Food Hydrocolloids 59 (2016) 50–58

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Improvement of chitosan production from Persian Gulf shrimp waste by response surface methodology

Marjan Nouri [a], Faramarz Khodaiyan [a, *], Seyed Hadi Razavi [b], Mohammad Mousavi [b]

[a] Bioprocessing and Biodetection Laboratory, Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, Campus of Agriculture and Natural Resource, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran
[b] Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, Campus of Agriculture and Natural Resources, University of Tehran, Iran

ARTICLE INFO

Article history:
Received 5 April 2015
Received in revised form
26 August 2015
Accepted 28 August 2015
Available online 2 September 2015

Keywords:
Chitosan
Molecular weight
Degree of deacetylation
Response surface methodology (RSM)

ABSTRACT

Chitosan is a water soluble polysaccharide that was extracted from the wastes of Persian Gulf shrimp. It has been a big challenge for the scientists to find different biological, mechanical, physical, enzymatic and microbial procedures to extract edible chitosan. In this study chitosan (isolated from waste of species of Indian white shrimp) with high functionality was produced using mild conditions (temperature (60, 80 and 100 °C), concentration of alkaline (30, 40 and 50%) and time (90, 195, 300 min) of reaction in chemical method and power of microwave (300, 600 and 900 W), concentration of alkaline (30, 40 and 50%) and irradiation time (20, 100, 180 S) in microwave method) and short duration which did not degrade the structure of chitosan. It was observed that the highest percent of chitosan preparation (19.47%), degree of deacetylation (89.34%) and molecular weight (806,931 Da) were achieved in microwave style at 50% NaOH solution, 720 W microwave power and 20 S reaction time.

1. Introduction

The Persian Gulf shrimp is one of the most valuable and delicious seafood resources of Iran and exported in a huge quantity (Parvaneh, 1977), but the shrimp waste is a major environmental safety problem in the country because the shells are very insoluble and resistant material for technological applications. To overcome the problem of shrimp waste a solution was proposed not only to solve industrial problems but also to change the biowaste to useful component (Nouri & Khodaiyan, 2014a; Mondal & Alam, 2013). In general, the shrimp waste contains (30–40%) protein, (30–50%) calcium carbonate and (20–30%) chitin on a dry basis (Nithya, Jothivenkatachalam, Prabhu, & Jeganathan, 2014). Chitin, β-(1 → 4) N-acetyl-D-glucosamine, is the second most abundant natural polysaccharide after cellulose and the major component of the exoskeleton animals like crustaceans, shrimps, insects and fungal cell walls (Arbia, Adour, Amrane, & Lounici, 2013; Ji, Wolf, Rodriguez, & Bowlin, 2012). Chitosan, β- (1 → 4) D-glucosamine, is a cationic amino polysaccharide which is a partly deacetylated form of chitin, if the percentage of acetyl glucosamine group is more than 50% it is called chitin, but if this percentage is less than it, the component is chitosan (Kamboj, Singh, Tiwary, & Rana, 2015; Xia, Liu, Zhang, & Chen, 2010). In recent times, more attention has been paid to the chitosan, because it has a variety of applications in medicine, pharmaceutical, biomedical, biological, agriculture, environment and in food technology such as, food formulations, biopackaging, binding, thickening, gelling, stabilizing, clarifying and antimicrobial agent (Arancibia et al., 2014; Benhabiles et al., 2012; Nouri, Khodaiyan, Razavi, & Mousavi, 2015; Ocloo et al., 2011).

The utilization of strong solution reagents at high temperature in traditional methods (deproteinization and demineralization) results in decreased quality and also increased costs and ecological problem (Hwang et al., 2002; Sahu, Goswami, & Bora, 2009; Synowiecki & Al-Khateeb, 1997). In recent years much attention has been given to microwave irradiation because it can accelerate the reaction time compared to other methods which transport temperature by conventional heating treatment and also heat transfer within the food structure can be done more homogeneously (Alishahi et al., 2011; Mahdy Samar, El-Kalyoubi, Khalaf, & Abd El-Razik, 2013).

Over the past years various crustaceans have been used to extract chitin, but here we focus on the extraction based on exoskeleton of the species shrimp. In the past, chitin was extracted

* Corresponding author.
E-mail address: khodaiyan@ut.ac.ir (F. Khodaiyan).

http://dx.doi.org/10.1016/j.foodhyd.2015.08.027
0268-005X/

from species of Brown shrimp (*Penaeus semisulcatus*), Pink shrimp (*Penaeus durarum*), Metapenaeus affinis (*Milne-Edwards*) from River Nile and Persian Gulf, respectively (Abdou, Nagy, & Elsabee, 2008; Sagheer, Al-Sughayer, Muslim, & Elsabee, 2009) however there is no existing report on the extraction of chitin from waste species of Indian white shrimp (*Penaeus indicus*). In Iran, Indian white shrimp is the major cultured shrimp species as a result of market demand, local availability and growth rate (Ouraji et al., 2009). The mineral, protein and chitin content of different types of shrimp exoskeleton are not the same, for example the protein content of *P. semisulcatus*, *P. durarum*, *Milne-Edwards* and *P. indicus* are 29.50, 34.02, 28.84 and 32.05% respectively, consequently different functional properties of chitosan may be produced (Abdou et al., 2008; Ravichandran, Rameshkumar, & Rosario Prince, 2009; Sagheer et al., 2009).

The attributes of biopolymer chitosan are influenced by the degree of deacetylation and their molecular weight, these parameters of chitosan influence not only biological characteristics but also solubility, biodegradability, antimicrobial, drug delivery behavior and scavenging reactive oxygen species (Czechwska-Biskup, Diana, Rokita, Ulanski, & Rosiak, 2012; Motta de Moura, Madeira Soares, & Almeida Pinto, 2011; Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013). Some of the past studies reported the roles of various factors, such as different extraction methods of chitosan, granularity and particle size of raw chitin, temperature and time of reaction, concentration and rate of reagents to chitin and atmospheric qualification on molecular weight and deacetylation reaction of chitosan (Hwang et al., 2002; Weska, Moura, Batista, Rizzi, & Pinto, 2007; Zhang et al., 2011).

Different methods such as titration, NMR spectrometer, spectroscopy UV/Vis and infrared spectroscopy have been used to measure the degree of deacetylation of chitosan and numerous detailed procedures exist in literature for each of these techniques, but potentiometric titration method could be easily perform in most laboratories and it is the most reliable and robust method among non-NMR methods (Czechwska-Biskup et al., 2012).

There are several ways to determine the molecular weight of chitosan such as using light scattering, gel permeation chromatography and FTIR spectroscopy but viscometry procedure is the most uncomplicated, fast, cheap, accessible and may be the most accurate determination method (Abdel-Salam, 2013; Weska et al., 2007). The Mark-Houwink exponent is usually used as a conformation indicator of chitosan in solution and it is related to the intrinsic viscosity of the molecular weight of polymer (Chen & Tsaih, 1998).

Response surface methodology (RSM) is a very useful statistical technique for complicated chemical, physical and food processes optimally (Nouri & Khodaiyan, 2014b).

The present study is an attempt to investigate the improvement of the extraction methods of chitin by using mild conditions from Persian Gulf shrimp waste. The chitin will be isolated and deacetylated with high yield by using different conditions of reaction by response surface methodology. The effect of the aforementioned experimental conditions on molecular weight and degree of acetylation of biopolymer chitosan will be explored too.

2. Materials and methods

2.1. Materials

Chitin, the most important ingredient in present article, was isolated from species of Indian white shrimp (*P. indicus*) from the Persian Gulf and purification leftovers. The dried samples were grained in blender and packaged in polyethylene bag to be stored at ambient temperature for further analysis. Other ingredients like sodium hydroxide, acetic acid, ethanol and acetone were purchased from Merck.

2.2. Extraction of chitin by chemical and physical methods

2.2.1. Deproteinization (DP)

The process of extraction involved deproteinization with 2% (w/w) sodium hydroxide solution for 2 h at 80 °C with constant stirring at a solid to solvent ratio of 30:1 (v/w). Separation of alkali-insoluble fraction (AIF) was done by centrifugation (4000 ×g, 15 min).

2.2.2. Demineralization (DM)

The deproteinization shells were demineralized with 10% (w/w) acetic acid 40:1 (v/w) for 4 h at 50 °C. Produced heterogeneous viscous slurry was mixed thoroughly to form insoluble particles (remaining from chitin) and remove them by centrifugation (4000 ×g, 15 min), precipitation was washed with water, (washing to pH = 7.0) ethanol and acetone respectively to remove any impurities and dried in oven to be used for production of crude chitin and the solution from centrifuge was used in next steps (Deacetylation of chitin).

2.3. Response surface optimization of chitosan production

2.3.1. Deacetylation of chitin (DA)

The conversion of chitin to chitosan was carried out through dissolution of chitosan which was produced by precipitation of sodium hydroxide solution (biopolymer chitin is dissolved in NaOH solution). The parameters employed at chemical and physical methods in this article were as follows in Table 1.

2.4. Analysis of the extraction yield

According to the Equation (1), the yield was calculated as the dry weight of the chitosan powder relative to the Persian Gulf shrimp waste.

$$\text{Chitosan extraction yeild} = \frac{\text{Dried chitosan extraction weight (g)}}{\text{Persian Gulf shrimp waste (40 g)}} \quad (1)$$

2.5. Determination of the degree of deacetylation (DDA) of chitosan

The degree of deacetylation was measured by the acid-base titration method (Zhang et al., 2011), through some modification. Chitosan (0.125 g) was dissolved in aqueous solution of HCL (30 mL, 0.1 mol/L), about 5–6 drops of methyl orange was added as indicator and stirred (30 min) until totally dissolved at room temperature. The solution was titrated with 0.1 mol/L NaOH solution until it turned orange. The degree of deacetylation of chitosan was calculated by the Formula (2) and (3):

Table 1
Levels of various independent variables at coded values of RSM experimental design.

Variables	Units	Symbol code	Levels		
			−1	0	1
NaOH concentration	(%) by weight	X_1	30	40	50
Reaction temperature	Celsius (°C)	X_2	60	80	100
Reaction time	Minutes (min)	X_3	90	195	300
Power of microwave	Watt (W)	X_4	300	600	900
Irradiation time	Second (S)	X_5	20	100	180