

# ANTIBACTERIAL ASSESSMENT OF ALOE VERA (*Aloe vera chinensis* Linn) CORTEX EXTRACT AND ITS APPLICATION AS INHIBITOR FOR KING PRAWNS (*Macrobrachium rosenbergii* Linn) SPOILAGE DURING STORAGE AT 5°C

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## Abstract

Aloe vera (*Aloe vera chinensis* Linn) cortex contains antibacterial compounds such as aloe emodin and aloin, that have potency as natural preservative for foods. The objectives of the research were to determine the minimum concentration of aloe vera cortex extract to inhibit bacterial growth, and the effects of soaking in the extract and storage at 5°C on the quality parameters of king prawns. The concentrations of extract were varied i.e. 125, 250, 500 and 1000 µg/ml, respectively, and minimum concentration of extract for inhibition of *Pseudomonas fluorescens* was determined by Minimum Inhibitory Concentration (MIC) method. Antibacterial compounds in the extract were analyzed by HPLC and TLC methods. TPC, TVB-N, TMA, pH and organoleptic characteristics of soaked king prawns were evaluated. The results showed 500 µg/ml aloe vera cortex extract was an optimum concentration, and soaking for 90 minutes was able to inhibit and to reduce one log cycle of bacteria population. Aloe vera cortex extracts contained 18.21 ppm aloe emodin and 15.5 mg/g aloin (1.55%). After 6 days storage at 5°C, therefore the king prawns was performed by TPC  $3.9 \times 10^5$ , TVB-N 28.6, TMA 4.60 and pH 7.7. The best quality of king prawns was resulted by 60 minutes soaking with organoleptic score 7.

**Keywords:** Aloe vera; antibacterial; aloe emodin; aloin; king prawns.

## 1. Introduction

Quality and safty of prawns and other processed products are affected by the degree of freshness, because prawns is highly perishable food stuffs or easily contaminated by spoilage bacteria. One of the efforts to preserve quality or freshness of prawns is preservation by using ice. Preservation by refrigeration method or use of ice, however the product is still contaminated by psychrophilic bacteria [1]. Therefore, pretreatments before refrigeration is needed with several reasons i.e. (1) refrigeration using ice is difficult to be controlled, (2) the quality of ice is not assured and free from bacterial contamination that come from environment and utensil, (3) psychrophilic bacteria is a group of microbes that are able to grow at low temperature. Microbes that are related to the spoilage of most fresh sea food products are generally reflection of microbe population in aquatic environment and its inhabitants such as prawns. During catching, however the prawns are in sterile condition, but after that soon they are contaminated by bacteria present in (a) the surface of prawns body and bacteria that surviving in the intestin, (b) water, utensil, and persons who are doing handling and processing. *Pseudomonas sp.* is belong to the spoilage bacteria that dominate

on fresh prawns, and Reference [2] stated that spoilage bacteria dominate on caught prawns particularly from cold sea water are psychrotrophic microflora such as *Pseudomonas sp.* The aloe vera (*Aloe vera chinensis* Linn) cortex as by product from aloe vera pulp processing industry as source of antibacterial agent have not been optimally utilized or studied, moreover the aloe vera cortex contain aloe emodin and aloin [3]. Reference [4] informed that aloe vera cortex powder have antibacteria activity, therefore have potency as natural preservative for foods. Antibacterial activity of aloe vera cortex powder is significantly affect on inhibition of *Pseudomonas aeruginosa* growth compared with *Eschericia coli* and *Staphylococcus aureus*. Based on several information above, therefore study on aloe vera cortex extract as source of antibacteria and its application as inhibitor for king prawns spoilage during storage at 5°C is really needed.

## 2. Materials and Methods

Aloe vera (*Aloe vera chinensis* Linn) cortex was obtained from aloe vera processing industry, Pontianak, West Kalimantan, and it was directly dried until the water content 5.66%. Fresh king prawns with standard size 30 pieces per kg was

purchased from prawn farmer, Minggir, Sleman, Yogyakarta. Culture of *P. fluorescens* was obtained from Laboratory of Microbiology, Food and Nutrition Study Center, Gadjah Mada University, Yogyakarta. All chemicals were Analytical Reagent (AR) grade and water was double glass distilled.

### 2.1. Preparation of aloe vera cortex extract.

Hundred (100) g aloe vera cortex powder was macerated in 1000 ml of ethanol 50% by using orbital shaker at ambient temperature for 8 hrs. Sample was filtered using vacuum filter with Whatman filter paper No. 42. The clean supernatant was concentrated using rotary vacuum evaporator and dried using freeze dryer for 48 hrs.

### 2.2. Preparation of sample for analysis of aloe emodin and aloin.

Methanol solvent was added to the 5 g dry extract sample and centrifuged at 3,500 rpm for 20 minutes. The supernatant was filtered by Whatman filter paper No. 42, and filtered by membrane with pore size of 0,45  $\mu\text{m}$ . Aloe emodin analysis was carried out by using HPLC method, while analysis of aloin was carried out by using TLC method.

### 2.3. Minimum Inhibitory Concentration (MIC) test of aloe vera cortex extract

Concentration of aloe vera cortex extract was varied as follow 125, 250, 500 and 1000  $\mu\text{g/ml}$ , respectively for soaking of king prawns in nutrient broth and inoculated with 50  $\mu\text{l}$  of *P. fluorescens* culture and incubated at 30°C for 24 hrs. Control sample (without extract) was also done. Minimum Inhibitory Concentration test of extract to the inhibition of *P. fluorescens* was assayed by using [5] method.

### 2.4. Preparation of king prawns for storage at 5°C

The soaked king prawn samples (30 pieces per kg) in 500  $\mu\text{g/ml}$  of aloe vera cortex extract were packed in sterofom box and covered by cling-wrap plastic and stored at 5°C for 8 days. Every 2 days periode the king prawn samples were taken and analysed for their TPC, TVB-N, TMA, and pH. Organoleptic characteristics (appearance, odor and texture) were also evaluated by panelists with scoring method for degree of prawn quality (1 = worst quality up to 9 = best quality).

### 2.5. Analysis of quality parameters of king prawn during storage at 5°C

Analysis of Total Plate Count (TPC) of king prawn after soaking in variation of aloe

vera cortex extract concentration and during storage at 5°C was assayed by SNI 01-3232.3-2006 method [6]. TVB-N, TMA and pH of king prawn during storage at 5°C were carried out by using [7] and [8] methods, respectively. Organoleptic evaluation for king prawn during storage at 5°C by using SNI 01-2346-2006 method [9] with scoring test. Statistical analysis was used for analysis of collected data by Anova, and to identify the difference between treatments, therefore Least Significant Different (LSD) test was conducted with 95% of honesty level.

## 3. Results and Discussion

### 3.1. Dry extract preparation of aloe vera cortex

Extraction of aloe vera cortex to produce crude extract with 50% ethanol (1:10) and subsequent purification by filtration, rotary vacuum evaporation and freeze drying produced dry extract of aloe vera cortex. This dry extract was considered satisfactory as source of antibacterial agent for all subsequent experiments.

### 3.2. Inhibitory effect of aloe vera cortex extract to *P. fluorescens* growth

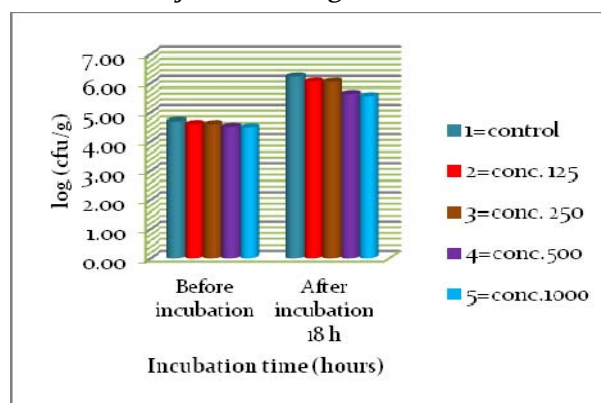


Figure 1 Concentration effect of aloe vera cortex extract on *Pseudomonas fluorescens* growth.

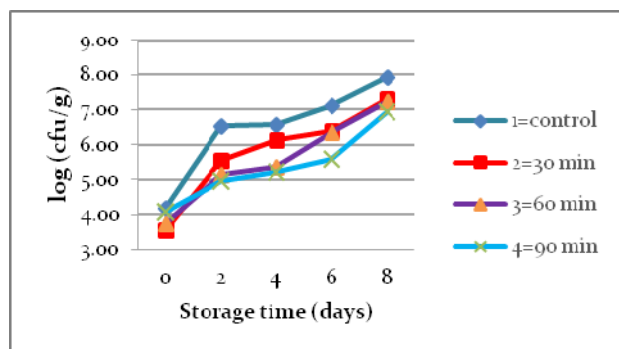
The effect of soaking in concentration variation of aloe vera cortex extract to the *P. fluorescens* growth is shown in Fig. 1. There was contrast condition in reduction of bacteria population between control (without extract) and treated samples with extract of aloe vera cortex after 18 hrs incubation. The optimum concentration of aloe vera cortex extract was 500  $\mu\text{g/ml}$ , although there was no significant different with concentration of 1000  $\mu\text{g/ml}$ . Before incubation there was very small difference 0.19 log cfu/g between control and treated samples and there was no significant different. While, after incubation for 18 hrs the different between control and treated samples was 0.58 log cfu/g or 1 log cycle.

Therefore soaking in concentration extract of 500 µg/ml after 18 hrs incubation was able to inhibit bacteria population 1 log cycle.

It was evident that inhibition by bioactive compounds in the extract of aloe vera cortex to the *P. fluorescens* growth occurred. It was suspectedly due to the breakdown or lysis of microbial cell or inhibition on synthesis of compounds of bacteria cell wall as confirmed by [10]. On the other hand, reaction between bioactive compounds and cell membrane might change the permeability of cytoplasmic membrane that can cause leaking of nutrient from inside cell, and it can inhibit substrate transport [11].

### 3.3. Effect of soaking in aloe vera cortex extract on bacterial growth based on Total Plate Count test

MIC test showed that the best concentration that was able to inhibit *P. fluorescens* growth was 500 µg/ml. Subsequent experiments were to use such concentration for king prawns preservation. Variations of soaking time were carried out i.e. 30, 60 and 90 minutes, respectively and control was also done. The bacteria growth of king prawns were determined before and during storage at 5°C for 8 days. The objective of this experiment was to study the effect of soaking in aloe vera cortex extract to the bacteria inhibition of king prawns during storage at 5°C, and the result of the experiment is shown in Fig. 2.



**Figure 2** Effect of soaking in aloe vera cortex extract to bacterial growth on king prawns based on TPC

Based on statistical analysis showed that variation of soaking time in 500 µg/ml aloe vera cortex extract was not significant difference ( $p \leq 0.05$ ). Subsequently, bacteria growth during storage of king prawns for 8 hrs were determined by TPC method (Fig. 2). This results are in accordance with [12] stated that more time for soaking in liquid smoke, so that the accumulation of benzopyrene in the soaked fish was also higher. Similar case in this study that soaking of king prawns

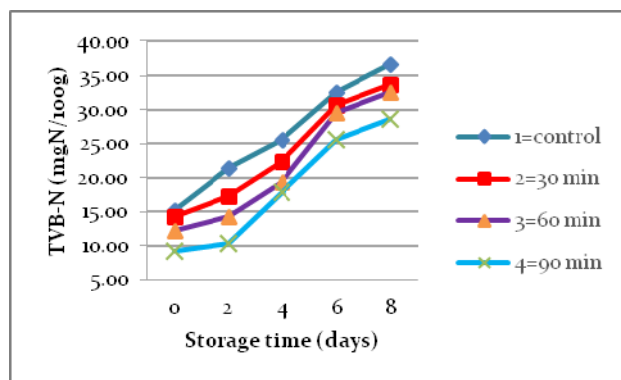
in the aloe vera cortex extract (500 µg/ml) cause the accumulation of bioactive compounds such as aloe emodin and aloin was also higher than control, therefore the inhibition of bacteria growth on king prawns occurred. Observation on second day of storage showed that soaking for 30, 60 and 90 minutes were also significant difference ( $p \leq 0.05$ ) with the control. It means that all of treatments showed the inhibition of *P. fluorescens*. The best treatment was soaking for 90 minutes and performed the lowest growth of *P. fluorescens* during storage at 5°C and all treatments were significantly difference ( $p \geq 0.05$ ), except for 2 days storage, there was no significant difference ( $p \leq 0.05$ ). Control sample had TPC 3.74 log (cfu/g) or increased 4 log cycle. While soaking for 90 minutes of course TPC increased 2.85 log (cfu/g) or equal to 3 log cycle. Inhibition of bacteria growth effectively until 6 days storage and TPC still under maximum requirement for consumption i.e.  $5 \times 10^5$  as regulated in SNI 01-2728.2-2006) [13].

### 3.4. Effect of soaking in aloe vera cortex extract to the king prawns spoilage based on TVB-N analysis

TVB-N is one of the methods to determine spoilage or freshness of fishery products based on volatility of base compounds as effect of protein degradation or its derivatives to release ammonia, histamine, hydrogen disulphide and trimethylamine having spoilage odor. More high of TVB-N value refer to the quality of fish products more decrease [14]. Products that are still good for consumption if they have TVB-N value of 35 - 40 mg N/100 g sample [2]. TVB-N test result is shown in Fig. 3. All treatments (soaking and storage time) and control were not significantly difference ( $p \geq 0.05$ ). At the beginning of observation on degradation of protein have not occurred, so that the TVB-N is relatively still low and they were not significant difference in between treatments. While observation of samples after 2, 4, 6 and 8 days storage were significantly difference ( $p \leq 0.05$ ). Soaking for 60 and 90 minutes, however the degree of prawns spoilage can be inhibited. Soaking for 90 minute was the best treatment, because inhibitory force was the highest or TVB-N value was the smallest.

Spoilage of prawns was indicated by detected spoilage odor as a result of bacterial degradation and released volatile compounds. According to [8] said that volatile base compounds such as ammonia and trimethylamine were resulted by spoilage bacteria. While [14] stated that increasing of TVB-N value during storage at 5°C was an effect of

protein degradation to produce volatile base such as ammonia, histamin and trimethylamine. Over all of the study showed that soaking in aloe vera cortex extract for 90 minutes was the best treatment (Fig. 3). It was performed by TVB-N value of 28,6 mg N/100 g after storage for 8 days and such TVB-N value was below minimum requirement for consumption i.e. 35 - 40 mg N/100 g [2].

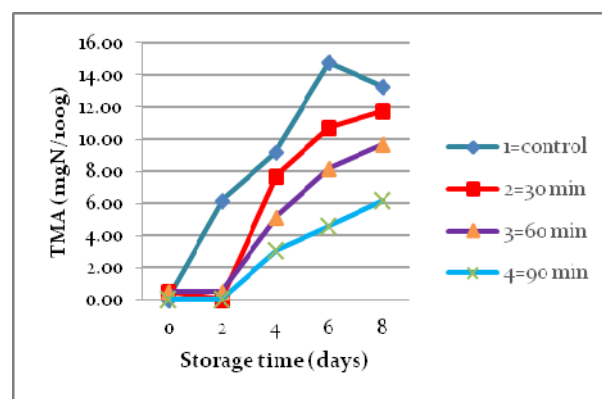


**Figure 3** Effect of soaking in aloe vera cortex extract to the Total Volatile Base-Nitrogen (TVB-N) of king prawns

### 3.5. Effect of soaking in aloe vera cortex extract on king prawns spoilage based on TMA analysis

Microbiological process is strongly related to the chemical process, and both process are occur together and affect each other on meat prawns. Microbiological activities cause degradation of several compounds in meat prawns to produce nitrogen base compounds such as TMA (trimethylamine) and ammonia from trimethylamine oxide (TMAO). Subsequent degradation of TMA will produce compounds with spoilage odor such as ammonia, putrescine, isobutylamine, isoamylamine and cadaverin. Therefore, the result of determination of prawns spoilage after soaking in 500 µg/ml extract and during storage at 5°C by using TMA analysis is shown in Fig. 4. All treatments (variation in soaking and storage time) including control were not significantly difference ( $p \geq 0.05$ ). It was due the beginning of storage, however microbial activity that breakdown the protein compounds to produce trimethylamine have not occurred. Beside this, the breakdown of protein compound by proteinase enzyme produced volatile compounds such as trimethylamine have not also occurred. Moreover, observation of TMA after storage for 2, 4, 6 and 8 days, respectively were significantly difference ( $p \leq 0.05$ ). It shows that microbial and enzymatic process to

produce volatile compounds such as TMA increased.



**Figure 4** Effect of soaking in aloe vera cortex extract to the bacterial growth based on Trimethylamines (TMA) of king prawns.

At the same time, however inhibitory process by bioactive compounds in the aloe vera cortex extract according to the difference of soaking time. Over all of the experiments showed that soaking of king prawns in the 500 µg/ml aloe vera cortex extract for 90 minutes was the best treatment. It was due to the soaking for 90 minutes in the aloe vera cortex extract was able to preventing microbial activity since 2 days storage until 6 days storage. Observation after 6 days storage performed TMA 4.6 mg N/100 g sample and this value was still below the maximum requirement for consumption i.e. 5 mg N/100 g.

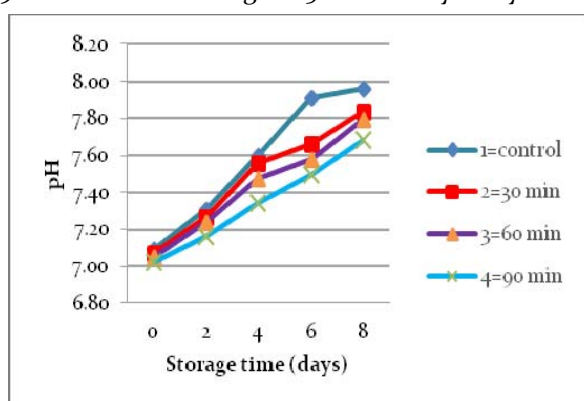
### 3.6. Effect of soaking in aloe vera cortex extract on pH of king prawns

The basic changes after killed prawns is rate of metabolism which uncontrol and destructive effect was continuously occurred. Metabolism reaction continuously occurred to breakdown complex compounds in the meat prawns to produce simple compounds that was easily consumed by bacteria as substrate for their growth. Reference [1] stated that pH is one of indicators for measuring freshness degree of fishery products such as prawns. Putrefaction of fishery products, therefore pH changes of meat prawns is due to the autolysis process and bacterial attack. Fig. 5. shows the pH of king prawns as results of soaking time variation in 500 µg/ml aloe vera cortex extract and during storage at 5°C.

All of the experiments show that the pH of king prawns increasing continuously bit by bit. Increasing of pH was due to nitrogen base accumulation [14]. But each treatment had different pH. Soaked king prawns had lower pH if compared to the control. It was due to the content of

bioactive compounds in the extract that was able to preventing bacterial spoilage that cause the accumulation of nitrogen base compounds. Higher the spoilage bacteria population will increase nitrogen base compounds accumulation, and subsequently will effect to the increasing of meat prawns pH.

Soaking in 500 µg/ml of aloe vera cortex extract for 90 minutes (Fig. 5) had similar trend with soaking for 60 and 30 minutes, but soaking for 90 minutes had lower pH if compared to the other treatments. Fresh prawns do have pH 7.25 – 7.50, and prawns with the marginal quality have pH 7.50 – 7.75, while spoilage prawns have pH over 7.75. The result of this study shows that control sample was able to stand until 4 days storage, because after 6 days storage the pH value have gained over 7.75 (i.e. 7.92). Soaking for 30 and 60 minutes was able to stand until 6 days storage, while soking for 90 minutes was able to stand until 8 days storage. Thus the best treatment was soaking in 500 µg/ml aloe vera cortex extract for 90 minutes and storage at 5°C for 6 days only.



**Figure 5** Effect of soaking in aloe vera cortex extract to the pH of king prawns.

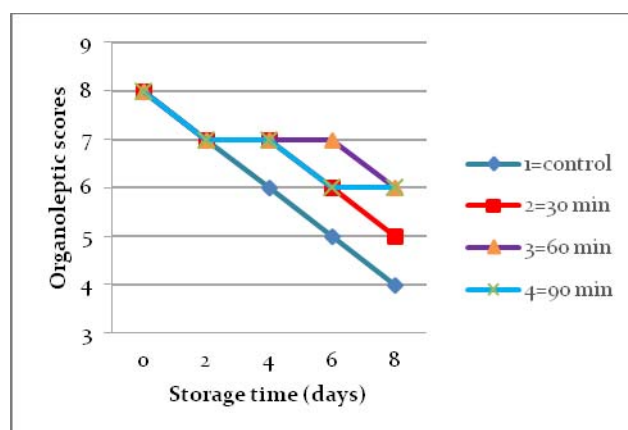
### 3.7. Effect of soaking in aloe vera cortex extract on organoleptic characteristic of king prawns

Organoleptic evaluation by using scoring test that is test method to determine quality level based on number scale that is 1 for lowest value and 9 for highest value. By using evaluation form (**score sheet**), evaluation was carried out to the evaluated samples by filling on score sheet according to the quality level of fresh prawns. Score sheet contains spesification of prawn quality including appearnce, odor and texture. Principle of organoleptic evaluation by using selected panelists in the amount of 20 panelists. The results of organoleptic evaluation are shown in Table 1 and Fig. 6. Table 1 shows that final score from 20 panelist tend to decrease proportionally with the increasing of storage time and or days of observation. It was occur due to microbial and

enzymatic activities which cause the meat prawns quality was decrease. Based on SNI 01-1728.2 – 2006 [13] stated that freshness level of prawns for good consumption have to perform minimal organoleptic score 7. Therefore, it can be concluded that based on organoleptic freshness of king prawns can last 2 days only for control sample, while soaking in 500 µg/ml aloe vera cortex extract for 30 and 90 minutes can last 4 days and six days for soaking time of 60 minutes (Fig. 6). Based on Tale 1. that before storage at 5°C and after 2 days storage had same organoleptic score. It showed that there were not significantly effects of the treatments (soaking and storage).

**Table 1** Organoleptic score of king prawn as result of soaking in aloe vera cortex extract and during storage at 5°C.

Soaking time (mins)	Time of observation (days)				
	0	2	4	6	8
Control	8	7	6	5	4
30	8	7	7	6	5
60	8	7	7	7	6
90	8	7	7	6	6



**Figure 6** Organoleptic scores of king prawns as result of soaking in aloe vera cortex extract with dose of 500 µg/ml

Soaking in 500 µg/ml aloe vera cortex extract for 30 minutes, however freshness level of king prawns can last 4 days with organoleptic score below 7. Soaking in the extract for 60 minutes had freshness level of treated king prawns was longer, but soaking for 90 minutes the freshness level of treated king prawns was shorter that can last 4 days. All of these reasons were due to the content of aloe emodin and aloin in the aloe vera cortex extract as strong anti-oxydant compounds. Naturally, prawns contain polyphenol oxydase that are able to cata-

lysed oxydation of monophenol to diphenol, and subsequent oxydation will produce o-quinon and it will react with amino acid to produce black color compounds namely black spot.

### 3.8. Aloe emodin and aloin as bioactive compounds in the cortex extract

Aloe vera cortex can be utilized as antibacterial agent because of the content of aloe emodin and aloin compounds. Analysis of aloe emodin by using HPLC method showed that the aloe vera cortex extract contained 18.21 ppm aloe emodin and 15.5 mg/g aloin (1.55%). Reference [15] stated that aloe emodin contained O-glycoside and C-glycoside, and Reference [4] also stated that aloe vera cortex have antibacterial activity, therefore it can be used as natural preservative. This potency was tried to preserve king prawns in order to prolong shelf life and to increasing economical value.

### 4. Conclusion

Minimum Inhibitory Concentraion (MIC) test showed that the best concentration of aloe vera cortex extract was able to inhibit *P. fluorescens* in concentration of 500 µg/ml. On such concentration bacterial growth was inhibited 1 log cycle. Soaking of king prawn in aloe vera cortex extract for 90 minutes at concentration of 500 µg/ml, however the king prawns was still palatable to be consumed up to 6 days storage at 5°C. It was performed by TPC  $3.9 \times 10^5$ , TVB-N 25.55 and TMA 4.60, while the pH was 7.5. All of such quality parameters meet to the quality requirement for fresh prawns. Although based on organoleptic evaluation that after 6 days storage showed that the best treatment was soaking in aloe vera cortex extract for 60 minutes with the organoleptic score 7. This organoleptic score was to agree with fresh prawns quality as regulated in SNI 01-2728.2-2006 [13].

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