of different heating temperatures on amino acid composition in pan-roasted pork patties (mg/100g)

	NH	LTH	HTH	SEM	P-value		NH	LTH	HTH	SEM	P-value
Histidine	0.98	0.95	1.21	0.121	0.0758	Arginine	1.38^{c}	1.61^{b}	1.93^{a}	0.109	0.0024
Isoleucine	0.94^{b}	1.06^{b}	1.36^{a}	0.113	0.0092	Cystine	0.16	0.16	0.21	0.028	0.1068
Leucine	1.75^{b}	2.00^{b}	2.49^{a}	0.158	0.0033	Glutamic acid	2.36	4.09	4.90	1.217	0.1012
Lysine	1.91^{b}	2.20^{b}	2.71^{a}	0.170	0.0033	Glycine	1.08	1.29	1.36	0.171	0.1855
Methionine	0.31	0.33	0.43	0.263	0.8250	Proline	0.56	1.12	1.32	0.362	0.0929
Phenylalanine	1.11^{b}	1.22^{b}	1.49^{a}	0.093	0.0057	Tyrosine	0.88^{b}	0.96^{b}	1.19^{a}	0.066	0.0024
Valine	1.11^{b}	1.23^{a}	1.53^{a}	0.099	0.0053	Alanine	1.29^{c}	1.52^{b}	1.76^{a}	0.108	0.0046
Threonine	0.69	1.13	1.35	0.357	0.1553	Aspartic acid	1.46	2.48	3.04	0.743	0.0977

NH: uncooked pork patty; LTH: pork patty cooked at 220±5°C; HTH: pork patty cooked at 320±5°C. $^{a-c}$Mean with a different letter within a different column are significantly different (P<0.05). SEM: Standard error of the mean.

IV. CONCLUSION

High-temperature heating promoted the formation of HCAs and changed the amino acid composition. High-temperature heating increased amino acid content and promoted the Maillard reaction. These changes provided more ideal conditions for the formation of HCAs.

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