Enzymatic Remediation in Standard Crude Palm Oil for Superior Quality

Haniza Ahmad
Sime Darby Research, Banting, Selangor, Malaysia
haniza.ahmad@simedarby.com

Norliza Saparin, Ahmadilfitri Mohd. Noor and Mohd Suria Affandi Yusoff
Sime Darby Research, Banting, Selangor, Malaysia
norliza.saparin@simedarby.com
ahmadilfitri.md.noor@simedarby.com
mohd.suria.yusoff@simedarby.com

***Abstract*—Free fatty acid (FFA) in crude palm oil (CPO) is varies depending on harvesting methods, storage conditions up to processing at oil mill. CPO with low FFA is contemplated as superior. Lowering the FFA level in high FFA CPO by lipase Candida Antartica enzyme in passed research by others is achievable. Enzyme is applied in standard CPO to investigate if further FFA reduction is able to take place and produce premium CPO (<1% FFA). There are four different lipase Candida Antartica brands used in this study. Samples submitted for enzymatic remediation using rotary evaporator under 100mbar vacuum with rotation at 260rpm. FFA less than 1% was achieved after 24 hours reaction by all 4 brands. The FFA reduction was intensified with the presence of glycerol that provides more sites for fatty acid attachment. At 2% glycerol, 71-88% FFA was reduced whereas at 1% glycerol, 46-75% FFA was reduced. However, partial glycerides was increased with the presence of glycerol. This study concluded that enzymatic remediation could further reduce FFA in CPO in order to obtain premium CPO (<1% FFA).**

***Index Terms*—crude palm oil, enzyme, free fatty acid, glycerol**

I. Introduction

Crude palm oil (CPO) is obtained by extracting the oil from the mesocarp of oil palm fruits (*Elais guineensis*). It has a unique fatty acid and triacylglycerol profile which makes it suitable for numerous food applications [1]. Palm oil general components are triglycerides (95%) as its major component and free fatty acids, partial glycerides, phosphatides & glycolipids, tocopherols & tocotrienols, carotenoids and sterols.

Free fatty acids (FFA) that normally present in CPO needs to be removed to ensure CPO quality and to meet the standards set by the industrial players. In Malaysia, the Palm Oil Refiners Association Malaysia (PORAM) standard for FFA is 5% max for CPO [2]. Higher than than that, a penalty shall be imposed and this is a liability to the business.

Free fatty acids presents in CPO is due to bruising of fruitlets during harvesting, transportation and discharge from lorry into hoppers and into sterilizer cages in refinery, which promoting lipase enzyme reaction [3]. The rate of FFA content released was significantly influenced by the level of fruit damage after chopping and it increased proportionally with the length of storage periods [4]. "Reference [5]" suggested that the FFA content is determined by length of storage of fruits prior to oil extraction process and also the length of storage of the oil after processing. Apart of storage length, the exposure to light also promotes FFA production by the increasing not only the rate of oxidation but also hydrolysis as well since light is a source of energy [6]. In light of FFA content in palm oil, lipase enzyme inactivation was also being study to control FA release, which concluded in laboratory scale that microwave sterilization is better to be opted from conventional steam bath during milling process [7].

In current palm oil industry, FFA is removed from CPO during physical refining process at deodorization stage. Deodorization is actually a combination of three-different operations: (a) distillation, i.e. stripping of volatile components (FFA, tocopherols, tocotrienols, sterols and contaminants); (b) actual deodorization, i.e. removal of odoriferous components; and (c) heating effect, i.e. thermal destruction of pigments (carotenoids) [8]. Removing FFA during refining, resulting in yield loss during deacidification and deodorization process. Alternatively, in chemical refining, neutralization by incorporating sodium hydroxide (NaOH) to remove FFA generated soaps which resulting greater loss of oil. To reduce oil loss during refining, "reference [9]" suggested enzymatic remediation to be applied to CPO. FFA in CPO could be re-attached with mono- and di-glycerides and these condition could reverse yield loss and recovered by enzymatic reaction and thus, no oil loss is occurred during removal of FFA process by enzymatic

Manuscript received January 21, 2016; revised June 1, 2016
To be presented in 7th International Conference of Biotechnology and Food Science ICBFS 2016 in Antalya, Turkey. This conference is organized by Asia-Pacific Chemical, Biological & Environmental Engineering Society (APCBEES).

doi: 10.18178/ijlbpr.5.1.22-25

means. Research on treatments of CPO by applying enzymatic remediation for FFA reduction found out that at deep vacuum (25mbar), the best remediation efficiency was observed after 24hours of reaction time [10]. "Reference [11]" suggested that enzymatic deacidification or esterification is effectively can be utilized for high free fatty acids rice brand oil in order to produce high quality oil. Further FFA reduction in addition to enzymatic remediation could be obtain by introducing glycerol in the material [9], [12]. The FFA reduction is improved with the presence of glycerol by providing more site for FFA re-attachment at the glyceride bones. "Reference [9]" explained that non-specific lipase reduced FFA in the oil more quickly due to the ability of the enzyme to attach a fatty acid at any position on the glycerol backbone.

This research was focusing on standard CPO (FFA<4%) to be further reduce the FFA % targeted to obtain premium CPO with <1% FFA by enzymatic remediation. In this study, four different brands of *lipase Candida Antartica* enzymes were used.

II. Materials and Methods

A. Materials

Low FFA CPO (<4%) were provided by Sime Darby Jomalina Sdn. Bhd. located at Telok Panglima Garang, Selangor, Malaysia. All chemicals used was either of analytical or chromatographic grades purchased from Merck (Darmstadt, Germany) or Fischer Scientific (Loughborough, UK). *Lipase Candida Antartica* enzymes were purchased from four different international companies.

B. Methods

Free fatty acid composition Free fatty acid (FFA) composition was determined based on the AOCS Official Method F 9a-40 (American Oil Chemists' Society 1997). The CPO (1.0g) is dissolved in an isopropanol solution which was titrated with sodium hydroxide (NaOH). The FFA content was calculated as palmitic acid percentage. Acylglyceride composition Acylglyceride composition was using gas chromatography as described in AOCS official method Cd 11b-91 (American Oil Chemists' Society 1997). CPO samples (0.05g) were dissolved in *n*-hexane (5mL) and then analyzed for triaclyglycerides (TG) composition using gas chromatography (Model : Clarus 500; Perkin, Elmer, Waltham, Massachusetts, USA). The TG were separated using a SP2380 (Supelco, Bellefonte, Pa., U. S. A.) capillary column (0.25 cm i.d. x 30 cm x 0.2 µm). Temperature maintained in the analysis were as follows: column oven, 180 ℃; injection block, 100 ℃; and detector temperature, 370 ℃. The carrier gas was nitrogen at 45mL/min. The injection volume was 1 µL. Enzymatic remediation method Using a rotary evaporator, CPO (250g) were heated (70 ℃) and, mixed with enzyme and glycerol. The flask were incubated (60 ℃), under 100mbar vacuum and rotation at 260 rpm. The feed oil and the treated oil were then analysed for FFA and acylglycerides compositions. The treated oil was analysed at 4, 8 and 24 hours interval.

III. Results and Discussion

There were FFA reduction occurred at all conditions of treatments at 4, 8 and 24 hours regardless of the glycerol percentage with the amount of reduction was related to the amount of glycerol percentage, time of reaction and enzyme brands. Glycerol at 2% showed better FFA reduction compared to 1% glycerol at all intervals of 4, 8 and 24 hours with varies results among brands.

At 4 hours with 2% glycerol, Enzyme D and Enzyme B showed nearly similar FFA reduction efficiency at 45% and 44% respectively whilst Enzyme A's showed 24% reduction, and Enzyme C's at 41% reduction. However, at 1 % glycerol, Enzyme C showed the best performance at 39% while Enzyme A showed 18% FFA reduction, 27% by Enzyme B and 33% by Enzyme D. At 8 hours of reaction time at 2% glycerol, Enzyme C and Enzyme D exhibit almost similar efficiency at 60% and 59% respectively while Enzyme A exhibited 50% FFA reduction and Enzyme B showed 53% reduction. At 1% glycerol, both brands Enzyme C and Enzyme D also showed nearly similar proficiency at 55% and 54%, Enzyme A showed 25% and Enzyme B 44%. At final 24hours with 2% glycerol, Enzyme D stands out to be the most effective enzymes at both 2% and 1% glycerol respectively at 88% and 75% FFA reduction whilst Enzyme A showed 72% and 46% FFA reduction, Enzyme B 81% and 71% reduction and Enzyme C 85% and 72% reduction.

This result is aligned with "reference [10]" who revealed that the most efficient reaction time is at 24hours, even though the vacuum pressure varies at 25mbar compared to this study at 100mbar. In term of FFA %, at 4 hours reaction time, working from at approximately 4% FFA, all conditions showed <3% FFA. At 8 hours, almost all conditions showed <2% FFA except for Enzyme A and Enzyme B at 1% glycerol. By 24 hours, all conditions with 2% glycerol achieved <1% FFA. Table 1 and Fig. 1 summarized the result.

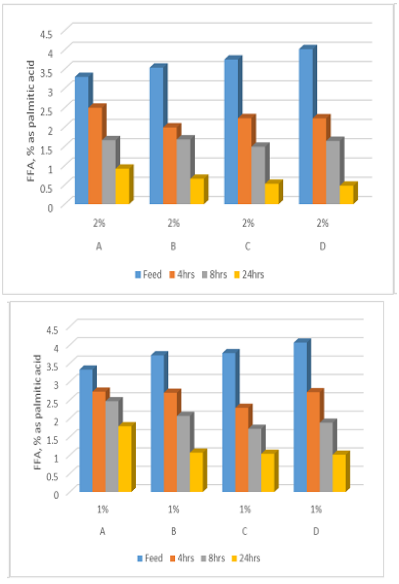

Figure 1. Ffa % expressed as palmitic acid at (a) 2% glycerol an (b) 1% glycerol

TABLE I. FFA % IN CPO DURING FEED AND AFTER TREATMENT

	Enzyme A		Enzyme B		Enzyme C		Enzyme D	
Glycerol%	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0
Feed	3.30 ±0.17 [1]	3.33 ±0.24 [2]	3.605 ±0.09 [2]	3.72 ±0.11 [2]	3.75 ±0.01 [2]	3.78 ±0.01 [2]	4.02 ±0.03 [2]	4.07 ±0.09 [2]
4 hours	2.50 ±0.03 [1]	2.73 ±0.04 [2]	2.12 ± 0.18 [2]	2.70 ±0.25 [2]	2.23 ±0.07 [2]	2.29 ±0.16 [2]	2.23 ±0.12 [2]	2.72 ±0.08 [2]
8 hours	1.66 ±0.12 [1]	2.47 ±0.08 [2]	1.61 ±0.10 [2]	2.07 ±0.20 [2]	1.49 ±0.04 [2]	1.72 ±0.09 [2]	1.64 ±0.01 [2]	1.89 ±0.02 [2]
24 hours	0.92 ±0.02 [1]	1.79 ±0.02 [2]	0.67 ±0.01 [2]	1.07±0.00 [2]	0.53±0.10 [2]	1.04 ±0.05 [2]	0.48 ±0.00 [2]	1.02 ±0.01 [2]

[1]Results expressed as mean ±standard deviation (n3)
[2] Results expressed as mean ±standard deviation (n2)

TABLE II. CHANGES OF GLYCERIDES WITH DIFFERENT GLYCEROL ADDITION PERCENTAGE

		Enzyme A		Enzyme B		Enzyme C		Enzyme D	
Glycerol %		2%	1%	2%	1%	2%	1%	2%	1%
MG	Feed	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00	0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
	4 hrs	2.94 ±0.96	2.25 ±0.16	2.81 ±0.03	2.38	2.63	2.84 ±0.21	2.68 ±0.01	2.77 ±0.11
	8 hrs	3.63 ±1.65	2.81 ±0.18	3.26 ±0.33	2.59	3.71	3.24 ±0.08	2.75 ±0.13	3.07 ±0.04
	24 hrs	3.31 ± 0.59	2.80 ±0.48	3.41 ±0.16	3.67	3.47	3.54 ±0.09	3.34 ±0.38	3.61 ±0.40
DG	Feed	2.98 ±0.58	3.28 ±0.59	2.71 ±0.18	3.16	2.85	2.49 ±0.18	2.16 ±0.01	2.71 ±0.28
	4 hrs	3.19 ±1.06	2.57 ±0.23	3.25 ±0.64	2.95	2.66	2.41 ±0.30	2.45 ±0.35	2.57 ±0.23
	8 hrs	3.01 ±0.43	2.98 ±0.06	3.14 ±0.33	2.85	3.03	2.66 ±0.26	3.03 ±0.21	2.81 ±0.26
	24 hrs	3.76 ±0.30	2.83 ±0.40	4.68 ±0.86	3.66	3.89	3.50 ±0.19	3.86 ±0.02	3.49 ±0.47
TG	Feed	92.26 ±0.69	92.89 ±1.56	93.12 ±1.56	92.40	91.40	92.68 ±1.00	92.62 ±0.18	92.33 ±0.00
	4 hrs	89.52 ±2.62	91.93 ±0.93	91.25 ±0.93	91.00	92.84	91.52 ±0.63	92.04 ±0.53	91.30 ±0.49
	8 hrs	90.85 ±1.00	91.50 ±1.03	92.15 ±1.03	92.26	91.28	91.90 ±0.45	92.47 ±0.06	91.62 ±0.18
	24 hrs	91.43 ±0.63	92.37 ±0.79	91.33 ±0.79	91.60	91.96	92.04 ±0.21	92.42 ±0.40	91.96 ±0.04

[1] Results expressed as mean ±standard deviation (n3)
[2] Results expressed as mean ±standard deviation (n2)

In "reference [9]", it was explained that adding in glycerol into the reaction would further increase the FFA reduction rate. However, this will increase partial glycerides (monoacylglycerides and diacylglycerides) as well as the triacyglycerides. This study agreed with "reference [9]" as demonstrated in Table 2. It was observed that monoacylglycerides and diacylglycerides were increased with addition of glycerol. At 2% glycerol, it showed higher partial glycerides compared to 1% glycerol. By comparison to enzymes, at 2% glycerol after 24 hours, Enzyme D showed the least partial glycerides increment at 30% from initial followed by Enzyme B at 33%, Enzyme C 39% and Enzyme A at 42%.

IV. CONCLUSION

In current conventional FFA removal method in the palm oil industry, FFA was removed either through chemical refining or physical refining. Both refining routes has significant yield loss during refining process and also resulted in effluents. Enzymatic treatment offered possibilities for a 'green' means of crude palm oil refining during the FFA removal.

Based on this study, FFA parameter for a quality CPO is achievable with reduction to <1% is observed by introducing liquid *lipase* Candida *Antartica* enzyme with glycerol into CPO before undergo conventional refining process. At 24hours of reaction with 1% enzyme and 2% glycerol, premium CPO (<1% FFA) is producible by all *lipase Candida Antartica* enzyme brands at 72-88% of FFA reduction. The reduction rate was dissimilar depending on the brands. In this study, Enzyme D showed the best reduction of FFA compared to others at 24hours by 88% reduction. Nevertheless, adding in glycerol changes glycerides composition with increase of partial glycerides

Further study need to be carried out to obtain further FFA reduction at less time by avoiding partial acylglycerides increment and possibility to recycle these enzymes in order to be cost effective for the industry to adopt this method of FFA removal.

REFERENCES

[1] O. I. Mba, M-J Dumont and M. Ngadi, "Palm Oil: Processing, characterization and utilization in the food industry – A Review," *Food BioScience* vol.10, pp. 26-41, January 2015.

[2] PORAM *Handbook*, 7th ed, The Palm Oil Refiners Association of Malaysia, Kelana Jaya, Selangor, Malaysia, 2012.

[3] S. Hadi, D. Ahmad and F. B. Akande (2009), "Determination of the bruise indexes of oil palm fruits," *Journal of Food Engineering* vol 95, pp. 322-326, May 2009.

[4] F. S. Ali., R. Samsuddin. and Y. Robiah (2014), "Effect of chopping oil palm fruit spikelets on the free fatty content release rate and its mechanical properties," *International Journal of Research in Engineering and Technology*, vol. 3, no. 1, pp. 511-516, January 2014.

[5] S. M. A., Tagoe, M. J. Dickson and M. M. Apetorgbor, "Factors influencing quality of palm oil produced at the cottage industry level in Ghana," *International Food Research Journal* vol. 19, no. 1, pp. 271-278, 2012.

[6] O. H. Henry, "Monitoring the free fatty acid level of crude palm oil stored under light of diff wavelength," *American Journal of Food Technology*, vol 6, no. 8, pp. 701-704, 2011.

[7] M. Sarah, M. R. Taib and A. Adamu, "Enzymatic Inactivation of Oil Palm Fruits: Comparison of Microwave Irradiation and Steam Bath Process", *Jurnal Teknologi*, vol. 65, pp. 55-60, 2014.

[8] D. Cowan, H. C. Holm, H. C. and H. S. Yee, "Reduction in free fatty acids in crude palm oil by enzymatic remediation," *Journal of Oil Palm Research*, vol. 24, pp. 1492-1496, December 2012.
[9] J. Maes, K. Sitharam, S. Danthine and V. Gibon, "Influence of enzymatic remediation on composition and thermal properties of palm oil and palm oleins from fractionation," *Journal of American Oil Chemical Society*, vol. 92, pp.821-831, May 2015.
[10] G. Misra, and S. Nandi, "Enzyme deacidification of rice bran oil containing high free fatty acids with recycling'" *Chemical Science Review and Letters*, vol 2, no. 5, pp. 376-381, January 2014.
[11] S. Bhattacharya, and D. K. Bhattacharya, "Biorefining of high acid rice bran oil", *Journal of the American Oil Chemist's Society,* vol. 66, pp.1469-1471, December 1989.

Haniza Ahmad is with the Sime Darby Research, Banting, Selangor, Malaysia.