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CORRELATION BETWEEN PROTEIN OXIDATION, FREE AMINO ACID COMPOSITION, AND HETEROCYCLIC AMINES FORMATION IN COOKED CHICKEN BREAST

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I. INTRODUCTION

Heating improves the palatability and digestibility of meat, while refrigeration ensures its safety during storage. However, even at temperatures below 300 °C, heating can lead to the formation of heterocyclic amines (HCAs), such as 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), and 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx), which are known carcinogens [1,2]. These HCAs are influenced by precursors such as carbonyl compounds and free amino acids present in meat. This study investigated the relationship between protein oxidation and HCA formation by analyzing changes in carbonyl content and free amino acid composition (tyrosine, histidine, lysine, and arginine) in chicken breast during refrigerated storage (0, 7, and 14 days).

II. MATERIALS AND METHODS

The freshly slaughtered chickens used in this study were purchased from a slaughterhouse located in Jinju, Republic of Korea. Chicken breasts were separated, vacuum-packed, and stored at 4 °C for 0, 7, and 14 days. After the storage period, the chicken breasts were grilled at 250 ± 20 °C for 5 minutes per side (a total of 10 minutes), ensuring that the internal temperature reached 75 °C. Protein oxidation during the storage period was assessed by analyzing carbonyl content using 2,4-dinitrophenylhydrazine (DNPH). Free amino acids were measured in raw chicken breast using an amino acid analyzer (Model II), and HCAs were measured in cooked chicken breast using high-performance liquid chromatography (HPLC). Both measurements were qualitatively and quantitatively determined using external standard methods. The experiment was independently repeated three times, using four chickens per experiment. Results were expressed as LSMeans with standard errors using SAS software 9.4 (SAS Institute Inc., Cary, USA), and statistical significance was determined by Tukey's post-hoc test ($p<0.05$). Pearson correlation analysis was conducted using the CORR procedure in SAS software 9.4 to further examine the relationships among experimental variables. The strength and direction of the correlations were interpreted based on the correlation coefficient (r), and statistical significance was evaluated at $p<0.05$, $p<0.01$, and $p<0.001$.

III. RESULTS AND DISCUSSION

The results are presented in Table 1. As the storage period increased, pH, cooking loss, and carbonyl content significantly increased ($p<0.05$), indicating that protein oxidation actively progressed over time. Pearson correlation analysis was conducted to further examine the relationships among these variables (Figure 1). Cooking loss showed a positive correlation with all four HCAs, with particularly strong correlations observed for IQx and 7,8-DiMeIQx ($r=0.65$, $p<0.05$). This suggests that various precursors released into the exuded moisture during heating may have been exposed to direct thermal reactions on the surface of the meat, thereby promoting the formation of HCAs [2]. Histidine showed a strong negative correlation with MeIQx ($r=–0.76$, $p<0.05$), whereas it was strongly positively correlated with IQx ($r=0.90$, $p<0.01$). This finding contrasts with previous studies that reported an inhibitory effect of histidine on IQx formation. This discrepancy likely results from the strong correlation between histidine and carbonyl compounds formed during protein oxidation ($r=0.92$, $p<0.01$). In fact, carbonyl content also showed a strong positive correlation with IQx levels ($r=0.92$, $p<0.01$). In contrast, lysine and arginine, which were negatively correlated with carbonyl compounds, also exhibited strong negative correlations with IQx formation ($r=–0.90$ and $r=–0.78$, respectively). These results suggest that histidine may not directly contribute to IQx formation, but rather promote its production indirectly by increasing the concentration of carbonyl compounds through protein oxidation. Tyrosine and arginine were strongly associated with the formation of IQx and 4,8-DiMeIQx. In particular, arginine showed strong positive correlations ($r=0.93$, $p<0.05$ for MeIQx; $r=0.92$, $p<0.01$ for 4,8-DiMeIQx). This strong association is likely due to arginine's function as a nitrogen donor during thermal processing and its role in contributing reactive intermediates essential for the formation of the quinoxaline ring structure in HCAs [3].

Table 1 - Changes in physicochemical properties and HCA levels of chicken breast during refrigerated storage.

Traits	Storage period (d)			SEM	p-value
	0	7	14		
pH	5.77^{b}	5.78^{ab}	5.87^{a}	0.03	0.0263
Cooking loss (%)	33.43^{b}	41.89^{a}	43.56^{a}	0.90	<.0001
Carbonyl content (ng/mg of protein)	2.02^{a}	2.49^{a}	5.34^{b}	0.35	< .0001
Tyrosine[1]	3.01^{b}	4.88^{a}	3.31^{ab}	0.54	0.0271
Histidine[1]	13.69^{b}	11.09^{c}	17.58^{a}	0.31	<.0001
Lysine[1]	31.50^{a}	26.66^{a}	16.65^{b}	1.74	0.0005
Arginine[1]	6.60^{b}	20.47^{a}	0.86^{c}	0.73	<.0001
IQx[2]	2.12^{b}	2.84^{b}	13.48^{a}	0.35	<.0001
MeIQx[2]	2.55^{b}	11.21^{a}	3.11^{b}	0.89	0.0014
4,8-DiMeIQx[2]	1.57^{c}	17.68^{a}	3.32^{b}	0.51	<.0001
7,8-DiMeIQx[2]	0.88^{b}	1.85^{ab}	2.60^{a}	0.55	0.0451

$^{a\sim c}$ Means within a column with different superscript letters differ significantly ($p<0.05$). SEM represents the standard error of the LSMeans. [1]Free amino acid (mg/100 g of meat); [2]HCAs (ng/g of freeze-dried sample).

	pH	Cooking loss	Carbonyl	Tyrosine	Histidine	Lysine	Arginine	IQx	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx
pH	1										
Cooking loss	0.51	1									
Carbonyl	0.63*	0.70*	1								
Tyrosine	0.05	0.39	-0.28	1							
Histidine	0.31	0.47	0.92**	-.49	1						
Lysine	-.62	-.85**	-.90**	0.02	-.81*	1					
Arginine	-.29	-.06	-.78*	0.74*	-.94**	0.54	1				
IQx	0.52	0.65*	0.96***	-.26	0.90**	-.93***	-.73*	1			
MeIQx	-.08	0.20	-.47	0.78*	-.76*	0.31	0.93*	-.42	1		
4,8-DiMeIQx	0.14	0.32	-.32	0.78*	-.74	0.13	0.92**	-.28	0.97***	1	
7,8-DiMeIQx	0.43	0.65*	0.61	0.52	0.41	-.59	-.10	0.58	0.07	0.08	1

Figure 1. Pearson correlation heatmap illustrating the relationships among carbonyl content, free amino acids, and the levels of heterocyclic amines in chicken breast samples. Indicates the strength and direction of correlation: red for positive, blue for negative, and white for no correlation ($r \approx 0$). *p<0.05, **p<0.01, ***p<0.001.

IV. CONCLUSION

Changes in precursor composition caused by protein oxidation influenced the formation of HCAs during heating. IQx was positively correlated with histidine and negatively correlated with lysine and arginine, whereas MeIQx and 4,8-DiMeIQx showed positive correlations with tyrosine and arginine. Critically, the correlations between histidine, lysine, and carbonyl content suggest that these amino acids may indirectly contribute to HCA formation by promoting the generation of reactive intermediates during protein oxidation.

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