The effect of addition mangrove leaves powder to lipid oxidation of chocolate bar during the shelf life

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Abstract. Mangrove leaves consist of antioxidants to covert the nutritional value during storage, to the handling of radical scavenging and lipid oxidation. Many kinds of mangrove species such as Avicennia officinalis (AO), Avicennia marina (AM), and Rhizophora apiculata (RA) with contains an antioxidant such as flavonoids, tannins, and saponin. The study was aimed to determine FFA Value, Peroxide Value (PV), vitamin C, and antioxidant activity of chocolate by adding mangrove leaves powder (A. officinalis, A. marina, and R. apiculata) during storage 14 days at ambient temperature. The design used in this research was Randomized Block Design (RBD) using three different treatments for variety mangrove leaves powder. The test method used in this study are testing FFA Value, Peroxide Value (PV), vitamin C, and antioxidant activity. The statistical analysis used in this study is the analysis of variance (ANOVA) with a confidence level of 95%. The addition of mangrove leaf powder affects biochemical changes in chocolate products during 14 days of storage. The addition of R. apiculata leaf powder can slow the increase in FFA and PV levels and decrease antioxidant activity in chocolate bar products during the shelf life. In addition, the addition of Avicennia marina leaf powder can slow down the decrease in vitamin C levels due to the storage process. The results of Vitamin C test from chocolate with the addition of Avicennia marina leaf powder during storage for 14 days decreased by 0.18%, Avicennia officinalis 0.19%, and Rhizophora apiculata 0.35%.

Keywords: antioxidant; chocolate; lipid oxidation; mangrove leaves

1. Introduction

Indonesia is rich in natural biological resources used as a source of natural antioxidants, one of which is mangrove plants. The potential of natural antioxidants in mangrove plants is widely used in industrial progress, both in the health, beauty, and food industry. Exploration of antioxidant potential in mangrove plants, especially in the leaves of mangroves, has been widely carried out. Mangroves thrive in extreme environments because of their ability to produce secondary metabolites and act as antioxidants to adapt to various extreme environmental factors on the beach. Antioxidants can protect plants against environmental stress, both biotic and abiotic and prevent the formation of Reactive Oxygen Species (ROS) free radicals that are harmful to plants [1]. The utilization of mangrove plants as natural antioxidants can replace the use of synthetic antioxidants. The continuous use of synthetic antioxidants in food can trigger disease because they are carcinogenic. The advantage of using synthetic antioxidants is their very strong anti-radical activity. However, synthetic antioxidants BHA and BHT are potentially carcinogenic. The search for natural sources of antioxidants is urgently needed to replace the role of synthetic antioxidants [2]. Several types of mangroves that have potential as sources of natural antioxidants include Avicennia officinalis, Avicennia marina, and Rhizophora apiculata. The bioactive components detected in Avicennia marina leaves were flavonoids, steroids,
and reducing sugars. *Avicennia marina* leaves have also been shown to have antioxidant activity and can inhibit the oxidation process in oil emulsions [2] In addition, *Avicennia officinalis* leaves also contain bioactive components through initial phytochemical screening, namely carbohydrates, reducing sugars, combined reducing sugars, glycosides, tannins, alkaloids, proteins, terpenoids, and flavonoids which have the potential as antioxidants, analgesics, and antibacterial activities [3]. In addition, another study also stated that *Rhizophora apiculata* leaf extract showed antioxidant activity with an IC50 value of 96.68 ± 0.68 µg/mL, which means that *Rhizophora apiculata* leaves have potential as natural antioxidants.

The nature of antioxidant compounds is to inhibit and prevent oxidation reactions in foodstuffs. Oxidation reactions are caused by the presence of free radicals that trigger changes in the quality of food during storage. Food products that contain high levels of unsaturated fatty acids are easily oxidized and easily go rancid. One product that contains unsaturated fatty acids is chocolate. Chocolate bar contains cocoa fat which consists of unsaturated fatty acids, namely oleic (18:1) 32.6%, linoleic acid (18:2) 2.8%, linolenic acid (18:3) 0.1% [4]. Although unsaturated fatty acids can reduce atherogenicity, at the same condition high levels of unsaturated fatty acids make the product susceptible to oxidation which is the main cause of off-flavor development [5]. In addition, chocolate is a processed food product whose constituent ingredients consist of chocolate paste, sugar, cocoa butter, and powdered milk. The process of making chocolate with the addition of milk powder which is a type of animal protein that has a high-fat content and it is easily damaged [6]. The oxidation process in a food ingredient can be inhibited by several methods, including preventing contact between food with oxygen and light, adding antioxidants, storing at low temperatures, inactivating enzymes that catalyze oxidation reactions, and using packaging. The addition of antioxidants is a method that is widely used because it is relatively easy and inexpensive [7]. Mangrove leaves are a source of natural antioxidants that can be added to food. Apart from being a natural antioxidant, the addition of mangrove leaf powder to chocolate bars affects the nutritional composition of chocolate bars. The addition of mangrove leaf powder from *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina* species can increase water content, protein content, ash content, carbohydrate content, dietary fiber, and vitamin C [8]. Research related to the effect of adding mangrove leaf powder to chocolate bars has not been done. The related research that carried out is that the addition of mangrove leaf powder can increase the value of sensory quality in chocolate bar products [9], the addition of mangrove leaf powder can affect the nutritional composition of chocolate bar products [8]. During the storage period, chocolate bars can experience a decrease in quality, so this needs to determine the effect of adding mangrove leaf powder to fat oxidation in chocolate bar products. This study aims to determine the FFA Value, Peroxide Value (PV), Vitamin C, and antioxidant activity of chocolate bar products added with mangrove leaf powder during the shelf life.

2. Materials and Method

2.1. Materials and equipments

The research materials were *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina* species leaves, bar chocolate, ether (Merck), ethanol (Merck), phenolphthalein indicator (Merck), sodium hydroxide (Merck), acetic acid (Merck), chloroform (Merck), potassium iodide (Merck), sodium thiosulfate (Merck), starch indicator, ascorbic acid, DPPH solution, aquadest. The equipment’s used in this research were Erlenmeyer (pyrex), beaker glass (pyrex), buret (pyrex), volumetric flask (pyrex), volumetric pipette (pyrex), analytical balance (Ohaus), blender (Philips), Spektrometer UV-Visible (UV mini-1240), and stative and clamps.

2.2. Sample preparation

Mangrove leaves powder prepared from *Rhizophora apiculata*, *Avicennia officinalis*, and *Avicennia marina* species. Samples of mangrove leaves that have been collected then washed using water. The leaves are cut into small pieces and dried using a black transparent cloth to dry. Dried mangrove leaves mashed with a blender and then filtered to get a fine and smooth powder [9].
Mangrove leaf powder and chocolate mixture and stirred until homogeneous, then tempered, molded, and put in the refrigerator. The testing method used in this study was to test the water content, fat content, protein content, ash content, and different carbohydrate, fiber, and vitamin C levels. This research used experimental method and completely randomized design (CRD) with the addition of mangrove leaf powder of different types namely Rhizophora apiculata, Avicennia officinalis, and Avicennia marina, and control (without addition of mangrove leaf powder). The experiment in this study was repeated 3 times, so this study consisted of 12 experimental trials. The chocolate bars were stored for 14 days then tested FFA, PV, Vitamin C, and antioxidant activity in day 0, 7, and 14 in room temperature [10].

2.3. Free fatty acid (FFA) content
Placed known weight (W), approximately 10 g of Chocolate with mangrove leaf powder sample into a 200 mL Erlenmeyer flask; added 50 mL ether, 50 mL 95% ethanol, 2 to 3 drops of 1% phenolphthalein indicator; and titrated the mixture with 0.005 mol/L sodium hydroxide ethanol solution until the mixture turns pink for at least 1 min. The volume of the titrant consumed was recorded as V. FFA content was calculated using the following equation:

\[
FFA (\%) = \frac{V \times C \times 282}{1000 \times W} \times 100
\]  

(1)

Where C is the calibrated concentration of sodium hydroxide ethanol solution and 282 is oleic acid molar mass in g/mol [11].

2.4. Peroxide value (PV)
Weigh 5.00 ± 0.05 g of Chocolate with mangrove leaf powder into a 250 mL Erlenmeyer flask and add 30 mL acetic acid–chloroform (3:2) solution, and 0.5 mL saturated potassium iodide (KI) solution. Allow the solution to stand with occasional swirling for 1 min and then add 30 mL distilled water. Slowly titrate with 0.01-N sodium thiosulfate (Na\(_2\)S\(_2\)O\(_3\)). Continue titrating until the color changes to light yellow. Add 0.5 mL of 1% soluble starch indicator. Continue titrating, shaking the flask vigorously near the endpoint which is a faint blue color. Add the sodium thiosulfate (Na\(_2\)S\(_2\)O\(_3\)) dropwise until the blue color just disappears. The PV as mEq of peroxide per kg of oil was calculated according to following equation:

\[
PV = \frac{V \times C \times 1000}{W}
\]

(2)

Where V is the volume of (Na\(_2\)S\(_2\)O\(_3\)) solution in mL, C is the concentration of the (Na\(_2\)S\(_2\)O\(_3\)) solution in mol/L, and W is the weight of the Chocolate with mangrove leaf powder sample taken in g [11].

2.5. Analysis vitamin C level
Measurement of vitamin C levels was carried out using UV-Vis spectrophotometry method. Analysis of vitamin C levels was carried out in several stages, namely making 100 ppm standard vitamin C solution, determining the maximum absorption wavelength of vitamin C solution, then preparation of calibration curve. The method of measuring vitamin C levels begins with weighing a sample of 2.5 g, then the sample is dissolved in 50 ml of aquadest to the mark on the volumetric flask. The solution was diluted then the absorbance determined at the maximum wavelength [12].

2.6. Antioxidant activity
For the determination of antioxidant activity, 0.2 mL of each sample with various concentrations was pipette with a micropipette and put into a vial, then add 3.8 mL of 50 M DPPH solution. The mixture
Figure 1. FFA content of chocolate bar with the addition of different mangrove leaf powder during the shelf life. KO: Control, AM: *Avicennia marina*, *Avicennia officinalis*, *Rhizophora apiculata*.

was shaken until homogeneous and left for 30 minutes in a dark place, then the absorption was measured by UV-Vis spectrophotometry at the maximum wavelength of DPPH. The antioxidant activity of the sample by the magnitude of the DPPH radical absorption inhibition can be determined by calculating the percentage of DPPH uptake inhibition [13].

2.7. Statistical analysis
Data were analyzed using SPSS 22 software with analysis of variance (ANOVA) 95% confidence level. Further tests were carried out using Duncan's test to determine the significant differences between each variable.

3. Results and discussion
3.1. Free fatty acid (FFA) content
FFA (Free Fatty Acid) test used to determine the level of free fatty acids contained in a chocolate bar product. Free Fatty Acid (FFA) levels were determined using the acid-base titration method. The level of FFA in a portion of food shows the level of damage to the food due to the breakdown of triacylglycerols and fatty acid oxidation [14]. High levels of free fatty acids in food will cause an unpleasant aroma and taste called rancidity. Oxidative rancidity can be caused by the oxidation of fatty acid bonds to form aldehydes, ketones, and other acidic compounds that have lower molecular weights than the fatty acids [15].

Based on Figure 1, the shelf life of chocolate for 14 days showed an increase in the FFA value. However, the addition of mangrove leaf powder as a source of antioxidants can suppress the increase in FFA values, while the control chocolate showed a significant increase. The best treatment that can inhibit the rate of increase in FFA is the addition of mangrove leaf powder of the *Rhizophora apiculata* (RA) species. FFA is one indicator of damage to fat or oil in a food ingredient. The role of mangrove leaf powder in chocolate is as a source of antioxidants, where antioxidants can inhibit free radicals and fat oxidation. Antioxidants are compounds or substances that can inhibit, delay, prevent oxidation reactions even in small concentrations. Oxidation is a chemical reaction that can produce free radicals, thereby triggering a chain reaction. In addition, *R. apiculata* contains flavonoid, tannin, and terpenoid antioxidants [16]. The results of the FFA test on the control treatment on the 14th day were 2.72%, while the results on the chocolate added with mangrove leaf powder had FFA levels of
Figure 2. Peroxide value (PV) of chocolate bar with the addition of different mangrove leaf powder during the shelf life. KO: Control, AM: Avicennia marina, Avicennia officinalis, Rhizophora apiculata.

1.64-1.93%. The permissible level of FFA in a food ingredient is not more than 3% [15]. The results of the FFA test in the control treatment were almost close to the threshold of 3%, while the treatment with the addition of mangrove leaf powder had FFA levels that were still below the maximum allowed FFA limit.

The shelf life of chocolate can be affected by several parameters, namely storage temperature, humidity, availability of oxygen in the environment, and other added ingredients [5]. The increase in free fatty acid levels is also affected by the length of storage. The test results showed that the levels of free fatty acids increased from storage days 0, 7, and 14. During storage, fats underwent physico-chemical changes caused by hydrolysis and oxidation reactions. Improper storage for a certain period can cause the breakdown of the triglyceride bonds in the oil and form glycerol and free fatty acids [17].

3.2. Peroxide value (PV)

Fat oxidation is one of the causes of changes in taste and aroma in chocolate. Peroxide Value is one of the primary forms of fat oxidation. Peroxide Value (PV) testing on chocolate bar samples with the addition of mangrove leaf powder was carried out by the titration method. The determination of the peroxide value is carried out by iodometric titration which calculates the amount of iodine liberated by KI through oxidation by peroxides in fats or oils [18]. The PV measurement determines the levels of peroxides and hydroperoxides formed at the beginning of fat oxidation. The high PV value indicates that the fat has been oxidized [19].

The increase in PV during the shelf life is one indicator of a decrease in the quality of a food ingredient. The increasing peroxide value during the shelf life indicates that the peroxide content increases due to the oxidation process [14]. Based on Figure 2, the PV levels in cocoa with the addition of mangrove leaf powder and control cocoa during the 14-day shelf life increased. However, the addition of mangrove leaf powder to chocolate can slow the increase in PV levels. In chocolate with the addition of leaf powder, Avicennia marina increased by 1.75 meqO2/kg, Avicennia officinalis 2.09 meqO2/kg, and Rhizophora apiculata 0.94 meqO2/kg. While, in the control treatment, the PV increase was 3.88 meqO2/kg. The addition of Avicennia marina, Avicennia officinalis, Rhizophora apiculata mangrove leaf powder had an effect on the rate of PV increase in cocoa during storage (P<0.05). The addition of natural antioxidants can slow down the oxidation of fat in food during its
shelf life. The addition of *Rhizophora apiculata* mangrove leaf powder can slow the increase in PV values the best because *Rhizophora apiculata* leaves contain bioactive components of flavonoids, tannins, and terpenoids and have strong antioxidant activity, which can be determined from the IC50 value < 50 ppm [16]. While, the antioxidant activity of *Avicennia marina* mangrove leaves inhibits the oxidation process in fat emulsions, but the antioxidant activity is still relatively weak [2]. The bioactive compounds contained in the leaves of *Avicennia Marina*, *Avicennia officinalis*, and *Rhizophora apiculata* are the same, namely flavonoids. Flavonoids are substances that contain polyphenolic compounds derived from plants. Flavonoids are antioxidants that are the potential to ward off free radicals, so they are often used as a prevention or treatment of cancer [20].

### 3.3. Vitamin C

Vitamin C is an essential compound commonly referred to as ascorbic acid, is an ordinary vitamin, and is easily damaged due to oxidation. Vitamin C is easily oxidized when exposed to oxygen and this process can be accelerated by heat and light and the length of storage [21]. Analysis of vitamin C levels in this study used the UV-Vis spectrophotometry method. Analysis of vitamin C levels carried out in this study aimed to determine the effect of adding mangrove leaf powder from different species to changes in the vitamin C content of chocolate bar products during storage.

The results of statistical tests showed that the addition of mangrove leaf powder was significantly different from changes in vitamin C levels during the storage process (P<0.05). Figure 3 shows that the addition of mangrove leaf powder can slow down the decrease in vitamin C levels in chocolate bars. The results of Vitamin C test from chocolate with the addition of *Avicennia marina* leaf powder during storage for 14 days decreased by 0.18%, *Avicennia officinalis* 0.19%, and *Rhizophora apiculata* 0.35%. While the control treatment decreased levels of vitamin C was greater, namely 0.61%. The decrease in vitamin C levels was due to an increase in the activity of the ascorbic acid oxidase enzyme which plays a role in the overhaul of vitamin C due to the length of storage. With a storage period of 2 to 3 days, ascorbic acid oxidase, which plays a role in the breakdown of vitamin C, decreased its activity. The reaction for the breakdown of vitamin C is still ongoing but is running slowly, resulting in a decrease in vitamin C levels. This means that the activity of enzymes that play a role in the reshuffling of vitamin C continues with increasing storage time [22]. A decrease in vitamin C levels in food is an indication of a decrease in the quality of the food. Vitamin C is an essential compound needed by the body. In addition, biochemically vitamin C has various roles, namely:
3.4. Antioxidant activity

Testing of antioxidant activity in this study used the DPPH method. Quantitative measurement of the antioxidant activity of a material can be seen from the occurrence of purple decay in the Diphenylpicrylhydrazyl (DPPH) material. If the DPPH solution is added to ingredients containing antioxidants, the intensity of the color of the DPPH solution will decrease according to the concentration and inhibition of materials containing antioxidants [23]. The results of the antioxidant activity test on chocolate with the addition of different mangrove leaf powder during 14 days of storage are presented in Figure 4.

Based on Figure 4 shows that the highest antioxidant activity was in the *Rhizophora apiculata* treatment. The lowest antioxidant value was in the control treatment without the addition of antioxidants from mangrove leaf powder. The level of antioxidant activity is affected by the constituent components of the antioxidant itself and its free radical inhibition ability. *Rhizophora apiculata* is a type of mangrove that has the potential as a source of natural antioxidants. The leaves of this plant contain secondary metabolites such as tannins, phenolics, chlorophyll, carotenoids, and alkaloids. The antioxidant ability of the observed extract was higher than the antioxidant activity of *Avicennia marina* that had an IC50 value of 182.33 ppm and was a weak antioxidant [2]. *R. apiculata* can adapt better than *A. marina*, and it can affect the active compounds in it. The antioxidant activity of plant extracts depends on the type of species, extraction method, season, and sampling location [24]. In addition, the decrease in antioxidant activity can be affected by environmental conditions of storage and storage time. Different storage conditions and storage time can affect the depression of antioxidant activity in purple yam flour [25].

4. Conclusions

The addition of mangrove leaf powder affects biochemical changes in chocolate products during 14 days of storage. The addition of *R. apiculata* leaf powder can slow the increase in FFA and PV levels...
and decrease antioxidant activity in chocolate bar products during the shelf life. In addition, the addition of Avicennia marina leaf powder can slow down the decrease in vitamin C levels due to the storage process. The results of Vitamin C test from chocolate with the addition of Avicennia marina leaf powder during storage for 14 days decreased by 0.18%, Avicennia officinalis 0.19%, and Rhizophora apiculata 0.35%.

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