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Physicochemical properties and antioxidant activity determination of jackfruit (*Artocarpus heterophyllus* Lam.) beverage with crab chitosan

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ABSTRACT

Post harvest interventions have been the key to transforming the jackfruit pulp into other food products to increase its economic value. In order to increase its shelf life, modern methods of preservation have been applied including the use of biopolymers. Chitosan because of its biological and chemical properties has been utilized and studied in different fields and industries. In this study, chitosan was added to the processing of jackfruit beverages at different levels. The physicochemical properties such as pH, TSS, and Titratable Acidity of jackfruit beverage with chitosan were determined for fifteen days at different storage conditions. The results showed that chitosan significantly affects the pH, TSS but not the Total Titratable Acidity of the jackfruit beverage at ambient condition. Moreover, chitosan significantly affects the pH, TSS and Titratable Acidity of jackfruit beverage stored at refrigerated condition. Consequently, the jackfruit beverage with increasing chitosan levels regardless of the storage periods and conditions showed an increasing free radical scavenging activity. The jackfruit beverage with 3g chitosan stored in refrigerated condition had the highest antioxidant activity from 421.94 to 470.71 µmol TE/100g after five days of storage. The study revealed that chitosan, when utilized, provides stability and consistency to the jackfruit beverage thereby enhancing its functional properties regardless of storage conditions. The jackfruit beverage that contains 3g of crab chitosan is more stable compared to other treatments and the presence of E. coli and Salmonella has not been detected.

Keywords: crab chitosan, jackfruit pulp, physicochemical properties, antioxidant activity, shelf life, *Portunus pelagicus*

INTRODUCTION

Chitosan exhibits a variety of physicochemical and biological properties, therefore it has found numerous applications in various fields such as waste and water treatment, agriculture, fabric and textiles, cosmetics, nutritional enhancement, and food processing. In addition to its lack of toxicity and allergenicity, its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields (Singla and Chawla, 2001 as cited by Senel and McClure, 2004)[1]. The consumption of foods that promotes a state of well-being, better health, and reduction of the risks of diseases has become popular as the consumer is becoming more health conscious (Ryan et. al., 2009) [2]. Today, preference of consumers for foods without chemical preservatives has led to the discovery of new natural microbial agents. The antimicrobial and antifungal of chitosan and its degradation products such as, chitooligomers

and low molecular weight chitosans have been studied by several researchers, with particular emphasis on their ability as a food preservative (Srinivasa and Tharanathan, 2007)[3].

Because chitin and chitosan cannot be degraded in the intestine, they can be used as dietary fibers for humans and livestock (Muzzarelli, 1996 as cited by Troger and Niranjan, 2010)[4]. These products have been shown to reduce lipid absorption and to have antigastritic properties (Zacour et al., 1992 as cited by Troger and Niranjan, 2010) [4].The growing awareness and demands towards microbiologically safe and nutritionally sound foods have made the food industry to develop strategies to meet the expectations of the consuming public. Chitosan has caught the attention and interests of researchers in various disciplines as a promising polymeric material with interesting applications and as an ingredient for the development of functional foods. It has a great potential for a wide range of application due to its biodegradability, biocompatibility, antimicrobial activity, nontoxicity, and versatile chemical and physical properties (Dutta et al., 2012)[5] making it worthy of the attention and the utmost interests it gets. In this study, the effects of crab chitosan on the physicochemical properties and antioxidant activity of jackfruit beverage was being determined.

EXPERIMENTAL METHODS

Collection and Preparation of Crab Exoskeleton

The crab exoskeletons were obtained from the processing plant of Eastern Visayas Fresh Seafood Incorporated located in Brgy. Silanga, Catbalogan City, Samar. They were cleaned by removing the remaining meat and other undesirable components. The cleaned crab exoskeletons were sun-dried for 3 to 5 days. The dried crab exoskeletons were crushed into smaller pieces using a mortar and pestle and then sieved. The powdered crab exoskeletons were kept in an air-tight container until used.

Extraction of Chitin and Chitosan

Chitin and chitosan were prepared from the crab shells according to the methods of (Abazinge et al., 2007)[6] with some modifications.

Deproteinization

The powdered crabs' exoskeleton was placed in a 250 ml beaker in sodium hydroxide (4% v/v) and boiled for 1 h in order to dissolve the proteins and sugars to isolate the crude chitin. After boiling the sample in sodium hydroxide, it was removed from the hot plate and allowed to cool inside the fumehood for 30 min at ambient condition.

Demineralization

A 25g sample of crude chitin was demineralized using 100 mL of 5% acetic acid concentration. The sample was soaked for 24 h to remove the calcium carbonate. The demineralized sample was treated with 50 mL of 2% sodium hydroxide solution for 1 h to decompose the albumen into water-soluble amino acids. The remaining chitin was washed with water and drained off. The chitin was further converted into chitosan by the process of deacetylation.

Deacetylation

The deacetylation process was carried out by adding 100 mL of 50% sodium hydroxide solution to the sample and boiled at 100^0C for 2 h on a hot plate. The sample was placed under the hood and allowed to cool for 30 min at room temperature. The supernatant liquid was decanted and the sample was washed continuously with distilled water and filtered in order to collect the solid matter, which is the chitosan. The chitosan was placed in a beaker and dried in a blow dryer at 50-60^0C for 48-72 h. The dried chitosan was kept in an air-tight container until used.

Preparation of jackfruit beverage

Fresh ripe jackfruit was washed with running tap water, brushed to remove the unwanted dirt and rinsed with chlorinated water. The fruit was then cut into halves. The rags and seeds were removed from the fruit and fruit pulp was collected and kept in the freezer until used. One hundred grams (100g) of jackfruit pulp was placed in a blender and added with 600 mL of water and homogenized for one to two minutes. The homogenized pulp was then strained using a cheese cloth and pressed to extract the juice. In a liter of juice, 150g of sugar, chitosan was incorporated into the mixture, and the pH was adjusted to pH 4.4 by adding citric acid. The mixture was then pasteurized at 80^0C for six minutes and hot-filled in a sterilized bottle. The bottles were stored for five days at ambient and refrigerated condition and subjected to Free Radical Scavenging Assay (FRSA).

Physicochemical Analysis

The optimum formulation was added with chitosan at different levels (0, 1, 2, 3, 4 grams) and was evaluated for its physicochemical properties. The Total Soluble Solids (TSS), Titratable Acidity (TA), and pH of the sample were the parameters to be considered for the analysis. The determination was done during the 5^{th}, 10^{th}, and 15^{th} day of storage under refrigerated and ambient conditions.

Total soluble solids (TSS) and pH

The TSS and pH of the beverage were determined using a hand refractometer and pH meter, respectively. A drop of sample was placed on the ATAGO hand-held refractometer and reading of the ˚Brix of the product for TSS was determined. On the other hand, using a pH600 pocket-sized pH meter, the pH of each sample was measured by dipping the electrode into a 15mL jackfruit beverage sample and then each reading was recorded.

Total Titratable acidity (TA)

The titratable acidity of the processed product was determined using a standard titration method of 0.1N NaOH solution (Hamilton and Simpson, 1971)[7].

Determination of Free Radical Scavenging Activity

The samples of jackfruit beverage were sent to the Department of Pure and Applied Chemistry, Visayas State University (VSU), Visca, Baybay City, Leyte for the determination of the Free radical scavenging assay (FRSA).

Ten (10) ml of each treatment samples were prepared. The ratio between the sample and the solvent was 1:10 (v/v). Samples were then homogenized using a blender with 100 ml of the solvent at room temperature. The solvents were 95% ethanol, vinegar (5% acetic acid) and water. The resulting mixture was transferred into a small beaker, allowed to stand for an hour at room temperature and was filtered using Whatman 42 filter paper. The beaker was then wrapped with carbon paper, to minimized degradation of pigments, and stored overnight in the refrigerator.

The antioxidant of all samples was determined using the DPPH radical scavenging assay. Stock solution of DPPH (22.5g/L) was prepared using ethanol/water solvent and the initial absorbance was measured at 517 nm by UV-Vis spectrophotometer (Shimadzu Double Beam Spectrophotometer, UV-210A). 0.1 ml of sample was added to 3.9 ml of DPPH solution in order to initiate the reaction. The resulting mixture was shaken for 5 minutes and allowed to stand at ambient temperature in the dark for 1 hour to complete the reaction of the cellular antioxidants with DPPH. Absorbance was read at 517 nm using 95% ethanol as blank and the antioxidant activity was calculated from the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard curve.

The amount of the sample necessary to react with one-half of the DPPH solution is expressed in terms of the micromole equivalents of the standard Trolox per millimetre of the sample or the Trolox units per gram or TE/100g.

TE/100g = [(μTE) (volume of solvent)/ (mass of sample)] (100)

Microbial Evaluation

The presence or absence of *Salmonella* and *Coliforms* were also determined to check the sanitary condition of each sample. The samples were sent to the Department of Veterinary Medicine Laboratory for the microbial evaluation.

RESULTS AND DISCUSSION

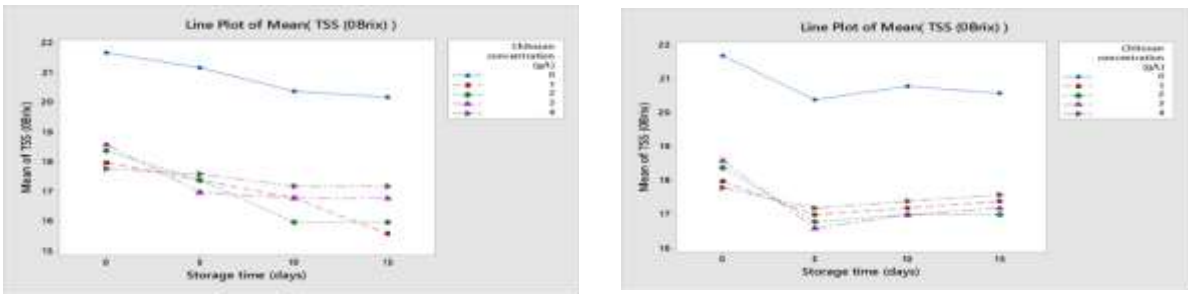
Physicochemical Properties of Jackfruit Beverage with Chitosan

pH. Figure 1-3 depicted the developments of the different physicochemical properties of jackfruit beverage with chitosan stored for 15 days at different conditions. The study conducted by Abd and Niamah (2012) utilizing apple juice revealed that their samples' pH increased over storage time however there is no interaction effect resulted from storage time and chitosan concentration. On the other hand, a study conducted by Ryan et al., 2009, on orange juice revealed that the pH of the juice increased significantly with increasing chitosan concentration. According to Imeri and Knorr, 1988 as cited by Ryan et al., 2009, this effect could be due to the capacity of the chitosan to reduce fruit juice acidity based on its acid-binding properties. When the pH is lower than 6.5, chitosan carries a positive charge along its backbone (Einbu and Varum, 2003 as cited by Ryan et al., 2009). Also, the pH increase undergone by the juices over storage time has been associated to microbial spoilage (Del Caro, Piga, Vacca, and Agabbio, 2004; Cortes et al., 2008 as cited by Ryan et al., 2009). As what has been observed in Figure 1, the pH of the jackfruit beverage is on the increasing rate as storage time and chitosan concentration increased but became stable on the 10^{th} and 15^{th} of storage. The same trend was observed in the beverage stored at refrigerated condition.

(a) (b)

Figure 1. pH of jackfruit beverage with chitosan stored for 15 days at (a) ambient and (b) refrigerated conditions

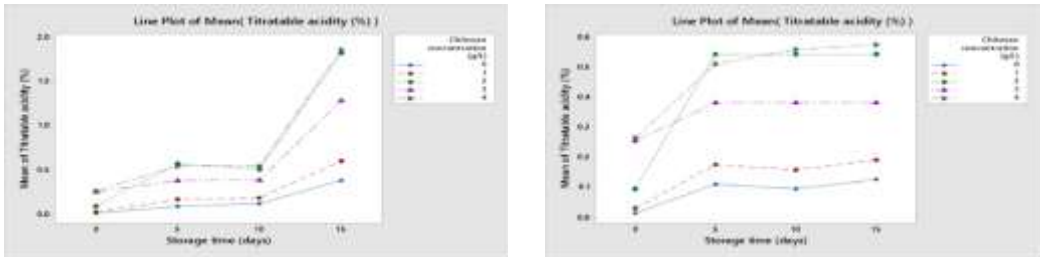
TSS. The TSS of the beverage stored in ambient condition decreases as chitosan concentration and storage time increases. However, in refrigerated condition, the TSS decreases after the 5^{th} day of storage but increases as chitosan concentration and storage time increases. The refrigerated condition, on the other hand, shows that chitosan concentration and storage time significantly affects the TSS of the beverage. An increase in every unit of chitosan concentration will have a corresponding decrease in TSS by 2.84 units while the increase in every unit of storage time will have a corresponding decrease in TSS by 0.27 units for the jackfruit beverage. As shown in Figure 2, the beverage untreated with chitosan has TSS higher compared with those treated ones which only suggests that chitosan reduced the ^{0}Brix value of the jackfruit beverage. These findings are in agreement with the study conducted by Ryan et al., 2009 on fresh orange juice which was observed to have a decreasing ^{0}Brix value. This could be explained by the ability of the positive charged polysaccharide to coagulate suspended solids, increasing the flocculation capacity of chitosan which could bind the sugar (negatively charged) (Sapers, 1992 as cited by Ryan et al., 2009).



(a) (b)

Figure 2. TSS of jackfruit beverage with chitosan stored for 15 days at (a) ambient and (b) refrigerated conditions

%TA. The organic acids present in foods influence the flavor (i.e., tartness), color (though their impact on anthocyanin and other pH-influenced pigments), microbial stability (via inherent pH-sensitive characteristics of organisms), and keeping quality (arising from varying chemical sensitivities of food components to pH) (Murphy and Sadler, 2010). As what has been shown in Figure 3, the %TA of jackfruit beverage stored in ambient condition increases over storage time although at refrigerated condition the %TA decreases on the 10th day but increases on the 15th day of storage. The %TA of the treated beverage at ambient condition, on the other hand, increases as chitosan concentration increases over time and the same was also observed in beverage treated with 4g of chitosan stored at refrigerated condition while those treated with 2g and 3g their respective %TA remains the same throughout the storage period. The beverage treated with 1g chitosan dropped its %TA from 0.176 on the 5th to 0.160 on the 10th but increases again on the 15th day to 0.192. An increase and decline was observed on the titratable acidity of jackfruit beverage during the entire storage period at different storage conditions. The reduction of the titratable acidity is due to the acid-binding properties of chitosan. Chitosan with a partial positive charge has been shown to possess acid-binding properties. The effect of chitosan treatment on the reduction of titratable acidity provides a potential for acidity control in other food systems (Imeri and Knorr, 1988).



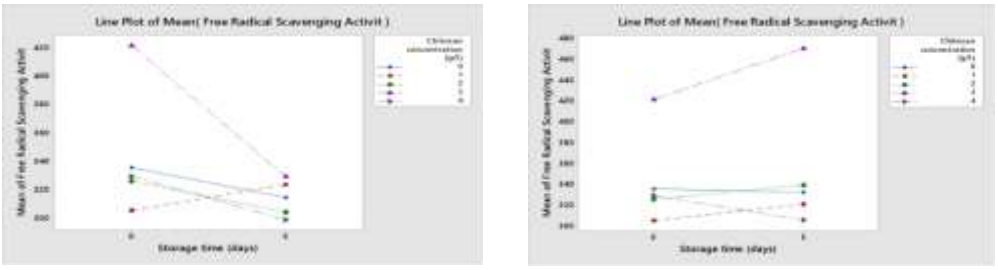
(a) (b)

Figure 3. %TA of jackfruit beverage with chitosan stored for 15 days at (a) ambient and (b) refrigerated conditions

Free Radical Scavenging Activity and Microbial Quality Evaluation

Natural polysaccharides are being utilized more and more in the markets for the reason that they show biodegradability, biocompatibility, versatility, and are found plenty in nature. Their diversity provides a broad spectrum of raw materials that can be used in many biological applications. Chitin and chitosan are important among such polysaccharides (Rajasree and Rahate, 2013). Figure 4 showed the antioxidant capacity of the jackfruit beverage with chitosan at various levels and stored at ambient and refrigerated condition. The initial antioxidant activity revealed that the activity is highest at the beverage treated with 3g of chitosan followed by the untreated, next is the beverage with 4g, 2g and 1g respectively. After five days of storage at ambient condition, antioxidant activity of both the treated and

untreated beverages have dropped except in jackfruit beverage treated with 1g of chitosan which increased. At refrigerated condition, the untreated and the beverage with 4g of chitosan decreased their antioxidant activity after five days of storage on the contrary with those treated with 1g, 2g and 3g. The beverage with 3g of chitosan had the highest antioxidant activity of 470.71 µmol TE/100g after five days of storage at refrigerated condition. According to Park et al., 2004 as cited by Mata et al., (2012) that the free radical activity of chitosan has been attributed to the presence of a protonated nitrogen on carbon number 2, which has the ability to simultaneously bind several free radicals. Some authors have shown that the molecular weight of the chitosan is also an important factor in its antioxidant capacity. Yen et al., (2008) reported that various crab chitosan prepared by alkaline *N*-deacetylation of crab chitin for 60, 90 and 120 minutes and antioxidative activity of the prepared chitosans exhibited antioxidative effects of 58.3%–70.2% at 1.0 mg/mL concentration. The Chitosan prepared for 120 minutes with more amino groups on C-2 position showed the highest antioxidative activity. No presence of *E. coli* and *Salmonella* has been observed in the beverage.



(a) (b)

Figure 4. Antioxidant activity of jackfruit beverage with chitosan stored for 5 days at (a) ambient and (b) refrigerated condition

CONCLUSION

As what has been observed in this study, chitosan produced from the exoskeleton of the blue crab (*Portunus pelagicus*) provided stability and enhanced the antioxidant activity of the jackfruit beverage. In this research, no characterization of chitosan was done such as the study on degree of deacetylation, viscosity, molecular weight and toxicity. These properties may contribute to further enhance the antioxidant activity of jackfruit beverage so it is therefore recommended that further research will be conducted.

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