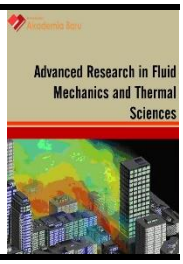
Penerbit Akademia Baru

Journal of Advanced Research in Fluid Mechanics and Thermal Sciences

Journal homepage: www.akademiabaru.com/arfmts.html
ISSN: 2289-7879



Periodic UV-C Light Treatment for Reducing the Formation of Free Fatty Acids in Crude Palm Oil During Storage

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ARTICLE INFO

Article history:
Received 4 April 2020
Received in revised form 19 May 2020
Accepted 20 May 2020
Available online 5 July 2020

Keywords:
UV-C light; free fatty acid; crude palm oil; lipase deactivation

ABSTRACT

The formation of free fatty acids (FFA) during long term storage of crude palm oil (CPO) is inevitable, and it can reduce the quality and losing the CPO. Therefore, this study aims to evaluate the effect of UV-C light on lipase enzyme deactivation and the effect of periodic UV-C light treatment to reduce the FFA formation in the CPO during storage. The lipase deactivation was evaluated by exposing UV-C light to the lipase solution in the range of 2 to 8 hours at various dose rates of 0.8528, 1.2318 and 1.7238 (mW/cm^2) and various periodic exposure times (2. 4 and 6 hours/day) for 7 to 28 storage days. The FFA level was analyzed using the NaOH titration method. The deactivation kinetics followed first-order model with deactivation rate constant in the range of 3.4 10^{-3}-5.8 10^{-3} min^{-1}. The half-life was 204.168, 150.586 and 120.024 (min) respectively. The highest value of FFA formation reduction is 85.6% at a dose rate of 1.7238 mW/cm^2 and a periodic exposure time of 6 h/day for 28 days storage. Overall, the experimental results show that UV-C treatments were able to deactivate lipase enzymes and reduce the rate of FFA formation in CPO during storage.

1. Introduction

In the long-term storage and transportation period, the formation of free fatty acids (FFA) caused by enzymatic triglyceride hydrolysis increase the FFA content and decrease the quality of crude palm oil (CPO) [1,2]. In addition, an increase in FFA will increase neutral oil losses and the cost of separation and refining processes in CPO-based industries [3,4]. Furthermore, FFAs in some foods may be responsible of undesired flavor and aroma and can act as precursors of other compounds such as alcohols, ketones, lactones or aldehydes [5,6]. Therefore, a reliable long-term CPO storage system is needed to guarantee the high quality and national strategic CPO reserve, and as a buffer for CPO when there is a global economic downturn. The level of FFA in CPO will affect its quality and selling price [4]. The standard quality of FFA content in CPO is below 5% [7]. The presence of lipase enzymes

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https://doi.org/10.37934/arfmts.73.1.2534

in CPO is derived from oil palm fruit mesocarp [8] and is caused by contamination from lipase-producing microorganisms at various stages of the process, transport and storage [1]. To maintain the FFA content in CPO in the desired range, it needs a treatment that can deactivate the lipase enzyme to stop the hydrolysis reaction. At present, thermal treatment method was applied to evaluate antioxidant properties on food spoilage and deterioration [9], and the temperature and light intensity was investigated on the growth of microorganisms [10]. Heating CPO and maintaining temperatures in the range of 40 and 60^{o}C in storage at elevated temperatures [11] can promote CPO quality deterioration [2,12] and relatively high energy consumption.

Recently, many studies have been carried out to develop methods of deactivating microorganisms and enzymes by using UV-C light in the process of preserving horticultural products such as fruits and fruit juices [13,14]. UV-C rays with a wavelength range of 200 to 280 nm effectively deactivate the growth of microorganisms and enzyme proteins (consisting of amino acids) because in this range UV rays are absorbed by ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and proteins, and which can cause cross-linking between constituent nucleic acids or break chemical bonds of amino acids which can damage RNA, DNA or enzymes [15,16] and has the potential to develop as one of the non-thermal preservation technologies in the food processing industry because it retains nutritional quality [17]. Lamikanra *et al.,* demonstrated that UV-C irradiation on fresh-cut cantaloupe melons can caused lipase activity contained in it to decrease during storage [18]. Jeon *et al.,* reported that intense pulsed light as an improved form of UV-C was able to deactivate lipase enzyme, and they discovered that the fluence and exposure time significantly decreased the lipase activity [19]. In general, the deactivation method with UV-C light has some advantages: simple, energy saving, low operating costs, and low investment capital [20]. Designing the UV-C light treatment system for CPO storage requires information relating to the deactivation of the lipase enzyme and the interaction between the light treatment and CPO. Since such kind of reports are still not available, a study related to these things needs to be done. Therefore, the main objective of this study was to investigate the effect of UV-C irradiation on lipase deactivation, and the influence of the periodic UV-C light treatment in reducing FFA formation in CPO during storage time. The reason for performing periodic UV-C treatments on CPO due to continuous treatment over a long period of time can increase the temperature and decrease the CPO quality.

2. Methodology

2.1 Crude Palm Oil (CPO)

Crude palm oil was obtained from a local palm oil mill company, PT. Karya Tanah Subur in Aceh Barat, Indonesia. CPO was placed in a closed plastic container, brought to the laboratory and stored in a room with average temperature of 30^{o}C. The FFA content in CPO was around 5%. Before being used for these experiments, the FFA content in CPO was increased from 5% to around 11.5% by placing 15 liters of CPO in a 20 liter round plastic container that was opened and exposed to the air. The container was placed in a room with an average temperature of 30^{o}C for several months. According to Tagoe *et al.,* CPO which is exposed to the air for a long time will contain high bacteria which produce high levels of FFA and lipase [1]. It was expected that the use of CPO with high lipase content as a sample would provide a significantly different response to FFA formation in untreated (control) and treated samples with UV-C light during storage.

2.2 Lipase Enzyme

The lipase enzyme was obtained from Sigma Aldrich Company with product number L3170. It comes from Candida sp. recombinant, expressed in *Aspergillus niger* in the form of aqueous solution with density = 1.2 g/ml.

2.3 UV-C Cabinet

Experiments of UV-C light treatment were carried out on the UV-C cabinet. The cabinet has a size: 94 (L) x 30.5 (W) x 25 (H) cm, and was built from an iron frame with top and bottom side walls made of iron plates while the other sides are made of cardboard. The entire side of the inner wall was painted black. The upper side of the bench was fitted with a UV-C lamp holder. The type of lamp used is a fluorescent lamp, which is a low-pressure mercury lamp with a wavelength of 253.7 nm, a power of 20 W and a tube length of 59 cm, and was manufactured by Sunkyo Denki Japan.

2.4 Lipase Assay Activity and UV-C Deactivation

The effect of UV-C light on the deactivation of the lipase enzyme was evaluated by comparing the activity of lipase treated UV-C with those not treated (control). Following definition of enzyme activity [21], one unit of lipase activity (U) is defined as that catalyzing the formation of 1 µmole FFA from CPO hydrolysis in 1 minute. Hydrolysis of CPO was carried out by adding 5 ml of CPO and 2 ml of Arabic gum as surfactant into the lipase solution of 100.5 ml in a 250 ml beaker glass and the mixture was stirred for 1.5 hours [22]. The FFA content produced from the hydrolysis was measured by NaOH titration method. The lipase solution was prepared by mixing 0.5 ml of the concentrated lipase liquid with 100 ml of the acetate buffer solution (pH 6). Acetate buffer and Arabic gum solutions were arranged as follows, acetate buffer solution with a total volume of 500 ml was prepared by mixing 356 ml of acetic acid solution (0.1 N) with 144 ml of sodium hydroxide solution (0.2 N) in a beaker glass (1000 mL). The mixture was then stirred until it reaches homogeneous. Arabic gum solution as surfactant was prepared by adding 100 g of Arabic gum to 200 ml of distillation water in a beaker glass (500 ml), the mixture was heated and stirred vigorously until homogeneous [22].

Evaluation of UV-C irradiation effect on lipase deactivation was carried out in the UV-C cabinet at temperature of around 30^{o}C. Lipase solution before being used to catalyze the hydrolysis of CPO was exposed to UVC light first by placing the sample under UV-C lamps in the cabinet. The distance between the surface of the solution and the UV-C light source was set at 15 cm. The solution was then irradiated with UV-C at different time of 2, 4, 6, and 8 hours and at various dose rates. All tests were carried out with replicate.

2.5 Periodic UV-C Light Treatments on CPO

The effect of UV-C irradiation treatment on the reduction of FFA formation in CPO during storage was evaluated by exposing UV-C irradiation to CPO samples at various exposure times and dose rates. The treatments were carried out in the UV-C cabinet at temperature of around 30^{o}C. CPO samples with a volume of 100 ml were put into a 250 ml beaker glass and placed under the UV-C lamp. The distance between the surface of the sample and the light source was set at 15 cm. UV-C irradiation values at a distance of 15 cm were measured using a UVC light meter (Lutron, UVC-254SD). CPO samples were irradiated periodically for 28 days with a periodic exposure time of 2, 4, and 6 hours/day and at various values of the average dose rate: 0.8528, 1.2318 and 1.7238 mW/cm^2.

Untreated (control) CPO samples of the same volume were placed in another cabinet and without irradiation treatment. FFA levels in all samples were analyzed every seven days during the course of experiments. UV-C light experiments were performed twice.

2.6 Method for Analyzing FFA Content in Samples

The FFA content in sample was analyzed by the NaOH titration method (Indonesian National Standard, SNI 10-2901-2006). The CPO sample from the container was preheated and stirred until the temperature reaches 50°C. 5 g of sample aliquot was poured into 250 ml Erlenmeyer flask. Then the aliquot was mixed with 50 ml of ethanol (95%). The mixture was regulated to temperature of 40°C by heating and stirring. After the mixture was completely emulsified, 1-2 drops of the phenolphtalein indicator were added and then titrated with NaOH solution (0.1 N) until the mixture turns pink. The percentage of FFA content in sample was calculated by the following equation

$$\%FFA = \frac{B(Vs-Vb)M}{10\ x\ S} \quad (1)$$

where B = Base (NaOH) normality; Vs = volume of base utilized in titration of sample (mL); Vb = volume of base utilized in titration of blank (mL); S = mass of sample (g); M = Relative molecular mass of palmitic acid (256).

3. Results

3.1 Effect of UV-C Irradiation on Lipase Activity

Table 1 presents the enzyme activity and residual enzyme activity (%) of lipase before and after treated with UV-C radiation at various exposure times and dose rates. Residual activity (RA (%)) is the ratio between enzyme activity in treated sample (A_t) and in untreated sample (A_0) [23]. UV-C treatment has a significant effect on lipase activity, higher dose rate and longer exposure times resulting in lower lipase activity. However, none of the UV-C treatment conditions applied can achieve completely lipase decay.

Table 1
The lipase activity (U/mL) before and after treated by UV-C radiation at 30°C and pH 6

Exposure	Enzyme Lipase Activity (U/mL)			Residual Activity (%)		
Time (h)	0.8528 mW/cm^2	1.2318 mW/cm^2	1.7238 mW/cm^2	0.8528 mW/cm^2	1.2318 mW/cm^2	1.7238 mW/cm^2
0	0.6739	0.6739	0.6739	100.00	100.00	100.00
2	0.4158	0.3685	0.3057	61.71	54.68	45.37
4	0.3184	0.2443	0.2058	47.25	36.25	30.53
6	0.2189	0.1737	0.1331	32.48	25.77	19.75
8	0.1211	0.0653	0.0277	17.97	9.69	4.11

Previous studies [22] had used first-order kinetic models to correlate the deactivation kinetics of the lipase enzyme. The same model was used to relate residual lipase activity with exposure time of UV-C at different dose rates. The expression is

$$Ln\frac{A_t}{A_0} = -k_d t \quad (2)$$

$$t_{1/2} = \frac{\ln(2)}{k_d} \quad (3)$$

$$D = \frac{\ln(10)}{k_d} \quad (4)$$

where A_t = lipase activity in treated sample after exposure time t, A_0 = initial lipase activity (untreated), k_d = deactivation rate constant, t = exposure time, $t_{1/2}$ = the half-life, the time needed to deactivate 50% of the initial activity and D = the decimal reduction time, the time needed to deactivate 90% of the initial activity.

Figure 1 shows the Ln (A_t / A_0) plot versus time which provides linear lines for various dose rates with a significant correlation coefficient of 0.92 - 0.98. This coefficient indicates that the first-order kinetic model fits the experimental data and characterizes a strong relationship between the rate of lipase deactivation and the time of UV-C exposure at various dose rates. The values (k_d) at different dose rates were calculated from the slope of the lines of Figure 1. The values of k_d, $t_{1/2}$ and D were tabulated in Table 2. From Figure 1, it can be seen that residual activity decreases proportionally with increasing exposure time. Likewise, the dose rate significantly influences deactivation lipase, increasing the dose rate increases the deactivation rate as indicated by an increase in deactivation rate constant (k_d) values or a decrease in values of ($t_{1/2}$) and (D) (Table 2). Lipase deactivation occurs due to UV light breaks peptide bonds between amino acids produce poly-peptide unit fragments which then changes the tertiary structure and leads to loss of lipase activity [17].

The activity retained by lipase decreased with increasing dose rate from 0.8528 to 1.7238 (mW/cm^2). The lowest residual activity (4.11%) occurred at a dose rate of 1.7238 mW/cm^2 and at an exposure time of 8 hours (Table 1). In comparison, Jeon *et al.,* applying intense pulsed light (IPL) in the range of wavelengths from 250 to 1,100 nm inactivating the lipase enzyme. They reported that IPL irradiation affected the lipase activity, increased exposure time and fluence decreased the lipase activity, the time needed to deactivate 50% of the initial enzyme activity ($t_{1/2}$) were 7.90, 12.65, 20.84, and 37.63 minutes at different fluence values of 14.86, 13.87, 11.89, and 8.79 (mJ/cm^2) respectively [17]. Sarah and Taib, used microwave irradiation for sterilization of fresh oil palm fruits bunch to deactivate lipase at treatment temperature range from 72 to 80^{o}C and at power density from the range of 239.51 to 1,199.63 (W/kg).They obtained D-value of lipase in the range of 8.333 to 16.949 minutes [24]. Both methods provide a faster deactivation rate compared to the results obtained in our work (Table 2). But UV-C systems with low pressure mercury lamps as a source of UV radiation have several advantages over IPL systems with xenon flash lamps, including high electricity efficiency, long lifetime, and simple to set up and operate [25]. In addition, high-temperature operations such as microwave irradiation systems are less preferred because they can affect the quality of heat-sensitive material [18].

Table 2
The values of kd, t1/2 and D

Dose Rate (mW/cm^2)	Deactivation constant k_d (1/min)	$t_{1/2}$ (min)	D-value (min)	R^2
0.8528	0.0034	204.168	678.228	0.9840
1.2318	0.0046	150.586	516.852	0.9647
1.7238	0.0058	120.024	398.718	0.9229

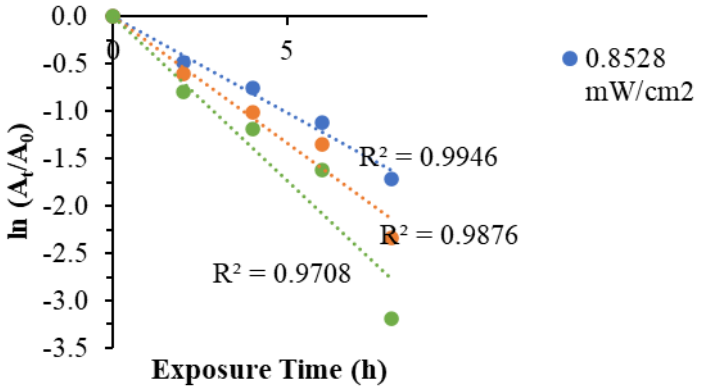

Fig. 1. Lipase residual activity as a function of dose rate of UV-C with a wavelength of 253.7 nm

3.2 Effect of Periodic UV-C Light Treatment on Reduction of FFA Formation in CPO

The experimental results of periodic UVC light treatment for 2, 4 and 6 hours every day at different doses was shown in Figure 2. It was observed a continuous increase in FFA levels in the untreated (control) sample for 28 days of storage time. FFA levels increased sharply from initial level of 11.45% to 15.74% or an increase of 37.47%. According to Frank *et al.,* the FFA formation rate in CPO during storage depend on its initial FFA level and the water content, they found an increased by 15 % and 110% within 4 weeks storage for CPO samples with the initial FFA level of 4.7 and 6.4%, and water content of 0.08 % and 0.22% respectively [10]. The difference in the profile of FFA levels in treated and untreated samples (Figure 2) suggested that the periodic UV-C treatment reduced the FFA formation over the course of the storage period. The amount of reduction in FFA in each sample due to UV-C treatment was tabulated in Table 3. The reduction was calculated as the ratio of an increase in FFA levels in treated samples (It) to an increase in FFA levels in untreated samples (Iu), and was written as RI (%) = (1- It /Iu) x100). The percentage of reduction increases with increasing exposure time or with increasing dose rate. At a constant dose rate of 1.2318 (mW/cm^2) and with an exposure time of 2, 4 and 6 hours/day, the reduction in the formation of FFA in each sample was 60.72%, 72.86% and 82.25% respectively. Likewise, for a constant periodic exposure time of 6 hours per day at dose rates of 0.8528 and 1.7238 mW/cm^2 the reduction in each sample was 71.64% and 85.63%. The reduction of FFA formation in treated samples is actually attributed by the destruction of lipase activity due to periodic UV-C irradiation during storage. These relationships need to be explained quantitatively in a mathematical model that can be useful for prediction of FFA levels and setting dose rates and expose time during UV-C treatment in CPO storage systems. The semi-log plot of (F_t / F_u), the ratio values between the FFA level in the treated and untreated samples versus the treatment time was shown in Figure 3 and 4. The straight lines with the high correlation coefficient in the range from 0.987 to 0.997 show a strong relationship between the treatment time and ratio of FFA level and suggested that the model can fit significantly the experimental data.

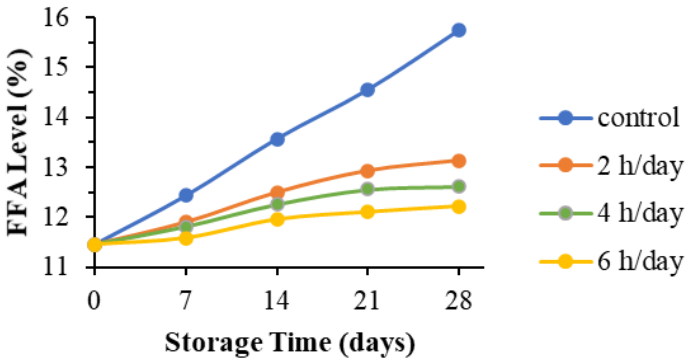

Fig. 2. The profiles of FFA level in CPO samples during periodic UV-C treatment at constant dose rate of 1.2318 mW/cm^2, at temperature of 30 ^{o}C, for 28 days storage

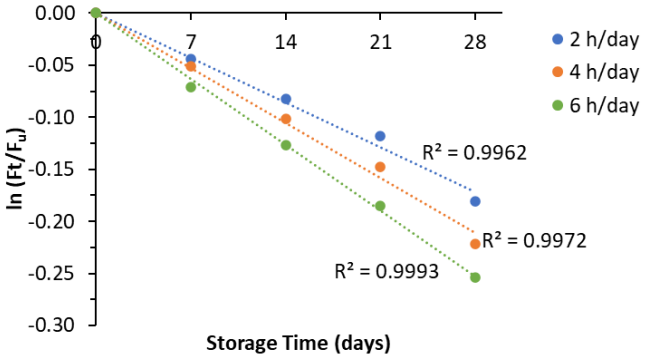

Fig. 3. The plot of the ratio of FFA levels in treated (F_t) and untreated samples (F_u) versus storage time at difference periodic exposure time and constant dose rate of 1.2318 mW/cm^2

Table 3
Reduction percentage of FFA formation due to periodic UV-C treatment at difference periodic exposure time and dose rates

Storage Time (days)	Increased FFA Level in untreated (control) samples (%)	Percentage of reduction at constant dose rate of 1.2318 mW/cm^2			Percentage of reduction at constant exposure time of 6 h/day	
		2 h/day (%)	4 h/day (%)	6 h/day (%)	0.8528 mW/cm^2 (%)	1.7238 mW/cm^2 (%)
7	0.9778	54.4680	63.5134	78.8500	59.7765	82.2100
14	2.1178	50.6627	61.9902	76.4260	57.0893	82.3174
21	3.1015	52.2719	64.4167	79.1428	62.8281	84.2139
28	4.2908	60.7219	72.8641	82.2553	71.6404	85.6374
h (1/day)		0.0061	0.0075	0.009	0.0073	0.0095
R^2		0.989	0.9919	0.9979	0.9879	0.9994

The model describing the plot is stated as follows

$$\ln \frac{F_t}{F_u} = -ht \tag{5}$$

where F_t = FFA levels in treated samples after treatment time t, F_u = FFA level in untreated (control) samples at treatment time t, *h* = inhibition constant, t = treatment time during storage.

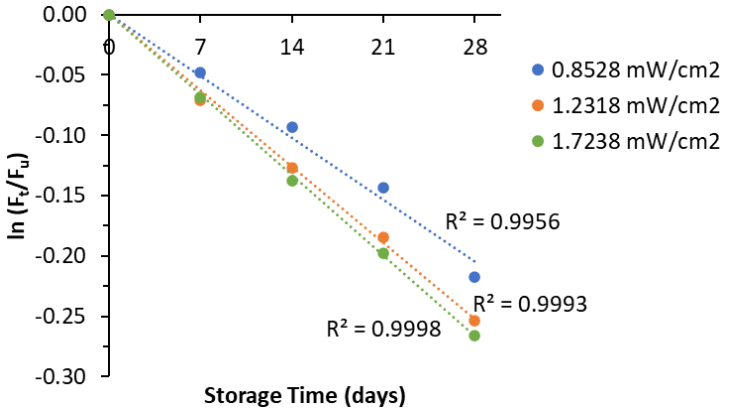

Fig. 4. The plot of the ratio of FFA levels in treated (F_t) and untreated samples (F_u) versus storage time at difference dose rate and constant periodic exposure time of 6 h/day

Inhibition constants (*h*) were obtained from the slops of the lines of Figure 3 and 4, and tabulated in Table 3. Following the Eq. (1), the constant of inhibition (*h*) in Eq. (4) can be proposed as a parameter associated to deactivation of lipase activity. The constant *h* is directly proportional to the reduction of FFA formation in treated samples (ln (F_t / F_u)), an increase of *h* value will increase the reduction rate. Similar to the deactivation rate constant (k_d), the inhibition constant (*h*) is also affected by the dose rate, increasing dose rate increases the *h* values. Compared to the constant k_d (see Table 2), the constant *h* has a much lower value (see Table 3) this is because of the transparency of the lipase solution where CPO hydrolysis occurred is much higher (average T% = 97) than the transparency of CPO (average T% = 0.0244). The small value of transparency prevents the dose delivery of UV-C light into CPO, and decreased the reduction rate of FFA formation. The samples transparency was measured using Spectrophotometer UV-Vis 1700 Shimadzu at wavelength of 253.7 nm.

4. Conclusions

FUV-C light treatment was able to decrease the activity of the lipase enzyme, and residual lipase activity significantly affected by dose rate and exposure time in proportion. The lowest residual activity (4.11%) was achieved at a dose rate of 1.7238 mW/cm^2 and at an exposure time of 8 hours. In addition, the first order kinetic model is suitable to present the experimental data of CPO hydrolysis. The periodic UV-C treatment can reduce the formation of FFA in CPO during the storage period. The amount of reduction depends proportionally on the specified dose rate and exposure time. A reduction in FFA formation of 85.6% was achieved at a dose rate of 1.7238 mW/cm^2 and a periodic exposure time of 6 hours per day.

Acknowledgement
The authors would like to fully thank Syiah Kuala University Research Grant and the Chemical Engineering Department of Syiah Kuala University for the technical support in the experimental work 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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