



รายงานวิจัยฉบับสมบูรณ์

โครงการ คุณค่าทางโภชนาการของเมล็ดต้นหูกวางและการศึกษาศักยภาพ
ในการเป็นน้ำมันประกอบอาหาร

โดย ดร.มณฑนา วีระวัฒนาการ

มิถุนายน 2556

บทคัดย่อ

รหัสโครงการ: MRG5080015

ชื่อโครงการ: คุณค่าทางโภชนาการของเมล็ดพันธุ์หูกวางและการศึกษาศักยภาพในการเป็นน้ำมันประกอบอาหาร

ชื่อนักวิจัย ดร. มณฑนา วีระวัฒนากร มหาวิทยาลัยนเรศวร

อีเมล: monthanac@nu.ac.th

ระยะเวลาโครงการ: 2 ปี

บทคัดย่อ:

เพื่อประเมินศักยภาพในการใช้เป็นแหล่งอาหารใหม่ เมล็ดหูกวางถูกวิเคราะห์ค่า

สารอาหาร สารต้านสารอาหาร และผลการให้ความร้อน ตัวอย่าง 100 กรัม

น้ำหนักแห้ง เมล็ดหูกวางประกอบด้วย 22.3 กรัม โปรตีน 60 กรัม ไขมัน 6 มิลลิกรัม

สังกะสี และ 2.5 กรัม ทองแดง กรดอะมิโนที่มีจำกัดได้แก่ ทริปโตเฟน และไลซีน หลังการ

อบที่ อุณหภูมิ 180 องศาเซลเซียส เป็นเวลา 4 นาที เกิดการสูญเสียกรดอะมิโนเพียง

เล็กน้อย ปริมาณสารฟีนอลิกและไฟเตต (อินโนซิทอลเตรตาฟอสเฟต (IP4) +

อินโนซิทอลเพนตาฟอสเฟต (IP5) + อินโนซิทอลเฮกซาฟอสเฟต (IP6)) คือ 64.6 กรัมของ

กรดแกลลิกต่อกรัมตัวอย่าง และ 2,110 มิลลิกรัมต่อ 100 กรัมตัวอย่าง น้ำหนักแห้งซึ่งมี

ค่าเทียบเท่ากับปริมาณในถั่วบราซิลอื่น ๆ ปริมาณสารที่ขัดขวางการทำงานของน้ำย่อย

โปรตีนทริปซินคือ 2.25 หน่วยทริปซินอินฮิบิเตอร์ต่อกรัมตัวอย่าง หลังการอบทั้ง

สารฟีนอลิกและค่าปริมาณสารที่ขัดขวางการทำงานของน้ำย่อยโปรตีนทริปซินลดลง

โดยอินโนซิทอลเฮกซาฟอสเฟตสลายไปเป็นอินโนซิทอลเตตราฟอสเฟตและอินโนซิทอล

เพนตาฟอสเฟต กรดไขมันพันธะคู่หนึ่งพันธะโอเลอิก (C18:1) และกรดไขมันพันธะคู่

หลายพันธะกรดไลโนเลนิก (C18:2) เป็นกรดไขมันหลักซึ่งมีปริมาณ 32.4 % และ30.0%

ตามลำดับ อัตราส่วนของกรดไขมันอิ่มตัว:กรดไขมันพันธะคู่หนึ่งพันธะ:กรดไขมัน

พันธะคู่หลายพันธะเป็นไปตามปริมาณที่แนะนำโดยสมาคมแพทยโรคหัวใจอเมริกัน

(AHA) คุณสมบัติทางเคมีกายภาพของน้ำมันมีค่าเหมาะสมเป็นไปตามมาตรฐาน

อาหารน้ำมันสำหรับใช้บริโภค

คำหลัก : หูกวาง กรดอะมิโน สารต้านสารอาหาร น้ำมัน กรดไขมัน

Abstract

Project Code : MRG5080015

Project Title : Nutritional properties and the potential study of *Terminalia catappa* Linn seed as edible oil

Investigator : Dr. Monthana Weerawatanakorn

E-mail Address : monthanac@nu.ac.th

Project Period : 2 years

Abstract:

To evaluate their potential use as a new dietary source, the seeds of *Terminalia catappa* Linn (TC) were analyzed for their nutritional and antinutritional properties, and to determine the effect of roasting. Per 100 gram (dry basis), the TC seeds were found to contain 22.3 g protein, 60 g oil, 6 mg Zn and 2.5 mg Cu. The limiting amino acids were tryptophan and lysine. After roasting at 180°C for 4 min, there was minimal loss of amino acids. Contents of phenolic compounds and phytates (inositol tetrphosphate (IP4) + inositol pentaphosphate (IP5) + inositol hexaphosphate (IP6)) found were 64.9 g of gallic acid equivalents per gram of sample and 2,110 mg/100g (dry basis), which is comparable to edible nuts. Trypsin inhibitor activity was 2.25 trypsin inhibitor units per sample milligram. After

roasting, both phenolic compounds and trypsin activity were reduced, while IP6 phytate degraded into IP4 and IP5 forms. Monounsaturated fatty acid, oleic acid (C18:1), and polyunsaturated fatty acid, linoleic acid (C18:2), are the main fatty acids which were determined at 32.4% and 30.3%, respectively. The ratio of saturated: monounsaturated: polyunsaturated fatty acid was found to be close to what is recommended in the dietary guideline of the American Heart Association (AHA). Physico-chemical properties of the oil extracted from TC showed that oil quality is under Thai industrial standards for edible oil.

Keywords : *Terminalia catappa*, amino acids, antinutritional properties, oil, fatty acids

เนื้อหางานวิจัยประกอบด้วย

1. บทคัดย่อ

Abstract

To evaluate their potential use as a new dietary source, the seeds of *Terminalia catappa* Linn (TC) were analyzed for their nutritional and antinutritional properties, and to determine the effect of roasting. Per 100 gram (dry basis), the TC seeds were found to contain 22.3 g protein, 60 g oil, 6 mg Zn and 2.5 mg Cu. The limiting amino acids were tryptophan and lysine. After roasting at 180°C for 4 min, there was minimal loss of amino acids. Contents of phenolic compounds and phytates (inositol tetraphosphate (IP4) + inositol pentaphosphate (IP5) + inositol hexaphosphate (IP6)) found were 64.9 g of gallic acid equivalents per gram of sample and 2,110 mg/100g (dry basis), which is comparable to edible nuts. Trypsin inhibitor activity was 2.25 trypsin inhibitor units per sample milligram. After roasting, both phenolic compounds and trypsin activity were reduced, while IP6 phytate degraded into IP4 and IP5 forms. Monounsaturated fatty acid, oleic acid (C18:1), and polyunsaturated fatty acid, linoleic acid (C18:2), are the main fatty acids which were determined at 32.4% and 30.3%, respectively. The ratio of

saturated: monounsaturated: polyunsaturated fatty acid was found to be close to what is recommended in the dietary guideline of the American Heart Association (AHA). Physico-chemical properties of the oil extracted from TC showed that oil quality is under Thai industrial standards for edible oil.

2. Executive summary

Terminalia catappa, Linn is belonging to the family Combretaceae and is naturally widespread in the subtropical and tropical zones of Indian and Pacific Oceans. It is widely planted extensively through the tropics especially from the Seychelles through India, the Andamans, and throughout Southeast Asia such as Myanmar, Thailand, the Malay Peninsula, Vietnam, the Philippines, and Indonesia. In Thailand, the medium to large tree has been found throughout the country both along the seashore and inland as a shade tree and the popular common name is Hu-kwang.

It has been known for a long period of time that the leave, fruits, and seeds are edible as well as they has been used as folk medicinal treatment like antipyretic, antidiabetes, prevention of hepatoma and hepatitis and treatment of sickle cell disorder. The research of *T. catappa* is still limited. Most of researches done so far are mainly focusing on using leaves as medicines while a couple of studies aims to analysis nutritional content and phytochemicals of fruits as database for medicinal benefit, not in field of food advantage. Although, there are widely accepted that the seed (kernel) of fruit at full maturity can be consumed as nut, there are, however, lacking knowledge about nutritive value including nutrient composition and antinutritional profiles of raw and cooked seed and the potential to develop for alternative food such as snack, seed oil.

Like other nut including peanuts, almond, cashew, hazelnut, macadamia, pecan, walnut, seed of *T. catappa* should be a good source of dietary protein and lipid. Globally, it is well accepted that not only nut are rich sources of lipid in particular unsaturated fatty acids and protein, but also consumers lately associate nut to healthy products due to their considerable amount of micronutrients like tocopherols,

unsaturated fatty acids, dietary fibre, and phytochemicals which possess the protective effect on degenerative diseases.

Obviously, several antinutritional factors including oxalic acid, phytate (phytic acid), trypsin inhibitor as well as amylase inhibitor, are present in various plant, especially in the raw one that may adversely affect on their nutritional quality such as minerals and protein absorption. However, there are also lots of evidences showed that thermal treatments like boiling, baking, and roasting reduce the antinutritional factors in various plants.

Only one study from Brazil had done to determine the nutritive content of raw seed from *T. catappa* Linn. From the point of human consumption, data on nutrient composition and antinutritional content of *T. catappa* seed are limited; especially, no published data in a journal are available on the effect of heat treatment on nutritional properties, and the possible use as optional food. Hence, the present work has been aimed at evaluate the macronutrients, antinutritional factors of raw and cooked *Terminalia Catappa* Linn seed and investigated the possible use as alternative food in terms of cooking oil.

3. Objectives

Objective

1. To determine the proximate composition including moisture content, crude protein, total lipid, carbohydrate, and ash of *T. catappa* seed.
2. To determine amino acid composition and fatty acid profile of *T. catappa* seeds.
3. To determine the presence of anti-nutritional contents including dietary fiber, phenolic content, phytate and trypsin inhibitor of *T. catappa* seeds.
4. To evaluate the effect of roasting on anti-nutritional content of *T. catappa* seeds.
5. To extract *T. catappa* seed oil and determine vitamin E and fatty acid compositions.
6. To evaluate chemical and physical properties of extracted oil.
7. To investigate the further application of *T. catappa* seeds as a new source of oilseed.

4. Materials and Methods

Sample Preparation.

Ripe (yellow) and partially dried fruits (with gray colored pericarp) of *T. catappa* were collected from the campus of Naresuan University, Phitsanulok, Thailand during July-August and October -November 2011. The fruits were cleaned and oven-dried at 60 °C for 12 hr. The dried fruits were shelled manually by using a stainless steel knife and Thai carving knife and then the shell was cracked to remove the single seed (kernel). The seeds were then ground in a coffee grinder (Model CBG5 series, Black and Decker Canada Inc., Brockville, ON) for 5 min to a fine powder. Powdered samples were packed into airtight sealed plastic bags and stored at - 20 °C until further analysis.

Raw almond (Heritage; Heritage Snacks & Food Co, Ltd), cashew nuts (Raitip; Raitanya Co, Ltd), and peanuts (KhaoThong; Food Industry (1964) Co, Ltd) were bought from a supermarket (Makro, Phitsanulok, Thailand). They were directly ground in a coffee grinder and were kept in the same condition as *T. catappa* fine powder. For cooking condition, the whole seeds were roasted on oven (House Worth, HW-B001/Thailand) at 180 °C for 4 min.

Proximate analysis

Moisture content of seed powders was determined by measuring constant weight at 100 °C (AOAC Official Method 925.40) (AOAC, 1995). The crude protein content ($N \times 6.25$) was determined by the Kjeldahl method (AOAC Official Method 950.48) (AOAC, 1995). Crude lipid (Soxhlet extraction), crude fibre and ash contents were determined by gravimetric method employing AOAC methods (AOAC, 1990). Total crude carbohydrate was based on calculating the following equation

% Carbohydrate = 100% - % of (protein + fat+ ash +moisture). Gross energy (kJ) was estimated by calculation using the following equation

$$\text{Energy (Kcal)} = (\% \text{ protein} \times 4) + (\% \text{ fat} \times 9) + (\% \text{ carbohydrate} \times 4)$$

Mineral contents

Iron, zinc, magnesium, calcium, potassium, and copper were characterized and quantified, in triplicate, by atomic absorption spectrophotometry, and Phosphorus was quantified by gravimetric method (AOAC Official Method 985.35, 945.46) (AOAC, 2005; Kolthoff, 1996).

Phenolic contents and antioxidant activity

Total phenolics were determined according to the Folin–Ciocalteu method (Caboni, 1997). This protocol gave a good idea of the total phenolic content. Briefly, diluted extracts (3.6 ml) were mixed with 0.2 ml of Folin–Ciocalteu reagent (Merck) and 3 min later, 0.8 ml of sodium carbonate (20% w/v) was added. The mixture was heated to 100 °C for 1 min. After cooling, the absorbance at 750 nm was measured. Gallic acid (Sigma) was used as standard, and results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight of sample

The antioxidant activity of nut was examined using the DPPH assay based on scavenging ability of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Kumaran & Karunakaran, 2006). Ground sample was extracted by methanol. Sample solution 0.5 ml and 1.5 ml of DPPH in methanol solution were mixed and keep in dark condition. The absorbance at 517 nm of samples was determined after 60 min. Methanol was used, instead of DPPH reagent as a blank to correct for any sample absorbance at 517 nm.

Fatty acid content (AOAC Official Method 932.06, 989.05, 963.22 and 969.33)

(Horwitz, 2005)

The seed lipid was obtained by AOAC 932.06, 989.05 and fatty acids were determined by gas chromatography according to AOAC method 963.22 and 969.33. Fatty acids were obtained by saponification of fats. Then free fatty acids were esterified by boron trifluoride catalyst. The methyl esters of fatty acids were extracted by petroleum ether. Then they were dissolved with chloroform and were injected into gas chromatograph (Varian 3600) equipped with flame ionization detector. A GC analysis was performed with the detector temperature programmed for 300 °C, and flow rate of 2 ml/min. The injector temperature was set at 250 °C. Nitrogen was used as the carrier gas. The identification of the peaks was achieved by comparing their relative and absolute retention times with authentic standards analyzed under the same conditions. P/M/S ratio was obtained from the sum of each type of fatty acids and calculated to proportion.

Amino acid content (Petritis, 2002; Bosch, 2006)

The quantification of amino acids was performed by acid hydrolysis of proteins and peptides following the derivatization approach (Petritis, 2002; Bosch, 2006). Since cysteine (Cys) and methionine (Met) are partially destroyed by acid hydrolysis, they were oxidized with performic acid (Sigma) to cysteic acid and methionine sulphone prior

to acid hydrolysis. Acid hydrolysis was performed with 6 M HCl (5 mL) for 22 h at 110°C in sealed glass tubes. The derivatization step consisted of esterification with AccQ-flour derivatization buffer and AccQ-flour reagent to derivatize. Aliquots were used for analysis by HPLC (Water Alliance 2695 with heater Jasco FP2020 fluorescence Detector (EX: 250, EM: 395 nm). Amino acid composition was reported as grams of amino acid per 100 g of protein, and essential amino acid (EAA) score was determined by the following formula:

Trypsin inhibitor

Trypsin inhibitor activity (TIA) was examined by a spectrophotometric method (method 22-40) (AACC, 1999), using α -N-benzoyl-DL-arginine-nitroanilide hydrochloride (BAPNA) as the trypsin substrate. Dried sample was extracted with 0.1 N NaOH, and the suspension was made up to the suitable concentration. Trypsin solution (in 0.001M HCl) was added to each test tube and kept in a water bath at 37°C. To each tube, BAPNA solution (Aldrich, 85711-4; purity $\geq 99\%$) in dimethyl sulfoxide was added, and the reaction was terminated after 10 min by adding 1mL acetic acid (30%). After

centrifugation, spectrophotometer (UV-Visible spectrophotometer, Shimadzu, Japan)

$$\text{EAA score} = \frac{\text{amount of EAA}}{\text{amount of reference EAA}}$$

was used to measure the absorbance of the clear solutions at 410 nm against reagent blank (30% acetic acid containing trypsin and distilled water+ BAPNA solution). Trypsin inhibitor activity (TIA) was expressed in term of trypsin inhibitor units (TIU) /mg sample. One trypsin unit was arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction

Phytate content

Phytates are inositol tetraphosphate (IP4), inositol pentaphosphate (IP5) and inositol hexaphosphate (IP6). Their contents were determined according to the method described by Hotz and Somsu (Hotz & Gibson, 2001; Somsu & Kongkachuichai, 2008). Briefly, 0.5 g of constant weight dried sample were ground in a coffee grinder (Kenwood CG 100), and were extracted with 0.67M HCl (5mL) for 30 min in an ultrasonic bath (Model 1510E-MT Branson). Following centrifugation at 3,000 rpm for 10 min, the supernatants were diluted with deionized water, and then were applied onto an anion exchange column (WAT023620, Sep-Pak Vac 1 cm³ Water Accell Plus QMA, Water, Milford, MA) and inositol phosphates were eluted with HCl and evaporated to dryness at 40°C in a centrifugal evaporator (Model CVE-2000; EYELA, Tokyo Rikakikai Co. Ltd.). The residues were dissolved in deionized water and analysis of IP4, IP5 and

IP6 was performed by reverse phase HPLC (Atlantis dC18, 5 mm, and 4.6 x 150 mm²) with refractive index detector (Waters IR 2414). The mobile phase was a mixture of methanol: H₂O (3:2) set at a flow rate of 0.8 ml/min.

Extraction and Physico-chemical properties of *Terminalia catappa* oil

Ripe (yellow) and partially dried fruits (with gray colored pericarp) of *T. catappa* were collected from the campus of Naresuan University, Phitsanulok, Thailand during July-August and October -November 2012. The fruits were cleaned and oven-dried at 60 °C for 12 hr. The dried fruits were shelled manually by using a stainless steel knife and Thai carving knife and then cracked to remove the seeds. The seeds were then ground in a coffee grinder (Model CBG5 series, Black and Decker Canada Inc., Brockville, ON) for 5 min to a fine powder. Powdered sample were subjected to solvent, hexane, extraction by Soxhlet extraction (C) and maceration using Grant OLS 200 orbital shaker with (B) and without temperature (C) as in **Table 1**. All oil samples were stored in the dark at -20 °C until tested. Yield of oil extracted in each method was calculated. Oil extracted from soy beans (the second most consumed commercial oil in the world) was used to compare for the properties.

1. Soxhlet extraction (Pena et al, 1992):. ground sample (50 gram) was extracted according to the soxhlet extraction method with hexane (250) and refluxed for 8 hour at a temperature of ~ 90 °C. Then the sample was removed and the solvent by rotary vacuum evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) at 60 °C for 1 hour. The TC oil was used for the physicochemical property determinations.

2. Maceration extraction with and without temperature (Ooi Yong and Jumat, 2006): ground sample was extracted by maceration extraction method using hexane. The ratio of ground sample 50 gram to solvent 250 milliliter, and then with shaker 200 rpm at room temperature (without temperature) and 55 °C (with temperature) for 4 hours. The sample was removed solvent by rotary vacuum evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) at 60 °C for 1 hour. The TC oil was used for the determinations.

Table 1 Solvent extraction method of TC oil by hexane

methods	times	condition
Soxhlet extraction	8 ชั่วโมง	-
Maceration (Grant OLS 200 orbital shaker)	4 ชั่วโมง	-
Maceration (Grant OLS 200 orbital shaker)	4 ชั่วโมง	shaking ที่ 175 rpm ที่ อุณหภูมิ 55 °C

Physical and chemical properties.

The oil obtained from the different extraction methods were characterized using the following physical and chemical parameters; Density (AOAC. Method 40.1.08, 1990), viscosity (Brookfield), melting point (Differential Scanning Calorimeters/DSC), color CIE (L^* , a^* , b^*) (spectrophotometer), saponification value (AOAC. Method 920.160, 1995), unsaponification value (AOAC. Method 933.08, 1995), acid value (AOAC. Method 940.28, 1990), peroxide value (AOAC. Method 965.33, 1990), fatty acid composition (AOAC. Method 932.06, 989.05, 963.22 and 969.33) (Horwitz, 2005), Vitamin E (Devries, 1997 ด้วยเครื่อง HPLC), Phytosterol (Laakso, 2005)

Oil refining

1. Degumming or water refining is to remove resin, protein, phosphatide and phospholipid from TC crude oil. Crude oil at temperature of 65-75 °C is treated with water at 2.5 % (w/w) of oil followed by centrifugal separation (6,000 rpm for 5 minutes) after mixing for 30-60 minutes.

2. Neutralization is to remove free fatty acid from crude oil using alkaline, NaOH

2N. The amount of sodium hydroxide is based on the following equation.

$$Q = \frac{Q1 \times P \times A \times 100}{100 \times M \times N}$$

Q = an amount of NaOH solution (L)

Q1 = an amount of crude oil (L)

P = Specific gravity of crude oil

A = Oil acid value (%)

M = Molecular weight of fatty acid (oleic acid = 282)

N = Concentration of NaOH

Sodium hydroxide solution is added to crude oil with 24-30 °C following heating at 54-60 °C after the mixture is stirred for 10 minutes. The soap is removed by

separator. Oil is washed with hot water until neutral followed by centrifugal separation

(6,000 rpm for 5 minutes)

5. Results and Discussions

From the point of view of human consumption, it is widely accepted that the seed (kernel) of *T. catappa* fruit at full maturity can be consumed. However, data on nutrient composition and antinutritional content of TC seeds is limited. There are no published data on nutritive value including nutrient composition and antinutritional profiles, and on the effect of heat treatment on nutritional properties. In this study, their mineral contents, fatty acid, protein patterns, and effect of heat treatment on antinutritional content were determined. The range in weight for individual fruit was 4.62 g to 7.68 g and the average weight was 6.26 g (from 32 fruits and seeds). The kernel or seed weight ranged from 0.29 g to 0.12 g and the average weight was 0.21 g. The percentage yield of seeds was 3.35.

Proximate composition

The proximate compositions of TC seeds in wet and dry weight were shown in **Table 2** and compared with other nuts in **Table 3**. Moisture and ash contents of TC seed were 43.36 g kg⁻¹ and 42.21 g kg⁻¹ dry matter respectively. Both values were significantly different ($p < 0.05$) from other seeds. The moisture content was lower than

almond and peanut, but higher than cashew nut. The low moisture content with low water activity (0.45) is beneficial for keeping quality and shelf life of the TC seeds because of a decreased rate of microbial growth, and other undesirable chemical and biochemical changes. The higher total ash value of TC seeds indicated the possibility of higher mineral contents. The mineral profile including calcium, iron, phosphorus, potassium, magnesium, copper, zinc, chloride of raw TC seeds was shown in **Table 4**.

Table 2 Proximate composition of seeds from *Terminalia catappa* on wet and dry weight basis (n=3)

Component	Raw seed (wet weight)	Raw seed (dry weight)
Moisture (%)	4.16 ± 0.13	4.34± 0.13
Protein (N x 6.25) (g/100 g)	22.31 ± 0.14	23.28± 0.14
Oil (g/100 g)	57.55 ± 0.01	60.04± 0.01
Ash (g/100 g)	4.05 ± 0.14	4.22± 0.14
Total carbohydrate ^a	11.93	12.45
Crude fiber	8.61±0.17	8.52±0.17
Energy value (Kcal/100 g)	654.91	683.34
Calorie from fat (Kcal/100 g)	517.95	540.44

^a Calculated by difference include crude fiber

Table 3 Proximate compositions (g kg⁻¹ dry matter), total phenolic, and DPPH**activity of seeds from *Terminalia catappa* compared with seed of almond, peanut,****and cashew nut**

Component (g Kg⁻¹ dry matter) ^a	<i>T. catappa</i> seed	Almond	Peanut	Cashew nut
Moisture	43.36 ± 1.40	59.01 ± 0.96	78.34 ± 1.77	33.26 ± 1.58
Protein (N x 6.25)	232.80 ± 1.42	237.99 ± 1.18	311.08 ± 1.47	190.07 ± 1.20
Oil	600.41 ± 0.10	556.75 ± 7.04	433.89 ± 0.03	501.68 ± 6.44
Carbohydrate ^c	124.58	174.79	229.78	285.25
Ash	42.21 ± 1.45	30.47 ± 1.0	25.25 ± 1.31	23.00 ± 0.78
Total phenolic mg GAE/g dw	46.24 ± 2.00	23.71 ± 0.22	48.65 ± 0.16	45.18 ± 0.30
% DPPH radical scavenging activity	58.76 ± 0.62	41.35 ± 0.57	92.70 ± 0.85	17.14 ± 1.15

^aMean of triplicate analyses^b*Terminalia catappa* = *T. catappa*^cCalculated by difference

paired t-test was used to analyze the data

Protein content of TC seeds was 232.8 g kg⁻¹ dry matter which was lower than other nuts except for cashew nut, and the value was comparable to almond protein content. Statistical tests showed that TC seed protein content is not significantly different ($p > 0.05$) from almond, but it was significant different from peanut and cashew nut.

Table 4 Mineral composition of raw seeds *Terminalia catappa* (dry weight)

Minerals (mg/100g)	Raw seed	almond ^a	peanut ^b
Calcium	325.23± 0.30	248	32.65
Iron	5.39± 0.28	4.3	1.89
Phosphorus	889.94± 7.56	474	856.29
Potassium	731.06± 5.24	728	668.11
Magnesium	364.72±2.04	275	190.47
Copper	2.51±0.01	1.11	-
Zinc	6.09±0.03	3.36	4.33

^a data by Alexiadou & Katsilambros, 2011

^b data by de Oliveria, 2011

Fat content of TC seed is significantly different ($p<0.05$) from other seeds, and were found to be the highest (600.4 g kg^{-1} dry matter) compared with almond, peanut, and cashew nut. (Table 3)

Although phenolic compounds such as tannin, chlorogenic acid are classified as antinutrients, they possess a benefit in terms of reducing the risk of degenerative chronic diseases due to antioxidant activity, anti-cancer, anti-inflammation, anti-bacterial activity. Antioxidant capacity of TC seeds was determined by scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and total phenolics were determined according to the Folin– Ciocalteu method (Caboni, 1997). The result showed that phenolic compounds as indicated by total phenolic content and antioxidant activity of TC seeds were 46.24 mg GAE/g dry matter, and 56.7 percent respectively (**Table 3**). Although

total phenolic content of TC seed was comparable to peanut (48.65 mg GAE/g dry matter) and cashew nut (45.18 mg GAE/g dry matter), statistical analysis revealed a significant difference ($p < 0.05$) from other seeds. Compared with almond and cashew nut, antioxidant activity of TC seeds was significantly higher. The antioxidant activities of peanut was shown to be the highest since its skin is known to be a rich source of powerful bioactive compounds, in particular resveratrol (Soares et al, 2003; King et al, 2005).

TC seeds showed an optimal nutritional density in minerals, and had the highest content compared to almond and peanut (**Table 4**). Mineral contents of TC seeds, including Fe, P, K, Mg, Cu and Zn, were the highest compared to those of almond and peanut. Considering the nutritional importance of essential elements such as Cu and Zn as an antioxidant in biological systems and limited amounts in plant foods, TC seed can be considered as a good source of zinc and copper.

Protein pattern

Table 5 registers the amino acid composition, and amino acid profile of TC seed compared with other nuts. Despite the small size of TC seed, its amino acid

concentrations were much higher. The predominant amino acids amongst the nonessential amino acids were glutamic acid and aspartate and those amongst the essential amino acids were arginine and leucine.

Table 5 Amino acid composition (g / 100 g protein) of raw seeds from *Terminalia*

***catappa* compared to raw almond, peanuts, and cashew nut**

amino acid	<i>T. catappa</i> seed ^a	Almond ^b	Peanut ^b	Cashew nut ^b
Isoleucine*	3.53 ± 0.15	3.79 ± 0.12	3.45 ± 0.04	4.15 ± 0.02
Leucine*	8.06 ± 0.18	7.19 ± 0.19	7.03 ± 0.03	8.00 ± 0.05
Phenylalanine*	5.28 ± 0.24	5.46 ± 0.11	5.38 ± 0.12	4.83 ± 0.03
Lysine*	2.43. ± 0.10	3.06 ± 0.30	3.88 ± 0.07	4.59 ± 0.08
Threonine*	3.18 ± 0.08	2.60 ± 0.10	2.21 ± 0.11	3.22 ± 0.26
Valine*	5.01 ± 0.08	4.41 ± 0.12	3.95 ± 0.02	5.65 ± 0.08
Arginine*	16.93 ± 0.39	10.09 ± 0.29	11.04 ± 0.19	9.84 ± 0.04
Histidine*	1.77 ± 0.13	2.97 ± 0.14	2.54 ± 0.07	2.68 ± 0.02
Methionine	1.02 ± 0.05	0.81 ± 0.20	1.30 ± 0.04	2.27 ± 0.11
Tryptophan	0.14 ± 0.00	0.70 ± 0.01	0.73 ± 0.01	1.31 ± 0.08
Cystine	3.12 ± 0.12	0.30 ± 0.10	0.33 ± 0.07	0.54 ± 0.02
Alanine	4.17 ± 0.05	4.85 ± 0.10	4.58 ± 0.20	4.44 ± 0.06
Aspartate	8.63 ± 0.04	9.18 ± 0.08	12.07 ± 0.26	8.53 ± 0.16
Glutamic acid	20.86 ± 0.32	26.78 ± 1.09	21.11 ± 0.17	22.43 ± 0.13
Glycine	6.30 ± 0.08	6.88 ± 0.07	6.43 ± 0.30	4.55 ± 0.25
Proline	4.12 ± 0.13	5.09 ± 0.38	5.81 ± 0.67	5.37 ± 0.04
Serine	4.29 ± 0.08	3.67 ± 0.18	4.81 ± 0.17	5.21 ± 0.21
Tyrosine	2.83 ± 0.15	2.21 ± 0.45	3.40 ± 0.19	2.43 ± 0.13

data are reported as mean ± standard deviation (n = 2).

^a*Terminalia catappa* = *T. catappa*

^bVenkatachalam & Sathe, 2006

*Essential amino acids

Although all essential amino acids of TC seeds were not comparable to whole egg (**Table 6**), they compare favorably with other edible nuts; typically almond, cashew nut, and peanuts with the exception of lysine and histidine that are slightly lower than other nuts. Like amino acid profiles of other nuts, the predominant amino acid composition of TC seeds are glutamic acid (20.86 g/100 g protein) followed by arginine (16.93 g/100 g protein). L-arginine is a semi-essential amino acid (must be exogenously supplied to specific populations under special conditions) that promotes a healthy cardiovascular system since it is a precursor of nitric oxide (NO) which plays an important role in many bioactivities including vasodilatation, antiplatelet effect, and antioxidant activities (Vallance & Chan, 2001; Wu & Meininger, 2002; Well et al, 2005). Relative to other nuts, TC seeds contain the highest cysteine and sulfur containing amino acid (Met + Cys) approximately 4% of total amino acid followed by cashew nut (2.8%), pea nut(1.6%), and almond (1.1%). Methionine is an indispensable sulfur amino acid in human nutrition, whereas cystine is considered to be a conditionally indispensable because it can be synthesized from serine through different pathways. Both sulfur containing amino acids are essential for the entire biological system in particular the sulfur sources. Cysteine participates in the synthesis of essential bio-

molecules like antioxidants, vitamins and co-factors (such as glutathione, thiamine, lipoic acid, biotin, coenzyme A for example) (Wirtzl & Droux, 2005).

Table 6 Amino acid composition of TC seeds and with the roasted seeds (g/100 g protein) compared to whole egg protein and to the FAO/WHO reference pattern (1991) for the essential amino acids.

Amino acid	Raw seeds	Roasted seeds	Whole egg protein ^a	FAO/WHO pattern ^b	EAA score ^c
Isoleucine	3.53 ± 0.15	2.84 ± 0.04	6.3	2.8	126/102
Leucine	8.06 ± 0.18	7.47 ± 0.20	8.8	6.6	123/114
Phenylalanine	5.28 ± 0.24	3.47 ± 0.04	5.7	6.3 ^e	129/104
Lysine	2.43. ± 0.10	2.40 ± 0.00	7	5.8	42/42
Threonine	3.18 ± 0.08	2.07± 0.04	5.1	3.4	94/61
Valine	5.01 ± 0.08	5.19 ± 0.09	6.9	3.5	144/149
Arginine	16.93 ± 0.39	10.38 ± 0.10	6.1		
Histidine	1.77 ± 0.13	2.07 ± 0.06	2.4	1.9	94/109
Methionine	1.02±0.05	1.58±0.11	3.4	2.5 ^d	166/146
Tryptophan	0.14±0.00	0.15±0.01	1.7	1.1	13/14
Cystine	3.12±0.12	2.07±0.00	5.9		
Alanine	4.17± 0.05	2.76± 0.02	5.9		
Aspartate	8.63 ± 0.04	7.77 ± 0.11	9.6		
Glutamic acid	20.86 ± 0.32	15.50 ± 0.04	12.7		
Glycine	6.30 ± 0.08	5.38 ± 0.10	3.3		
Proline	4.12 ± 0.13	5.44 ± 0.00	4.2		
Serine	4.29 ± 0.08	3.44 ± 0.09	7.6		
Tyrosine	2.83 ± 0.15	3.06 ± 0.17	4.2		

^a Whole egg protein (FAO,1968)

^b FAO/WHO recommended pattern for 2-5 year old children (FAO/WHO,1991)

^c Essential amino acid score of raw seeds/ roasted seeds based on FAO/WHO recommended pattern

^d Methionine + cystine

^e Phynylalanine + tyrosine

Amino acid compositions of raw or roasted seed, whole egg protein (FAO, 1968), FAO/WHO reference pattern EAA, and EAA score are given in **Table 6**. There were minimal losses of all amino acids from heat treatment, roasting, (except arginine and glutamic acid), contributed to the slight difference of EAA score between raw and roasted seeds. The EAA score of raw and roasted seeds, ranged between 13 (tryptophan) and 166 (methionine), and almost all EAA are above the suggested pattern of requirement by FAO/WHO, 1991 with the exception of tryptophan, histidine, threonine, and lysine.

The limiting amino acids in most legumes are sulphur amino acid (methionine + cysteine) and tryptophan (Oliveria, 2000). Venkatachalam et al. reported tryptophan to be the first limiting essential amino acid in nuts including macadamia, pecan and pistachio, while Ruggerinet et al (1998) found that lysine was the first essential amino acid in almond, hazelnut, pecan, pine nut, pistachio and walnut (Venkatachalam & Sathe, 2006; Ruggeri et al, 1998)

Like other nuts, the first and second limiting essential amino acid of TC seed are tryptophan (EAA score 13%) and lysine (EAA score 42%). This suggested that TC seeds are deficient in both amino acids. Similar result was reported by Oliveira et al

(2000) who concluded that lysine and tryptophan are limiting amino acid in raw TC seeds (Oliveria et al, 2000). While, EAA score of lysine (42%) from the current study was close to that of the Oliveria study (40%), the one of tryptophane (13%) was quite different from the Oliveira study (93%). Part of this variation may be due to the difference between HPLC and colorimetric methods for tryptophan measurement and cultivar differences.

Fatty acid composition

The data showed that TC seeds mainly consisted of unsaturated fatty acids with a percentage of 63.90, and the most abundant unsaturated fatty acids fatty acids in seed oil were oleic acid (C18:1) (32.4%), followed closely by linoleic acid (C18:2) (30.3%), indicating good sources of essential fatty acids. The fatty acid profiles found were consistent with Ajayi et al, 2008, but were different in the lipid content in terms of total saturated lipid (36.15% and 43.92%), and unsaturated lipid (63.90% and 56.08%). Similar to other nuts and other vegetable oil (**Tables 7 and 8**) except coconut oil, palmitic acid is the predominant saturated fatty acid (29.95 %) in TC seeds followed by

stearic acid (6.10 %). The oil contained phytosterol (β -sitosterol, campesterol) contents in TC seed oil was 67.97 mg/100 g (data not shown).

Table 7 Fatty acid composition of TC seeds compared with other nuts (g/100 g lipid)

Fatty acid	TC seeds	Almond*	Peanut*	Cashew nut*
<i>Saturated fatty acids</i>				
Myristic acid (C14:0)	0.10	0.06	0	0.03
Palmitic acid (C16:0)	29.95	7.36	6.20	10.70
Margaric acid (C 17:0)	0	0.05	0.07	0.12
Stearic acid (C18:0)	6.10	1.56	2.06	9.33
Arachidic acid (C 20:0)	0	0.06	1.03	0.63
Behenic acid (C22:0)	0	0	2.22	0.12
Lignoceric acid (C24:0)	0	0	1.28	0
Total	36.15	9.09	12.86	21.12
<i>Monounsaturated fatty acid</i>				
Palmitoleic acid (C16:1)	0.40	0.66	0.07	0.54
Oleic acid (C18:1)	32.4	60.93	81.28	61.15
Eicosenoic acid (C20:1)	0.70	0	0	0
Total	33.50	61.60	81.49	61.68
<i>Polyunsaturated fatty acid</i>				
Linoleic acid (C18:2), n-6	30.30	29.21	3.87	16.88
Gamma linolenic acid (C18:3),n-6	0.10	0.10	1.79	0.32
Total	30.40	29.31	5.66	17.19
P/S**	1.77	10.01	6.78	3.74
S:M:P***	1.2:1.1:1	1:6.8:3.2	2.3:14:1	1.2:3.6:1

*The data by Venkatachalam and Sathe, 2006

** Unsaturated fatty acids/saturated fatty acids

***Saturated fatty acid/Monounsaturated fatty acid/Polyunsaturated fatty acid

Oleic acid, the monounsaturated fatty acid, showed the highest percentage of composition of 32.40 %. Many studies suggest that high intake of monounsaturated fat (MUFA) has been associated with protection against CHD. The mechanisms are thought to be their effect on plasma LDL (bad cholesterol) and HDL (good cholesterol) cholesterol levels, and involvement in the oxidative modification of LDL particles (Pe´rez-Jime et al, 1999). Diet rich in PUFA causes LDL particles to be more susceptible to oxidation than a diet rich in MUFA (Reaven et al, 1991, 1994; Bonanome et al, 1992; Aviram & Elias, 1993). Apart from sesame oil, TC seed contained the highest MUFA level, in particular oleic acid (18:1, n-9) relative to vegetable oil including coconut, palm, and soybean oil (**Table 8**). Nevertheless, it was the lowest MUFA content when compared with other nuts (**Table 7**).

Total saturated fatty acid content of TC was higher than other nuts and soybean oil (**Table 7 and 8**), but it was lower than coconut, and palm oil. The palmitic acid (C16:0) content of TC seed was also the highest compared to other nuts and other vegetable oils except palm oil. Although abundant evidence showed that some dietary saturated fatty acids raise serum TC (triglyceride), LDL, and HDL, palmitic acid (C16:0) is the second impact strength among three types of cholesterol-raising saturated fatty

acids including lauric acid (C12:0), palmitic acid (C16:0) and myristic acid (C14:0)

(Grundy, 1997; Wahrburg, 2004). It has been accepted that PUFA fatty acids have a

positive impact on the LDL/HDL ratio, reducing the risk of heart disease However, the

effects of lipid on a healthy heart is not only the types of lipid either saturated fatty acid

or unsaturated fatty acid, but also their content or the proper balance of fatty acids ratio.

Table 8 Fatty acid profile of TC seed compared with oilseeds (vegetable oil)

Fatty acid	TC seeds	Coconut oil*	Sesame oil*	Soybean oil*	Palm oil*
<i>Saturated fat</i>					
C6:0	0	0.5	0	0	0
C8:0	0	8.0	0	0	0
C10:0	0	6.4	0	0	0
C11:0	0	0	0	0	0
C12:0	0	48.5	0	0	0.3
C14:0	0.10	17.6	0	0.1	1.1
C16:0	29.95	8.4	9.9	11	45.1
C 17:0	0	0.05	0.00	0	0
C18:0	6.10	2.5	5.2	4	4.7
C19:0	0	0	0	0	0
C 20:0	0	0.1	0	0.3	0
C22:0	0	0	0	0.1	0
C24:0	0	0.06	0	0	0
Total	36.15	92.11	15.10	15.5	51.20
<i>Monounsaturated fat</i>					
C14:1	0	0	0	0	0
C16:1	0.40	0	0.3	0.1	0.1
C18:1	32.4	6.5	41.2	23.4	38.8
C20:1	0.70	0	0	0	0
Total					

	33.50	6.5	41.50	23.5	38.9
<i>Polyunsaturated fat</i>					
C18:2, n-6	30.30	1.5	43.3	53.2	9.4
C18:3,n-3	0.10	0	0.2	7.8	0.3
Total	30.40	1.5	43.5	61	9.7
P/S**	1.8	0.1	5.6	5.5	0.95
S:M:P	1.2:1.1:1	61.4:4.3:1	1:2.8:2.9	1:1.5:3.9	5.3:4:1

* Data from Chow, 1992

** Unsaturated fatty acids/saturated fatty acids

***Saturated fatty acid/Monounsaturated fatty acid/Polyunsaturated fatty acid

According to the fat dietary guideline of the current National Cholesterol Education Program (NCEP) and American Heart Association (AHA) for the heart health promotion by dietary fat, the optimum fat intake is recommended at percentage of total dietary calories which are 30 to 40%. Beside of fat intake amount, the optimum fatty acid balance (ratio of saturated fatty acids (SFA)) to monounsaturated fatty acid (MUFA) to polyunsaturated fatty acid (PUFA) is recommended at approximately 1:1:1 or 1:1.5:1 to keep the best LDL/HDL ratio (Hayes, 2002). Significant deviation from optimum fatty acid ratio such as too low SFA or too high MUFA or PUFA induces an undesirable lipoprotein profile. Fatty acid balance of TC seed, 1.2:1.1:1 for SFA: MUFA: PUFA is

relatively close to fat dietary guideline of NCEP and AHA compared with other vegetable oil (**Table 8**).

With respect to individual fatty acid results from current studies, fatty acid profile of TC seeds was more similar in composition to conventional vegetable edible oil especially sesame and soybean oil than other nuts including peanut, cashew nut and almond. There is a promising potential capacity to develop TC seeds in terms of dietary oil, rather than as indigenous snacking nuts just like, peanut, cashew nuts. Further study is needed to investigate on the TC oil quality for potential use as an alternative edible oil.

Antinutritional compounds

Seed often contains antinutritional components such as phytate, oxalate, trypsin inhibitor, tannin and polyphenol which are toxic to animals and humans. These compounds need to be removed or inactivated by various steps in processing, including washing and heat treatment, prior to an application in diet. Some of antinutritional factors such as phytate, trypsin inhibitor, goitrogen, antivitamin factors, cyanogens, and tannin are heat-labile, while others such as alkaloids, flavonoids, saponin, and oxalate are heat-stable (Seena et al, 2005).

Table 9 Nutrient and antinutritional components of seed of *Terminalia catappa***in dry weight basis (n=3)**

Components	Raw seeds	Roasted seeds*
Protein (%)	19.800±0.36 ^a	19.16±0.14 ^a
Fat (%)	55.08±1.13 ^a	53.47±0.35 ^a
Ash (%)	4.87±0.10 ^a	4.83±0.13 ^a
Total phenolics (mg GAE/g)	64.91±2.67 ^a	51.09±0.67 ^b
Trypsin inhibition activity (TIU/mg)	2.25±0.35 ^a	1.60±0.01 ^b
Phytate (IP6, mg/100g)	1,762.71±8.39 ^a	1,508.82±10.19 ^b
Phytate (IP5, mg/100g)	299.40±1.06 ^a	600.68 ± 3.4 ^b
Phytate (IP4, mg/100g)	48.87± 0.61 ^a	166.56 ± 0.85 ^b

* Roasting temperature at 180 °C for 4 minutes.

Means within each row with different letters (a, b) are significantly (P < 0.05) different

Despite all their nutritional potential, there is no data available on the effect of heat treatment conditions on trypsin inhibitor activity (TIA), phytate content (myo-inositol phosphate), and phenolic content present in the TC seeds, which have a detrimental effect on mineral absorption such as Fe and Zn. Generally, Inositol hexaphosphate (IP6) is a major form of phytate in raw food (Lehrfeld & Morris, 1992; Phillippy et al, 2004), and many data indicated that those inositol phosphates in the forms of inositol mono-, di-, tri- and tetraphosphates (IP1, IP2, IP3, and IP4) have no detrimental effect on mineral

absorption probably due to their lesser capacity to bind metal ions as well as the more soluble complexes (Brune et al, 1992; Somsab et al, 2008). Nevertheless, IP5 and IP6 have stronger inhibitory effects on micro-nutrient absorption than the other inositol forms (Sandberg et al, 1999). By anion-pair reverse phase high-performance liquid chromatography measurement, the current result showed that initial total phytate content (IP4+IP5+IP6) in TC seeds was $2,110.98 \pm 10.07$ mg/100 g (dw). The phytate contents of TC seeds were comparable with peanut (170-4,470 mg/100 g Dw), almond (350-9,420 g/100 g Dw), and cashew nut (190-4,980 g/100 g Dw) (Schlemmer et al, 2009). According to Sandberg and Anderine (1986), phytate elimination or reduction is to degrade IP6 into other forms of phytate (Sandberg, 1986). Roasting the TC seeds at 180°C for 4 min had decreased IP6 level while increasing the IP 5 level and the IP4 level (**Table 9**).

Trypsin inhibitor (TI) occurs naturally in many plant foods, notably soybean and peanut protein products, and has impact on reducing protein digestibility and is associated with growth inhibition and hypertrophy of the pancreas (Liener, 1994). Heat treatment is widely used to inactivate TI, improving nutritional quality. It was reported that *T catappa* seeds contained only negligible amount of trypsin inhibitor (37.3 g/Kg)

(Grant, 1995). The current result indicated that trypsin inhibitor activity (TIA) of raw seeds was initially 2.25 TIU/mg sample. The activity of trypsin inhibitor was reduced by 29 % by roasting at 180°C for 4 mins.

Furthermore, the study showed that roasting treatment reduced the antinutritional factor phytate, trypsin inhibitor, and phenolic content, but did not alter nutritional content including the protein, fat, and ash. Evaluation of nutritional properties of raw and roasted TC seeds in the current study revealed that these seeds are promising for food application due to the comparable nutritional content and quality to other food mentioned in the study. However, considering nutrient absorption, the antinutritional, heat treatment process including wet and dry heat on antinutritional properties such as oxalate, hemagglutinating activity, alkaloids, proteolytic activity need to be further investigate.

Extraction and Physico-chemical properties of *Terminalia catappa* oil

Solvent extraction of TC seed oil was conducted by three methods which are Soxhlet extraction and maceration using Grant OLS 200 orbital shaker with and without

temperature. Hexane was used as extraction solvent. The extraction yield percentage registered in **Table 10**.

Table 10 Extraction percentage yield of TC oil from different condition

Extraction methods	% Yield
Maceration (A)	49.49 ^b
Maceration (Grant OLS 200 orbital shaker) at 55 °C (B)	51.16 ^b
Soxhlet extraction (C)	57.5 ^a

^{ab}Values in the same row with different superscripted letters are significantly different ($p < 0.05$)

The oil extraction yield by Soxhlet extraction (C) is the highest (57.5%) compared with maceration methods (A, B), and the oil extraction yield of the maceration extraction with and without the accelerated condition are not statistically significant. Accelerated condition by temperature at 55 °C together with shaking are not affected the yield of TC oil.

Physicochemical analysis of extracted oil

Physicochemical properties of TC crude oil extracted by three different methods are presented in **Table 11** and **Table 12**. Both physical and chemical characteristics of the oil showed its quality as good as vegetable oil. Physical properties of TC oil by three different methods are not significant different.

Table 11 Physical properties of TC oil extracted by different methods

Chemical properties	Extraction methods		
	Maceration (A)	Maceration at 55 °C (B)	Soxhlet extraction (C)
Specific gravity (at 25 °C)	$0.9^b \pm 0.001$	$0.9^b \pm 0.001$	$0.9^b \pm 0.001$
Moisture (%)	$5.4^a \pm 0.2$	$5.4^a \pm 0.3$	$5.4^a \pm 0.2$
Viscosity (cP)	$37.7^a \pm 1.1$	$36.3^a \pm 1.0$	$33.5^b \pm 1.1$
Color			
L	$13.60^b \pm 0.47$	$15.84^a \pm 1.15$	$6.74^c \pm 0.40$
a	$1.71^a \pm 1.15$	$-0.06^{an} \pm 2.01$	$-0.20^{as} \pm 0.99$
b	$6.17^a \pm 1.23$	$4.46^a \pm 0.51$	$5.21^a \pm 1.27$

^{ab} Values in the same row with different superscripted letters are significantly different ($p < 0.05$)

The chemical characteristic of TC crude oil extracted by three methods showed are not significant different as shown in Table 12 except for peroxide value. Soxhlet extraction caused peroxide value higher than maceration since it is involved in high temperature.

Oil extracted by A methods and C method were chose to study the effect of degumming and neutralization since oil properties extracted by three methods are not significant different.

Table 12 chemical properties of TC crude oil extracted by different methods

Chemical properties	Extraction methods		
	Maceration (A)	Maceration at 55 °C (B)	Soxhlet extraction (C)
Saponification value (mgKOH/oil 1 g)	179.7 ^b ± 10.2	179.8 ^b ± 7.2	178.7 ^b ± 8.9
Unsaponification value (% by weight)	0.8 ^b ± 0.3	0.8 ^b ± 0.5	1.0 ^b ± 0.3
Acid value (mg KOH/oil 1 g)	2.0 ^a ± 0.3	2.1 ^a ± 0.2	2.3 ^a ± 0.3
Free fatty acid (% oleic acid)	1.0 ^a ± 0.1	1.1 ^a ± 0.1	1.2 ^a ± 0.1
Peroxide Value (milliequiv / oil 1 kg)	0.3 ^a ± 0.1	0.3 ^a ± 0.1	0.7 ^b ± 0.1

^{ab} Values in the same row with different superscripted letters are significantly different (p < 0.05)

Table 13 Characteristics of *T cattapa* seed oil compared with soybean oil

Properties	Soybean oil	TC crude oil	Standards of oil ^a
%Total lipid content	20.59 ^b ±0.72	58.90 ^a ±0.71	
Acid value (mg KOH/oil 1 g)	2.62±1.2	2.33±0.21	< 4
% FFA as oleic acid	1.31±0.61	1.17±0.13	
Saponification value (mg KOH/oil 1 g)	172.02±11.4	178.7±8.9	189-198
Unsaponification value (% by weight)	2.15±0.29	1.93±0.33	< 15
Peroxide value (milliequiv / 1Kg)	1.13±0.25	0.65±0.10	< 10
Color			
a*	1.38 ^a ±0.19	(-)0.95 ^b ±0.03	
b*	10.50 ^a ±0.22	3.54 ^b ±0.23	
L*	10.90 ^b ±0.11	19.73 ^a ±0.04	
Metal (mg/kg)			
Fe		< 0.1	< 2.5
Cu		< 0.1	< 0.1
Pb		< 0.1	< 0.1
As		< 0.1	< 0.1

^{ab}Values in the same row with different superscripted letters are significantly different (p < 0.05)

¹ Thai industrial standards for edible oil

Color

In this coordinate system, the a^* value is a measure of greenness ranging from -100 to +100 of redness, the b^* value ranges from -100 (blueness) to +100 (yellowness) while the L^* value indicating the measure of lightness range of 0 (black) to

100 (white). L^* value of TC seed oil by Soxhlet extraction (19.7) was significantly different from those of soybean oil (10.9). The higher L^* of TC seed oil make the oil lighter in color than soybean oil, while greenness and redness (a^* and b^* value) of TC oil were significantly lower than those of soybean oil. However, the oil obtained is crude oil which does not undergoes any refined, bleach and deodorized (RBD) processes. The other physical properties including the viscosities, refractive index as well as melting point of the extracted seed oil are in the process.

Chemical properties

TC seed contains a high percentage of total lipid content of 58.9 % compared to soybean (20.6%). The acid value of oil is dependent on the amount of free fatty acids present or on the degree of hydrolysis of the oil. Acid value of TC seed oil shows a comparative low (2.3) due to its low in free fatty acid (1.71), and both acid value and free fatty acid value of TC seed oil were significantly lower than soybean oil. This suggests their application as good edible oil. Peroxide value of TC seed oil exhibited a very low value (as crude seed oil) of 0.65, acceptable levels of PV which were less than 10 units, proving the oxidative stabilities of the seed oil. The high oxidative stability of TC seed oil compared to soybean oil shows that TC seed oil may possess the good

qualities as edible oil purposes or can be stored for a long period without deterioration.

Normally, oils become rancid when the peroxide value ranges from 20.0 to 40.0 mg/g oil

(Babalola & Apata, 2011)

The saponification number of TC seed oil showed significantly high number of 178.7 compared to soybean oil with the saponification number of 172, suggesting that TC oil contain high proportion of fatty acids of low molecular weight. The saponification values of TC oil was comparable to the one of safflower, sunflower and corn oil with average saponification numbers ranging between 191 and 250 (Babalola & Apata, 2011) Nonetheless, the TC seed oil showed a low unsaponifiable matter of 1.93%.

According to the food standard regulation of edible oil based on ministry of industry, the concerned metal amounts in edible oil including iron, arsenic, copper, lead have to be less than 2.5 mg/Kg for iron and 0.1 mg/Kg oil for arsenic, copper and lead.

These metal levels of TC oil extracted by soxhlet extraction are less than 0.1 mg/Kg.

Table 14 fatty acids profiles of oil in different extraction condition

Fatty acid	Maceration (A)	Maceration with temperature (B)	Soxhlet extraction (C)
<i>Saturated fat</i>			
C14:0	0.10	0	0
C16:0	29.95	33.08±0.81	31.28± 10.26
C 17:0	0	0	0.00
C18:0	6.10	6.06±0.21	8.46± 0.41
C19:0	0	0	0
C 20:0	0	0.75±0.03	1.03±0.06
Total	36.15 ^a	39.89 ^b	40.77 ^b
<i>Monounsaturated fat</i>			
C16:1	0.40	0	0
C18:1	32.4	24.65±1.97	31.72±13.56
C20:1	0.70	0	0
Total	33.50 ^a	24.65 ^b	31.72 ^a
<i>Polyunsaturated fat</i>			
C18:2, n-6	30.30	31.07±0.92	22.92±3.77
C18:3,n-6	0.10	0	0.11±0.01
Total	30.40 ^a	31.07 ^a	23.03 ^b

^{ab}Values in the same row with different superscripted letters are significantly different ($p < 0.05$)

Fatty acids of oil extracted by different methods

Fatty acid contents of TC oil extracted from three different methods is shown in

Table 14 Total saturated fatty acids from soxhlet (C) and maceration with temperature and shaking extraction (B) were significantly give higher than maceration extraction (A). However, unsaturated fatty acid contents of TC oil extracted by both soxhlet (C) and maceration with temperature and shaking extraction (B) were significantly lower than of maceration extraction (A). It is possible that extraction condition with temperature involving cause loss of unsaturated fatty acids since unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids.

Oil Refining

TC crude oil extracted by maceration and soxhlet extraction were refined by degumming and neutralization. The result as in **Table 15** showed reduction of acid value and free fatty acid value, but increase in peroxide value. The extraction method did not significantly have effect on the chemical properties after partially refining. Acid values of these extractions were reduced by 86 % and free fatty acid value was reduced by 88%. Both acid value and free fatty acid value of TC oil extracted by two methods

were significantly less than soybean oil, leading to reduction percentage of acid value

and free fatty acid value of soybean oil was statistic significantly less than TC oil.

Peroxide value of soybean oil (5.25 milliequiv/oil 1 Kg) was significantly higher than TC

oil (1.75, 2.42 milliequiv /1 Kg). The increasing of PV after refining may be attributed to

the open system of refining process which increases an exposure to oxygen.

**Table 15 chemical properties if TC oil after degumming and neutralization
compared with soybean oil**

Oil type	Extraction methods	Acid value (mg KOH /oil 1 g)	% acid value reduction	Free fatty acid value (oleic acid)	% free fatty acid reduction	Peroxide Value (milliequiv/oil 1Kg)	Increasing of peroxide value
TC	Hexane extraction	0.27±0.02 ^b	-86	0.14±0.01 ^b	-86	1.75±0.27 ^b	+6.7
	Soxhlet extraction	0.27±0.03 ^b	-88	0.14±0.02 ^b	-88	2.42±0.46 ^b	+3.6
Soybean	Hexane extraction	0.534±0.02 ^a	-76	0.27±0.01 ^a	-77	5.25±1.02 ^a	+7.0

^{ab}Values in the same row with different superscripted letters are significantly different (p < 0.05)

Table 16 Physico-chemical properties of TC oil extracted by soxhlet extraction after degumming and neutralization

Properties	Standards of edible oil ^a	TC crude oil	Refined oil
Acid value (mg KOH/oil 1 g)	< 0.6	2.34±0.02	0.27±0.03
Saponification value (mg KOH/oil 1 g)	189-198	178.71±8.91	-
Unsaponification value (% by weight)	< 15	0.99±0.33	-
Peroxide value (mequiv/1Kg)	< 10	0.65±0.10	2.42±0.46
Metal (mg/kg)			
Fe	< 2.5	< 0.1	< 0.1
Cu	< 0.1	< 0.1	< 0.1
Pb	< 0.1	< 0.1	< 0.1
As	< 0.1	< 0.1	< 0.1

Table 16 presents chemical properties of crude oil extracted by soxhlet extraction after degumming and neutralization. Oil extracted by soxhlet extraction was chosen due to the highest percentage yield as well as good chemical properties compared with two methods. The results showed that TC oil chemical properties after partially refining by degumming and neutralization are under the Thai industrial standards for edible oil.

6. Conclusion

In summary, the current results obtained have shown that *Terminalia catappa* seeds (locally known as Hu-Kwang in Thailand) are a good source of lipid, and contain micronutrients and amino acid pattern comparable to widely consumed nuts. Heat treatment by roasting the seed causes reduction of IP6, trypsin inhibitor activity, and phenolic compound value, but did not alter the protein and fat contents.

The fatty acid profile, compared with other oilseeds and nut oil, exhibited a 1:1:1 optimum ratio of S: M: P, which relatively close to the dietary recommendation of National Cholesterol Education Program (NCEP) and American Heart Association (AHA). Regarding the Physico-chemical properties of TC oil, there is a promising candidate as a dietary-oil.

Further study is needed to evaluate are other dietary toxic substances including oxalate, hemagglutinin, alkaloids, proteolytic activity, saponin and amylase inhibitor. In addition, the nutritional quality or protein digestibility including protein deficiency ratio (PER), relative PER (RPER), net protein utilization (NPU), and true nitrogen

digestibility (DIG) have to be investigated., and more research concerning on an application as dietary oil is required.

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APPENDIX A

Pictures of *Terminalia catappa* fruits and seeds (Kernel) and the kernel yield of

***Terminalia catappa* fruits**



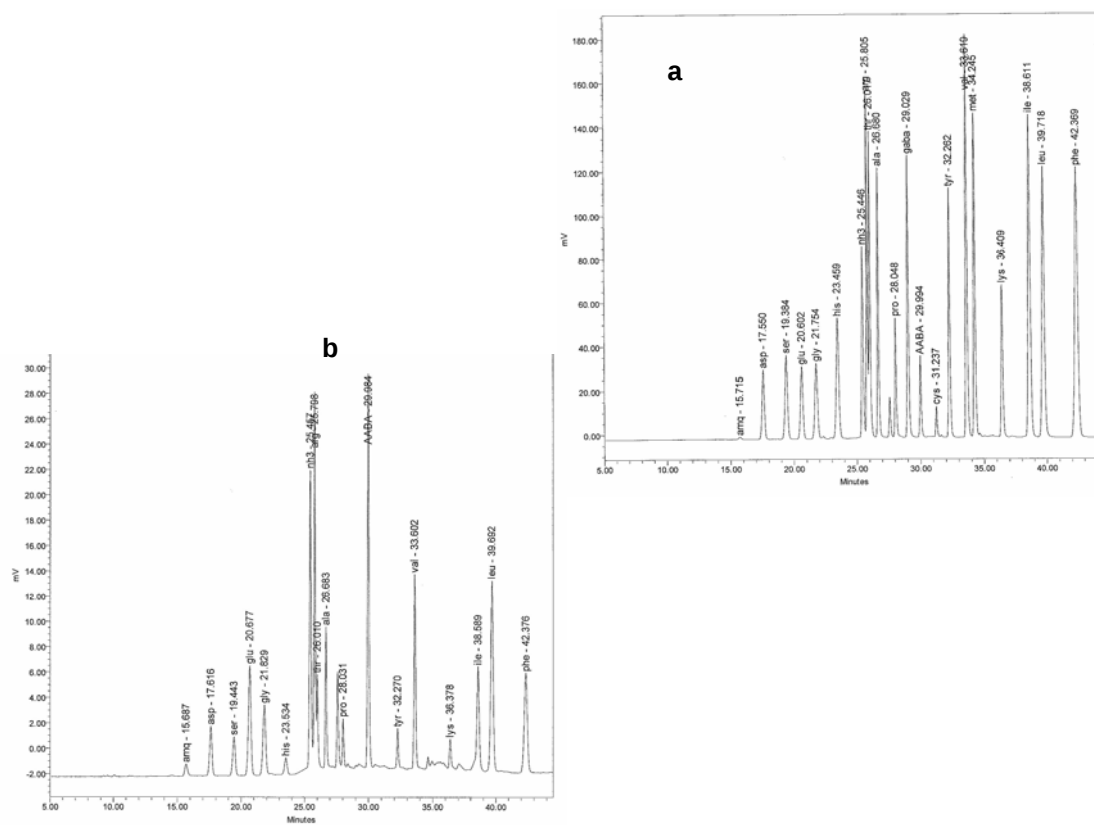
Fruit weight (g)	Kernel weight (g)	% yield	Width	Length
6.26 ± 0.34	0.21 ± 0.04	3.35	18.17±0.89	41.17±2.20

n=32 samples

APPENDIX B

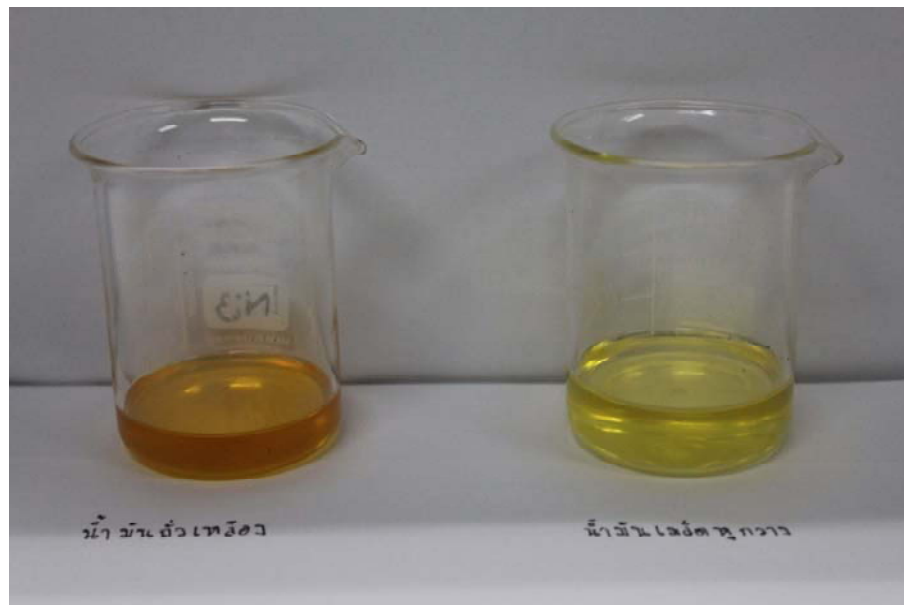
HPLC chromatograph of amino acid composition of TC seed; (a) standard,

(b) *TC* seeds.



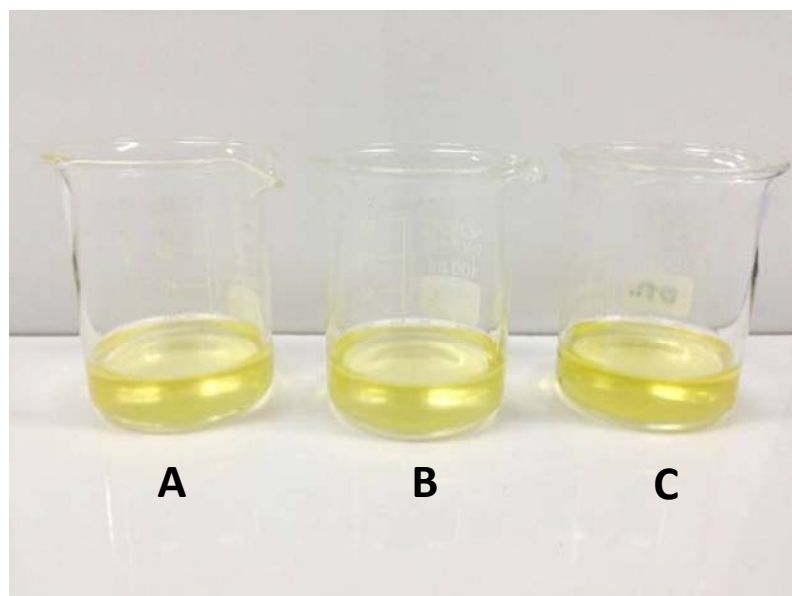
APPENDIX C

Picture of oil extracted from *Terminalia catappa* seeds compared with oil
extracted from soybean oil by soxhlet extractor



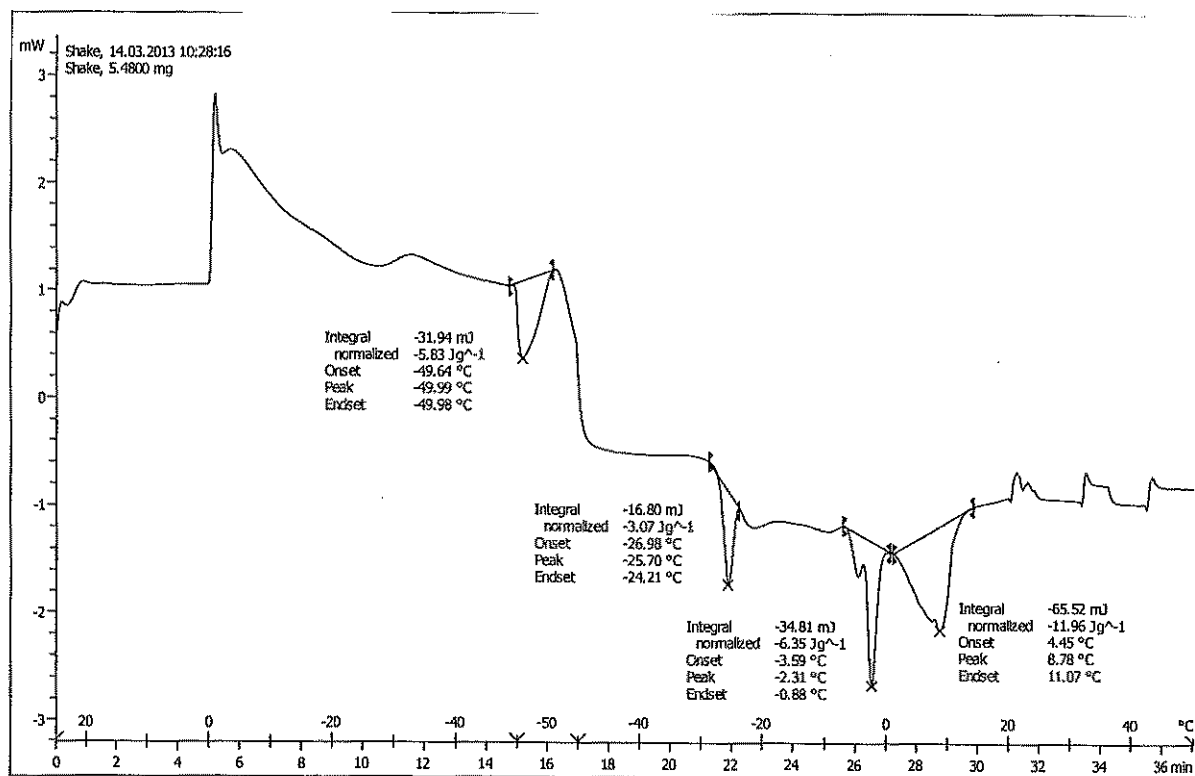
APPENDIX D

Picture of oil extracted from *Terminalia catappa* seeds by three different hexane extraction; A: hexane extraction, B: hexane extraction with accelerated condition, C: soxhlet extraction



APPENDIX E

Differential scanning calorimetry (DSC) melting curves of TC oil



Output

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Weerawatanakorn M, Chavasit V and Ho, CT. Physico-chemical properties of Terminalia catappa Linn seeds oil as edible oil. In process.