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Optimization of medium composition for enhanced chitin extraction from *Parapenaeus longirostris* by *Lactobacillus helveticus* using response surface methodology

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Chitin extraction by biological way, using the lactobacilli *Lactobacillus helveticus*, is a non-polluting method and offers the opportunity to preserve the exceptional qualities of chitin and its derivatives. However, the major disadvantage of the fermentative way is the low efficiency of demineralization and deproteinization. The aim of our study is to improve the yield of extraction.

Many factors, such as the initial concentration of carbon source, fermentation time, incubation temperature, inoculum size, shell size, volume and medium composition have been reported to influence the fermentation process and consequently demineralization and deproteinization efficiency. Based on the use of central composite design and response surface methodology ten factors with three levels each were examined to determine the optimal operational conditions of demineralization and deproteinization.

The analysis of the obtained results showed that the optimal conditions of 98% of demineralization and 78% of deproteinisation are 171.4 g L^{-1} of reducing sugars, 2.03 g of nitrogen source [$(NH_4)_2Fe(SO_4)_2$] and 1.29 g of calcium source ($CaCl_2$), used to ferment 4.84 g of shells, of 1.053 mm size heat treated at 120 °C, with 10 mL of inoculum (*L. helveticus*) incubated at 32.1 °C in 100 mL of juice date for 254.38 h (15 days).

1. Introduction

Chitin is the second most abundant structural biopolymer found in nature (Ji, Wolf, Rodriguez, & Bowlin, 2012; Khuoshab, Jaruseranee, Tanthanuch, & Yamabhai, 2012). It occurs in a multitude of organisms from bacteria and fungi to molluscs and others, but is certainly most prominent in the largest and most diverse group of the animal kingdom particularly, arthropod group (Fabritius et al., 2011).

In arthropods, chitin is used together with various proteins and inorganic salts such as calcium carbonate, to form the exoskeleton. The actual chitin content varies depending on physiological stage of the organism (Benhabiles et al., 2012), harvesting season (Nitar, Tetsuya, & Hiroshi, 2011), health of the animals and geographical location (Kjartansson, Zivanovic, Kristberg, & Weiss, 2006). To date, the major source of industrial chitin comes from wastes of marine food production mainly crustacean shells, *e.g.* shrimp and crab shells or krill (Jayakumar et al., 2010; Mojarrad, Nemati, Valizadeh, Ansarin, & Bourbour, 2007; Xia, Liu, Zhang, & Chen, 2010; Xu, Gallert, & Winter, 2008).

The traditional processes of chitin production consist of the use of strong acids and bases under high temperature for demineralization and deproteinization, respectively. These processes, however, may cause pollution (Zakaria, Hall, & Shama, 1998) and significantly lower intrinsic viscosities of chitin (Rødde, Einbu, & Vårum, 2008). An alternative way to solve these problems is the use of biotechnological methods. The calcium and the protein in the shell waste were dissolved mainly by organic acids and proteases produced by microorganisms, respectively. Many factors, such as inoculum level (Shirai et al., 2001), shell content in medium, shell size (Oh, Kim, Nguyen, Jung, & Park, 2008), carbon source such as glucose (Rao, Muñoz, & Stevens, 2000; Shirai et al., 2001), sucrose (Choorit, Patthanamanee, & Manurakchinakorn, 2008), cassava flour (Rao and Stevens, 2006), molasses (Seda, Sebnam, Yekta, & Ali Fazil Yenidunya, 2004) and date juice (Adour, Arbia, Amrane, & Mameri, 2008), initial pH and its evolution during fermentation

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