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Use of a fractional factorial design to study the effects of experimental factors on the chitin deacetylation

Islem Younes[a,*], Olfa Ghorbel-Bellaaj[a], Moncef Chaabouni[b], Marguerite Rinaudo[c], Florence Souard[d], Cécile Vanhaverbeke[e], Kemel Jellouli[a], Moncef Nasri[a]

[a] Laboratory of Enzyme Engineering and Microbiology, National School of Engineering, PO Box 1173-3038, Sfax, Tunisia
[b] Laboratory of Industrial Chemistry, National School of Engineering, BP W 3038 Sfax, Tunisia
[c] Biomaterials Applications, 6, rue Lesdiguières 38000 Grenoble, France
[d] Univ. Grenoble Alpes, DPM, F-38000 Grenoble, France
[e] CNRS, DPM, F-38000 Grenoble, France

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ABSTRACT

Chitosan is obtained by deacetylation of chitin. Chitosan versatility is directly related to the polymer's characteristics depending on the deacetylation process. The aim of this research was to study the parameters influencing deacetylation and to elucidate their effect on acetylation degree (DA) and molecular weight (MW). The effect on chitosan DA was investigated using a fractional factorial design 2^{7-3} with seven factors and two variation levels. The tested factors were: X_1 = number of successive baths, X_2 = reaction time, X_3 = temperature, X_4 = alkali reagent, X_5 = sodium borohydride, X_6 = the atmospheric conditions and X_7 = alkali concentration. A mathematical model was investigated corresponding to the following relation $\hat{y} = 7.469 - 1.344X_1 - 1.094X_2 - 3.094X_3 + 1.906X_4 + 0.656X_5 + 0.906X_6 - 1.031X_7 + 0.469X_1X_2 - 0.781X_3X_4 + 0.906X_1X_3X_4$ with $R^2 = 0.99$. This model allows fixing experimental conditions for each desired DA. To study the effect on chitosan MW, only atmospheric conditions and use of sodium borohydride as an oxygen scavenger were investigated. The use of sodium borohydride and nitrogen atmosphere was found to have a protective effect against chitosan degradation during deacetylation.

1. Introduction

Chitosan is a natural polysaccharide comprising glucosamine and *N*-acetylglucosamine monomeric units. It can be obtained by partial deacetylation of chitin, a major component of the shells of crustaceans [1,2]. Chitosan has received considerable attention due to its wide range of applications in diverse fields, ranging from waste management to food processing, medicine and biotechnology [3].

The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the two types of residues, is employed to differentiate between chitin and chitosan. When the DA (expressed as molar percentage) becomes lower than 50 mol%, the product is named chitosan and becomes soluble in acidic aqueous solution [4]. During deacetylation process, not only acetyl groups are removed but also a depolymerization reaction occurs affecting chitosan molecular weight.

Chitin can be converted to chitosan by enzymatic preparations [5–8] or chemical process [9,10]. Chemical methods are used extensively for commercial purpose of chitosan preparation because of low cost and suitability to mass production [10]. From a chemical point of view, either acids or alkalis can be used to deacetylate chitin. However, glycosidic bonds are very susceptible to acid; therefore, alkali deacetylation is used more frequently [5,11,12]. Chemical process in chitosan preparation has been developed by many researchers using concentrated sodium hydroxide solution (40–50%) in various conditions. Rarely, potassium hydroxide solution is used [13]. The variations in preparation methods of chitosan result in differences in its acetylation degree, the distribution of acetyl groups along the chains, the viscosity of chitosan solution and its molecular weight [14,15]. These variations affect chitosan properties and its behavior toward many applications [16,17]. It has been reported that the acetylation degree is considered as the most important characteristics of chitosan that control its properties and especially solution properties [18].

* Corresponding author.
E-mail address: islem.younes@gmail.com (I. Younes).

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Many factors in the deacetylation process can influence the characteristics of the final product [18]. Rege and Block [19] investigated the influence of reaction temperature, processing time, and mechanical shear and found that the reaction temperature and processing time have a significant impact on the acetylation degree and molecular weight of chitosan. Tolaimate et al. [13] reported that acetylation degree of the chitosan is greatly affected by temperature and repetition of alkaline steps. Wu and Bough [20] studied the effects of time and NaOH concentration. Tsaih and Chen [21] also studied the effect of time reaction and temperature. All these studies were conducted using a classical one-variable-at-a-time experimentation. These reports indicate that molecular weight and acetylation degree of chitosan are mainly affected by NaOH concentration, reaction time, temperature and repetition of alkaline steps. Additional factors are reaction reagent, atmosphere, particle size, chitin and chitosan solvent ratio, and source of raw material [10,13,22,23]. These multiple factors pose a challenge to understanding and controlling the outcome of the products. Therefore, there is a need for process optimization.

Response surface methodology (RSM) is useful for analyzing the effects of several independent variables [24,25]. The main advantage of RSM is the reduced number of experimental trials needed to evaluate the effect of multiple factors on the response. In order to determine a suitable polynomial equation that describes the response surface, RSM can be employed to optimize the process for gathering research results better than classical one-variable-at-a-time or full factorial experimentation. The RSM statistical technique was previously used in investigating chitosan production while controlling MW and/or DA. Weska et al. [26] introduced RSM to optimize the deacetylation process of chitin using temperature and reaction time as factors. Hwang et al. [27] studied the effects of temperature, time and NaOH concentration on the deacetylation. Chang et al. [23] reported the influence of NaOH concentration, temperature and solution/chitin ratio and found that chitosan acetylation degree was decreasing with increasing temperature and NaOH concentration. All previous studies were investigated using NaOH as alkali but there are no reports investigating the use of KOH. Other parameters could influence deacetylation but were not considered previously mainly the use of alkali successive baths, the atmospheric conditions and the addition of oxygen scavenger.

For that purpose, the present research was developed to elucidate the effect on deacetylation of the alkali reagent as well as its concentration, the temperature, the reaction time, the use of successive baths, the atmospheric conditions and the use of sodium borohydride as oxygen scavenger. For this, a fractional factorial design was applied and a mathematical model was established allowing fixing experimental conditions for each desired chitosan acetylation degree.

2. Materials and methods

2.1. Raw material and chitin preparation

The raw material used in this study was shrimp (*Metapenaeus monoceros*) waste obtained from a shrimp processing plant located in Sfax, Tunisia.

Chitin was obtained from grounded material, through the stages of demineralization using successive baths of 0.55 M HCl with a ratio of 1/10 (w/v) at ambient temperature and deproteinization where proteins were digested using an enzymatic process. Chitin was then dried in a tray dryer until reaching moisture content of 5.0–6.0% on wet basis and milled (chitin was sieved from 2 to 4 mm). This chitin is an α-chitin isomorph 100% acetylated. This chitin was treated with 50% NaOH at a ratio of 1:20 (w/v) under air atmosphere at 120 °C and 30 rpm stirring for 3 h. After this step, chitin acetylation degree was about 54%. This chitin was used for the preparation of chitosans using fractional factorial design.

Table 1
Variables and experimental domains.

Variables	Level (−)	Level (+)
X_1: number of successive baths	1	2
X_2: reaction time (h)	3	6
X_3: temperature (°C)	90	140
X_4: alkali reagent	NaOH	KOH
X_5: atmospheric conditions	Nitrogen	Air
X_6: use of sodium borohydride	With	Without
X_7: alkali concentration (mol/L)	7	10

2.2. Reactor design for deacetylation of chitin

For each experiment, 5 g of dried chitin was placed in a reactor (5 g of $NaBH_4$ was added for reactions carried out in presence of sodium borohydride), in which alkali solution was added under heating and stirring. The ratio of chitin and alkali solution was 1:20 (w/v). The contents of the reactors were stirred at 30 rpm [19]. Temperature was maintained constant (at ±0.5 °C) and controlled by a Fluke data logger system (model 2640A, Everett, WA). One neck of the reactor was fitted to a nitrogen bottle to ensure atmospheric conditions (under air or nitrogen). A second neck of the reactor was fitted with a condenser for refluxing vapors.

2.3. Statistical design for deacetylation of chitin

A two-level fractional factorial design 2_{IV}^{7-3}, with seven factors and two variation levels, was used for the study of the deacetylation reaction, being analyzed by the ANOVA test. The studied factors were: number of successive baths (X_1), reaction time (X_2), temperature (X_3), alkali reagent (X_4), atmospheric conditions (X_5), use of $NaBH_4$ (X_6) and alkali concentration (X_7). The variation levels in the codified form (X_n), based on literature data, are presented in Table 1 [26–28].

In a fractional factorial design, we assume that interaction effects between three or more factors are negligible and thus useful information on the main effects and second order interactions may be obtained by running only a fraction of the complete factorial design.

In a 2_{IV}^{7-3} fractional factorial design, seven variables (factors) are studied each of them at two levels. The effects of three variables (extra variables) are confounded with high order interactions between the four other variables (basic variables).

In this work, the 2_{IV}^{7-3} fractional factorial design is obtained by writing down the complete 2^4 factorial as the basic design (with the four variables X_1, X_2, X_3 and X_4) and then equating factors X_5, X_6 and X_7 to the $X_1X_2X_3$, $X_1X_2X_4$ and $X_1X_2X_3X_4$ interactions, respectively.

The last conditions allow us to identify the independent generators (1) and the corresponding complete defining relation (2) which is the set of all columns that correspond to the identity column I:

$$I = X_1X_2X_3X_5 = X_1X_2X_4X_6 = X_1X_2X_3X_4X_7 \quad (1)$$

$$I = X_1X_2X_3X_5 = X_1X_2X_4X_6 = X_3X_4X_5X_6 = X_1X_2X_3X_4X_7 = X_4X_5X_7 = X_3X_6X_7 = X_1X_2X_5X_6X_7 \quad (2)$$

From this set of generators, it is possible to develop the complete alias structure for this design as indicated in Table 2. It is to be noticed that three and four-factor interactions are considered as negligible.

To simplify the calculations, coded variables X_j are used instead of natural variables U_j. The range of variation of each U_j ($U_{j,\text{low}}$ and