

Effect of *aloe vera* (*Alloe vera*) and crown of god fruit (*Phaleria macrocarpa*) on sensory, chemical, and microbiological attributes of Indian mackerel (*Restrelliger neglectus*) during ice storage

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Abstract: The effects of *Aloe vera* (*Aloe vera*) and crown of god fruit (*Phaleria macrocarpa*) on the sensorial, chemical, and microbiological attributes stability of Indian mackerel (*Rastrelliger neglectus*) during chilled storage for 12 days were studied. Treatment with blended 20% blended *Aloe vera* reduced the deterioration rate of the sensorial attributes, but treatment with 1% dried powder from crown of god fruit produced brownish color. Moreover, these treatments reduced total plate count (TPC) and total volatile bases nitrogen (TVBN) during the 12-day storage. This study showed that treatments with *Aloe vera* and crown of god fruit can prolong the shelf-life of Indian mackerel by four days during chilled storage.

Keywords: Indian mackerel, chilled, storage, total plate count, total volatile bases nitrogen

Introduction

Quality of fish depends on its catching methods and post-harvest handling. Chilling is usually considered as the most effective method for keeping fish quality (Opara *et al.*, 2007). Alternatively, ice is used to lower the temperature of fish and it is considered as one of the effective methods for preserving fish. Currently, fishermen in the developing countries are facing difficulty in using ice due to the increase of its price since energy cost is increasing. Consequently, they are trying to find simple, cheap, and effective methods to maintain fish quality.

Handling of fish at low temperatures such as freezing (Fagan *et al.*, 2003; Fagan *et al.*, 2004), using ice (Hernández *et al.*, 2009), chilling (Gould, 1996; Siskos *et al.*, 2007; Chen *et al.*, 2007) and using slurry ice (Rodríguez *et al.*, 2004a; Losada *et al.*, 2005; Múgica *et al.*, 2008) were reported. Moreover, to extend the shelf-life of fish at low temperature, chemical preservatives such as chlorotetracycline, sodium chloride, ellagic acid, L-ascorbic acids, and chlorine dioxide (ClO₂) have been used (Frag *et al.*, 1986; Shin *et al.*, 2004; Zambuchini *et al.*, 2008). Furthermore, some natural preservatives such as liquid smoke from coconut shell, seaweeds extract of *Gracillaria* sp, *Parinarium glaberimum* HASSK, cashew powder and red galangal, thyme powder, plant extract and tea polyphenols have also been used (Moniharapon *et al.*, 1993; Agustini *et al.*, 2003; Agustini *et al.*, 2007; Swastawati *et al.*, 2008; Fan *et al.*, 2008; Attauchi and Saloua, 2009;

Quitral *et al.*, 2009). Natural compounds are used as preservatives to obtain 'green label' product and secure food safety (Roller, 1995; Devlieghere *et al.*, 2004). In this respect, natural compounds extracted from different fruits showed wide spectrums of antibacterial activity (Ordogh *et al.*, 2010; Jadhav *et al.*, 2010; Kahriman *et al.*, 2010; Razavi and Ebrahimi, 2010; Ibrahim *et al.*, 2010; Basile *et al.*, 2010). Plants and fruits exhibit a broad range of biological activities including antibacterial (Goun *et al.*, 2003; Matu, and Staden, 2003; Das *et al.*, 2011), anti-inflammatory (Matu and Staden, 2003), antifungal (Goun *et al.*, 2003; Rodríguez *et al.*, 2005; Das *et al.*, 2011) antioxidant activities (Choi and Hwang, 2005; Patthamakanokporn *et al.*, 2008; Andarwulan *et al.*, 2010).

Aloe vera is one of the natural substances that contains antibacterial (Lorenzetti *et al.*, 2006), anti-inflammatory (Langed *et al.*, 1964), anti-viral (Hamman, 2008), antioxidant (Hamman, 2008), and antifungal (Rodríguez *et al.*, 2005; Saks and Golan, 1994; Joseph and Raj, 2010) substances. Thus, it could be used to reduce fish microbial spoilage. Moreover, crown of god fruits (*Phaleria macrocarpa*) also contains some natural active compounds, which can function as antibacterial substances. The potential of using natural preservatives has not been fully exploited (Roller, 1995). Therefore, The aims of this study were to optimize the conditions of using *Aloe vera* and crown of god fruits as preservatives and study their effects on the sensorial, chemical, and microbiological attributes stability of Indian

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Mackerel (*Rastrelliger neglectus*) during ice storage for 12 days.

Materials and Methods

Fish

Indian mackerel were caught from the northern coast of central Java Sea. 24 kg of fresh fish samples were selected randomly and evaluated for its freshness based on Indonesian National Standard Number SNI-01-2719.2006 (NSB, 2006). The fresh fish were kept in ice and immediately transported to the laboratory within 1 hour. Fish samples from each treatment were taken for subsequent analysis during 0, 3, 6, 9 and 12 days, as well as for control treatment.

Aloe vera and crown of god fruits

Aloe vera was harvested in Semarang, Indonesia and crown of god fruit were harvested in Klaten, Indonesia. Crown of god fruit was brought to Semarang without any treatment as fresh fruit. Both natural substances were taken to laboratory for subsequent preparation.

Aloe vera and crown of god fruits preparation

Due to practical methodology in natural substances preparation, *Aloe vera* and crown of god fruit were prepared in different method. *Aloe vera*, (2.4 kg) and crown of god, (1kg) were washed and cut into pieces followed by draining of water. Crown of god was dried under sunlight at average temperature of $33 \pm 2^\circ\text{C}$ for three days and then ground it into powder (from 1000 g fresh crown of god fruit produced 250 gr powder). Fresh *Aloe vera* were blended to make a thick solution. The fresh *Aloe vera* concentration was used based on samples weight (w/w) of 2.4 kg of *Aloe vera* flesh/12 kg fish sample, and crown of god were used based on solution (w/v) of 50 g crown of god powder/ 5 L distilled water.

Optimization the conditions of using Aloe vera and crown of god fruits in Indian mackerel

Initial studies were conducted to obtain the most effective treatments of each natural substances on the sensory and microbial attributes of Indian mackerel. *Aloe vera* was used at 16, 18, and 20 g *Aloe vera* flesh/100 g fish sample, whereas crown of god was used at 0.5, 1, and 1.5 g crown of god fruit powder/100 ml distilled water. The Indian mackerel fish were soaked for 2 hours in solution containing the natural substance, fish were drained and stored in styrofoam box with ratio of ice:fish of 1:1 for 7 days in a chilled storage (4°C). During this storage, the sensory and microbiological attributes of Indian mackerel were determined. Sensory analysis of the fish was conducted daily, whereas Total Plate Count (TPC) was conducted at the initial day (0 day) and final days (day 7) of storage. The best concentration of natural substances was determined based on sensory and microbiological characteristics of fish.

Storage study with Aloe vera and crown of god fruits treatments

The *Aloe vera* and crown of god fruits solutions were prepared and fish samples were soaked at the best concentrations obtained from the first stage experiments (Tables 1 and 2). Fresh Indian mackerel were treated using the following treatments as: (i) ice-fish ratio as 1:1 without natural substance treatment (T0), (ii) ratio as 1:1 (T1); 1:3 (T2) and 1:5 (T3) treating with solution containing natural substances. The samples were soaked for 2 hours in solution, drained and placed in ice in styrofoam box for 12 days. Ice was replaced after every 8 hours. Sensory evaluation of the samples was conducted everyday by ten panel members whereas, total bacterial count TPC and TVB-N (Total Volatile Base Nitrogen) were carried out in 3 days interval of 0, 3, 6, 9 and 12 days of storage.

Table 1. Changes in sensory and total bacteria (cfu g⁻¹) of Indian mackerel fish (*Rastrelliger neglectus*) with crown of god fruit treatment during storage in order to get optimum concentration.

Concentration (%)	Sensory (days)								TPC (CFU/g)	
	0	1	2	3	4	5	6	7	0	7
0	8.78	8.04	6.99	6.81	6.00	5.69	4.85	3.99	2.5×10^3	4.7×10^6
0.5	8.78	8.23	7.48	7.20	6.70	6.50	5.48	4.33	3.8×10^2	5.7×10^5
1	8.78	8.18	7.75	7.48	7.23	6.84	5.93	5.10	2.5×10^2	3.6×10^3
1.5	8.78	8.24	7.67	7.38	7.09	6.63	5.83	4.98	3.1×10^2	3.9×10^3

Table 2. Changes in sensory and total bacteria (cfu g⁻¹) of Indian mackerel fish (*Rastrelliger neglectus*) with aloe vera treatment during storage in order to get optimum concentration

Concentration (%)	Sensory (days)								TPC (CFU/g)	
	0	1	2	3	4	5	6	7	0	7
0	8.78	7.92	6.67	6.33	5.83	5.62	4.79	4.01	3.1×10^3	6.8×10^6
16	8.78	8.03	7.23	6.93	6.73	6.28	5.60	4.23	4.2×10^3	7.8×10^5
18	8.78	8.20	7.50	7.01	6.92	6.63	5.83	4.69	5.8×10^2	3.8×10^4
20	8.79	8.35	7.59	7.41	6.98	6.72	6.30	4.87	4.3×10^2	3.2×10^4

Sensory analysis

Overall sensory quality of fish was assessed on a nine point scale based on the changes in eye, gill, appearances, belly integrity, odour, texture and slime by using the Indonesian National Standard 2006 Number 01-2346-2006 freshness grading scheme of raw fish (INSB, 2006). The assessment was conducted using a 1 to 9 scale; 1 for extremely poor, The organoleptic, 2 for very poor, 3 for poor, 4 for fair, 5 for very fair, 6 for very fair to good, 7 for good, 8 for very good, and 9 for extremely good. Ten trained sensory panelists from Diponegoro University participated in the sensory analysis. The assessment was conducted at Laboratory of Fisheries Processing Technology, Department of Fisheries, Faculty of Fisheries and Marine Science, Diponegoro University.

Determination of total volatile bases nitrogen

Total volatile base nitrogen (TVB-N) content was determined using Conway micro-diffusion method Indonesian National Standard SNI 01-4495-1998 (NSB, 1998) and was expressed as mg/100 g samples according to the following formula :

$$TVBN \text{ (mg/100 g sample)} = \frac{(\text{titre sample} - \text{titre blank}) \times N \text{ HCl} \times 14.007 \times 2 \times 100}{\text{sample weight}}$$

Total Plate Count Analysis

Total Plate Count was determined by the method of Indonesian National Standard number SNI -01-2332.3-2006 (NSB, 2006). Fish muscles were cut into very small pieces using sterile knife and forceps then minced. Minced fish of 20 g and Butterfield’s phosphate buffered BFB (Merck Group, Frankfurter, Dramstatdt, Germany) solution 180 ml were mixed and blended it in a laboratory stomacher 400 (Seward Medical London UK) for 2 min. After serial dilutions, 1 ml was plated in plate count agar (Merck Group, Frankfurter, Dramstatdt, Germany) and the plates were incubated aerobically at 35°C(±1) for 24 hours.

Experimental design

This research was experimental laboratory by comparing one or more group subject to treatment with one or more group without treatment. Experimental design used was Completely randomized design with split plot in time. Each treatment was replicated two times. The main plot was the different ratio of fish and ice and the sub plot was the length of storage time.

Statistical analysis

Data analysis was performed by statistical package SPSS 14.0 software (IBM Company, Chicago, Illinois USA). The effects of Aloe vera and crown of

god fruits solutions on TVB-N and microbiological properties of the samples were determined by one-way Analysis of Variance (ANOVA) at the significance level of 0.05. Sensory data was analyzed using statistics nonparametric of Friedman test (Steel and Torrie, 1991).

Results and Discussion

Effective treatments of Aloe vera and crown of god fruits

In the initial stage of this study, Indian mackerel was treated with different concentrations of natural substances which resulted in wide variations in sensory and TPC value (Tables 1 and 2.) Different concentration of natural substances gave different effect to both sensory and TPC value. Aloe vera at 0.5% and crown of god fruit at 16% gave little effect on both parameters. The optimum concentration of Aloe vera and crown of god fruit treatments used were 1% and 20%, respectively based on the changes in sensory score and TPC. This concentration of the natural substances were used for the subsequent study.

Storage study with Aloe vera and crown of god fruits treatments

Sensory changes

Sensory analysis of all treatments were assessed during 12 days of storage are shown in Figure 1. Fresh Indian mackerel exhibited shiny appearance, bright eyes, reddish gill, seaweedy odor and very firm texture. Changes in sensorial properties varied among treatments, but as the storage time increased, all sensory attributes decreased. The fastest decreasing rate of sensory attribute was observed in the untreated samples followed by treated samples with 1% crown of god fruit. Whilst the least decrease in sensory attribute was observed in the samples treated by 20% *Aloe vera* and ice ratio (1:1), and its sensory attributes remained at the highest score.

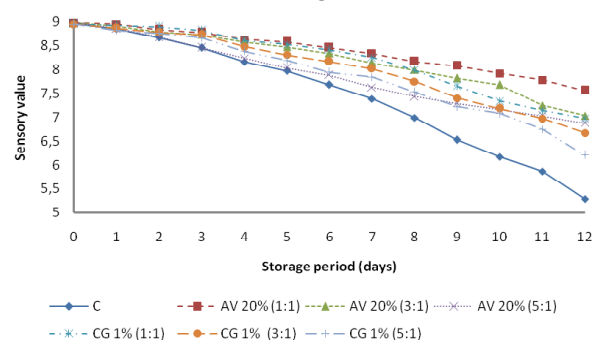


Figure 1. Changes in Sensory of Indian Mackerel (*Rastrelliger neglectus*) during storage.

Control treatment fish were rejected by the panelists at 8th day of storage. In contrast, none of treated Indian mackerel were rejected by the panelist until at 11th day of storage. These results indicated that treatment with Aloe vera and crown of god fruits provided preservative effects and stabilized the sensorial properties of Indian mackerel, however, this stability varied among type of fruits. Treating fish with crown of god fruits affected fish appearance. Crown of god fruit treatment made fish-eye more opaque. This treatment not only influenced eyes but also the gills and the appearance of the fish which became more brownish due to tannin content of this fruit. In contrast, *Aloe vera* had no effect on fish appearance.

Treatment with 20% *Aloe vera* was considered as the most effective treatment. At the end of storage time (12 days), fish treated at such concentration were not rejected by the panelist because sensory value of sample was higher than the Indonesian National Standard No. 01-2346-2006 for raw fish. This effect was possibly due to the complex substances of *Aloe vera* such as aloin which has anti bacterial (Reynold and Dweck, 1999), antifungal and antiinflammatory activities (Das *et.al.*, 2011), carboxypeptidase that inactivates bradykinin and salicylate that inhibits thromboxane formation (Klein and Neal, 2008). Results of the Friedman non-parametric test confirmed that from 3th day of storage until the end of storage there was significant difference on sensory attribute between the samples treated by different natural substances and the different fish and ice ratio during storage time.

Total Volatil Base Nitrogen (TVB-N) changes

Results of TVB-N changes during storage time are showed on Figure 2. All of fish treatments exhibited an initial TVB-N content below 10 mg/100 g. Whereas, during storage period, TVB-N content progressively increased but the rate of the TVB-N change varied on samples and it did not exceed the limit of acceptability (30-35 mg N%) for ice stored cold-water fish (Ozogul, 2010). Total volatile nitrogen base on untreated Indian mackerel was significantly increased ($P < 0.05$). Ocaño-Higuera, *et al.* (2009) reported that TVB-N and TMA-N on Cazon fish stored on ice (0°C) significantly increased ($p < 0.05$).

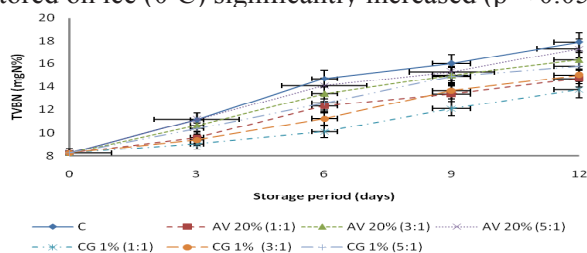


Figure 2. Changes in TVB-N contents of Indian Mackerel (*Rastrelliger neglectus*) with treatment

During fish deterioration bacterial growth increases and produces vapour gasses such as ammonia. Furthermore, the increasing of TVB-N concentration is caused by proteolytic enzyme that change substance to carbocilate acid, sulfid acid, ammonia and the other compounds (Botta, 1995). In marine fish, the rate of increase in TVB-N concentration varies considerably from spesies to spesies (Huss, 1988). Based on the result a wide variations of TVB-N concentration on fish samples are caused by ratio between fish and ice treatment; and antimicrobial effect resulted from different natural substances.

In contrast, TVB-N slowly increased in the treated fish during the storage which could show the effect of *Aloe vera* and crown of god fruits on the TVB-N increasing rate. Attouchi and Sadok (2009) reported that thyme powder addition on sea bream fish significantly reduced the level of TVBN. The result of one-way ANOVA confirmed that there was significantly difference ($p < 0.05$) between different treatment of natural substances and storage time. It also been observed in this study that the changes in TVB-N concentration is almost in line to total plate count resulted from the samples.

Microbiological changes

The bacterial changes in Indian mackerel can be seen in Figure 3. The initial TPC was different from one fish treatment to the others. TPC can be used an one indicator representing bacteria. TPC is not the measure of the 'total' bacterial population, only a measure of the fraction of the microflora that is able to produce colonies under the conditions of the growth medium and the incubation (Lyhs, 2009). Total bacteria on untreated Indian mackerel significantly ($p < 0.05$) increased during storage and it reached 6 log cfu g⁻¹ at the end of storage. Tzikas *et al.* (2007), reported that microflora on Mediteranian mackerel and blue jack mackerel which were stored in ice at 10 days was $< 6 \log_{10} \text{cfu g}^{-1}$. Also, Ozyrut *et al.* (2009) reported that microbiological content on goldband goatfish exceeded 7 log cfu g⁻¹ after 8 days, and 11 days for red mullet. The bacteria that grow on fish during ice storage are *Shewanella putrefaciens* and *Pseudomonas* spp. (Gram and Hans, 1996).

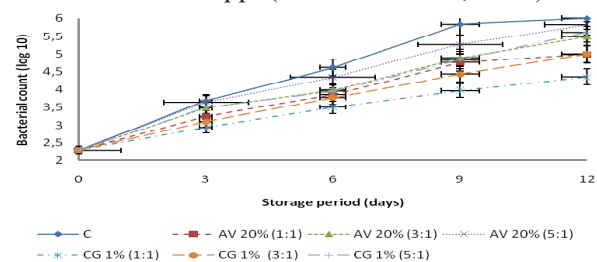


Figure 3. Changes in total bacterial population (CFU g⁻¹) of Indian Mackerel (*Rastrelliger neglectus*)

In treated Indian mackerel, the samples which were treated with 1.5% crown of god fruit showed the lowest rate of bacterial increasing. In contrast, treatment with 20% *Aloe vera* did not affect on bacterial growth. The results indicated that different of microbiological growth were affected by different treatments of natural substances. Crown of god fruit contains flavonoid, tannin, and polyphenol compound. These compounds were shown antimicrobial activity against *Escherichia coli*, *Bacillus substilis*, *Pseudomonas aeruginosa* and *Bacillus cereus* (Rusmiarti *et al.* (2006); Wirahardja *et al.* (2006). In addition, *Aloe vera* contains aloesin, 8-C-glucosyl-7-O-methyl-(S)-aloesol, neoaloesin A, 8-O-methyl-7-hydroxyaloin A and B, 10-hydroxyaloin A, isoaloesin D, aloin A and B, aloeresin E and aloemodin from *A. barbadensis*: and aloenin, aloenin B, 10-hydroxyaloin A, aloin A and B, and aloemodin from *A. arborescens* (Park *et al.*, 1998). *Aloe vera* gel has antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Flavobacterium-rignese*, *Trichophyton mentagrophytes* and *Aloe vera* leaf is able to against on *Pseudomonas aeruginosa* and *Candida albicans* (Agarry *et al.*, 2005) The difference chemical compounds of *Aloe vera* and crown of god fruits caused different rate of fish deterioration due to microbial activity. The principle of antimicrobial is disrupts the barrier properties of the outer membrane of gram-negative bacteria. The mechanism of action appears to derive in part from the ionic interaction between the cationic groups of the natural substances molecules and the anionic groups of the microbial cell membrane, which can rupture the cell membrane.

At the end of storage, TPC reached at a value below 5×10^5 cfu g⁻¹ in samples treated with 1% crown god whereas, at the same time in samples treated by 20% *Aloe vera* TPC reached a value more than 5×10^5 cfu g⁻¹, indicating spoilage and possible rejection. Some studies showed that TPC on milk fish that used cashew and red galanga were below 5×10^5 cfu g⁻¹ at the end of storage time (12 days) and there was significant effect on different ice:fish ratio on TPC. (Agustini *et al.*, 2007). Results of the one-way ANOVA of TPC were significantly difference (P<0.01) between treatment of natural substances and ice:fish ratio and time storage.

Conclusion

Changes in the chemical, sensorial and microbiological attributes of treated Indian mackerel varied among concentrations and type of fruits. 20% *Aloe vera* and 1.5% crown of god fruits treatments were found to be the best treatments to reduce changes

in sensorial and microbial attributes respectively during storage. In addition, the best effectiveness of these fruits was found at 1:1 ice and fish ratio.

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