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PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF EXTRACTED GLUCOSAMINE FROM CRUSTACEAN SHELL WASTE

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ABSTRACT : The purpose of present study is to extract glucosamine from shrimp and crab chitosan and assess their proximate composition, functional properties were studied. The analyses were carried out in three treatments namely T_1 (1:10), T_2 (1:20) and T_3 (1:30) with their replicates. 1:10, 1:20 and 1:30 these ratio showed glucosamine with different HCl concentration with extraction time of 50 min, 75 min and 100 min, respectively. The results were summarized the proximate compositions of shrimp Glc and crab Glc were recorded in different treatments *i.e.* T_1, T_2 and T_3; in shrimp Glc, the protein content; 10.57±0.12 %, 10.87±0.09% and 10.47±0.18%, the fat content; 1.7±0.12%, 2.17±0.22% and 2.33±0.15%, the carbohydrate content; 82.31±0.01%, 83.03±0.12% and 81.1±0.06%, the ash content; 4.3±0.06%, 3.59±0.02% and 4.47±0.03% and the moisture content; 0.74±0.05%, 0.78±0.01% and 0.80±0.05% and in crab Glc, the protein content; 10.78±0.04%, 10.97±0.01% and 10.89±0.05%, the fat content; 6.77±0.09%, 5.67±0.19% and 6.2±0.2%, the carbohydrate content; 76.99±1.73%, 78.18±1.19% and 77.43±0.03%, the ash content; 4.03±0.03%, 3.5±0.12% and 3.5±0.03% and the moisture content; 1.13±0.14%, 1.37±0.18% and 1.67±0.12%, respectively. The yield of shrimp Glc and crab Glc were recorded 13.10±0.08% and 14.30±0.17% respectively. The viscosity of shrimp and crab Glc were recorded in the T_1, T_2 and T_3 are 2.8±0.15cP, 3.87±0.15cP & 3.07±0.09cP and 1.43±0.02cP, 1.6±0.01cP & 1.46±0.02cP respectively. The solubility of shrimp and Glc with treatment T_1, T_2 and T_3 are 99.32±0.01%, 99.64±0.02% & 97.63±0.23% and 99.43±0.02%, 99.60±0.01% & 99.46±0.02%, respectively. The emulsifying capacity and stability of shrimp Glc in the treatments were recorded in T_1, T_2 and T_3; 38.4±0.25%, 52.43±0.48% & 45.8±0.7% and 25.5±0.30%, 28.2±0.25% & 26.9±0.11%, respectively. Emulsifying capacity and stability of crab Glc in T_1, T_2 and T_3 were recorded 47.27±0.54%, 53.27±0.64% & 49.27±0.43% and 28.33±0.38%, 29.17±0.33% & 29.57±0.26% respectively. The foaming capacity and stability of shrimp Glc were recorded in T_1, T_2 and T_3 are 10.63±0.23%, 12.64±0.02% & 12.32±0.01% and 9.53±0.14%, 10.6±0.26% & 9.63±0.14% respectively. Foaming capacity and stability of crab Glc were recorded in T_1, T_2 and T_3 are 12.43±0.02%, 12.6±0.01% & 12.46±0.01% and 9.47±0.17%, 10.63±0.21% & 9.73±0.26%, respectively. This study demonstrated that procedure modification or by changing concentration of HCl and extraction time may affect the production and functional properties, antibacterial and antioxidant activity of shrimp Glc and crab Glc. This study revealed that, use of shrimp Glc and crab Glc has a positive impact in the field of medical and cosmetics and helps to reduce environmental and ocean pollution by utilizing the shrimp and crab shell waste.

Key words : Shrimp and crab shell waste, chitin, chitosan, glucosamine and physicochemical properties.

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INTRODUCTION

Glucosamine is a naturally occurring substance; it is derivative of Chitin and Chitosan, which has remarkable uses. As its name represents, it is made by the synthesis of glucose and amino acid glutamine. Also, glucosamine is found in hard shell animals like crab, shrimp and prawn or crustaceans. Currently, industrial waste like shrimp and crab shell waste is major factor affects the ocean life and environment; this waste can be utilized for preparation of glucosamine which may helps to reduce the environmental pollution.

Significance of environmental safety is currently being realized worldwide. Researchers throughout the world are striving hard to implement sustainability goals in every aspect of development. Waste minimization is one of the most important factors to be considered for sustainable development. Fish processing industry generates enormous amount of waste. Globally, around

6-8 million tonnes of shells of shrimp lobster and crabs are generated as waste annually. Approximately 1.5 million tonnes of this is solely contributed by Southeast Asia. Total production of shrimps and crabs reported during year 2018-19 is 4,43,519 (CMFRI Annual report 2018-19). The total production of major species of shrimp was reported 30% and total production of major species of crab was reported 18% out of total crustacean group (FAO, 2020). Therefore, shrimp has more production which creates more shell waste and available easily from processing industry as compared to crab production.

In the present era of Covid-19 pandemic, human bodies subjected to extreme strain. To counter act excessive strains on human bodies, it is advisable to use active pharmaceutical ingredients that would directly participate in the metabolism, having an acto-protective effect without exhausting the body. Glucosamine has such properties. Unsurprisingly glucosamine is accessible in human body, including the cartilage that covers the ends of bones in the joints. When one gets older, the production of glucosamine in body slows down, and then supplements are needed to regenerate it. When taken orally, glucosamine hydrochloride and glucosamine sulphate is absorbed readily into the system and can be traced to cartilage as soon as four hours after consumption.

Glucosamine is a naturally occurring substance; it is derivative of Chitin which has remarkable uses. D-glucosamine ($C_6H_{13}NO_5$) or 2-amino-2-deoxy-D-glucose is an amino sugar (hexamine). As its name represents, it is made by the synthesis of glucose and amino acid glutamine. Also, glucosamine is found in hard shell animals like crab, shrimp and prawn or crustaceans. Extraction of glucosamine was first initiated in 1876 by Georg Ledderhose. He used the process where chitin was hydrolysed with concentrated hydrochloride acid. At 21st century, in the period of 2004-2018, the FDA declared there was not enough evidence for supplement manufacturers to declare that glucosamine was effectual for joint degeneration, treating arthritis or cartilage deterioration. Since then researchers are still working on the properties and system of glucosamine to make the production more efficient. A present study attempt has been made to assess physiochemical properties of Glucosamine extracted from crustacean shell waste and further study and make the process of production of glucosamine robust.

MATERIALS AND METHODS

Collection of shrimp and crab shell waste

Samples (shrimp and crab shell waste) were collected from the local vendor of Mirkarwada jetty, Ratnagiri. Inexact 1000-1300gram (weight with viscera and tomalley) of each sample was collected. Then washed with running tap water and dried in cabinet solar dryer for 3 days to remove moisture. Dried shrimp shell waste and crab shell waste weighted 780 gram and 820 gram, respectively. These samples grind well and estimate the proximate composition (which are reported in result) then used for further procedure of chitin.

Isolation of glucosamine

Extraction of glucosamine was done by a method adopted from Gandhi and Laidler (2002) with some changes in method. Glucosamine was prepared in three different treatments which vary with concentrations and extraction time. The treatments are T_1, T_2 and T_3. Where, T_1 shows the 1:10 ratio with Chitosan: concentration of HCL and 50 min of extraction time, T_3 shows the 1:20 ratio with Chitosan: concentration of HCL and75 min of extraction time and T_3 shows the 1:30 ratio with Chitosan: concentration of HCL and 100 min of extraction time. The isolation method was used for all the treatments. The chitosan was grinded. Then chitosan was hydrolyzed with conc. HCL.The resultant brownish black material was dissolved in distilled water (1:1 v/v). The solution was filtered using Whatman filter paper to remove insoluble particles and other impurities. Distillate 10% activated charcoal is added and the solution was heated at 60^{0}C at 30 min. Then the filtrate was evaporated at 40-45°C to recover glucosamine hydrochloride (Glu-HCl). The crystals were washed with 96% ethanol and to obtained glucosamine were dried under the mechanical air drier at 50^{0}C (as shown in plate 1 to 4).

Proximate compositions

Proximate compositions such as moisture, protein, fat, ash were determined according to AOAC (2005) method.

Functional properties of glucosamine

Viscosity

The shrimp and crab glucosamine sample were determined according to the method of Cho *et al* (2006). Glucosamine solutions at the concentration of 6.67% (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60^{0}C for the determination of viscosity. The viscosity (cP) of 10ml of the solution was determined using Brookfield digital viscometer (Model DV – E Brookfield Engineering, USA) equipped with a No. 1 spindle at 40^{0}C.

Solubility

The method of solubility of shrimp and crab glucosamine adopted from the Tungtong *et al* (2012). To

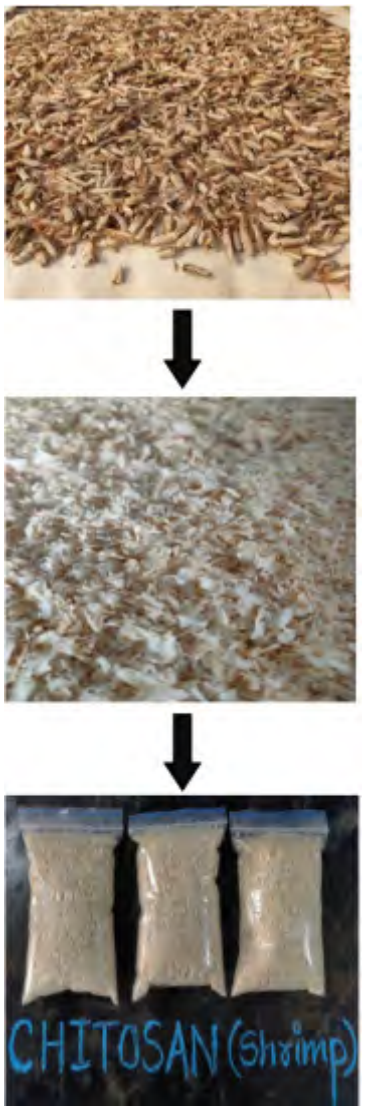

Plate 1: Shrimp shell waste, Shrimp chitin and Chitosan.

Plate 2 : Crab shell waste, Crab chitin and chitosan.

estimate the solubility of shrimp and crab glucosamine, 100 mg of sample were suspended in 10.0 mL of a solvent (distilled water, 0.1N HCl or 0.1N NaOH) and the suspension was stirred at 25°C for 5 hr. Then the mixture was filtered through filter paper to retain the undissolved portion, which was then washed with acetone and dried at 50^0C in a mechanical dryer or oven for all night. The total weight of sample was subtracted by the weight of the insoluble portion to obtain the weight of the soluble portion. The solubility of samples was expressed as g/ 100 mL. The total weight of sample was subtracted by the weight of the insoluble portion. This formula was used to obtain the weight of the soluble portion.

Emulsifying capacity and stability

Method of Yaumatsu *et al* (1972) was adapted to measure emulsifying capacity and stability of shrimp and crab glucosamine. For the measurement of emulsifying capacity of shrimp and crab glucosamine, 7 g of soybean product was suspended in 100 ml of water and then 100 ml of soybean salad oil (cooking oil) was added to it. The mixture was emulsified with homogenizer (Remi Electrotechnik Ltd.) at 10,000 rpm for 1 min. The emulsion obtained was divided evenly into four 50 ml centrifugal tubes and centrifuged at 1, 300 g for 5 min. The "emulsifying capacity" was calculated as:

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer in centrifuge tube}} \times 100$$

To measure the "emulsion stability," the emulsion prepared by the procedure for emulsifying capacity measurement was heated for 30 min at 80°C, cooled with tap water for 15 min, divided into four centrifugal tubes evenly and centrifuged at 1, 300 g for 5 min. "Emulsion stability" was expressed as:

Plate 3 : Shrimp glucosamine (T_1, T_2 and T_3).

Plate 4 : Crab glucosamine (T_1, T_2 and T_3).

$$\text{Emulsifying stability (\%)} = \frac{\text{Height of remaining emulsified layer}}{\text{Height of whole layer in centrifuge tube}} \times 100$$

Foaming capacity and stability

Measurement of foaming capacity was done by the method used by Miller and Groninger (1976). A 2g sample was added with 100 ml deionised water measured by using measuring cylinder. Then, it was whipped for 5 min in a Homogenizer (Remi Elektro technik Ltd.) at 8,000 rpm and was poured into a 250 ml graduated measuring cylinder. The total volume was noted. Volume increase (%) was calculated according to the following equation:

$$\text{Foaming capacity (\%)} = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Foaming stability is an extension of foaming capacity. After measuring the foam volume, the foam was transferred to an 1 liter funnel supported in a 250 ml calibrated cylinder. A small amount of glass wool was placed in the outlet of the funnel before transferring the foam. The liquid from syneresis was measured at 15min intervals for 60 mm.

$$\text{Foaming stability (\%)} = \frac{\text{Height of remaining emulsified layer}}{\text{Height of whole layer in centrifuge tube}} \times 100$$

RESULTS AND DISCUSSION

Recently, Glucosamine has worldwide demand as its tremendous uses in medical and cosmetic field. It helps to cure osteoarthritis. In present investigation includes the baseline information on physicochemical, antioxidant and microbiological analysis of shrimp glucosamine and crab glucosamine in different treatments (T_1, T_2 and T_3) extracted from shrimp and crab shell waste. Limited researches have been studied on functional properties of

glucosamine. Therefore, in the present study attempt has been made to cover physicochemical and functional properties of glucosamine and same discussed herewith.

Physicochemical and functional properties

Proximate compositions

In the present study, the proximate composition of raw material *i.e.* shrimp shell waste was found the values of moisture, ash, protein and fat are 13.9%, 33.74%, 29.46% and 3%, respectively. The proximate composition of crab shell waste was found the values of moisture, ash, protein and fat are 12.57%, 44.56%, 26.2% and 1.23%, respectively (Table 1).

According to Ushakumari and Ramanujan (2012) the proximate composition of dried shrimp shell was shown moisture (15.5%), ash (28.4%), protein (30.0%) and fat (2.1%). Aim of study was to propose an easy and proficient method for the isolation of high-value pigment, astaxanthin, from a low-value raw material, shrimp waste. Varun *et al* (2017) was reported their study found the proximate composition of crab shell on dry matter basis was shown ash (61.66%), protein (14.42%) and fat (0.20%). The studies focus on the extraction of chitin, chitosan and chito-oligosaccharides from crab shell waste. Chitin and chitosan yield from crab shell waste was found to be 12.2% and 10.54%, respectively.

In the present investigation, proximate composition of shrimp glucosamine of three treatments showed in Table 2. In which out of three treatments, T_2 showed the highest percentage of protein (10.87±0.09) and T_1 showed highest percentage of carbohydrate (82.31±0.01), T_3 showed highest no. of fat (2.33±0.15), ash (4.47±0.03) and moisture (0.8±0.05). Proximate composition of crab glucosamine of three treatments showed in Table 2. Where, T_2 showed the highest percentage of protein (10.97±0.01) and carbohydrate (78.18±1.19), T_1 showed highest percentage of fat (6.77±0.09) and ash (4.03±0.03) and T_3 showed highest percentage of moisture (1.67±0.12).

Wanichpongpan and Attasat (2016) reported that carbohydrate was the major component found in the crab shell, indicating chitin as a major component. Chitin is the most abundant polysaccharide, containing a derivative of glucose as a backbone and has nitrogen and acetamido groups in its molecule. According to Pachapur *et al* (2016), generally, the chemical composition of crab / shrimp shells varies with species, seasons, and many other factors.

Yield

In the present study, yield of chitin and chitosan was calculated on the dry weight basis. Yield of crab chitin (23.36±0.26) from crab shell was more than the yield of shrimp chitin (22.36±0.31), from shrimp shell yield of shrimp chitosan (67.44±0.28) is more than that of crab chitosan (64.60±0.03 (Table 3). The yield of shrimp and crab Glc were recorded 13.80% and 14.97% respectively (Table 3). Differences shown in the yield of present study and earlier research references because in present study the raw material *i.e.* shrimp shell waste and crab shell waste was used in mixture of various species shells of shrimp and crab. Present study has not focused on the same species for raw material.

Sibi *et al* (2013) has reported in their study the glucosamine hydrochloride (Glu-HCl) from various crustacean shells namely *P. monodon* (Indian shrimp), *P. sanguinolentus* (three spot crab) and *Portunus pelagicus* (blue crab). The yield of chitin was 87.83%, 89.18% and 51.11% and yield of chitosan was 68.91%, 75.67% and 30% and yield of glucosamine hydrochloride (Glu-HCl) was 21.64 mg g^1, 21.83 mg g^{-1} and 3.32 mg g^{-1} for *P. sanguinolentus*, *P. pelagicus* and *P. monodon*, respectively.

According to Islam *et al* (2011), the yields of the product mainly depend on reaction conditions. For optimizing glucosamine hydrochloride, chitin was hydrolysed with concentrated HCl with different reaction time and temperature. Different reaction time and temperature gave dissimilar yield (%). Chitin was

Table 1 : Proximate compositions of dried Shrimp and Crab shell waste.

S. no.	Proximate compositions	Composition in % (Shrimp Shell)	Composition in % (Crab shell)
1.	Moisture	13.9± 0.12	12.57±0.62
2.	Ash	33.74±0.33	44.56±0.01
3.	Protein	29.46±0.28	26.2±0.11
4.	Fat	03.00±0.06	1.23±0.09

Analyses were done in three replications and values are mean ± S.E. (Standard Error) of three replicate.

Table 2 : Proximate compositions of Glucosamine extracted from shrimp shell.

S. no.	Proximate compositions (%)	T_1	T_2	T_3
1.	Protein	10.57±0.12	10.87±0.09	10.47±0.18
2.	Fat	1.7±0.12	2.17±0.22	2.33±0.15
3.	Carbohydrate	82.31±0.01	83.03±0.12	81.1±0.06
4.	Ash	4.3±0.06	3.59±0.02	4.47±0.03
5.	Moisture	0.74±0.05	0.78±0.01	0.8±0.05

T_1 = (1:10) Chitosan: concentration of HCL and 50 min of extraction time, T_2 = (1:20) Chitosan: concentration of HCL and 75 min of extraction time and T_3 = (1:30) Chitosan: concentration of HCL and 100 min of extraction time. Analyses were done in three replications and values are mean ± S.E. (Standard Error) of three replicates.

Table 3 : Percentage yield of Chitin, Chitosan and Glucosamine extracted from shrimp and crab shell.

S. no.	Raw material	Chitin (%)	Chitosan (%)	Glucosamine (%)
1	Shrimp shell	42.36±0.31	67.44±0.28	13.80±0.12
2	Crab shell	49.30±0.26	64.60±0.03	14.97±0.12

Analyses were done in three replications and values are mean ± S.E. (Standard Error) of three replicates.

Table 4 : Proximate composition of Glucosamine extracted from crab shell.

S. no.	Proximate compositions (%)	T_1	T_2	T_3
1.	Protein	10.78±0.04	10.97±0.01	10.89±0.05
2.	Fat	6.77±0.09	5.67±0.19	6.2±0.20
3.	Carbohydrate	76.99±1.73	78.18±1.19	77.43±0.03
4.	Ash	4.03±0.03	3.5±0.12	3.5±0.03
5.	Moisture	1.13±0.14	1.37±0.18	1.67±0.12

T_1 = (1:10) Chitosan: concentration of HCL and 50 min of extraction time, T_2 = (1:20) Chitosan: concentration of HCL and 75 min of extraction time and T_3 = (1:30) Chitosan: concentration of HCL and 100 min of extraction time. Analyses were done in three replications and values are mean ± S.E. (Standard Error) of three replicates.

Viscosity

In the present study, shrimp Glc viscosity of sample T_1, T_2 and T_3 are 2.8 cp, 3.87cp and 3.07cp, respectively. The viscosity of crab Glc sample T_1, T_2 and T_3 are 1.43cp, 1.6cp and 1.46cp, respectively. In both shrimp and crab Glc viscosity showed highest in T_2 as compared to T_1 and T_3 (Table 5). Viscosity of glucosamine and chitosan are somewhat similar values recoded. Bansal *et al* (2011) has reported that the viscosity of chitosan was less than 5 cps. Chitosan was psedoplastic material and an excellent viscosity enhancing agent in acidic environment.

Solubility

In the present study, solubility of shrimp Glc of sample T_1, T_2 and T_3 are 99.32%, 99.64% and 99.63%, respectively. Also, viscosity of crab Glc sample T_1, T_2 and T_3 are 99.43%, 99.6% and 99.6%, respectively. In both shrimp Glc and crab Glc solubility showed highest in T_2 as compared to T_1 and T_3 (Table 5).

Cahyono *et al* (2014) has reported in their study the solubility of glucosamine produced with the pressure hydrolysis method with different HCl concentration are

Table 5 : Functional properties of shrimp and crab glucosamine.

Treatments	Shrimp Glucosamine					
	Viscosity (cP)	Solubility (%)	Emulsifying Capacity (%)	Emulsifying Stability (%)	Foaming Capacity (%)	Foaming Stability (%)
T_1	2.8±0.15	99.32±0.01	38.4±0.25	25.5±0.30	10.63±0.23	9.53±0.14
T_2	3.87±0.15	99.64±0.02	52.43±0.48	28.2±0.25	12.64±0.02	10.6±0.26
T_3	3.07±0.09	97.63±0.23	45.8±0.7	26.9±0.11	12.32±0.01	9.63±0.14
Treatments	**Crab Glucosamine**					
T_1	1.43±0.02	99.43±0.02	47.27±0.54	28.33±0.38	12.43±0.02	9.47±0.17
T_2	1.6±0.01	99.6±0.01	53.27±0.64	29.17±0.33	12.6±0.01	10.63±0.21
T_3	1.46±0.02	99.46±0.02	49.27±0.43	29.57±0.26	12.46±0.01	9.73±0.26

T_1 = (1:10) Chitosan: concentration of HCL and 50 min of extraction time), T_2 = (1:20) Chitosan: concentration of HCL and 75 min of extraction time and T_3 = (1:30) Chitosan: concentration of HCL and 100 min of extraction time. Analyses were done in three replications and values are mean ± S.E. (Standard Error) of three replicates.

prepared from shrimp shell waste and when chitin was hydrolysed with concentrated HCl for 1.30 h then maximum yield was obtain which was 63.5%.

Cahyono *et al* (2014) was reported in their findings, the yield of glucosamine was depending on the acid concentrations and heating time. In this study, the chitosan made from tiger prawn (*P. monodon*) was used as a raw material to produced glucosamine. The highest glucosamine yield was 65.33%, obtained with a hydrochloric acid highest on treatment concentration of 5% HCl followed by 63.98% with 8% HCl and 64.34% with 10% HCl, all at a pressurised heating time of 60 minutes.

96.33% at 5% HCl, 95.87% at HCl 8% and 93.53% with 10% HCl, all for the 60 minute heating treatment. In this study, the chitosan made from tiger prawn (*Penaeus monodon*), which was later used to produced glucosamine. Study concluded that the solubility increases or decreases with the temperature of the solvent.

Santhosh and Mathew (2007) were reported that the glucosamine produced from shrimp shell waste was readily soluble in water. If a substance can be readily dissolved at low temperatures, this indicates that it is highly soluble. Also, Prasetyo *et al* (2019) concluded that the glucosamine is freely soluble in demineralised water.

Emulsifying capacity and stability

Emulsifying capacity and stability of both shrimp Glc and crab Glc in current was showed in Table 5, respectively. Emulsifying capacity of shrimp Glc of sample T_1, T_2, T_3 are 38.4%, 52.43%, 45.8% and emulsifying stability of shrimp Glc of sample T_1, T_2, T_3 are 25.5%, 28.2%, 26.9%. Emulsifying capacity and stability of crab Glc of sample T_1, T_2, T_3 are 47.27%, 53.27%, 49.27% and 28.33%, 29.17%, 29.57%, respectively (Table 5).

Miller and Groninger (1976) has reported the emulsifying activity of fish protein derivatives that have been acylated and hydrolyzed at various levels and found the results of control ranges between 53-100, acetylation with different treatments are between 64-72 and succinylation with different treatments are between 70-93.

Foaming capacity and stability

In present study showed the foaming capacity and stability of shrimp Glc of sample T_1, T_2, T_3 are 10.63%, 12.64%, 12.32% and 9.53%, 10.6%, 9.63%, respectively. Also, the foaming capacity and stability of crab Glc of sample T_1, T_2, T_3 are 12.43%, 12.6%, 12.46% and 9.47%, 10.63%, 9.73%, respectively (Table 5). In present study foam capacity and stability was recorded lower than earlier researcher reported.

Miller and Groninger (1976) has reported that the foam stability of fish protein derivatives that have been acylated and hydrolyzed at various levels and found the results of control ranges between 52-77 ml, acetylation with different treatments are between 59-72 ml and succinylation with different treatments are between 18-57 ml.

CONCLUSION

Shrimp shell waste and crab shell waste were cheap source, tons of shell waste discarded by processing industry every season; which were used in this study as a raw material for the production of shrimp and crab chitin, shrimp and crab chitosan and shrimp Glc, crab Glc. Successfully extracted glucosamine from shrimp shell waste with yield of three treatments T_1, T_2 and T_3 and are 13.10%, 13.80% and 13.40, respectively and yield of glucosamine from crab shell waste of treatments T_1, T_2 and T_3 and are 14.1%, 14.97% and 14.37%, respectively. Shrimp Glc and crab Glc showed desirable physicochemical and functional properties. In the comparison of shrimp Glc and crab Glc; shrimp Glc showed more advantageous functional properties than that of crab Glc. Taken as a whole, the current study enlightens the utilization of shrimp Glc and crab Glc in medical and cosmetics industries.

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