Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(2): 123 – 131 (2019)
www.ejabf.journals.ekb.eg

Effect of some extraction techniques on properties and economic of chitosan obtained from shrimp shells waste

Ibrahim, M. A. [1]**; Mostafa, S. M.** [2] **and Ibrahim, S. M.** [1]

1- Fish Processing Technology Lab.,
2- Economic and Fish Statistics Lab.,
National Institute of Oceanography and Fisheries, Cairo, Egypt.
Corresponding Author: Saber_mostafa0@yahoo.com

ARTICLE INFO

Article History:
Received: March 30, 2019
Accepted: April 29, 2019
Online: May 18, 2019

Keywords:
Chitosan
Extraction techniques
Antioxidant
Shrimp wastes
Economic evaluation

ABSTRACT

The present study was planned to investigate the effect of three techniques; traditional, microwave and autoclave on properties and economic evaluation for chitosan obtained from shrimp shell waste. Results showed that sun dried Suez-shrimp wastes composed (wet weight) 5.28% moisture, 7.09% (total nitrogen), 7.77% lipid and 36.15ash content. Microwave technique improved the water binding capacity (WBC) property and antioxidant properties whereas traditional technique improved the oil binding capacity (OBC) property. Also, there are variation in degree of deacetylation (DDA) values; 95.5% for autoclaved, 93.0 for microwaved and 88.5 for traditional chitosan samples. Economically, the traditional method is the best economic methods. Also, it provides about 45.7 thousand pounds/ton and it reduces the chances of imported chitosan and modifies of the Egyptian trade balance. In conclusion, this study recommends that shrimp shells waste should be utilized to produce chitosan as an economic and value added product and to encourage local production and reduce imported chitosan.

INTRODUCTION

Seafood by-products play an important role in production of high valuable products. Shrimp waste could be used as a source of protein and prebiotic in feedstuffs (Khempaka *et al.*, 2011). Many techniques; acids and alkali, enzymes, ensiling, bioremediation were applied to obtain of chitosan from its natural resources (Jag Pal *et al.*, 2014). Chitosan is the deacetylated product of chitin and is linear, polycationic and heteropolysaccharide mainly composed of β-1,4-2-deoxy-2-amino-D-glucopyranose and β-1,4-2-deoxy-2-acetaamido-D-glucpyranose glycol-sideic linkages (Costa *et al.*, 2012). In case of chitin, the content of N-acetyl-D-glucosamine in polymer is higher than the biopolymer while in case of chitosan; the glucosamine content is higher than the biopolymer (Ramirez *et al.*, 2010). Major reactive functional groups; amino/acetamide group and primary and secondary hydroxyl group are found at C-2, C-3, and C-6 positions, respectively where the amino group of chitosan properties is varied (Kumirska *et al.*, 2011). Degree of deacetylation (DDA) of chitosan widely ranged 75% and 95% and molecular weight (MW) ranged 50 and 6000 KDa. And, the biological properties such as antioxidant of chitosan and its oligomers are varied (Raafat and Sahl, 2009; Younes and Rinaudo, 2015).

Moreover, the solubility of chitosan depends on distribution of N-acetyl and free amino groups where it is soluble in dilute organic acid like acetic acid, formic acid, etc., and high viscous solution, due to protonation of amino groups occurs at pH<6.0 and it is insoluble in water, basic pH solutions and organic solvents, due to basic pH protonation does not occur and pH value of ~6.5 leads to solubility and insolubility transition (Mano *et al.*, 2008; Padmanabhan and Nair, 2016; Varun *et al.*, 2017). Therefore, the current work was designed to investigate the effect of three techniques; traditional, microwave and autoclave on characterization, antioxidant effects and economy for chitosan produced from shrimp shell waste.

MATERIALS AND METHODS

Shrimp shell waste

5 kg of sun–dried shrimp shell waste was obtained from Al-Fayoum Fish market during December, 2018. They were transferred to the Fish Processing and Technology Lab., National Institute of Oceanography and Fisheries (NIOF) within 2h. After that, they were sorted to estimate the percentage of impurities, dried at 50°C for overnight, grinded, sieved using 50mesh and subjected to different three techniques; traditional, microwave and autoclave to obtain chitosan.

Extraction of chitin and chitosan

To obtain purified chitin; dried shrimp waste was demineralized by 2%HCl (10:1 v\w at 30°C) and proteinized by 4% NaOH (10:1 v\w at 90°C for 12 h), centrifuged (4000 rpm for 15min) and washed twice with distilled water in each phase and dried at 40°C for overnight (Synowiecki, 1997). Besides, to obtain chitosan; chitin was deacetlated using three techniques; traditional by 50%NaOH (10:1 v\w at 100°C for 8-10 h) (Trung *et al.*, 2006), Sumsung oven Microwave by 50% NaOH (10:1 v\w at 1400 watts for 10 min) (Sahu et al., 2009) and Autoclave by 50% NaOH (10:1 v\w at 15psi and 121°C for 20) (Youn *et al.*, 2007) were used. Deacetylated chitosan was washed and dried at 40°C for overnight. Figs. (1&2) show steps used and final chitosan products.

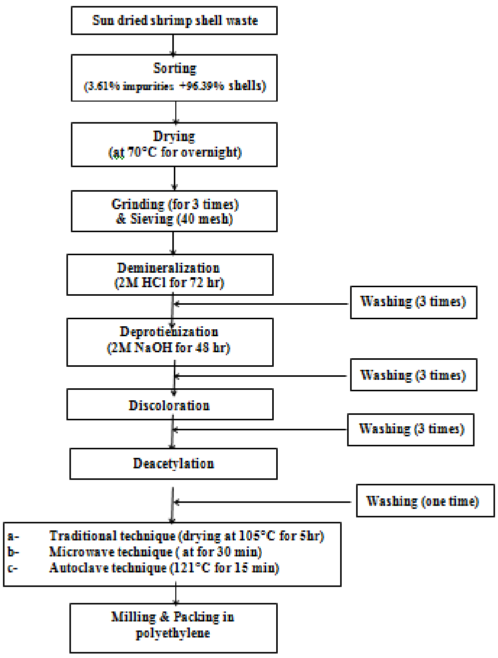

Fig. 1: Flowchart of chitosan extracted by different techniques.

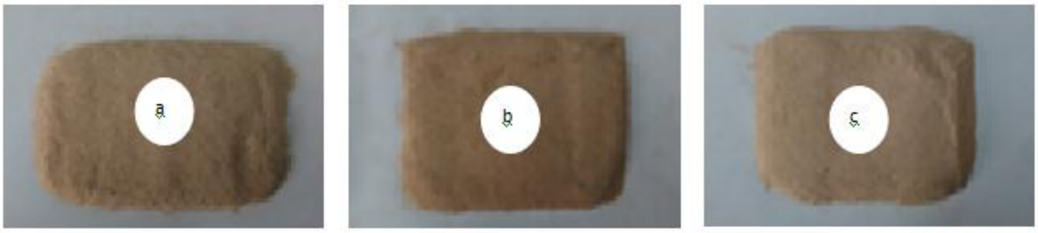

Fig. 2: Chitosan extracted from shrimp shells by different techniques; a- traditional technique, b- microwave technique and c- by autoclave technique.

Analytical methods

Chemical composition; moisture, crude protein, lipid, and ash content of shrimp waste and chitosan were determined according to the methods described by AOAC (2005). Physic-chemical properties; water binding capacity (WBC, g water\g chitosan) and oil binding capacity (OBC, g oil\g chitosan) (Wang and Kinsella, 1976) and solubility (Fernandez-Kim, 1976), degree of deacetylation (DDA) (Qin *et al.*, 2004) and molecular weight (MW) (No *et al.*, 2003) were determined. Antioxidant properties; scavenging ability on 1, 1-diphenyl 1-2-pieryl-hydrazyl radicals (DPPH) (Shimada *et al.*, 1992) and antioxidant activity (Lingnert *et al.*, 1979) at concentrations (0.5 and 1.0 mg\ml) were determined. Results (n=3) were expressed as mean and standard deviation (Mean ± SD).

RESULTS AND DISCUSSION

Chemical composition of chitosan

Table (1) shows the proximate analysis of raw shrimp shells waste and chitosan extracted using three chemically techniques; traditional, microwave and autoclave and compared with commercial chitosan. Raw shells waste contained 5.28% moisture, 7.09% total nitrogen (TN), 7.77% lipid and 36.15% ash content. Mahdy Samer (2013) reported that shrimp wastes contained 45.65%, 32.46% and 32.77 of moisture, ash and protein content, respectively. On the other hand, raw crayfish shell waste contained 63.80% moisture, 9.24% protein, 3.25% fat, 11.07 % ash and 12.64% carbohydrate content (Ibrahim, 2017).

Table 1: Chemical composition of raw shrimp shells waste and chitosan.

Extraction technique	Chemical composition of chitosan;				% Yield
	% Moisture	% Total nitrogen (T.N)	% Lipid	% Ash	
Raw shrimp shells waste	**5.28±0.16**	**7.09±0.13**	**7.77±0.30**	**36.15±**	**-**
Traditional chitosan	**1.59±0.05**	**4.02±0.15**	**-**	**4.09±**	**31.8**
Microwaved chitosan	**0.86±0.04**	**3.65±0.02**	**-**	**3.73±**	**31.4**
Autoclaved chitosan	**1.18±0.06**	**2.50±0.19**	**-**	**3.16±**	**29.4**
Commercial chitosan	**2.71±0.02**	**10.78±0.00**	**-**	**5.08±0.00**	**-**

- **: Not determined.**

Concerning proximate analysis of chitosan, these values were markedly decreased where moisture content ranged 0.86-1.59%, 2.50-4.02% T.N, and 3.16-4.09% lipid compared with commercial chitosan. Li (1992) showed that the commercial chitosan products contained less than 10% moisture. Hossain and Iqbal (2014) found that the shrimp shell chitosan samples had moisture ranged 7.69-8.25%. Besides, our results are more (exception moisture content in microwaved chitosan) than those findings by Ghannam Hala *et al.*, (2016), who showed that the shrimp chitosan contained moisture 0.8% and ash 0.5%. This variation in chemical composition of chitosan is due to the extraction technique and size of particles (Mahdy Samer *et al.*, 2013). Also, the data obtained showed that chitosan extracted from shrimp waste is considered a low quality based on No *et al.,* (2002), who mentioned that high quality chitosan should be contained < 1% ash content.

From the same table, the yield of chitosan obtained was ranged 29.4-31.8%. This data is less than the range (33.77-46.00%) of chitosan obtained from shrimp shells (Puvvada *et al.*, 2012; Mahdy Samer, 2013 and Premasudha *et al.,* 2015) and it is higher than reported by Hossain and Iqbal (2014), they found that the yield of purified chitosan was15.4% and Ghannam Hala *et al.,* (2016), who showed that the yield of shrimp chitosan was 18.7%. However, it is in agreement with Ibrahim (2017), who found that the yield chitosan obtained from crayfish shell waste was 30.37%. Generally, the variation in chemical composition of crustacean shells waste and yield of chitosan are due to several factors; resource of raw material, moisture content, deproteinization and demineralization, concentration of acid and alkali, time of reaction …etc.

Physico-chemical properties of chitosan

Physico-chemical properties of chitosan extracted from shrimp shells waste are presented in Table (2). The values of water binding capacity (WHC) were 412.45%, 454.45% and 631.24% of autoclaved, microwaved and traditional chitosan samples, respectively. All of these values were higher than commercial chitosan (307.67%). Concerning fat binding capacity (FBC), its values recorded 393.75% in microwaved, 466.90% in autoclaved and 587.76% in traditional chitosan samples. Also, FBC is taken the same trend certain commercial sample. Also, WBC and FBC values of chitosan samples obtained by different techniques were higher than that commercial chitosan.

Table 2: Physico-chemical properties of chitosan extracted from shrimp shells waste.

Extraction technique	Physico-chemical properties of chitosan;				
	*WBC%	**FBC%	Color intensity	***DDA%	****MW (KDa)
Traditional chitosan	631.24 ±3.21	587.76 ±5.10	0.682 ±0.08	88.5±0.70	21.1
Microwaved chitosan	454.45 ±2.10	393.75 ±7.80	0.675 ±0.01	93.0±1.40	18.8
Autoclaved chitosan	412.45 ±2.07	466.90 ±2.38	0.703 ±0.01	95.5±0.70	11.4
Commercial chitosan	307.67	213.04	-	88.0	-

*WBC: Water binding capacity. **OBC: Oil binding capacity. ***DDA: Degree of deacetylation. ****MW: Molecular weight.

This data of WBC is higher (230-440%) than those reported by Knorr (1982) and 1.32% for shrimp chitosan as findings by Ghannam Hala *et al.*, (2016). However, it is lower (458-805%) than those findings by Young *et al.,* (1998) and (581-1150%) Rout (2001). Results of FBC are the highest than those (170-315%) reported by Knorr (1982) and (314-535%), Young *et al.*, (1998) and lower than those (706%) findings by Rout (2001). In addition to, these results are in agreement with those reported by Hossain and Iqbal (2014), they found that purified chitosan extracted from shrimp waste was characterized for WBC (537.29%) and OBC (427.98%). OBC of traditional shrimp chitosan (587%) is higher than those reported by Ghannam Hala *et al.,* (2016), who found that the FBC of shrimp chitosan was 539%. Based on these results, it could be found that microwave technique improved the WBC property followed by traditional and autoclave techniques. However, traditional technique improved the FBC property followed by autoclave and microwave techniques. From the same table (2), the values of color intensity were similar in all investigated chitosan; 0.675, 0.682 and 0.702 for microwave, traditional and autoclave techniques, respectively.

With regard to DDA, there are variation in DDA values; 95.5% for autoclaved, 93.0 for microwaved and 88.5 for traditional chitosan samples. These results are higher than that ranges (45.5- 81.24%) of DDA of shrimp chitosan samples (Hossain and Iqbal, 2014) and it within (56- 99%) as reported by No and Meyers (1995) and Gannam Hala *et al.*, (2016). From the same table, the MW values recorded 21.1, 18.8, and 11.4 KDa of traditional, microwaved and autoclaved chitosan samples, respectively. These results are lower than those (1050 KDa) as showed by Hossain and Iqbal (2014). High temperature, concentration of alkali, reaction time, previous treatment of the chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may influence on DDA and MW of chitosan (Li *et al*., 1992 and Roberts, 1997).

Antioxidant properties

Antioxidant properties (DPPH and conjugated Diene at levels of 0.5 and 1%) of chitosan extracted from shrimp shells waste are presented in Table (3). All values of DPPH and conjugated Diene were higher in chitosan prepared by different techniques than commercial one. Also, they were taken trends as the following order: autoclaved > microwaved > traditional chitosan at 0.5% and 1.0%. High effective of DPPH and conjugated Diene are due to low MW (Anraku *et al.*, 2008), DDA (Park *et al.*, 2004), and content of hydroxyl and amino groups and different substituting groups (Xie *et al.*, 2001).

Table 3: Antioxidant properties of chitosan extracted from shrimp shells waste.

Item	Antioxidant properties of chitosan prepared by different techniques;							
	Traditional		Microwaved		Autoclaved		Commercial	
	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%
DPPH	**41.86±1.42**	**47.67±0.67**	**42.72±0.62**	**58.53±0.07**	**45.87±0.09**	**59.16±0.35**	**26.82**	**33.92**
Conjugated Diene	**29.18±1.29**	**32.50±0.36**	**36.75±0.45**	**41.26±0.40**	**39.01±0.26**	**46.50±1.04**	**22.41**	**34.21**

DPPH: 1, 1-diphenyl 1-2-pierylhydrazyl radicals

Moreover, variation in percentage of DPPH and conjugated Diene increased with increasing chitosan concentration as mentioned by Ghannam Hala *et al.,* (2016) and Ibrahim (2017) and it is due to different techniques conditions.

Economic evaluation

FAO (2014) pointed that by products of crustacean (shrimp, crab, lobster…etc.) ranged from 6 to 8 million metric tons, about 1.5 million metric tons produced from Asia east south. GAFRD (2016) reported the Egyptian total local production of shrimp recorded 12.3 thousands metric tons; 7.2, 1.4, 3.6 and 0.1 from Mediterranean Sea, Red Sea, Lakes Aquaculture, respectively in addition to imported quantities. Also, unpublished data as set by Ministry of Industry and Trade, Egypt (2017) mentioned that shrimp wastes estimated 8.3 thousands metric tons per year. Total shrimp production from the Red Sea during the period of 2000-2016 is shown in Fig. (1). Total shrimp production ranged from 501 to 2655 tons during the periods of 2000 – 2016. The highest catch (2655 tons) of shrimp was found during the period of 2000 while the lowest was 501 tons during the period of 2012. Among periods (2000 – 2016), total shrimp catch was fluctuated. A decrease in shrimp catch was found till

2013. However, it was increased during the period of 2014 to record 1946 tons and decreased (1146 tons) during the period of 2016. In general, crustacean shells waste represent about 80% of total catch as reviewed by Ibrahim (2017). On the other side, these wastes are representing as a risk source for our environmental conditions.

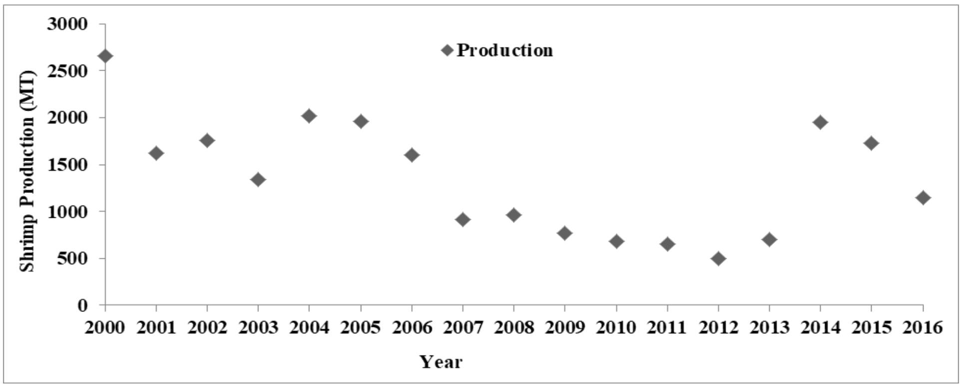

Fig. 3: Total shrimp production from the Red Sea during the period of 2000- 2016.
Source: GAFRD (2016).

Table (4) exhibits the economic evaluation of chitosan extracted from shrimp shells waste by different techniques. All items; raw material and transportation, chemicals, water, electricity and other were estimated based on marketing prices during this work. Although the methods used in this study to produce chitosan but the traditional method which is the best economic methods where 3.1 kg of shrimp shells could be produced one kg chitosan with stability of other items. Also, the economic gain was about 45.65 Egyp. Pounds/kg chitosan, and thus the production of tons of chitosan from shrimp shells provides about 45.7 thousand pounds/ton. In addition, it will be reduced the chances of imported chitosan and modifies of the Egyptian trade balance.

Table 4: Economic evaluation of chitosan extracted from shrimp shells waste.

Item	Unit	Price (Egy. Pound) for chitosan extracted by techniques;		
		Traditional	Microwave	Autoclave
Raw shrimp shells waste and transportation	**1 Kg**	**2.00**	**2.00**	**2.00**
NaOH	**1 Kg**	**108.00**	**108.00**	**108.00**
HCl	**500 ml**	**20.00**	**20.00**	**20.00**
Ethanol	**500 ml**	**17.00**	**17.00**	**17.00**
Water	**10 Lt**	**30.00**	**30.00**	**30.00**
Electricity	**KW**	**5.28**	**0.46**	**0.23**
Other	**-**	**1.00**	**1.00**	**1.00**
Yield	**Gm.**	**318**	**314**	**294**
Total cost		**183.28**	**178.46**	**178.23**
Commercial chitosan for the same quantity	**-**	**198.10**	**195.6**	**183.2**
Quantity of raw material for 1 kg chitosan	**Kg**	**3.1**	**3.2**	**3.4**
Chitosan	**Kg**	**576.35**	**568.34**	**606.22**
Commercial chitosan	**Kg**	**623**	**623**	**623**
Gain	**Egy. pound**	**45.65**	**53.66**	**16.78**

CONCLUSION

The variation in chemical composition of crustacean shells waste and yield of chitosan are due to several factors; resource of raw material, moisture content, deproteinization and demineralization, concentration of acid and alkali, time of reaction …etc. Microwave technique improved the WBC property and antioxidant properties whereas traditional technique improved the OBC property. Economically, the traditional method is the best economic methods. Also, it provides about 45.7 thousand pounds/ton and it reduces the chances of imported chitosan and modifies of the Egyptian trade balance. Based on this study, shrimp shells waste should be utilized to produce chitosan as an economic and value added product and to encourage local production and stop imported chitosan.

REFERENCES

Anraku, M.; Kabashima, M. and Namura, H. (2008). Antioxidant protection of human serum albumin by chitosan. Inter. J. Biol. Macromol., 43: 159-164.

AOAC. 2005. Official Methods of Analysis. 15th Edition. Association of Official Analytical Chemists. Washington, D.C.

Costa, E.M.; Silva, S.; Pina, C.; Tavaria, F.K. and Pintado, M.M. (2012). Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens. Anaerobe, *18(3): 305-309.*

FAO, (2014). Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture.

Fernandez–Kim, S.O. (2004). Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols. Dissertation, Louisiana State University: Baton Rouge, La, USA.

Fernandez-Kim, S.O. (2004). Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols.

GAFRD (2016). General Authority of Fish Resources Development. Fish Statics Year Book. Ministry of agriculture, Egypt.

Ghannam Hala E.; Talab, A. S.; Natalia V. Dolganova; Hussein, A.M.S. and Nahed M. Abdelmaguid (2016). Characterization of chitosan extracted from different crustacean shell wastes. J. Applied Science, 10 (16): 454-461.

Hossain, M. S. and Iqbal, A. (2014). Production and characterization of chitosan from shrimp waste. J. Bangladesh Agril. Univ. 12(1): 153–160.

Ibrahim, M.A. (2017). Utilization of crayfish in some fishery products. Ph.D. Thesis, Faculty of Agriculture, Ain Shams Univ.

Jag Pal; Hari Om Verma; Vijay Kumar Munka; Satyendrakumarmaurya; Deepayan Jameela; Misra, A. S R and Jayakrishnan, A. (1994). Cross-linked chitosan microspheres as carriers for prolonged delivery of macromolecular drugs. J. Biomater. Sci. Polym. Ed. 6:621632.

Khempaka, S.; Chitsatchapong, C. and Molee, W. (2011). Effect of chitin and protein constituents in shrimp head meal on growth performance, nutrient digestibility, intestinal microbial populations, volatile fatty acids, and ammonia production in broilers. Poultry Science Association, Inc. J. Appl. Poult. Res. 20:1-11.

Knorr, D. (1982). Functional properties of chitin and chitosan. J. Food Sci. 47:593-595.

Kumirska, J.; Weinhold, M.X.; Thöming, J. and Stepnowski, P. (2011). Biomedical activity of chitin/chitosan based materials—Influence of physicochemical properties apart from molecular weight and degree of N-acetylation. Polymers, 3: 1875-1901.

Li, Q.; Dunn, E.T.; Grandmaison, E.W. and Goosen, M.F. (1992). Applications and properties of chitosan. Journal of Bioactive Compatible Polymers, 7: 370-397.

Lingnert, H.; Vallentin, K. and Eriksson, C.E. (1979). Measurement of antioxidative effect in model system. Journal of Food Processing and Preservation,3:87-104.

Mahdy Samar, M.; El-Kalyoubi, M.H.; Khalaf, M.M. and Abd El-Razik, M.M. (2013). Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique. Annals of Agricultural Science, 58(1): 33–41.

Mano, J.F.; Hugerford, G. and Ribelles, J.L.G. (2008). Bioactive poly (l-lactic acid)-chitosan hybrid scaffolds. Mater. Sci. Eng. C, 28(8): 1356-1365.

Ministry of Industry and Trade, Egypt (2017). Unpublished data, February, 2017.

No, H.K. and Meyers, S.P. (1995). Preparation and characterization of chitin and chitosan-A review. J. Aquatic Food Product Technology.4(2):27-52.

No, H.K.; Cho, I. Y.; Kim, H.R. and Meyers, S.P. (2000). Effective deacetylation of chitin under conditions of 15 psi/121ºC. J.Agric, Food Chem., 48(6):2625-2627.

No, H.K.; Park, N.Y.; Lee, S.H. and Samuel, P.M. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbial, 74: 65-72.

Padmanabhan, A. and Nair, L.S. (2016). Chitosan hydrogels for regenerative engineering. In. Dutta, P.K., editor. Chitin and Chitosan for Regenerative Medicine. Springers, New Delhi. p3-40.

Park, P.J.; Je, J.Y.; Byun, H.G.; Moon, S.H. and Kim, S.K. (2004). Antimicrobial activity of hetero chitosans and their oligosaccharides with different molecular weights. J. Microbiol. Biotechnol., 14: 317-323.

Premasudha, A.P.; Vanathi, P. and Abirami, M. (2015). Extraction and characterization of chitosan from crustacean waste: A Constructive waste management. International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064.

Puvvada, Y.S.; Vankayalapati, S. and Sukhavas, S. (2012). "Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry," International Current Pharmaceutical Journal, 1(9), pp. 258 - 263, 2012.

Qin, C.Q.; Zhou, B.; Zeng, L.; Zhang, Z.; Liu, Y.; Du, Y.M. and Xiao, L. (2004). The physicochemical properties and antitumor activity of cellulose-treated chitosan. Food chem., 84: 107-115, ISSN 0308-8146.

Raafat, D. and Sahl, H.G. (2009). Chitosan and its antimicrobial potential - A critical literature survey. Microb. Biotechnol., 2 (2): 186-201.

Ramirez, M.A.; Rodriguez, A.T.; Alfonso, L. and Peniche, C. (2010). Chitin and its derivatives as biopolymers with potential agricultural applications. Biotechnol. Appl., 27: 270-276.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications.Progress in Polymer Sci., 31: 603-632.

Roberts, G.A.F. (1997). Chitosan production routes and their role in determining the structure and properties of the product. In: Domart, (Eds.) Advance in Chitin Sci., Taiwan, National Taiwan Ocean University, 2: 22-31.

Rout, S.K. (2001). Physicochemical, Functional, and Spectroscopic analysis of crawfish chitin and chitosan as affected by process modification. Dissertation.

Shimada, K.; Fujikawa, K.; Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food chem., 40: 945-948.

Singh, R. and Balange, A. (2005). Characteristics of pink perch *Nemipterus jaaponicus* Surimi at frozen temperature. Journal of Food Processing and Preservation, 29 (1): 75-83.

Singh, S. and Singh, R. P. (2008). In-vitro methods of assay of antioxidants: An overview. Food Reviews International, 24: 392–415.

Synowiecki, J. and Al-Khateeb, N.A., (1997). Mycelia of Mucor rouxii as a source of chitin and chitosan. Food Chem. 60, 605–610.

Trung, T.S; Thein-Han, W.W.; Qui, N.T.; Ng, C.H. and Stevens W.F. (2006). Functional characteristics of shrimp chitosan and its membranes as affected by the degree of deacetylation. Bioresource Technol., 97: 659-663.

Varun,T.K.; Senani, S.; Jayapal, N.; Chikkerur, J.; Roy; S.; Tekulapally, V.B.; Gautam, M. and Kumar, N. (2017). Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect. Veterinary World, EISSN: 2231-0916. Available at ww.veterinary world. org/Vol.10/February-2017/6.

Wang, J.C. and Kinsella, J.E. (1976). Functional properties of novel proteins: Alfalfa leaf protein. J. Food Sci. 41, 286–292.

Xie, W.; Xu, P. and Liu, Q. (2001). Antioxidant activity of water soluble chitosan derivatives. Bioorganic and Medical Chemistry Letters., 11: 1699-1701.

Youn, S.K.; Her, J.H.; Kim, Y.J.; Choi, J.S.; Park, S.M. and Ahn, D.H. (2004). Studies on the improvement of shelf-life in spicy beef meat using chitosan. J. Korean Soc. Food Sci. Nutr., 33(1): 207-11.

Youn, S.K.; Her, J.H.; Kim, Y.J.; Choi, J.S.; Park, S.M. and Ahn, D.H. (2004). Studies on the improvement of shelf- life in spicy beef meat using chitosan. J Korean Soc. Food Sci. Nutr., 33(1): 207-11.

Younes, I. and Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs, 13(3): 1133-1174.*

Young, I.C.; No, H.K. and Meyers, S.P. (1998). Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. J. Agric. Food Chem., 46(9): 3839-3843.