DETERMINATION OF ADENOSINE TRIPHOSPHATE BREAKDOWN PRODUCTS IN COOKED PORK LOIN BY THE ISOCRATIC HPLC-UV METHOD

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I. INTRODUCTION

Nucleotide compounds or adenosine triphosphate (ATP) metabolites are important meat flavor components [1]. In postmortem (PM) muscles, ATP dephosphorylation results in the production of adenosine diphosphate (ADP), adenosine 5´-monophosphate (AMP), and inosine 5´-monophosphate (IMP), which further breakdown to inosine and hypoxanthine [1, 2]. In addition, 5′-ribonucleotides, AMP, IMP, and guanosine monophosphate (GMP) provide umami taste in cooked meat [1]. The available methods [1, 2, 3] for measuring nucleotides in meat samples utilized high performance liquid chromatography (HPLC) with gradient elution. The equipment is costly and may not always be available in all laboratories. Although the gradient system enhances separation efficiency, it often results in salt accumulation causing system clogging and high pressure. With isocratic elution, the composition of a solvent mixture remains the same for the entire run. By solution mixing and filtering prior to introducing into the system, isocratic elution helps eliminate salt deposition. In addition, some laboratories only have a basic HPLC equipped with an ultraviolet (UV) detector available. Therefore, the objective of this study was to develop a simple nucleotide compound determination method for cooked pork *Longissimus dorsi* (LD) muscle with adapted method of [1] by using isocratic elution for basic HPLC-UV equipment.

II. MATERIALS AND METHODS

Sample collection and preparation

For each of five replications, three boneless pork LD muscles were obtained from left side of Duroc castrated male carcasses (110.0±10 kg slaughter weight) at a commercial packing plant. At 24-h PM, each LD was fabricated, and visually evaluated for marbling (score=1 or 2) and color (score=2 or 3) between the 10th and 11th ribs according to [4]. LD was vacuum-packaged and placed in styrofoam containers with ice (3.0±2.0°C, EBI 20, Ebro data logger, Germany), transported to Meat Technology Research Network Center, KMITL and stored in a walk-in chiller (2.0±1.0°C). At 48-h PM, each LD was evaluated for pH value (Mettler Toledo, Germany), cut into chops, individually vacuum packaged (K-Nylon/LLDPE, Packmart, Thailand), and kept frozen (-20°C) until analysis. Frozen LD chops were thawed at 2.0±1.0°C for 24 h and evaluated for fat content [5]. Another set of three thawed LD chops were cooked according to [6] and used for determination of nucleotide components.

Sample extraction

Cooked pork LD was extracted for nucleotide compound determination following a method of [1] with slight modifications. Briefly, a centrifugation speed of an extracted mixture was at 3,900 rpm for 5 min at 4.0°C (Beckman Coulter; Avanti J-E, USA). The filtered supernatant was adjusted to a pH value of 5.5, using 0.5 M Potassium hydroxide or 3.5% Perchloric acid. The final extracted mixture was then transferred to a vial and frozen at -20°C until further analysis.

Apparatus and chromatographic conditions

Frozen final extracted mixture was thawed at 25°C for 10 min and centrifuged at 10,000 rpm for 5 min at 4°C before injected into the HPLC (Thermo Separations Constametric 4100 BioSystem, Thermo Separation Products, USA) using a UV detector (SpectraSYTEM™ UV1000, Thermo Separation Products, USA) with a modified method of [1]. The chromatographic separation was accomplished on an Amide 80 (25.0 cm×4.6 mm) analytical column (TSKgel®, Tosoh Bioscience, Germany) with particle sizes of 5.0 µm. The column was protected by a guard column (Amide-80, 1.0 cm×4.6 mm, TSKgel®, Tosoh Bioscience, Germany) and thermostatized at 40°C under isocratic conditions. The mobile phase consisted of 100 mM Potassium dihydrogen phosphate buffer: Acetonitrile (30:70). The mobile phase was filtered through a 0.45-µm pore size nylon millipore membrane (47-mm-diameter, Whatman®, China) and degassed with ultrasonic bath (U500H,

Ultrawave, UK) for 30 min before using. The flow for optimal separation was at 1.2 ml/min for 10.0 min and increasing to 1.4 ml/min for 10.1-20.0 min. Injection volume was 20 µl. The UV detector wavelength was set at 254 nm. Identification and quantification of hypoxanthine, Inosine, IMP, and GMP were based on standard curves obtained by using external standards (Clarity chromatography data system software, DataApex, Czech Republic). Data are presented as mg/g of cooked meat.

III. RESULTS AND DISCUSSION

From Table 1, the pH value of pork LD samples was 5.60 and crude fat was 2.55%. The pH value indicated that the samples are in normal pH range. Using our method, the elution times for hypoxanthine, inosine, IMP, and GMP were at 4.5, 5.0, 9.5, and 11.3 min, respectively. The resulted hypoxanthine, inosine, IMP, and GMP contents were 0.19, 0.55, 1.71, and 0.18 mg/g of cooked sample, respectively. Previous study [3] reported that hypoxanthine, inosine, IMP, and GMP of cooked red non-exudative pork loins were 0.24, 2.7, 7.79, and 0.12 µmol/g, respectively. However, another study [1] indicated that IMP, inosine, and hypoxanthine in fresh (uncooked) pork were 3.76, 1.75, and 0.52 nmol/mg, respectively. In addition, fresh pork loin was reported to have IMP, inosine, hypoxanthine, and GMP in the amount of 2.39, 0.66, 0.08, and 0.04 mg/g, respectively [7]. The contents of nucleotides reported by [1] and [7] are higher than the levels found in cooked pork LD from our study. According to [8], increasing cooking temperatures (60, 100, and 120°C) resulted in the increase in inosine and hypoxanthine contents in cooked goat and sheep meats. The small range of standard deviations (0.01-0.15) reported in our study indicated to the precision of the method.

Table 1 pH, crude fat, and nucleotide metabolite contents in cooked *Longissimus* muscles (N=15)

parameters	pH	crude fat (%)	nucleotide compounds (mg/g of cooked sample)			
			hypoxanthine	Inosine	IMP[2]	GMP[3]
means±SD[1]	5.60±0.11	2.55±0.36	0.19±0.02	0.55±0.06	1.71±0.15	0.18±0.01

[1]SD = standard deviation, [2]IMP = inosine monophosphate, [3] GMP = guanosine monophosphate

IV. CONCLUSION

Nucleotide compound determination in cooked pork LD by using an isocratic HPLC-UV method offers short run time (20 min), low detection limit with good precision (bias < 0.5%), and high reproducibility. This simple method without gradient condition eliminates salt accumulation problem in the system and is also cost effective.

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