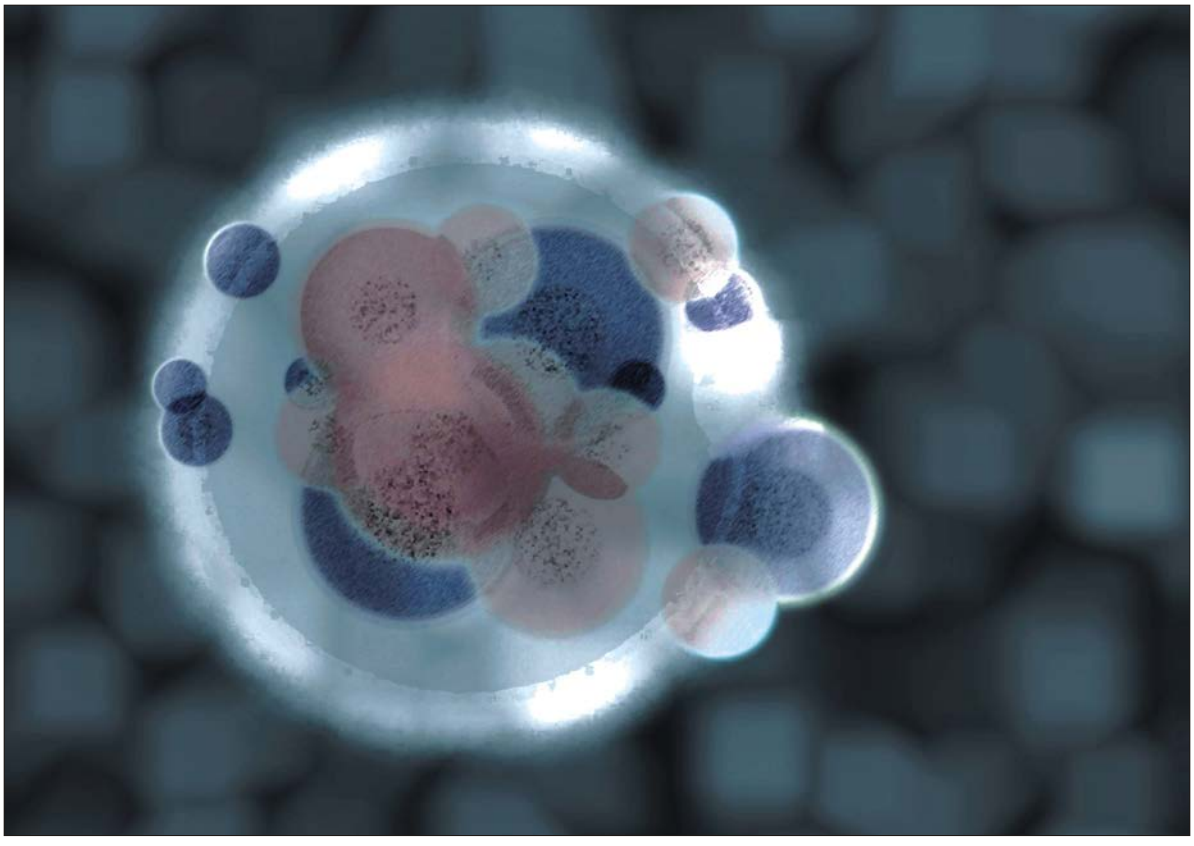

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 13 (7): 640-644, 2013
ISSN 1727-3048 / DOI: 10.3923/jbs.2013.640.644
© 2013 Asian Network for Scientific Information

The Use of *Gracilaria* sp. Extract on Refrigerated Red Tilapia Fillet

Amir Husni, Ustadi and Hendra Wijaya
Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada,
Yogyakarta 55281, Indonesia

Abstract: Inhibition efforts for quality deterioration in fish can be performed using any preservatives. Preservatives that used must be sourced from natural ingredients, in order to minimize the effects of hazardous chemicals when using synthetic materials. One of natural preservatives from marine is the extract of *Gracilaria* sp. The fresh red tilapia were prepared to form the fillets and then soaked for 30 min in *Gracilaria* sp. extract solution with a concentration of 0, 0.5, 1, 1.5 and 2% then drained for 15 min. Red tilapia fillets stored at low temperature (6°C) for 10 days with an interval of observation every 2 days. Observations made on the organoleptic, measurement of pH, Total Volatile Bases (TVB) and Total Plate Count (TPC). Based on the value of pH, TVB and TPC, the extract of *Gracilaria* sp. it enabled to extend the storage life of the fillet from 6-10th day. Based on organoleptic, extract of *Gracilaria* sp. enabled to maintain the parameters of appearance, texture and smell of red tilapia fillet from 4-6th day of storage. The use of extracts of *Gracilaria* sp. 2% gave the best results based on the parameters of odor, pH, TVB and TPC.

Key words: Red tilapia, fillet, preservatives, *Gracilaria* sp. refrigerated

INTRODUCTION

Fresh fish (including red tilapia) are more susceptible compared to red meats and chicken and its quality decreases due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated (Gonzalez-Fandos *et al*., 2005). Enzymatic and chemical reactions are responsible for the initial loss of freshness whereas microbial activity is responsible for spoilage (Gram and Huss, 1996). Therefore, it needs to be done attempt to increase the shelf life of fishery products through processing and preservation, one of which is low-temperature storage. Low-temperature storage besides to inhibiting the activity of microbes and enzymes also can retain the original properties of the fresh fish. However, storage of fresh fish at low temperatures still have a limited shelf life (Santoso *et al*., 1999). Therefore we need safe of preservatives in the product in order to have a good quality with a longer shelf life.

Seaweed is one of the many marine biota known to contain active compounds that are useful as antibacterial, antivirus and antitumor (Atmadja, 1992). According to Husni (2006), *Gracilaria* sp. is one of macroalgae that has potential as antibacterial. This type of marine algae are found on the south coast of Yogyakarta. This study aimed to determine the effect of extracts of *Gracilaria* sp. as preservatives of red tilapia fillet stored at refrigerator.

MATERIALS AND METHODS

Materials: Red tilapia (size±250 g fish^{-1}) were obtained from freshwater fish of Mina Kepis Sleman Yogyakarta. *Gracilaria* sp. obtained from the Drini beach Gunungkidul Yogyakarta. *Staphylococcus aureus* obtained from the Laboratory of Agricultural Microbiology, Faculty of Agriculture, University of Gadjah Mada and *Pseudomonas aeruginosa* from the Laboratory of Food Microbiology, University Centre of the University of Gadjah Mada. Trypticase Soya Broth (TSB), Tryptone Soya Agar (TSA), TCA, K2CO3 obtained from Difco (Detroit, MI, USA).

Extraction procedures: Extraction *Gracilaria* sp. using the method by Husni (2006) with modifications. A total of 750 g of *Gracilaria* sp. washed with clean water and then rinsed using PBS and then cut into pieces and added to 96% ethanol (1:4) further blended for 30 min. Extracts have been obtained is evaporated using a rotary evaporator with a temperature of 40°C. Further drying process by using a freeze dryer.

Antibacterial activity test: The test antibacterial activity of extracts of *Gracilaria* sp. against *S. aureus* and *P. aeruginosa* used Kirby-Bauer method as described by the Fadhlan (2010).

Corresponding Author: Amir Husni, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora Gedung, A4 Bulaksumur Yogyakarta 55281, Indonesia Tel: +62 274 551218 Fax: +62 274 551218

The use of extract *Gracilaria* sp. on red tilapia fillet: Preparation of red tilapia fillet using the method described by NSA (2006a). The use of extracts of *Gracilaria* sp. i.e., by tilapia fillet soaked in the extract solution. Extract solution made by adding powdered extract *Gracilaria* sp. into distilled water at various concentration of 0, 0.5, 1, 1.5 and 2%. Fillet was prepared then immersed into the extract for 30 min at room temperature. After soaking for 30 min then drained and then put in the refrigerator with low temperature (6°C) for 10 days. Each treatment was repeated 3 times. Testing parameters include: pH (AOAC, 1990), Total Plate Count (TPC) (NSA, 2006a), Total Volatile Base (TVB) (AOAC, 1995) and sensory analysis (NSA, 2006b).

Statistical analysis: SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as Mean±SD (SD). The significance of differences ($p<0.05$) among the corresponding mean values was determined by using one-way analysis of variance (ANOVA) followed by Duncan's new multiple-range test.

RESULTS AND DISCUSSION

Antibacterial activity: The *in vitro* antimicrobial activities of *Gracilaria* sp. extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were qualitatively and quantitatively assessed by the zone diameters and MIC values. The results were given in Table 1 show that *Gracilaria* sp. from the Drini beach Gunungkidul Yogyakarta has antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) which is higher antibacterial activity on *S. aureus* than *P. aeruginosa*. The maximum inhibition zones and MIC values of *Gracilaria* sp. extract were 13.7 mm and 30 mg mL^{-1} for *S. aureus* and 8.4 mm and 300 mg mL^{-1} for *P. aeruginosa*, respectively. Research conducted Maduriana and Sudira (2009) showed that *Gracilaria arcuata* from Batu Bolong beach Canggu Bali and *Gracilaria lichenoides* from the coast of Serangan Bali also have higher antibacterial activity against Gram-positive (*Micrococcus luteus*) than Gram-negative (*E. coli*). According Pelczar and Chan (1977) the growth of Gram-positive bacteria more easily inhibited than Gram-negative bacteria. Weaker antibacterial activity in *P. aeruginosa* is possible because the active compounds from the extract of *Gracilaria* sp. rather difficult to penetrate the bacterial cell wall of *P. aeruginosa*. Gram-negative bacteria have an outer membrane that protects bacteria from toxic substances (Prescott *et al*., 1993). Outer membrane has narrow pores that adds protection for Gram-negative bacteria. Outer membrane in Gram-negative bacteria may hinder antibacterial, dyes and detergents into the inside of the bacterial cell. The different in the composition and structure of the bacterial cell wall of Gram-positive and Gram-negative bacteria cause different responses in the two groups of bacteria (Prescott *et al*., 1993).

Table 1: Inhibition activity of extract of *Gracilaria* sp. against *S. aureus* dan *P. aeruginosa*

Bacteria	Inhibition zone (mm^2)	MIC (mg mL^{-1})
Staphylococcus aureus	13.7	30
Pseudomonas aeruginosa	8.4	300

P. aeruginosa is a Gram-negative bacteria that have a cell wall structure is more complex and contains more lipid components (11-12%) compared to the structure of the cell wall in bacteria *S. aureus*. Thus, the cell wall of bacteria *S. aureus* will be more easily damaged by bioactive compounds contained in the extract of *Gracilaria* sp. Inhibition of cell wall synthesis would lead to the bacterial cell wall is weakened and becomes lysis. Lysis of cells caused by cell wall no longer function in maintaining the shape and protect bacteria that have a high osmotic pressure. In addition, the bacteria *S. aureus* has an osmotic pressure in the cells 3-5 times larger than Gram-negative bacteria, making it more susceptible to lysis (Jawetz *et al*., 2001).

Degree of acidity (pH): The changes in the pH of red tilapia fillet were given extracts of *Gracilaria* sp. during stored at cold temperatures are described in Fig. 1. The initial pH of the fish samples was 6.6. The pH of the samples decreased initially and then increased. The initial pH decrease may be attributed to the dissolution of CO_2 in the fish samples. Several authors have reported a decrease in the pH of fish samples with increase in the

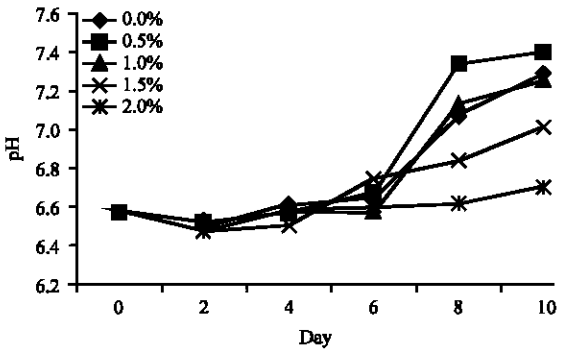

Fig. 1: Effect of *Gracilaria* sp. on pH of red tilapia fillet during storage at low temperature

concentration of CO_2 in the atmosphere (Manju *et al*., 2007; Lannelongue *et al*., 1982). The increase of pH may be caused to the production of volatile basic components, such as ammonia and trimethylamine by fish spoilage bacteria (Ruiz-Capillas and Moral, 2001).

The results of the analysis showed that the pH occurred significant difference between treatments after 8 and 10 days, were the 0-6 days there is no significant difference and all the fillet still in good condition. At the end of the observation (10 days) showed treatment of extract of 2% has a pH of 6.70 which means by treatment with 2% red tilapia fillet able to maintain the pH within the threshold of fresh fish while other treatments showed pH values above the threshold pH of fresh fish.

Total volatile base (TVB): Total Volatile Base (TVB) which is mainly composed of ammonia and primary, secondary and tertiary amines, resulted from degradation of proteins and non-protein nitrogenous compound which is caused by microbial activity (Ruiz-Capillas and Moral, 2005). The TVB content of red tilapia fillet during storage indicates that the value is always increasing (Fig. 2). Purwaningsih *et al*. (2005) said that TVB changes can be caused by temperature and length of storage time. The increase in TVB red tilapia fillet with treatment of extract of *Gracilaria* sp. relatively small compared to the control (0%). This is because of the antibacterial compounds in extracts of *Gracilaria* sp. capable of inhibiting bacterial decomposition of protein components into a volatile compound. Suptijah *et al*. (2008) suggested that the increased value of TVB in fish during storage due to degradation of the protein or its derivatives to produce a range of volatile base such as ammonia, histamine, hydrogen sulfide and trimethylamine.

TVB content on the first day of observation does not happen real difference. This is because red tilapia fillet is still in a state of fresh and TVB content is very low (4.2 mgN/100 g). At the end of the observation, the extract of 2% has the lowest value of TVB (21.64 gN/100 g). According to Connell (1995) when the content of TVB in fish above 30 g gN/100 an indicator of decaying fish. Extract *Gracilaria* sp. 2% are able to maintain the tilapia fillet 4 days longer than without the extract, for treatment without extract (0%) fillet shelf life only until 6 days.

Total plate count (TPC): Figure 3 shows that the total bacteria on red tilapia fillet increased along with the length of storage, but the number of bacteria on a red tilapia fillet were given the extract *Gracilaria* sp. 2% lower than other treatments. It shows that the use of extract of *Gracilaria* sp. can inhibit the activity of bacteria on red tilapia fillet. The increase in total bacteria on red tilapia because fish fillet is a suitable medium for bacterial growth.

The maximum limit for total bacterial fish fillet products is 5.69 log CFU g^{-1} (NSA, 2006c). After 6 days, total bacteria in all treatments still in the acceptable threshold, but after 8 days the red tilapia fillet with the addition of extract 0 and 0.5% have exceeded the maximum number of total bacteria. Extract with a concentration of 2% showed the lowest TPC during storage and until the end of the observation showed a concentration of 2% had 5.40 log CFU g^{-1} and below of maximum allowable TPC. Thus it can be said that the best treatment is to give the extract at a concentration of 2% because it is able to inhibit the growth of bacteria until day 10. According to Fenical and Paul (1984), red algae contain compounds terpenoids, acetogenin and halogen compounds are a group of compounds that function as plant antibakteri. Terpenoid have significant benefits as a traditional medicine, antifungal and antibacterial (Bohlmann *et al*., 1998).

Sensory analysis: The acceptability of fish and fishery product during cold temperature storage depends on the

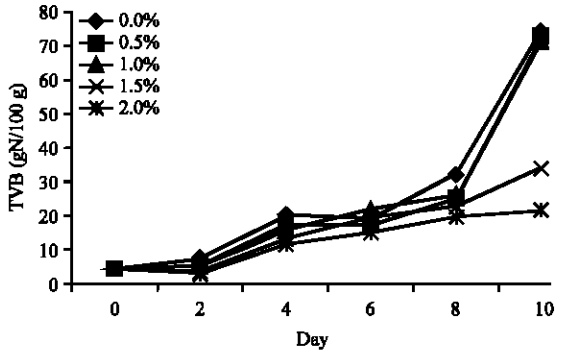

Fig. 2: Effect of *Gracilaria* sp. on TVB of red tilapia fillet during storage at low temperature

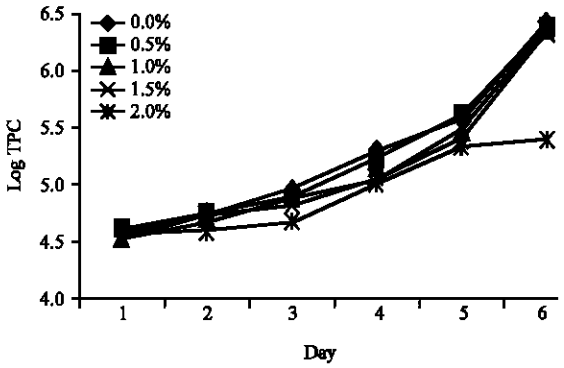

Fig. 3: Effect of *Gracilaria* sp. on TPC of red tilapia fillet during storage at low temperature

Table 2: Effect of *Gracilaria* sp. on score of appearance of red Tilapia fillet during storage at low temperature

	Score of appearance at the day					
Gracilaria sp. (%)	0	2	4	6	8	10
0.0	8.58^{b}	7.62^{a}	7.14^{a}	5.71^{a}	4.39^{a}	3.56^{a}
0.5	8.08^{a}	8.10^{b}	7.71^{bc}	6.00^{ab}	4.29^{a}	3.86^{a}
1.0	8.17^{a}	8.05^{b}	7.38^{ab}	6.05^{ab}	3.95^{a}	4.05^{b}
1.5	8.00^{a}	8.00^{b}	7.48^{ab}	7.29^{c}	4.81^{a}	3.95^{a}
2.0	8.33^{a}	8.28^{b}	8.29^{d}	7.48^{cd}	6.00^{b}	4.71^{c}

Values in the same column with different letters are significantly different at $p<0.05$

Table 3: Effect of *Gracilaria* sp. on score of texture of red Tilapia fillet during storage at low temperature

	Score of texture at the day					
Gracilaria sp. (%)	0	2	4	6	8	10
0.0	8.67^{a}	8.00^{a}	7.24^{a}	5.43^{a}	3.33^{a}	2.33^{a}
0.5	8.25^{a}	8.05^{a}	7.10^{a}	5.38^{a}	3.71^{b}	3.29^{b}
1.0	8.25^{a}	8.14^{a}	7.14^{a}	6.05^{b}	3.71^{b}	3.48^{b}
1.5	8.17^{a}	8.24^{a}	7.90^{b}	7.14^{c}	4.05^{bc}	3.24^{b}
2.0	8.08^{a}	8.00^{a}	8.14^{bc}	7.38^{cd}	5.71^{c}	4.48^{c}

Values in the same column with different letters are significantly different at $p<0.05$

changes in their sensory attributes including appearance, texture and odor. Based on Table 2 it can be seen that the appearance of red tilapia fillet after 6 days who were given the extract with a concentration of 1.5 and 2% had a good appearance score (more than 7). According to INS-01-2346-2006, fish is still fresh when the sensory test value ranges between 9-7 and not fresh when the value of sensory tests ranged from 4-1 (NSA, 2006c). Wicaksono (2010) reported that the addition of extracts of *Sargassum* sp. 1% in the red tilapia fillet is the best treatment in maintaining the highest organoleptic values for red tilapia fillet appearance parameters are stored at low temperatures. Wibowo (1993) suggested that the extract *Sargassum* sp. 2% is added to the mackerel fillet is the best treatment to preserve the value of the highest organoleptic appearance mackerel fillet during storage in ice.

Table 3 shows that treatment with a concentration of 1.5 and 2% had a good texture score (more than 7) after 6 days, whereas treatment without the extract (0%), 0.5 and 1% only until 4 days. Wicaksono (2010) reported that the extract of *Sargassum* sp. 1% is added to the red tilapia fillet is the best treatment to preserve the highest value of texture red tilapia fillet stored at low temperatures.

Table 4 shows that the storage up to 4 days, the odor of all red tilapia fillet still within the limits of acceptance, but after the 6 days of treatment only with a concentration of 2% which indicates an acceptable odor score (7.1) while for other treatments have scores odor below 7. Wicaksono (2010) reported that the extract of *Sargassum* sp. 1% is added to the red tilapia fillet is the best treatment in maintaining the highest organoleptic

Table 4: Effect of *Gracilaria* sp. on score of odor of red Tilapia fillet during storage at low temperature

	Score of odor at the day					
Gracilaria sp. (%)	0	2	4	6	8	10
0.0	8.58^{a}	7.47^{a}	7.05^{a}	4.90^{a}	3.02^{a}	1.43^{a}
0.5	8.08^{a}	7.85^{b}	7.05^{a}	5.14^{a}	2.95^{a}	1.57^{a}
1.0	8.17^{a}	7.90^{b}	7.03^{a}	5.67^{b}	3.03^{a}	1.76^{a}
1.5	8.00^{a}	7.97^{bc}	7.13^{a}	6.95^{cd}	3.70^{a}	1.48^{a}
2.0	8.00^{a}	8.00^{c}	7.49^{b}	7.10^{d}	4.00^{a}	2.43^{b}

Values in the same column with different letters are significantly different at $p<0.05$

values for red tilapia fillet were stored at low temperatures. Wibowo (1993) mentions that the extract *Sargassum* sp. 2% was added to the mackerel fillet is the best treatment to preserve the highest value for the parameter organoleptic odor mackerel fillet during storage in ice. The factors that cause the difference in odor scores for each treatment because of the difference of extract of *Gracilaria* sp. Extract *Gracilaria* sp. contain antibacterial that can inhibit the growth of bacteria (Atmadja, 1992).

CONCLUSION

Extract *Gracilaria* sp. have potential as an antibacterial. Based on the content of TVB, TPC and pH, the use of extracts of *Gracilaria* sp. 2% were able to extend the shelf life of red tilapia fillet during low-temperature storage from day 6 to day 10 of storage.

ACKNOWLEDGMENT

The research was carried out with funding from the Community Fund of the Faculty of Agriculture, Universitas Gadjah Mada Research Grant program through the Faculty of Agriculture Universitas Gadjah Mada 2011, Number: 1556/PN/TU.

REFERENCES

AOAC, 1990. Official Method of Analysis of the Association of Official Agriculture Chemists. 13th Edn., AOAC, Washington, DC, USA.

AOAC, 1995. The Official Method of Analysis of the Association of Official Analytical Chemists. 16th Edn., Association of Official Analytical Chemists, Washington DC.

Atmadja, W.S., 1992. Rumput laut sebagai obat. J. Oseana, 17: 1-8.

Bohlmann, J., G. Meyer-Gauen and R. Croteau, 1998. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. Proc. Nat. Acad. Sci. USA., 5: 4126-4133.

Connell, J.J., 1995. Control of Fish Quality-Proposed Limit of Acceptability for Marine Species. 4th Edn., Fishing News Books Ltd., Surrey, UK.

Fadhlan, A.H., 2010. Antimicrobial activity of methanolic extract of *Sygyzium cordatum* leaf agains *Escherecia coli* dan *Staphylococcus aureus* by Kirby-Bauer method. Jimki, 1: 4-6.

Fenical, W. and V.J. Paul, 1984. Antibiotic and cytotoxic terpenoids from tropical green algae of the family Udoteaceace. Hydrobiologia, 116-117: 135-140.

Gonzalez-Fandos, E., A. Villarino-Rodriguez, M.C. Garcia-Linares, M.T. Garcia-Arias and M.C. Garcia-Fernandez, 2005. Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. Food Control, 16: 77-85.

Gram, L. and H.H. Huss, 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol., 33: 121-137.

Husni, A., 2006. Identifikasi dan uji antibakteri rumput laut dari pantai Gunungkidul [Identification and antibacterial test of seaweed from Gunungkidul beach]. Proceedings of the 4th Seminar of Marine and Fisheries, (SMF'06), Gadjah Mada University, Yogyakarta Indonesia.

Jawetz, E., J.L. Melnick and E.A. Adelberg, 2001. Medical Microbiology Twenty. 2nd Edn., Salemba Medika, Universitas Airlangga, Jakarta, Indonesia.

Lannelongue, M., M.O. Hanna, G. Finne, R. Nickelsen and C. Vanderzant, 1982. Storage characteristics of finfish fillets (*Archosargus probatocephalus*) packaged in modified gas atmospheres containing carbon dioxide. J. of Food Prot., 45: 440-444.

Maduriana, I.M. and I.W. Sudira, 2009. The screening and activity test of antibacteria from some seaweeds, in batu bolong canggu and serangan beach. Bul. Vet. Udayana, 2: 1-6.

Manju, S., L. Jose, T.K. Srinivasa-Gopal, C.N. Ravishankar and K.V. Lalitha, 2007. Effects of sodium acetate dip treatment and vacuum-packaging on chemical, mirobiological, textural and sensory changes of Pearlspot (*Etroplus suratensis*) during chill storage. Food Chem., 102: 27-35.

NSA, 2006a. Fillet of tilapia (*Tilapia* sp.) frozen-part1: Specification. National Standardization Agency (NSA), Indonesian National Standard (INS)-01-4103-2006.

NSA, 2006b. Microbiology test method-part 3: Determination of Total Plate Count (TPC) in fishery products. National Standardization Agency (NSA), Indonesian National Standard (INS)-03-4103-2006.

NSA, 2006c. Organoleptic assessment sheets frozen fish fillet. National Standardization Agency (NSA), Indonesian National Standard (INS)-01-2346-2006.

Pelczar, M.J. and E.C.S. Chan, 1977. Laboratory Exercise in Microbiology. 3rd Edn., McGraw-Hill, New York, USA., ISBN: 9780070492271, Pages: 478.

Prescott, L.M., J.P. Harley and D.A. Klein, 1993. Microbiology. 2nd Edn., Brown Publisher, Dubuque, USA.

Purwaningsih, S., W. Josephine and D.S. Lestari, 2005. Effect of storage time of Rajungan (*Portunus pelagicus*) at room temperature. Bull. Fish Prod. Tech., 8: 42-50.

Ruiz-Capillas, C. and A. Moral, 2001. Residual effect of CO_2 on hake (*Merluccius merluccius* L.) stored in modified and controlled atmospheres. Eur. Food Res. Technol., 212: 413-420.

Ruiz-Capillas, C. and A. Moral, 2005. Sensory and biochemical aspects of quality of whole bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. Food Chem., 89: 347-354.

Santoso, J., Nurjanah, Sukarno and S.R. Sinaga, 1999. Deterioration of red nile (*Oreochromis* sp.) during storage at chilling temperature. Bull. Fish Prod. Tech., 6: 1-4.

Suptijah, P., Y. Gushagia and R.S. Dadi, 2008. Inhibition effect of kitosan on quality of patin (*Pangasius hypopthalmus*) fillet at room temperature. Bull. Fish Prod. Tech., 11: 89-101.

Wibowo, T.W., 1993. Effect of *Sargassum* sp. on quality of mackerel (*Rastrelliger* sp.) fillet. M.Sc. Thesis, Faculty of Fisheries Bogor University of Agriculture, Bogor, Indonesia.

Wicaksono, A.A., 2010. Effect of *Sargassum* sp. on quality of red nile (*Oreochromis niloticus*) fillet during storage at low temperature. M.Sc. Thesis, Faculty of Agriculture Universitas Gadjah Mada, Yogyakarta, Indonesia.