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Influence of the addition and storage time of crude extract of tea leaves (*Camellia sinensis* L.) toward value of free fatty acid in crude palm oil

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Abstract: The purpose of this study was to determine the effect of the crude extract of tea leaves (*Camellia sinensis* L.) and storage time on the content of free fatty acid in palm oil. The dried tea leaves were macerated and concentrated by rotary evaporator. The extract obtained was added to crude palm oil with various added mass of the extract and various storage times. Phytochemical tests indicated the presence of secondary metabolites including alkaloids, triterpenoids, steroids, phenolics and flavonoids. The ANOVA test showed a decrease in free fatty acid content in crude palm oil with the addition of tea leaves extract. The LSD (Least Significant Difference) test showed the best influence on ALB of palm oil is on the total extract mass of 2 grams and the storage time of 20 days.

1. Introduction

Tea is widely consumed as a beverage, also to provide a fresh flavor and is also used as an additive to food, cosmetics and drugs, and does not cause negative effects [1]. Tea derivative products can be used for body slimming, herbal tea, preventing the formation of dental caries, ingredients of medicine for treating diabetes, hypertension and cancer [1-2].

Studies on the efficacy of the tea leaves have been carried out. Tea extract has high antioxidant activity [3-6]. Tea extract has potential as antimutagenic, anticarcinogenic and protective agents against cardiovascular diseases [3]. Tea can be used in a precautionary measure for cancer, prostate cancer, kidney or liver disease [4]. Chemical compound content of leaves tea are polyphenols, flavonoids, flavones, flavonone, isoflavones, anthocyanins and catechins. In addition, tea also contains alkaloids (caffeine, theobromine and theofolin) of about 7-8% [1-2,7].

Caffeine contained in tea leaves has many negative effects if consumed in excess, can increase stress, accelerate bone damage, to affect heart health and stomach acid [2]. Caffeine compounds in tea can be used to lower free fatty acid content in palm oil, and can increase its economic value. Moreover, in tea



leaves contain many basic metabolite compounds that are alkaline so it is expected to reduce free fatty acids in palm oil.

Based on the above description, the research to reduce free fatty acid in crude palm oil by utilizing crude extract of tea leaves (*Camellia sinensis* L.) as a base source has been done. Some important parameters such as the total amount of crude extract of tea leaves (*Camellia sinensis* L.) and the various of storage time are also studied. This research method is environmentally friendly and can increase the economic value of crude palm oil and tea.

2. Material and Methods

2.1. Material

Equipment used in this study are rotary evaporator, glass funnel, beaker glass, pipette, test tubes, press equipment, tube, centrifuge, vial, spatula, measuring flask, analytical balance, Erlenmeyer flask, magnetic stirrer, electric water bath, the stand poles, clamps, measuring cups and buret. The materials used in this study include crude palm oil, dried tea leaves, ethanol, distilled water, cotton, oxalic acid, KOH and phenolftalein.

2.2. Metodology

2.2.1. Preparation of crude palm oil. The fruit of palm oil is separated between the seeds and the flesh. The flesh is smoothed using a mortar to a pulp and filtered with a thin cotton cloth into an oil press. The extract obtained was centrifuged for 60 minutes at a speed of 6000 rpm. The oil produced is ready for use.

2.2.2. Extraction of tea leaves. The amount of 678 g of powder dried tea leaves (*Camellia sinensis* L.) was macerated with ethanol for 3 times 24 hours at room temperature. The filtrate obtained was concentrated using a rotary evaporator at a temperature of 30-40 °C and obtained crude extract of tea leaves [8-9].

2.2.3. Alkaloids test. The crude extract of tea leaves (*Camellia sinensis* L.) were dissolved in ethanol, added with a few drops of 2N H₂SO₄, while shaken. The formation of orange to red brown deposits after the addition of Dragendorff reagent indicated the presence of alkaloids [10].

2.2.4. Triterpenoid and steroid test. The crude extract was dissolved in ethanol, put in a glass tube following by the addition of 10 drops of glacial acetic acid and 2-3 drops of concentrated sulfuric acid (Liebermann Burchard reagent). The formation of reddish orange or purple colour indicated the presence of triterpenoids, while green color indicated the presence of steroid [8].

2.2.5. Saponin test. A certain amount of crude extract was dissolved in 2 mL of distilled water then shaken strongly in a test tube. The presence of saponin was characterized by the formation of durable foam on the surface of the liquid. Foam has remained stable after the addition of a few drops of concentrated HCl [11].

2.2.6. Phenolic test. A certain amount of crude extract was dissolved in ethanol and 3 drops of 1% iron (III) chloride was added to a total of 1 mL of the extract. The presence of phenolic was indicated by the appearance of green, red, purple, blue or black colors [12]

2.2.7. Quinoline test. A total of 5 mL of extract solution was added 1N sodium hydroxide. The presence of quinone was indicated by the formation of red color [13].

2.2.8. Flavonoids test. Flavonoid test was done by Wilstater method. A certain amount of extracts is dissolved in a suitable solvent. A total of 1 mL of extract was added with a small amount of Mg powder and 5 drops of concentrated HCl. The presence of flavonoids gives the color orange, red to dark red [14].

2.2.9. Addition of crude tea leaves into crude palm oil. Amount of 5 g of crude palm oil is inserted into each of 63 vials. 1st - 21st (negative control), 22st-42st, 43rd -63rd vial were added 0, 1, and 2 gram crude extract of tea leaves, respectively. Each vial was homogenized with a stirrer. Furthermore, the samples were moved into a dark glass bottle sealed and stored at room temperature. Determination of free fatty acids performed on the storage of varied time of 0 – 30 days.

2.2.10. Determination of Free Fatty Acid. A total of ± 2 g of oil is weighed into the Erlenmeyer flask. The oil is then added 25 mL of neutral alcohol and 2 drops of phenolphthalein indicator. The solution was titrated with a 0.1N KOH-alcoholic solution until a pink color was obtained and not dissipated for 30 s.

3. Result and Discussion

3.1. Extraction and Phytochemical Test

The extraction process in this study, from 678 g of tea leaves (*Camellia sinensis* L.) was obtained from Bogor, West Java which has been dried and macerated with ethanol of 1.9 L for several days and then concentrated with a rotary evaporator to obtain a total was 65.62 g (9.72%). The resulting total extract tested its secondary metabolite content, as shown in Table 1.

Table 1. The chemical compound content crude extract of tea leaves

Secondary metabolites	Alkaloid	Triterpenoid	Steroid	phenolic	Quinoline	Flavonoid	Saponin
result	+	+	+	+	+	+	+

Based on test results obtained information that the phytochemical extracts containing coarse alkaloids, triterpenoids, steroids, phenolic, quinone, and flavonoids.

3.2. Free Fatty Acid (FFA) Test

Determination of free fatty acids was done by using titration of acidimetry. In this study, the amount of free fatty acids in palm oil was determined for various variations in total amount of added extract of tea leaves (*Camellia sinensis* L.) and storage duration time. The determination of free fatty acid in palm oil is done by triplo respectively.

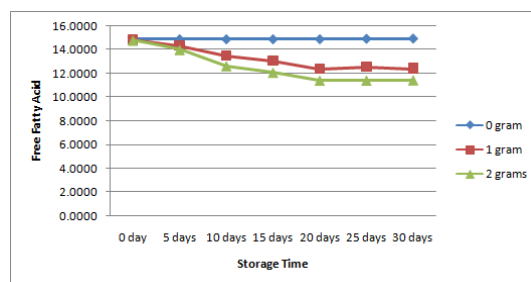


Figure 1. FFA content at various variations addition of crude extract tea leaves and duration of storage on crude palm oil

The results of the study (**Fig. 1**) showed the comparison of FFA value in crude palm oil which was not added with crude extract of tea leaves (*Camellia sinensis* L.) (as negative control) and with added crude extract of tea leaves. The value of FFA in negative control is higher than that of the added crude palm oil with crude extract of tea leaves. The increase in FFA is directly proportional to the length of storage, this is due to good hydrolysis caused by water and lipase enzymes during the treatment and storage [15-16]. The FFA value of the controlled palm oil increases with the length of storage time. This is due to the increased FFA caused by oxidation and enzyme hydrolysis reaction during processing and storage time [17,18]. In contrast to the oil that has been added to the crude extract tea leaves (*Camellia sinensis* L.), the FFA value of the oil decreases with the amount of total extract added to the oil.

It can be concluded that oil storage is good only up to 20 days. After 20 days there is an increase in the value of free fatty acids. The oil will be physically damaged in the form of a color that turns into turbid and is chemically a formed FFA. The color of the oil will become turbid, because the salt deposition of the reaction between alkaloids and free fatty acids (shown in **Fig. 2**). This is presumably because the alkaloid content of the crude extract tea leaves (*Camellia sinensis* L.) added in palm oil has reacted with free fatty acids, so that no alkaloids neutralize the free fatty acids formed as oil hydrolysis. Alkaloids are natural bases that have basic Lewis properties because they contain N atoms in heterocyclic rings that can contribute a single pair [19].

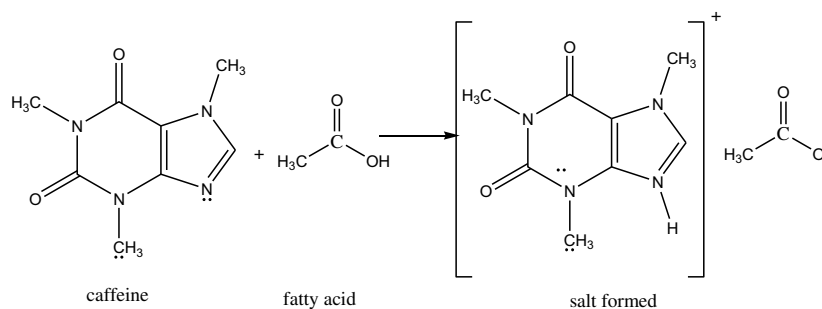


Figure 2. Reaction between caffeine with free fatty acid

To prove the hypothesis, is there any effect of addition of the crude extract tea leaves (*Camellia sinensis* L.) toward free fatty acid on palm oil done by ANOVA method. After the test is obtained $F_{\text{count}} > F_{\text{table}}$. $F_{\text{count}} = 19.9148$ and $F_{\text{table}} = 9.33$ with significance level of 1%. If $F_{\text{count}} > F_{\text{table}}$. H_0 is accepted and H_a is rejected, it is concluded that there is an effect of addition of the crude extract tea leaves (*Camellia sinensis* L.) and variation of storage time to free fatty acid in crude palm oil.

LSD (Least Significant Difference) test is performed to determine parameters that give the best effect to free fatty acid value. The best amount of the crude extract tea leaves (*Camellia sinensis* L.) was 2 g. The average value of FFA on the addition of 2 g of extract significantly different with LSD value, was $2.3686 > 1.0343$ with significance level of 1%. This indicates that the addition of mass of the crude extract tea leaves (*Camellia sinensis* L.) 2 g can lower the larger free fatty acids. From the calculation of LSD to the storage period, it is obtained at 20 days day storage. This can be seen from the average value of FFA on 20 days of storage is significantly different to the LSD value, was $1.954 > 1.0343$ with a significance level of 1%. It can be concluded that the length of storage of oil mixture with the crude extract tea leaves (*Camellia sinensis* L.) is a maximum of 20 days. After 20 days of storage, palm oil will undergo physical changes and FFA on palm oil will increase so that the quality of the palm oil is damaged.

4. Conclusion

Based on this research, the optimum mass addition of the crude extract of tea leaves was 2 g with a reduction of free fatty acids of 22.81% with the optimum length of storage time of 20 days.

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