Inhibition of Free Fatty Acids generation in Crude Palm Oil during storage by using UV-C Light treatment

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Abstract. During the lengthy storage of crude palm oil (CPO), free fatty acid (FFA) concentration generated in the oil tends to increase which will affect the quality of CPO as the result of lipase enzymatic degradation of triglycerides. Currently, the conventional thermal processes in CPO storage tanks still applied to reduce the FFA formation. However, the thermal method is not very effective in reducing FFA for prolonged heat exposed due to deteriorate the CPO quality and high energy consuming. The alternative UV-C light technique gives some advantages compared with thermal treatment i.e less energy consumption, simple method, cheaper investment and low-cost operation. This study is aimed to evaluate the effects of UV-C light on inhibiting FFA generation in CPO during storage. The evaluation of UV-C light effect on the inhibition of FFA generation was conducted by irradiating CPO samples with various UV-C light exposure time. The FFA level of CPO samples was analyzed by using sodium hydroxide solution before and after treatment. The results show that UV-C light has the ability to deactivate lipase enzyme and consequently inhibit FFA generation in CPO.

1. Introduction

The world palm oil trade is a $60 billions industry with Indonesia and Malaysia produce 85% of the world’s supplies [1]. It is predicted in years 2040-2050 CPO production in Indonesia will reach 65 million tons per year [2]. In order to respond the over capacity, the utilization of CPO as a renewable raw material for biorefinery scheme production system tend to increase continuously considering of its environmental benign. Conversion of CPO into biodiesel fuel in Indonesia and in other main CPO producing countries like Malaysia is reaching at a spectacular increase recently. This fact is mostly driven by implementing new paradigm of sustainable development in order not only to reduce the utilization of fossil fuel which produce greenhouse gases (GHGs) emission affecting global warming, but also in term of their national trade and fiscal policy [5].

Quality of CPO is determined by parameters, including free fatty acid (FFA), which needs to be between 2 – 5% [3]. High FFA level may degrade the CPO [4]. The CPO storage tank system of a palm oil mill is very important to maintain the level of FFA in CPO not increasing and being in the desired level of maximum 5% (maximum standard specification) during long storage or transportation time. The greater than 5% FFA level in CPO would decreased its selling price [2]. The average FFA content in CPO products when leaving palm oil mill is 2.3 – 7.2% [3,6]. The increased levels of FFA in CPO are caused by the triglyceride hydrolysis reaction which is catalyzed by lipase enzyme. Lipase enzyme existed in CPO is originated from microbes activities in damage oil-palm fruits during harvesting [4] and the contamination of fungi in CPO [6]. In order to overcome the increase in FFA level, the current
enzyme deactivation treatment which has been widely used is by heating the CPO with steam coil and maintaining its temperature in the range of 40–60 °C [7,8]. Unfortunately, the conventional process has not been able to completely stop the increase in FFA formation. On the other hand, the prolonged heating treatment could destruct the composition of CPO [9].

Recently, UV-C light has been widely utilized to maintaining the quality and content of food ingredients. UV-C light has capability on killing microbes and destructing enzymes structure that acting as destructive agents in food [10]. The use of UV-C light in preventing changes in material quality has advantages compared to the use of heat treatment, i.e the simple system option, cheaper and energy efficient. However, the utilization of UV-C light for destruction of lipase enzyme in CPO is still rarely reported by researchers. The aim of this research is to investigate the effect of UV-C light exposure treatment on CPO in inhibiting the generation of FFA during storage period. In addition, the destruction of lipase enzyme as the effect of UV-C light exposure treatment was also evaluated.

2. Materials and Method

2.1. Experimental set up
Experiments were carried out in a cube shaped cardboard box with an iron frame. The size of the box is 20 (h) x 94 (l) x 30,5 (w) cm. the inside part is painted black. A low pressure UVC lamp of 20 W, wavelength of 253.7 nm, and the lamp length 59 cm (Sankyo Denki Japan) is placed on the top side of the box. The inside temperature of the box when irradiation took place was around 33 °C. Each CPO sample of 100 ml in a beaker glass of 300 ml was placed on the bottom side of the box. The distance between the light source (UV-C lamp) and the surface of the samples is 15 cm.

2.2. UV-C light and lipase enzyme decay
The following steps are arranged to evaluate the effect of UV-C light exposure on lipase enzyme decay:
1. Preparation of acetate buffer and Arabic gums solution (as surfactant)
   Acetate buffer solution was prepared by mixing 178 ml of acetic acid solution (0.1 N) with 72 ml of sodium hydroxide solution (0.2 N) in a beaker glass and then stirring until homogeneous. A surfactant solution was prepared by mixing 50 g Arabic gum into 100 ml of distilled water. Then the mixture was stirred while heated until homogeneous [11].
2. CPO hydrolysis with unexposed and exposed enzyme to UV-C light
   For CPO hydrolysis using unexposed enzyme, an enzyme solution which is a mixture of 0.5 ml lipase enzyme and 100 ml of acetate buffer was poured into a 300 ml beaker glass, then 5 ml of CPO and 2 ml of Arabic gum solution was added into the solution. Subsequently, the mixture was stirred for 1.5 h and then the FFA level generated in CPO was analyzed. For CPO hydrolysis using exposed enzyme, the enzyme solution was exposed first to UV-C light at desired time prior to adding CPO and Arabic solution [11].
   Lipase enzyme decay as an effect of UV-C light exposure was evaluated. The percentage of enzyme decay is calculated by the following equation:

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   \% \text{Enzyme decay} = \frac{E_u - E_e}{E_u}
   \]

   Where: \( E_u \) = increased level of FFA due to hydrolysis of CPO catalysed by unexposed enzyme
   \( E_e \) = increased level of FFA due to hydrolysis of CPO catalysed by exposed enzyme

2.3. UV-C light treatment and exposure time
The UV-C light treatment on the inhibition of FFA generation in CPO was conducted in two ways. Firstly, CPO samples were exposed to UV-C light at various time then the samples were placed in a room temperature of 30 °C and kept for 3 weeks. FFA level in CPO were analysed every 1 week (7 days). The experiment was carried out as follows; a 100 ml CPO sample filled in a 300 ml glass beaker then each sample was placed under a UV-C lamp to be exposed for 2, 4, 6, and 8 hours. After the light
exposure process, the sample was then placed into a room with a temperature of around 30 °C for 3 weeks and the level of FFA generated in CPO was analysed. For blank test, 100 ml CPO sample in a 300 ml beaker without UV-C light exposure was placed directly in the same room for 3 weeks. Secondly, CPO samples were exposed repeatedly to UV-C light for 2, 4, 6, and 8 hours every day for 3 weeks and then the FFA level generated in CPO was analysed. The effect of the UV-C light treatment on the inhibition of FFA generation was determined as the value of the percentage of FFA inhibition, which is defined as the ratio between the value of the difference of the FFA levels increased in unexposed CPO and that of exposed one to the value of the increase in FFA levels in unexposed CPO. The equation of percentage of FFA inhibition calculation is written as:

$$\text{% FFA inhibition} = \frac{Y_u - Y_e}{Y_u}$$

where:

- $Y_u$ = increased level of FFA in unexposed CPO
- $Y_e$ = increased level of FFA in exposed CPO

2.4. Determination of FFA level in CPO
The FFA level was determined by sodium hydroxide titration. Prior to analysis, the CPO stock in 10 L plastic container was poured into a 5 L beaker glass and heated to 60 °C and then stirred until homogeneous. The 5 g of CPO sample from a glass was placed into an Erlenmeyer flask and added with 50 mL of 95% ethanol. The mixture was heated to 40 °C and stirred until all the CPO was completely emulsified. Subsequently, the 1-2 drops phenolphthalein indicator was added into the mixture. The sample was titrated by adding 0.1 N NaOH solution until pink colour developed. The titration volume which indicated the percentage of FFA (w/w) content in the oil was recorded and FFA concentrations was calculated using the following equation [12]:

$$\text{% FFA} = \frac{N M (V_a - V_b)}{W}$$

where:

- $N$ = NaOH normality;
- $V_a$ = the volume of NaOH used in sample titration (mL);
- $V_b$ = the volume of NaOH used in blank titration (mL);
- $W$ = sample mass (g);
- $M$ = molecular mass of the predominant fatty acid (25.6).

3. Results and Discussion

3.1. Effect of UVC light exposure on lipase enzyme deactivation
Lipase enzyme catalyzes hydrolysis of CPO into FFA [4], and UV-C light has an ability to decay enzymes [5]. Prior to evaluating the effect of UV-C light on inhibition of FFA generated in CPO during storage it was needs to characterize the effect of UV-C light on lipase decay. The percentage of lipase decay determined by measuring FFA levels formation in CPO due the addition of pure lipase (control) and UV-C light exposed lipase. Lipase activity test under various UV-C exposed time was conducted to investigate its ability on hydrolysing triglycerides in CPO. Figure 1 revealed that FFA levels increased from its initial value of 7 up to 30%, with an increasing of 23%. Irradiation of UV-C light on lipases decreased its ability in activating CPO hydrolysis. The effect of UV-C light exposure on enzyme deactivation is shown in Figure 1. The longer the exposure time of UV-C light to the enzyme, the lower the increase in FFA levels. The Increased levels of FFA with exposure times of 2, 4, 6, and 8 h was 21.56, 8.33 and 2.24 and 0.56%, respectively. The formation of FFA tends to terminated for exposure time greater than 6 hours. These evidences indicated that UV-C light have decayed the enzyme structure and deactivated enzyme to catalyzing more triglycerides for further hydrolysis. The percentage of enzyme decay depends on exposure time. The longer the exposure time the more enzymes decay. The percentage of enzyme decay with exposure time of 2, 4, 6, and 8 h was 6.52, 63.78, 90.26 and 97.56%, respectively. In comparison, Jeon et al reported the use of intense pulsed light (IPL) irradiation in lipase
deactivation. Increasing pulse fluence and exposure time decreases activity. IPL induced deactivation causes fragment which then causes changes in tertiary structure of lipase [3].

3.2. Effect of UV-C light irradiation on inhibition of FFA generation

The effect of UV-C light irradiation on the inhibition of FFA generation in CPO was carried out by exposing UV-C light to CPO samples for various times, after that all samples were stored in the room with a temperature of around 30 °C. The FFA generation during storage was monitored by analysing FFA level in each sample every week for 3 weeks of storage. The generation of FFA in CPO increased over time during storage. Figure 2 shows the profile of FFA levels in CPO. The increased FFA levels in CPO that were not exposed to UV-C light after 3 weeks (21 days) of storage time is 1%, this value is higher than those of the exposed one. The longer exposure time of UV-C light to CPO resulted the lower FFA levels. The increased FFA levels in the exposed samples (6, 12, 18, 24 h) after 3 weeks of storage time was 0.8, 0.7, 0.55 and 0.39%, respectively. The percentage inhibition of FFA generated in CPO as an effect of UV-C light treatment was increased with the increasing UV-C light exposure time. For the exposure time of 6, 12, 18 and 24 h, the percentage FFA inhibition were 10, 22, 38, and 56% respectively.

![Figure 1. Effect of UV-C exposure time on lipase enzyme decay](image)

**Figure 1.** Effect of UV-C exposure time on lipase enzyme decay
3.3. Effect of repeated UV-C light exposure on inhibition of FFA generation in CPO

UV-C light was exposed to each CPO sample repeatedly for; 2, 4, 6 and 8 hours per day for 3 weeks of storage time. The FFA level in each sample was determined by sodium hydroxide titration. During 3 weeks of storage, the not exposed (control samples) FFA content in CPO was increased from 8.12% to 9.2% or an increase of 1.1% from its initial value. Figure 3 shows a decrease in the generation of FFA in CPO as the effect of repeated UV-C light exposure. The values of decreased in FFA levels in CPO depend on time of exposure. Increased exposure time inhibit the increase in FFA generation. The increase in FFA levels in each CPO sample subjected to UV-C exposure time of 2, 4, 6 and 8 hours per day was 0.60, 0.38, 0.23, and 0.13%, respectively. This evidence showed that the UV-C treatment was able to inhibit the increase of FFA level in CPO. Regarding to the inhibition, increasing the exposure time would increase the percentage of inhibition of FFA generation in CPO. The percentage of inhibition of each CPO sample exposed to UV-C light at varied exposure time of; 2, 4, 6, and 8 hours per day was 45.45, 65.45, 79.10, and 87.27%, respectively. The experimental results showed that the inhibition profile of FFA generated in CPO (Fig.3) is in agreement with lipase enzyme deactivation test (Fig.1). These results confirmed that the inhibition of FFA generation in CPO is as a result of lipase enzyme decay by UV-C light exposure.
4. Conclusions

UV-C light can affect lipase enzyme decay to inhibit FFA formation in CPO. The experimental results revealed that the observed variables; UV-C light exposure time significantly contributed on lipase enzyme decay. The percentage of enzyme decay was increased with the increasing UV-C light exposure time. The percentage inhibition of FFA generated in CPO at various UV-C light exposure time of 2, 4, 6 and 8 hours per day for 3 weeks was 45.45, 65.45, 79.10, and 87.27%, respectively. It can be concluded that UV-C light treatment is one of a promising simple method which is able to inhibiting excessive amount of FFA formation in CPO during storage.

Acknowledgements

The authors would like to express our deep gratitude to Mr. M.A Peruzzi and Mr. K. Jodi for their valuable assistance with experimental works at Bioprocess Engineering Laboratory of Chemical Engineering Department, Syiah Kuala University and our thanks are also extended to PT. Karya Tanah Subur, West Aceh, Indonesia for providing CPO sample for this works.

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Figure 3. Effect of repeated UV-C exposure time on the increased FFA level in CPO
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