Optimization of Fish Waste Extracted by Biosynthesis Methods

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Abstract

The fish 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 fish waste a solution was proposed not only to solve industrial problems but also to change the biowaste to useful component. In this study chitosan with high functionality was produced using mild conditions (power of microwave (200, 300 and 400 W), concentration of alkaline (30, 40 and 50 %) and irradiation time (30, 60, 90 S) in microwave method). DPPH radical scavenging activities of chitosan samples were observed.

Keywords: Fish, Waste, Microwave, Scavenging activities

Introduction

A great amount of food has been dumped as commercial and domestic waste. Fish is widely eaten, which, for its preparation, requires the removal of skin, bones and fins (Arvanitoyannis and Kassaveti, 2007). Moreover, great quantities of these wastes are also produced in fish shops and fish-processing factories (Nagai and Suzuki, 2000).

A technological point of view, it would be economic to converting the by-products of seafood processing because of its richness in high value compounds (Ocloo et al, 2011). Chitosan, ß- (1 → 4) D-glucosamine, is a cationic amino polysaccharide which is a partly deacetylated (more than 50%) form of chitin. Traditional chemical method for the commercial isolating of chitin from shrimp shell involves alkali and acidic treatments (Youn et al, 2009). In recent studies much more interested has been observed in the electromagnetic irradiation because it can accelerate the reaction of conventional heating treatment compared to traditional methods (Mahdy samar et al, 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 (Zhang et al, 2011). Chitosan has been interested in medicine, pharmaceutical, biomedical, biological, agriculture, environment and in food technology such as, food formulations, binding, thickening, gelling, stabilizing, clarifying and used as antimicrobial biopackaging due to its effectiveness of inhibiting the growth of not only bacteria (gram-positive and gram-negative bacteria) but also yeasts and molds (Arbia et al, 2013).

To prevent lipid oxidation of food products can be added synthetic chelating agents and antioxidants but in new day searchers focused on new natural preservatives. Until now, several sources of natural antioxidants, antibacterial and antifungal were recognized both biopolymer chitin and chitosan have been shown to activate the antioxidants, antifungal and antibacterial which are affected by its molecular weight and concentration (Mahdy samar et al, 2013).

The present study is an attempt to investigate the improvement of the microwave extraction methods of chitin by using mild conditions from fish waste.

Material and method

Fish were procured from a local region in Persian Gulf. The shrimp shells were washed, dried and grained. Other ingredients like sodium hydroxide, acetic acid, ethanol and acetone were purchased from Merck.

The process of extraction involved deproteinization (1 % (w/w) sodium hydroxide solution 30:1 (w/v) for 2 h at 70 ºC) and demineralized (10% (w/w) acetic acid 40:1 (w/v) for 4 h at 40 ºC). After each step separation was done by centrifugation (4,000 g, 10 min). The deacetylation of chitin was produced by precipitation of sodium hydroxide solution (Nouri and Khodaiyan, 2013).

The parameters employed at physical methods ,all reaction under microwave irradiation was carried out in the microwave device Butane microwave (M 245, Iran), are listed in Table 1. Response surface methodology (RSM) is a very useful statistical technique for complicated chemical, physical and food processes optimally.

Table 1. Levels of various independent variables at coded

Microwave method	**Variables and Units**		
Run order	NaOH concentration (%): x_1	Power of microwave: (Watt): x_2	Irradiation time Second (S): x_3
1	-1 (30)	-1 (200)	0 (60)
2	1 (50)	-1 (200)	0 (60)
3	-1 (30)	1(400)	0 (60)
4	1 (50)	1(400)	0 (60)
5	-1 (30)	0 (300)	-1 (30)
6	1 (50)	0 (300)	-1 (30)
7	-1 (30)	0 (300)	1 (90)
8	1 (50)	0 (300)	1 (90)
9	0 (40)	-1 (200)	-1 (30)
10	0 (40)	1(400)	-1 (30)
11	0 (40)	-1 (200)	1 (180)
12	0 (40)	1(400)	1 (180)
13	0 (40)	0 (300)	0 (60)
14	0 (40)	0 (300)	0 (60)
15	0 (40)	0 (300)	0 (60)

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 fish waste (Nouri et al, 2015).

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

Antioxidant activity

The antioxidant/ free radical-scavenging activity of chitosan was evaluated using DDPH according to the method of Blois (1958) whit some modification. The chitosan sample of various (2.5, 5, 7.5 and 10 µg/mL) were prepared. The absorbance was then measured at 517 nm using a spectrophotometer (CECIL 2502-Insturuments Cambridge England Serial 2502-Instruments Cambridge England Serial no 125- 624). Free radical-scavenging activity was calculated by following equation 2:

$$\text{Scavenging activity} = 1 - \frac{\text{Absorbance sample}}{\text{absorbance control}} \quad (2)$$

Results and discussion

Optimisation of chitosan production yield

In the present study Box–Behnken design in RSM was applied in order to show an optimum combination of parameters for chitosan extraction with three independent variables NaOH concentration (X_1), power of microwave (X_2) and irradiation time (X_3) in microwave process for each treatment and a set of 15 experiments were carried out as shown in Table 1. The results indicated that the extracted chitosan from 40 g of dried waste of fish at microwave conductions was in the range of 3.49-6.04 (Table 2).

Table 2. The optimum conditions by the BBD design on yield, of chitosan extraction.

Run order	Yield of chitosan (g/40 g)	Run order	Yield of chitosan (g/40 g)
1	3.49	9	4.25
2	4.44	10	5.01
3	3.21	11	3.24
4	6.47	12	4.27
5	4.21	13	6.05
6	5.84	14	6.01
7	4.20	15	6.04
8	6.00	-	-

Results showed that in both methods using strong condition such as more time, temperature or microwave power had a negative effect on the yield and properties of the purified. Chitosan. Chitosan extracted from fish waste was crystalline powder, non-harmful and odorless, white and off-white. Microwave heating leads to short the reaction time, less harmful to the environment and also reduces a certain degree of depolymerization thus better chitosan quality compared to chemical method.

Antioxidant activity of chitosan

The results show that maximum antioxidant activity of different concentration of chitosan was observed at 50 % NaOH concentration (14.82 to 16.89 %), at 400 W power of microwave (15.83 to 17.55 %) and 60 S reaction time (14.95 to 16.42 %) was received the maximum antioxidant activity (Figure 1).

Scavenging activity of the samples was produced increased with increase in concentration of chitosan (from 2.5 until 10 µg/mL). This circumstance may be due to the fact that the activity of microwave samples did not contribute to increasing of concentration more than 7.5 µg/mL and a similar result has been reported by Ocloo et al (2011). Arbia et al reported that 80% scavenging activity of 30 kGy oligosaccharide sample in a solution form of chitosan (Youn et al, 2009).

However our results showed that chitosan had a low antioxidant activity. The scavenging activity of samples may be due to the reaction between free radicals with the hydrogen ion form the ammonium ion (NH_3^+) to form a stable molecule (Mahdy samar et al, 2013). The lower molecular weight or viscosity of chitosan demonstrated increase mobility and the chance of exposure of their residual free amino group and inhibition of lipid peroxidation (Arbia et al, 2013).

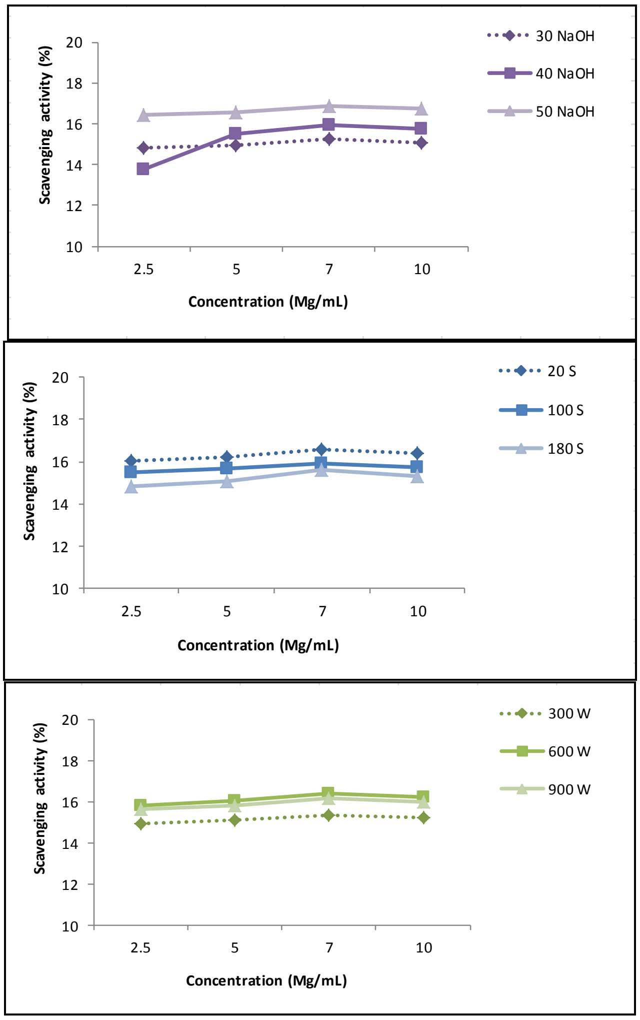

Figure 1. Scavenging activity (%) of chitosan sample of various concentrations (2.5, 5, 7.5 and 10 µg/mL).

Conclusions

We demonstrated the most advantageous statistical technique for investigating the effect of major independent factors such as NaOH concentration, power of irradiation and time of reaction on the biological, antioxidant properties of chitosan for choosing the best biosynthesis treatment with the highest inhibitory influence of the growth of organism.

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