Effect of Ultraviolet Photooxidation A and C on the Quality of Natural Antioxidant Cooking Oil of Tapak Dara Leaves (*Catharanthus roseus* L.)

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Abstract: Cooking oil is one of the commodities in Indonesia with a total production of 49.7 million tons in 2021, the largest of which is produced from palm oil extraction. Most of the use of cooking oil is still dominated by household consumption, restaurants, and biodiesel fuel purposes. Cooking oil for the household sector is generally obtained from traditional markets. Sellers in traditional markets leave the oil in an open container, place it in a damp place and expose it to sunlight. This causes it to not be durable, spoil quickly, and smell rancid due to oxidization to form aldehydes. Actions to slow down oxidation include adding synthetic and natural antioxidants like Tapak Dara. Tapak Dara is a natural herbal plant that contains 90.27% higher antioxidants than bitter melon and brotowali plants. The active compounds contained in tapak Dara include alkaloids, flavonoids, and terpenoids that can behave as antioxidants so that they can be added to the oil. This research was observed the decrease in oil quality within the addition of tapak Dara leaves under ultraviolet A (365nm) and C (254nm) exposure. Photooxidation was carried out with time variations of 1, 6, and 12 hours. The oxidation resistance can be seen from the damage value of the oil by measuring into several tests, namely the water content, peroxide number, and free fatty acids. The measurement results of tapak Dara leaf after photooxidation showed that there was a linear increase in the formation of free fatty acids (%FFA) and peroxide number with the length of time of photooxidation. The decreased quality was greater in UV-C photooxidation compared to UV-A. However, the curve tends to be flatter than the results of UV-A photooxidation. The water content test showed the opposite activity, UV-C photooxidation tends not to have an impact on increasing water content compared to UV-C photooxidation results.

Keywords: Photooxidation, Ultraviolet, Cooking oil, Tapak Dara.

Abbreviations: UV is ultraviolet, FFA is a free fatty acid

Introduction

Cooking oil is one of the trade commodities in Indonesia with a total production of 49.7 million tons in 2021, the largest of which is produced from palm oil extraction. (Direktorat Jenderal Pertanian, 2019). Palm oil tends to be used as raw material for cooking oil because the oil produced is 59% more effective in producing products than ordinary coconut (Listia et al., 2015). The cooking oil consumption sector is mostly found in the food industry, restaurants, and hotels where the average use of oil for frying ingredients is only 3 times, after which it is disposed of as used cooking waste. (Ayu et al., 2016). In addition, the use of oil is also used to produce biodiesel, but specifically for this purpose, used cooking waste can be used as a raw material (Efendi et al., 2018; Suirta, 2009).

In Indonesia, many oil consumers, especially the household sector, obtain oil from purchases in traditional markets. This is because the purchase price of cooking oil in the traditional market is much cheaper and the quantity of goods obtained is greater (Anggraini et al., 2017; Shavana et al., 2014). In contrast to the cheaper selling price of oil, bulk oil traders in traditional markets often do not

pay attention to the quality of the cooking oil they sell. Neglecting the quality of this oil includes leaving cooking oil in an open container, placing it in a place that has high humidity, and often leaving it exposed to sunlight. This causes the cooking oil to be easily damaged by oxidation due to exposure to oxygen in the air. Moreover, oil breakdown can be accelerated by the presence of water and photooxidation of ultraviolet light (Mannucci et al., 2019). The damage experienced in the oil can cause changes in the chemical structure of the oil so that it can change the physical properties of the oil such as a paler color, an unnatural taste, and the appearance of a pungent rancid odor. (Jacobsen, 2018). Damage to the oil that is allowed to continue will cause the cooking oil to be short-lived in use, easily damaged when used, and smelly due to the oil being oxidized to form aldehydes.

Actions to slow down oil oxidation include providing antioxidants as oxidation inhibitors in addition to physically treating the oil container tightly, avoiding exposure to light, and reducing humidity. (Asap & Augustin, 1986; Nainggolan et al., 2016; Noriko et al., 2012; Wan et al., 2018). To deal with oil damage in terms of adding antioxidants, namely through the mechanism of inhibiting electron radicals contained in the chemical structure of the oil. Although naturally cooking oil contains antioxidants beta carotene, tocopherol, phytosterols, and phenolic compounds that are useful for overcoming oil damage, these natural antioxidants are not strong enough to overcome further damage. (Teixeira et al., 2013). Therefore, it is necessary to make efforts to strengthen the antioxidants of the oil so that the oil is not easily oxidized. The addition of synthetic antioxidants into the oil is often done to maintain its quality. The added antioxidants such as beta hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) which are strictly controlled in their use must comply with the Indonesian National Standard (SNI) not more than 200 ppm. (BPOM, 2013). The use of synthetic substances is not the best solution, because the prolonged use of synthetic antioxidant compounds can cause cardiovascular disorders and can increase the risk of liver cancer. (Elshafie et al., 2012; Narayanankutty et al., 2018). Therefore, as an antioxidant solution that can be added to the oil, use natural antioxidants, one of which uses extracts from tapak dara leaves.

Tapak Dara is an ornamental plant that contains alkaloids, flavonoids, phenolics, tannins, and terpenoids. (Verrananda M et al., 2016). As well as having more than several types of alkaloids that are useful as anti-cancer. Tapak Dara antioxidant was reported to be higher than some plants such as bitter melon and brotowali which reached 90.27% activity. The high activity causes the tapak Dara plant to have a high potential to be used as an alternative to synthetic antioxidants (Kristianto et al. 2014). To determine the antioxidant activity of tapak Dara leaf extract in cooking oil, it is necessary to observe the type of light that can cause oil damage. One of the rays that can damage the structure of the oil is ultraviolet light. Andarwulan's research states that the presence of light in oil can increase the peroxide value, causing the oil to have a short shelf life of only 1.32 hours. (Andarwulan et al., 2016). Meanwhile, Monteiro tried to observe the effect of UV-C irradiation on lipids in tuna. The results showed that ultraviolet light was able to affect the formation of free fatty acids. From these observations, there has been no similar research on the type of ultraviolet current used as photooxidation. In this study, a comparison of the types of ultraviolet A and C rays exposed in tapak Dara leaf extract oil will be studied for a certain period. This ultraviolet light, as it is known, is the light that is commonly used for UV-C sterilization, while UV-A is the light that is abundantly emitted by the sun. The results of this study can then be used as a foothold to handle and anticipate damage to bulk oil due to ultraviolet light sterilization so that the shelf life of bulk oil can be longer in processing.

Materials and Methods

Materials and tools

The ingredients in the research used were sodium hydroxide, sodium thiosulphate, glacial acetic acid, potassium iodide, chloroform, amylum indicator, phenolphthalein indicator, ethanol 96%. The material has a pro-analysis degree with the

trademark Merck and Smart-lab Indonesia. Materials for the manufacture of extracts are made from fresh tread simplicia. Cooking oil samples were taken from the traditional market of the Bojonegoro district. The tools used are standard oven, Ohaus PA224 digital scale, B-one 772 spectrophotometer, vortex. The glassware used is a standard general tool.

Preparation Procedure
Making oil with the addition of tread Dara
The tapak dara plant is taken about 2 kg of wet weight and then washed with clean water. After that, the selection of tapak Dara leaves was sorted to be dried using a 50°C oven for 5 hours of drying. The dried palm leaves were mashed using a blender and then sieved using a 100mesh sieve. As much as 1 gram of sifted powdered palm leaves was added to 250mL of oil. The cooking oil is then stirred using a magnetic stirrer at a speed of 1000 rpm (set of tools) for 1 hour of stirring. Cooking oil with the addition of tapak Dara leaves can be used for further analysis,

Sample Photooxidation
The oil sample with the addition of dried tapak Dara leaf Simplicia was then put into a container for photooxidation of ultraviolet light for 1, 6, and 12 hours of photooxidation. Ultraviolet light used is a tube lamp (TL) beam with two maximum wavelengths produced in each lamp. The use of ultraviolet light for photooxidation uses 254 and 365 nm waves with a lamp consumption of 15 watts. The irradiation is carried out in a plastic container with an area of 30x20cm and an irradiation distance of 20 cm as shown in Figure 1.
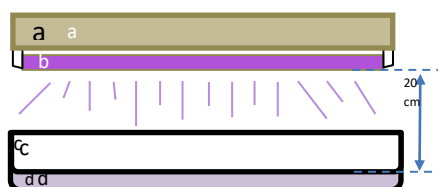
Figure 1. Ultraviolet photooxidation design. (a). ballast, (b)UV-C/A tube lamp, (c) sample container, (d) test oil

Variable Measurement
Variable measurements to determine antioxidant activity in oil were repeated twice each time after being exposed to ultraviolet light. The treatment of giving ultraviolet light to the sample was carried out with variations in time of 1, 6, and 12 hours of exposure. The results of the sample exposure were then measured by three parameters of the oil quality test including the measurement of free fatty acid levels, determination of peroxide number, and measurement of water content (Suroso, 2013).

Measurement Procedure
Free Fatty Acid Analysis
The cooking oil is stirred evenly and cultivated in a liquid state so that it is stable. The sample was weighed 28.2 ± 0.2 g into a 250mL Erlenmeyer. A total of 50 mL of neutral hot alcohol was added to the Erlenmeyer and then 2 mL of phenolphthalein (PP) indicator was added. The solution was titrated using 0.1 N NaOH which had been standardized with the oxalic acid solution until the color changed to pink which did not disappear for 30 seconds. If it is lost before this time, it is titrated again slowly. Measurements were made twice in each sample. Free fatty acid levels can then be calculated following the formula.

$$\% \text{ Free Fatty Acid} = \frac{\text{V NaOH (ml) x N NaOH x BM Fatty Acid}}{\text{Sample Weight (gr)}} \times 100\%$$

Determination of Peroxide Number
A total of 5 g of the oil sample was put in a 250 mL Erlenmeyer and 20 mL of chloroform–glacial acetic acid (4:6) mixture was added to it. The solution was then shaken until homogeneous and 0.5 mL of 6M KI solution was added. The solution was shaken and allowed to stand for 2 minutes. The solution was titrated with 0.1N sodium thiosulfate until the yellow color almost disappeared. Three drops of the starch indicator were added and then titrated again until the blue color disappeared. The determination of the peroxide number was carried out twice. The calculation of the peroxide number follows the following equation.

$$\text{Peroxide number} = \frac{\text{V Na}_2\text{S}_2\text{O}_3\text{(ml) x N Na}_2\text{S}_2\text{O}_3}{\text{Sample Weight (gr)}} \times 1000$$

Moisture Test
The water content test method is used to monitor oil quality due to exposure to ultraviolet light. The

high water content in the oil can trigger a hydrolysis reaction in the oil so that the quality of the oil will decrease due to the formation of free fatty acids. Measurement of water content using the gravimetric analysis method, namely weighing the difference in the weight of the evaporation of water in the oven at a temperature of 100°C with the weight of the oil before being heated in the oven. The weight of the oil used to be tested is 2 grams. The reduction in the initial weight of the oil is calculated from the result of the reduction of the evaporated water. The calculation of this water content follows the following equation.

$$\% \text{ Water content} = \frac{m_1 - m_2}{m_0} \times 100\%$$

Description:

m_1 = sample mass + cup mass before drying (gr)

m_2 = mass of sample + mass of cup after drying (gr)

m_0 = sample mass (gr)

Results and Discussion

### Ultraviolet Photooxidation Results

The results of ultraviolet light irradiation on the test oil at the highest time interval of 12 hours showed a significant color change from the original color of the golden-yellow oil to pale yellow. This was observed in the photooxidation of the oil to UV-C, while in the UV-A photooxidation of the oil, the color change almost did not appear as shown in Figure 2. When viewed from the quality of the smell of the irradiated oil, it showed that the oil was simplicia of tapak Dara leaf with UV photooxidation. UV-C has a more pungent odor than UV-A radiation. This pungent odor is caused by changes in the chemical structure of the oil to form radical compounds as a result of reacting with free air triggered by ultraviolet C rays. UV-A rays show a relatively less pungent odor compared to UV-C because these rays have lower energy.

Figure 2. The results of irradiating cooking oil using UV-A and UV-C rays at a maximum photooxidation of 12 hours

Free Fatty Acid Measurement Results

Testing of free fatty acids using the titrimetric method using the principle of saponification in measuring the presence of free fatty acids. Free fatty acids are formed due to the presence of a certain amount of water in the cooking oil, causing a hydrolysis reaction in the oil with the final product forming glycerol and fatty acids. The reaction for the formation of free fatty acids is shown in the following reaction:
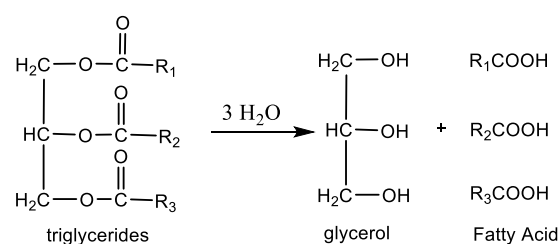
Three water molecules react with one triglyceride molecule to form three free fatty acid molecules. The formation of free fatty acids indicates the formation of structural damage in the oil which can result in the formation of aldehyde compounds. The measurement results can be shown by the graph in Figure 3. It shows an increase in the formation of free fatty acid levels in UV-A photooxidation from 10% to a maximum of 40%, which is from 1 hour to a maximum of 12 hours. Meanwhile, if you look at the results of irradiating oil with the addition of tapak Dara leaves, it shows an unstable value with a value that tends to fluctuate and is read at 6 hours of irradiation. Compared to 1 and 12 hours of irradiation, the autoxidized oil using UV-A light tends to have a more linear (flat) value compared to photooxidation using UV-C light.
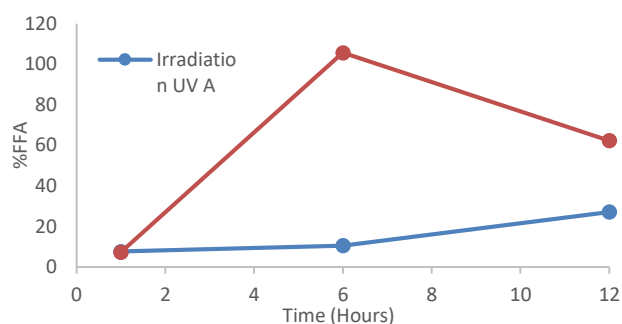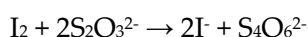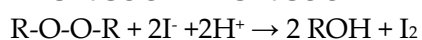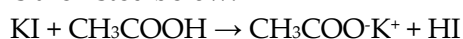

Figure 3. the results of measuring the free fatty acid content in cooking oil with the addition of tapak Dara leaves after photooxidation

Peroxide Number Determination Results

Measurement of peroxide value in oil as oxide in fat structure using a redox titration method. The decrease in oxidation number is caused by the presence of parallel oxygen atoms to form peroxides in the chemical structure. Peroxide oil will react with iodide acid resulting from the reaction between acetic acid and potassium iodide. The formation of iodine resulting from oxidation by peroxide will then be measured by the titrant of sodium thiosulfate added to the solution. The reactions are listed below.

$$KI + CH_3COOH \rightarrow CH_3COO^-K^+ + HI$$
$$R\text{-}O\text{-}O\text{-}R + 2I^- + 2H^+ \rightarrow 2\ ROH + I_2$$
$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

The results of determining the number of peroxides in the oil-containing additional tapak Dara leaf powder due to ultraviolet exposure are shown in Figure 4. It can be seen that the irradiation curve using ultraviolet A and C has a different increase in curve. Radiation using ultraviolet C light showed a value that was not flat (flat) compared to irradiation using ultraviolet A light. Ultraviolet C irradiation on the oil sample added with palm oil powder showed the highest value was found in the 12 hour photooxidation period, which is around 25 numbers peroxide. This is almost the same as irradiation at 6 hours which has almost the same value. The inverse of the results with the lowest irradiance of 1 hour showed a drastic increase in the value from about 5 to 20 peroxide values which were not the same as above. In contrast to irradiation using ultraviolet A, the peroxide value tends to be more stable and flat. The increase was only slightly observed, there was a change in the increase in the irradiation treatment at 6 hours which was almost similar to the photooxidation treatment for 1 hour.
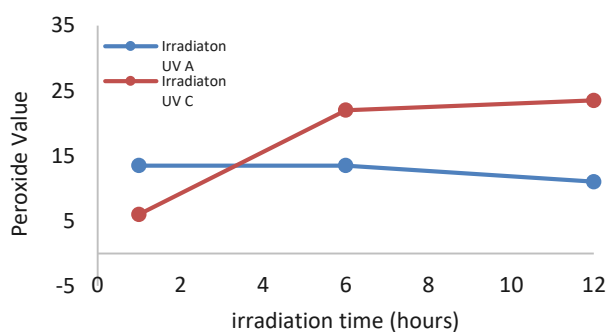
Figure 4. the results of measuring peroxide value in cooking oil with the addition of tapak Dara leaves after photooxidation

Water Content Measurement Results

The amount of oil mass is 2 grams into the evaporating dish by ensuring the cup is free from water that is absorbed into it. The oil in the evaporating dish is then heated in an oven for 2 hours at 110ºC to evaporate the water content that may be absorbed into the oil. The water will evaporate leaving the oil because the boiling point of water is 100ºC while the oil does not evaporate due to the boiling point of the oil which is more than 100ºC. The results of heating in the oven are shown in Figure 5, there are black deposits under the oil. This precipitate comes from simplicia powder which is burnt due to oil heated at 110ºC. In the initial preparation, pre-analytical filtration was not carried out in the sample treatment as a dependent variable with the aim that during irradiation the antioxidant content in simplicia released gradually from tapak Dara leaf powder to inhibit the process of oil damage by ultraviolet light.

Figure 5. the results of oil samples at a temperature of 110 ºC. there is a black precipitate in the middle of the sample which is the powder of the charred leaves of the tapak dara due to heating

The results of the measurement of water content are shown in the graph in Figure 5 with three variations of irradiation, namely 1, 6, and 12 hours. The irradiation results showed that there was a change in the water content that varied with the length of the oxidation time. It is observed in the graph in Figure 5 that there is an erratic (nonlinear) increase in the ultraviolet A exposure treatment. The reading value was high in the 6-hour photooxidation treatment with the percentage of water contained reaching more than 3%. It was read at the beginning of the 1-hour irradiation treatment that the water content value was almost as low as 0.5% as well as the maximum irradiation treatment which was 12 hours which had almost

the same value. On the other hand, this is not the case for irradiation using ultraviolet C, where the water content measured during the analysis tends to show a more linear curve with a flatter slope. As measured by the analysis of water content in exposure to ultraviolet light for 1 hour photooxidation was higher than in the subsequent treatment, which had a value of more than 1%. While in the longest photooxidation, the water content in the oil was measured which only reached no more than 0.5%.
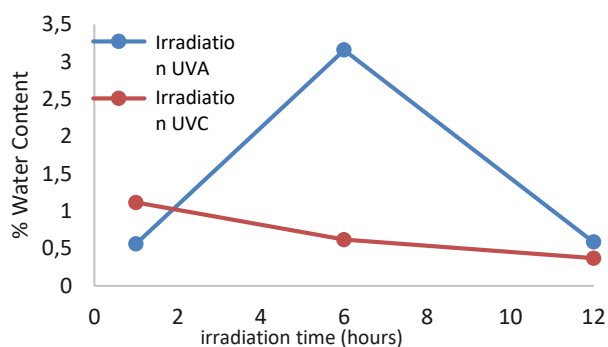
Figure 6. the results of measuring water content in cooking oil with the addition of tapak Dara leaves after photooxidation

Discussion

The measurement of the quality of the oil can be monitored from three measurements of the content in the oil, namely the measurement of free fatty acid levels, the measurement of the oil peroxide contained in the sample as the value of the peroxide value of the oil and the measurement of the water content in the oil. In the measurement of fatty acid levels based on the results in Figure 3, there is a graph that ultraviolet C irradiation tends to be unstable in oxidizing oil, while photooxidation in samples using ultraviolet A is more stable. This is influenced by the presence of ultraviolet C light which has higher energy so that it can hydrolyze oil at a higher rate. Although in general, ultraviolet C light is used for sample sterilization, this seems to need to be considered for the optimal time of sterilization of materials from bacteria because the longer the irradiation causes the oil to easily decompose to form free fatty acids. Therefore, as a side of the discussion, UV-C ultraviolet light is not suitable for use as a sterilizer for oil-based foodstuffs because it has been shown to cause damage to oil by forming free fatty acids. Apart from the feasibility of using

ultraviolet light which can be used as a food sterilizer in its application, the overall measurement shows that the percentage of free fatty acid levels after UV photooxidation still shows a value above 0.3%. This shows that the quality of the oil is still far from the Indonesian national standard (SNI) 01-3741-2002 even though additional antioxidants have been added to it, which are derived from tapak Dara leaf powder.

The measurement of free fatty acid levels in Figure 3 with UV-C exposure to ultraviolet light seems to have a relationship with the measurement of water content in Figure 6. According to the reaction for the formation of fatty acids above, it takes 3 molecules of water for one molecule of triglyceride from oil to decompose. The measurement curve for water content is flatter at UV-A compared to UV-C, proving that water is not measured higher so that the loss of water molecules is used to form the hydrolysis reaction of oil. The measurement of the peroxide value in the oil as has been observed shows the same increasing pattern characteristic as the measurement of the free fatty acid content. This phenomenon is closely related to the formation of free fatty acids which will be more easily oxidized by ultraviolet light to form radicals rather than the complex form of triglyceride bonds (Warabi et al., 2004).

Conclusions

Ultraviolet rays A and C affect the quality of the oil even though it has been given natural antioxidants from tapak Dara leaves. UV-A irradiation gives a more linear reduction in oil quality than UV-C irradiation. From the measurement of the results of UV-A exposure, it shows that the lowest value that found in the fatty acid level test and the peroxide number test compared to UV-C. on the other hand, the water measurement result is in contrast to other analyzes UV-C light shows the lowest value than UV-A irradiation.

Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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