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EFFECT OF CRAB CHITOSAN ON THE ANTIOXIDANT ACTIVITY OF JACKFRUIT (*Artocarpus heterophyllus* Lam.) BEVERAGE

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ABSTRACT

Chitosan is a very potential biopolymer for the beverage industry because of its chemical and biological properties. In this study, chitosan was obtained from the dried exoskeleton of blue crab (*Portunus pelagicus*) with a recovery yield of 55.35% and was utilized in the production of jackfruit beverage. Determination of the free radical scavenging assay (FRSA) of chitosan-treated jackfruit beverage kept for five days at ambient and refrigerated condition was undertaken. The FRSA of chitosan-treated beverage increased as the level of chitosan increased regardless of the storage conditions employed. The jackfruit beverage with 3g chitosan and stored in refrigerated condition had the highest antioxidant activity from 421.94 to 470.71 µmol TE/100g after five days of storage. Increasing the chitosan level to 4g, a lower FRSA was noted in samples stored at ambient and refrigerated conditions.

Keywords: jackfruit beverage, free scavenging activity, chitosan

INTRODUCTION

The huge amount of seafood wastes generated by the seafood industry has contributed a lot to the on-going problem in waste management. The wastes comprised of heads, bones, and skin from finfish and the exoskeleton, cephalothorax, and carapace from crab, shrimp, and lobster. It is estimated that the annual discards from the world fisheries exceed 20 metric tons, equivalent to 25% of the total production of marine capture fisheries (Venugopal, 2011). Crustacean processing produces about 40% of shell waste (Gildberg and Stenberg, 2001 as cited by Troger and Niranjan, 2010) while the global annual production of shell waste from crustacean processing is estimated to be 1.44 million metric tons dry weight (Knorr, 1991; Rodde et al., 2008 as cited by Troger and Niranjan, 2010).

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The seafood wastes not only generate unpleasant smells but also become an eye sore to the residents living in the community and tourists as well. The constant dumping of these wastes in the landfills creates management and environmental concerns associated with ground and drinking water pollution (Abazinge et al., 2007). Since the biodegradation of these wastes is very slow, accumulation of large quantities has become a major concern in the seafood processing industry. The use of these wastes for renewable products such as biopolymers is a dual-purpose opportunity (Troger and Niranjan, 2010). These shell wastes are potential sources to isolate chitin and are currently utilized for the commercial-scale chitin production as well as production of chitosan and their oligomers (Kim and Mendis, 2006).

Chitin and its deacetylated product chitosan, as well as their derivatives, have found varied applications in agriculture, food processing, biotechnology, chemistry, cosmetics, dentistry, medicine, textiles, veterinary medicine, and environmental sciences. Their food uses cover a wide range of applications, including control of microbial deterioration, inhibition of lipid oxidation, emulsification, thickening, stabilization of color, and as dietary supplements (Venugopal, 2011). Figure 1 shows the chemical structure of chitosan.

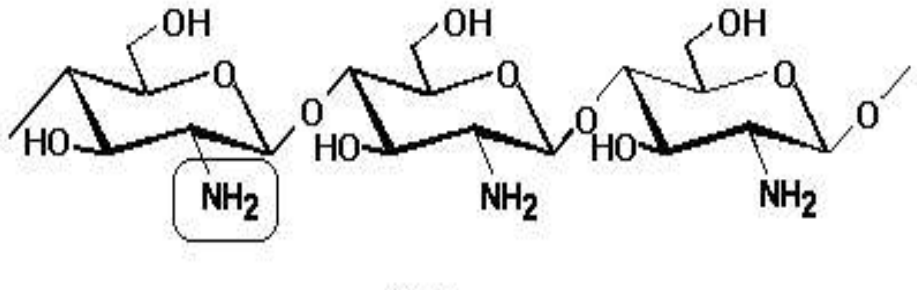

Figure 1: Chemical structure of chitosan

The 'Oxidative Stress' can be defined as the imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage. In humans, oxidants are commonly formed during aerobic metabolism but their amount increases in presence of pathophysiological conditions. Among oxidants, free radicals are highly reactive species capable to oxidise nucleic acids, proteins and lipids, causing degenerative diseases such as cancer, heart diseases, dermal disorders and ageing (Heim et al., 2002 as cited by Genova et. al., 2012).

Antioxidant activity is one of the well-known functions of chitosan. Many studies have shown that chitosan inhibits the reactive oxygen species (ROS) and prevent the lipid oxidation in food and biological systems. Several mechanisms about the antioxidant action of chitosan have been proposed (Kim and Thomas, 2007 as cited by Rajalakshmi et. al., 2013). Potent sources of

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natural food additive compounds have been found in several types of natural material. One of these is chitosan (Mahae et al., 2011). The trend to go for potent, naturally derived antioxidant molecules over those of synthetic origin is ever increasing. To this class belong chitosan and several of its derivatives, which being safe and non-toxic offer protection from free radicals, thus retarding the progress of numerous chronic diseases (Tiwari, 2004 as cited by Prashanth and Tharanathan, 2007).

Microbial contamination, microbial growth and oxidation of lipids in foods during processing and storing are the major causes of food-borne illnesses and loss of shelf life. A considerable number of antimicrobial agents and antioxidants are therefore permitted by regulatory agencies to minimize the deterioration of food quality. But, synthetic preservatives are suspected of being responsible for some severe toxic effects. Thus, natural preservatives such as chitosan against different groups of microorganisms, such as bacteria, yeast and fungi have received considerable attention in recent years (Roller, 2001 as cited by Toan et al., 2013).

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. In particular, its antioxidant activity has been well-documented by different researchers in different fields and has been found to have a promising effect. However, there is still a little documentation on the application of chitosan in the beverage industry.

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). This study reports the enhancement of crab chitosan on the antioxidant activity of the jackfruit beverage.

METHODOLOGY

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

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Chitin and chitosan were prepared from the crab shells according to the methods of Abazinge et al., 2007 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). Figure 2 shows the process flow for the production of jackfruit beverage.

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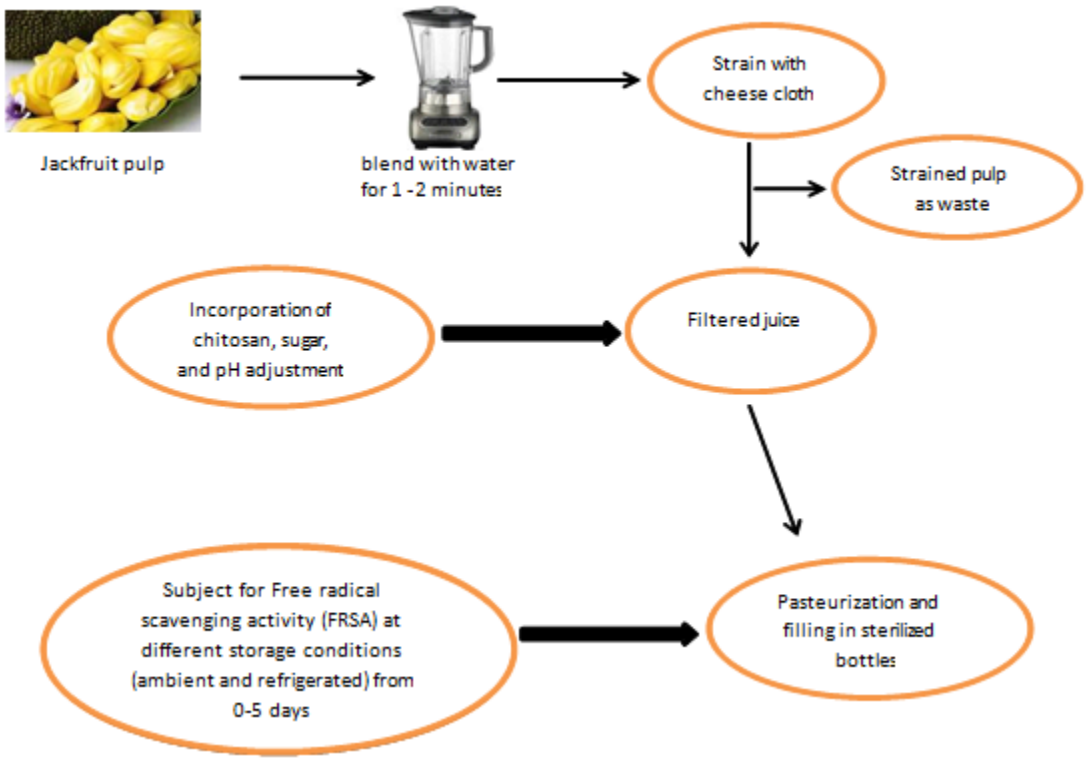

Figure 2: Process flow for the production of jackfruit beverage

Determination of Free Radical Scavenging Activity

The initial and after five days of storing period at ambient and refrigerated condition for the jackfruit beverage was conducted. The samples 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

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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)

Statistical Analysis

The data for the antioxidant activity of the jackfruit beverage was analyzed using the Statistical Analytical Software version 9 (SAS, 2008) for the RS REG and Analysis of Variance (ANOVA) while Statistica version 6 software was used for the graphical presentation of the contour plots, and minitab17 for the line plots.

RESULTS AND DISCUSSION

Production and Utilization of Chitosan

A 55.35g of dried chitosan was obtained from a 100g dried blue crab's exoskeleton yielding a 55.35% recovery lower than what is reported by Ladres, 2004, which has a 69.8% recovery. According to Abd and Niamah, 2012, the slight decreased in the chitosan production could be attributed when acid and base concentrations are increased during the extensive demineralization and deproteinization processes, the removal of protein, lipid, pigments and other inorganic acid which result in the production of a whiter colored end product. Shown in Figure 3 are the images of crab shell, chitin and chitosan powder respectively.

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A. Crab shell B. Chitin C. Chitosan

Figure 3: Images of crab shell (A), chitin (B), and chitosan (C)

Free Radical Scavenging Activity

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). In this study, the chitosan produced was utilized in the production of jackfruit beverage.

Shown in Table 1 is 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 (Figure 4).

Apparently, there is no significance in the linear, quadratic and cross product terms of the antioxidant activity for the jackfruit beverage stored at ambient and refrigerated condition as shown in Table 2. It was also confirmed in the parameter estimates (Table 3) that antioxidant activity of the jackfruit beverage is not significantly affected by chitosan and storage time since no interaction was observed. Although, there was no statistical significance but the data shows that crab chitosan added at a certain level in the jackfruit beverage has the capacity to enhance and increase the antioxidant activity of the beverage. Figure 5 shows that over time, a lower level

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of chitosan will result to a lower antioxidant activity of the jackfruit beverage but an increased level of chitosan will also increase the antioxidant activity of the beverage both at ambient and refrigerated conditions.

There have been many documented studies about the antioxidant capacity of chitosan. 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.

Table 1: Free Radical Scavenging Activity of jackfruit beverage as influenced by the levels of chitosan at different storage condition

Storage time (days)	Chitosan level (g)	FRSA (µmol TE/100g)
At ambient condition:		
0	0	335.84
0	1	305.75
0	2	326.39
0	3	421.94
0	4	329.82
5	0	314.93
5	1	324.11
5	2	304.52
5	3	330.07

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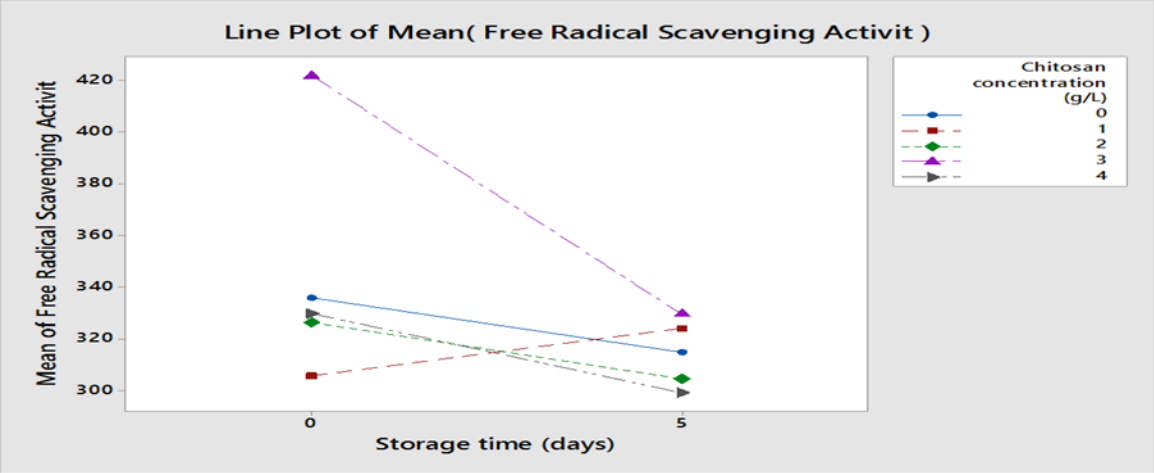
5	4	299.42
At refrigerated condition:		
5	0	332.94
5	1	321.30
5	2	339.53
5	3	470.71
5	4	306.06



(a)

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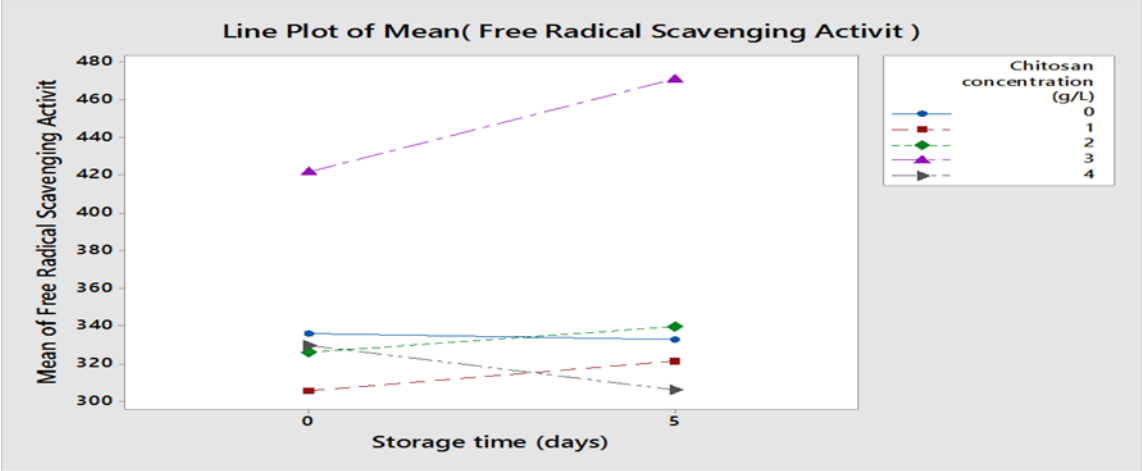

(b)

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

Table 2: Analysis of Variance (ANOVA) of the free radical scavenging activity of the jackfruit beverage with chitosan at different storage condition

Regression	Free radical scavenging activity
At ambient condition:	
Linear	0.83^{ns}
Quadratic	0.17^{ns}
Crossproduct	0.57^{ns}
Total Regression	0.60^{ns}
At refrigerated condition:	
Linear	0.26^{ns}
Quadratic	0.48^{ns}
Crossproduct	0.00^{ns}
Total Regression	0.25^{ns}

ns=not significant

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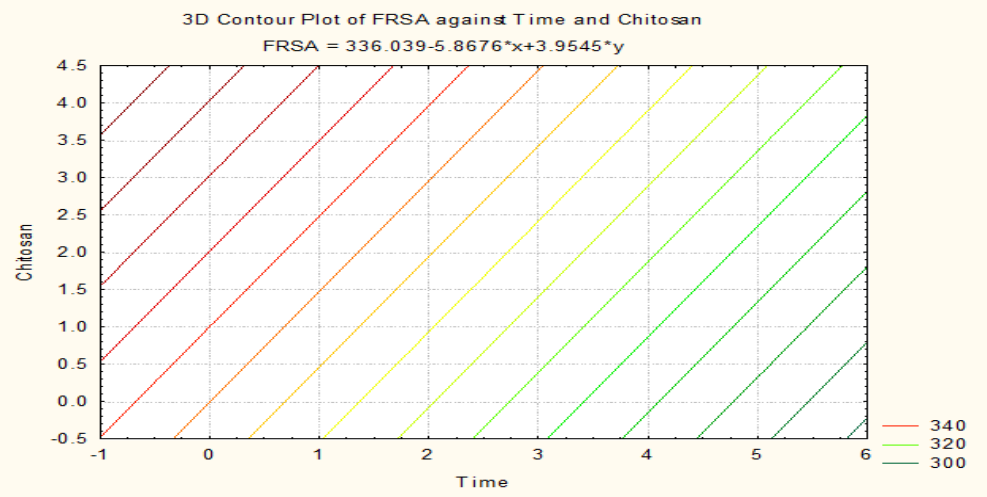
Table 3: Parameter estimates of the free radical scavenging activity of the jackfruit beverage with chitosan at different storage condition

Regression	Free radical scavenging activity
At ambient condition:	
Intercept	9.58**
Time	-0.08^{ns}
Chitosan	0.71^{ns}
time*time	-
chitosan*time	-0.75^{ns}
chitosan*chitosan	-0.41^{ns}
At refrigerated condition:	
Intercept	5.39**
Time	0.16^{ns}
Chitosan	0.83^{ns}
time*time	-
chitosan*time	-0.03^{ns}
chitosan*chitosan	-0.70^{ns}

**=significant at 1% ns=not significant



(a)

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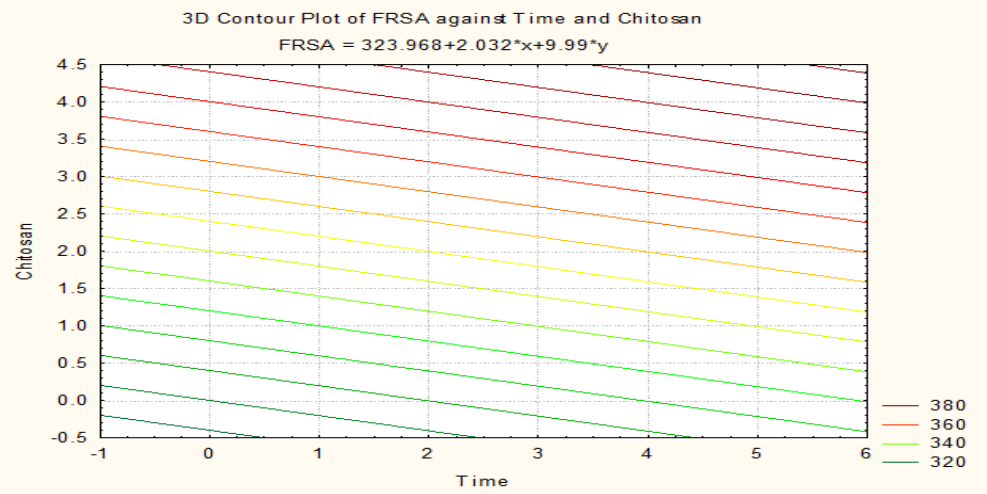

(b)

Figure 5: Contour plots for the antioxidant activity of jackfruit beverage with chitosan at (a) ambient and (b) refrigerated conditions

CONCLUSION

Chitosan was produced from the exoskeleton of the blue crab (*Portunus pelagicus*) with a yield of 55.35%. It 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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