Application of Edible Coating Bases Extract of Lindur (*Bruguiera gymnorrhiza)* and Chitosan on Peeled Off Shrimp

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ABSTRACT- ***Shrimp, one of fishery product commodities has been processed in dried and freezed in the forms of whole fresh, head-off tail on and peeled off. Peeled off shrimp is usually sold at supermarkets and displayed at an air conditioned equipped with lights. This condition causes the temperature of display room increase more than 10°C from its initial temperature (-1.6°C), therefore the products presented will suffer a quality degradation. Edible coating can be applied as a packaging to protect shrimps from any damage during storage, to extend the storage period and maintain products' quality. Besides chitosan, the extract of fruit of lindur plant (that belongs to mangrove group) is suggested as have preservative and antimicrobial activities. In the present study, edible coating was formulated using a combination of chitosan (0 %, 1 %, 2 %) and Lindur fruit extract (0 %, 1 %, 2 %). The observation were conducted at one day and seven days of storage using the parameters of TVB value, pH, TPC, and organoleptic characteristics. The results showed that edible coating with 2 % of lindur extract without chitosan (K0L2) is able to inhibit bacterial growth at storage temperature of ±10°C . The values of TVB, TPC, pH, and sensory (texture, colour, odor and general acceptance) characteristic are significantly different from those of other treatments (p < 0.05) after one week storage. Lindur extract of 2% is the optimum concentration to inhibit the increase of microbial count. This study demonstrated that lindur's extract can be an alternative as antibacterial materials and it is effective as a natural preservative for peeled shrimp.***

Keywords— edible coatings, lindur (*Bruguiera gymnorrhiza*), chitosan, peeled off shrimp.

1. INTRODUCTION

Peeled off shrimp is one product of shrimp that usually sold at supermarkets and presented at an air conditioned food display equipped with lights. This condition causes the temperature of display room increase more than 10°C from its initial temperature (-1.6°C), therefore the products presented will suffer a quality degradation. Other conditions being also a problem in shrimp is the occurrence of color change, the increase in volatile base nitrogen, change to structure and the release of some liquid containing shrimp's flesh solid known with the term of drip (Erdogdu et al. 2004). Micro-organism will change the flesh protein structure during storage and will generate an undesirable smell (Serdaroglu and Felekoglu 2005).

Edible coating is important to extend the storage period and maintain products' quality. This edible coating incorporating antimicrobial compounds provides a novel way to improve the safety and shelf-life of ready-to-eat foods and can also be applied to inhibit the growth of microorganisme on the surface of fresh processed products (Cagri et al. 2004). This edible coating is currently also widely applied as a biodegradable packaging material since it is environmentally friendly and harmless (McHugh and Krochta 1994).

Chitosan has widely attracted much interest since it was obtained a good film and coating on food by improving the quality and timelength of a food consumption (Abugoch et al. 2011). In addition, as it does with the other natural polymers, chitosan also offers a great opportunity in supplementing the degree of food nutrition, biodegradability, and compatibility towards environment. Chitosan and its derivative compounds are having anti-bacterial activities (Bourbon et al. 2011; López-Caballero et al. 2005). Chitosan characteristics will form a film and anti-microba activity as a potential source for coating material and a natural food products preservative.

Beside chitosan, another material might have a characteristic of natural anti-microba source can be generated from mangrove plant. A high content of bioactive components such as steroid, triterpen, saponin, flavonoid, alkaloid and tannin can inhibit the growth of various bacterial species (Sivaperumal et al. 2010). One of mangrove plants not widely applied in extract form is lindur. Fruit from mangrove species (*Brugiera gymnorrhiza*) called lindur has a great potential to developed as food of carbohydrate source and anti-oxidant. Lindur fruits contain antinutrient, namely tannin and hydrogen cyanide (HCN) that of which concentration should be reduced first before processing to be safe for consumption.. In this study, it is expected that the generated edible coating is capable of extending the storage period of peeled off shrimp. The objective of this study is to find out the effect of the application of lindur's extract and chitosan to generate the best edible coating in maintaining the quality of peeled off shrimp.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study is shrimp of black tiger species (*Penaeus monodon*) were obtained from local markets, lindur fruit (*Bruguiera gymnorrhiza*) were obtained from Kei Island, South-east Mollucas (Indonesia), chitosan from biotech surindo Co. (Indonesia), dietile ether, pH indicator paper, Whattman paper no. 41 and 42, ethanol 96%, acetic acid 100%, phosporic acid 85% (H_3PO_4), NaOH, HCl 37%, glycerol 87%, sorbitol 70%, trichloroacetate acid (TCA) 7%, nutrient agar, and nutrient broth.

The determination used in this study were pH, TPC and TVBN, completed with organoleptic test to find out the consumers favorable test (reference).

2.2 Methods

The methods of this study consist of two experimental phases, namely preliminary and mainly experiments. Preliminary experiments includes characterization of raw materials (chemical analysis), characterization of chitosan to get the good deasetilation degree (≥80%), the making flour lindur from lindur fruits to make maseration process efectively, the making of extract from LINDUR flour, and testing of antimicrobial activity. The main experiments includes the application of chitosan by adding lindur extract for the making of edible coatings as well as the application of edible coatings on peeled shrimp. Below is the description of each experimental phase.

2.2.1. Lindur sample preparation

The lindur skin fruit peeled and soaked in water, then boiled for 60 minutes. After the boiling process, lindur crushed and dried fruit to dry. Then, once it is dry, lindur is blended till it transforms into a powder and sifted using a 65 mesh sieve. The extraction of sample is done by maseration method. 20g of flour is weighed, soaked in 100 ml of ethanol 95% for 24 hours. This treatment is done for 48 hours. Filtrate obtained is put together then evaporated to get an ethanol's extract. Weighing the empty flask and the flask after the initial weight derived extracts. Extracts evaporated in a flask and then dissolved in 10 ml ethanol and sonication the extract soluble in ethanol. The extract resulting from evaporation is refrigerated in a dessicator to be analyzed further (Malangi et al. 2012).

2.2.2. Properties of chitosan

Chitosan was obtained from Biotech surindo Inc. (Indonesia). Chitosan prepared was analyzed for sensory appearance to assess its color as white or yellowish. Moisture, protein and ash content were determined according to the method of AOAC (2000). Degree of deacetylation of the chitosan was determined following the method of Shigemassa, Matsuura, and Sashiwa (1996) to assess its DD ≥ 80%. DD chitosan measurement using FTIR (Fourier Transform Infrared Spectroscopy PerkinElmer C69526) with the frequency of 4000-400 cm-1.

2.2.3. Determination of antimicrobial activity

This study was conducted to determine the antibacterial activity of chitosan and extracts lindur against Salmonella thypii and Escherichia coli using the well diffusion method. Isolate of *Salmonella thypii* and *Escherichia coli* respectively inoculated into 9 ml of NB medium, and then incubated at 37°C for 24 hours. Culture with bacterial count 10^5 cfu / ml in 1 ml put into a petri dish and poured as much as 20 ml agar medium, allowed to set and then made a well with a diameter of 5 mm, incubated at 37 ° C and observed clear zone formed after 24 hours (Bharathidasan and Panneerselvaam 2012).

2.2.4. Biochemical quality evaluation of lindur and peeled shrimp

Moisture content was determined by drying a known amount of homogenized sample to constant weight in an air oven at 105 °C for 16 h (AOAC, 2000). Percentage crude protein was determined by total nitrogen method (AOAC,

2000). Crude fat content was extracted with petroleum ether using AOAC method (2000). Ash content was determined by heating at 550°C in a muffle furnace (AOAC, 2000). Free fatty acid (FFA) value was determined as per AOCS (1989) to assess the hydrolytic rancidity. Carbohydrate content was determined by luff schroll methods (AOAC 2000). Total volatile base (TVB) analysis was carried out according to the method proposed by Conway (1950). pH was measured by homogenizing the sample in distilled water (1:2 w/v) by using a glass electrode digital pH meter (*Autech Instrument*) as described in AOAC (2000).

2.2.5. Microbiological analysis

Peeled shrimp sample (10 g) was transferred aseptically to a stomacher bag (Seward Stomacher circulator bag, Model No.400, England) to which 90 ml of sterile normal saline (0.85%) was added and homogenized for 60 s at 230 rpm using a lab stomacher blender (Seward Stomacher 400 Circulator, England). Tenfold serial dilution was prepared with normal saline (1:10 with 0.85%) and used for total mesophilic counts analysis. For this, shrimp homogenate sample (0.5 mL) of appropriate dilutions were spread evenly on the surface of dry plate count agar media (PCA, HiMedia, HiMedia Laboratories Pvt. Ltd., Mumbai, India). Total mesophilic count was determined after incubating the plates at 37°C for 48 h as per Fardiaz (1993).

2.2.6. Application of edible coating on peeled off shrimp

In this study the anti-microba activity of chitosan (0%, 1%, 2%) is searched, then followed by any anti-microba activity of lindur's extract (0%, 1% and 2%). Each solution that is previously prepared is then converted into coating by soaking it into a solution of 40°C for 5 seconds. Then a draining is done at ambient temperature for 1 minute. After soaking, the excess of material after the coating is left to disposal. The product is then let to become cold till edible coating adhered and it is kept at a chilling temperature (±10°C).

2.3. Sensory analysis

Sensory analysis of the stored peeled off shrimps was carried out by thirty semi-trained researchers. The samples were served in a coded and covered plate. The sensory attributes evaluated were texture/appearance, color, odor and overall. The panelists were asked to assign a score of 0-7 as prescribed by Meilgaard, Civille, and Carr (1999). The overall impression of the product on the assessor was estimated as overall acceptability, by adding the scores for all the attributes and dividing by the total number of attributes. A high score (7-6) was given to fish with no off-odors, a score 5 to fish with a flat and neutral odor and scores below 5 to fish with offodors. An overall acceptability score of below 5 corresponded to unacceptable quality. The test is done from the beginning (day 1) up to the end of product storage (the 7th day).

2.4. Statistical analysis

The statistical analysis of the data was performed through an analysis of variance (ANOVA) using IBM SPSS Statistics Software (Version 20.0, IBM SPSS Inc, Armonk, NY, USA) (SPSS 2000). Duncan's multiple range test was used to detect differences among mean values of films. The p-value of the test was ≤ 0.05.

3. RESULTS AND DISCUSSION

3.1 Chemical analysis of Flour and lindur fruit

Based on the test results of the chemical analysis which includes moisture content, ash content, protein, fats and carbohydrates found that fruit and flour lindur having the specifications listed in Table 1.

Table 1. Chemical analysis of lindur fruits and lindur flour

Analysis	Lindur fruits	Lindur flour
moisture (%) (wb)	59.49	13.75
ash (%) (wb)	1.35	1.05
protein(%) (wb)	2.17	4.46
fats (%) (wb)	0.27	0.03
carbohydrates (%) (wb)	14.85	47.43
Hydrogen cyanide (ppm)	19.26	5.59
Tannin (mg)	34.11	25.25

3.2 Characterization of chitosan

Tabel 2 Characterization of chitosan

Character	Parameter	
	Chitosan commersial*	Chitosan used**
Colour	Light brown to white	Light brown to white
Particle size	Flake to powder	Flake
Deasetilation degree	≥ 80-85%	80,25 %
Moisture content	≤ 10%	13,60%
Ash content	≤ 2%	1,67%
pH (1%)	7-8	7

Source : * Protan Laboratories *in* Suptijah (1992)
** Proximate Analysis of chitosan used

The result of this analysis showed that chitosan has moisture content 13.60%, ash content 1.67%, and deasetilation degree 80.25%. Particle size will affect the solubility of chitosan, more smaller is more easily soluble of the solvent. The ash content affected by demineralization of water used when neutralizing pH. Demineralization process that will effectively eliminate many minerals (Angka and Suhartono 2000), so that impurities can be reduced so the performance of chitosan will be optimal. Water used for neutralization must not contain minerals because it can increase the mineral content in the material, thus increasing the amount of impurities (Suptijah 2006). Degree of deacetylation is one of the important quality parameters for chitosan. High degree of deacetylation shows purity (Bastaman 1989). DD of chitosan determine how many acetyl groups were loss during the process of deacetylation of chitin. Analysis of test results show dd of chitosan was 80.25 %, according to the quality standards set by the Laboratories PROTAN ≥ 70%. Muzarelli and Peter (1997) states that the greater the degree of deacetylation, the chitosan will be more active because of the amine group which replaces the acetyl group. Amine group is more reactive than acetyl group because of the lone pair on the nitrogen atom in the structure of chitosan is well known as antibacterial agent.

3.3 Antimicrobial Activity

Based on table 3, the result of this analysis showed that inhibition zone diameter of lindur extract and chitosan on the growth of bacteria *S. aureus* and *Salmonella* is too small, so its effectiveness is low, especially at low concentrations. The inhibition zone diameter of chitosan 1% is found to be 8.30 mm on *Salmonella* and 6.98 mm on *S. aureus*. Inhibition zone diameter of 2% chitosan is found to be 6.48 mm on *Salmonella* and 6.57 mm on *S. aureus*. Whereas chitosan 3% has no inhibition zone diameter. The results showed that the ethanol inhibit the growth of test bacteria, so it is not affecting the antibacterial activity of the extract compounds lindur. This study aims to determine the antibacterial activity of chitosan and lindur extract on the growth of test bacteria. Inhibition zone diameter lindur extract and chitosan on the growth of bacteria *S. aureus* and *Salmonella* is too small so its effectiveness is low, especially at low concentrations. Greater inhibition of chitosan against *Salmonella* compared with *S. aureus* at concentrations of 1 % and 2 %. This suggests that *Salmonella* is more resistant to antibacterial compounds in chitosan compared with *S. aureus*. Antibacterial activity of chitosan can be influenced by the degree of deasetilation. This is consistent with research conducted by Hongpattarakere and Riyaphan (2008), chitosan has the highest DD shows the lowest of MIC (minimum inhibitory concentration), both against *Escherichia coli, S. Aureus* and *C. Albicans*. Yuliana (2011) in his research also revealed that the chitosan with DD 99.36 % inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* greater than the dd 87.81 %. Chitosan which has a high value of deacetylation degree will be increase the antibacterial activity, with the increase of total NH_2 protonated (Tsai 2002). Chitosan that used in testing having the degree of deacetylation 80.25%, is not high enough so that the antibacterial activity has not showed up.

Tabel 3 diameter inhibition of chitosan in acetic acid to *Salmonella* and *S. aureus*

Concentration of chitosan in acetic acid	*Salmonella*	*S. aureus*
0%	0 mm	0 mm
1%	8.30 mm	6.98 mm
2%	6.48 mm	6.57 mm
3%	0 mm	0 mm

Based on table 4, inhibition zone diameter of lindur's extract 1% is found to be 7.55 mm on *Salmonella* and 7.47 mm on *S. aureus*. Inhibition zone diameter lindur extract 2% is found to be 6.75 mm on *Salmonella* and 6.77 mm on *S. aureus*. Inhibition zone diameter of lindur extract 3% is found to be 6.42 mm on *Salmonella* and 6.23 mm on *S. aureus*. Lindur extract inhibit against *Salmonella* compared with *S. aureus*. This suggests that *Salmonella* is more resistant to antibacterial compounds in the extract lindur compared with *S. Aureus*. The low value of the diameter of the inhibition

caused by the extraction process of extract lindur is not going perfectly. Extraction process using ethanol have not been able to separate both the active compounds from fruit lindur. The results showed that the higher concentration of extract lindur will be increase the diameter of bacterial inhibition test. The concentrations of extracts lindur can accumulate active phenolic compounds such as steroids, flavonoids, tannins so the better to destroy the bacterial cell wall. The interaction of these compounds in large quantities can cause lysis of bacterial cell walls with greater effectiveness .

Tabel 4 diameter inhibition of lindur's extract in ethanol solvent to *Salmonella* and *S. aureus*

Concentration of lindur's extract in ethanol solvent	*Salmonella*	*S. aureus*
0%	0 mm	0 mm
1%	7.55 mm	7.47 mm
2%	6.75 mm	6.77 mm
3%	6.42 mm	6.23 mm

Phase II study was to use the best treatment of the phase I study as an antibacterial material peeled shrimp .

3.4 TVB

Total Volatil Base is defined as an easily evaporating alkali compound such as *trymethylamine* or TMA ($(CH_3)_3N$) and *dimethylamine* or DMA ($(CH_3)_3NH$) formed in fish's muscle tissue (Songsaeng et al. 2010). Farber (1965) classify fish freshness based on the value of TVB. Very fresh fishes has TVB values < 10 mgN/100 g, fresh fish 10-20 mgN/100g, fish feasible to consumption is 20-30 mgN/100 g and spoiled fish has TVB values> 30 g mgN/100.A change to TVB degree of peeled off shrimp during storage is shown in Table 5.

Table 5. Changes in the value of total volatile base

Design		**Code**	**One day storage**	**Seven days storage**
Chitosan (%) (w/v)	**Lindur (%) (v/v)**			
0	0	K0L0	26.02 ± 0.99^{f}	189.02 ± 0.26^{f}
	1	K0L1	18.48 ± 1^{a}	44.23 ± 0.06^{c}
	2	K0L2	19.99 ± 0.01^{b}	34.82 ± 0.76^{a}
1	0	K1L0	19.57 ± 0.04^{b}	37.86 ± 0.05^{b}
	1	K1L1	23.02 ± 1^{c}	48.43 ± 0.01^{de}
	2	K1L2	25.56 ± 1^{d}	38.97 ± 0.04^{b}
2	0	K2L0	17.62 ± 1^{a}	48.28 ± 0.79^{de}
	1	K2L1	22.43 ± 0.03^{c}	46.44 ± 0.02^{d}
	2	K2L2	26.61 ± 1^{e}	50.29 ± 0.01^{e}

In each column, TVB value with the same alphabet, were found to be significance level ($p = 0.05$)

The results of diversity analysis shows that there is an actual TVB degree in the nine treatments at the first day storage and the last day of storage ($p. \leq 0.05$). The TVB value of peeled off shrimp increases in line with the length of storage period. The increase of shrimp's TVB degree during storage results in the degradation of protein or its derivative, generating a number of easily vaporable alkali such as ammonia, hystamine, hydrogen sulfide and trimethylamine (Suptijah et al. 2007). According to Songsaeng et al. (2010) the increase in TVB value is the result of an increasingly faster of microbial growth involved in the production of volatile alkali. However, treatment with *edible coating* is proven as capable to protect products from the building of undesired volatile alkali. At 7 day storage, it is found out that a treatment chitosan 0%; lindur's extract 2 % (K0L2) is capable of maintaining shrimp freshness so it is still feasible to consume after being stored in ±10°C temperature.

3.5 pH value

pH degree of peeled off shrimp in this study ranges between 5.88 and 7.76. The change of peeled off shrimp's pH degree during a storage is shown in Figure 3. A diversity analysis on pH degree shows that at the first day of storage there is an actual difference in pH degree ($p \leq 0.05$). Obtained at pH best treatment are without chitosan concentration and extract lindur 2% (K0L2) on the first day of storage. The added lindur's extract treatment is alleged as acid nature so it may decrease pH degree, while an excessive addition of chitosan (2%) may increase the pH degree of products to become more alkali since chitosan contains many amino clusters which has alkali characteristic. At the end of storage,

chitosan 0%; 2% lindur's extract has a pH value of pH is best but the shrimp's ($p \leq 0.05$). The changes in the value of pH on peeled shrimp during storage is shown in Table 6.

Table 6. Changes in the value of pH on peeled off shrimp during storage

Design		Code	One day storage	Seven days storage
Chitosan (%) (w/v)	Lindur (%) (v/v)			
0	0	K0L0	7.73 ± 0.22^{d}	7.76 ± 0.15^{b}
	1	K0L1	6.42 ± 0.39^{ab}	6.11 ± 0.49^{a}
	2	K0L2	6.17 ± 0.37^{a}	6.23 ± 0.02^{a}
1	0	K1L0	6.49 ± 0.12^{abc}	6.37 ± 0.12^{a}
	1	K1L1	6.78 ± 0.24^{abc}	6.31 ± 0.35^{a}
	2	K1L2	6.57 ± 0.01^{abc}	6.32 ± 0.21^{a}
2	0	K2L0	6.98 ± 0.06^{bcd}	6.76 ± 0.07^{a}
	1	K2L1	6.98 ± 0.02^{bcd}	6.67 ± 0.58^{a}
	2	K2L2	7.11 ± 0.37^{cd}	6.63 ± 0.51^{a}

In each column, pH value with the same alphabet, were found to be significance level ($p = 0.05$)

3.6 Total number of bacterial (TPC)

The results of analysis show that TPC of shrimp during the storage ranges between 3.79 log cfu/g and 89 log cfu/g. The coating layer on the surface of peeled off shrimp products allegedly functions as barrier or preventer of bacterial to enter from outside. Peeled off shrimp with no coating applied (K0L0) will be more easily suffering a microbiological contamination from its environment so the bacterial content in the product is higher compared to product with coating applied. Based on TPC score it is said that the number of micro-organism tends to get higher along with the storage period. Graphically, the score of shrimp's TPC during storage is shown in Table 7.

Table 7. Changes in the value of TPC on peeled off shrimp during storage

Design		Code	One day storage	Seven days storage
Chitosan (%) (w/v)	Lindur (%) (v/v)			
0	0	K0L0	7.72 ± 0.23^{e}	6.24 ± 0.02^{c}
	1	K0L1	4.87 ± 0.72^{ab}	4.13 ± 0.18^{a}
	2	K0L2	5.65 ± 0.14^{d}	5.42 ± 0.53^{ab}
1	0	K1L0	5.96 ± 0.03^{bcd}	4.85 ± 0.03^{ab}
	1	K1L1	5.73 ± 0.36^{d}	5.50 ± 0.71^{ab}
	2	K1L2	5.61 ± 0.38^{cd}	5.11 ± 0.09^{ab}
2	0	K2L0	5.55 ± 0.61^{cd}	5.24 ± 0.09^{ab}
	1	K2L1	5.63 ± 0.53^{a}	3.87 ± 0.03^{ab}
	2	K2L2	6.35 ± 0.87^{abc}	4.51 ± 0.18^{b}

In each column, pH value with the same alphabet, were found to be significance level ($p = 0.05$)

A diversity analysis for the nine treatments conducted shows a significant difference in TPC score at the first day storage and the seventh day storage ($p \leq 0.05$). Meanwhile at one-week storage it is found out that the one most effective in preventing bacterial growth is chitosan 0%; lindur's extract 2% (K0L2). It indicates that lindur's extract have a capability to protect shrimp from contamination of bacteri during the storage of shrimp. This is because lindur's extract contains antibacteri substance from phenolic compound. Phenolic compound forms the main antibacteri component in plants' essential oil (Nychas et al. 1995). The application of *edible coating* combined with bio-active components generates the function of food additional ingredient and is able to extend the storage period of highly perishable products (Bourbon et al. 2011).

3.7 Sensory tests

3.7.1. Texture

Overhaul of shrimp meat spoilage by microbial enzymes and may cause changes texture sensory of shrimp meat. Best quality of shrimp have elastic texture, dense and compact. When the deteriorated quality, the texture becomes soft and destroyed.The highest sensory value before storage and during storage is found in the treatment is chitosan 0%; lindur's extract 2% (K0E2) provides significant differences before and after 7 day storage. It might be due that lindur's extract was found to be significantly ($p \leq 0.05$). lindur's extract with agar can form a gel to protect the coating from absorbance of solution into the pores or layers of shrimp flesh. It is this gel formating characteristic that is believed as capable of increasing the sensory value of peeled off shrimp's texture. Tseng et al. (2005) says that secondary oxidation of lipid products (e.g. carbonyls) can cause cross-linking and oxidative modification of proteins, thereby adversely affecting the texture of the muscle tissue. In addition, lindur's extract also has a characteristic of antibacteri substance capable to block the decaying of flesh so it is able to maintain the flesh texture during storage. The change to sensory value on peeled off shrimp during storage is shown in figure 1.

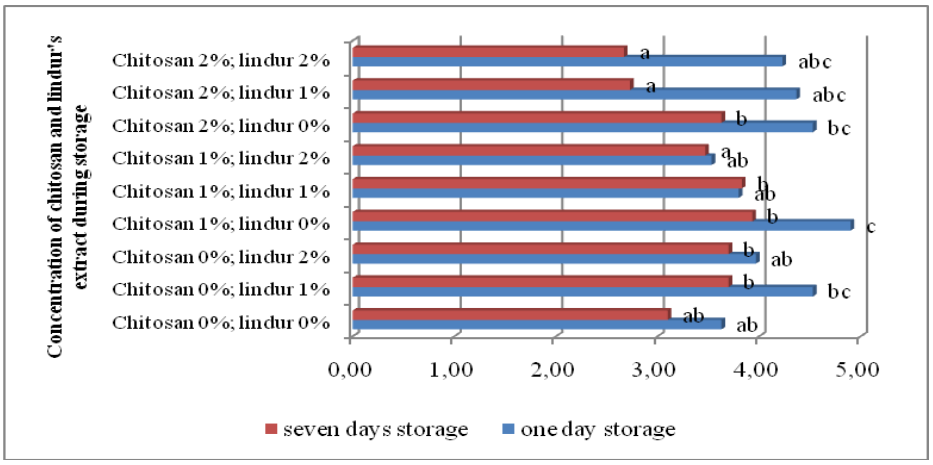

Figure 1. Changes in the value of organoleptic texture peeled shrimp at various concentrations during storage

The results of ANOVA conducted on the organoleptic value of peeled off shrimp's texture with chitosan treatment during cold temperature storage provides a real different value one to another (Sig.< 0.05). It shows that the application of chitosan solution and LINDUR's extract gives a real impact on the organoleptic value of peeled off shrimp's texture before storage and during storage at cold temperature (10°C).

3.7.2. Color

During storage, shrimp will suffer a color change from red to opaque. Niamnuy et al. (2008) explained that the condition was caused by an oxidation to astaxanthin (red pigment) so the red color constituting the astaxanthin's characteristics gone. The change to organoleptic value of peeled shrimp's color during storage is shown in figure 5. Application of a combined chitosan 0%; lindur's extract 2% (K0L2) are capable of maintaining the color of peeled off shrimp's flesh better than in control treatment (without chitosan and lindur's extract) (K0L0) before and during 7 day storage ($p \leq 0.05$). It shows that the concentration of 2% lindur's extract is capable to protected shrimp flesh from discoloration due to an interaction occurring between shrimp flesh and the surrounding environment.

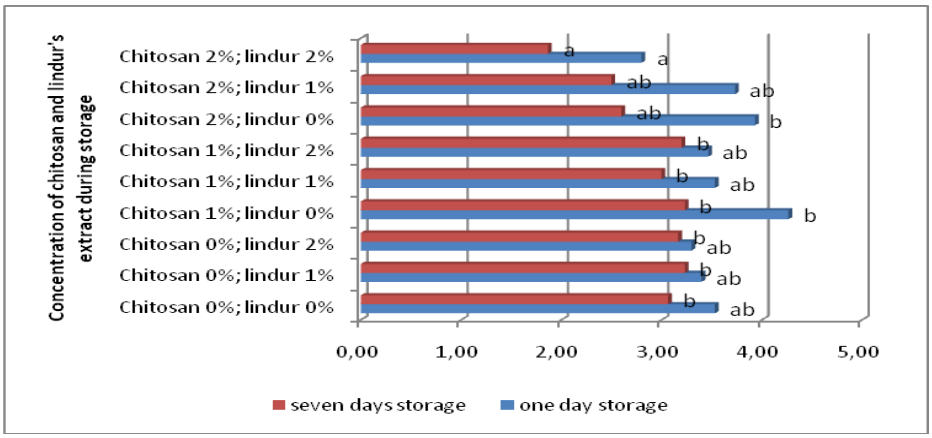

Figure 5. Changes in the value of colour peeled shrimp at various concentrations during storage

3.7.3. Odor

The sensory value of peeled off shrimp odor during storage ranges between 2.03 and 4.53. At 24-hour storage, the lowest sensory value is found in control treatment (K0L0) (without chitosan and lindur's extract) and the highest sensory value is in the treatment of concentration chitosan 0%; lindur's extract 1% (K0E1). Meanwhile after it is stored for 7 days, shrimp's aroma commencing to decay and shows a deterioration of quality, i.e. at control. The bad odor emerged is caused by a degradation of protein into volatile compounds due to decomposing bacterials' activity. The highest sensory value during storage is at chitosan 1%; lindur's extract 1% (K0L1) as well as at the treatment chitosan 0%; lindur's extract 2% (K0L2). This shows that the application of lindur's extract 2% constitutes an optimum concentration capable of keeping any bad smell from shrimp caused by the growth of decomposing microba in shrimp's body. In addition, this condition shows also that anti-bacterial compounds contained in lindur's extract is capable of blocking the work of decomposing bacterial. It is also explained that lindur's extract can inhibit degradation of protein and its derivative (the lowest TVB) so it can maintain the fish's fresh smell. Change to organoleptic value of peeled off shrimp's aroma during storage is shown in figure 6.

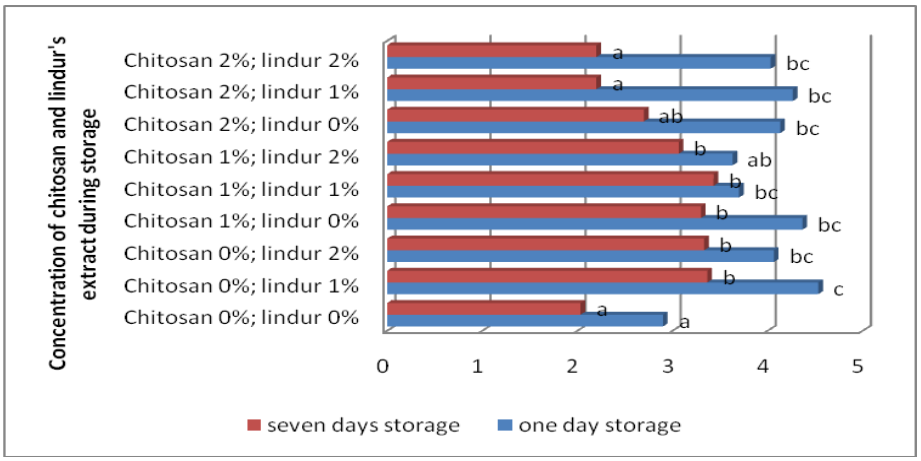

Figure 6. Changes in the value of odor of peeled shrimp at various concentrations during storage

3.7.4. Overall Acceptability

Overall acceptability attribute describes a panelists' total scoring on peeled off shrimp with chitosan and lindur's extract treatment and control. The change to sensory value of peeled off shrimp general acceptance during storage is shown in figure 7.

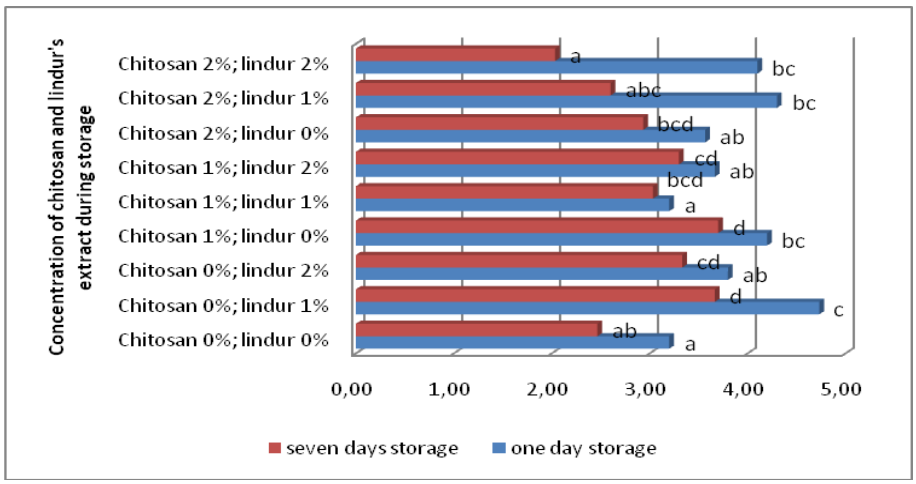

Figure 7. Changes in the value of over-all sensory peeled shrimp at various concentrations during storage

Control treatment (K0L0) and 1% chitosan; 0% lindur's extract (K1L0) are not a panelists' favor, while after 7 day storage it is control (K0L0) and 2% chitosan; lindur's extract 2% (K2L2) that are the most unfavored by panelists. As for in overall term, panelists favor most the concentration treatment of chitosan 0%; lindur's extract 2% (K0L2) (p<0.05) on the scoring of general acceptance by panelists. This condition implies that differences in term of texture, color and aroma have been able to give a real influence to the panelists' fondness level upon the overall peeled shrimp products before and during 7 day storage.

4. CONCLUSION

Interaction between lindur's extract and chitosan was not optimale than no interaction both of them to extend the shelf life during storage. Edible coating from chitosan 0%; lindur's extract 2% (K0L2) are capable of minimizing the growth of bacterial at storage ±10°C with TVB, TPC, pH, and sensory (texture, colour, odor and general accpetance) significantly difference from other treatments ($p \leq 0.05$) after seven days storage. Lindur's extract 2% are the optimum concentration to inhibit the increase in the number of microbes. This study demonstrated that lindur's extract can be an alternative antibacterial materials and effective as a natural preservative used on peeled shrimp.

5. REFERENCES

- Abugoch LE, Cristián T, Maria CV, Mehrdad YP, Mario DD, Characterization of Quinoa Protein-Chitosan Blend Edible Films, Journal of Food Hydrocolloids 25: 879-886, 2011.
- Angka SI, Suhartono MT, Utilization of Marine Waste: Result of Marine Biotechnology, Bogor: Centre of Coastal and Marine Resources, Bogor Agricultural University, 2000.
- AOAC, Official methods of analysis, Vol. II Maryland, USA: Association of Official Analytical Chemists International. Chapter 391-27 (17th ed.), 2000.
- AOCS, Official Methods and Recommended Practices of American Oil Chemists Society (5th ed.). Champaign, USA: A.O.C.S, 1989.
- Bastaman S, Studies on Degradation and Extraction of Chitin and Chitosan from Prawn Shells, England: The Departement of Mechanical Manufacturing Aerononticaland Chemical Engineering.,The Queen University, 1989.
- Bharathidasan R and Panneerselvam, Antibacterial Activity and Bioactive Compounds Analysis od Mangrove Endophytic Fungi *Penicillium janthinellum*, *Aspergillus conicus* and *Phomosis* sp, *International J of Pharma Research and Dev* (IJPRD) Vol 4(04): June-2012 (159-168), 2011.
- Bourbon AI, Pinheiro AC, Cerqueira MA, Rocha CMR, Maria C, Avides MC Quintas MAC, Vicente AA, Physico-chemical Characterization of Chitosan-based Edible Films incorporating Bioactive Compounds of Different Molecular Weight, *J of Food Eng* 106: 111-118, 2011.
- Cagri A, Uspunol Z, Ryser E. Antimicrobial Edible Films and Coatings, *J of Food Prot*, 67: 833-848, 2004.
- Conway EJ, Micro-diffusion Analysis and Volumetric Error. London: Crosby, Lockwood and Son Ltd. p. 220, 1950.
- Erdogdu F, Balaban MO, Otwell WS, Garrido L, Cook-related Yield Loss for Pacific White (*Penaeus Vannamei*) Shrimp Previously Treated with Phosphates: Effects of Shrimp Size and Internal Temperature Distribution. *J of Food Eng*. 64: 297-300, 2004.
- Farber L, Freshness tests in fish as food. (ed. George Borgstrom), 4, 73-75. New York: Academic Press, 1965.
- Fardiaz S, Food Analyze Microbiological, Bogor: Centre of university of Food and Nutrition, Bogor Agricultural University, 1993.
- Hongpattakere T, Riyaphan O, Effect of deacetylation condition on antimicrobial activity of chitosan prepared from carapace of black tiger shrimp (*Penaeus monodon*), *Songklanakarin J Sci and Tech*, 30, 2008.
- López-Caballero ME, Gómez-Guillén MC, Pérez-Mateos M, and Montero P, A Chitosan-Gelatin Blend as a Coating for Fish Patties, *Food Hydrocol*, 19:303-311, 2005.
- Meilgaard M, Civille GV, Carr BT, Sensory Evaluation Techniques (3rd ed.). Boca Raton, Fla: CRC Press. p. 387, 1999.
- McHugh TH, Aujard JF, Krochta JM, Plasticized Whey Protein Edible Films: Water Vapour Permeability Properties. *J of Food Sci*, 59: 416-419, 1994.
- Muzarelli RAA, Peter MG, Chitosan Handbook, European Chitin Society, 1997.
- Niamnuy C, Devahastin S, Soponronnarit S, Vijaya Raghavan GS, Kinetices of Astaxanthin Degradation and Color Changes of Dried Shrimp during Storage, *J of Food Eng*, 87(4): 591-600, 2008.
- Nychas GJE, Tassou CC, Antimicrobial Activity of the Essential Oil of Mastic Gum (*Pistachia lenticus* var. *Chia*) on Gram-positive and Gram-negative Bacteria in Broth and in Model Food System, *International J of Food Microbiol*, 28: 221-232, 1995.
- Serdaroglu M, Felekoglu E, Effects of using Rosemary Extract and Onion Juice on Oxidative Stability of Sardine (*Sardina pilchardus*) Mince. *J of Food Qual*, 28: 109-120, 2005.
- Sivaperumal P, Ramasamy P, Inbaneson SJ, Ravikumar S, Screening of Antibacterial Activity of Mangrove Leaf Bioactive Compounds Against Antibiotic Resistant Clinical Isolates. *World J of Fish and Marine Sci*, 2(5): 348-353, 2010.
- Shigemassa Y, Matsuura H, SashiwaH, Evaluation of Different Ratios from Infrared Spectroscopy for Analysing the Degree of Acetylation in Chitin, *International J of Biol Macromolecules*, 18, 237-242, 1996.
- Songsaeng S, Sophanodora P, Kaewsrithong J, Ohshima T, Effect of Different Storage Conditions on Quality of White-Scar Oyster (*Crassostrea belcheri*). *International Food Research J*, 17: 491-500, 2010.

- SPSS, Statistical Package for Social Sciences for Windows Release 10. Chicago, IL: SPSS Inc.. Headquarters, Wacker Drive, 2000.
- Suptijah P, Descriptive Characteristics and Applications of Chitin-Chitosan, In the Proceedings of National Seminar on Chitin-Chitosan, Bogor: Department of Fisheries Product Technology, Bogor Agricultural University, 2006.
- Suptijah P, Gushagia, Sukarsa DR, Inhibitory Effects Study Power Quality Chitosan against Setback Fillet Catfish (Pangasius hypothalamus) at Room Temperature Storage. Bulletin of Fisheries Technology 11 (2): 89-101, 2007.
- Townley RR, Lanier T, Effect of Early Evisceration on the Keeping Quality of Atlantic Croaker (*Micropogon undulates*) and Grey Trout (*Cynoscion regalis*) as Determined by Subjective and Objective Methodology. *J of Food Sci*, 46, 863-867, 1981.
- Tsai GJ, Su WH, Antibacterial Activity of Shrimp Chitosan Againts *Escherichia coli, J Food Prot*, 68: 170-177, 2002.
- Tseng YC, Xiong YL, Webster CD, The Preservation of the Quality of the Muscle in Frozen Australia Red Claw Crayfish (*Cherax quadricarinatus*) by Pre-storage Anti-oxidant Dipping Treatments. *International J of Food Sci and Tech*, 40: 841-848, 2005.
- Yuliana IK, Antibacterial activity of chitosan by deacetylation degree difference and Molecular Weights [thesis]. Bogor: Master Program, Bogor Agricultural University, 2011.