ผลของวิธีการสกัดที่มีต่อองค์ประกอบกรดไขมัน และคุณลักษณะทางเคมีและกายภาพของน้ำมันจากจิ้งหรีดทองแดงลาย (*Acheta domesticus*)

Effect of Extraction Methods on Fatty Acid Composition, and Chemical and Physical Characteristics of Oil from House Crickets (*Acheta domesticus*)

จิตราพร งามพีระพงศ์[1], กาญจนา นาคประสม[1], วิวัฒน์ หวังเจริญ[1], นันท์นภัส ระหงษ์[1], ธาดา ช่างประดิษฐ์[1], ธวัลรัตน์ วาสีอนุรักษ์[1], นัฐพล ตั้งสุภูมิ[2,*]

Chitraporn Ngampeerapong[1], Kanjana Nakprasom[1], Wiwat Wangcharoen[1], Nunnapat Rahong[1], Tada Changpradit[1], Thvunrud Waseeanuruk[1], Nattapol Tangsuphoom[2,*]

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บทคัดย่อ

จิ้งหรีดทองแดงลาย (*Acheta domesticus* (L.)) เป็นแหล่งโปรตีนคุณภาพสูง และมีไขมันเป็นองค์ประกอบที่มีปริมาณมากที่สุดรองจากโปรตีน น้ำมันจิ้งหรีดเป็นผลิตภัณฑ์พลอยได้จากกระบวนการผลิตโปรตีนจากจิ้งหรีด ซึ่งมักจะสกัดไขมันออกจากผงจิ้งหรีดก่อนนำไปสกัดโปรตีน การศึกษานี้ได้ประเมินคุณภาพของน้ำมันที่สกัดจากจิ้งหรีดทองแดงลายด้วยวิธีการสกัดต่างกัน ได้แก่ การสกัดด้วยตัวทำละลายเฮกเซนและการบีบอัดด้วยสกรู โดยเปรียบเทียบองค์ประกอบกรดไขมันและสมบัติทางเคมีกายภาพของน้ำมันที่สกัดได้ ผลการศึกษาพบว่าการสกัดด้วยตัวทำละลายทำให้ได้ผลผลิตน้ำมัน มากกว่าการบีบอัดประมาณ 1.6 เท่า องค์ประกอบกรดไขมันของน้ำมันที่สกัดได้ทั้ง 2 วิธี ไม่แตกต่างกัน โดยกรดไขมันที่มีปริมาณสูงสุดสามอันดับ ได้แก่ กรดลิโนเลอิก กรดโอเลอิก และกรดปาล์มมิติก น้ำมันจิ้งหรีดทองแดงลายมีสีเหลืองใส มีค่าดัชนีหักเห 1.465-1.466 อย่างไรก็ตามน้ำมันที่ได้จากการบีบอัดด้วยสกรูมีค่าความสว่าง (L*=19.63 vs. 13.92) มากกว่าน้ำมันที่สกัดด้วยเฮกเซน แต่มีค่าความเป็นสีเหลืองน้อยกว่า (b*=18.76 vs. 26.54) น้ำมันที่สกัดโดยการบีบอัดด้วยสกรูมีค่าเพอร์ออกไซด์ (7.93±0.02 vs. 25.12±2.24 meqO_2/kg) และค่าของกรด (4.95±0.03 vs. 5.83±0.19 mg KOH/g) ต่ำกว่า แต่มีค่าแอคติวิตีของน้ำ (0.357±0.004 vs. 0.245±0.002) สูงกว่าน้ำมันที่สกัดด้วยเฮกเซน ผลการศึกษาทั้งหมดสรุปได้ว่าการสกัดน้ำมันด้วยตัวทำละลายและการบีบอัดด้วยสกรูส่งผลต่อคุณภาพของน้ำมันจากจิ้งหรีดทองแดงลายในลักษณะเดียวกัน คุณลักษณะทางเคมีและกายภาพของน้ำมันจิ้งหรีดแสดงให้เห็นถึงศักยภาพในการใช้เป็นน้ำมันบริโภคในอาหาร

คำสำคัญ: จิ้งหรีดทองแดงลาย แมลงที่กินได้ การสกัดน้ำมัน น้ำมันบริโภค ส่วนผสมอาหารใหม่

ABSTRACT

House crickets (*Acheta domesticus* (L.)) are recognized as a high-quality protein source with fat being the second abundant component. Cricket oil is the by-product of cricket protein production, in which dried cricket powder is defatted prior to protein extraction. This study assessed the quality of oils extracted from house crickets using different methods, namely solvent extraction by hexane and screw pressing. Fatty acid composition and physicochemical properties of the obtained oils were compared. It was found that solvent extraction gave 1.6 times higher oil yield than screw pressing. The fatty acid composition of both oils was similar, of which linoleic acid, oleic acid and palmitic acid were the three most abundant fatty acids. Cricket oils were clear yellow with similar refractive indices (1.465-1.466). However, the oil from screw pressing was lighter in color (L*=19.63 vs. 13.92) than that extracted using hexane but exhibited less yellowness (b*=18.76 vs. 26.54). The screw-pressed oil also had a lower peroxide value (7.93±0.02 vs. 25.12±2.24 meqO_2/kg) and acid value (4.95±0.03 vs. 5.83±0.19 mg KOH/g), but higher water activity than its counterpart (0.357±0.004 vs. 0.245±0.002). In conclusion, solvent extraction and screw pressing had similar effect on cricket oil quality. The chemical and physical characteristics of the cricket oils suggest their potential as edible oils for use in food products.

Keywords: *Acheta domesticus* (L.), edible insect, oil extraction, edible oil, novel food ingredient

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้

[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

INTRODUCTION

Edible insects have been used as a food source around the world for centuries. Among these, house cricket (*Acheta domesticus*) is particularly notable for its economic value and is commonly farmed in Thailand for human consumption. Crickets are rich in protein, fats, minerals, vitamins, and some bioactive compounds such as sterols [1]. Fat is the second largest component in crickets after protein. Magara et al. [2] reported that fat content in cricket ranges from 9.80-22.80%, with linoleic (C18:2), palmitic (C16:0) and oleic (C18:1) being the major fatty acids present. Moreover, cricket oil contains the beneficial omega-3 and omega-6 fatty acids which offer health benefits including anti-inflammatory effects, as well as reducing the low-density lipoprotein (LDL) cholesterol levels while high-density lipoproteins cholesterol, hence lowering the risk of cardiovascular disease [3, 4]. Furthermore, cricket oil is also a source of phytosterols, like campesterol, stigmasterol, and beta-sitosterol, which are known to improve the lipid profiles by lowering LDL cholesterol and reducing intestinal cholesterol absorption [3].

Insect oil can be used as an ingredient in mayonnaises, vinaigrettes, as well as frying oil. Tzompa-Sosa et al. [5] used the oil from *Tenebrio molitor* instead of vegetable oil in crackers and houmous formulations. The results showed that replacement of vegetable oil by *Tenebrio molitor* oil did not affect the liking and visual appearance but impacted the flavor acceptability. Smetana et al. [6] studied the substitution of oils from *Hermetia illucens and Tenebrio molitor* for plant lipids in margarines. The results showed that the replacement at 75% did not have negative effects on spreading abilities but improved product color. However, studies on the use of cricket oil as food ingredient and edible oil are currently scarce.

Fat content, fatty acid composition, beneficial bioactive compounds and physicochemical properties of cricket oil depend on factor like sex, life stage and diet [2], as well as the fat extraction method used. Several lipid extraction methods like conventional solvents, or green novel techniques such as ultrasound-assisted extraction, pressurized liquid extraction, alternative novel techniques, microwaves extraction or supercritical fluid extraction have been studied [7]. Since lipids and fat-soluble compounds are primarily targeted, organic solvents with nonpolar and polar properties are likely be the most effective extracting medium. Various organic solvents such as aqueous solutions, chloroform, methyl-tert-butyl ether, and hexane-isopropanol, can also be used to extract fat from insects [8, 9]. Extraction using organic solvents gives the highest oil extraction yield but may cause disadvantageous effect on fatty acid composition of the extracted oil [10]. Conventional solvent extraction is also time-consuming, requires high purity solvent and carries the risk of solvent residue in the oil cake. As a result, physical extraction methods like pressing are often preferred. The screw press method, in particular, is cost effective and simple [11]. While pressing is commonly used to extract oil from seeds like walnut, flex seeds, and ground nuts, its application in extracting fat from insects is still limited.

As aforementioned, insect oils emerge as a sustainable and nutrient-rich food. In this study,

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University
[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

we determined the fatty acid composition and physicochemical properties of cricket oils extracted using different extraction methods, namely conventional hexane extraction and screw pressing. The information obtained from this study would fill a gap of knowledge on cricket oil extraction and its potential in compliance with existing standards and regulations on edible oils.

MATERIALS AND METHODS

1. Materials

House crickets (*Acheta domesticus*) aged 35-40 days were obtained from a local farm in Sukhothai, Thailand, at which they were fed with chicken feed and starved for 24 h prior to harvesting. Crickets were killed by blanching in water at an insect-to-water ratio of 1:5 at 95°C for 3 minutes, packed in sealed plastic bags and stored frozen at -20°C. The frozen crickets were transported in a temperature-controlled vehicle to our laboratory. Within 24 hours after receiving, drying was performed at 70°C in a hot air oven (FD115, BINDER GmbH, Tuttlingen, Germany) until the final moisture content of 10% was obtained. The drying yield was about 35%. Dried crickets were packed in vacuum-sealed plastic bags and kept at room temperature until being used for oil extraction. The proximate composition of dried crickets as analyzed by AOAC official methods was, 10% moisture, 17% fat, 52% protein, 4% ash, and 17% carbohydrate. Dried crickets were pooled and divided into two parts for subjecting to extraction using 2 different methods. All chemical reagents used in this study are analytical grade and obtained from Sigma-Aldrich (St. Louis, Mo., U.S.A.), unless stated otherwise.

2. Extraction of cricket oil

2.1 Screw pressing

Dried crickets were pressed by oil press machine (Mellix, Bangkok, Thailand). A 200g dried crickets were fed from the hopper to the screw press on demand by gravity. The temperature of screw press was 140°C and temperature of oil was not exceed than 65°C. After pressing, oil was centrifuged (XC-2450, Premier Scientific, Belfast, U.K.) at 4,000 rpm for 10 minutes to remove the residue and the oil was collected in screw-capped amber bottles, flushed with nitrogen and stored in a refrigerator at 10°C. Extraction was performed in triplicates and oil samples were analyzed within 2 weeks after preparation.

2.2 Solvent extraction

Dried crickets were ground using an electrical blender (HR2118/02, Philips, Jakarta, Indonesia) and sieved through a 20 Mesh screen. Ground crickets were mixed with *n*-hexane at a cricket-to-solvent ratio of 1:10 (w/v) in glass beakers. Extraction was performed at room temperature for 2 hours with continuous agitation by a magnetic stirrer (RT basic, IKA, Staufen, Germany). After extraction, the mixture was vacuum filtered through Whatman No. 1 filter paper and the obtained mixture was evaporated using a vacuum rotary evaporator (OSB-2200, EYELA, Shanghai, China) at 50°C to remove hexane. The oil was kept in nitrogen-flushed screw-capped amber bottles and stored in a refrigerator. Extraction was performed in

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้
[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

triplicates and oil samples were analyzed within 2 weeks after extraction.

3. Analyses

3.1 Oil yield

Oil yield of each extraction was calculated from Eq. (1).

% Oil yield
= (oil weight/dried cricket weight) x 100 (1)

3.2 Proximate analysis of residue from oil extraction

Moisture content was determined by a hot air oven at 105°C according to AOAC Official Method 925.40 [12]. The protein content was determined by the Kjeldahl method according to AOAC Official Method 948.22 [12], and a nitrogen to protein conversion factor of 6.25 was applied. The fat content was conducted by Soxhlet extraction method using hexane as solvent according to AOAC Official Method 950.48 [12]. The crude ash was determined by gravity method using the muffle furnace at 550°C as described in AOAC Official Method 950.49 [12]. Finally, the total carbohydrate was calculated from the following Eq. (2).

% Carbohydrate
= 100 – (%moisture+%protein+%fat+%ash) (2)

3.3 Fatty acid composition

Fatty acid composition was analyzed using an in-house method TE-CH-208 based on the AOAC Official Method 996.06 [12]. Analyses were performed by Central Laboratory (Thailand) Co., Ltd. (Chiang Mai, Thailand).

3.4 Chemical and physical characteristics

3.4.1 Color

The color of oil was measured with a colorimeter (Colorflex EZ 45-0 LAV, HunterLab, Reston, Va., U.S.A.). The results were expressed in the CIELAB system for which L* (0 = black, 100 = white), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness) were determined.

3.4.2 Water activity

Water activity (a_w) was measured with a water activity meter (Lab Touch-aw, Novasina AG, Lachen, Switzerland) at 25°C.

3.4.3 Refractive index

Refractive index was measured with an Abbe's refractometer according to AOAC Official Method 921.08 [13]. Cricket oil was dropped on the prism of Abbe's refractometer and recorded refractive index at 40°C.

3.4.4 Specific gravity

Specific gravity was determined using a 25 mL volumetric flask following the AOAC Official Method 920.21 [14]. An empty 25 mL volumetric flask was weighed then filled with cricket oil up to the mark and the flask was weighed again. In a similar procedure, water was used instead of cricket oil, and the weight of filled 25 mL volumetric flask was measured. The specific gravity was calculated as Eq. (3).

Specific gravity = (W2 - W1) / (W3 – W1) (3)

where: W1 is the weight (g) of empty 25 mL volumetric flask; W2 is the weight (g) of 25 mL volumetric flask filled with cricket oil weight;

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University
[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

and W3 is the weight (g) of 25 mL volumetric flask filled with water.

3.4.5 Peroxide value

Peroxide value (PV) was determined according to AOAC Official Method 965.33 [15]. Briefly, 5 g of cricket oil was dissolved in 25 mL of a 3:2 (v/v) glacial acetic acid and chloroform mixture. Then 1 mL of saturated potassium iodide was added into the flask. The solution was kept in the dark for 1 minute, prior to adding 30 mL of water and 2-3 drops of starch solution. The mixture was titrated with 0.01 N of sodium thiosulphate until the color changed from blue to colorless (end point). A parallel blank was done with the same procedure. The calculation of PV ($mEqO_2/kg$) was performed using Eq. (4).

$$PV = (VS - VB) \times N \times 1000) / W \quad (4)$$

where: VS is the volume (mL) of sodium thiosulphate titrated with the cricket oil; VB is the volume (mL) of sodium thiosulphate titrated with the blank; N is the normality (N) of sodium thiosulphate solution; and W is oil weight (g).

3.4.6 Acid value

The determination of acid value (AV) was performed following the AOAC Official Method 969.17 [15]. Cricket oil (2 g) was dissolved in 50 mL of neutralized ethyl alcohol then titrated with 0.1 N potassium hydroxide and phenolphthalein was used as the indicator. The calculation of AV (mg KOH/g) was performed using Eq. (5).

$$AV = (VS - VB) \times M \times 56.1) / W \quad (5)$$

where: VS is the volume (mL) of potassium hydroxide titrated with the oil; VB is the volume (mL) of potassium hydroxide titrated with the blank; N is the normality of potassium hydroxide solution (N); and W is oil weight (g).

3.4.7 Iodine value

Iodine value (IV) was determined according to AOCS Official Method To 1b-64 [16]. Cricket oil (0.3 g) was dissolved in 10 mL of chloroform, and 25 mL of a Wijs solution was added. The mixture was kept in the dark for 30 minutes, then mixed with 20 mL of 20% potassium iodide solution and 100 mL of water, followed by titration with 0.1 N sodium thiosulfate using a 1% starch solution as an indicator. The calculation of IV (g of iodine/g) was performed using Eq. (6).

$$IV = (VB - VS) \times N \times 12.69) / W \quad (6)$$

where: VB is the volume (mL) of sodium thiosulfate titrated with the blank; VS is the volume (mL) of sodium thiosulfate titrated with the oil; N is the normality of sodium thiosulfate solution; and W is oil weight (g).

3.4.8 Saponification number

Saponification number (SN) was determined using AOAC Official Method 920.160 [15]. Cricket oil (1 g) was weighed into a round-bottom flask. Then 25 mL of 0.5 M alcoholic potassium hydroxide was added, and the mixture was boiled under a reflux condenser for 30 minutes. The mixture was cooled and titrated with 0.5 M hydrochloric acid using phenolphthalein as an indicator. A blank was similarly prepared and analyzed. The calculation of SN (mg KOH/g) was performed using Eq. (7).

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้
[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

$$SN = (VB - VS) \times M \times 28.05 / W \quad (7)$$

where: VB is the volume (mL) of hydrochloric acid titrated with the blank; VS is the volume (mL) of hydrochloric acid titrated with the oil; M is the molarity of hydrochloric acid (M); and W is oil weight (g).

3.4.9 Unsaponifiable matter

The determination of the unsaponifiable matter (USM) was performed according to AOCS Official Method Ca 6b-53 [17]. Cricket oil (2 g) was weighted into weighed into round bottom prior to the addition of 25 mL of 95% ethanol and 1.5 mL of 50% KOH. Then the mixture was boiled under a reflux condenser for 30 minutes and the mixture was cooled and transferred to a separating funnel. Hot water (50 mL) was added into the flask then transferred to the same separating funnel. To extract USM, 50 mL of diethyl ether was added in to the same separating funnel three times. The mixture was left at room temperature until separation occurred. The diethyl ether phase was collected and washed with 0.5 N KOH three times followed by washing with water several times to obtain a neutral pH value. The diethyl ether phase was transferred into glass beaker then placed in the hot air oven until dry. The weight of residue was weight and USM was calculated using Eq. (8).

$$\% USM = (100 \times A) / W \quad (8)$$

where: A is the residue weight; and W is the oil weight (g).

4. Statistical analysis

All analyses were performed in triplicates and results were presented as means ± standard deviations of the three replicates. Data was analyzed by independent sample t-test at $p<0.05$. Statistical analysis was carried out using a statistical software (IBM SPSS Statistics for Windows Version 20, IBM, Armonk, N.Y., U.S.A.). Only significant different results are discussed in the text.

RESULTS AND DISCUSSION

1. Oil yield

As shown in Table 1, oil yield of solvent extraction using hexane was 12.39% while that obtained from screw pressing was 7.44%. Compared with screw press, solvent extraction using hexane gave 1.7 times higher oil yield than screw pressing. Previous studies also reported that solvent extraction using hexane had significant higher oil yield than screw pressing in various samples such as beauty leaf seed kernel (12%) [18], rapeseed (10-13%) [19], and walnuts (2%) [11]. Hexane is a non-polar organic solvent that is usually used to extract fat from food and non-food [20]. Oil extraction using hexane is relatively simple and easy, repeatable and reproductible results and process but the use of hexane had some limitations such as a limited solubility of the protein extracted from residue [21, 22], safety issues due to solvent contamination, environmental concerns and high processing cost unless hexane is recovered [11]. On the other hand, screw pressing destroys the cellular structure and making the oils be easier to extract by crushing and pressing. The efficiency of oil extraction using screw press

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University
[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

depends on various factors such as temperature, pressure, and speed of the screw press, size of the nozzle, and moisture content in sample [18].

Table 1 Oil yield of different extraction methods

Extraction method	Oil yield (%)
Screw press	7.44 ± 1.86^{b}
Solvent extraction	12.39 ± 0.78^{a}

Results are presented as means ± standard deviations of 3 replicates (n=3).

[a,b] means with different lower letter superscripts within the same column are significantly different ($p<0.05$).

2. Proximate composition of residue from oil extraction

The moisture content of cricket residue from solvent extraction method was about 2 times lower than that of residue from screw pressing (3.08 vs. 6.67 g/100 g). This is because the residue was dried in hot air oven to remove the residual hexane. In addition, the remaining fat in cricket residue from solvent extraction was about 3.5 times lower than its counterpart (3.04 vs. 10.60 g/100 g). The results confirmed that the solvent extraction was more effective in removing fat from the cricket. Other components such as ash and carbohydrate found in similar amount in residues from both extraction methods (Table 2).

Table 2 Proximate of cricket residues obtained from different oil extraction methods

Composition	Content (g /100 g residue)	
	Screw Press	Solvent extraction
Moisture	6.67 ± 0.01^{a}	3.08 ± 0.11^{b}
Protein	68.58 ± 0.79^{b}	76.33 ± 2.20^{a}
Fat	10.60 ± 0.95^{a}	3.04 ± 0.16^{b}
Ash	4.68 ± 0.06	5.32 ± 0.06
Carbohydrate	10.44 ± 0.38	12.22 ± 2.87

Results are presented as means ± standard deviations of 3 replicates (n=3).

[a,b] Means with different lower letter superscripts within the same row are significantly different ($p<0.05$).

3. Fatty acid composition

The fatty acid composition of cricket oils is listed in Table 3. Fatty acids in both screw press and solvent extraction method were present in similar pattern, and there was no significant difference in content of any of fatty acids in cricket oils obtained from both extraction methods ($p>0.05$). Linoleic acid, oleic acid, and palmitic acid were the three most abundant fatty acids in both extracted oils. These results are consistent with the fact that the major fatty acids in cricket were linoleic acid, palmitic acid, and oleic acid) [2]. Screw-pressed and solvent extracted cricket oils showed the ratio of saturated fatty acids (S): monounsaturated fatty acids (M): polyunsaturated fatty acids (P) of 1:0.8:1 and 1:0.7:1, respectively, which is close to the recommendation for healthy diet. To reduce risk of cardiovascular disease and non-communicable diseases, World Health Organization recommends limiting the saturated

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้
[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

fatty acids and increasing unsaturated fatty acids consumption and the ideal of S:M:P in diet is 1:1:1 up to 1:1.5:1 [23]. Both cricket oils contained similar amounts of omega-3 and omega-6 fatty acids, with the omega-3 fatty acids content of <1 g/100 g. The ratio of omega-3 to omega-6 fatty acids was about 1:32 for screw-pressed oil and 1:36 for solvent-extracted oil (Table 3). The recommendation ratio of omega-3 and omega-6 fatty acids for a healthy diet is 1:4 to 1:10 [23]. Both cricket oils contained <0.5 g *trans* fat in 100 g, allowing them to be claimed as "no *trans* fat" or "*trans* fat free", as per the Thai regulation [24].

Table 3 Fatty acid composition of cricket oils obtained from different extraction methods

Fatty acids	Content (g/100 g oil)	
	Screw press	Solvent extraction
Butyric acid (C4:0)	ND	ND
Caproic acid (C6:0)	0.06 ± 0.03	0.06 ± 0.02
Caprylic acid (C8:0)	ND	ND
Capric acid (C10:0)	0.01 ± 0.00	0.02 ± 0.00
Undecanoic acid (C11:0)	ND	ND
Lauric acid (C12:0)	0.11 ± 0.01	0.13 ± 0.01
Tridecanoic acid (C13:0)	ND	ND
Myristic acid (C14:0)	0.70 ± 0.06	0.63 ± 0.02
Pentadecanoic acid (C15:0)	0.13 ± 0.00	0.12 ± 0.01
Palmitic acid (C16:0)	25.88 ± 2.64	24.37 ± 1.01
Heptadecanoic acid (C17:0)	0.32 ± 0.02	0.31 ± 0.04
Stearic acid (C18:0)	8.49 ± 0.10	8.92 ± 0.16
Arachidic acid (C20:0)	0.51 ± 0.62	0.96 ± 0.06
Heneicosanoic acid (C21:0)	1.22 ± 0.00	1.08 ± 0.00
Behenic acid (C22:0)	0.27 ± 0.13	0.42 ± 0.13
Tricosanoic acid (C23:0)	0.01 ± 0.00	0.04 ± 0.00
Lignoceric acid (C24:0)	0.06 ± 0.04	0.09 ± 0.00
Myristoleic acid (C14:1)	0.03 ± 0.00	0.03 ± 0.00
cis-10-Pentadecenoic acid(C15:1n10)	ND	ND
Palmitoleic acid (C16:1n7)	1.03 ± 0.07	0.90 ± 0.04
cis-10-Heptadecenoic acid (C17:1n10)	0.11 ± 0.00	0.10 ± 0.00
Trans-9-Elaidic acid (C18:1n9t)	0.21 ± 0.08	0.30 ± 0.01
cis-9-Oleic acid (C18:1n9c)	26.08 ± 2.86	24.21 ± 1.27
cis-11-Eicosenoic acid (C20:1n11)	0.54 ± 0.64	0.49 ± 0.58
Erucic acid (C22:1n9)	0.75 ± 1.03	0.70 ± 0.84
Nervonic acid (C24:1n9)	0.13 ± 0.12	0.16 ± 0.06

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University

[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

Fatty acids	Content (g/100 g oil)	
	Screw press	Solvent extraction
trans-Linolelaidic acid (C18:2n6t)	ND	ND
cis-9,12-Linoleic acid (C18:2n6)	32.57 ± 1.42	35.05 ± 0.22
gamma-Linolenic acid (C18:3n6)	0.02 ± 0.01	0.05 ± 0.02
alpha-Linolenic acid (C18:3n3)	0.64 ± 0.02	0.61 ± 0.01
cis-11,14-Eicosadienoic acid (C20:2)	0.15 ± 0.12	0.16 ± 0.13
cis-8.11.14-Eicosatrienoic acid (C20:3n6)	0.12 ± 0.04	0.13 ± 0.06
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.08 ± 0.00	0.04 ± 0.03
Arachidonic acid (C20:4n6)	0.28 ± 0.18	0.34 ± 0.04
cis-13,16-Docosadienoic acid (C22:2)	0.07 ± 0.00	0.06 ± 0.02
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.25 ± 0.30	0.25 ± 0.25
4,7,10,13,16,19 Docosahexaenoic acid (C22:6n3)	ND	ND
Saturated fatty acids (S)	37.13 ± 1.00	36.54 ± 0.66
Monounsaturated fatty acids (M)	28.80 ± 1.17	26.81 ± 0.09
Polyunsaturated fatty acids (P)	34.08 ± 2.16	36.66 ± 0.57
S:M:P ratio	1:0.8:0.9	1:0.7:1
Trans fatty acids	0.21 ± 0.08	0.30 ± 0.01
Omega 3 fatty acids	0.92 ± 0.34	0.89 ± 0.28
Omega 6 fatty acids	32.98 ± 1.65	35.55 ± 0.18
Omega 9 fatty acids	26.96 ± 1.72	25.05 ± 0.37

ND Not detected

Results are presented as means ± standard deviations of 3 replicates (n=3).

4. Chemical and physical characteristics

Cricket oils obtained from both screw pressing and solvent extraction were clear yellow color liquids at room temperature (Figure 1).

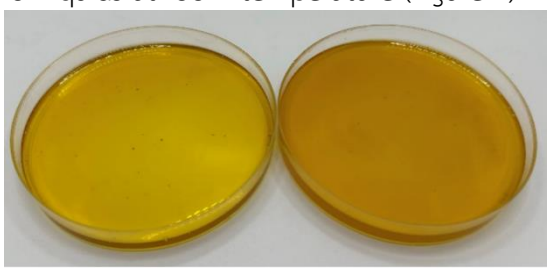

Figure 1. Photograph taken of cricket oils obtained from extraction using (a) screw pressing and (b) solvent extraction

Oil from screw pressing appeared in a less intense color than its counterpart. This was confirmed by its higher L* (19.63 vs. 13.92), although the less yellowness (lower b*) was observed for screw pressed cricket oil (18.76 vs. 26.54) (Table 4). The color of cricket oils resulted from lipid-soluble pigments such as carotenoids, anthraquinonoids, xanthoaphins, and melanin [25] that could be co-extracted with the oil by hexane better than screw pressing. Therefore, oil from solvent extraction method exhibited greater yellowness (higher b*) than that obtained from screw pressing.

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้

[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

Table 4 Chemical and physical characteristics of cricket oils obtained from different extraction methods

Chemical and physical characteristics	Screw Press	Solvent extraction
Color		
L*	19.63 ± 0.51[a]	13.92 ± 1.60[b]
a*	3.90 ± 0.80	4.77 ± 1.09
b*	18.76 ± 2.51[b]	26.54 ± 0.68[a]
Water activity	0.357 ± 0.004[a]	0.245 ± 0.003[b]
Refractive Index at 40°C	1.46 ± 0.00	1.46 ± 0.00
Specific gravity	0.88 ± 0.02	0.87 ± 0.03
Peroxide value (mEq O_2/kg)	7.93 ± 0.02[b]	25.12 ± 2.24[a]
Acid value (mg KOH/g)	4.95 ± 0.02[b]	5.83 ± 0.19[a]
Iodine value (g of iodine/g)	49.56 ± 0.89	49.99 ± 4.40
Saponification number (mg KOH/g)	273.24 ± 11.05[a]	224.45 ± 7.84[b]
Unsaponifiable matter (%)	6.60 ± 0.51[b]	8.15 ± 0.67[a]

Results are presented as means ± standard deviations of 3 replicates (n=3).

[a,b] means with different lower letter superscripts within the same row are significantly different ($p<0.05$).

Water activity (a_w) of cricket oil obtained from solvent extraction was significantly lower than that from screw pressing (0.245 vs. 0.357) (Table 4). Water activity is important for the shelf life of food products. Rate of lipid oxidation is known to be lowest in foods with a_w of 0.2-0.3 [26, 27]. Therefore, both methods could provide oils with appropriate a_w. Refractive index (RI) of both cricket oils were similar at 1.46, indicating the absent effect of extraction methods (Table 4). Interestingly, RI of both cricket oils were closely the common vegetable oils, as outlined in the Codex Alimentarius for fats and oils [28, 29]. Previous studies in cricket oil also reported the RI of 1.460-1.470 [30, 31]. RI of oil depends on the number of carbon atoms and number of double bonds of fatty acids and type of triacylglycerol. The high RI of cricket oils in this study confirmed the presence of unsaturated long chain fatty acids. Specific gravity of both cricket oils was also not significantly different ($p>0.05$) (Table 4). Similar findings were also previously reported for cricket oil [31]. Compared with oils from other edible insects, both cricket oils had lower specific gravity than caterpillar, termites and grasshoppers, of which the specific gravity ranged 0.93-0.94 [30, 32]. In addition, the specific gravity of cricket oil in this study was lower than those of common vegetable oils [28].

The peroxide value (PV) and acid value (AV) are presented in Table 4. Screw-pressed cricket oil had significantly lower PV (7.93 vs. 25.12 mEq O_2/kg) than that obtained from solvent extraction. A slightly lower AV was also observed in cricket oil obtained from screw pressing than that of hexane-extracted oil (4.95 vs. 5.83 mg KOH/g). PV is the index of lipid peroxidation, while AV indicates the occurrence

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University

[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

of hydrolysis of triacylglycerols to liberate free fatty acids. It is evident that less amounts of peroxides and free fatty acids were produced during the process of obtaining cricket oil using screw pressing, which also resulted in a lower degree of lipid oxidation. According to the Codex standards for vegetable oils and animal fats, the appropriate edible oil should not exceed 10 mEq O_2/kg for PV and 4.0 mg KOH/g for AV [28, 29]. So, AV of cricket oils of both extraction methods exceeded the standard, but PV of screw-pressed oil was within the limit. It should be noted that PV and AV of extracted oils also depend on various internal and external factors, especially the condition used for oil extraction. The higher PV found in cricket oil extracted using solvent extraction in this study might be due to the longer exposure to oxygen for longer time during extraction than screw pressing.

Iodine value (IV) of both cricket oils were similar at about 50 g of iodine/g (Table 4). Hence, the extraction method did not affect IV of the obtained cricket oils. IV of oil is dependent on the number of double bonds of fatty acids. This is well consistent with the similar composition of fatty acids in cricket oils obtained from screw pressing and solvent extraction (Table 4).

Saponification number (SN) of cricket oil obtained from screw pressing was significantly higher than that of solvent extraction (273.24 vs. 224.45 mg KOH/g) (Table 4). SN of both cricket oils in this study was close to that previously reported by Murugu et al. that SN of black cricket oil was in the range of 234-246 mg KOH/g [31]. SN of an oil reflects the average molecular weight of the fatty acids in its triacylglycerols and the amount of triacylglycerol present in the oil. A high SN indicates low molecular weight of fatty acid and high triacylglycerol content in oil. In the food industry, SN is a critical parameter for assessing oil quality. The increase of SN is related to the high prevalence of short and medium fatty acids which are more benefits for health [33]. Moreover, high SN is also an indication of high degree of unsaturation in an oil sample. However, in this study, both cricket oils contained about similar amounts of monounsaturated and polyunsaturated fatty acids (Table 4).

Unsaponifiable matter (USM) of cricket oil from screw pressing was significantly lower than that of conventional solvent extraction (6.60 vs. 8.15%) (Table 4). These values were comparable to the USM of cicada oil extracted with hexane, which was approximately 8. 1% [34]. USM of oil depends largely on its sources and thus is used for characterization and authentication of edible oils. According to the standard values of USM for different edible oils, vegetable oils contain 1.0-2.0% USM, while animal fats contain lower USM of 1.0-1.2%, which is much lower than those of the cricket oils in this study. USM includes content of hydrocarbons, alcohols, fat soluble vitamins and sterols [35]. In this study, the use of hexane in solvent extraction might dissolve these substances more efficiently than screw pressing. In generally, hexane is usually used to extract fat from various foods without effect from the hexane residue in meal such as beauty leaf seed kernel [18], rapeseed [19], and walnuts [11]. It is a simple and repeatable method with

*Corresponding author e-mail: nattapol.tng@mahidol.ac.th
[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้
[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

high efficacy. Additionally, cricket oils in this study were not subjected to purification and refining process, which could result in their high USM.

CONCLUSIONS

The method of extraction greatly affected the yield and color of the obtained cricket oil. Solvent extraction with hexane yielded more oil with darker and more intense color than screw pressing. Screw-pressed oil had higher water activity and saponification value but lower peroxide value, acid value and unsaponifiable matter than oil from conventional extraction. There was no significant difference in refractive index, specific gravity and iodine value between both oils. When compared with the Codex standard for edible fats and oils, most of the chemical and physical characteristics of both cricket oils in this study passed the standard, except for peroxide value, acid value, and unsaponifiable matter, although the oils were not refined. According to its high efficiency of extraction, solvent extraction may be suitable for extraction of cricket oil, but further refining process is required for quality improvement. Our findings reveal that cricket oil extracted using screw pressing and hexane could be an alternative oil for human consumption, as its the physical and chemical characteristics are in compliance with the standard for edible oils.

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*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

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[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

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*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

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[2]กลุ่มวิชาการและวิจัยด้านอาหารและโภชนาการ สถาบันโภชนาการ มหาวิทยาลัยมหิดล

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*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]Food Science and Technology Division, Faculty of Engineering and Agro-Industry, Maejo University

[2]Food and Nutrition Academic and Research Cluster, Institute of Nutrition, Mahidol University

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*Corresponding author e-mail: nattapol.tng@mahidol.ac.th

[1]สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้

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