Aqueous enzymatic extraction of palm kernel oil

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Abstract

Inexpensive enzyme with high hydrolitic activity is required to bring the aqueous enzymatic extraction (AEE) of seed / biomass oil into commercialization. In this study a commercial digestive drug which is mainly composed of amylase and protease enzymes has been applied to extract the palm kernel oil. A single factor experiment evaluated the effect of the ratio palm kernel to water, concentration of digestive drug used, pH, incubation time and temperature on extraction efficiency, free fatty acid (FFA) content and lipid profile in form of monoglyceride, diglyceride and triglyceride. A significant effect was observed for all the parameters and the highest extraction efficiency of 96% with FFA content less than 0.68% was observed in the incubation condition of 1:5 ratio palm kernel to water, 9% w/w of digestive drug, pH of 9, 90 min of incubation time at 45°C. Green metrics assesment confirmed that the AEE process is more green process than the soxhlet method. In comparison with other existing extraction methods, the AEE showed better extraction efficiency against the screw press and supercritical carbon dioxide methods.

Keywords: digestive drug, palm kernel, lipid profile, green metric.

1. Introduction

The world vegetable oil consumption has increased 2.3% per annum during the past two decades of 21st century, with China and Brazil as the highest consumers at 30 and 24 kg/capita. Consumption is predicted to continue to rise by 0.9% in the coming years (OECD et al. 2020). Currently mechanical pressing and solvent based extraction methods were used in the oil industry (Cheng and Rosentrater 2017). Hexane is the most popular extraction solvent due to its high extraction yield and low cost (Cheng and Rosentrater 2017). However, hexane is toxic, flammable and classified as a pollutant (Potrich et al. 2020; Toda et al. 2016). Therefore, greener extraction processes have been developed recently using green solvents such as carbon dioxide, ethanol, ethyl acetate, etc (He et al. 2020; Dal Prá et al. 2018; Castejón et al. 2018; Castro-Puyana et al. 2017; Toda et al. 2016). The carbon dioxide extraction process is usually conducted in a supercritical condition to produce a good yield and pure oil (Costa et al. 2019; Mouahid et al. 2018). Even though the polar solvents could not dissolve oil, some researchers have confirmed the oil could be extracted with a comparable yield using a polar solvent (de Oliveira et al. 2013; de Jesus et al. 2019; Kumar et al. 2017). For example, Castejòn *et al* found that ethanol could extract more oil from *Echium plantagineum* L seeds than hexane assisted by ultrasound particularly at 55°C (Castejón et al. 2018). However, those green processes required a high temperature and pressure in a prolonged reaction time and consume a lot of solvent (de Jesus et al. 2019; Sitepu et al. 2020).

Another green extraction process was using enzyme in aqueous environment. The extraction efficiency of AEE on some oilseeds materials such as soybean, peanut, sesame, and *Moringa oleifera*, as well as shrimp by-product has been reported as higher (Liu et al. 2020; Wenwei et al. 2019; Mat Yusoff et al. 2016; Mat Yusoff et al. 2015; Latif and Anwar 2011). Enzymes could hydrolize the cell wall of oil bearing materials releasing oil to the system (Liu et al. 2020; Mat Yusoff et al. 2016; Mat Yusoff et al. 2015; Li et al. 2011; Latif and Anwar 2011). The use of enzyme in an AEE process has some advantages including being environmentally friendly, inexpensive because of the use of water as solvent, easy to separate, and finally, having better appearance and taste of the oil product (Mat Yusoff et al. 2015). Moreover, valuable materials contained in the residue such as protein and carbohydrate could be used for other food purposes (Wenwei et al. 2019). Latif and Anwar concluded that the protein which is also extracted in the AEE process has better quality and could be used for human consumption (Latif and Anwar 2011). In addition the degumming oil process which is energy intensive (Yao et al. 2020) has been simultaneously processed in AEE as water dissolves the phospholipid (Zhao et al. 2020; Teixeira et al. 2013).

The compounds composed in the cell walls affect the choice of enzyme type and or its combination (Mat Yusoff et al. 2015). The extracted soybean oil was observed to increase when using Alcalase, endo-protease enzyme, while the extracted oil was decreased when using cellulase enzyme (Jung and Mahfuz 2009; Lamsal et al. 2006). Indeed the rapeseed cell walls were easier to break by pectinase than other types of enzymes, which proved that the main component of their cell walls is pectin (Zhang et al. 2007). Some oilseed cell walls required a mixture of enzyme to disrupt. For example sunflower oil increased significantly when using Viscozyme to rupture the cell walls (Latif and Anwar 2009) and a similar effect was also observed on the AEE of bush mango kernel flour (Womeni et al. 2008). Long *et al* obtained a high oil yield of flaxseed when using a combination of cellulase, pectinase and hemicellulase in same ratio as the enzyme itself (Long et al. 2011). However, enzyme is expensive and requires a significant amount to hydrolyze the cell walls for oil extraction. Yusoff *et al* reported that more than 1% of enzyme based on the weight of oil extracted, was needed to proceed the hydrolysis reaction, which therefore raising the production cost (Mat Yusoff et al. 2015).

An immobilization enzyme for the AEE process has been developed to overcome the cost issue. The main advantage of using an immobilized enzyme is the possibility of using it several times without losing its activity (Wan et al. 2008; Santos et al. 2020). Long *et al* have successfully immobilized a mixture of cellulase, pectinase and hemicellulase onto alginate-glutaraldehyde matrix to hydrolyze cell walls of flaxseed. Results showed that the enzyme could be reused and lowering the production cost (Long et al. 2011). However, the result of enzyme reusability has not been cleared yet. Other researchers observed that papain enzyme could be used several times in the AEE of Sacha inchi seed oil with only a slight decrease in the oil yield. It was suggested to add more enzymes to hold the hydrolytic activity same as the first stage (Nguyen et al. 2020). Therefore, finding a low cost enzyme is necessary to enable implementation of the AEE process on an industrial scale.

The pancreatin drug which contained a mixture of enzyme lipase, amylase and protease has been used worldwide to repair human digestion (Lebenthal et al. 1994). Many manufacturers have produced the pancreatin drug with different brand names and doses of enzymes (Drugbank 2005). This drug opens the possibility of use in the AEE process. To the best of our knowledge, no literatures have been reported the utilization of a digestive drug as an enzyme source for the AEE process. Therefore, this present study aimed to extract palm kernel oil using a mixture of the enzymes contained in pancreatin drug in an aqueous system. The single effect of the palm kernel : water ratio, pH, temperature, incubation time, and the concentration of digestive drug used was studied in order to achieve the optimized condition. The yields of extracted oil, FFA content, and lipid composition (monoglyceride (MG), diglyceride (DG) and triglyceride (TG)) were determined. Also, the reusability of the enzymes was measured. Finally the green chemistry metrics were calculated to determine how green the AEE process is.

2. Materials and methods

2.1. Materials

The palm kernel was collected from a local palm oil processing plant in Medan, Sumatera Utara – Indonesia. All chemicals were purchased from a local chemicals distributor and were used without pre-treatment. The digestive drug (brand name Vitazym) used in this study contains Amylase 10,000 IU, protease 9,000 IU, lipase 240 IU, DHA 30 mg, simeticone 25 mg, vit B_1 10 mg, vit B_2 5 mg, vit B_6 5 mg, vit B_{12} 5 mcg, niacinamide 10 mg, Ca pantothenate 5 mg (MIMS 2020) and was bought from local pharmacies.

2.2. Oil extraction using soxhlet method

The traditional extraction soxhlet method was used to determine the quantity of palm kernel oil and the result was compared with the AEE. The procedure we followed for the soxhlet extraction has been reported elsewhere (Al-Hamamre et al. 2012; Tarigan et al. 2019). About 20 g of grounded palm kernel was extracted in the soxhlet apparatus using hexane as an extracting agent under reflux condition for 30 minutes. The oil was collected and weighed after solvent evaporation and stored in a desiccator for gas chromatography (GC) analysis.

2.3. Aqueous enzyme extraction of palm kernel

The ground palm kernel seeds were mixed with water at investigated ratio ranging from 1:3 – 1:7 (w/v) and the digestive drugs was added according to explored concentration (%, w/w). Next, the pH was adjusted with NaOH or HCl following investigated value and incubated in the shaking incubator (Vision model VS8480SN) at an appropriate temperature and time. The oils were separated from water and residue using centrifuge running at 8000 rpm for 5 minutes and was stored in a desiccator for analysis. The acid value was determined by the standard titration method following ASTM D664. The AEE oil yield was calculated following equation 1. The extraction efficiency was measured based on the comparison between AEE and soxhlet oil yield.

$$Yield_{AEE} = \frac{W_{AEE}}{W_{palm\ kernel}} \quad (1)$$

$$Extraction\ Efficiency\ (\%) = \frac{Yield_{AEE}}{Yield_{Soxhlet}} x\ 100\% \quad (2)$$

where W_{AEE} is the weight of AEE oil extracted and $W_{palm\ kernel}$ is the weight of palm kernel used.

2.4. Lipid content and fatty acid profile analysis

The lipid profile i.e. monoglyceride, diglyceride and triglyceride containing in each palm kernel oil extracted was determined using gas chromatography. A 1 μL of sample was injected to GC Shimadzu type 2010 equipped with a capillary column (length 15 m ID 0.25 mm) and a flame ionization detector with temperature set to 370°C. The carrier gas was helium with a constant delivered flow of 30 mL/min and both the injection port and detector temperature was set to 370 °C.

2.5. Green metrics calculation

In order to determine how green the AEE process is, the green metrics equations were adopted from previous published report (Sheldon 2018) and used to calculate the environment impact of this study.

$$E - factor = \frac{Total\ mass\ of\ waste}{Mass\ of\ oil\ extracted} \tag{3}$$

$$Process\ mass\ intensification = \frac{Total\ mass\ in\ process}{Mass\ of\ oil\ extracted} \tag{4}$$

$$Solvent\ Intensity = \frac{Mass\ of\ solvents}{Mass\ of\ oil\ extracted} \tag{5}$$

$$Waste\ water\ intensity = \frac{Mass\ of\ process\ water}{Mass\ of\ oil\ extracted} \tag{6}$$

$$Effective\ mass\ yield = \frac{Mass\ of\ oil\ extracted}{Mass\ of\ hazardous\ reactants} \tag{7}$$

2.5. Statistical analysis

The significant effect of the ratio of palm kernel to water, pH, temperature, incubation time, and concentration of digestive drug used to the independent parameters such as extraction efficiency, free fatty acid content and lipid composition as MG, DG and TG were statistically analysed using Statistica v13.3 software at significance level set to $\alpha = 0.05$. The results are expressed as mean ± SD. One way analysis of variance (ANOVA) was used to compare the means.

3. Results and discussion

The traditional soxhlet method was used to determine the quantity of oil contained in palm kernel. The oil extracted was 39.8 ± 3.04 % which is in the range of reported oil content in palm kernel (Costa et al. 2019; Tarigan et al. 2017) and the acid value was 1.01 ± 0.22 mg/g KOH. The oil dominated by saturated fatty acid was 82.52% while monounsaturated and polyunsaturated fatty acids were 15.34% and 2.14%, respectively. Table 1 presents the fatty acid profile of palm kernel oil with lauric acid as the main component dominating more than half.

Table 1. Fatty acids profile of palm kernel oil

Fatty Acid		Concentration (g/100 g)	Percentage
$C_{6:0}$	Caproic	0.05	0.12
$C_{8:0}$	Caprylic	0.94	2.36
$C_{10:0}$	Capric	1.10	2.77
$C_{12:0}$	Lauric	20.32	51.07
$C_{14:0}$	Myristic	6.54	16.43
$C_{16:0}$	Palmitic	3.15	7.92
$C_{18:0}$	Stearic	0.71	1.78
$C_{20:0}$	Arachidic	0.03	0.08
$C_{18:1}$	Oleic	6.11	15.34
$C_{18:2}$	Linoleic	0.82	2.05
$C_{20:1}$	Gondoic	0.03	0.07
Total fatty acids		**39.8**	
Saturated		32.84	82.52
Monounsaturated		6.11	15.34
Polyunsaturated		0.85	2.14

3.1. Effect of ratio palm kernel : water

The amount of solvent use to extract oil is important as it affects the oil extracted quantity and the following procedure for oil-solvent separation (Halim et al. 2012). Zakaria and Harvey explained that, based on Fick's law of diffusion, increasing the volume of solvent will increase the quantity of oil extracted (Zakaria and Harvey 2012). Therefore, in this study the effect of the ratio of palm kernel to water on the AEE intensified extraction efficiency. The FFA content and lipid profile were investigated at ratio of 1:3 to 1:7 (w/v) with pH of 9, concentration of digestive drug of 7% w/w, incubation time of 90 min and temperature of 45°C. A ratio below than 1:3 is inadequate to immerse the palm kernel seeds. Even though water cannot dissolve lipids, as they have different polarity, the seed to water ratio had a significant effect on all the measured variables. The extraction efficiency ranging from 57.91 ± 1.60% to 82.04 ± 1.60% with ratio of 1:5 was the highest, as shown in Fig. 1A. This result supports the previously published results which concluded that insufficient water volume prevents the enzyme from penetrating the cell wall while an excess of water volume decreases the contact of the enzyme and substrate causing low yield (Zhang et al. 2007; Mat Yusoff et al. 2015). The FFA content of the palm oil extracted ranged from 0.39 ± 0.03% to 0.66 ± 0.03%.

Water had a significant effect on MG, DG and TG content with the yield ranging from 0.35 ± 0.29% to 0.60 ± 0.62%, 1.37 ± 0.02 % to 2.19 ± 0.13% and 19.91 ± 0.37 to 26.16 ± 0.70%, respectively. As shown in Fig. 2 the highest yield of MG was obtained using ratio of 1:6, while for DG occurred in a ratio of 1:5, and TG had maximum yield when the ratio of 1:4 was applied. The average yield of MG, DG, and TG in this study was 0.47, 1.17, and 22.94%, respectively.

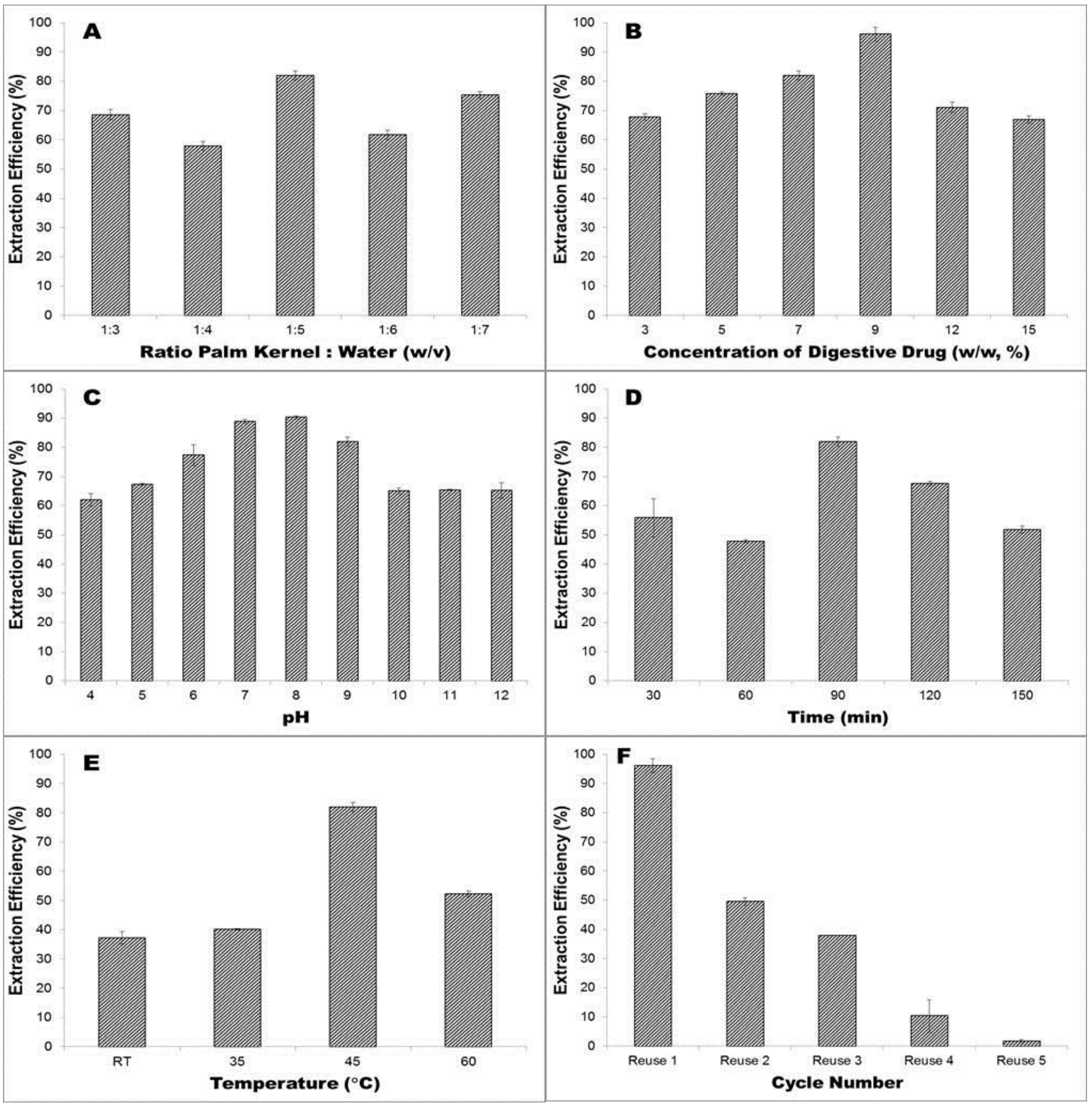

Fig. 1 The effect of (A) ratio palm kernel to water; (B) concentration of digestive drug; (C) pH; (D) incubation time; (E) temperature and (F) reusability of digestive drug on extraction efficiency of AEE intensified.

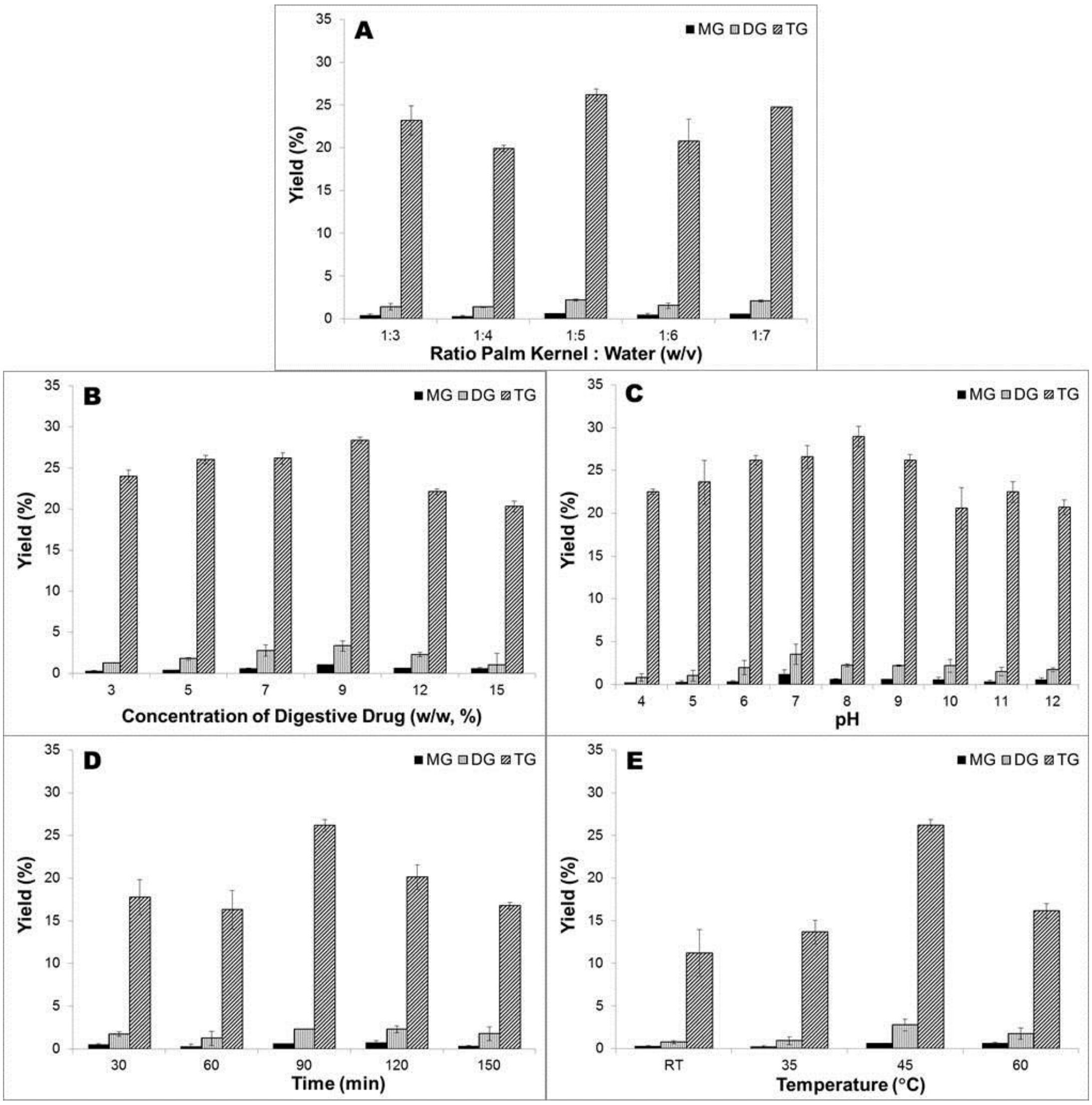

Fig. 2 The effect of (A) ratio palm kernel to water; (B) weight of digestive drug; (C) pH; (D) incubation time and (E) temperature on MG, DG and TG yield of AEE intensified.

3.2. Effect of concentration of digestive drug

Finding a significant concentration of enzyme in AEE is critical as it affects the hydrolysis of cell walls releasing oil to the environment. Hence the effect of the concentration of the digestive drug used in this study was investigated at six different concentration (3, 5, 7, 9, 12 and 15% w/w) in reaction condition: ratio of palm kernel : water of 1:5, pH of 9, temperature of 45°C and at 90 min incubation time. As observed, the average extraction efficiency of 76.63% occurred for all the weights of the digestive drug used. The ANOVA test revealed that the concentration of digestive enzyme had significant effect on the extraction efficiency and free fatty acid content. The highest extraction efficiency occurring at the weight of 9% w/w was 96.11 ± 2.31%. Figure 1B shows that the pattern of this parameter is in agreement with some published results, which supports the conclusion that the extraction efficiency increased to reach the maximum levels and decreased when increasing the concentration of enzyme used (Zúñiga et al. 2003; Jiang et al. 2010; Mat Yusoff et al. 2015; Mat Yusoff et al. 2016). This was presumably due to the increasing saturation of substrate rendering less contact of enzyme to cell walls (Jiang et al. 2010).

The same trend has obtained for the effect of the weight of digestive drug to MG, DG or TG yield (Fig. 2B). The yield of each lipid profile climbed to reach 1.05 ± 0.04%, 3.34 ± 0.61%, and 28.37 ± 0.41%, respectively, and then dropped to 0.30 ± 0.07%, 1.01 ± 1.42% and 20.29 ± 0.67%, respectively. Therefore, the univariate test identified a significant effect of the concentration of drug used to lipid profile.

3.3. Effect of pH

The effect of pH on extraction efficiency, FFA content and lipid profile were determined based on different pH values from 4 to 12 with an increment of 1 value. There were significant effects for all independent parameters. Furthermore, the Post-hoc Tukey test analysis determined that the significance occurring in extraction efficiency was driven by high efficiency of >70% at pH of 6 to 9, achieving extraction efficiencies of 77.39 ± 3.55%, 88.94 ± 0.71%, 90.23 ± 0.53%, and 82.04 ± 1.60%, respectively. In contrast, the significance of FFA content was forced by pH of 6, 10, and 12 with percentage of 0.76 ± 0.02%, 0.62 ± 0.07% and 0.57 ± 0.01%, respectively.

The average extraction efficiency for this parameter was 73.76% with the highest occurring at pH 8 (Fig. 1C). The FFA content ranged from 0.26 ± 0.01% to 0.76 ± 0.02% which is lower than the minimum standard allowed for biodiesel production (Britton and Raston 2015). The highest yield of MG was extracted using pH 7 with 1.19 ± 0.51%, while DG yielded of 3.53 ± 1.17% at the same pH. The maximum yield of TG was obtained using pH 8 with valued at 28.96 ± 1.16%.

3.4 Effect of incubation time

Another important variable that could affect the extracted oil yield is incubation time. Hence five different incubation times were studied starting from 30 min to 150 min using the ratio of palm kernel to water of 1:5, 7% w/w of weight of the digestive drug, a temperature of 45°C and a pH of 9. As shown in Figure 1D, incubation time had significant effect against extraction efficiencies and FFA content. The average extraction efficiencies are 61.06%, ranging from 47.86 ± 0.53% to 82.04 ± 1.60%. Prolonged incubation time observed did not increase the yield. This becomes constant indicating that the solubility of oils is saturated or the limitation of active enzymes. Contrary to expectations, this result does not support the previous research. Some studies showed that addition incubation time could increase the oil yield (Jiang et al. 2010; Rui et al. 2009). However, the rate of increase was not economic and the quality of oil also decreased. Furthermore, increasing the incubation time increased the difficulty in oil purification (Liu et al. 2016). A main significant effect was detected for an incubation time of 90 min that yielded the highest extraction efficiency. In contrast, the significant effect for FFA content was driven by an incubation time of 150 min that produced the highest percentage (0.97 ± 0.01%).

Incubation time had a significant effect on the MG, DG and TG (Fig. 2D). Extraction of MG, DG, and TG was significantly lower at the incubation time of 60 min achieving yields of 0.28 ± 0.31%, 1.24 ± 0.82 and 16.31 ± 2.26%, respectively. In contrast, highest extractions of MG and DG were obtained at the incubation time of 120 min giving yields of 0.72 ± 0.26% and 2.29 ± 0.40%, while the maximum yield for TG of 26.16 ± 0.70% occurred at incubation time of 90 min.

3.5. Effect of temperature

Recent evidence suggested that the high hydrolytic activity of enzymes on AEE intensifies the process at a temperature range of 40°C - 55°C (Mat Yusoff et al. 2015; Rui et al. 2009). However, considering production cost, low incubation temperature is preferable, as maintaining thermal conditions in prolonged reaction time is cost intensive. Therefore, the effect of temperature in this study was investigated ranging from room temperature to 60°C.

A significant effect was observed in this parameter for extraction efficiency and FFA content. This was mainly driven by high extraction efficiency at a temperature of 45°C, while FFA content was significantly influenced by high concentration of 0.73 ± 0.08% at a temperature of 60°C. The average extraction efficiency was 51.93% ranging from 37.19 ± 2.13% to 82.04 ± 1.60% (Fig. 1E). The FFA content was observed to increase in the increasing incubation temperature. This result supports previous research as mentioned above. Average extraction yield of MG, DG and TG for the effect of temperature was 0.44%, 1.55% and 16.52%, respectively. The highest MG concentration of 0.65 ± 0.09% was observed at a temperature of 60°C, while DG and TG had highest yield at 45°C of 2.78 ± 0.70% and 26.17 ± 0.70%, respectively.

3.6. Reusability of the digestive drug

One factor for reducing production cost in enzymatic method is reusing the enzyme (Santos et al. 2020). To assess the reusability of the enzymes contained in a digestive drug, the incubation was set based on the condition giving the highest oil yield which is ratio palm kernel to water of 1:5, weight of digestive drug of 9% w/w, pH of 9, incubation time of 90 min at 45°C. Figure 1F illustrates the extraction efficiency decrease in the second cycle and almost nothing after the fifth cycle. It seems the enzymes lost contact with the cell walls, as the residue from first cycle remains in the environment. Even though the result of this study is similar to previous published results (Nguyen et al. 2020) regarding the decrease of oil yield after a second cycle, the reduced yield in this study after a second cycle is too high, presumably due to the mixture of enzymes used in this study compared to using a single enzyme.

3.7. Green metrics assessment

Environmental concern has driven the demand to determine how green the chemicals process is (Curzons et al. 2001; Sheldon 2018). The term ‘green chemistry’ was used to describe the chemical process which generates less waste in the production process and avoids the use of hazardous chemicals. In this study the green chemistry metrics such as environmental factor, process mass intensification, solvent intensity, wastewater intensity and effective mass yield were calculated following equations 3 – 7 mentioned above. Due to insufficient published data for other palm kernel extraction methods such as screw press and supercritical carbon dioxide (SCO) methods, a comparison was made using data from the extraction of linseed oil (Pradhan et al. 2010). Table 2 shows the raw data for the green metrics calculation in this study and in other researcher published results.

Table 2. The mass reactant used in various oil extraction methods.

Reactants (g)	Soxhlet	AEE	Screw Expeller	Supercritical CO_2
Seed	Palm kernel*		Linseed(Pradhan et al. 2010)	
Seed weight	20	20	20	20
Seed residue	12.04	12.35	14.9	12.94
Hexane	131	0	0	0
Water	0	100	0	0
Oil	7.96	7.65	5.1	7.06

* This study

The e-factor which determines the ratio of waste product per oil yield showed that the AEE method has less 22% of waste product than the soxhlet method. The use of organic solvents in soxhlet extraction is harmful, requires a vast quantity of solvent, and has been identified as energy intensive for evaporation solvents (Halim et al. 2012).

Table 3. The green metric of different oil extraction methods.

Green Metrics	Soxhlet	AEE	Screw Expeller	Supercritical CO_2
E-Factor (g/g)	17.97	14.69	2.92	1.83
Process mass intensification (g/g)	18.97	15.69	3.92	2.83
Solvent intensity (g/g)	16.46	0	0	0
Waste water intensity (g/g)	0	13.07	0	0
Effective mass yield (%)	6.08	0	0	0

It also apparent in Table 3 that the AEE processes performed better in the process of mass intensification, solvent intensity and effective mass yield than the soxhlet method. However due to the use of water as a solvent, the AEE method had a higher waste water intensity than the soxhlet method. The screw expeller and SCO methods which have been categorized as green processes (Herrero and Ibáñez 2015; Sheldon 2018) showed better green metrics in all the parameters calculated. This is because no toxic solvents were used and no waste water was produced. In general, the SCO method has better green metrics indicator than other methods.

However, related to the direct cost of production such as energy consumption and time consumption, the SCO is the disadvantage method (Nguyen et al. 2020). For example, Pradhan *et al.* required 180 min of extraction time to obtain an oil yield of 35.5% at a temperature of 50°C and a pressure of 30 MPa while in only 12 min 25.5% oil yield was produced using the screw press process. However, the extraction efficiency, calculated based on the soxhlet-oil yield, of the AEE method is actually better than the other two green processes. The extraction efficiency of 96% was observed in the AEE process while 65% and 91% occurred from screw press and SCO methods, respectively.

4. Conclusions

The utilization of a commercial digestive drug as enzyme source for the AEE of palm kernel has been developed using various production variables. The extraction efficiency of 96% with FFA content less than the standard required for vegetable oil and biodiesel raw material was achieved in an incubation condition of 1:5 ratio of palm kernel to water, 9 w/w% digestive drug, pH of 9, and 90 min incubation time at 45°C. In contrast, the highest TG yield was observed in a pH of 8 and with 7 w/w% digestive drug. There is a significant effect on all parameter studied. Even though the screw press and SCO methods have better green metrics indicators, the extraction efficiency of the AEE process was 31% and 5%, respectively, better than those processes.

List of abbreviations

AEE Aqueous enzymatic extraction

FFA	Free fatty acid
MG	Monoglyceride
DG	Diglyceride
TG	Triglyceride
GC	Gas chromatography
NaOH	Sodium hydroxide
HCl	Hydrochloride acid
KOH	Potassium hydroxide
ANOVA	Analysis of variance
SCO	Supercritical carbon dioxide

Declarations

- Ethics approval and consent to participate: Not applicable
- Consent for publication: Not applicable
- Availability of data and materials: All data generated or analysed during this study are included in this published article and its supplementary information files.
- Competing interests: The authors declare that they have no competing interests.

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- Authors contributions:
  FS conducting the research work and writing the manuscript assisted by MR, FCU and TAH, M assisted in data analysis, JAK and JBT assisted in results and discussion section, SC supported in data analysis and draft revision, and EKT assisted in finalisation and submission manuscript.

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Figures

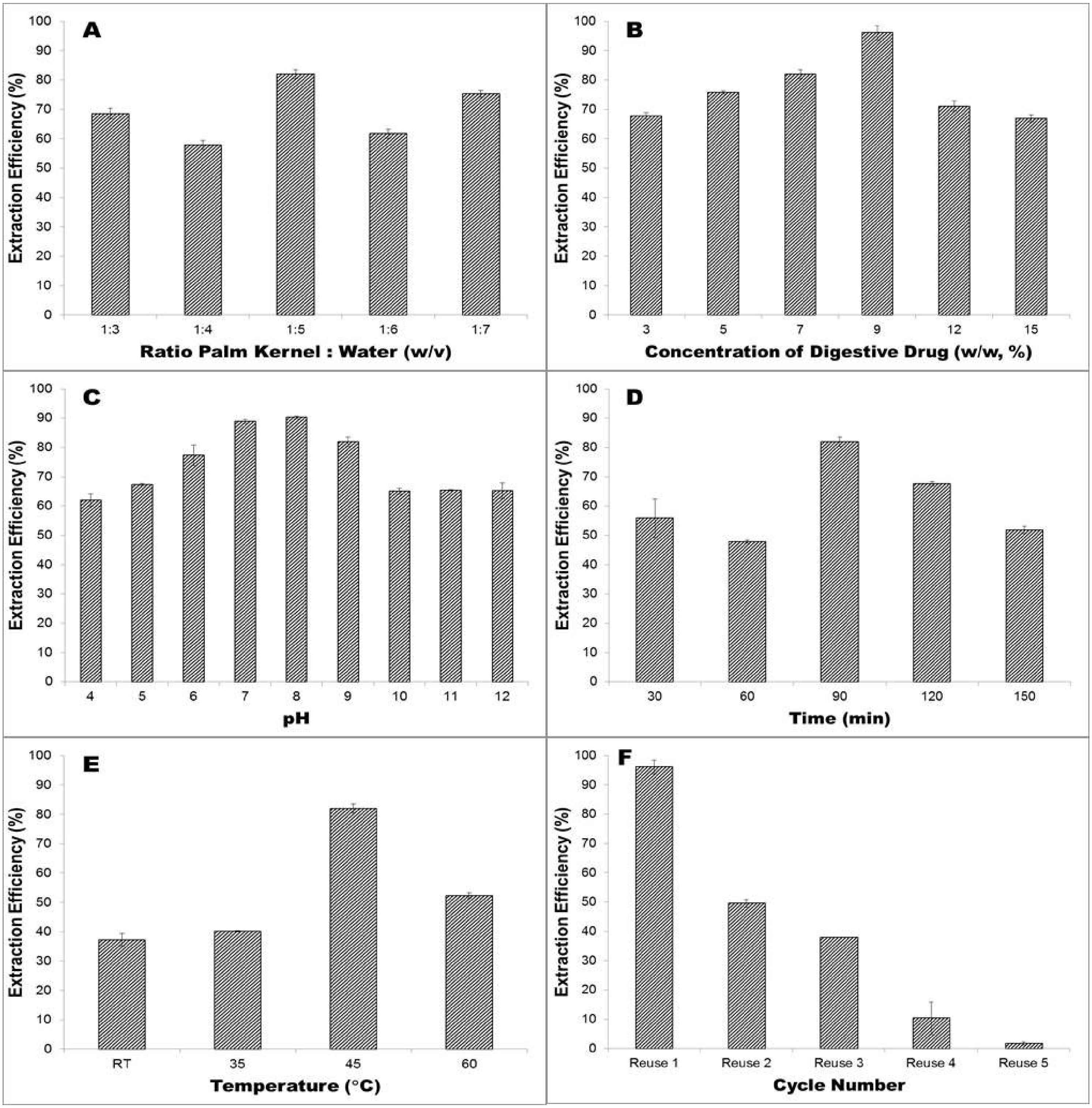

Figure 1

The effect of (A) ratio palm kernel to water; (B) concentration of digestive drug; (C) pH; (D) incubation time; (E) temperature and (F) reusability of digestive drug on extraction efficiency of AEE intensified.

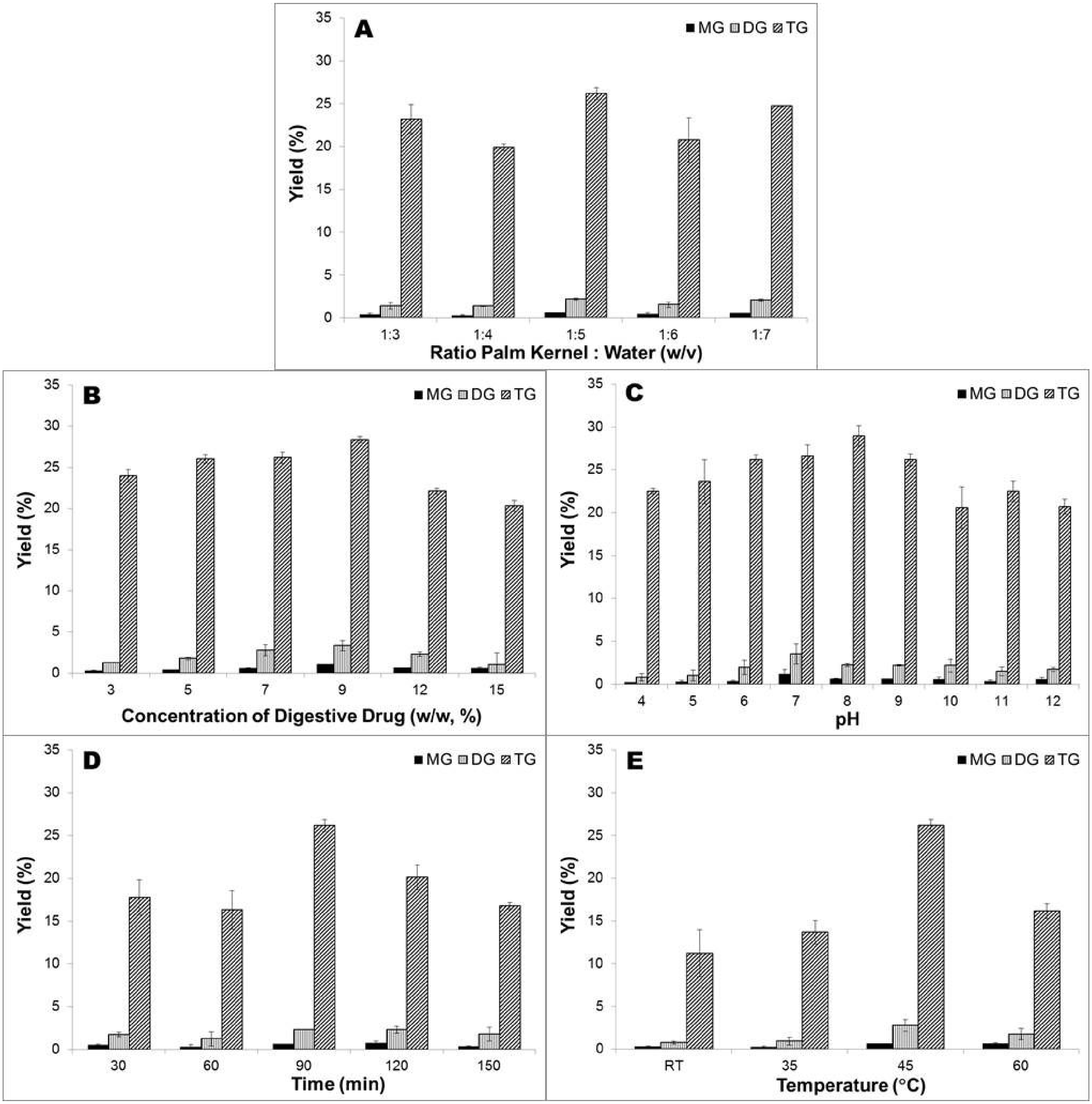

Figure 2

The effect of (A) ratio palm kernel to water; (B) weight of digestive drug; (C) pH; (D) incubation time and (E) temperature on MG, DG and TG yield of AEE intensified.

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