Antioxidant and antimicrobial potency of *Eupatorium inulifolium* leaf extract for fish preservation

D R A Muhammad*, D T Miraningrum*, N H R Parnanto

*Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Jalan Ir. Sutami 36A Kentingan, Surakarta 57126, Jawa Tengah, Indonesia.

*Corresponding Author: dimasrahadian@staff.uns.ac.id

Abstract. Fish is generally known as a perishable product. This study aimed to investigate the antioxidant and antimicrobial potency of *Eupatorium inulifolium* leaf extract for fish preservation. The effect of different levels of the leaf extracts (0.1%, 0.25%, 0.5%) on the pH value, aw, Total Volatile Bases (TVB), Total Plate Count (TPC), and texture of tongkol fish (*Euthynnus Affinis*) during 4 days cold-storage was studied. The results showed that after 4 days of storage, the level of TVB was found at 28,745 mg/100 g in the control sample and 16,022 mg/100 g in the sample of fish with 0.5% of *Eupatorium inulifolium* leaf extract. On the last day of storage, the lowest number of microbe was found at 10,100 CFU/g in a sample of fish with 0.5% of *Eupatorium inulifolium* leaf extract while the highest number of microbe was 53,500 CFU/g in the control sample. The addition *Eupatorium inulifolium* leaf extract resulted in a harder texture. On the contrary, the *Eupatorium inulifolium* leaf extract addition had no significant effect on the aw value of the fish during storage. This study provided a new perspective of using *Eupatorium inulifolium* leaf extract for a natural preservative for fish.

1. Introduction

Indonesia is an archipelagic country with a total land area of 1,860,360 km², a sea area of 5,800,000 km², and a total of 81,000 km of coastal line. More than 76% of the area is water making this country one of the biggest fish producing countries in the world [1,2]. *Euthynnus affinis* which is locally well-known as tongkol fish is one of the main popular aquatic foods in Indonesia. This country produced about 291,863 tonnes tongkol fish in 2014 while the consumption level of tongkol fish in Indonesia is considered high, particularly in Papua (0.589 kg/week), East Kalimantan (0.308 kg/week), and West Sumatra (0.303 kg/week). Aside from its use for domestic purposes, tongkol fish is also a potential export commodity [3,4]. However, this commodity, in fact, is perishable, mainly because of the activity of bacteria, oxidation, and autolysis resulting in a short shelf-life [5]. To preserve fresh tongkol fish, many people still use non-food grade additives with the excessive dosage because of economic reasons. Natural antioxidants and the antimicrobial agent have gained high attention nowadays as they are considered safer and healthier than synthetic ones [6,7,8,9]. Therefore, finding an alternative preservative agent for fresh fish which is still affordable for people is crucial.

*E. inulifolium* leaf, also known as “kirinyuh”, has a long history as a traditional remedy in many ethnic groups in Indonesia [10]. However, up to date, there is still a very limited number of scientific reports regarding the antioxidant, and antibacterial activity of *E. inulifolium*. In a study by Saito and his co-worker, *E. inulifolium* has been testified to contain terpenoid compounds, monoterpenes, and sesquiterpene [11]. These compounds in fact can act as antioxidants and antibacterial agents [12]. Due
to that fact, *E. inulifolium* is potential as a natural preservative agent. Besides, as it is already well-known that cold temperature can slow down the deterioration of fresh fish [13], a study on the effectiveness of *E. inulifolium* leaf extract to preserve tongkol fish in low-temperature condition is required. Thus, this study aims to investigate the antioxidant and antimicrobial activity of *E. inulifolium* leaf extract and its potential for tongkol fish preservation in cold condition.

2. Material and methods

2.1. Preparation of *E. inulifolium* leaf extract

The fresh leaves of *E. inulifolium* were collected from nature around the rice field in Mojolaban, Sukoharjo. The leaves were dried in the oven at 50°C for 2 days. After that, the dry leaves were powdered using a kitchen blender and then sieved at 50 mesh. The extraction was carried out using a maceration method for 6 h in ethanol 80% with a ratio of 1:10 (w/v). Finally, it was filtered and then precipitated for 24 h at 26-27°C. This process was repeated three times. All the filtrate was mixed and then were vacuum-evaporated in a rotary evaporator at 50°C.

2.2. Preservation method and experimental design

The fillet tongkol fish was cleaned and cut became 3 parts. The extract of *E. inulifolium* leaf was dissolved in distilled water until reaching concentrations of 0.1 %; 0.25 % and 0.5%. Tongkol fish (600 g) was soaked in the *E. inulifolium* leaf extract for 30 minutes. After that, it was drained for 15 minutes and then packaged in PE 0.5 mm plastic. Finally, the sample was put in a jar and then saved in the chiller at 0°C for 4 days. The sample was frequently taken form the storage for further analysis. This research was designed by Completely Randomized Design (CRD) with one factor.

2.3. Analysis

In this study, the antioxidant activity of *E. inulifolium* leaf extract and the tongkol fish after 4-days storage were evaluated using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method which is by measuring the reduction of the purple-colored ethanolic solution of DPPH [14]. Briefly, 2 ml of 400 µM ethanolic solution of the DPPH was mixed with 1 ml of *E. inulifolium* leaf extract. Afterward, 7 ml ethanol 96 % was well-mixed and the mixture was kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a Jenway 6305 Spectrophotometer.

To test the antioxidant activity of the fish during storage, 5 g of tongkol fish was dissolved in ethanol 96 % (25 ml) and then homogenized using a vortex for 5 min. The mixtures were centrifuged at 5000 rpm for 2 min. The supernatant was sampled (1 ml) and then mixed with 7 ml of ethanol 96 % and 2 ml of DPPH 400 µM. The mixtures were well shaken and kept at room temperature in the dark for 30 min. Finally, the absorbance was measured at 517 nm using a spectrophotometer. Antioxidant activity was calculated using the Eq 1 where Ao is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the test compound.

\[
\text{Inhibition} \, (\%) = \left(1 - \frac{A_s}{A_0}\right) \times 100 \quad \text{(Eq. 1)}
\]

The total Plate Count (TPC) method was used to investigate the antimicrobial activity of *E. inulifolium* leaf extract in tongkol fish samples. In short, 10 g of the sample was dissolved in 900 ml distilled water. After that, the mixtures were homogenized for 5 min using a vortex. Each sample was made to one series of dilutions (1:100, 1:1000, 1:10000, 1:100000, and 1:1000000) and the 3rd, 4th, and 5th dilutions were 0,1 ml inoculated in the 1st and second day then the 4th, 5th, and 6th dilutions were 0.1 ml inoculated in the 4th, 5th, and 6th days. All of the samples were conducted twice and the incubation was conducted at 37°C for 2 days. Lastly, the colony of each sample was calculated [15].

To determine the ability of *E. inulifolium* leaf extract to preserve tongkol fish, an analysis of Total Volatile Base (TVB) was conducted. Accurately-weight of the sample (25 g) was mixed with 75 ml of TCA 7 %. The mixture was screened until got clear solutions. Boric acid (1 ml) was added into the inner part of the chamber of the Conway cup. The mixture (1 ml) was put in the outer part of the
chamber, and then 1 ml of K2CO3 was added in the outer part of the chamber. The sample was shaken for 1 min and then the incubation was done at 35°C for 2 h. Titration of the boric solutions with HCl N/70 was conducted until the pink color was reached. TVB value was calculated using Eq. 2.

\[ TVB = (\text{ml test titration} - \text{ml blank titration}) \times 0.2 \times 100/1 \times 100/25 \text{ mg N/100 g of meat fish} \]  
(Eq. 2)

The texture of tongkol fish was measured using Zwick/Z 0.5 Llyod Instrument Universal testing Machine. Each sample was tested by the tensile penetration test method. The water activity (aw) was measured using the Aqualab water activity meter. The calibration of the water activity meter was carried out using NaCl until showing a value of 0.5 + 0.02. The samples were firstly milled to reduce the sample size prior to water activity analysis. The statistical analysis was performed using ANOVA \((\alpha = 0.05)\) and followed by a test of the Duncan Multiple Range Test (DMRT) \((\alpha = 0.05)\) in case any significant different.

### 3. Result and discussion

The total Volatile Bases (TVB) value of tongkol fish during storage is presented in Table 1. It was shown that after stored in cold temperature for 4 days, the highest of TVB levels was 28,745 mg/100 g which was the control sample and the lowest was 16,022 mg/100 g which was the sample with 0.5 % \(E.\ inulifolium\) leaf extract. Theoretically, the increase of TVB value was correlated with proteolytic bacteria activity degrading proteins into amino acids. As shown in Table 2, the aw value on the fillet of tongkol fish was in the range of 0.96-0.97 which was suitable for bacteria growth. Some enzymes involved in the degradation process are category, peptidase, transaminase, amidase, amino acid decarboxylase, and glutamate dehydrogenase. A higher level of degradation resulted in a higher volatile compound such as putresin, isobutilamines, isoamilamines, and cadaverine \([16]\). \(E.\ inulifolium\) leaf extract was reported to contain sesquiterpenes \(\alpha\)-caryophyllene which have antibacterial activity and monoterpens \(\beta\)-pinene has antioxidant activity \([17,18]\). Thus, the higher the concentration of \(E.\ inulifolium\) leaf extract, the lower the increase of TVB value. During storage, the TVB value of the control sample increased from about 20 to 28.7 mg/100 g while the TVB value of tongkol fish with 0.5% of \(E.\ inulifolium\) leaf extract increased from about 15 to 16 mg/100 g.

#### Table 1. Total Volatile Bases (TVB) value of tongkol fish during storage (mg/100 g).

|                 | 0        | 1        | 2        | 3        | 4        |
|-----------------|----------|----------|----------|----------|----------|
| \(Eupatorium\ inulifolium\) leaf extract |           |          |          |          |          |
| Control         | 20.188\textsubscript{a} | 25.554\textsubscript{b} | 26.144\textsubscript{c} | 28.356\textsubscript{c} | 28.745\textsubscript{d} |
| 0.1 %           | 19.205\textsubscript{ab} | 18.649\textsubscript{a} | 21.076\textsubscript{bc} | 22.668\textsubscript{c} | 25.554\textsubscript{d} |
| 0.25 %          | 16.962\textsubscript{b} | 13.776\textsubscript{a} | 20.256\textsubscript{bc} | 20.561\textsubscript{c} | 21.278\textsubscript{b} |
| 0.5 %           | 14.996\textsubscript{a} | 12.032\textsubscript{a} | 14.353\textsubscript{ab} | 14.647\textsubscript{c} | 16.022\textsubscript{b} |

Superscript with the same small letters in the same row and the subscript with the same capital letter in the same column indicates no significant different at level \(\alpha = 0.05\).

#### Table 2. Water activity value of tongkol fish during storage.

|                 | 0        | 1        | 2        | 3        | 4        |
|-----------------|----------|----------|----------|----------|----------|
| \(Eupatorium\ inulifolium\) leaf extract |           |          |          |          |          |
| Control         | 0.967\textsubscript{ab} | 0.963\textsubscript{a} | 0.976\textsubscript{c} | 0.968\textsubscript{ab} | 0.977\textsubscript{c} |
| 0.1 %           | 0.975\textsubscript{ab} | 0.967\textsubscript{b} | 0.969\textsubscript{ab} | 0.977\textsubscript{b} | 0.976\textsubscript{b} |
| 0.25 %          | 0.971\textsubscript{b} | 0.969\textsubscript{b} | 0.963\textsubscript{a} | 0.970\textsubscript{b} | 0.977\textsubscript{c} |
To confirm the microbial growth during the storage. Total Plate Count analysis was carried out (Table 3). It was shown that the lowest number of bacteria was 10,000 CFU/g in the sample with 0.5% of *E. inulifolium* leaf extracted while the highest was 53,500 CFU/g found in the control sample after 4-days of storage. Also, it was remarkable that the higher the concentration of *E. inulifolium* leaf extract. The lower the increasing of the number of microbes. During storage, the TPC value of the control sample increased from about 780 to 53,500 CFU/g while that of tongkol fish with 0.5% of *E. inulifolium* leaf extract increased from about 150 to 10,100 CFU/g. Decreasing the microbial growth in the tongkol fish was because *E. inulifolium* leaf extract contained antibacterial compounds. The sesquiterpenes α-caryophyllene could react with porin (transmembrane proteins) on the outer of the bacteria cell wall causing damage of porin. Damaged porin substantially reduces the permeability of bacteria cell walls leading to nutritional deficiencies. In this case therefore the bacteria growth could be inhibited or even the bacteria could be killed [19]. In this research, Plate Count Agar (PCA) was used as a medium for TPC analysis and hence it allowed all microbes to grow. However, in the calculation of TPC analysis, a colony of bacteria with shiny, yellow, spherical colony shape as well as large and small size and also flat surface colony characteristic was counted.

Table 3. Total Plate Count (TPC) of Tongkol Fish during storage (CFU/g)

| Eupatorium inulifolium leaf extract | 0     | 1     | 2     | 3     | 4     |
|-------------------------------------|-------|-------|-------|-------|-------|
| Control                            | 780\(^a\) \(_C\) | 2,150\(^b\) \(_A\) | 7,500\(^a\) \(_AB\) | 18,500\(^b\) \(_B\) | 53,500\(^a\) \(_C\) |
| 0.1 %                              | 630\(^b\) \(_BC\) | 1,400\(^b\) \(_A\) | 6,700\(^a\) \(_B\) | 13,000\(^b\) \(_AB\) | 28,000\(^c\) \(_B\) |
| 0.25 %                             | 495\(^a\) \(_B\) | 720\(^b\) \(_A\) | 5,250\(^b\) \(_B\) | 7,550\(^b\) \(_AB\) | 21,500\(^a\) \(_AB\) |
| 0.5 %                              | 150\(^a\) \(_A\) | 348\(^a\) \(_A\) | 2,100\(^b\) \(_A\) | 5,200\(^b\) \(_A\) | 10,100\(^a\) \(_A\) |

Superscript with the same small letters in the same row and the subscript with the same capital letter in the same column indicates no significant different at level α = 0.05.

As *E. inulifolium* leaf extract was hypothesized to retard the oxidation process of tongkol fish, in this research the antioxidant activity of the extract as well as the treated-tongkol fish was investigated (Table 4). It was found that the DPPH radical scavenging activity of the extract was 46.53 %. In the *E. inulifolium* leaf extract-treated tongkol fish fillet, the highest antioxidant activity was found at 13.78 % which was in the sample with *E. inulifolium* leaf extract at a concentration of 0.5% whereas the lowest antioxidant activity was found at 0.51% which was in the control sample. *E. inulifolium* leaf extract contained terpenes possessing antioxidant activity. According to Muhammad [12], the monoterpenes β-pinene was known to have antioxidant capacity. Nevertheless, the antioxidant activity of plant extract was strongly influenced by their structure compounds [14]. It was noteworthy in all of the samples that the longer storage the tongkol fish fillet, the lower the antioxidant activity. This phenomenon indicated that there was an oxidation reaction during the storage. The phospholipids and fat breaking enzymes were still active even if the tongkol fish were kept at low temperature. The results of the oxidation process were free radical compounds, and then upon the reaction, peroxides, aldehydes, and carbonyls were formed.

Table 4. Antioxidant activity of tongkol fish during storage (%).

| Eupatorium inulifolium leaf extract | 0     | 1     | 2     | 3     | 4     |
|-------------------------------------|-------|-------|-------|-------|-------|
| Control                            | 10.791\(^a\) \(_A\) | 10.002\(^a\) \(_A\) | 5.667\(^b\) \(_A\) | 1.179\(^a\) \(_A\) | 0.510\(^a\) \(_A\) |
| 0.1 %                              | 21.655\(^a\) \(_B\) | 16.214\(^a\) \(_B\) | 13.686\(^c\) \(_B\) | 11.822\(^b\) \(_B\) | 8.428\(^a\) \(_B\) |
The physical properties of tongkol fish fillet, in terms of texture, were analyzed during the storage (Table 5). All of the samples with *E. inulifolium* leaf extract had higher textural value than the control sample. The sample with *E. inulifolium* leaf extracted at a level of 0.5% had the highest textural value which was at the level of 0.2689 N. Decreasing of the hardness of the tongkol fish fillet was related to cell damage of tongkol fish especially the sarcolema. The damaging was also caused the loss of water holding capacity in the tongkol fish. *E. inulifolium* leaf extract exhibited antioxidant and antimicrobial activity. It could further prevent cell damage during storage. Thus, the decrease in the textural properties of the fish could be prevented. It was noticeable that the addition of *E. inulifolium* leaf extract could even increase the hardness of the sample during storage. A similar trend was also found in the study of a previous study using *E. odoratum* leaf extract [20]. However, the mechanism was still unclear. Therefore, further research for revealing this phenomenon was required.

### Table 5. Texture of Tongkol Fish during storage (N).

| *Eupatorium inulifolium* leaf extract | Day   | 0     | 1     | 2     | 3     | 4     |
|--------------------------------------|------|------|------|------|------|------|
| Control                              |      | 0.2156<sup>a</sup> | 0.2051<sup>b</sup> | 0.1947<sup>ab</sup> | 0.1793<sup>ab</sup> | 0.1640<sup>a</sup> |
| 0.1 %                               |      | 0.2184<sup>a</sup> | 0.2258<sup>a</sup> | 0.2573<sup>b</sup> | 0.2709<sup>bc</sup> | 0.2913<sup>b</sup> |
| 0.25 %                              |      | 0.2335<sup>a</sup> | 0.2589<sup>ab</sup> | 0.2974<sup>bc</sup> | 0.3139<sup>c</sup> | 0.3268<sup>c</sup> |
| 0.5 %                               |      | 0.2689<sup>a</sup> | 0.2946<sup>ab</sup> | 0.3130<sup>c</sup> | 0.3172<sup>b</sup> | 0.3337<sup>b</sup> |

Superscript with the same small letters in the same row and the subscript with the same capital letter in the same column indicates no significant different at level α = 0.05.

### 4. Conclusion

The extract of *E. inulifolium* leaf exhibited antioxidant activity and had a significant effect on the reduction of the TVB level of tongkol fish. The extract could also inhibit microbial growth. The addition of *E. inulifolium* leaf extract resulted in harder fish texture after 4-days storage in cold conditions. The use of extracts of *E. inulifolium* leaf at a level of 0.5% had a better preservation effect than the extract at a lower concentration. A trajectory study of the application of *E. inulifolium* leaf extract in tongkol fish at room temperature is required for validating the preservation effect of the extract.

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