Extraction of Chitosan From Fish Scale for Food Preservation and Shelf-Life Enhancer

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Research Article

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Abstract

With the growing demand for chitosan in a variety of applications and the drawbacks associated with crustacean shell-based chitosan, there is a need to look for alternative sources of chitosan extraction and production. Chitosan was extracted from the scales of red snapper (*Lutjanus johnii*) in this study. It was discovered that the concentration of solvent (HCl and NaOH) and temperature at various stages of the extraction process influenced the yield of extracted fish scale chitosan. The characterization result revealed that the commercial crustacean-based chitosan and the extracted fish scale chitosan had similar Fourier-transform infrared spectroscopy (FTIR) spectra, indicating that the two had similar functional groups. The degree of deacetylation (DDA) of the extracted fish scale chitosan was determined to be 76.9%, with the ash value, loss on drying, and solubility being 1.28%, 3.62%, and 88.8%, respectively. The extracted and commercialised chitosan were found to be similar in all characterization results. The potential of fish scale chitosan as a food preservative and shelf-life enhancer was then investigated in this study. Strawberries coated with chitosan were stored at various temperatures, and their physical appearance and moisture loss were recorded. When used in conjunction with traditional preservation techniques such as storage in a cool environment, fish scale chitosan was found to be capable of preventing up to 50% moisture loss in strawberries.

1.0 Introduction

Chitin, a form of natural polysaccharide, is one of the most abundant and important compounds found mainly in two types of crustacean shell, which are shrimp and crab. Chitosan is derived from chitin by removing its acetyl group through chemical treatments such as deacetylation. Both chitin and chitosan offer a unique set of physicochemical and biological characteristics, such as biocompatibility, biodegradability, nontoxicity, physiological inertness, immunological activity, antibacterial properties, wound-healing activity, heavy metal ions chelation, gel-forming properties and hydrophilicity, and affinity to protein. These properties make chitin and chitosan appealing materials for a variety of applications. Their applications are widely distributed in many fields, such as wastewater treatment, biotechnology, medical and pharmaceutical, food industry, agriculture, cosmetics and pulp and paper industries (Kumari et al. 2017).

According to Gokulalakshmi et al. (2017), about 130 million tons of fish waste is being generated every year around the world. Most of these wastes come from the seafood industry. While the wastes generated are biodegradable, the speed at which it degrades is considered slow. An effective method to reduce the amount of waste being generated is to utilize and transform it into a more biologically sustainable material, in this case, chitosan. In a research by Boarin-Alcalde and Fonseca (2016), the production of fish scale chitosan from a fish species Nile tilapia (*Oreochromis niloticus*) was performed and it was found that the extracted chitosan showed comparable properties to the commercial crustacean shell-based chitosan. In another report by Santos et al. (2017), the usage of chitosan coating to increase the shelf life of liquid smoked Nile tilapia fillet was investigated. The authors reported that chitosan possesses strong

antimicrobial and antifungal activities, which are useful in preserving seafood products that are highly susceptible to both microbiological and chemical deterioration.

Chitosan is found to inhibit the growth of spoilage microorganisms in food, which suggests its potential to be used as a food preservative (Abdeltwab et al. 2019). Based on the recent research, development of chitosan from fish wastes provides a two-way benefit where the waste produced from the fish species can be subsequently used as a preservative to extend the shelf-life of the higher valued food products in the market. The present research aims to extract chitosan from fish scales obtained from the fish bred in Malaysia. The effect of extracting solvent and temperature at different stages of extraction process (demineralization, deproteinization and deacetylation) on the chitosan yield were studied. Finally, the feasibility of utilizing the extracted fish scale chitosan in the application of food preservation as shelf-life enhancer of strawberries was investigated.

2.0 Materials And Methodology

2.1 Extraction of Fish Scale Chitosan

The fish scale wastes of red snapper (*Lutjanus johnii*) were obtained from a local wet market in Kuala Lumpur, Malaysia. They were washed with water and dried in the oven at 50°C for 24 hours. The dried fish scales were then grinded into smaller pieces and kept in an air-tight container prior to the extraction processes. The extraction of fish scale chitosan was performed based on the procedures described by Kumari et al. (2017) and Premasudha et al. (2017) with slight modifications.

2.2 Demineralization

Demineralization process was done by soaking the dried fish scales in 1 (w/v) % hydrochloric (HCl) at room temperature for 24 hours. Then, the fraction of acid-insoluble fraction was separated from the acid solvent by centrifugation at 3500 rpm for 20 minutes. After that, the separated fraction was washed with distilled water to rinse off the remaining acids and subsequently dried at 40°C overnight to yield chitin that will be subjected to deproteinization.

2.3 Deproteinization

Deproteinization process was performed by adding the chitin into 1 (w/v) % sodium hydroxide (NaOH) solution at 25°C for 24 hours to remove the protein. Separation of alkali-insoluble fraction was done by centrifugation at 3500 rpm for 20 minutes. The separated fraction was then washed repeatedly using distilled water until the pH was neutral.

2.4 Deacetylation

The isolated chitin from deproteinization was added into 50 (w/v) % NaOH solution at 100°C for 2 hours for the deacetylation process. The chitosan was then separated from the alkali solvent by centrifugation at 3500 rpm for 20 minutes. The products were washed with distilled water until the pH was neutral. They were then dried at 40°C overnight to yield the fish scale chitosan.

2.5 Effect of Extracting Solvent and Temperature of Different Stages

The effect of extracting solvent and temperature at different stages of extraction process on the chitosan yield were investigated based on the values tabulated in Table 1.

Table 1 The extraction parameters investigated

Extraction Stage	Parameters	Unit	Value
Demineralization	Concentration of HCl	(w/v) %	0.05, 1, 3
	Extraction Temperature	°C	25, 40, 60
Deproteinization	Concentration of NaOH	(w/v) %	1, 2, 3
	Extraction Temperature	°C	25, 40, 60
Deacetylation	Concentration of NaOH	(w/v) %	40, 50, 60
	Extraction Temperature	°C	60, 80, 100

2.6 Characterization of Chitosan

The degree of deacetylation (DDA) value of the extracted fish scale chitosan was measured using pH metric titration as described by Perez-Alveraz et al. (2018). The extracted chitosan was also characterized using Fourier-transform infrared spectroscopy (FTIR) from 4000 to 400 cm⁻¹ with potassium bromide (KBr) pellets to identify the functional groups present. Subsequently, the ash value, loss on drying and solubility tests were performed to identify the physical properties of the extracted chitosan. The ash value was determined by heating the chitosan in a furnace at 650 °C for 3 hours and then weighed. However, prior to heating, the extracted chitosan was weighed beforehand. The ash value was then calculated using eq. (1).

$$Ash \% = \frac{Weight\ of\ residue\ (g)}{Weight\ of\ sample\ (g)} \times 100\ \% \tag{1}$$

The percentage of loss on drying was calculated using eq. (2), after weighing the wet weight of extracted fish scale chitosan and the oven dried weight of the fish scale chitosan.

$$\text{Loss on drying \%} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Dry weight (g)}} \times 100 \quad (2)$$

Lastly, the solubility of the fish scale chitosan was determined through incubated shaking 0.1 g of the chitosan sample at 25 °C for 20 minutes (250 rpm). However, it was first dissolved in 10 ml of 1 % acetic acid. Subsequent to shaking, the solution was then immersed in a boiling water bath for another 20 minutes, and then was let to cool down to room temperature. Afterwards, the solution underwent centrifugation at 3500 rpm for 30 minutes for solid-liquid separation. The acetic acid (liquid) was discarded, whereas the chitosan sample (solid) was weighed. The solubility percentage was calculated using eq. (3)

$$\text{Solubility \%} = \frac{(\text{Initial weight of tube + chitosan})(g) - (\text{Final weight of tube + chitosan})(g)}{(\text{Initial weight of tube + Chitosan})(g) - (\text{Initial weight of tube})(g)} \times 100 \% \quad (3)$$

2.7 Food Preservative and Shelf-Life Enhancer

Chitosan coating was prepared by dissolving 1 g of the extracted fish scale chitosan in 100 ml of 1 % acetic acid to produce a 1 % chitosan solution. Strawberries were then immersed into the solution for 1 minute and air dried for 1 hour. The strawberries were kept in plastic container and stored at 10°C for 7 days. A control set of strawberries without chitosan coating was prepared as the control. The appearance and weight of the strawberries were monitored and recorded on the 1st, 3rd and 7th day of storage. The fruit quality was evaluated based on several parameters that were inclusive of peel color and acceptability (physical condition, mold growth). The loss of moisture content within the strawberries was measured as well.

3.0 Results And Discussion

3.1 Effect of Extracting Solvent and Temperature of Different Stages

It was observed that as the concentration of HCl solution used in the demineralization increased from 0.05 % to 3 %, the yield of extracted chitosan decreased from 49.05 to 32.96 % (**Fig. 1**). This was consistent with the findings by Ait et al. (2018) who reported that the yield of chitosan extracted enhanced from 2.1 % to 4.4 % when fish and shrimp wastes were treated with 3.5 M and 0.5 M of HCl solution respectively. The increase in hydrochloric acid concentration resulted in an extensive demineralization step that removed minerals such as calcium carbonate and led to higher weight loss in the process. From **Fig. 1**, it was observed that the extraction temperature did not affect the yield of chitosan significantly. Nevertheless, when the temperature was increased beyond 40°C, the yield started

to drop. According to Younes and Rinaudo (2015), demineralization reaction can be accelerated at higher temperatures as it drives the penetration of solvent into the chitin matrix. However, at elevated temperatures, the final physicochemical properties of the product chitosan can be affected, which may explain the results obtained in this study.

Fig. 2 shows the effects of NaOH concentration and temperature on the yield of chitosan at the deproteinization process. The results showed that the yield of chitosan increased as the concentration of NaOH increased. In another report by Tokatlı and Demirdöven (2017), 0.5 M and 0.95 M of NaOH solution yielded 9.64 % and 10.13 % shrimp-based chitin respectively. They explained that higher concentration of NaOH removed complex protein structures which were difficult to separate as they functioned to retain the chitin structure. Increased of sodium hydroxide solution concentration was found to provide better protein removal efficiency and thus resulted in the higher chitosan yield. On the other hand, positive correlation was obtained between the temperature of deproteinization with the yield of extracted chitosan, consistent with the results reported by Kader and Aslıhan (2017). In the deacetylation process, the yield of chitosan extracted improved from 45.13 % to 53.54 % as the NaOH concentration increased from 40 % to 60 %, as shown in **Fig. 3**. At high concentration of NaOH, amide hydrolysis reaction occurred rapidly with the presence of NaOH as the base and chitin as the amide. NaOH introduced -OH group into the chitin structure which promoted the elimination of the acetyl group, hence promoting the deacetylation process. Nevertheless, as the temperature was increased from 60°C to 100°C, the difference between the yield obtained was approximately 3 % only. It was suggested not to perform the deacetylation process at high temperature as the chitosan structure may be disrupted from the large amount of heat and energy supplied (Kader and Aslıhan 2017).

3.2 Characterization of Chitosan

The comparison of properties between the commercialized crustacean-based chitosan and the extracted fish scale chitosan in this study is presented in Table 2.

Table 2 The properties of commercialized crustacean-based and the extracted fish scale chitosan

Properties	Commercialized Chitosan	Extraction Chitosan
Degree of Deacetylation (%)	75.6	76.9
Ash Value (%)	0.99	1.28
Loss on Drying (%)	7.95	3.62
Solubility (%)	86.8	88.8

The standard commercialized chitosan DDA values typically range from 66 – 95 % (Kumari et al, 2017). The extracted fish scale chitosan of red snapper in this study was within the DDA range and was found to be consistent with the results reported by Takarina et al. (2017) whereby DDA for the extracted white

snapper chitosan ranged between 75 – 85 % in their study. Ash value of chitosan represents the effectiveness in the demineralization process in removing inorganic materials. The result of this study revealed that the extracted chitosan was within the range of standard value 1.26 – 1.56 %. The loss of moisture content was also within the range reported that was lesser than 10 % (Pérez-Álvarez et al. 2018). According to Premasudha et al. (2017), chitosan of higher quality shows higher solubility in acidic solution. From Table 2, solubility of 88.8 % obtained for fish scale chitosan indicated it to be categorized as high solubility. The Fourier Transform Infrared (FTIR) Spectra of both chitosan samples are presented in **Fig. 4**. The major functional groups were found to be similar in both spectra. The presence of peaks and bands based on **Fig. 4** is tabulated in Table 3.

Table 3 Wavenumber of the peaks identified from FTIR spectra and its functional group

Commercialized Chitosan	Fish Scale Chitosan	Bond	Functional Groups
3359 cm ⁻¹	3349 cm ⁻¹	N-H bend	Primary amines [4]
2877 cm ⁻¹	-	C-H stretch	Alkane [4]
1646 cm ⁻¹	1646 cm ⁻¹	C=O vibration	Amide [4]
1417 cm ⁻¹	1416 cm ⁻¹	C-N stretch	Secondary amines [4]

3.3 Food Preservative and Shelf-Life Enhancer

The effect of fish scale chitosan coating on strawberries that were kept at 10°C and 25°C was compared to the control batches. The results are shown in **Fig. 5**. For the strawberries that were kept at 10°C, as the storage duration was prolonged to day 3, the control strawberry showed a slightly darker peel colour compared to the coated one whereby the colour was still noticeably vibrant. However, on the 7th day, black spots were observed on the control strawberry (suspected to be mold), which was not found on the coated strawberry. This observation could be due to the antimicrobial characteristics of chitosan as reported by Khan et al. (2019). On the other hand, for the strawberries that were kept at 25°C, a similar observation was recorded for both the control and chitosan-coated batches. Signs of spoilage were found on the surface of the strawberries on the 3rd day. The peel colour of both batches turned dark and signs of mould growth were observed at certain areas on the surface of the strawberry. White fibres were also observed showing signs of mould growth. On the 7th day, sour smell was detected from both batches with white molds observed on the surface of the strawberries along with several holes.

In addition to the physical appearance, the feasibility of using fish scale chitosan as a food preservative and shelf-life enhancer was also investigated using moisture loss analysis. The weight loss recorded for controlled and chitosan- coated strawberries kept at 10°C and 25°C for 7 days are tabulated in Table 4. The results revealed that the chitosan-coated strawberry had improved preservation outcome as the weight loss was significantly lesser than the control batches, especially the one kept at 10°C. Reduction

of up to 50 % and 40 % of moisture loss was observed when the fish scale chitosan was applied to the strawberries that were kept at 10°C and 25°C, respectively, compared to the control counterpart. Though the weight loss was enhanced at the 25°C storage environment with the presence of chitosan coating, based on the physical appearances, it was suggested that the performance of chitosan as a shelf-life enhancer was more apparent when it was applied concurrently with other critical storage parameters such as storage temperature. The preservative effect was more obvious at the storage temperature of 10°C. Hence, further investigation to purify the fish scale chitosan or manipulate its coating concentration can be explored in improving its performance as a food preservative.

Table 4 The comparison of weight loss between uncoated and chitosan-coated strawberries

Storage Temperature (°C)	Strawberry	Weight Loss (%)
10	Control (uncoated)	2.07
	Chitosan coated	1.04
25	Control (uncoated)	7.69
	Chitosan coated	4.43

4.0 Conclusion

The present work attempted to extract chitosan from fish scales originating from red snappers (*L. johnii*) as an alternative raw material for the production of chitosan. The effect of two process parameters in different stages of the chitosan extraction process was investigated. Generally, it was found that higher solvent concentrations and higher extraction temperatures were able to increase the yield of fish scale chitosan. Based on the characterization results, comparable characteristics were observed between the extracted fish scale chitosan in this study with the commercialized crustacean-based chitosan. In terms of the performance of fish scale chitosan as a food preservative and shelf-life enhancer, the preliminary results demonstrated that reduction of moisture loss in chitosan-coated strawberries was possible when they were kept at lower room temperature, indicating the possibility of exploiting it as a green-food preservative.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication (include appropriate statements)

This paper is a contribution to the Special Issue of “Green Technology and Industrial Revolution 4.0 for a Greener Future” as recommended by the 7th International Conference on Environment 2021 (ICENV2021).

This paper has been agreed upon by all authors to be submitted to Environmental Science and Pollution Research.

Availability of data and material (data transparency)

All data and materials provided in the manuscript originated from the experimental work performed by the authors.

Conflicts of interest/Competing interests

Not applicable.

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Authors' contributions

Hon Min Ooi: First author of the manuscript (write-up), performed experimental work.

Mohamad Huzair Munawar: Improvement of manuscript in terms of supporting evidences and in-depth explanation.

Peck Loo Kiew: Corresponding author of the manuscript.

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Figures

Figure 1

Effect of HCl concentration and temperature at the demineralization process

Figure 2

Effect of NaOH concentration and temperature at the deproteinization process

Figure 3

Effect of NaOH concentration and temperature at the deacetylation process

Figure 4

The FTIR spectra of (a) Commercialized crustacean-based chitosan and (b) Fish scale

Figure 5

The physical appearance of strawberries on the 1st, 3rd and 7th day of storage