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Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells

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Abstract

A Box–Behnken design with three variables (sucrose concentration, initial pH value and soaking time) and three levels were used for studying the demineralization efficiency in fermented shrimp shells by *Pediococcus* sp. L1/2. First, the bacterial cells were inoculated into the media with various concentrations of sucrose and initial pH values, and fermentation took place under static conditions at 37 °C for 24 h. Significant differences in the levels of total titratable acid were observed. This was followed by adding shrimp shells and soaking them in the fermentation media for 12, 24 and 36 h. The results showed that when the sucrose concentration was 50 g/L, and the initial pH value was 6.00, soaking for 36 h gave a demineralization efficiency of 68.38%. By solving the equation and also analyzing the response surface contour plots, optimum conditions occurred when the sucrose concentration was 50 g/L, the initial pH value was 7.00 and the soaking time was 36 h with a predicted value of demineralization of 83.03% whereas our experiment gave 83.47%.

Keywords: Box–Behnken design; Demineralization; Lactic acid bacteria; Response surface method; Shrimp waste

1. Introduction

Shrimp production is a major agro-industry in tropical and subtropical countries. These products are frozen raw shrimp, frozen cooked shrimp and frozen value-added shrimp products which generate vast quantities of solid waste. As reported by Islam et al. (2004) the head, shell and tail portions of shrimp are removed during processing and these account for approximately 50% of the volume of raw materials. Shrimp waste is considered to be a source of protein, pigments (e.g. astaxanthin), flavour compounds (Fanimo et al., 2000; Sachindra and Mahendrakar, 2005; Handayani et al., 2008; Sachindra et al., 2007; Babu et al., 2008) and chitin (Rødde et al., 2007). Protein and pigments found in shrimp waste have been proven to be an excellent animal feed supplement (Coward-Kelly et al., 2006; Cavalheiro et al., 2007). Moreover, chitin, and its de-*N*-acetylated derivative chitosan, has numerous applications in the food, chemical, cosmetic, paint and textile industries (Synowiecki and Al-Khateeb, 2003; Daum et al., 2007).

The traditional processes of chitin production consist of the use of strong acids and bases under high temperatures for demineralization and deproteinization, respectively. These processes, however, may cause pollution (Zakaria et al., 1998; Lertsutthiwong et al., 2002) and significantly lower intrinsic viscosities of chitin (Rødde et al., 2007). An alternative way to solve these problems is to use biotechnology methods. Under mild conditions, demineralization efficiency can be achieved by organic acids in a culture medium of lactic acid bacteria. Moreover, the separated liquor containing proteins and lipids (Shirai et al., 2001) can be directly used for animal feed. Accordingly, the lactic acid bacteria have been used for recovery of value-added by-products, such as chitin and protein liquor (Rao et al., 2000; Shirai et al., 2001; Cira et al., 2002; Rao and Stevens,

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2006; Bhaskar et al., 2007). Since shrimp waste is characterized by its high perishable waste, it easily undergoes spoilage and transforms into a public health hazard. In order to avoid the development of spoilage microorganisms, fermentation with lactic acid bacteria is recommended (Rao et al., 2000; Bhaskar et al., 2007).

Many factors, such as inoculum size, initial pH value, carbon concentration and carbon/nitrogen ratio have been reported to influence the fermentation process and consequently demineralization efficiency. In the present study, demineralization efficiency of shrimp shells was determined by fermenting shrimp shells with the selected strain of lactic acid bacteria at various sucrose concentrations, initial pH values and soaking times. A response surface Box–Behnken design was performed and for optimization purposes, the predicted and experiment values are reported.

2. Methods

2.1. Lactic acid bacteria and starter culture preparation

Lactic acid bacterial strain L1/2 used in this study was isolated from fermented vegetable products by Associate Professor Wilawan Jalernjiratrakul, Faculty of Science, Prince of Songkla University, Thailand. In brief, 10 g of a sample were homogenized with 90 ml of 0.9% NaCl (w/v) and serially diluted in the same dilutent. One milliliter of these dilutions was pour plated in the MRS agar supplemented with 1% $CaCO_3$, and incubated in an Anaerobic Gas-Pack system, at 37 °C. Identification of the strain L1/2 was performed (Badis et al., 2004). Microscopic observation of the bacterial cells showed a cocci characteristic form into tetrad. The cells were catalase negative, showed no spore formation, and gas was not produced. Accordingly, the bacterial strain L1/2 was called homofermentive *Pediococcus* sp. L1/2.

The starter culture was prepared by adding a loopful of the bacterial cells to 7 ml of MRS broth and incubating at 37 °C for 24 h. Subsequently, 5 ml of the culture was transferred to 45 ml of the MRS broth and incubated at 37 °C for 24 h. The culture was adjusted to an optical density of 1.0 (approximately 10^8 cells/ml) at a wavelength of 660 nm with the MRS broth.

2.2. Preparation of shrimp shell

Shrimp (*Litopenaeus vannamei*) head waste was obtained from Taksin Sa-mutr Company (Songkla, Thailand) and was kept frozen during transportation. After removing meal, the shrimp shell was soaked in a 1% chlorine solution for 30 min, and washed with distilled water four times before being stored at −20 °C until required. The surface area of twenty samples of shrimp shell was measured. Calculation of the surface area of the shrimp shell was achieved by drawing an outline of the shrimp shell onto paper and then using a digital planimeter (Placom, Japan), which followed the outline of the shrimp shell, the area was measured. Shrimp shells with an average surface area of 13.20 ± 0.57 cm^2 and a weight of 0.2757 ± 0.0003 g/shell were used throughout this study, unless otherwise mentioned.

2.3. Design of experiments

Studies on the effects of sucrose concentration, initial pH value and soaking time for demineralization were carried out in the fermentation media containing x g/L sucrose (commercial grade), peptone 5, yeast extract 5, K_2HPO_4 5 and $MnSO_4 \cdot H_2O$ 0.3, as described by Chronopoulos et al. (2002). After sterilization of the medium at temperature of 121 °C for 15 min, the initial pH of the medium was adjusted by addition of 1 N NaOH or 1 N HCl using a pH meter (ORION, model 420A). After cultivation in a vessel containing 1 L of the medium at 37 °C for 24 h, *Pediococcus* sp. L1/2 reached $3.1–8.2 \times 10^9$ cells/ml. Then 5% wet weight of shrimp shell (about 18–19 shells) was added. The shrimp shells were soaked in the culture broth at 37 °C under static conditions.

In order to describe the nature of the response surface in the experimental region, a Box–Behnken design was applied (Box and Behnken, 1960). As presented in Table 1, the experimental design involved three parameters (x_1, x_2 and x_3), each at three levels, coded −1, 0, and +1 for low, middle and high concentrations, respectively. Table 2 represents the design matrix of a 17 trials experiment. For predicting the optimal point, a second order polynomial function was fitted to correlate the relationship between independent variables and response. For the three factors this equation is

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2, \quad (1)$$

where Y is the predicted response, b_0 model constant; x_1, x_2 and x_3 are independent variables; b_1, b_2 and b_3 are linear coefficients; b_{12}, b_{13} and b_{23} are cross product coefficients and b_{11}, b_{22} and b_{33} are the quadratic coefficients.

The software Design Expert (Version 7.0.1, Stat-Ease Inc., Minneapolis, USA) was used for experimental design, data analysis and quadratic model building. The optimal fermentation conditions for enhanced yield of demineralization were obtained by solving the regression equation and also by analyzing the response surface contour plots using the same software.

Table 1
Design of experiment-levels of various process parameters

Parameter	Level		
	−1.0	0.0	1.0
x_1: Sucrose concentration (g/L)	10	30	50
x_2: Initial medium pH	5	6	7
x_3: Time (h)	12	24	36