Biotechnology and Bioprocess Engineering 2009, 14: 819-827
DOI/10.1007/s12257-009-0105-0

Determination of Fungal Glucosamine Using HPLC with 1-napthyl Isothiocyanate Derivatization and Microwave Heating

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Abstract A rapid method for the determination of fungal glucosamine (GlcN) from *Aspergillus sp* BCRC 31742 was developed. The hydrochlorination process using microwave effectively reduced reaction time needed for GlcN analysis. The analytical method consisted of two steps: (1) hydrochlorination of fungal cells and (2) derivatization process. Fungal GlcN hydrochloride was reacted with 1-napthyl isothiocyanate (1-NITC) as the derivatizing agent to enhance the sensitivity of GlcN and so to achieve high resolution. This method was specific for quantification of GlcN hydrochloride at the wavelength of 230 nm. The standard deviation and relative error of the analytical results were less than 5%. By using microwave heating, the reaction time of hydrochlorination process was shortened from 24 h to 3 min. Thus, the overall time needed for analyzing GlcN from fungal sources was reduced from 5 h (thermal method) to 2 h (microwave method). © KSBB

Keywords: microwave, fungal glucosamine, microbial, kinetics, chromatography, optimization

INTRODUCTION

Glucosamine (GlcN), or 2-amino-2-deoxy-D-Glucose, is a molecule, which is produced naturally in the body to promote healthy cartilage. GlcN and its acetylated form, *N*-acetyl glucosamine (GlcNAc) can be found in the cell wall of fungi as the monomer of chitin and chitosan. These fungi include subdivisions of Ascomycotina (*e.g. Aspergillus sp*) [1] and Zygomycotina (*e.g. Rhizopus sp, Mucor sp, Gongronella butleri*) [2-6]. GlcN and GlcNAc can also be obtained from the exoskeleton of the crustaceans [7,8].

The studies on GlcN production using microorganisms have increased recently because of some limitations of its production from shellfish or other marine sources, due to allergic reactions, metal contamination, and supplies limitation of marine sources [8]. Productions of GlcN using chemical processes are usually carried out by chemical and or enzyme hydrolysis, wherein the energy expenditure leads to increased production cost and also need longer time [7,9]. Several studies have reported the production of GlcN from microbial fermentations. Using *Monascus pilosus* the GlcN obtained was up to 264 mg/L [10] and using *Rhizopus oligosporus* the GlcN content obtained was 107 mg/gdw cells [11]. An *Aspergillus sp* was utilized to produce GlcN in a solid state fermentation process which produced the GlcN content of about 24.4 mg/gdw cells [12]. Later, a production of GlcN using an Ascomycotina, *Aspergillus sp* BCRC 31742, the GlcN obtained was 3,428 mg/L [1].

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A reliable method of determination of GlcN from fungal fermentation is needed to endorse a successful fungal fermentation. Several papers have reported about the determination of GlcN and acetyl GlcN from supplements, and chitosan or chitin from microbial biomass [13-16]. Generally, the quantitative analysis of GlcN uses colorimetric assay [17], radioactivity [18], gas chromatography [19], capillary electrophoresis [20], and high performance liquid chromatography (HPLC) techniques [1]. A recent paper reported that the time needed for analyzing GlcN from biomass takes about 8 h, the procedure includes hydrolysis at room temperature, hydrolysis at high temperature, depolymerization, and deamination to obtain deaminated GlcN [21]. This process converts chitin and chitosan into anhydromannose and acetic acid, where anhydromannose used as the marker of GlcN amount in the sample and acetic acid represents GlcNAc.

As reported in the literature, the determination of chitosan in cells (including GlcN) using enzyme is a complex process. The process usually consists of cell disruption, deproteination, deacetylation, and extraction using acetic acid. In each