การเปรียบเทียบปริมาณเคอร์คิวมินจากสารสกัดขมิ้นชันด้วยน้ำมันมะพร้าว ด้วยวิธีให้ความร้อนที่แตกต่าง
Comparisons of Curcumin Content in Turmeric Extract in Coconut Oil using Two Heating Methods

นิพนธ์ต้นฉบับ **Original Article**

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บทคัดย่อ

วัตถุประสงค์: เพื่อการเปรียบเทียบปริมาณเคอร์คิวมินจากสกัดขมิ้นชันด้วยน้ำมันมะพร้าวตามวิธีการแพทย์แผนไทยดั้งเดิมและวิธีสมัยใหม่ (วิธีตามประกาศฯ ของสำนักงานคณะกรรมการอาหารและยา) **วิธีการศึกษา:** เตรียมสารสกัดขมิ้นชันโดยนำขมิ้นชันไปทอดในน้ำมันมะพร้าวตามวิธีการแพทย์แผนไทยดั้งเดิม โดยทอดนาน 3 และ 4 ชั่วโมง เตรียมสารสกัดตามวิธีการสมัยใหม่ โดยทอดขมิ้นชันนาน 35, 40 และ 45 นาที แล้วนำสารสกัดขมันชันที่ได้ไปตรวจวิเคราะห์หาปริมาณสารเคอร์คิวมิน ด้วย UV-Visible spectrophotometer ดัดแปลงวิธีการตรวจวิเคราะห์จาก Thai Herbal Pharmacopoeia 2020 โดยใช้ absolute ethanol เป็น blank และวัดค่าการดูดกลืนแสงที่ความยาวคลื่น 425 nm **ผลการศึกษา:** เมื่อนำข้อมูลที่ได้ไปวิเคราะห์ข้อมูลทางสถิติด้วย one way ANOVA พบว่า วิธีการสกัดขมิ้นชันในน้ำมันมะพร้าว นาน 3 ชั่วโมง มีค่าเฉลี่ยของปริมาณสารเคอร์คิวมิน เท่ากับ 0.400 ± 0.014 %w/v ซึ่งมากกว่าวิธีการสกัดขมิ้นชันในน้ำมันมะพร้าว นาน 4 ชั่วโมง 35 นาที 40 นาที และ 45 นาที ที่มีค่าเฉลี่ยเท่ากับ 0.038 ± 0.006, 0.155 ± 0.006, 0.144 ± 0.006, 0.116 ± 0.006 %w/v ตามลำดับ อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) แต่วิธีการสกัดขมิ้นชันในน้ำมันมะพร้าว นาน 35 นาที มีค่าเฉลี่ยของปริมาณสารเคอร์คิวมิน น้อยกว่าวิธีการสกัดขมิ้นชันในน้ำมันมะพร้าว นาน 40 นาที เท่ากับ 0.011 ± 0.006 %w/v แต่ไม่มีนัยสำคัญทางสถิติ ($P > 0.05$) **สรุป:** วิธีการสกัดขมิ้นชันด้วยน้ำมันมะพร้าวนาน 3 ชั่วโมง ซึ่งเป็นวิธีตามองค์ความรู้การแพทย์แผนไทยดั้งเดิม มีปริมาณเฉลี่ยของสารเคอร์คิวมิน มากกว่าวิธีการสกัดวิธีอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$)

คำสำคัญ: ขมิ้นชัน, เคอร์คิวมิน, น้ำมันมะพร้าว, วิธีการทอด

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Abstract

Objective: The purpose of the present study was to compare curcumin content between Thai traditional extraction method of turmeric using coconut oil with modern extraction method (the method mentioned by Thai Food and Drug Administration). **Methods:** Turmeric extract was prepared by deep - frying turmeric (*Curcuma longa* L.) in coconut oil for 3 and 4 hours according to Thai traditional medicine practice. For modern extraction method, turmeric was deep fried in coconut oil for 35, 40, and 45 minutes. The UV-Visible spectrophotometer was carried out to determine curcumin in the turmeric extract, which the quantitative determination was modified from the Thai Herbal Pharmacopoeia 2020 using absolute ethanol as a blank and measured the absorbance at 425 nm. **Results:** The statistical analysis performed by using one-way ANOVA demonstrates that the average content of curcumin in the 3-hours turmeric extract was found to be 0.400 ± 0.014 % w/v, which is significantly higher ($P < 0.05$) than the average content of curcumin in 4-hours, 35-minutes, 40-minutes, and 45-minutes extracts (0.038 ± 0.006, 0.155 ± 0.006, 0.144 ± 0.006, 0.116 ± 0.006 % w/v, respectively). However, the 35-minutes extract has less average Curcumin content than 40-minutes extract for 0.011 ± 0.006 %w/v with no significant difference ($P > 0.05$). **Conclusion:** The turmeric extract obtained from deep - frying turmeric in coconut oil for 3 - hours, which is the extraction method of Thai traditional medicine practice yielded significantly higher average curcumin content than other sample extracts.

Keywords: turmeric, curcumin, coconut oil, deep-frying method

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Introduction

Turmeric (*Curcuma longa* L.) is a plant in the Zingiberaceae family. The active constituents present in the rhizome of turmeric is curcuminoids, of which the most found substances include curcumin (71.5%), desmethoxycurcumin (19.4%), and bisdemethoxycurcumin (9.1%), respectively.[1] The plant also contains other compounds such as volatile oils, carbohydrates, proteins, fats, etc. Curcumin is reported to possess varied pharmacological activities, for instance, gastroprotective, anti-inflammatory, antioxidant, and anticancer properties.[2] According to Thai traditional medicine (TTM) knowledge, turmeric has been used for a long time in TTM herbal formularies for the treatment of many different health diseases, for example, turmeric in carminative, anti-flatulent, and anti-diarrhea herbal formularies. Turmeric also appears in TTM herbal formularies for external use such as relief of insect bites and stings formularies and relief of

muscular pain formularies. The turmeric oil extraction method for using as active ingredient in traditional external analgesic oil is mentioned by The Thai Food and Drug Administration (FDA) in the notification of the Ministry of Public Health[3] for herbal product registration (the modern method). This method is done by deep-frying sliced turmeric in coconut oil until crispy and its color changes into brown, then remove the residue and add the remain sliced turmeric and deep fried again. Nevertheless, the extraction procedure of turmeric using coconut oil in the mentioned monograph is different from TTM practice. Pursuant to the meeting resolution of TTM experts at the Department of Thai Traditional and Alternative Medicine (DTAM), traditional Thai practice of turmeric extraction is prepared by deep-frying thin-sliced turmeric in coconut oil for 3 to 4 hours. It is noticeable that the two procedures are different in the duration of deep-frying turmeric. So far, no data of curcumin content analysis for both techniques are available; thus, that is why we would like to prove the highest curcumin content between the two extraction methods.

The main objective of this research is to compare the curcumin content from Thai traditional turmeric extraction technique using coconut oil as solvent with the modern one in order to determine whichever gives the highest yield, and apply the procedure to form a guideline for consideration of herbal product registration of FDA and DTAM. However, if the two techniques yield no difference, the modern technique can substitute for the Thai traditional one which will reduce time, chemical reagent, and production cost in the industry.

Methods

Chemicals and reagents

Standard curcumin was purchased from Sigma. Absolute ethanol was purchased from MERCK. All chemicals and reagents were analytical grade, except coconut oil which was the commercial grade from Lotus brand.

Plant Material

The rhizomes of *C. longa* were collected from Chiang Mai Province in the north area of Thailand during March 2022 and identified by Assoc. Prof. Dr. Sarin Tadtong. A voucher specimen number “CL_2022_001” of this plant was deposited in herbarium at the Faculty of Pharmacy, Srinakharinwirot University, Nakhonnayok, Thailand.

Instruments

The quantitative determination of curcumin was performed using UV-VIS spectrophotometer Model UV-1800. Analytical balance 4 digits from Mettler Toledo and precision balance model MS1602TS were used in this study.

Preparation of Turmeric Extracts

TTM practice[4] of turmeric extraction are as follows: 1) Fresh rhizomes were cleaned, air-dried, cut into small pieces (approximately 1.0 x 3.0 x 2.0 cm) and the samples were then weighed at 200 g; 2) Heat 100 ml of coconut oil to 150-160 °C using medium-high heat (the ratio of turmeric to coconut oil is 2:1); 3) Put the 200 g of sliced turmeric and deep-fry for 3 and 4 hours; 4) Remove the fried turmeric residue and let the oil cool down; and 5) Filter the oil and record the amount of the filtrate. Three independent turmeric extraction (N = 3) was done.

Modern practice[3] of turmeric extraction are as follows: 1) Fresh rhizomes were cleaned, air-dried, cut into small pieces (approximately 1.0 x 3.0 x 2.0 cm) and the samples were then weighed at 200 g; 2) Heat 100 ml of coconut oil to 150-160 °C using medium-high heat (the ratio of turmeric to coconut oil is 2:1); 3) Put the 100 g of sliced turmeric and deep-fry until dry, crispy, and brown. According to the extraction procedure in the FDA’s monograph of herbal products that can be listed, the exact duration of deep-frying is obscure. Thus, this experiment is set to assess the turmeric extract at 35, 40, and 45 minutes of deep-frying. This time setting is in line with TTM experts’ opinion which explained that frying turmeric in coconut oil will expel moisture in the turmeric and frying it for more than 45 minutes will turn the turmeric into black. On the other hand, turmeric will become crispy at 30 minutes of frying and the air bubbles will disappear at 45 minutes); 4) Remove the fried turmeric residue and add the rest turmeric slices (100 g), then deep-fry again; 5) Let the oil cool down, filter the oil and record the amount of the filtrate. Three independent turmeric extractions (N = 3) were done.

Determination of curcumin content

The quantitative determination of curcumin was modified from the Thai Herbal Pharmacopoeia 2020.[5] The UV-Vis spectrophotometry was carried out using absolute ethanol as a blank (due to the immiscible of turmeric extract in methanol) and detected absorbance at 425 nm[6] which the wavelength is from the maximum absorption spectra of curcumin. The

determination method was verified in order to check linearity, accuracy, precision, and range following an analytical characteristic used in verification method.

Linearity

Stock standard curcumin solutions at concentration of 100 µg/ml was prepared using absolute ethanol as a solvent. For the linearity, six concentrations were prepared (1, 2, 3, 4, 5, and 6 µg/ml) by pipetting 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of stock solution into a 10 ml-volumetric flask and dilute with absolute ethanol to the proposed concentration.

Accuracy

The preparation of standard curcumin solutions at concentration of 1, 3 and 6 µg/ml. The stock solution was pipetted 0.1, 0.3 and 0.6 ml into 10 ml-volumetric flask each and dilute with absolute ethanol to the proposed concentration.

Precision

Standard curcumin solutions at concentration of 4 µg/ml were prepared for 6 times. The stock solution was pipetted 0.4 ml into 10 ml-volumetric flask, 6 volumetric flasks each and dilute with absolute ethanol to the proposed concentration.

Preparation of Sample Solutions

The sample solution was diluted by 2,000 times using absolute ethanol to the proper concentrations for turmeric extract, and test the sample.

Data analysis

The statistical analysis of curcumin content was performed by one-way ANOVA (IBM SPSS Statistics 26). The result shows that deep-frying turmeric in coconut oil for 3 hours yields the highest average curcumin content (0.400 ± 0.014 %w/v).

Results

The color of the turmeric extracts from TTM and modern techniques related to the duration of extraction, the longer extraction duration the more intense color (Table 1). They become more intense as it changes from yellow to brown. The quantities of raw material and turmeric extract yield were shown in Table 2.

The curcumin determination method was performed to verify linearity, range, accuracy, and precision (Figure 1, Table 3, and Table 4). Curcumin content in turmeric extracts from TTM and modern methods were calculated using its linear regression equation (y = 0.1369x + 0.007) with correlation coefficient (R^2) of 0.9991 (Figure 1). Moreover, 5 turmeric extracts were analyzed for curcumin content by UV-Vis Spectrophotometry as shown in Table 5.

Table 1 Turmeric extraction by coconut oil follows TTM and modern practices.

Extraction	Modern method			TTM method	
	35 min	40 min	45 min	3 hours	4 hours
Appearance Characteristics	 Oil is a yellow +, have an odor of turmeric and coconut oil	 Oil is a yellowish orange ++, have an odor of turmeric and coconut oil	 Oil is a yellowish orange ++, have an odor of turmeric and coconut oil	 Oil is an orange ++, have an odor of turmeric and coconut oil	 Oil is a dark orange ++, have an odor of turmeric and coconut oil

Remark: +, ++ is intensity color of extraction

Table 2 Quantity of raw material and turmeric extract.

	Modern method						TTM method			
	35 min		40 min		45 min		3 hours		4 hours	
N	W (g)	V (ml)	W (g)	V (ml)	W (g)	V (ml)	W (g)	V (ml)	W (g)	V (ml)
1	200.000	82.000	200.030	80.000	200.020	84.000	200.040	82.000	200.030	82.000
2	200.010	84.000	200.010	80.000	200.030	82.000	200.020	80.000	200.030	84.000
2	200.010	82.000	200.010	82.000	200.020	84.000	200.040	84.000	200.040	82.000
Mean	200.007	82.667	200.017	80.667	200.023	83.33	200.033	82.000	200.033	82.667
SD	± 0.005	± 0.943	± 0.009	± 0.943	± 0.005	± 0.943	± 0.009	± 1.633	± 0.005	± 0.943

Remark: W = weight of turmeric; V = volume of turmeric extract.

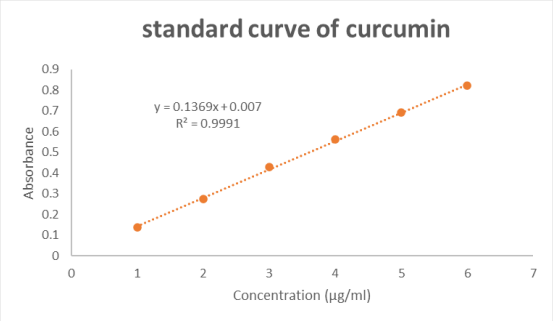

Figure 1 Standard curve of curcumin (n = 3, SD of the measured absorbances at all concentrations are ± 0.000).

Table 3 Result of accuracy test.

Concentration	Absorbance (Mean ± SD) N= 3	Concentration of curcumin added (µg/ml)	Concentration of curcumin measured (µg/ml)	% Recovery (Mean ± SD)
1 µg/ml	0.138 ± 0.000	1.000	0.957	95.690 ± 0.000
3 µg/ml	0.428 ± 0.000	3.000	3.075	102.508 ± 0.000
6 µg/ml	0.820 ± 0.000	6.000	5.939	98.977 ± 0.000

Table 4 The absorbance of precision test

Replication	Absorbance
1	0.553
2	0.555
3	0.551
4	0.553
5	0.553
6	0.549
%RSD	0.374

Table 5 Quantity of curcumin in each extraction technique (%w/v)

Replication	Modern method (%w/v)			TTM method (%w/v)	
	35 min	40 min	45 min	3 hours	4 hours
1	0.242	0.252	0.287	0.387	0.365
2	0.248	0.257	0.283	0.399	0.368
2	0.246	0.259	0.283	0.415	0.355
Average yield (%w/v) ± SD	0.245 ± 0.003	0.256 ± 0.003	0.284±0.002	0.384 ± 0.014	0.363 ± 0.007

On the contrary, the method of deep-frying for 35 minutes yields the lowest average curcumin content which is 0.245 ± 0.003 %w/v. For the difference of the five extraction methods in the present study, there is at least one pair of turmeric extraction that different from each other with statistical significance ($F = 258.309$; $df = 4, 10$; $P < 0.001$).

Therefore, pairwise comparison was conducted using Bonferroni correction in order to determine which pairs of extraction method is dissimilar. The result indicates that the average content of curcumin in the 3-hours turmeric extract is significantly higher ($P < 0.05$) than the average content of curcumin in 4-hours, 35-minutes, 40-minutes, and 45-minutes extracts (0.038 ± 0.006, 0.155 ± 0.006, 0.144 ± 0.006, 0.116 ± 0.006 % w/v respectively). Nonetheless, the 35-minutes extract has less average curcumin content than 40-minutes extracts for 0.011 ± 0.006 %w/v with no significant difference ($P > 0.05$).

Discussions and Conclusion

As shown in Table 1, preparation of turmeric extract using coconut oil with the same temperature (150-160 °C) but different frying times could result in the different curcumin content. We observed that the more frying time, the extract color become more intense as it changes from yellow to yellowish-orange to orange, and to dark orange or brown. More duration of extraction yields more curcumin content, except the 4-hours turmeric extract. This may because longer exposing of turmeric to the heat leads to degradation of curcumin.

The determination of curcumin method modified from the THP 2020. The results show that all the four analytical characteristics were within the acceptance criteria. The linear regression equation of curcumin has correlation coefficient more than 0.99 with the concentration range of 1 – 6 µg/ml, % recovery within 95-105% and the precision results in the percentage of the relative standard deviation (%RSD) is less than 2. Thus, the curcumin determination by UV-Vis spectrometric in this study is suitable and reliable.

Average curcumin content (%w/v ± SD) in the 35-minutes, 40-minutes, 45-minutes, 3-hours, and 4-hours turmeric extracts was found to be 0.245 ± 0.003, 0.256 ± 0.003, 0.284 ± 0.002, 0.400 ± 0.014 and 0.363 ± 0.007, respectively. The 3 hours extraction, which is TTM technique, acquires the most yield of curcumin when compare with other extracts. In contrast, the lowest amount of curcumin was obtained from 35-minutes extraction which is the practice in modern method. According to the study by Bachar Zebib and colleagues[7], curcumin stability temperature is approximately 150 – 160 °C and at 400˚C curcumin is totally decomposed. Therefore, deep-frying turmeric in coconut oil at 150 – 160 °C can maintain curcumin stability. Nevertheless, deep-frying turmeric in coconut oil for 4 hours or more result in less curcumin yield than 3 hours extraction with statistical significance ($P < 0.05$). The curcumin content from the TTM method was higher than the modern method due to the duration of extraction. The

modern method needs shorter extracting duration (35-45 min) while the TTM method needs longer extraction duration (3-4 h) which suggested that the duration of extraction affect the curcumin content.

Extraction of turmeric with coconut oil for 3 hours significantly gives the highest average curcumin content ($P < 0.05$) follow by 4-hours, 45-minutes, 40-mintues and 35 minutes turmeric extraction method, respectively. The 40-minutes extract has 0.011 ± 0.006 %w/v higher average curcumin content than 35-minutes extract with no significant difference ($P>0.05$). In conclusion, the most effective way to extract curcumin by coconut oil is to deep-fry turmeric at 150 - 160 °C for 3 hours in coconut oil. This technique can be useful for entrepreneurs in herbal product industry, as well as herbal product regulatory organization such as FDA in order to form a guideline for consideration of herbal product registration. The further study of other curcumin contents in turmeric extract using coconut oil should be conducted with the aim to study their stability. Furthermore, biological activities study of turmeric extracted by coconut oil is recommended, for example, anti-inflammatory and anti-oxidant properties.

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References

1. Ashraf K and Sultan S. A comprehensive review on *Curcuma longa* Linn.: Phytochemical, pharmacological, and molecular study. *Int J Green Pharm* 2017;11(4):672-685.
2. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of turmeric (*Curcuma longa*). *J Altern Complement Med* 2003;9(1):161-168.
3. The Notification of the Ministry of Public Health Re: Name, category and type or characteristics of herbal products whose production or importation for sale requires a certificate of herbal product formula registration, a listing receipt, and name, quantity and condition of the materials. Thai Government Gazette. 2021. No. 138;116:19-20. (in Thai)
4. Group of supporting regulation for medicine and herbal product. The meeting of Thai medicine practice and pharmaceutical experts “The preparation of oil extract in Thai traditional formula”. Department of Thai traditional and Alternative Medicine, Ministry of Public Health. Bangkok. 2021. (in Thai)
5. Department of Medical Science. Turmeric: Thai Herbal Pharmacopoeia 2020. Bureau of Drug and Narcotic, Department of Medical Science, Ministry of Public Health, Thailand. 2020: pp.177-185. (in Thai)
6. Kim HJ, Kim DJ, Karthick SN, et al. Curcumin dye extracted from *Curcuma longa* L. used as sensitizers for efficient dye-sensitized solar cells. *Int J Electrochem Sci* 2013;8:8320-8328.
7. Zebib B, Mouloungui Z, Noirot V. Stability of curcumin by complexation with divalent cations in glycerol/water system. *Bioinorg Chem Appl* 2010;10:1-8.