การศึกษาองค์ประกอบทางเคมีของผลปาล์ม *(Elaeis guineensis)* หลังการเก็บเกี่ยว และการประยุกต์ใช้ไลเปสจากปาล์มในการผลิตไบโอดีเซล

Study of Chemical Constituents from Palm Fruit *(Elaeis guineensis)* After Harvesting and the Application of Palm Lipase for Biodiesel Production

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บทคัดย่อ

เมื่อนำผลปาล์มหลังการเก็บเกี่ยวที่ 0 ชั่วโมงมาสกัดด้วยเมทานอลจะให้สารสกัดหยาบสูงสุด คิดเป็นค่าประสิทธิภาพการสกัด 21.64 เปอร์เซ็นต์ ในสารสกัดเมทานอลจากผลปาล์มหลังการเก็บเกี่ยว 0 ชั่วโมงจะพบสารกลุ่มซาโปนิน ฟีนอล และแทนนินในปริมาณสูง เมื่อวิเคราะห์องค์ประกอบทางเคมีหลังการเก็บเกี่ยวของผลปาล์ม 0-240 ชั่วโมง พบว่า ปริมาณสารฟีนอลิก วิตามินอี โปรตีน และแคโรทีน จะลดลงตามระยะเวลาการเก็บเกี่ยวที่เพิ่มขึ้น ในขณะที่กิจกรรมของไลเปสจะค่อย ๆ เพิ่มขึ้น โดยจะมีกิจกรรมของเอนไซม์สูงสุดที่ 120 ชั่วโมง เมื่อนำไลเปสมาแยกและทำบริสุทธิ์บางส่วนพบว่ากิจกรรมของเอนไซม์จะเพิ่มเป็น 4.76 ยูนิต/มิลลิกรัม ไลเปสจากผลปาล์มจะถูกนำมาใช้เป็นตัวเร่งในการผลิตไบโอดีเซลโดยมีน้ำมันใช้แล้วเป็นสารตั้งต้น จากการทดลองพบว่าการใช้เอนไซม์ทำบริสุทธิ์บางส่วนในการผลิตไบโอดีเซล ปฏิกิริยาจะให้ผลผลิตสูงสุดที่ 6 ชั่วโมง ซึ่งใกล้เคียงกับการเร่งปฏิกิริยาด้วยไลเปสทางการค้า ในขณะที่การใช้สารสกัดหยาบของเอนไซม์จะใช้เวลาสูงกว่า (12 ชั่วโมง) ผลผลิตไบโอดีเซลที่ได้มีปริมาณเมทิลเอสเทอร์เท่ากับ 96.5-98 เปอร์เซ็นต์ และคุณสมบัติของไบโอดีเซลจากการทดลองนี้ยังผ่านมาตรฐานกรมธุรกิจพลังงาน และมาตรฐาน American Society for Testing and Materials (ASTM)

คำสำคัญ: ชีวมวลรุ่นที่สอง ทรานส์เอสเทอริฟิเคชัน พลังงานทางเลือก เมทิลเอสเทอร์

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Abstract

Palm fruit after 0 h of harvesting was collected and extracted. Extraction with methanol gave the highest yield with the value of recovery yield at 21.64%. High saponin, phenol and tannin were also detected. The chemical composition of palm fruit after 0-240 h of harvesting was characterized. The results showed that phenolic, vitamin E, protein and carotene decreased as harvesting time increased. In contrast, enzyme activity in lipase increased as harvesting time increased. The highest lipase activity was achieved at 120 h of harvesting and the activity increased to 4.76 U/mg after partial purification. Therefore, palm lipase was used as the catalyst for biodiesel production using waste cooking oil as substrate. Purified enzyme reached the optimal reaction time for biodiesel production after 6 h. A similar result was also obtained from commercial lipase. A longer incubation time (12 h) was observed in crude enzyme. Highest methyl ester content (96.5-98%) was achieved in this study and biodiesel specifications were characterized and passed Thailand's fuel and American Society for Testing and Materials (ASTM) standards.

Keywords: Second Generation Biomass, Transesterification, Alternative Energy, Methyl Ester

Introduction

Currently, the highest yielding edible oil crop in the world is oil palm (*Elaeis guineensis* Jacq.). It is cultivated in 42 countries, consuming approximately 11 million ha worldwide [1]. Palm oil contains both saturated and unsaturated fatty acids [2] and also 1% of minor constituents with nutritional and beneficial health properties [3]. Carotenes in palm oil range between 400-3,500 mg/L. However, it contains about 15-300 times more retinol equivalents (vitamin A) than carrots and tomatoes [4]. Small amounts of phytosterols (300-620 ppm), squalene (250-540 ppm), phospholipids (20-100 ppm), co-enzyme Q10 (20-80 ppm) and polyphenolics (40-70 ppm) are also observed in palm oil.

Intense development of the palm oil industry in Thailand contributes to the country's economic growth mainly through the trading of crude palm oil and its products [5]. However, there is an abundance of illegal entry of palm from Malaysia and Indonesia which makes palm prices in Thailand volatile. In addition, the other problem in the palm oil industry is habitat degradation. Once the oil palms have started to produce, the fruit must be harvested at the right moment to avoid natural degradation. If palm fruit becomes too ripe suddenly after harvest, many clusters will drop and the quality of the fruit will be poor. To our knowledge, many researchers have reported about chemical compositions in palm oil [1-5]. However, there have been no reports documenting the chemical compositions, including phenolic acids, tocols (tocopherol and tocotrienol), carotene and lipase profile of the palm fruit after the harvesting and ripening stages. Such information is necessary for better understanding of the physical and biochemical events. Therefore, this paper aims to determine the biochemical constituents, phytochemical compositions and antioxidant activity of oil palm fruit cultivated in southern Thailand after 0-240 h of harvesting. In addition, lipase profile of oil palm fruit was investigated and the possible application of enzyme for biodiesel production was also determined.

Materials and methods

Plant Collection

Oil palm fruit (*E. guineensis*) were collected from the Faculty of Science, Thaksin University, Thailand and used as the sample for this study. Oil palm fruit was collected following an oil palm fresh fruit bunch (FFB) grading standard guideline (ARDA, Thailand). Orange mesocarp with a weight around 15.29 ± 3.0 g were classified during the ripen stage. The ripen fruits were picked from different parts of bunches and left for 0-240 h after harvesting under room temperature (28 ± 2°C). Afterward, the samples were separated under specific time, washed thoroughly under running tap water, cut into smaller pieces and blended under suitable solvent [6].

Solvent Extraction

Five organic solvents (acetone, ethanol, methanol, chloroform and hexane) and 0.05 mM Tris-HCl buffer (pH 8) were used to extract chemical compositions in oil palm. Fifty grams of sample were dissolved in respective solvents (500 mL) using sonication for 10 minutes. The extract was filtered through filter paper (Whatman No.1 with pore size of 11 μm) using a vacuum pump and the extraction process was repeated two times. The extract from organic solvent was concentrated at 40°C and 90 rpm using a rotary evaporator [6]. The samples obtained were kept at -20°C for further study. Extraction yields were weighed and calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant powder}} \text{x100}$$

Chemical Composition Study

Chemical composition studies were determined for qualitative analysis to identify the presence of biochemical constituents. Total phenolic content was measured by the Folin-Ciocalteu method [6]. Qualitative analysis was done following the basic protocols [7]. Ash and moisture were analyzed by oven assay [8]. The total amount of palm oil was determined by solvent extraction [9]. Protein was determined following AOAC procedures [10]. Vitamin E, tocopherol, tocotrienol and carotenes content were analyzed by High Performance Liquid Chromatography (HPLC) followed the method of Schieber et al. [11]. The activity of lipase was determined for the absorbance at 410 nm compared with *p*-nitrophenol standard curve at concentrations ranging from 0-1.0 μg/mL [12].

Scavenging Activity

DPPH scavenging radical activity of oil palm extract against stable DPPH was assessed [13]. In addition, the ABTS and ABTS radical cations (ABTS+) and the FRAP were determined at 734 and 593 nm, respectively. The results were expressed as grams of Trolox equivalents per 100 g of dry weight (DW) [13-14].

Lipase Preparation for Biodiesel Production

Lipase was extracted by 50 mM Tris-base buffer (pH 8.0) from oil palm fruit after 120 h of harvesting [12]. The enzyme was determined for protein and lipase activity. Afterward, enzyme solution was freeze dried and utilized as “crude enzyme”. For partial purification of enzyme, Aqueous Two Phase System (ATPS) with 20% of polyethyleneglycol (PEG) 1000 and 15% of NaH_2PO_4 were employed. Therefore, purified lipase was

collected and characterized. The enzyme solution after ATPS was freeze dried and utilized as "partial purification enzyme".

Transesterification Reaction for Biodiesel Production

Waste cooking oil and lipase from oil palm fruit was utilized as substrate and catalyst, respectively. The enzymatic transesterification reactions were carried out in a test tube that contained 2 g of waste frying oil, 5.6 Unit of enzyme/10 g oil and 6:1 alcohol-to-oil molar ratio. The reaction was carried out in an incubator at 35ºC with constant stirring at 200 rpm. Experiments were carried out to optimize the reaction conditions varying reaction time. At the end of the reaction period, samples were taken from the reaction mixture and centrifuged in order to obtain the upper layer that was analyzed.

Determination of Biodiesel Properties

Biodiesel from lipase was characterized for viscosity, acid value and free fatty acid content. The viscosity was analyzed by the gravity method. In addition, acid value and free fatty acid content were determined by the titration method [12]. The Fatty Acid Methyl Ester (FAME) content in the product liquid was analyzed by a Shimadzu GC-2010 equipped with a hydrogen flame ionization detector. The separation was carried out on a DB-1HT capillary column (30 m × 0.25 mm id, Agilent Tech). The operating conditions were set as following: the temperature of sampling inlet was 370°C and detector temperature was 375°C; the starting temperature of the column was 150°C, increasing at 10 °C/min up to 205°C, then increasing at 2°C/min up to 215°C, finally increasing at 10 °C/min up to 360°C and then maintained at 360°C for 10 min. The split ratio was 1:50 and the carrier gas was nitrogen at a pre-column pressure of 100 kPa. Peaks in the chromatograms were identified by comparing retention times with the standards of known substances such as methyl salicylate, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate and methyl linoleate [14].

Results and Discussion

Effect of Solvent on Extraction Yield

Oil palm fruit gave the highest amount of recovery yield (21.64%) with methanol extraction. However, the other solvents yielded only 4.80-14.32% (Table 1). Bioactive compounds from plants belong to various chemical groups. Methanol is an amphiphilic compound which has a polarity index of 5.1. The molecule of methanol consists in a single atom of a tetrameric carbon, linked to 3 hydrogens, and a hydroxyl (-OH) group. The hydroxyl group is the polar group, and the three hydrogens, the water-insoluble hydrocarbon chain [15]. Therefore, methanol gave the highest extraction yield because it can dissolve both polar and also non-polar molecules. The biologically active constituents of the plants were extracted using methanol through standard methods. During extraction, plant material was dispersed and solubilized by solvents with similar polarity [6]. Polyphenols were frequently recovered from the plants by polar solvents such as ethanol and methanol. Ethanol is safe for human consumption. Methanol and aqueous acetone have been recognized to be suitable for lower molecular weight polyphenols and higher molecular weight flavanols, respectively [17].

Chemical Composition

The highest chemical composition was observed in oil palm fruit after 0 h of harvesting. The sample contained moisture, carbohydrate and protein content at 30.24%, 9.86% and 5.12% on a dry matter (DM) basis, respectively. While the lipid content and ash were 52.94% and 1.84%. The level of moisture, protein and ash were similar to those of African oil palm fruit as reported by Atchley [16]. It has been known that nutritional composition and antioxidant activity are influenced by several parameters such as cultural practices, climatic conditions, locations, soil types, temperature, water stress, nutrient availability and fertilization parameters [18].

Table 1 Yield of oil palm fruit (50 g) extracted using different types of solvents.

Solvents	Yield		Solvents	Yield	
	gram	% recovery		gram	% recovery
Acetone	$4.84 \pm 0.10^{(c)}$	$9.68^{(c)}$	Methanol	$10.82 \pm 0.25^{(e)}$	$21.64^{(e)}$
Ethanol	$7.16 \pm 0.10^{(d)}$	$14.32^{(d)}$	Chloroform	$2.40 \pm 0.11^{(a)}$	$4.80^{(a)}$
0.05 mM Tris-HCl buffer (pH 8)	$7.16 \pm 0.10^{(d)}$	$14.32^{(d)}$	Hexane	$2.90 \pm 0.10^{(b)}$	$5.80^{(b)}$

(a), (b),(c),(d),(e) - Statistical analysis. Different letter(s) in the same column indicate significant differences ($p<0.05$).

Phytochemical Study

The qualitative analysis of phytochemical constituents of oil palm fruit with methanol extraction are indicated in Table 2. The presence of different biochemical constituents in oil palm fruit may indicate their possible use in pharmacology application. For example, alkaloids can increase blood circulation and nutrient absorption. In addition, phenolic compounds are playing an important role in food and medical applications due to their capacity to stimulate some cellular enzyme functions [19]. Likewise, flavonoids are a significant compound that affects various biological systems [6].

Table 2 Group of phytochemical, method and phytochemical content in oil palm fruit at 0 h of harvested.

Group of phytochemical	Method	Unit	Oil palm fruit (This study)	Oil palm fruit[a]	Oil palm leaves[b]
Alkaloids	Bromocresol green method	mg/g dry weight, DW	$1.60 \pm 0.10^{(b)}$	5.15	5.00
Saponin	Uematsu method		$3.50 \pm 0.20^{(c)}$	108.24	24.00
Flavonoid	The Dowd method		$0.30 \pm 0.10^{(a)}$	118.03	257.00
Phenolic	Folin-Ciocalteu method		$3.60 \pm 0.01^{(c)}$	Not report	70.07
Tannin	Konate and Souza method		$3.50 \pm 0.10^{(c)}$	85.87	165.00

a, b Data obtained from [22] and [6], respectively.
(a), (b),(c) - Statistical analysis. Different letter(s) in the same column indicate significant differences ($p<0.05$).

The Chemical Composition of Oil Palm Fruit at Various Time of Harvesting

Phenolic contents were also determined from oil palm fruit after 0-240 h of harvesting. The highest phenolic content for methanolic extract was 3.60 ± 0.01 mg gallic acid equivalents (GAE)/g DW after 0 h of harvesting. The phenolic content gradually decreased during 0-240 h after harvesting to a relatively low level (2.80 ± 0.01 mg GAE/g DW) (Table 3). Oil palm fruit at 0 h of harvesting yielded the highest vitamin E content (0.10 ± 0.01 mg/g DW) and protein (2.40 ± 0.01 mg/g DW). The level of tocols and carotene during various times after harvesting were also investigated. The highest tocopherol (850 mg/kg), tocotrienol (800 mg/kg) and carotene (790 mg/kg) were observed at 0 h after harvesting. However, oil palm fruit (this study) contained low amounts of tocopherol when compared to the other reports [1-4]. It was due to many physical and environment factors such as palm varieties and cultivation area that affected the tocols contents [2, 6].

Table 3 Phenolic content, vitamin E, lipase, tocopherol, tocotrienol and carotene of the methanolic extract from oil palm fruit after 0-240 h of harvesting.

Hour after harvested (h)	Phenolic content (mg GAE/g DW)	Vitamin E content (mg/g DW)	Protein content (mg/g DW)	Lipase activity (U/mg)	Total tocopherol (mg/kg)	Total tocotrienol (mg/kg)	Total carotene (mg/kg)
0	$3.60 \pm 0.01^{(d)}$	$0.10 \pm 0.01^{(d)}$	$2.40 \pm 0.01^{(e)}$	$0.30 \pm 0.01^{(a)}$	$850 \pm 1.20^{(h)}$	$800 \pm 2.40^{(h)}$	$790 \pm 7.00^{(k)}$
24	$3.20 \pm 0.01^{(c)}$	$0.09 \pm 0.01^{(d)}$	$1.00 \pm 0.01^{(d)}$	$0.60 \pm 0.01^{(b)}$	$845 \pm 2.10^{(g)}$	$789 \pm 2.10^{(g)}$	$720 \pm 1.30^{(j)}$
48	$3.20 \pm 0.01^{(c)}$	$0.09 \pm 0.01^{(d)}$	$0.90 \pm 0.01^{(d)}$	$0.80 \pm 0.01^{(c)}$	$820 \pm 2.00^{(f)}$	$750 \pm 1.90^{(f)}$	$680 \pm 2.00^{(i)}$
72	$3.00 \pm 0.01^{(b)}$	$0.08 \pm 0.01^{(c,d)}$	$0.80 \pm 0.01^{(c,d)}$	$1.00 \pm 0.01^{(d)}$	$780 \pm 1.50^{(e)}$	$751 \pm 2.40^{(f)}$	$650 \pm 4.00^{(h)}$
96	$3.00 \pm 0.01^{(b)}$	$0.08 \pm 0.01^{(c,d)}$	$0.80 \pm 0.01^{(c,d)}$	$1.20 \pm 0.01^{(e)}$	$700 \pm 2.10^{(d)}$	$700 \pm 2.40^{(e)}$	$580 \pm 8.00^{(g)}$
120	$3.00 \pm 0.01^{(b)}$	$0.07 \pm 0.01^{(c)}$	$0.70 \pm 0.01^{(c)}$	$1.40 \pm 0.01^{(f)}$	$650 \pm 1.80^{(c)}$	$680 \pm 2.10^{(d)}$	$530 \pm 5.00^{(f)}$
144	$3.00 \pm 0.01^{(b)}$	$0.07 \pm 0.01^{(c)}$	$0.70 \pm 0.01^{(c)}$	$1.10 \pm 0.01^{(d,e)}$	$650 \pm 1.80^{(c)}$	$650 \pm 1.50^{(c)}$	$450 \pm 7.00^{(e)}$
168	$2.80 \pm 0.01^{(a)}$	$0.06 \pm 0.01^{(b)}$	$0.60 \pm 0.01^{(b)}$	$1.00 \pm 0.01^{(d)}$	$600 \pm 1.00^{(b)}$	$600 \pm 2.10^{(b)}$	$340 \pm 2.00^{(d)}$
192	$2.80 \pm 0.01^{(a)}$	$0.05 \pm 0.01^{(b)}$	$0.60 \pm 0.01^{(b)}$	$1.00 \pm 0.01^{(d)}$	$600 \pm 1.50^{(b)}$	$600 \pm 1.90^{(b)}$	$260 \pm 2.00^{(c)}$
216	$2.80 \pm 0.01^{(a)}$	$0.03 \pm 0.01^{(a)}$	$0.50 \pm 0.01^{(a)}$	$1.00 \pm 0.01^{(d)}$	$600 \pm 1.00^{(b)}$	$600 \pm 1.80^{(b)}$	$200 \pm 3.00^{(b)}$
240	$2.80 \pm 0.01^{(a)}$	$0.03 \pm 0.01^{(a)}$	$0.50 \pm 0.01^{(a)}$	$0.80 \pm 0.01^{(c)}$	$580 \pm 1.50^{(a)}$	$589 \pm 1.90^{(a)}$	$130 \pm 1.00^{(a)}$

(a), (b),(c)...(j) - Statistical analysis. Different letter(s) in the same column indicate significant differences ($p<0.05$).

Antioxidant Activity using Radical Scavenging Activity

The activity of methanolic extract from oil palm fruit after 0-240 h of harvesting was determined by three *in vitro* methods. The highest content of antioxidant activity was observed in oil palm fruit at 0 h after harvesting. The level of antioxidant from oil palm fruit based on FRAP, ABTS and DPPH assay (IC50) was 10.05 mmol TE/100 g DW, 3.21 mmol TE/100 g DW and 5.0 µg/mL, respectively. High antioxidant activity corresponded with high phenolic as well as flavonoid content in oil palm fruit. Phenolic compounds, carotenoids, tocols and other phytochemicals from oil palm fruit exhibit antioxidant capacity causing the reduction of oxidative stress

attributed to chronic disease, heart disease, neurodegenerative disease, cancers and diabetes [20]. Palm carotenes have been known for their antioxidant activities such as preventing chromosomal damage in bone marrow, reducing on white blood cell counts and enhancing survival after X-ray irradiation [20].

Lipase Activity in Oil Palm and Its Application for Biodiesel Production

The lipase significantly increased from 0-120 h after harvesting (Table 3). The highest lipase activity (1.40 U/mg) was observed at 120 h after harvesting then the activity remained constant up to 216 h. Therefore, lipase was extracted from palm fruit at 120 h after harvesting. Afterwards, 20% of polyethylene glycol (PEG) 1000 and 15% sodium dihydrogen phosphate (NaH_2PO_4) were utilized to purify lipase from oil palm fruit. Lipase activity increased significantly from 1.40 U/mg to 4.76 U/mg after the purification step (data not shown). Crude and partial purified enzymes were utilized as catalysts for biodiesel production using the transesterification method. The optimal reaction time for biodiesel production using crude enzyme was 12 h while partial purified enzyme reached the highest methyl ester content (96.5%) after 6 h of incubation time. Generally, the suitable time for enzymatic reaction was around 6-24 h due to the source of enzyme and substrate. In this study, the highest methyl ester content (98%) was achieved from biodiesel using crude lipase as the catalyst. It may due to the stable of enzyme in transesterification process. Normally, the longer an enzyme is incubated with its substrate, the greater the amount of product that will be formed. However, the rate of formation of product is not a simple linear function of the time of incubation. All proteins suffer denaturation, and hence loss of catalytic activity, with time. Some enzymes, especially in partially purified preparations, may be noticeably unstable, losing a significant amount of activity over the period of incubation. Purified enzymes in diluted solutions are denatured more rapidly than enzymes in crude extracts [20]. Biodiesel from lipase catalyst was characterized for the specifications according to Thailand's fuel and ASTM standards. An acid value (0.45 mg/g KOH), the viscosity (3.97 mm^2/s) and free fatty acid content (0.21%) of biodiesel from lipase were not significantly different from commercial biodiesel and it also passed Thailand's fuel and ASTM standards.

Conclusion

The presence of different biochemical constituents in oil palm fruit may indicate their possible use in pharmacology application. Palm fruit contained high content of antioxidant activity, saponin, phenol, tannin, phenolic, vitamin E, protein and carotene. In addition, lipase activity (1.40 U/mg) was achieved in palm fruit at 120 h of harvesting and the activity can be increased to 4.76 U/mg after partial purification. Palm lipase showed a possibility to catalyst transesterification reaction using waste cooking oil as substrate. The biodiesel with 96.5% methyl ester content was achieved after 6 h of reaction and biodiesel specifications were characterized and passed Thailand's fuel and ASTM standards.

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