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Physiochemical properties of chitosan extracted from shrimp shells caught in Libyan coast

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Abstract :

This study aims to assess the possibility of extracting chitosan extracted from shrimp collected from the Libyan coast, to study some of its physiochemical properties, and to classify it compared to those extracted from imported shrimp available in the Libyan market in Tripoli. The physical and chemical properties of chitosan were studied including fat-binding capacity (FBC), water-binding capacity (WBC), throughput, solubility, moisture content, ash, protein, lipid, and degree of deacetylation of chitosan. The results showed that the moisture content was 28.025% in the untreated shrimp shells. The chitin sample contained 10.166% of moisture, compared to 2.677% only in the chitosan sample. Fresh local shrimp shells contained 4.70% ash, whereas the ash content in the imported sample was 53.62%. The extracted chitin and chitosan samples recorded 0.45% and 2.05% ash content, respectively. Local shrimp shells contained 28% of protein. 4.16% protein content was found in chitin extract from locally collected shrimp shells, against 0.098% in extracts from imported shrimp shells. Moreover, local shrimp shell samples contained 0.668% of fat, against 5.86% fat content in

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imported samples. Chitin and chitosan extracted from local shrimp shells contained 0.74% and 2.50% fat, respectively. Chitosan extracted from imported shrimp shells had a fat content of 7.307%. Fresh shrimp yielded 14.04% of chitosan, with a 0.329% the chitosan extracts from the local shrimp shells, compared to 8.5% extracts from the imported shrimp shells. A deacetylation score of 9.944 was found in chitosan extracted from both local and imported chimpanzees. The solubility of chitosan extracted from domestic shrimp shells was 0.329%, against an 8.5% solubility of chitosan extracted from imported shells. The solubility of locally extracted chitosan is, therefore, comparatively weaker. The water-binding capacity (WBC) of chitosan extracted from local and imported shrimp shells were found to be 485.6% and 483.2%, respectively. The ability of chitosan extracted from local shrimp shells showed a fat binding capacity (FBC) of 611% against a capacity of 764% of imported chitosan. Although this study succeeded in extracting chitin and chitosan from local and imported shrimp shells using chemical treatment, chitosan production was significantly poor, compared to previous studies.

Keywords: Fat binding capacity, Fresh shrimp, Physiochemical properties, Solubility of chitosan

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الخصائص الفيزيوكيميائية للكيتوزان المستخلص من قشور الجمبري المصطادة على طول الساحل الليبي

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الملخص

تهدف هذه الدراسة إلى تقييم إمكانية استخلاص الكيتوزان المستخرج من الجمبري المستخرج من الساحل الليبي ، لدراسة بعض خواصه الفيزيوكيميائية ، وتصنيفه مقارنة بتلك المستخرجة من الجمبري المستورد المتوفر في السوق الليبي بطرابلس. تمت دراسة الخصائص الفيزيائية والكيميائية للكيتوزان بما في ذلك قدرة ربط الدهون (FBC) ، وسعة ربط الماء (WBC) ، والإنتاجية ، والقابلية للذوبان ، ومحتوى الرطوبة ، والرماد ، والبروتين ، والدهون ، ودرجة نزع الأسيتيل من الكيتوزان. أظهرت النتائج أن المحتوى الرطوبي كان 28.025٪ في قشور الجمبري غير المعالجة. احتوت عينة الكيتين على 10.166٪ رطوبة مقارنة بـ 2.677٪ فقط في عينة الكيتوزان. احتوت قشور الجمبري الطازج على 4.70٪ رماد بينما كان محتوى الرماد في العينة المستوردة 53.62٪. سجلت عينات الكيتين و الكيتوزان المستخرجة 0.45٪ و 2.05٪ محتوى رماد على التوالي. قشور الجمبري المحلية تحتوي على 28٪ من البروتين. تم العثور على 4.16٪ بروتين في مستخلص الكيتين من قشور الجمبري التي تم جمعها محليًا ، مقابل 0.098٪ في مستخلصات قشور الجمبري المستوردة. كما احتوت عينات قشور الجمبري المحلية على 0.668٪ دهون مقابل 5.86٪ دهون في العينات المستوردة. احتوى الكيتين و الكيتوزان المستخلصان من قشور الجمبري المحلية على 0.74٪ و 2.50٪ دهون على التوالي. الشيتوزان المستخرج من قشور الجمبري المستورد يحتوي على دهون 7.307٪. بلغ إنتاج الجمبري الطازج 14.04٪ من الكيتوزانو 0.329٪ مستخلص الكيتوزان من قشور الجمبري المحلي مقابل 8.5٪ مستخلصات قشور الجمبري المستورد. تم العثور على

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درجة نزع الأسيتيل 9.944 في الكيتوزان المستخرج من كل من المحلي والمستورد. كانت قابلية ذوبان الكيتوزان المستخرج من قشور الجمبري المحلي 0.329٪ ، مقابل 8.5٪ ذوبان الكيتوزان المستخرج من قشور مستوردة. وبالتالي ، فإن قابلية ذوبان الكيتوزان المستخرج محليًا أضعف نسبيًا. وجد أن قدرة الارتباط بالماء (WBC) للكيتوزان المستخرج من قشور الجمبري المحلية والمستوردة بلغت 485.6٪ و 483.2٪ على التوالي. أظهرت قدرة الكيتوزان المستخرج من قشور الجمبري المحلية قدرة ربط الدهون (FBC) بنسبة 611٪ مقابل قدرة 764٪ من الشيتوزان المستورد. بالرغم من نجاح هذه الدراسة في استخلاص الكيتين و الكيتوزان من قشور الجمبري المحلية والمستوردة باستخدام المعالجة الكيميائية ، إلا أن إنتاج الكيتوزان كان ضعيفاً بشكل ملحوظ مقارنة بالدراسات السابقة.

الكلمات المفتاحية: القدرة على ربط الدهون، الجمبري الطازج، الخواص الفيزيائية والكيميائية، ذوبان الكيتوزان.

Introduction

Chitosan is an amino polysaccharide biopolymer primarily derived from chitin. It consists of two mono saccharides, GlcNAc and D–glucosamine (GlcN), linked together by β – (1□ 4) gylcosidic bonds (see figure 1; Raafat & Sahl, 2009). It is a versatile nontoxic, biodegradable film–forming polymer widely used in food, biomedical and chemical industries (Shahidi et al., 1999) and has been recognized as GRAS (generally regarded as safe) (FDA, 2001) .

Sources of chitosan include crustacean shells, insects, and the cell walls of certain fungi such as mucor rouxxi (Bento et al., 2009). with many unique biological properties, chitosan exhibits a wide spectrum of antimicrobial activity against both gram–positive and gram–negative bacteria as well as fungi (Dutta et al., 2009; Helander et al., 2001; Ziani et al., 2009) and generally has a stronger impact on gram–negative than on gram–positive organisms (Chung & Chen, 2008; Devlieghere et al., 2004). Chitosan is a natural bactericide (Chung & Chen, 2008). The presence of the positively charged amino groups in C2 position (below pH 6) is suggested to provide major functional structure (NH_3^+ groups of chitosan

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acetate) expected to interact with the predominantly negatively charged cell surface of bacteria (Holappa et al., 2006; Liu et al., 2004; Nikaido, 1996) .

Through binding and disrupting the normal functions of cell membranes, chitosan promotes the leakage of intracellular components (including enzymes and nucleotides, Chung & Chen, 2008) through damage of the cell membrane (Liu et al., 2004) and cell wall (Chung et al., 2004) and also by inhibiting the transport of nutrients into the cells (Chen & Chou, 2005). However, the exact mechanism of the antimicrobial effect of chitosan is still inconclusive. Chung and Chen (2008) suggest a two–step sequential mechanism of the antimicrobial activity of chitosan on an E. coli cell, initially separating the cell wall from its cell membrane, and then destroying the cell membrane .

The production of Chitosan from shrimp waste involves three chemical treatments, namely demineralization, deproteinization and deacetylation (Pranee et al., 2002). Several conditions, including the age of shrimps, the concentration of chemical during the extraction process, the soaking time, and the sequence of the treatments, could affect the quality of the produced chitosan. The aggressive nature of the chemical treatments could damage the final product, as well as corrode the equipment and yield environmentally harmful waste in large quantities (Pranee et al., 2002) .

The physical and chemical properties of chitin are related to its origin. The results of the study (Carlos et al. 2016) showed that the properties of chitin differ in different body parts (carapace, abdomen, legs and tails)

There are no previous studies on the possibility of extracting chitosan from shrimp shells caught from the Libyan beach. This study was conducted with the aim of assessing the possibility of extracting chitosan and studying some of its physiochemical properties and its classification, in comparison to that extracted from imported shrimp available in the local market.

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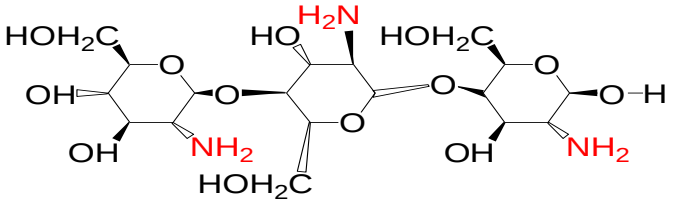

Figure 1. Polymer of B–(1–4)–D–Glucosamine Units

Materials and methods

Sample Collection and Preparation

Fresh shrimp was obtained from a Libyan Company for the manufacture and packing of fish in city Komes, Libya. Imported shrimp, was obtained from a Market in Tripoli. Packing in plastic bags and storing at -20° C. Before and during transportation to the laboratory. Shells were scraped free of loose tissue from the shrimp in the laboratory. The collected shrimp wastes were then cleaning and washing with cold water several times to remove any insoluble material on the shell. Drying at temp. 65 C/6 h. Grinding into 2-4 mm in size. Putting in brown bottle and storing at room temp (Shrimp Shell powders).

Extraction of Chitin and chitosan

Deproteination process

Samples of raw shrimp shell waste were added with 2.0 M NaOH in the ratio 1:16 (w/v) heating at 100° C for 1 hr, with pH ranged from (11-13). After that, the solution was filtered and the samples were washed with distilled water until neutral pH was achieved (pH6.5-8.0). Water from the samples was removed before performing the demineralization process by dewatering at 65 °C for 6 h.

Demineralization process

Samples from deproteination process were soaked in 2.0 N HCl in the ratio 1:16 (w/v) and allowed to stand for 1 h. With pH value

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ranged pH 1.0-2.5 at room temperature (~25oC). After that, the solution was filtered and the samples were washed with distilled water until neutral pH was achieved (pH6.5-8.0). Dewatering at 65 °C for 6 h. The dried sample is now known as chitin. Keeping in brown bottle and storing at room temperature.

Deacetylation process

Deacetylation process was done with soaking in 50% NaoH (1g chitin/15 ml NaoH) at 100°C for 2 h. Washing with tap water until neutral (pH 6.5-8.0) and dewatering at 65 °C for 6 h.

Determination of chemical composition

The chemical composition is based on examining moisture, crude fat and total ash content in chitosan. Experiments were conducted according to Association of Official Analytical Chemists (AOAC, 2007).

Chitosan Yield

The chitosan yield (%) was calculated as the dry weight of the chitosan flakes relative to the wet weight of Tripoli's shrimp shells. Chitosan extraction yield (%)= Dried extracted chitosan weight (g)x100/Tripoli shrimp shells(g).

Solubility in acid solution

One gram of chitosan obtained from the deacetylation process was dissolved in 100mL of 1% acetic acid solution and stirred by magnetic stirrer until a homogeneous solution was obtained. The chitosan acidic solution was then filtered using a vacuum pump. The procedure was repeated three times, the percentage of the solubility was calculated using the following:

Insoluble (%)= Insoluble (g)x100/sample weight (g)

Insoluble (g)= final weight if filter paper(g)-initial weight of filter paper(g) (g)×100/sample weight (g)

Solubility (%) = 100 - % insoluble.

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Degree of Deacetylation

Dried chitosan (0.2 g) was dissolved in 20 cm3 0.1 M hydrochloric acid and 25 cm3 deionized water. After 30 minutes continuous stirring, next portion of deionized water (25 cm3) was added and stirring continued for 30 minutes. When chitosan was completely dissolved, solution was titrated with a 0.1 mol·dm-3 sodium hydroxide solution using automatic burette (0.01 cm3 accuracy). Degree of deacetylation (DA) of chitosan was calculated using formula:

$$DA\ (\%)=V2-V1\times 2.03/M+0.0042$$

where: m is weight of sample, V1, V2 are volumes of 0.1 mol·dm-3 sodium hydroxide solution corresponding to the deflection points, 2.03 is the coefficient resulting from the molecular weight of chitin monomer unit, and 0.0042 is the coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

Data analysis

Data were analysed through IBM SPSS Statistics version 16.0 for Windows.

Results and discussion

Although shrimp shells mainly contain chitin, they also have proteins and minerals contents. Protein is removed by deproteinization and carbon and other mineral are removed by demineralization.

Moisture

Table (1) shows the results of moisture content in both imported and local fresh shrimp shells, as well as in extracted chitin and chitosan samples. The moisture content was 28.025% in the untreated shells, 10.166% in the chitin sample, and 2.677% in the chitosan sample.

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These results are lower than the 69.30% content reported in Hossain and Iqbal (2014). The low moisture content found in this study may be due to heterogeneity of the sample, the source of chitin, and/or the drying conditions.

Table 1. Characteristics of Fresh shrimp and chitosan

Sample	Moisture content (%)		Fat content (%)		Protein content (%)		Ash content (%)	
	Fresh	Imported	Fresh	Imported	Fresh	Imported	Fresh	Imported
Fresh shrimp	28.025	0.42591	0.668	5.86	28	-	4.7044	53.62
Chitosan	2.677	6.28	2.50	7.307		0.09756	2.05	0.6894

Ash content

The ash ratio is an indicator of the effectiveness of the demineralization process, aiming to remove calcium carbonate normally found in large quantities in shrimp shells. Fresh local shrimp shells were found to contain 4.70 % of ash. The percentage of ash in the imported sample was 53.62%. The extracted chitin and chitosan samples recorded 0.45% and 2.05% ash content, respectively. Ash content is due to the presence of calcium carbonate. The latter should be less than 1% for a higher quality chitosan. The presence of some of the ash residues in the extracted chitosan may affect its solubility, and thus the reduction of viscosity and some important properties of the extracted chitosan (La et al., 1995).

Protein content

Table (2) shows that the percentage protein content in local shrimp shells is 28%. The pigmentation method estimated a protein content of 4.16% in chitin extract from local shrimp shells and 0.09756% in chitosan extracted from imported shells.

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Fat content

The local shrimp shell sample contained a fat ratio of 0.668 while imported one contained 5.86% fat. Chitin and chitosan extracted from local shrimp shell had a 0.74% and 2.50% fat contents, respectively. Chitosan extracted from imported shrimp shells contained 7.307% fat.

Yield

The yield of shrimp of chitin and Chitosan is as shown in Table 1. It was found that the yield of chitosan from fresh shrimp is 14.04 times less than that reported by other studies, e.g. 12.03% and 15% found by Varun al. (2017) and Abdulkarim et al. (2013), respectively. Moreover, it was also found that repeating the deproteinization and demineralization processes twice helped increase the yield of chitin extracted from shrimp shells. Performing the deacetylation procedure on the final chitin at room temperature for a 3-days period led to higher yield of chitosan by 46%. The results show that a 4% hydroxide sodium concentration is appropriate to remove protein at 25 °C temperature. During deproteination, lime removal with 4 % HCL acid for two hours was enough to produce chitin with lower ash content. Generally, the yield and quality of the extracted chitosan depends on several factors including the conditions of chemical extraction, the concentration used chemicals, soaking time, and the sequence treatments to remove alcohol, lime, and deacetylation. The yield of chitosan could be reduced by several factors, including the deproteinization process to extract chitosan from polymer chitosan, and the mass/sample weight loss leading to an increased removal of acetyl groups from the polymer during deacetylation. Moreover, some chitosan molecules could be loss during washing. The decrease in chitosan yield may also be due to the age and size of the source shrimp. Kumar et al. (2017) showed that factors such as the

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hydrogen ion concentration (pH), time, temperature, solids-to-acids ratio, could improve the process of chitosan extraction. They also reported that, in order to increase the extracted chitin and reduce the level of impurities to an acceptable level, acids like lactic and/or acetic acids could be used during the demineralization process as alternative to stronger acids. Deproteinization could be achieved using alkalis or biologically. These are innovative techniques to extract chitin using microorganisms which work to degrade proteins, fungi, and/or enzymes, resulting in the production of oligomers with optimal polymerization. Such technique could be used in several other applications (Kumar et al., 2017).

Table 2. Yield of fresh and imported shrimp

Chitosan	Yield
Fresh shrimp	14.04
Imported shrimp	19.00

Solubility

The solubility of chitosan is one of the most important measurements of its quality. The higher the solubility, the higher the quality of chitosan. In this study, the solubility of the extracted chitosan was estimated after washing and drying the extracted chitosan and dissolving it in a 1% acetic acid solution, following prior literature. There is a number of critical factors which affect the solubility of chitosan, among which are temperature, deacetylation time, alkaline concentration, previous chemical processes of chitin extraction, chitin concentration in the alkaline solution, and particles' sizes. Yet, the degree of solubility is related to the degree of deacetylation as it is estimated that deacetylation should be at least 85% complete in order to achieve the desired solubility. In this study, the solubility ranged as shown in Table (3), where the solubility of chitosan extracted from local shrimp shells was 32.9,

while it was 8.5% for chitosan extracted from imported shell, a very weak solubility. Austin (1981) noted that low solubility values could be due to remaining proteins and acetyls owing to an incomplete deproteinization. Others like Lertsutthiwong et al. (2002) argued that the lack solubility of the extracted chitosan is due to an inefficient deacetylation.

Table 3. Percentage of soluble and non-soluble in Fresh and imported shrimp

Fresh shrimp		Imported shrimp	
Non-soluble	Soluble	Non-soluble	**Soluble**
0.167	32.9	91.5	**8.5**

The degree of deacetylation

The degree of deacetylation is an important factor affecting the solubility of chitin and chitosan (Arbia et al., 2015). In the present study Table (4), this measure was found to be 9.944 in the chitosan extracted from both local and imported shells.

Table 4. Degree of deacetylation of chitosan

Type	Degree of Deacetylation% (DD)
Fresh shrimp	9.944
Importedshrimp	9.954

Water binding capacity (WBC)

The water binding capacity (WBC) of the chitosan extracted from local shrimp shells was estimated at 485.6%, and 483.2% for chitosan extracted from imported ones. Rout (2001) mentioned that sequence of three chemical treatments influences the chitosan's capacity to bind water.

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Fat binding capacity (FBC)

The fat binding capacity (FBC) of shrimp shells is estimated using sunflower oil. The chitosan extracted from local shrimp shells exhibited a capacity of 611 % against a capacity of 764% of imported-shells extracted chitosan Table (5). Rout (2001) argued the impact of the sequence of demineralization and deproteinization on the chitosan's capacity to bind fat.

Table 5. Water and fat binding capacity of chitosan

Chitosan	Water(%)	Oil (%)
Fresh shrimp	485.6	611
Importedshrimp	483.2	764

Conclusion

Despite the various sequences and methods of chitosan extraction applied and the diversity of the chitosan sources, prior literature fails to identify a single optimal sequence/method of chitosan extraction. Generally, all methods suggested are expensive and time consuming, and produce low yields. Therefore, microbiological techniques for chitosan extraction seem more efficient and cost-effective. The results of this study show that shrimp shells can be effectively used in the extraction of chitin and, thus, chitosan. This study successfully extracted chitin and chitosan from local and imported shrimp shells using chemical treatment. However, the yield of the chitosan was very weak compared to previous studies reports.

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