POTENTIAL OF 2D QUANTITATIVE NUCLEAR MAGNETIC RESONANCE (2D qNMR) SPECTROSCOPY ANALYSIS FOR CHICKEN BREAST MEAT METABOLITES

H. C. Kim[1*], Y.-J. Ko[2], K. H. Baek[1], and C. Jo[1,3],

[1]*Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul, Republic of Korea*,

[2]*National Center for Inter-University Research Facilities, Seoul National University, Seoul, Republic of Korea*,

[3]*Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang, Republic Of Korea*,

*eoenhc@naver.com

I. OBJECTIVES

Acquiring accurate metabolic information is important to understand and/or elucidate biological changes in meat samples. Among the different quantitative analyses, one-dimensional (1D) 1H nuclear magnetic resonance (NMR) analysis is rapid, reproducible, and simultaneous for various metabolites without derivatization. However, despite these advantages, 1D ^{1}H NMR analyses still need to overcome the problem of chronic overlap, which is critical in cases of mixtures such as meat extracts. For this problem, the application of two-dimensional quantitative nuclear magnetic resonance (2D qNMR) can be helpful to reduce overlap issues via its dimensional expansion. Hence, in this study, 2D qNMR analytical methods were applied for metabolite analysis in meat, investigating its potential in comparison to high-performance liquid chromatography (HPLC) and 1D ^{1}H NMR.

II. MATERIALS AND METHODS

Chicken breasts meat were purchased from a local market and pooled prior to the analyses. The breast meat (5 g) was extracted with 0.6 M perchloric acid, neutralized with potassium hydroxide, and lyophilized for elimination of the water. Lyophilized samples were reconstituted with 1 mL of 20 mM phosphate buffer in D_2O solution (1 mM 3-(trimethylsilyl)propionic acid, pH 7.0) and applied for 1D ^{1}H NMR, 2D qNMR (heteronuclear single nuclear quantum coherence [HSQC]), and HPLC. Prior to setting up 2D qNMR, artificial free amino acid mixture was prepared manually and acquired on HSQC for quantification. All results were triplicated, and statistical analysis was performed using the procedure of the general linear model. Significance of differences among mean values was determined by a Student-Newman-Keul test ($P < 0.05$).

III. RESULTS

A total of 18 free amino acids were found in chicken breast meat extracts using 3 different analyses (Table 1). Compared to HPLC and HSQC, 1D ^{1}H NMR showed good precision with lower variation. However, some metabolites (arginine, glutamate, proline, histidine, lysine, serine, and tryptophan) were quantified in 1D ^{1}H NMR because of its overlap. Unlike 1D ^{1}H NMR, HSQC could qualify and quantify all metabolites without overlap. Different quantification in 2D qNMR was observed in the contents of arginine, proline, glutamine, histidine, leucine, phenylalanine, tyrosine, and valine, while other free amino acids were not different when compared to the conventional HPLC method ($P < 0.05$). This inconsistency can be improved by further optimization as it is possibly due to difference of ionic strength between breast meat extracts and artificial standard mixture, different 90° pulse (p1) of metabolites, and/or NMR acquisition parameters. In addition, although quantification of was

not consistent on HSQC, standard of each free amino acid showed good linearity (R^2 = 0.97 in proline and R^2 > 0.99 in others).

Table 1. Metabolite identification and quantification of chicken breast meat from HPLC, ^{1}H NMR, and 2D HSQC spectra

Compound	HPLC	^{1}H NMR	2D HSQC	$R^{2,1)}$
	(mg/kg)			
Alanine	304.80 ± 8.24	308.88 ± 5.08	306.58 ± 14.48	0.9964
Arginine	215.83 ± 8.03^a	nd$^{2)}$	85.36 ± 5.23^b	0.9957
Asparagine	77.67 ± 5.08^a	76.10 ± 2.38^a	44.82 ± 4.22^b	0.9954
Aspartic acid	181.26 ± 12.00	186.05 ± 2.45	170.75 ± 6.11	0.9972
Glutamate	324.28 ± 9.03	525.65 ± 5.12	363.23 ± 16.43	0.9955
Proline	116.81± 23.91^b	(Glu + Pro)$^{3)}$	158.85 ± 3.37^a	0.9678
Glutamine	269.27 ± 11.57^b	269.11 ± 1.32^b	430.98 ± 12.20^a	0.9950
Glycine	214.95 ± 4.79	216.57 ± 6.42	222.36 ± 14.49	0.9950
Histidine	178.23 ± 3.61^a	nd	126.59 ± 3.76^b	0.9942
Isoleucine	82.65 ± 1.45^c	100.81 ± 2.46^a	92.40 ± 1.42^b	0.9900
Leucine	165.09 ± 1.31^a	168.49 ± 3.02^a	153.85 ± 6.01^b	0.9939
Lysine	87.98 ± 6.17	nd	68.74 ± 13.14	0.9957
Methionine	73.00 ± 1.01	73.08 ± 3.72	75.00 ± 0.92	0.9912
Phenylalanine	87.87 ± 0.86^b	89.43 ± 0.28^b	151.52 ± 14.72^a	0.9964
Serine	197.97 ± 5.54	Nd	193.15 ± 11.37	0.9981
Tryptophan	56.27 ± 2.59	Nd	58.94 ± 9.80	0.9968
Tyrosine	135.63 ± 1.25^a	135.04 ± 6.52^a	112.70 ± 4.94^b	0.9969
Valine	125.70 ± 2.37^a	127.10 ± 2.42^a	83.32 ± 3.35^b	0.9955

$^{a-c}$Mean values (n=3) with different letters within the same row differ significantly ($p < 0.05$).

$^{1)}$ R^2: R-squared was calculated based on HSQC using artificial standard mixture

$^{2)}$ nd : not detected in the 1D ^{1}H spectrum.

$^{3)}$ 1D ^{1}H NMR data of both glutamate and proline were excluded from the calculation.

IV. CONCLUSION

Based on the results, 2D qNMR can be helpful in acquiring interactive and accurate information, which could be advantageous when compared to traditional chromatographic analysis. However, further optimization is needed for more accurate numerical quantification.

Keywords: one-dimensional quantitative nuclear magnetic resonance, two-dimensional quantitative nuclear magnetic resonance, chicken breast, high-performance liquid chromatography, heteronuclear single nuclear quantum coherence