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Chitosan- functional food from marine waste evaluation of antioxidant, antimicrobial properties and application in food matrices

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ABSTRACT

Seafood is divinely rich by virtue of its high nutritional value. With the increasing knowledge of bio-functional properties associated with marine foods, utilization of the marine derivatives has been accelerated which will also prevent pollution in coastal areas. The objective of the study was to process chitosan by deacetylating chitin of crab and prawn shells. The degree of deacetylation was determined by FTIR. The fresh chitosan has been analyzed for its antioxidant and antimicrobial property that helps to determine the health benefits of the derived product. The chitosan was also incorporated in a food product to develop value added products and the shelf life of the products was evaluated. The antioxidant and antimicrobial activity of the chitosan was directly propositional to the concentration used. Value added product was prepared i.e. Cereal bar, Beetroot Squash, Orange Squash and Ice cream with three variations. 5% chitosan from crab and prawn was added in two variations and compared with the control one. The sensory evaluation was done with semi- trained panel members. The proximate analysis and quality indices was done with standard methods. The shelf life of the product was determined by the total aerobic microbial count in the period of 15 days. There was increase in the antioxidant and antimicrobial activity level in the chitosan as concentration was increased. The sensory acceptability was good in Crab based chitosan when compared with other variations. Similarly proximate and quality enhancement was observed in prawn based chitosan products than the other variations. The shelf life of the product was also increased. Hence, chitosan can be encouraged in value addition, since it acts as a functional compound and does not interferes with the sensorial factors. It also helps to improve the shelf life of the product.

Introduction

The consumption and popularity of seafoods has consistently increased during recent years since seafoods are increasingly recognized as important sources of nutrients for human health (Sloan, 1986). Seafood processing industry in India is contributing tonnes and tonnes of waste materials and amongst them crab and prawn waste contributes more than one lakh tonnes every year. The utilization of marine processing co-products to generate bioactive ingredients for use in foods is both an economical and environmentally attractive option. The waste of the natural polymers is a major source of surface pollution in coastal areas.

Chitin is considered as underutilized resource which has got high potential in new functional biomaterial in various fields. It is a renewable biopolymer on earth that can be obtained as a cheap renewable biopolymer from marine sources.It is the second most abundant natural polysaccharide on earth after Cellulose and it has become a great interest as a new functional material of high potential in various fields. It is obtained from the exoskeleton of shellfish, including crab, lobster, and shrimp by chemical extraction (Mojarrad et al., 2007). Chitosan is a polysaccharide obtained by deacetylating chitin. The degree of deacetylation (DDA) influences the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristics, biodegradability, self aggregation, sorption properties, and the ability to chelate metal ions. Chitin is made up of a linear chain of acetylglucosamine groups while chitosan is obtained by removing enough acetyl groups.

Today chitin and its derivatives have diverse applications in agriculture, biotechnology, chemistry, dentistry, food product development, medicines, textiles and veterinary sciences. Properties that makes chitosan very useful includes inhibition of tumor cells, antifungal effects, acceleration of wound healing, stimulation of the immune system and acceleration of plant germination (Goosen, 1997). Antioxidant activity is well-known chitosan functionality. The antimicrobial activity of chitin, chitosan and their derivatives against different groups of micro- organisms, such as bacteria, yeast and fungi has received considerable attention in recent years (Dutta, 2009). Owing to the high chelating and coagulating ability of chitosan, the polymer has been widely used in food industry. Since chitosan is also edible, it can be applied to fabricated foods, encapsulating agents, or packaging materials (Jeon et al., 2001).

In this context Chitosanhas been evaluated for its antioxidant and antimicrobial properties. It was usedfor value addition of different food products and their acceptability, quality and stability are reported in this paper.

Materials and Methods:

Collection of samples. Shells were obtained from the wastes of crab from the Everest Biotechnology, Bangalore and prawns from Indian Seafoods, Cochin, India. The shells were dried and packed in plastic bags. All the ingredients were purchased

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locally and the reagents were of analytical grade from standard companies.

Preparation of Chitosan. Chitosan was prepared by the process of deproteinization (4% NaOH for 7 hours at 65-100°C), demineralization (1.25N HCl for 4 hours), decolouration (0.315% sodium hypochloride), deacetylation (40:60% NaOH for 2 hours at 100-120°C) and sterilized.

Degree of Deacetylation of Chitosan. The degree of deacetylation (DDA) of chitosan samples was determined by Fourier transform infrared (FTIR) spectrophotometer using KBr disk samples. The KBr disk was prepared according to the method of Sabnis and Block with slight modification. Approximately 20 mg of chitosan powder and 120 mg of KBr was blended and triturated with mortar and pestle for approximately 10 min. The mixture was compacted to form a disk. The disk was conditioned in a desiccator placed in an oven at 80°C for 16 hours before analysis. The spectra of chitosan samples in the form of KBr disk were obtained using an IR instrument (Shimadzu, Model: FTIR8400S) with a frequency range of 4000-400 cm^{-1}. The degree of deacetylation (DDA) was evaluated by recording absorbance at 1655 cm^{-1} for amide-I and at 3450 cm^{-1} for OH group in chitosan. The absorbance of chitosan was used to calculate the degree of deacetylation (DDA) using the following equation (Baxter et al., 1992):

$DDA(\%) = [(A_{1655}/ A_{3450}) \times 115]$.

Determination of antioxidant activity. The antioxidant activity of the raw chitosan were determined using DPPH (1, 1-diphenyl-2-picryl-hydrazyl radical). This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. The effect of extract on DPPH radical was assayed using the method of Mensor et al. (2001). A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1ml of the different concentrations of extract. The solution was shaken strongly. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 518 nm. The purple color bleaching of the DPPH reagent showed as positive antioxidant activity. For control, methanol solution with DPPH was used. The absorbance was converted into percentage radical scavenging activity as follows,

$$\%Radical\ Scavenging\ activity = \frac{(Control\ OD - Sample\ OD) \times 100}{Control\ OD}$$

Total Aerobic Microbial Count. The aerobic plate count was carried out on the raw chitosan according to the manual given by Cappucino and Sherman, (2009) to determine the quality of the product. Serial dilution and pour plate techniques were used. Nutrient agar (NA) was used for bacteria and the viable count was recorded for bacteria as colony forming units (cfu/g).

Value added products from chitosan. The products were developed with addition of 5% chitosan derived from crab and prawn. The products developed were cereal bars, beetroot squash, orange squash and ice cream. The value added products were compared with their respective control ones by organoleptic evaluation using five score hedonic scale.

Quality parameter assessment of Chitosan products. The developed products were analysed for proximate and quality principles, viz., moisture, ash, carbohydrate, protein, fat, crude fibre, pH, total solids, total acidity, ascorbic acid and milk fat. The moisture and ash content were analysed according to AOAC (1990), protein by Kjeldahl, Carbohydrate by anthrone method (Hedge, 1962), fat by difference method, crude fibre (Raghuramulu, 2003), pH and total acidity (AOAC, 1999), ascorbic acid (AOAC, 1990) and milk fat (Weisshaar, 2012).

Shelf life determination. The total aerobic microbial count was used to determine the bacterial population formed in the products which were enumerated for stability of the product.

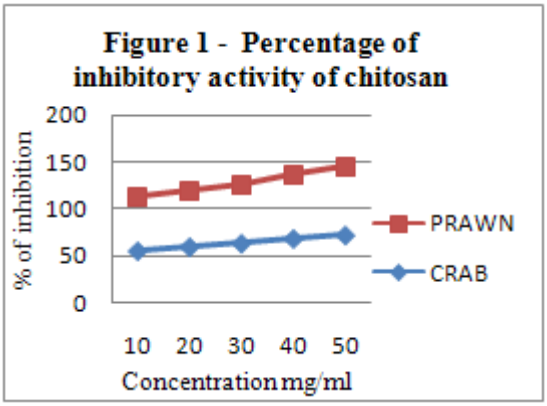
Statistical Analysis:

Data collected were subjected to analysis of variance (ANOVA) using the QI macros Statistical Package. T-test was used to compare the antioxidant activities of the two samples.

Results and Discussion:

Degree of Deacetylation of Chitosan. The degree of deacetylation obtained during the processing of crab and prawn shells were 78 and 82 per cent respectively. The percentage of the degree of deacetylation was obtained from the frequency range of 4000-400 cm^{-1}. The degree of deacetylation (DDA) was evaluated by recording absorbance at 1655 cm^{-1} for amide-I and at 3450 cm^{-1} for OH group in chitosan. The DOD depends mainly on the method of purification and reaction conditions such as concentration of chemicals used, time and temperature factor.

Determination of antioxidant capacity. The antioxidant activity level of the two different chitosan was determined at a concentration range of 10-50mg/ml.The activity increased gradually when there was increase in the concentration level. Among the two chitosan, prawn chitosan (71%) had greater activity when compared to the crab chitosan (73%). The percentage of inhibitory activity of chitosan has been shown in figure 1.



Total Aerobic Microbial Count. The microbial quality of the chitosan was estimated by the total aerobic microbial count. Microbial examination revealed that the initial total surviving microorganisms in the raw chitosan was 39.0cfu/gram for crab based chitosan and 41.0cfu/gram for prawn based chitosan. The total aerobic microbial count was within the standard value given by ASTM F2103-01 for food grade chitosan. The standard total aerobic count was less than 500cfu/gram. Thus this revealed that the raw material was good for use in product development.

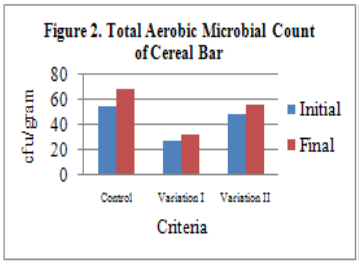
Organoleptic evaluation of Value added products from chitosan. The value added products that were developed included Cereal Bar, Beetroot Squash, Orange Squash and Ice cream. From each product, a control and two variations with 5% crab and prawn based chitosan was added. Sensorial analysis of food products were done with the semi trained panel members. The different criteria of the products included viz. Appearance, Color, Texture, Flavor, Taste and Overall acceptability. The result pertaining to sensory evaluation showed no significant differences between the products ($p>0.05$). Cereal bar and Ice cream with variation I (addition of crab based chitosan) scored maximum among the product. In Beetroot squash, Variation II (addition of prawn based chitosan) scored more and in Orange Squash, Control scored maximum.

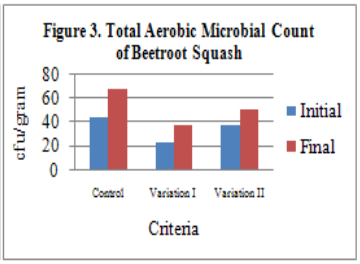
Quality parameter assessment of Chitosan products.

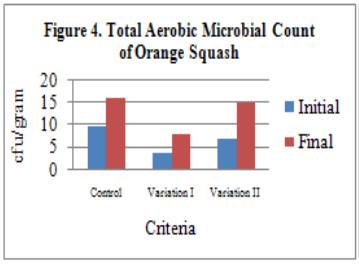
Nutrient analysis. The proximate analysis was done for the developed products i.e. control, variation I (crab based chitosan) and variation II (prawn based chitosan). The moisture content of the Cereal Bar with value addition of prawn had comparatively low content (2.75 gram per cent) when compared with the other two products. The moisture content of the Beetroot squash, Orange Squash and Ice cream were high since they are liquid based products and the value ranged from 48.08 to 63.25 gram per cent.The Ash content was low except in Ice cream. The ash content of the ice cream was between 2.58 to 3.45%. The other variations of the developed products had ash content ranging from 0.2 to 2%. The carbohydrate content of the developed products was high in comparison with other proximate components. Carbohydrate content in the variation II had the maximum level of carbohydrate followed by variation I and Control. The carbohydrate content of the Cereal bar was 88.12g/100 grams for the variation I, 84.55g/100 grams for variation II and 76.90g/100 grams. Similarly Beetroot squash and Orange squash had 9 to 14g/100 grams. The increase in the carbohydrate level may be due to the polysaccharide nature of the chitosan. The protein content of the developed products was in the normal range. There was a slight increase in protein content in the products with variation I. The fat content in the Ice cream was more when compared with the other products (12.60 to 13.80 g/ 100 grams). Within each product the variations had similar fat content. The crude fiber was assessed for cereal bar and there was not much difference between the control and variations. The values ranged from 4.0 to 4.60 grams per cent.

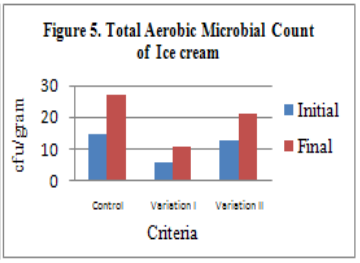
Quality Indices. Quality of the product was determined with various parameters such as moisture, total solids, total acidity, pH, ascorbic acid and milk fat. The initial and final analysis was done in 20 days for Cereal Bar and 15 days for rest of the products. The moisture content of the products did not much variation on storage. The value of the cereal bar ranged from 4.0 to 4.60 grams. The moisture content of the beetroot squash was high and the total solids content were between 36.75 to 37.45 gram per cent. The acidity level of the control was more when compared with the other variations. Similarly the ascorbic content in both the variations were more when compared with the control. Thus the prawn shells based chitosan was stable when compared with control and Variation I. The moisture content of the orange squash in the variations was more than that of control. The pH of the variations was within the standard limit of BIS i.e. 3.30 to 6.60. There was only a slight variation in pH after 15 days in the variations. The ascorbic acid content in the control was more i.e. 18.6mg/100ml whereas in both variations I and II it was 13.5mg/100ml. The pH of the ice cream increased after 15 days. Final acidity had increased. The moisture content was more in variations I and II when compared with the control. The milk fats of the variations was decreased.

Shelf life determination. Total Aerobic Microbial Count was assessed for the developed products. The count was more in the control samples when compared with the variations. Among the chitosan based products, variation I had more activity level than that of variation II. Orange squash had the minimal microbial load when compared with other products. The initial and final total aerobic microbial count result are represented graphically in figure 2, 3, 4 and 5 for each product. The resulted increase may be due to the proliferation of aerobes due to the utilization of nutritional compounds. The increase in microbial load after storage emphasize the need of proper storage.









Conclusion:

FTIR is an easy method for the evaluation of Degree of Deacetylation but the accuracy of the result depends upon the purity of the product. The antioxidant and antimicrobial activity of the chitosan can be increased with the increase in the concentration level.

Chitosan can be encouraged in the value addition, since it acts as a functional compound and does not interferes with the sensorial factors. It also helps to improve the shelf life of the product.

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