Biotecnología

Revista Mexicana de Ingeniería Química

Vol. 14, No. 2 (2015) 393-403

EFFECT OF INITIAL SUBSTRATE CONCENTRATION AND AGITATION ON XYLITOL PRODUCTION BY FERMENTATION OF HYDROLYZED TAMARIND SEED MEDIA WITH *Kluyveromyces marxianus*

EFECTO DE LA CONCENTRACIÓN INICIAL DE SUSTRATO Y AGITACIÓN SOBRE LA PRODUCCIÓN DE XILITOL POR FERMENTACIÓN DE MEDIO HIDROLIZADO DE SEMILLA DE TAMARINDO CON *Kluyveromyces marxianus*

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Received July 27, 2014; Accepted April 21, 2015

Abstract

Tamarind seed consists of 50 to 72 % of a branched heteropolysaccharide, called xyloglucan. By fragmenting xyloglucan with a coupled process of acid hydrolysis and thermal treatment, it is possible to obtain considerable amounts of fermentable sugars, glucose and xylose being the most abundant. Xylose is the precursor of xylitol, a sugar with similar characteristics to sucrose. Chemical synthesis of xylitol is very expensive and of low-yield. On the other hand, xylitol can be obtained by fermentation, using yeasts that incorporate xylose in their metabolism, such as *Kluyveromyces marxianus*. Being a biological process, xylitol production by fermentation depends on different environmental factors. In this paper, the effect of two factors on xylitol production was evaluated based on a 3^2 factorial experimental design: initial substrate concentration (20-80 g/L) and agitation (120-240 rpm). Both factors considerably influenced xylitol production of *K. marxianus*, where the optimization of the experimental design predicted a yield of 0.57 g of xylitol/g of xylose, with an initial substrate concentration of 50 g/L and an agitation of 177 rpm, from a source substrate of which there are no reports of its use in this field, such as tamarind seed.

Keywords: xylose, fermentation, xylitol, initial substrate concentration, agitation.

Resumen

La semilla de tamarindo está compuesta en un 50 a 72 % por un heteropolisacárido ramificado, llamado xiloglucano. Mediante su fragmentación con un proceso acoplado de hidrólisis ácida y tratamiento térmico, es posible obtener cantidades considerables de azúcares fermentables, siendo la glucosa y la xilosa los más abundantes. La xilosa es el precursor del xilitol, un azúcar con características similares a la sacarosa. La síntesis química del xilitol es costosa y de bajo rendimiento. Por otro lado, se puede obtener xilitol por fermentación usando levaduras que incorporan la xilosa a su metabolismo, como *Kluyveromyces marxianus*. Al ser un proceso biológico, la producción de xilitol depende de diferentes factores ambientales; en este estudio, se evaluó el efecto de dos factores usando un diseño experimental factorial 3^2: concentración inicial de sustrato (20-80 g/L) y agitación (120-240 rpm). Ambos factores tuvieron un impacto considerable sobre la producción de xilitol de *K. marxianus*, donde la optimización del diseño experimental predijo un rendimiento de 0.57 g de xilitol/g de xilosa, a una concentración inicial de sustrato de 50 g/L y agitación de 177 rpm, usando una fuente de sustrato que no ha sido reportada: la semilla de tamarindo.

Palabras clave: xilosa, fermentación, xilitol, concentración inicial de sustrato, agitación.

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WULFENIA JOURNAL KLAGENFURT, AUSTRIA ISSN:1561-882X

Vol 22, No. 1;Jan 2015

Gradient Reversed-Phase C_{18} High-Performance Liquid Chromatography of Important Gibberellins.

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Abstract

Most important gibberellins, i.e. GA_3, GA_4 and GA_7, are produced by means of fermentation processes. For their quantification, a gradient reversed-phase HPLC method was applied with good resolution between peaks of isomers. A 1.86 resolution was obtained between GA_3 and GA_1 peaks and a 2.29 resolution was obtained between GA_4 and GA_7 peaks. This method allows quantification of important gibberellins from fermentation cultures using UV detection. The method requires 40 min for quantification and equilibration and chromatograms are reproducible. The cultures came from fermentations of the fungus *Gibberella fujikuroi* CDBB-H984. In this paper a new method of extraction and purification for subsequent analysis in high resolution chromatography is presented.

Introduction

Gibberellins are growth hormones that appear in higher plants where regulate several growth-associated processes. Currently, gibberellins are produced via cultivation of certain fungus, i.e. *Gibberella fujikuroi*, in fermentative processes [1,2]. They are widely used in agriculture and in brewery industry [3].
Gibberellins identification is typically made by thin layer chromatography [4] and their quantification is carried out by several ways, outstanding reversed phase C_{18} high performance liquid chromatography. Many workers using several mobile phases has been described this procedure by [5-8].
Barendse et al. [5] described a gradient method for gibberellin analysis for several gibberellins, including GA_1, GA_3, GA_4 and GA_7. Castillo et al. [6] modified this method to obtain a better resolution between GA_1 and GA_3 peaks working with an isocratic fashion. Lin et al. [7] also describes a gradient method for gibberellin analysis that uses acetic acid.
In this work, we start from a slightly modification of Castillo´s method to obtain a better resolution between GA_1 and GA_3 peaks that continues with a gradient for GA_4 and GA_7 quantification.

Experimental

High performance liquid chromatography

The HPLC system was composed of the following modules from Varian: a pump (Model 9012), a variable wavelength UV-Vis detector (Model 9050) and an auto-sampler system (Model 9100). The column was a reversed-phase C_{18} ChromSpher (250×4.6 mm I.D., 5 μm, Varian). The column is provided with a guard column ChromGuard (50×3 mm I.D., 5 μm, Varian) and both are placed in a column heater (Timberline 101) maintained at 28 °C. The mobile phase consisted of a methanol-water mixture containing 10 mM