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Comparative characteristics of chitosan extracted from shrimp and crab shell and its application for clarification of pineapple juice

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ABSTRACT

The research has been carried out to compare chitosan extracted from shrimp and crab shell waste and to investigate its effect as a clarifying agent as well as preservative on the pineapple juice. Chitosan has been extracted from shrimp and crab shells by chemical process such as demineralization (2%HCl, 16hr), deproteinization (4% NaOH, 20hr) and deacetylation (60% NaOH, 65^{0}C, 20hr). Yield study showed that crab shells contained more chitosan (20.31%) than shrimp offal (16.29%). Physicochemical properties of chitosan extracted from both sources studied for fat binding capacity (FBC), water binding capacity (WBC), solubility, average molecular weight, ash content, moisture content and degree of deacetylation. The degree of deacetylation and solubility were obtained as 76 and 65% for shrimp shell chitosan and 91 and 70% for crab shell chitosan, respectively. However, WBC and FBC of shrimp and crab shells chitosan were found to be 448 and 265 % and 363 and 212%, respectively. The average molecular weight of shrimp (8.76×10^5Da) and crab (5.61×10^5Da) chitosan was found to be very close to each other. Chitosan was added to pineapple juice as a clarifying agent at a concentration of 0.2, 0.4 and 0.6g/L. It was observed that shrimp chitosan at a concentration of 0.6g/L was more effective at (28±2^{0}C) than crab chitosan after 90 min of addition. Statistical analyses revealed that source of chitosan possess significant effect on the perceived sensory characteristics of juices when chitosan has been added at a range from 0.2 to 0.6 g/L of pineapple juice. Chitosan also inhibited the growth of some spoilage bacteria. The analyses showed that increases in chitosan concentration extended the quality of the pineapple juice significantly (0.05) by increasing pH values range, reducing TSS value, reducing enzymatic and non-enzymatic browning and controlling the spoilage during the storage time. This study showed that shrimp chitosan was more effective than crab chitosan as a preservative and clarifier on the quality of pineapple juice.

Introduction

Chitosan is deacetylated derivatives of chitin and one of the richest polysaccharides taking place in nature next to cellulose (Ramasamy and Shanmugam, 1885). Chitin is originated on the outer surface of the arthropod body, in the mushrooms cell wall and in cell structures of algae and yeast (Kaya *et al.,* 2015). Crustacean waste from crab shells is composed of chitin which forms a chitin-protein complex with proteins. Fish scales, shrimp and crab shells are composed of proteins (15–50%), minerals (30–50%) and chitin (15–30%) (Hajji *et al.,* 2015, Hossain and Iqbal, 2014). The growing quantities of shell wastes from processing of crustaceans have become a major concern for seafood processing plants since their biodegradation is very slow. Global annual production of crustaceans shell wastes is estimated at 1.41 million metric tons (Pérez Roda *et al*., 2019). These processing byproducts, however, may be used as an important source for production of value-added product such as chitin and its deacetylated derivative chitosan. As a food additive, chitosan have been used in the processing of fruit juices, for reducing the turbidity (Soto-Peralta *et al*., 1989), reduction of acidity (Imeri and Knorr, 1988), reduction of browning (Sapers, 1992). It also possessed antimicrobial activity against some bacteria, yeasts and molds, with low toxicity in mammalian cells (Martín-Diana *et al.,* 2009), antioxidant activity (Chien *et al.*, 2007) as well as shelf life extender for different fruit and fruit juice (Martín-Diana *et al.*, 2009, Hossain and Iqbal, 2016). Pineapple juice is very widespread and consumed all over the world for its pleasant aroma and tastes. It contains high contents of antioxidant and phenolic compounds such as tyrosine, tryptophan, serotonin etc. (Wen and Wrolstad, 2002). Pineapple juice also contains phytosterols such as ergostanol, stigmastanol and antioxidant vitamin C which lessens the danger of cardiac disease by averting

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the oxidation of low-density lipoprotein (LDL) cholesterol (Laorko *et al.*, 2013). The microbial deterioration, production of unpleasant odors and degradation of ascorbic acid are the main causes for loss of quality and shelf-life (Raybaudi-Massilia *et al.*, 2009) of such juices.

To extend the shelf life of juices, various chemical additives have been used for a decade. Different countries limited their use because of their negative effect of public health. However, consumer demand is being increased for additives of natural origin, and hence boosted the Food scientists to increase new preservatives that could meet their expectations without deteriorating the quality (Raybaudi-Massilia *et al.*, 2009). Fruit juices are usually treated with various agents such as gelatins, silica, bentonite, sol, tannins or combinations of these compounds (Soto-Peralta *et al.*, 1989) for the clarification purposes. It has been revealed that Chitosan could be effective as an agent for the separation of colloidal and dispersed particles from food processing wastes (No and Meyers, 2000) due to having its partial positive nature. Although thermal pasteurization of juice decreases the risk of food borne illness, it also diminishes the nutrient content of juices. Considering these motives, the researchers and industry personnel recently focused their devotion on non-thermal emerging technologies and alternative novel treatments to control unwanted microorganisms with minimum effects on the product tastes and quality. Therefore, in this study, it is attempted to compare the characteristics of chitosan extracted from shrimp and crab shell and subsequently to study the suitability of applying chitosan as a clarifying agent to extend the quality of pineapple juice.

Materials and Methods

Shell of shrimp and crab were separated from the whole body using a sharp knife. The collected wastes were then washed with tap water and crushed with mortar-pastel. All the reagents (HCl, NaOH) and utensils required were collected from the laboratory stocks of the Department.

Extraction of chitosan

Fresh shrimp and crabs were collected from local market. Shell of shrimp and crab were separated from the whole body using a sharp knife. The collected wastes were then washed with tap water and crushed with mortar-pastel. Clean and crushed shrimp and crab wastes were kept in a polyethylene bags at ambient temperature (28±2^{0}C) for 24 hr for partial autolysis to facilitate chemical extraction of chitosan and to improve the quality of chitosan (Toan, 2009). The isolation of chitosan was carried out following the 3 (three) steps namely- demineralization, deproteinization and deacetylation as described by Toan (2009) and Hossain and Iqbal (2014). The resulting chitosan was then dried at cabinet dryer for 4 hr at (65±5^{0}C) and stored in polyethylene bag until they are used as a preservative in pineapple juice.

Physicochemical and functional properties

Physicochemical and functional properties of the crab and shrimp chitosan were determined as per standard methods such as moisture content by the gravimetric method (Black, 1965), ash content by AOAC (2004), solubility by Fernandez-Kim (2004), intrinsic viscosity and molecular weight by Wang *et al*. (2007), water binding capacity, fat binding capacity and degree of deacetylation by Hossain and Iqbal (2014). Bulk density of chitosan samples was determined as per method described by Cho *et al*. (1998) and calculated as grams per milliliter of the sample.

The color of chitosan powder, expressed in L*, a*, b*and whiteness values were measured (five readings) using a Chroma meter (CR-400, Konica Minolta, Japan). The whiteness was calculated using a formula from:

$$\text{Whiteness} = 100 - \left[(100 - L^{*})^{2} + a^{*2} + b^{*2}\right]^{\frac{1}{2}}$$

Preparation of pineapple juice

Fresh and mature pineapple *(Ananas comosus)* fruit were purchased from a wholesale market in Madhupur, Tangail, Bangladesh. Whole fruit was washed with 500 mg/l chlorine solution. Pineapple stem ends were discarded and the peel and core were removed. Then, cut into small pieces to facilitate blending using a sanitized sharp knife and cutting board. All knives, cutting boards and other equipment which may come into contact with the pineapple were sanitized by immersion in 1000 mg/l chlorine solution for 30 min before using. The pineapple pieces were ground by blender and juice was collected by pressing methods. Collected juice was filtered through fresh cheese cloth and then stored at 4^{0}C for further analysis.

Application of chitosan for the clarification

Clarification of pineapple juice was performed by addition of three different concentrations of shrimp and crab chitosan shrimp sample 1 (S1), crab sample 1 (C1), shrimp sample 2 (S2), crab sample 2 (C2), shrimp sample 3 (S3), crab sample 3 (C3) including a control sample P (0). The clear juice was obtained by centrifugation at 5000 rpm for 10 min. Unclarified juice was used as a control for comparison among clarification treatments using chitosan obtained from both the sources. All the juices were stored at 4^{0}C until further analysis (Oszmianski and wojdylo, 2007).

Quality evaluation of clarified juice

Total soluble solids (TSS) and pH

Total soluble solids (TSS) were determined by using Abbe Refractometer (Model no. 8987, Puji Kuki Ltd. Tokyo, Japan). pH value was determined using pH meter (Model no. hi-8424 Hanna Instruments, USA). TSS and pH value were measured as per methods of Ranganna (2011).

Potential browning

Potential browning was measured according to the methodology of Vina and Chaves (2006), 10 ml of fresh pineapple juice was treated with ethanol (95%) for 60 min and then centrifuged at 4000 rpm at 5^0C for 10 min. After retaining the supernatant, ethanol was added to bring the final volume to 25 ml. Absorbance at 420 nm of aliquots of these extracts were measured.

Microbiological test

For the total viable count of microorganism present in pineapple juice, standard plate count (SPC) method was done for three times and followed according to the method described in "Recommended method for the microbiological examination of food in American Public Health Association" (Marth, 1978).

Sensory evaluation

The consumer's acceptability of developed or clarified pineapple juice was evaluated by a taste tasting panel. The hedonic rating test was used to determine this acceptability (Larmond *et al.,* 1970; Fellers *et al.,* 1985). The data was then statistically analyzed by using statistical software (Stat Graphics Plus v5.1, 1999).

Results and Discussion

Physiochemical and functional properties

Various physicochemical and functional properties of shrimp and crab chitosans have been shown in Table 1. Shells from different parts of crab contained more chitosan (20.31%) than shrimp offal (16.29%). Brzeski (1982) reported yield of chitosan 14% from krill which is lower than this study value. Alimuniar and Zainuddin (1992) reported 18.6% yield from prawn waste.

Chitosan's moisture content, obtained from shrimp shells and crab was found as 4.25% and 3.79%, respectively. The result is consistent to commercial crab chitosan, Vanson75, and Sigma91 which have relatively higher moisture content, 4.5%, and 3.5%, respectively. (Fernandez-Kim, 2004). Moisture content of commercial chitosan was less than 10% (Li, 1992).

Ash content of crab and shrimp shell chitosan samples were found to be 2.28% and 0.31%, respectively, which indicates that crab chitosan has more ash content compared to shrimp chitosan. Ash content indicates the efficiency of demineralization step and also depends on the composition of shell. Mohanasrinivasan *et al.* (2014) found that shrimp and commercial chitosan consists 2.28% ash. The yield of chitosan varies from 0.31% to 2.28% for shrimp shell and crab shell, respectively. The variation may be due to the differences in chemical composition and structure of shells considered.

Table 1. Physiochemical and functional properties of chitosan

Parameter/properties	Chitosan from	
	Shrimp	Crab
Moisture content (%)	4.25	3.79
Ash content (%)	0.31	2.28
Yield (%)	16.29	20.31
Solubility (%)	91	70
Degree of deacetylation (%)	76	65
Bulk density (g/ml)	0.085	0.19
Intrinsic viscosity (dl/g)	11.48	8.23
Average molecular weight (Da)	8.76×10^5	5.61×10^5
Water binding capacity (%)	448	265
Fat binding capacity (%)	363	212

The solubility of chitosan from crab and shrimp was 70% and 91% respectively. Shrimp chitosan was more soluble than crab chitosan. The temperature during deacetylation process affect the solubility and usually decreases it. The quality of chitosan is determined by the degree of deacetylation which in-turn affects its solubility (Madhavan and Nair, 1974*)*. The degree of deacetylation in this study has been found as 76% and 65%, respectively for shrimp and crab sources. Solubility of crab chitosan was found lower than crab chitosan because crab chitosan has lower degree of deacetylation than shrimp chitosan. There are several critical factors that usually affects solubility of chitosan such as including temperature and deacetylation reaction time, alkali concentration, the ratio of chitin to alkali solution, and particle size (Hossain and Iqbal, 2014). The degree of deacetylation determines mainly the content of free amino groups in the polysaccharide. It was found that crab chitosan's degree of deacetylation was lower than shrimp chitosan. The degree of deacetylation obtained in this study is within the range of the value reported by No *et al.* (1989) who reported the range from 56% to 99% with an average of 80%.

The bulk density found for chitosan from shrimp and crab were 0.085 g/ml and 0.19 g/ml, respectively. The result indicates that shrimp chitosan is more porous than crab chitosan. Cho *et al.* (1998) showed that Bulk density of chitin and chitosan products was in the range of 0.20-0.38 g/ml where chitin and chitosan from shrimp were more porous than crab chitin and chitosan.

Intrinsic viscosity

Intrinsic viscosity is an important rheological parameter which is used to characterize the hydrodynamic properties of polymers and also to determine the weight average molecular weight of polymers. The intrinsic viscosity of chitosan obtained from shrimp and crab in this study was found to be 11.48 dl/g and 8.23dl/g respectively. Lower intrinsic viscosity of crab chitosan indicates that shrimp chirosan is superior quality than crab chitosan. Hossain and iqbal (2014) obtained intrinsic viscosity from shrimp chitosan 13.2dl/g which is consistent or close to our study.

Average molecular weight

The average molecular weight of chitosan derived from shrimp and crab was 8.76×10^5Da and 5.61×10^5Da respectively. Crab chitosan has less average molecular weight compared to that of shrimp. Average molecular weight of chitosan varies with the source, extraction method, and the residual aggregates in the solution. The molecular weight of commercial chitosan products fall between 100,000 to 1,200,000 Daltons (Li *et al*., 1992). Hossain and Iqbal (2014) reported average molecular weight of shrimp chitosan 1.05×10^6 Dalton which result is consistent with our present study.

Water binding capacity

Water binding capacity (WBC) of chitosan derived from shrimp and crab was found to be 448% and 265% respectively (Table 1). It was observed that water binding capacity of shrimp chitosan was better than that of crab chitosan.WBC for five commercial chitosan from shrimp and crab shell was found at a range of 458% to 805% and production step sequence has a pronounced effect on WBC as reported by Cho *et al*., (1998). WBC of shrimp chitosan was found 537.29% by Hossain and Iqbal (2014) which is higher than the present study.

Fat binding capacity

The fat binding capacity (FBC) of shrimp and crab chitosan was measured using soybean oil. The fat-binding capacity of shrimp and crab was investigated, and the values found to be 363% and 212% respectively (Table 1). No *et al.* (1989) reported that fat binding capacity values of chitosan were ranging from 314% to 535%. Hossain and Iqbal (2014) found FBC of shrimp chitosan 427.98%.

Color

The color of chitosan samples was expressed in L*(Lightness), a*(Redness), b*(Yellowishness), whiteness (Table 2) values. Chitosan powder is quite flabby in nature and its color varies from pale yellow to white (No *et al.*, 2000). Based on visual observation, the color of chitosan samples extracted in this study varied from white to extremely pink or yellow.

From the calculated value, it was found that chitosan prepared from crab shells was whiter compared to that prepared from shrimp shells. It seems that chitosan prepared from both shrimp and crab showed slight red and significant yellow color.

Table 2. Color property of chitosan

Sample	L* (Lightness)	a* (Redness)	b* (yellowishness)	Whiteness
Shrimp	73.94	5.86	13.13	70.237
Crab	76.98	4.74	13.27	73.0096

Effect of chitosan on pineapple juice quality

Clarification effect of chitosan

The effect of chitosan at different concentration (0.2, 0.4, 0.6 g/l) on the clarification of pineapple juice is shown in Table 3. Increasing the chitosan concentration increased the luminosity. At 90 min. the optical density of shrimp chitosan (0.6 g/l con.) added juice was found less than that of crab chitosan added juice.These results were explained by the clarification effect described in fruit juices associated with chitosan (Soto-Peralta *et al.*, 1989; Chatterjee *et al.*, 2004). Yuanjun *et al.* (2016) also found similar clarification effect using 0.2 g/l chitosan after one hour at 25^{0}C. Salts which carry a strong positive charge, have been shown to be effective as debasing agents, chitosan is a good clarifying agent for grapefruit juices (Chen and Li, 1996) and highly effective fining agent for pineapple juice, which can afford zero turbidity products with 0.8 kg/ m^3 of chitosan (Soto-perlata *et al.,* 1989). Chatterjee *et al.* (2004) observed a reduction of 73, 76, 72, and 61% of color using chitosan to clarity apple, grape, lemon and orange juices, respectively. Domingues *et al.* (2012) found that treatments with chitosan reduces100% turbidity of raw juice.

Table 3. Effect of Chitosan on clarification of Pineapple juice

Time (min)	Optical density (540 nm)						
	Control (P)	Shrimp chitosan conc.			Crab chitosan conc.		
		S1 (0.2g/L)	S2 (0.4g/L)	S3 (0.6g/L)	C1 (0.2g/L)	C2 (0.4g/L)	C3 (0.6g/L)
30	1.2	1.17	1.16	1.15	1.16	1.15	1.13
60	1.2	0.7	0.68	0.65	0.88	0.89	0.86
90	1.2	0.41	0.38	0.35	0.65	0.64	0.61

Total soluble solids (ºBrix)

The effect of chitosan on the total soluble solid of pineapple juice presented in Table 4. The result showed that chitosan reduced the TSS value of the pineapple juice, significant changes over storage time were observed. This result showed that the TSS reducing effect of Shrimp chitosan is higher than crab chitosan. This result is an agreement with Rivas *et al.* (2006) who found reduction of TSS with the increasing concentration of the positive charged polysaccharide occurs due to coagulate suspended solids, increasing the flocculation capacity of chitosan which could bind the sugar (Sapers, 1992).

pH

The effect of chitosan extracted from shrimp and crab shell on the pH of pineapple juice was significantly affected by chitosan concentration and storage time. pH value of pineapple juice was increased more rapidly at crab and shrimp chitosan treated sample than control during storage period (Table 4).

This effect could be due to the capacity of chitosan to reduce fruit juice acidity (Imeri and Knorr, 1988) based on its acid-binding properties.

Table 4. Effect of Chitosan on TSS and pH of Pineapple juice at different storage time

Time (day)	Control (P)	Shrimp chitosan conc. S1 (0.2g/L)	S2 (0.4g/L)	S3 (0.6g/L)	Crab chitosan conc. C1 (0.2g/L)	C2 (0.4g/L)	C3 (0.6g/L)
Total soluble solid (˚Brix) of pineapple juice at different days							
0 day	7.3	7.2	7.15	7.1	7.19	7.17	7.15
2nd day	7.2	7.08	6.98	6.92	7	6.94	6.84
4th day	7.2	7.02	6.92	6.88	6.91	6.88	6.78
6th day	7.1	6.97	6.81	6.71	6.9	6.81	6.73
8th day	7.1	6.91	6.77	6.68	6.87	6.79	6.7
pH of pineapple juice at different days							
0 day	3.7	3.7	3.7	3.7	3.7	3.7	3.7
2nd day	3.76	3.78	3.85	4	3.8	3.83	3.9
4th day	3.81	3.82	3.88	3.9	3.84	3.86	4
6th day	3.88	3.9	4	4.1	3.9	3.92	4.01
8th day	3.9	3.95	3.97	4.12	3.92	3.94	4.02
10th day	3.92	4.02	4.05	4.18	3.94	3.98	4.1

Browning potential

An interactive effect between chitosan concentration and storage time on the Browning potential (BP) was also observed. In samples with low or without chitosan concentration, the BP increased rapidly over storage time, while it remained almost constant in samples with higher chitosan concentration (Table 5). Chitosan has been found to enhance the control of enzymatic browning in apple and pear juice (Sapers, 1992). This datum showed a significant relationship between chitosan concentration and inhibition of browning. The control of browning could be associated with the capacity to coagulate solids to which browning related enzymes are bound. The antioxidant capacity of chitosan, similar to the capacity associated with phenolic compounds (Park *et al.*, 2004), could also explain this browning reduction by inhibiting the oxidative process. From Table 5 it is showed that crab chitosan is more effective in browning reduction than shrimp chitosan.

Table 5. Effect of chitosan on BP of pineapple juice in different time

Time (day)	Control (P)	Shrimp chitosan conc. S1 (0.2g/L)	S2 (0.4g/L)	S3 (0.6g/L)	Crab chitosan conc. C1 (0.2g/L)	C2 (0.4g/L)	C3 (0.6g/L)
Browning potential							
0 day	7	6.8	6	6.1	6.4	5.98	6
2nd day	7.5	7	6	6.1	6.8	6	6
4th day	8	7	6.5	6.3	6.8	6.4	6.2
6th day	8.2	7.2	6.8	6.4	7	6.4	6.5
8th day	8.5	7.5	6.9	6	7.2	6.8	6.6
10th day	9	7.8	7	6.8	7.6	6.8	6.6

Microbiological tests

The log numbers of the total viable count in pineapple juice during storage at 4ºc for 30 days were also inhibited by the presence of chitosan as shown in Table 6. The result showed that the microbial growth inhibited by shrimp chitosan more effectively than crab chitosan. The mode of inhibition of chitosan on the growth of bacteria and fungi might be due to the interaction of chitosan with membranes or cell wall components, the mechanism underlying the inhibition of bacterial growth is thought to be that the cationic ally charged amino-group many combine with anionic components and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition metal ions and inhibiting enzymes, due to the positive charge , resulting in increased permeability and leakage of cell material from tissue, or due to water binding capacity and inhibition of various enzymes by chitosan. Chitosan also has bioabsorption activity (Knorr, 1991) and can absorb nutrients of bacteria and may inhibit their growth.

Table 6. Changes of microbial count (log CFU/ml) of pineapple juice with added chitosan during storage of 30 days

Storage (Days)	Control (P)	Shrimp chitosan conc. S1 (0.2g/L)	S2 (0.4g/L)	S3 (0.6g/L)	Crab chitosan conc. C1 (0.2g/L)	C2 (0.4g/L)	C3 (0.6g/L)
Changes of TVC (log CFU/ml)							
0	0.97[a]	0.97[a]	0.67[b]	0.42[c]	0.98[a]	0.65[b]	0.46[c]
15	3.35[a]	3.31[a]	3.00[b]	2.90[b]	3.21[a]	3.09[b]	2.97[b]
30	3.70[a]	3.50[b]	3.10[c]	2.96[c]	3.58[a]	3.21[b]	3.00[b]

Sensory evaluation

These data showed increased significantly with increasing chitosan concentration were observed for color taste and overall acceptability of the control. This result agrees with Chatterjee *et al.* (2004) was found improvement of acceptability and appearance when treated with chitosan (Table 7). The result showed that Shrimp chitosan added juice is more acceptable than crab chitosan added juice.

Table 7. Sensory evaluation scores of pineapple juice enriched with chitosan

Treatment	Control (P)	Shrimp chitosan conc. S1 (0.2g/ L)	S2 (0.4g/ L)	S3 (0.6g/ L)	Crab chitosan conc. C1 (0.2g/ L)	C2 (0.4g/L)	C3 (0.6g/L)
Sensory evaluation scores							
Color	6.15[a]	6.82[b]	7.19[b]	7.85[c]	6.43[a]	7.15[b]	7.76[c]
Taste	7.02[a]	7.11[b]	7.13[b]	7.00[b]	7.06[a]	7.12[b]	6.85[c]
Overall acceptability	6.65[a]	7.08[b]	7.18[b]	7.56[c]	7.02[b]	7.17[b]	7.20[b]

Conclusion

Comparative studies on chitosan extracted from shrimp and crab waste showed that shrimp shell is the best choice for extraction of chitosan based on all physicochemical properties. Chitosan application on pineapple juice showed increased in luminosity with increasing chitosan concentration. Chitosan reduced the total soluble solid content of pineapple juice. The pH increased significantly with increasing chitosan concentration and shrimp chitosan added juice showed greater pH than crab chitosan added juice. Chitosan retarded potential browning of pineapple juice during storage. Shrimp chitosan showed lower microbial count than crab chitosan at 30 days storage period of pineapple juice. The sensory evaluation it was seen that shrimp chitosan added juice was more acceptable than crab chitosan added juice. As the outcome of this research is promising and optimistic, it will not be confined only to pineapple juice, but also for other fruit juices like apple, orange and litchi etc. juices.

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