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Fruit Stalk Extract from Chili Peppers (*Capsicum annum* L.) as a Natural Antioxidant to Inhibit Oxidation in Crude Palm Oil

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Abstract

Fruit stalk of chili pepper (*Capsicum annum* L.) so far is still an untapped waste. The content of active compounds in fruit and fruit stalk of chili pepper (*C. annum* L.) is known to have good antioxidant activity. The purpose of this study was to evaluate the extract of fruit stalk of chili pepper in inhibiting the oxidation of crude palm oil (CPO). In this study, the extraction of fruit stalk of chili pepper (*C. annum* L.) was carried out with n-hexane (from now on referred to as CHE) solvent and with ethanol solvent (from now on referred to as CEE). CHE and CEE were analyzed for total phenolic and flavonoid analyzes. Next, an evaluation of antioxidant activity was carried out using the DPPH method. The effectiveness test of adding the two extracts to the quality of CPO was carried out for ten days using a Completely Randomized Design (CRD) analysis. The parameters observed were peroxide numbers, free fatty acids, and iodine numbers. Data were analyzed by ANOVA and followed by a Least Significance Different (LSD) test. The results show that the total phenolic value is 0.257 mg QE/g dry weight in CEE and 0.003 mg QE/g dry weight in CHE. Total flavonoid values are 0.155 mg QE/g dry weight in CEE and 0.003 mg QE/g in CHE. IC_{50} values for DPPH test extract are 223.72 µg/mL in CEE and 953.77 µg/mL in CHE. The test results of the effectiveness of the two extracts against CPO show thin CEE, and CHE significantly ($P < 0.05$) influences to CPO free fatty acids, peroxide number, and iodine number. Both extracts can protect CPO from oxidation. CEE is more effective in maintaining CPO quality for ten days with free fatty acid values (2.1%), peroxide value values 0.48 meq/kg, and iodine number 54.8. Furthermore, this result meets the CPO quality standard, according to SNI-01-2901-2006.

1. Introduction

The development of oil palm agribusiness in Indonesia has had a very positive impact on national development because oil palm is one of the important foreign exchange-earners from the non-oil and gas sector. The benefits of oil palm development include increasing farmers' income, providing raw materials for other downstream industries, increasing employment opportunities, and supporting regional development efforts to be more advanced and developed [1].

The yield of crude palm oil (CPO) and the kernel is an essential industrial material because of its wide use [2]. Oil yield is the percentage of oil in a fruit bunch that is affected by several factors, including processing, fruit type, and harvesting techniques [3]. In the industrial sector, CPO is used as a material for cooking oil, margarine, soap, cosmetics, steel industry, wire, and pharmaceutical industries. Based on its application, CPO is the largest export commodity in Indonesia, where CPO production continues to increase.

Timeliness in the transport and processing of oil palm is related to the levels of free fatty acids (FFA) contained in oil palm fruit. Even though the harvested bunches are of good quality, if transportation is not managed properly, takes too long to travel or is long accumulated in the factory; it will automatically raise FFA. While a high FFA requires higher costs in the bleaching process [4]. Transporting fruit from the garden to the factory must be done as soon as possible. Palm fruit that is cut must be processed on the same day so that free fatty acids are not high. Peak harvest usually occurs when it rains every day. So transportation facilities and infrastructure must be considered because usually, the transportation of harvested fruit lasts for 24 hours [2].

Chili pepper (*Capsicum annum* L.) is one of the important plants in the food industry and the pharmaceutical industry because it is widely used for cooking spices. At this time, many researchers began to analyze the content of chili. Chili pepper (*C. annum* L.) contains the chemical compound capsaicin (8-Methyl-N-vanillyl-trans-6-nonenamide), which can cause spicy taste and capsaicin has antioxidant activity [5].

From the literature search, studies of stem extracts from chili pepper (*C. annum* L.) have not been found. While the availability is quite a lot and only becomes waste. Therefore, in this research, the extract of chili pepper (*C. annum* L.) fruit stalk was made, and total phenolic and flavonoid extracts were determined. Then the antioxidant activity was evaluated using the DPPH method. The effectiveness test of the addition of ethanol extract and n-hexane extract to CPO quality was carried out for ten days using a Completely Randomized Design (CRD) analysis. The parameters observed were peroxide numbers, free fatty acids, and iodine numbers. Data were then analyzed using ANOVA.

2. Methodology

In this research, ethanol extracts (from now on referred to as CEE) and n-hexane (from now on referred to as CHE) were made from fruit stalk of chili pepper (*C. annum* L.). In both extracts, total phenolic and flavonoid contents were determined, followed by the measurement of antioxidant activity using the DPPH method. The effectiveness of the addition of the two extracts to the quality of CPO was then tested and observed for ten days, where every 2 (two) days, the samples were analyzed. The parameters analyzed were peroxide numbers, free fatty acids, and iodine numbers.

The treatment consisted of:

A_0 = Control or without extract (250 mL CPO)
A_1 = 0.1 g of ethanol extract (CEE) in 250 mL CPO
A_2 = 0.1 g of n-hexane extract (CHE) in 250 mL CPO

2.1. Equipment and Materials

The equipment used in this study was Heidolph UV 2000 rotary evaporator, distillation equipment, incubator (Memert), microplate reader, and glassware. While the materials used were: fruit stalks from ripe chili pepper (*C. annum* L.) (at least three months old) obtained from Arengka market at Pekanbaru city, crude palm oil (CPO), NaOH, Wijs solution, ethanol, n-hexane, chloroform, potassium iodide, $Na_2S_2O_3$, HCl, indicator pp, acetic acid, starch solution, Folin-Ciocalteu reagent, Na_2CO_3, $NaNO_2$, $AlCl_2$, and DPPH (1,1-diphenyl-2-picrylhydrazyl) and distilled water.

2.2. Experiment

5000 g of fruit stalk of chili pepper (*C. annum* L.) were sorted and separated from the dirt, washed with running water and air-dried. Then sorted again to separate the dried fruit stalks with dirt or materials that were carried during the drying process. After that, the fruit stalks were blended and sifted with a B30 sieve. As much as 1480 g of powder was obtained and then stored in a clean container and protected from light.

2.2.1. Sample Extraction

1480 g chili stalks that have been finely divided into 700 g each for macerated with n-hexane and 780 g for macerated with ethanol. N-hexane and ethanol were used as much as ± 4000 mL each, and the sample was completely submerged. The mixture was stirred for 2 hours, then allowed to stand for 3x24 hours in a tightly closed container. Then, the filtrate was filtered and concentrated with a rotary evaporator. Maceration was repeated until a clear (colorless) maceration solution was obtained. Concentration results are then collected, dried, and weighed. The weight of n-hexane extract (CHE) was 4.93 g (yield of 0.68%), and ethanol extract (CEE) was 4.770 g (yield of 0.37%). Next, each extract was tested qualitatively and quantitatively [6].

2.2.2. Determination of Total Phenolic Content

The total phenolic content was determined by the visible spectrophotometric method according to the method [7], in the following way: Some extracts or test fractions were put into a 10 mL flask, added with 0.4 mL of the Folin-Ciocalteu reagent, and left for 5-8 minutes. The next solution was added 4 mL Na_2CO_3 7% and added double distilled water to the boundary mark. After 2 hours, the absorbance was read at a wavelength of 765 nm. As a blank, double distilled water and Folin-Ciocalteu reagent were used. The total phenolic content is expressed as grams of gallic acid equivalent (GAE) per 100 grams of the dry weight of the subfraction (% w/w GAE).

2.2.3. Determination of Total Flavonoid Content

The total flavonoid content was determined using visible spectrophotometry according to the method [7]. Some extracts or fractions of the test were put into a 10 mL measuring flask, plus 4 mL of distilled water and 0.3 mL of $NaNO_2$ solution, then left for 6 minutes. After that, the solution was added with 0.3 mL of 10% $AlCl_2$ and left for 5 minutes. The next solution was added 4 mL of 10%

NaOH and distilled water to reach a volume of 10 mL. The solution was left for 15 minutes, and then the absorbance was read at a wavelength of 510 nm. The same was done with blanks (consisting of all reagents used but did not contain quercetin or test samples). The total flavonoid content is expressed as grams of quercetin equivalent (QE) per 100 grams of sub-fractions (% w/w QE).

2.2.4. Antioxidant Activity Test

The antioxidant activity test was carried out using a two-fold dilution microplate reader with the DPPH method (1,1-diphenyl-2-picrylhydrazyl). Antioxidant measurements were carried out in a dark atmosphere measured at a wavelength of 520 nm [8]. A total of 2 mg of CHE was dissolved in 2 mL methanol (1000 µg/mL). Row A was put into a sample of 100 µL (the plate consisted of row A-H totaling 12 holes each). 50 µL of methanol was added to each well in row B-F. Line A was pipetted 50 µL and put into line B. Line B was pipetted 50 µL put into line C and carried to line F. Line F was pipetted 50 µL, then discarded to concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL was obtained. Meanwhile, the G-H line was filled with 100µL methanol. Row H was only filled in wells 1-6. Row A-G was added as much as 80 µL DPPH with a concentration of 40 µg/mL, then incubated for 30 minutes. The free radical activity was measured by decreasing the absorbance of DPPH with a microplate reader and then processing the data. The positive control used as a comparison was vitamin C, with a concentration of 50 µg/mL. The same procedure was carried out for ethanol extract (CEE)

The% inhibition value is calculated using the following formula:

$$\%\ Inhibition\ = \frac{absorbance_{control} - absorbance_{sample}}{absorbance_{control}} \times 100\%$$

2.2.5. Quality Testing of Crude Palm Oil (CPO)

The CPO quality tests, including free fatty acids, peroxide numbers, and iodine numbers, were conducted in reference to SNI 7709: 2012.

2.2.6. Data Analysis

The data obtained were analyzed using analysis of variance. Variance analysis results were tested with the Least Significant Difference (LSD) test at 5% level. The complete randomized linear design model is as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where: Yij = Observation value in the i^{th} to j^{th} treatment

µ = General average value
τ_i = Effect of i^{th} treatment
ε_{ij} = Effect of errors on the i^{th} test of the j^{th} replication

3. Results and Discussion

3.1. The total phenolic and flavonoid content of the fruit stalk of chili pepper extract (*C. annum* L.)

The results of total phenolic and flavonoid measurements, it is observed that the content of CEE is greater than the content of CHE, as presented in Table 1.

Table 1. Total phenolic and flavonoid content of the fruit stalk of chili pepper extract

Sample	Total Phenolic Content (mgGAE/g)	Total Flavonoid Content (mg QE/g)
CEE	0.257	0.1547
CHE	0.003	0.0025

Polar compounds will dissolve in polar solvents, and vice versa or better known as the principle of "like dissolves like". Phenolic and flavonoid compounds are secondary metabolites that are semipolar - polar in plants. In previous studies, phenolic and flavonoid compounds were known to have various biological effects as antioxidants [9], protecting cell structure, anti-inflammatory, and antiseptic [10]. The benefits of flavonoids include protecting the cell structure, increasing the effectiveness of vitamin C, and as an antibiotic [7].

3.2. Antioxidant values obtained by the DPPH Method

The inhibition percentage of CEE and CHE antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method is presented in Table 2.

Table 2. Antioxidant activity of fruit stalk extracts from chili pepper (*C. annum* L.)

Sample	IC_{50} (µg/mL)
CEE	223.7
CHE	953.7

Table 2 explains that the polar CEE antioxidant activity is better than the non-polar extract activity. The antioxidant activity of this extract is probably caused by the presence of phenolic compounds and flavonoids that are extracted. The ability of antioxidant activity of a sample is due to the presence of compounds that have active groups, i.e., hydroxy groups, which can be free antiradical. Antioxidant activity is performed by donating unpaired electrons to free radical compounds so that free radicals become stable. Compounds that have the potential as natural antioxidants are phenolic compounds, one of which is a flavonoid compound. The more flavonoid and phenolic compounds in a sample, the higher the antioxidant activity [10].

3.3. Effectiveness of CEE and CHE Addition to CPO Quality

The results show that the addition of CEE and CHE to CPO was able to inhibit the oxidation process in CPO. From the three test parameters, including free fatty acids, iodine number, and peroxide number, it was observed

that with the addition of both extracts, the quality of CPO samples was included in the SNI-01-2901-2006 Quality Standard range, as presented in Table 3.

CHE is non-polar, but from the total phenolic and flavonoid test results, this extract still has phenolic and flavonoid content. When CHE is added to the semipolar CPO, it also shows good activity. This is because the content of the active compound can be spread evenly in the sample. Meanwhile, polar CEE has a greater total phenolic and flavonoid content than CHE, but its solubility is smaller in CPO than CHE, so its effectiveness is almost the same as CHE in inhibiting the oxidation process of CPO samples. The results of the complete random design analysis continued with the Least Significant Difference Test (LSD) at the 5% level.

3.3.1. Free fatty acids in CPO

The provision of fruit stalk extract from C. annum L significantly affects free fatty acids on CPO at days 0, 2, 4, 6, 8, and 10. Results of the Least Significant Difference test (LSD) at 5% is presented in Figure 1.

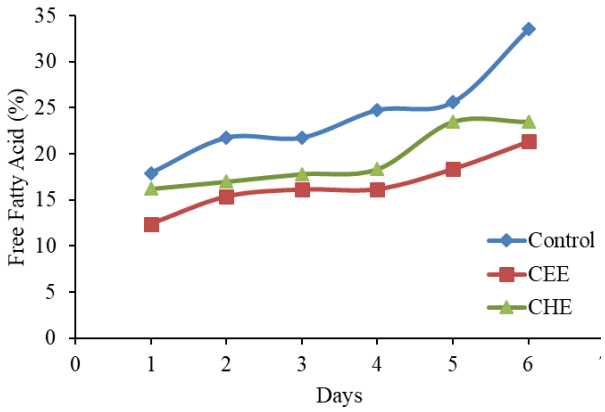

Figure 1. Effectiveness of fruit extract from C. annum L on CPO free fatty acid values measured in 10 days

Figure 1 shows a decrease in the value of free fatty acids after the addition of CEE and CHE. During storage, oils and fats undergo physical-chemical changes caused by hydrolysis and oxidation processes. Storage for a certain period can cause the triglyceride bonds to break in the oil, forming glycerol in free fatty acids.

The addition of CEE and CHE to CPO shows that the value of free fatty acid does not change much as a storage time function. This is because when adding chili fruit stalk extracts, there was contact between CPO and the extract caused diffusion between the two, so CEE and CHE were distributed in CPO.

3.3.2. CPO Iodine Number

The provision of fruit stalk extract of chili pepper significantly affects iodine number on CPO on day 0, whereas on days 2, 4, 6, 8, and 10 had no significant effect. The results of the Least Significant Difference (LSD) at the 5% level are shown in Figure 2.

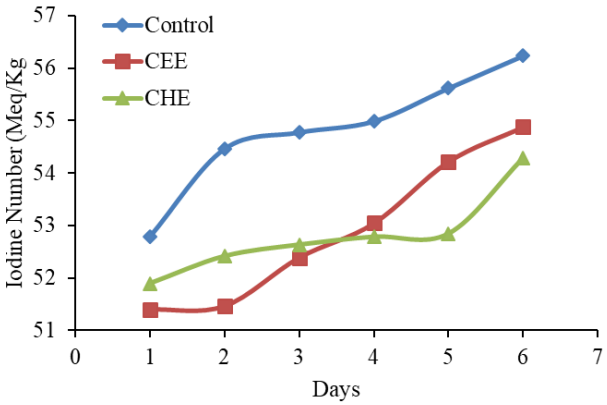

Figure 2. Effectiveness of fruit stalk extracts of chili pepper against CPO iodine numbers measured within ten days.

The addition of CEE and CHE to CPO can maintain the value of iodine number in the quality standard. This is because of the phenolic and flavonoid content in the extract, which can block oxygen from binding to the double bonds of fatty acids in CPO.

3.3.3. CPO Peroxide Numbers

The provision of fruit stalk extract of chili pepper significantly affects the peroxide number on CPO days 2, 4, and 6, while on days 0, 8, and 10 have no significant effect. The results of the Least Significant Difference (LSD) at the 5% level are shown in Figure 3.

Table 3. Effectiveness of fruit stalk of chili pepper extract (*C. annum* L.) on the value of free fatty acids, iodine numbers and peroxide numbers of CPO measured within ten days

Day	Free fatty acid (%)			iodine numbers			Peroxide number (meq/kg)		
	Control	CEE	CHE	Control	CEE	CHE	Control	CEE	CHE
D-0	1.7918 a	1.2370 b	1.6212 a	52.780 a	51.399 b	51.892 b	0.4414 a	0.3906 a	0.4245 a
D-2	2.1750 a	1.5356 b	1.6989 b	54.462 a	51.463 b	52.414 a	0.4584 a	0.3907 b	0.3395 b
D-4	2.1754 a	1.6125 b	1.7811 b	54.778 a	52.388 b	52.633 a	0.4925 a	0.4417 b	0.4755 b
D-6	2.4740 a	1.6161 a	1.8343 b	54.989 a	53.043 b	52.784 a	0.5094 a	0.4586 b	0.4756 b
D-8	2.5599 a	1.8343 b	2.3464 b	55.621 a	54.219 b	52.845 a	0.5095 a	0.4755 a	0.4925 a
D-10	3.3524 a	2.1330 b	2.3465 b	56.239 a	54.882 b	54.291 a	0.5096 a	0.4756 a	0.4926 a
SNI-01-2901-2006	< 5%			iodine number = 50 – 55			Maximum peroxide number= 10 Meq/Kg		

Note: Numbers followed by different letters on the same line are significantly different according to the LSD test at a 5% level.

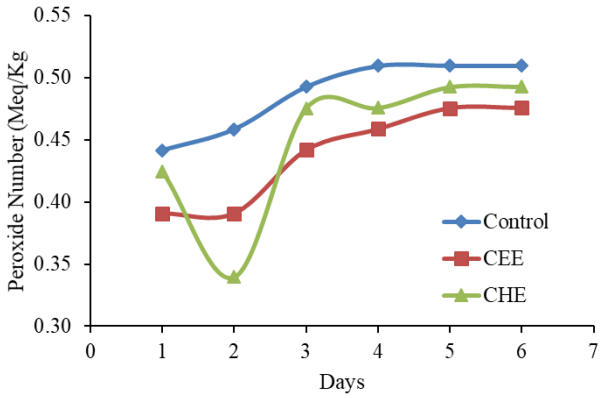

Figure 3. Effectiveness of fruit stalk extracts of chili pepper against CPO peroxide numbers measured within ten days.

Rancid odors in oils and fats occur due to oxidation reactions. The effect of temperature and light also causes the increase in peroxide numbers. In this treatment, it seems that the peroxide number does not change much due to the presence of phenolic and flavonoid active compounds in CEE and CHE.

From all measurements of free fatty acid parameters, iodic numbers, and peroxide numbers, it is observed that up to the 10th day, it is still included in the quality standard [11].

4. Conclusions

From the results of the study, it can be concluded that the total phenolic value and CEE flavonoids are better than CHE. Testing of antioxidant activity with the second DPPH extract method showed activity, with IC_{50} values of CEE better than CHE. The test results of the effectiveness of both extracts on CPO showed that CEE and CHE significantly ($P < 0.05$) influenced the value of free fatty acids, peroxide rate, and iodine rate on CPO. Both extracts have good antioxidant activity to inhibit oxidation in CPO. CEE is more effective in maintaining the quality and quality of CPO for ten days with free fatty acid values (2.1%), peroxide value of 0.48 Meq/Kg and iodine number of 54.8 and included in the CPO quality standard according to SNI- 01-2901-2006

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