Engineering solutions for the palm mill of the future: Increasing extraction rate and sustainability through biotechnology

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Abstract. The edible oil industry involving extraction and processing of oils and fats from vegetable sources has witnessed a tremendous growth over the decade accounting for rapid expansion of urbanization and industrialization. Consequently, concerted efforts have been undertaken to increase the palm oil extraction rate (OER) at processing mills. This paper explores the commercial application of enzymatic treatment of sterilised palm fruitlets and diluted crude oil (DCO) stream in a 60 t/hr oil palm mill. Incubation of sterilised palm fruitlets mixed with enzymes in a pre-digester vessel at 70 °C and retention time of 30 minutes resulted in an increment of 1.15 % OER. Parallelly, dosing of enzyme into DCO with simpler system yielded in OER increment up to 1.19 %. This study offers insight into the potential oil left in pressed fibre using different methods, such as prolonged Soxhlet extraction, ultrasonic treatment, and supercritical fluid extraction. Additionally, confocal microscopy images of treated samples showed disintegration and thinning of cell walls resulting in higher free oil droplets.

1. Introduction

The global oils and fats consumption is forecasted to increase to 250 million metric tonnes to fulfil the growing population of over 8.5 billion by 2050 [1]. Palm oil acts as the most relevant and crucial component of this food supply chain and accountable for nearly half of the global edible oil consumption. Oil palm is by far the most efficient biological source of oil-based hydrocarbons, outperforming any other commercial oil crop [2]. In short, of 300 million hectares for global oil crop cultivation, palm oil only accounts for 6 % of this land use with a significant contribution of 36 % global consumption [2, 3]. However, with limited land allowable to be planted under the RSPO requirement, with weather changes that has negatively impacting the yield and with very long development of higher yielding planting material, it is essential for palm oil producers to strengthen the role and address the substantial increase in demand. Therefore, ways are needed to increase the sustainability aspects of palm
oil and productivity by limiting the expansion of agriculture areas that could jeopardize these
biodiversity-rich lands [4].

The palm oil milling industry that is based on conventional mechanical extraction has almost
reaching the optimum extraction efficiency rate with the extraction efficiency of approximately between
92 to 94%. Palm oil mills use oil extraction rate (OER) to measure its efficiency and is calculated based
on the mass ratio of the crude palm oil (CPO) extracted to the fresh fruit branches (FFB) processed. For
the past 40 years, Malaysia's average palm OER has remained stagnant between 19 and 21%. Globally,
for every 1% rise in the OER, nearly 3 million tonnes of palm oil can be produced [5].

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\text{Oil extraction rate, OER (\%) = \left\{ \frac{\text{total oil produced}}{\text{total fresh fruit bunches processed}} \right\} \times 100\%
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With sustainable biotechnology solutions, this greater amount of oil may be generated without the
requirement of additional land, water, energy, or fertilizer for crop production. Solvent extraction that
supposedly will provide the highest means of extraction has not been acceptably recognized for palm
oil extraction by the consumer, therefore will have to compromise with the conventional mechanical
means of extraction. This will require palm oil milling to modify the process by either altering the
process condition or using process catalyst [6–8].

Some of the developmental works are in the area of sterilization whereby sterilization at higher
pressure and temperature will hydrolyze the hemi-cellulose that is part of the cell wall protecting the oil
globules in oil palm fruit mesocarp, thus it will improve the oil availability for the extraction [9]. Another
team of researchers has been exploring for the separation of oil palm fruit mesocarp from the nut that has
improved the digestion of the oil-bearing cell, giving a potential increment of OER of 0.5 to 0.7% [10].
As a result, alternative techniques to the aforementioned solvent extraction methods are being
investigated to meet the increased need for product purity, nonpolluting, and energy-efficient processes,
with the added benefit of requiring less solvent. Extraction assisted by pulsed electric field, microwave
extraction, instant controlled pressure drop technology, microwave hydro diffusion and gravity,
ultrasound assisted extraction, ionic liquid extraction, subcritical water extraction, high pressure assisted
extraction, aqueous two phase extraction, and enzyme assisted aqueous extraction are some of the most
recent extraction techniques developed in the quest to create commercially viable, efficient, energy-
saving, safe, compact, and sustainable extraction processes [6].

Enzymatic extraction of palm oil which involves addition of hydrolytic enzymes during maceration
process, has gained increased attention in deciphering the nation’s OER stagnation issue [11]. The
synergetic hydrolysis of complex constituents of cellulose, hemicellulose, lignin, and pectin, together
with the digestion and screw pressing steps could efficiently results in liberation of higher oil and
reduction in oil losses in pressed fibre. The enzymatic extraction has been widely explored with various
oil-bearing materials such as soybean, grape seed, sunflower, peanut, and olive, and proven to improve
oil extraction rates by more than 90% and promises superior quality [12–16].

The research and development of enzymatic technology for extraction in palm oil milling began in
year 1990 with an increment of 6.6% extraction rate that was obtained by treating palm mesocarp with
pectinase followed by hydraulic press [17]. Teixeira et al. (2013) have reported highest oil extraction
efficiency of 90 - 93% total oil utilizing 4% of enzyme cocktail of tannase, cellulase and pectinase, pH
4, ratio of solution to pulp of 2:1 and 30 minutes incubation at 50°C. Recently, Javvadi et al. (2012)
presented an invention for extracting oil from the mesocarp of oil palm fruit using enzymes that includes
exocellulolytic, pectinolytic, mannanolytic and glucanolytic activity with improved efficiency and
increased yield of 90%. In another study by Eshtiaghi et al. (2015), samples treated with 0.15% celluase resulted in distinctly 95% oil extraction yield and reduction of remaining press pulp by 5%.

Therefore, in this study, we have conducted laboratory evaluation of potential oil extractable and
with positive outcome, the plant trials were carried out. During the mill trial and subsequent commercial
implementation, we evaluated the application of enzyme to sterilized oil palm fruitlets as well as diluted
crude oil (DCO) with the objective of enhancing the palm oil extraction.
2. Materials and Method

2.1. Enzymatic treatment
PALMORA OER, a carbohydrase cocktail of xylanase and cellulase was obtained from Novozymes Malaysia Sdn Bhd. The Palmora OER is a thermotolerant enzyme, suitable to be used at processing temperature between 80 to 90 °C. Cellulases and xylanases catalyze the hydrolysis of the 1,4-beta-D-glycosidic bonds in cellulose, hemicellulose, lignin and cereal beta-D-glucans to break down the cellulose found in plants cells.

2.2. Enzymatic Treatment
Approximately 20 grams of sterilized palm mesocarp pulp was dissolved in enzyme solution (enzyme diluted with water) and extraction was carried out for 1 hour in a 100 mL conical flask placed in a water bath shaker operating at 80 to 90 °C and constant shaking of 200 rpm, followed by enzyme deactivation [11]. Three times serial centrifugation at 6500 rpm for 30 minutes was used to separate the oil, liquid, and solid fractions from the mixture, followed by hexane washing and filtration. The samples were dried overnight at 103 °C and oil content was quantified using Soxhlet extraction method.

2.3. Soxhlet Extraction
Total amount of oil in samples was determined by solvent extraction procedure to compare the extraction efficiency of palm mesocarp and pressed fibre treated with different methods. 10 g sample was extracted using n-hexane in a Soxhlet apparatus of 100 mL capacity between 4 to 16 hours. The extract was then filtrated, and rotary evaporator was used to remove the n-hexane contained in the filtrate.

2.4. Ultrasonic treatment
20 g palm pressed fibre was weighed into a 250 mL conical flask, 100 mL of distilled water was added, and the mixture was placed inside the Brasonic® Ultrasonic Cleaner Model 5510 (Emerson, USA) for ultrasonic pre-treatment at 485 W with operating hours ranging from 0 to 5 hours. Three times serial centrifugation at 6500 rpm for 30 minutes was used to separate the oil, liquid, and solid fractions from the mixture, followed by hexane washing and filtration. The samples were dried overnight at 103 °C and oil content was quantified using Soxhlet extraction method of 4 hours.

2.5. Supercritical Fluid Extraction (SFE)
This study was conducted using Supercritical fluid extraction (SFE) facility in UKM based on the method by Mohamed et al. (2018) with minor modifications. The SFE system comprise of a carbon dioxide pump (PU-2080, JASCO Corporation, Japan), BP 1580-81 model back pressure regulator (BPR, JASCO Corporation, Japan), series 111 solvent pump (Lab Alliance, USA), pressure transmitter (model 682-8, Dwyer Instrument, USA), extractor vessel enclosed in a FX2-2 model air circulating oven (Sheldon Manufacturing, USA) and sample collector. Before the extraction process began, a chiller (Protech Electronic, Malaysia) was utilized to keep the liquefied carbon dioxide (CO₂) in a liquid form at - 4 °C. The extraction was done in a controlled environment. The liquid CO₂ flow rates were set at 4 mL/minutes, 60 °C, and 200 barg. 4 g palm fibre samples (at 10 % moisture content) were inserted in the extractor vessel, and the extraction was begun using the dynamic extraction mode, with each fraction collected every 30 minutes. Each portion was weighed after drying in a 45 °C oven.

2.6. Plant Scale trial
The enzyme dosing rate has been optimized during the laboratory trials was used during the plant trial. Two different dosing strategies have been studied in 60 metric t/hr palm oil mills (figure 1). A methodology of alternate dosing for two (2) weeks for a period of six (6) cycles were carried out. Process performances were evaluated based on Delta OER as described in equation (2);

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\text{Delta OER (\%)} = \text{OER Enzyme} - \text{OER Control}
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In milling process, sterilized FFB are transferred to rotating threshing drums to remove the fruitlets from the bunches. These loose fruitlets are then passed to a digester and screw press for oil extraction, which is known as mass passing to digester (MPD). The first strategy was dosing of enzymes to sterilized palm loose fruitlets, a patented process developed by Sime Darby Plantation Research [22]. The enzyme was dosed into the MPD from closed enzyme spraying system to ensure longer contact time between enzymes and the fruitlets and a more homogenous mixing to enable penetration of enzymes into the mesocarp. Thereafter, the mixture of MPD and enzymes were fed into a Pre-Digester vessel to obtain an additional retention time of 30 minutes and followed by conventional maceration in digester. Due to mixing limitation, second strategy of dosing of enzymes to diluted crude oil (DCO) was evaluated. DCO is liquid stream post digestion and press, comprising of oil, water, non-oily solid. The enzyme was dosed into the DCO pipeline, a patented process developed by Sime Darby Plantation Research [23]. A much-simplified dosing system at lower capital cost was installed for this method.

![Diagram](image.png)

**Figure 1.** Plant scale trial enzyme dosing strategy.

### 2.7. Reducing sugar content

Reducing sugars (monosaccharide) in the clarifier were analysed using HPLC (Agilent Technologies, USA) equipped with refractive index (RI) detector and Rezex column (ROA Organic acids H+ (8 %) 4E, 7.8 mm × 300 mm) (Phenomenex, USA). The HPLC was run at 60 ºC using a mobile phase of 0.005 N sulphuric acid with 0.6 mL/minutes flowrate [24].

### 2.8. Confocal Microscopic Imaging

Control and enzyme treated DCO were skimmed of oil and incubated at 90 ºC for 1 hr. Oil and cells were subsequently labelled by the addition of an equal volume of 3.2 µM Calcofluor white (Sigma Aldrich) and 20 mM BODIPY493/503 (Invitrogen), incubated at room temperature for 10 minutes prior to imaging in 96 well imaging plates (Corning). Images were captured on an Olympus FV3000 confocal laser scanning microscope (CLSM) (Olympus, model FV3000 Japan) with a 20x objective. Image processing and visualization was performed with Imaris 9.6.
2.9. Statistical Analysis
The lab experiments were repeated at least three times. The data was statistically analysed by computing mean and standard deviation values for various parameters. The mean data was further analysed using one-way ANOVA at a 5 % significance level to see if there were any significant differences between extraction strategies.

3. Results and Discussion

3.1. Potential oil in Palm pressed fibre and palm mesocarp

In traditional palm oil milling, an average of 20 % of the oil is obtained from fresh fruit bunches (OER equivalent) while also producing 23 % empty fruit bunch (EFB), 15 % fibre, and 12 % nut. Oil is lost in a variety of by-products, including the fibre that remains after a screw press extracts the mesocarp oil. Oil content of pressed fibre ranges from 5 to 11 % w/w oil, however the fibre is usually burned as fuel to power the mill. Several studies were conducted using different methods such as prolonged soxhlet extraction, ultrasonic treatment, and supercritical fluid extraction to determine the potential oil left in pressed fibre as shown in figure 2. Results represent the mean values and error bars indicate the standard deviation of the mean (n = 3). The baseline for control comparison is 5.44 % w/w oil content by wet basis in pressed fibre sample. Extending the duration of soxhlet extraction, about 27 % of increment of oil content is obtained as compared to the usual 4 hours. Generally, increase of extraction duration and temperature is proportionate to the increase of oil yield. Solvent extraction using non-polar solvent, namely hexane has been widely utilized in various vegetable oil extractions such as rapeseed and soybean. In palm oil milling sector, soxhlet extraction with hexane is used as oil content analysis for product (mesocarp) and by-product (fibre, EFB, raw effluent) as process monitoring.

Ultrasound-assisted extraction is a new simple technique for the recovery of oil and bioactive compounds from different sources [25, 26]. Ultrasound intensity presented a positive effect on the extraction, indicating that its exposure overtime can lead to higher yields. The highest oil content, 8.3 % w/w was obtained at the prolonged ultrasound treatment of 4 hours, an increment potential of 27 %. Similar extraction trend was observed for supercritical fluid extraction. Supercritical fluid technology using carbon dioxide (CO2) has been applied in extraction, purification and fractionation of crude palm oil. An increment of 50 % higher and proves the existence of more oil than solvent extracted method (figure 2c). By comparing to the control sample, SFE treatment of 5 hours yields additional oil content of 2.77 % w/w. All our findings regarding potential palm oil extraction with emerging green technology are in similar accordance to review by Chew et al. (2021b).

Extended treatment with higher diffusivity and mass transfer of oil from complex plant matrices shows that additional oil as high as 3 % w/w are extractable, however left unextracted and contained as intact oil bodies due to the limitation of traditional thermo-mechanical extraction process. These oil bodies are either trapped in an insoluble extracellular denatured protein matrix or coalesced oil droplets that are too big to pass through the partially disrupted cellular matrix [27]. Alternatively, this additional potential oil can be extracted by deploying feasible biotechnology means, i.e., enzymatic technology due to enzymes' ability to successfully break down plant cell walls. As a result, enzymes could be the palm oil milling industry's next great operational breakthrough.

According to Silvamany et al. (2015), cellulose and hemicellulose make up the majority of the polysaccharides constituents in the palm mesocarp cell wall, followed by soluble lignin. The lignin layer on the mesocarp fibre functions as a physical barrier, preventing cellulose and hemicellulose from being accessed.
Figure 2. Potential oil left in pressed fibre by treatments of (a) extended soxhlet extraction, (b) ultrasonication and (c) supercritical fluid extraction.

Hence, to enable the release of oil from vacuoles and cytoplasmic membranes, the cellular wall of the fruit mesocarp must be degraded and ruptured. Palm mesocarp samples were treated with enzymes and subjected to extended soxhlet extraction alongside control samples as shown in figure 3. Results represent the mean values and error bars indicates the standard deviation of the mean (n = 3). Enzymatic treatment followed by the standard 4 hours soxhlet extraction showed only extra 1 % w/w oil content. Nevertheless, extending the solvent extraction of treated palm mesocarp to 16 hours yielded an additional 10.7 % w/w oil content, while omitting the solvent extraction contribution, enzyme alone could give 6 % w/w additional oil (1.2 % OER equivalent).

The findings are supported by the basics where enzyme acts as a pre-treatment step before the extraction procedure. During soxhlet treatment, the addition of the enzyme clearly breaks the cells and enables the escape of intact oil. Perez et al. 2013 reported a substantial oil recovery of 110.85 % from sunflower seeds using pectinase assisted solvent extraction in comparison to solvent extraction alone.
Similarly, enzyme cocktail of cellulase, xylanase, protease, and pectinase were used as a pre-treatment before solvent extraction of grape seed oil. Solvent extraction for 24 hours and 120 hours showed oil yield recovery of 106 % and 163 %, respectively. The increased oil extraction efficiency was attributable to the additional breaking of the cotyledon cells, which released the oils bodies and made the cell structure more porous, allowing for more efficient hexane percolation and hence improved oil liberation [13].

![Enzymatic treatment of palm mesocarp](image1)

**Figure 3.** Enzymatic treatment of palm mesocarp followed by extended soxhlet extraction.

3.2. Plant scale trials

Commercial trial of 6-months period with alternating sequences of enzyme cycles and control cycles conducted in Sime Darby palm oil mill achieved an average increment OER of 1.15 % (figure 4). The conclusion is based on mean OER on enzyme vs. control runs, with a rigorous statistical analysis to confirm 95 % statistical degree of confidence. The nature of enzymatic hydrolysis requires incubation time of at least 30 minutes to enable effective reaction, thus, a pre-digester was installed to hold the MPD before digester and screw pressing. It was observed that additional maceration time of MPD with predigester for 40 minutes yielded delta OER of 0.32 %, while addition of enzyme effect to the processing resulted in 0.83 % delta OER. The synergetic hydrolysis of complex constituents of cellulose, hemicellulose, lignin and pectin, together with the digestion and screw pressing steps has resulted in more oil being liberated when dosing the enzyme into the MPD. It is evident that enzyme integration into mechanical processing effectively breaks down plant cell walls to facilitate release of trapped oils.

![Enzyme treatment on MPD](image2)

**Figure 4.** Oil extraction rate increment by enzyme treatment on MPD in plant.
To further minimize mechanical limitation of enzyme technology in palm oil milling, another method of dosing the enzyme mixture into DCO was explored. Enzyme dosing to DCO is more feasible as it requires a very simplified dosing system of enzyme into liquid stream while eliminating limitation in effective mixing and installation of pre-digester. Some unbroken cells could be still present in DCO, thus addition of enzyme will rupture these cell walls enabling more oil to be released and recovered. The moisture level in DCO assists the hydrolytic reaction by increasing diffusivity, mobility of the enzymes and products, thus shortening the reaction time required to obtain higher oil yields [28].

Two methodologies of single line (mill 1 & 2) and dual line (mill 3 and 4) system monitoring were used for evaluation. Single line approach involves the dosing of enzymes alternately two weeks and control two weeks (1 cycle), with a total of 6 cycles based on normal harvesting interval to ensure both periods will get crops from the similar area. Monitoring was based on OER declared by mill on daily basis. On the other hand, dual line involves set up of two lines with identical equipment to ensure similar efficiency and direct comparison of dosing line to control at minimal variation. Evaluation was based on the accumulated OER which is equivalent to pure oil (PO) produced over DCO generated on daily basis. OER comparison for dual line mill was much simpler as the input materials are the same for both enzyme and control line. Summary of delta OER obtained for 4 mills is shown in figure 5. Results represent the mean values and error bars indicate the standard deviation of the mean (n=40). Delta OER ranged between 0.71 to 1.19 %, with an average of 0.91 %.

![Figure 5. Oil extraction rate increment by enzyme treatment on DCO in plant.](image)

Enzymatic treatment could potentially facilitate coalescence of small oil droplets to bigger oil drops that float up and improve clarification process by reducing viscosity and breaking emulsion [29, 30]. Therefore, an improved and shorter clarification time will be more favourable, given the importance of energy expenditure and time in industrial extraction operations. Reduction in consumption of process water, on the other hand, reduces overall processing costs, eliminates larger reaction and storage systems, and creates a significant waste management challenge [31, 32].

### 3.3. Reducing sugar content

Reducing sugar analysis is an indication to evaluate the degree of enzymatic hydrolysis. Figure 6 shows the reducing sugar content profile in clarifier underflow. Reducing sugar yield indicates the breakdown of carbohydrate polymer (xylan and glucan). Reducing sugar comprise of monomers and dimers mainly glucose, xylose, fructose, sucrose, mannose and arabinose. Total reducing sugar content in biocatalyst and control samples were 12.29 g/L and 10.13 g/L, respectively. With the presence of enzyme, the difference in reducing sugar is 2 g/L. The increase in reducing sugar is close to 21 %. This increment is considered high considering the available substrate.
DCO composition during the sampling were in the range of; moisture content: 52.47 %, oil content: 40.8 %; NOS (substrate): 6.76 % and structural carbohydrate of 13.8 % [10]. For 1 L of DCO, the substrate available is about 67.6 g of NOS and of that, cellulose and hemicellulose polymer make up about 10 g. Theoretically 1 g of xylan hydrolysed will yield 1.1 g of xylose. Back calculating, for release of 12 g/l monomers, substrate available should be 10.9 g, tallying the findings. Based on this, we can prove the hydrolytic enzymes acted on unbroken cell walls, causing an increase in the release of total reducing sugar content in the reaction media, indicating that the hydrolysis process was efficient during clarification process [33]. However, the value doesn’t really represent the complete reaction as oligomers such as cellobiose was not quantified and further testing is required.

3.4. Confocal Microscopy Imaging

Most of the oil bodies are found in the vacuoles of plant cells as free oil, as well as dispersed in the cytoplasm [34]. Confocal microscopy on fluorescently labelled DCO reveals that enzyme treatment results in larger oil bodies free of cells and that remaining cells display weaker Calcofluor fluorescence, suggesting degradation of the cell wall (Fig 7 a, b). This degradation is clearly visible when the surface of the cells are 3D rendered with Imaris (Fig 7 c-f), with predominantly intact cells visible in control samples while the majority of cells in the enzyme treated sample have large areas devoid of any cell wall staining. It can be observed that the cellular architecture of cell wall is disrupted with absolute release of oil globules while leaving only some traces of cell wall. The structural alteration in the cell wall of the palm tissue demonstrated the action of enzymes [11].
Figure 7. Visualization of oil-bearing cells. (a,b) CLSM fluorescent imaging of DCO stained with 1.6 μM Calcofluor white (Cyan) and 10 mM BODIPY 493/503 (Green) in the absence (a) or presence (b) of enzyme. (c,d) 3D rendering of stained surfaces. (e,f) Zoomed view of indicated area.
4. Conclusion

Deforestation, biodiversity loss, and greenhouse gas (GHG) emissions due to agriculture expansion are all ongoing environmental and sustainability challenges that require urgent attention and solutions. New breeding technologies such as genomics and gene editing, promise to increase yielding cultivars, oil profiles, disease resistance, and crop architecture, but they are time consuming. In terms of yield and delivery of a variety of specialised oils for human use, there are no viable alternatives to palm oil. Rather than demanding bans or boycotts on oil palm goods, it is critical to use appropriate biological extraction methods to boost palm oil processing yield and productivity. A green and novel process is developed to decipher the OER stagnation issue of the palm industry. Dosing of enzyme into MPD yielded in OER increment of 1.15%. Meanwhile, dosing of enzyme into DCO with a simple system yielded in OER increment of 0.71 to 1.19%. Cell wall degrading enzymes are used to hydrolyse complex structures of the oil-bearing cells to liberate more oil, reducing losses, and improving downstream processing. A 1.0% OER increase can potentially generate additional revenue of RM2 billion/year for the palm oil industry in Malaysia.

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