Free fatty acid formation in oil palm fruits during storage

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Free fatty acid formation in oil palm fruits during storage

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Abstract. Free fatty acids (FFAs) are derived from the undesired hydrolysis reaction of glycerides with the presence of lipase, and quantified as acid value for crude palm oil (CPO) grading. Since FFA content is directly proportional to time, duration from harvest to sterilization of fruits is not more than a day. This paper reports peculiar trend of FFA formation over time when the analysis of FFA was carried out differently. Analysis results of FFA and glyceride contents by timely picking the fruitlets (R-fruit) from fresh fruit bunch (FFB) were compared with the fruitlets from spikelet (S-fruit) that were initially removed all for 7 days. The results showed that the increment of FFA content of the latter was 10 hour faster. This implies that the fruitlets from spikelet resemble the detached fruits which having higher rate of FFA formation compared to the fruitlets that attached to FFB. By using SigmaPlot, the graphs of R-fruit and S-fruit were best fitted into damped sine, 5 parameter with linear and rational with 4 parameters respectively. Nevertheless, lower R^2 value was obtained for the fruitlets from readily-removed spikelet compared to the fruitlets from FFB, indicating that other factors might have also affected the formation of FFA.

1. Introduction

Free fatty acid (FFA) is a common indicator used for grading the palm oil quality. Rancidity caused by the FFA through oxidation reaction makes it unfavourable at high concentration [1, 2]. According to Food and Agriculture Organization (FAO) and World Health Organization (WHO), the standard requirement of FFA in the final product of crude palm oil (CPO) should not be more than 5% for food purposes [3]. Otherwise, the excess FFA will be removed during the refinery through the stripping or neutralization process and this will be considered as oil losses. It was reported that FFA content is highly associated with the lipolytic activity in the oil palm fruits [4, 5]. This enzyme is naturally present in the oil bodies and proportionally activated according to the maturity of the fruits [6, 7]. Contamination by microorganisms was also reported as one of the lipase sources which allow the hydrolysis reaction and subsequently forming the FFA compound [8, 9]. Other lipase inducing factors include the fruit bruising [10, 11] and post-harvest storage time [12, 13]. Apart from the lipolytic activities, the presence of moisture as source of water could be another thing to be considered. It was reported that autocatalytic reaction tends to occur at moisture level above 0.2% [14].

Based on previous studies, the ripeness or maturity level of FFB showed a significant effect on the FFA content in the extracted palm oil [15, 16]. It was reported by the researchers that the under ripe FFB has the lowest FFA content followed by the ripe, overripe and loose fruits. Common practice in the mill is that the harvested FFBs will be stored ideally for 24 hours prior to sterilization process. However, there is high possibility to have longer storage time due to massive loading of FFB from plantation. Long storage time is not preferred as the FFBs may turn over ripe, loosening more fruitlets and

increasing the FFA content. The possibility of FFA content is affected by the method of storage is also unclear. Based on the literature review of the authors, many previous studies had been focusing on the palm FFB storage time [12, 13, 15, 17, 18]. However the effect of storage method is yet to be done.

Hence, this research was executed to study the effect of different storage conditions by having a randomly picked fruitlets and a pre-removal spikelet from the bunch on the FFA content in the oil palm fruits. The experiment was conducted for a week to study the trend of FFA content over storage time as well. Fourier transform infrared spectroscopy (FTIR) was used in this study to quantitatively analyse the FFA content in the samples due to its repeatability and rapid analysis [19]. This method has been widely used in evaluating the quality and authenticity of edible oils [20-26].The intensity of the bands generated due to the vibrational mode of molecular groups in a sample indicates the corresponding concentration of particular functional groups, according to Lambert-Beer's law [27].

2. Materials and methods

2.1. Material

The ripe and the least visible bruising palm FFBs were harvested from LCSB Lepar, Pahang and the time was immediately recorded.

2.2. Storage conditions

The FFBs were distinctly stored indoor for a week whereby the first bunch with fruitlets remained intact was labelled as R-fruit as shown in Figure 1(a). The second bunch was stored indoor as well by performing pre-removal spikelets from the middle and top parts of the FFB using a sharp knife and was labelled as S-fruit which is shown in Figure 1(b). For R-fruit, the samples were randomly picked from the bunch regardless the part of FFB while for S-fruit, a batch of sample was taken from a whole spikelet from the middle part of FFB. All of these samples were triplicated. Starting from the harvesting process, at each 12 hours' time interval, the sample of palm fruits were chopped using knife and chopper to separate the mesocarp and the kernel. The palm oil was then extracted from the mesocarp by using a screw presser or a plier and was collected in a sample tube.

(a) (b)

Figure 1. Storage method of (a) R-fruit samples; (b) S-fruit samples.

2.3. *Analysis of FFA and glyceride content*

FFA content was studied using FTIR spectroscopy that is based on molecular absorption and transmission resulting in a different spectrum according to the functional group presents. A calibration model was used to determine the concentration of FFA and glycerides based on the COOH and COOC groups respectively. For each run, the crystal surface of the attenuated total reflectance (ATR) system of Nicolet™ iS™ 5 FTIR spectrometer was thoroughly cleaned with 70% ethanol solution and dried with a lens tissue as outlined by instructions in the equipment manual for a new analysis. The background spectrum was first calibrated before placing the oil sample onto the diamond crystal. All

spectra were collected from the range of 4000 to 400 cm^{-1} by using 32 scans [3]. The spectra were analysed by using Omnic software (Thermo Scientific USA). The FFA content in the extracted palm oil was determined at the wavelength between 1712 and 1710 cm^{-1} which representing the functional group of COOH [28, 29]. Meanwhile, the peak for COOC was observed at the wavelength of 1746–1743 cm^{-1} [29, 30]. Each run was triplicated and the average of absorbance readings for both functional groups were converted into molarity and best fitted into several equations in SigmaPlot 10.0 based on the calibration curve of FFA and glyceride concentrations against absorbance as regressed by using Equations (1) and (2):

$$C_{COOH} = 1.0275h^{0.6582} \qquad R^2 = 98.72 \qquad (1)$$

$$C_{COOC} = 0.0287 + 214.78h \qquad R^2 = 98.74 \qquad (2)$$

3. Results and discussion

Figure 2 (a) and (b) are separately showing the graphs of FFA concentration over storage time for R-fruit and S-fruit. The R-fruit was best fitted using a damped sine with 5 parameters and linear while the S-fruit was best fitted using a rational with 4 parameters equation as shown Table 1. For these models, high R^2 values were obtained where 95.2 % and 86.1 % of the FFA content can be explained by the length of storage time, respectively. Different trend shown by the graphs in Figure 2 was due to the sampling methods. Fruits obtained by random sampling tend to have different level of ripeness. The rate of ripeness differs between the top, middle and bottom parts of FFB [31] whereby the fruits start to mature from top, the farthest part from the stem [32]. This damping trend was also reported in several papers [33-35] suggesting the dynamic acclimatization of lipase to micro-aqueous environments. Meanwhile, the S-fruit is displaying a more stable trend of FFA concentration because of homogenized sampling. The FFA content had sharply increased after 72 hours instead of damping in a wave form. This result resembles the characteristics of the loose fruits, in which the formation rate of FFA is higher compared to others [16].

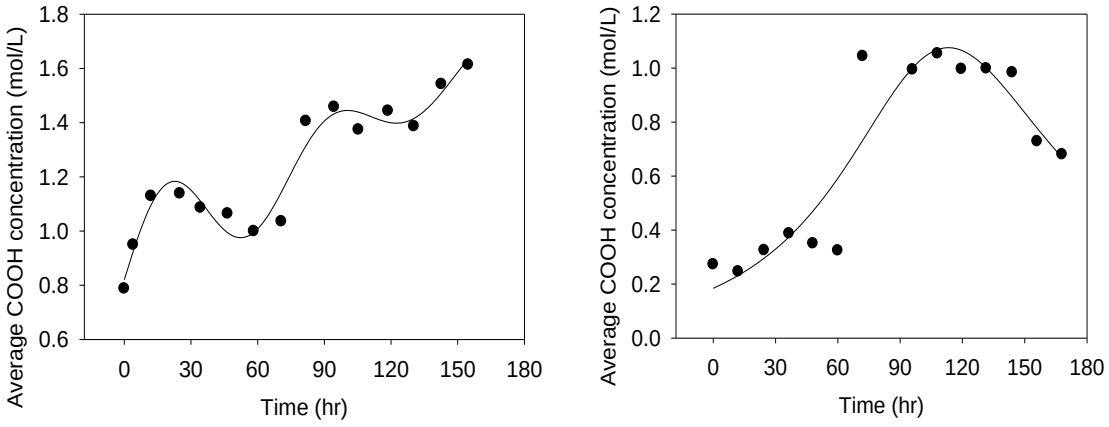

Figure 2. FFA concentration in oil extracted from (a) R-fruit; (b) S-fruit.

The graphs basically can be divided into two parts whereby for the first part, both storage methods show an upward trend of FFA. This is probably related to the ripening of fruitlets and the softening of pericarp, the outer layer of the fruits. The oil bearing cells tended to be disrupted and disintegrated as the fruit ripened. As the oleosomes ruptured due to the natural occurrence, ripening or bruising, more oil or triglycerides will be released and thus provide a sufficient substrate for the lipolytic activity to

occur [5, 36]. It was observed that the fruitlets soften over prolong storage and less resistance for rough handling. This condition increased the susceptibility of microbes' contamination which eventually produced exogenous lipase. It can be supported by a previous study which found that higher contamination and deterioration was observed on the longer storage fruits compared to freshly harvested fruits [15]. However, it was reported that at the early post-harvest stage, the lipolytic activity was highly dominated by the endogenous lipase [37]. This study is in agreement with the study conducted by España, Mendonça [18] who had analysed the effect of different storage time on another species of oil palm fruit, *E. oleifera* where the fruitlets were maintained connected to the spikelet to imitate the conditions at palm oil mill. FFA concentration was observed to escalate from day 1 to day 7 and further increased until day 14. From the result, R-fruit starts off with higher FFA concentration which is double the FFA in S-fruit. Regardless the scattered points, the trend clearly display a steady increase over a week of storage. However for the S-fruit, the FFA concentration increases steadily only up to 60 hours or 2.5 days before sharply increases on 72 hours after harvest. The FFA concentration then begins to be constant afterwards until 144 hours of storage. Similarly, it was reported in another study that the FFA content exceeded the standard requirement as it left unprocessed by 72 hours after harvest [12].

The second part of the graph covers from 156 to 168 hours after harvest. At these last two points, the FFA concentration declines for both R-fruit and S-fruit probably because of the endogenous lipase activity when the equilibrium reaction was achieved. According to Le Chatelier's principle, as the concentration of one reactant changes, the position of the equilibrium will shift to counteract the change. This might explain the depletion of FFA which could be due to the backward reaction and subsequently producing glycerides. This finding is somewhat similar to another study on the effect of long-term storage of different palm germplasms on FFA [17]. The study had found the increasing trend of FFA until a certain peak before starting to decrease gradually. It was further explained that any increment in FFA afterwards are probably due to the exogenous lipase activity [4].

Table 1. Equations generated for FFA concentration in extracted oil.

Sample	Best fit using Sigma Plot	Equation	R^2
R-fruit	Damped sine, 5 parameters linear	$C_{FFA} = 0.8670 + 0.2569^{(-t/112.03)} \times \sin\left(\frac{2\pi t}{74.25} - 0.1834\right) + 0.0048t$	0.95
S-fruit	Rational, 4 parameters	$C_{FFA} = \frac{0.1847 + 0.0009t}{1 - 0.0138t + 6.461\times10^{-5}t}$ $C_{FFA} = \frac{0.1847 + 0.0009t}{1 - 0.0138t + 6.4610 \times 10^{-5}t}$	0.86

Since the FFA is formed through hydrolysis reaction, glyceride content which is the main reactant was analyzed as well at different wavelength in the spectra. The band that was generated between 1746 and 1743 cm^{-1} denotes the functional group of carbonyl ester C=O which representing the glycerides [29, 30]. The trend of glyceride content in both samples contradicts to each other. The graph of R-fruit in Figure 3 (a) was best fitted into an exponential rise to maximum equation while for S-fruit; the graph in Figure (b) was best fitted into a rational with 5 parameters equation. Table 2 summarizes the parameters obtained from both graphs where the coefficients of determination are 68.7 % and 96.1% respectively.

The increment of glyceride content in the R-fruit was probably due to incomplete hydrolysis reaction, similar to the explanation for the second stage of Figure 2 (b), in which the water became a limiting reactant. From an observation study, the oil palm fruits were drying off over the time. According to previous researchers [38], moisture content in palm mesocarp decreased along with the ripening process and this will continue until the fruits reaching maximum oil content. This is also in line with the findings by Tagoe, Dickinson and Apetorgbor [15]. Later study conducted by Suresh and Behera [39] agrees the

trend of moisture content decreases as fruit ripen. Apart from the reverse hydrolysis, disintegration or rupture of oil-bearing call was probably the cause of glyceride rise which released free oil to the extract.

However, different trend of glyceride content is shown by the S-fruit. The initial concentration of the sample is much higher than the R-fruit. This is probably due to the resemblance of characteristics between the S-fruit and the loose fruits. Loose fruits were reported to have the highest oil content compared to others and so the FFA content [6, 31, 40]. Once the first fruit started to loose from the bunch, oil yield could increase by 7% [41]. High concentration of triglycerides which constitute the most part in the oil bodies provides abundant substrate for hydrolysis reaction [17, 36] hence causing the depletion of COOC content as exhibited by the S-fruit. It was reported in another study that oil losses is proportional to the ripeness level of the fruits [12] indicating high oil concentration presents. The reduction of COOC in S-fruit matches the upward trend of FFA content in Figure 2 (b). Conversely, lower oil content was reported for the fruitlets that are still attached to the bunch [40] suggesting that lesser oil would be released from the oil bearing cells in the R-fruit. Therefore, the contrast behavior displayed by R-fruit was probably due to the limiting substrate as well as the incomplete hydrolysis reaction. Later increment of glyceride content in S-fruit might be due to slower ripening process of some inner fruits compared to outer fruits that attached to the spikelet.

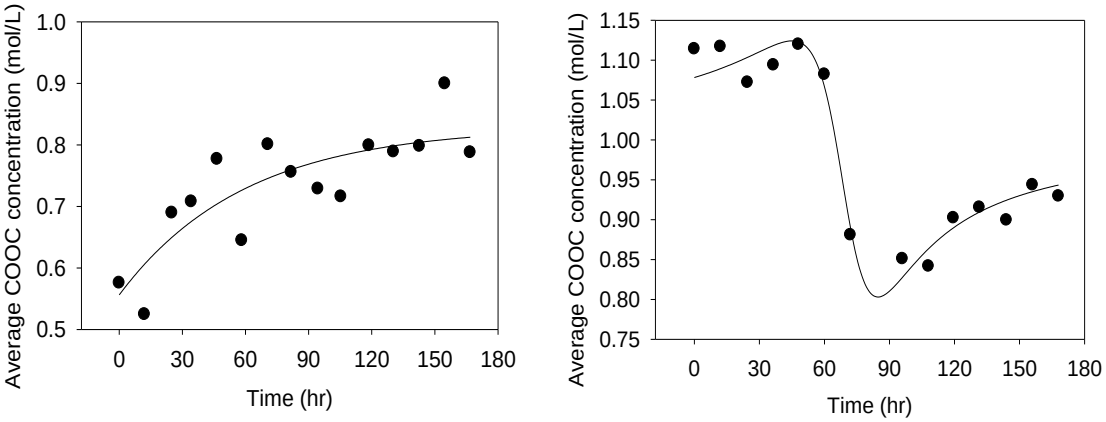

Figure 3. Glyceride concentration in oil extracted from (a) R-fruit; (b) S-fruit.

Table 2. Equations generated for glyceride concentration in extracted oil.

Sample	Best fit using SigmaPlot	Equation	R^2
R-fruit	Exponential rise to maximum	$C_{COOC} = 0.5563 + 0.2730[1 - 10^{-0.0168t}]$	0.69
S-fruit	Rational, 5 parameters	$C_{COOC} = \frac{1.0794 - 0.0279t + 0.0002t^2}{1 - 0.0266t + 0.0002t^2}$ $C_{cooc} = \frac{1.0784 - 0.0279t + 0.0002t^2}{1 - 0.0266t + 0.0002t^2}$	0.96

4. Conclusion

FFA content in oil palm fruits is affected by the method and the duration of the storage. The FFA concentration increases together with time for both R-fruit and S-fruit yet were best fitted into different models: damped sine and rational equations respectively. The glyceride content however contradicts to

each other whereby it had gradually increased in R-fruit and sharply decreased in S-fruit. The pre-removal spikelet fruit (S-fruit) resembles the characteristics of loose fruit in which higher oil concentration was initially observed compared to R-fruit. This provides abundant substrate for the hydrolysis reaction and reduced glycerides in prolonged storage. The results in this study were subjected to the fruit clone of DP-Chemara and DP-Felda, and may vary if other clones of oil palm fruit are tested.

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