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Development and validation of an analytical method for the determination of 4-hexylresorcinol in food

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ABSTRACT

This study presents a method validation for extraction and quantitative analysis of 4-hexylresorcinol residues in shrimp and crab meat using HPLC–FLD. We were focused on the collaboratively analysis of each shrimp and crab meat samples, and developed LC–MS/MS method for the correct confirmation of the identity of compound. Validation parameters; selectivity, linearity, LOD, LOQ, accuracy, precision, and measurement of uncertainty were attained. The measurement of uncertainty was based on the precision study, data related to the performance of the analytical process and quantification of 4-hexylresorcinol. For HPLC–FLD analysis, the recoveries of 4-hexylresorcinol from spiked samples at levels of 0.2–10.0 ppm ranged from 92.54% to 97.67% with RSDs between 0.07% and 1.88%. According to these results, the method has been proven to be appropriate for extraction and determination of 4-hexylresorcinol, and can be used to maintain the safety of shrimp and crab products containing 4-hexylresorcinol residues.

1. Introduction

Color change called "melanosis (black spots)" exists in crustaceans such as shrimp and crab due to polyphenol oxidase activity during storage. Melanosis causes a shorter shelf-life and poor quality, leading to a decrease in commercial value and organoleptic properties. Inhibitors such as sulfite derivates were used to prevent this phenomenon. However, sulfites have been replaced with 4-hexylresorcinol due to their association with health problems, in particularly for sensitive asthmatics (Collins-Williams, 1983; Gunnison, Jacobsen, & Schwartz, 1987; Taylor, Higley, & Bush, 1986).

The molecule 4-hexylresorcinol is a dihydroxybenzene with a hexyl group in the 4 position and hydroxyl groups on positions 1 and 3 of the aromatic ring is a well-known food additive. The molecule 4-hexylresorcinol is used as a satisfactory alternative for preventing melanosis in shrimp and crab (Iyengar, Bohmont, & Mcevily, 1991; Montero, Martínez-Álvarez, & Gómez-Guillén, 2004; Montero, Martínez-Álvarez, Zamorano, Alique, & Gómez-Guillén, 2006). The maximum residue levels of 4-hexylresorcinol in raw materials from China and Canada is 1.0 ppm, whereas 2 ppm is acceptable in the European Union (European Commission, 2011; Food and Drug Regulation, 1998; USDA Foreign Agricultural Service, 2011). Although 4-hexylresorcinol is "generally recognized as safe" (GRAS) (Frankos et al., 1991), its use is not permitted in South Korea. Consequently, a validated analytical method is needed to detect the use of unauthorized 4-hexylresorcinol in foods commonly consumed in South Korea.

The methods mainly reported for the determination of 4-hexylresorcinol in foods and pharmaceuticals were based on thin layer chromatography (TLC) (Robbins & Wesson, 1931), high performance liquid chromatography (HPLC) (Jonker & Dekker, 2000; King, McEvily, & Iyengar, 1991; Smallwood, Ranieri, & Satzger, 1998), and high performance liquid chromatography time-of-flight mass spectrometry (HPLC–TOF/MS) (Xiu-Qin, Chao, Yan-Yan, Min-Li, & Xiao-Gang, 2009). King et al. (1991) and Smallwood et al. (1998) conducted the determination of 4-hexylresorcinol in shrimp and crab meat using high performance liquid chromatography and UV detection (HPLC–UV). In particular, a silica Sep-Pak cartridge and C_{18} solid-phase extraction column were used to prepare the shrimp and crab meat samples, respectively.

Previously published papers efforts have focused on the analysis of each shrimp and crab meat samples. But these studies have

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not collaboratively tested with complex matrices such as uncooked frozen shrimp, cooked frozen shrimp, and crab meat. Also, the correct identity of identified peak in HPLC chromatogram was not confirmed. Because it is necessary to determine rather low levels of 4-hexylresorcinol in support of legislation, validated methods, reduction of analysis time, and correct confirmation of the identity of compound, are needed for the analysis of these matrices.

The aim of this study was to develop and validate a HPLC–FLD method applicable for the determination of various food matrices in uncooked frozen shrimp, cooked frozen shrimp, and crab meat as the primary domestic and imported product representations of crustaceans. We were developed LC–MS method for the correct confirmation of the identity of compound. Additionally, to demonstrate the effective application of the established method on real samples, various shrimp and crab were collected from grocery markets in South Korea and other countries and were monitored for their 4-hexylresorcinol contents. The identified peaks by HPLC–FLD were confirmed by LC–MS.

2. Materials and methods

2.1. Reagents

Standard 4-hexylresorcinol (209,465) with a purity of >98% and phosphoric acid were obtained from Sigma-Aldrich (St Louis, MO, USA). Potassium dihydrogen phosphate was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). All solvents were suitable for LC analysis and were purchased from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Food materials

The following shrimp and crab were collected from grocery markets in South Korea, Europe (Czech), and the USA (San Diego): 87 shrimp (uncooked or cooked frozen shrimp) and 20 crabs (or crab meat). To validate the procedure, shrimp and crabs that were found to be free of 4-hexylresorcinol were selected. All shells of the samples were removed, and the samples were homogenized using a food blender (SFM-656CS, Shinil Industrial, Seoul, South Korea). The homogenate was stored in an airtight plastic zipper bag at −20 °C until analysis.

2.3. Optimization of HPLC conditions

4-Hexylresorcinol was tested using HPLC. Analytical conditions were chosen for 4-hexylresorcinol analysis, as it achieved the best results. Parameters such as detection modes, mobile phases, various columns, and column temperatures were evaluated and until optimum results were obtained for the optimum separation condition of 4-hexylresorcinol from shrimp meat. The sensitivity of the analytical method was based on the maximum admissible levels of 4-hexylresorcinol in foreign (European Union, China, and Canada).

2.4. Optimization of extraction method

Based on the optimum condition of HPLC–FLD analysis method, the preparation methods for 4-hexylresorcinol in three matrices (uncooked frozen shrimp, cooked frozen shrimp, and crab meat) were optimized. The recovery rate was used as an index of the optimization of the sample preparation method. Sample preparation for 4-hexylresorcinol analysis was optimized by the previously reported method (Jonker & Dekker, 2000; Smallwood et al., 1998).

2.5. Sample preparation

The samples were prepared according to the modified method of Jonker and Dekker (2000). A total of 10 mL of water was added to 5 g of homogenate in a 50 mL conical tube, and the tube was shaken vigorously for 30 s to disperse the shrimp or crab meat in water. A total of 15 mL of acetonitrile was added and homogenized for 30 s with an ultrasonic cleaning bath (JAC 4020, KODO, Seoul, South Korea). Subsequently, the solution was agitated for 15 min using a mechanical shaker (SI-600, JEIO TECH, Seoul, South Korea) at 300 rpm and centrifuged (FLETA 5, Hanil, Incheon, South Korea) for 3 min at 3000*g*. The supernatant was separated into a 50 mL volumetric flask. The extraction procedure was repeated twice, each time with 10 mL of acetonitrile, and the extracts were combined. The supernatant was diluted to a volume of 50 mL with acetonitrile and filtered through a 0.45 μm syringe filter (Millex-HV, Millipore, Bedford, MA, USA).

2.6. HPLC instrument conditions

The HPLC apparatus was an iLC3300 HPLC system (Labogene, Eresing, Germany) equipped with a binary pump, an autosampler, a column heater, and a fluorescence detector (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on a Phenomenex Gemini 5 μ C_{18} 110 A (Phenomenex, 4.6 mm × 250 mm, 5.0 μm, Torrance, CA, USA) column maintained at 30 °C using an isocratic mobile phase of 0.01 M KH_2PO_4 (pH of 3.0 with 25% H_3PO_4)/acetonitrile (40:60, v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 μL, and the detector was set for an excitation wavelength of 280 nm and emission wavelength 310 nm (Jonker & Dekker, 2000). Data acquisition and remote control of the HPLC system were performed using DataApex Clarity™ software (DataApex, Praha, Czech Republic).

2.7. LC–MS/MS

LC–MS/MS was performed using a Finnigan Surveyor HPLC system and TSQ Quantum mass spectrometer (Thermo Finnigan, San Jose, CA, USA) with electrospray ionization (ESI) capabilities. Liquid chromatography separation was performed on a Thermo Syncronis C_{18} column (Thermo Scientific, 2.1 mm × 100 mm, 5.0 μm, San Jose, CA, USA) maintained at 30 °C using a gradient program consisting of mobile phase A (acetonitrile) and mobile phase B (water) at a flow rate of 0.3 mL/min. The initial conditions (<1 min) were 10% A–90% B followed by 100% A–0% B (1–6 min), 10% A–90% B (8–9 min), and finally holding for 3 min. The total run time was 12 min. The injection volume was 10 μL. The MS/MS data for 4-hexylresorcinol was collected in negative ion mode using selected reaction monitoring (SRM) of transition m/z 193.10 → 149.153 (Collision energy, 17 eV), 122.037 (Collision energy, 22 eV). The optimized parameter settings for ESI were: spray voltage, 3000 V; sheath gas pressure, 50; aux gas pressure, 10; capillary temperature, 270 °C; tube lens, 59; collision pressure, 1.2.

2.8. Method validation

The HPLC method for the determination of 4-hexylresorcinol in three food matrices (uncooked frozen shrimp, cooked frozen shrimp, and crab meat) was validated in terms of linearity, specificity, LOD and LOQ, precision, and accuracy according to the guidelines of the International Conference on Harmonization (2005). Matrix-matched calibration curves were prepared by spiking extracts of blank uncooked frozen shrimp, cooked frozen shrimp, and crab meat in seven concentrations from 0.005 to 1.0 μg/mL. Matrix-matched calibration curves from each validation run were evaluated to ensure that linearity and sensitivity were