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Research Article

Total phenolic content and antioxidant activity of coconut oil enriched with some extracts of curcuma species in Thailand

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Abstract

Coconut oil enriched with phenolic compounds from some curcuma species can be used in cosmetic products or food supplement. The objectives of this work were to produce coconut oil incorporated with some extract of curcuma species including *Curcuma aromatica* Salisb*, Curcuma longa* L., *Curcuma xanthorrhiza* Roxb., *Curcuma Zediaria* Roscoe*, Cucuma aeruginosa* Roxb*, Curcuma aurantiaca* Van Zijip*.,* and *Cucuma parviflora* Wall H., and to determine the antioxidant activity and the total phenolic content of the coconut oil obtained. The antioxidant activity of resulting coconut oil was assayed with DPPH (2,2-diphenyl-1-prohydrozyl), and ABTS. Antioxidant activity with ABTS and DPPH calculated in IC_{50} (the concentration of an antioxidant at with 50% inhibition of free radical activity), IC_{50} values at 30 min was found in the range of 0.35 to 13.58 mg/ml and 2.66 to 179.40 for ABTS and DPPH, respectively. The results showed that the coconut oil with extracted *Curcuma Zediaria* Roscoe had the highest antioxidant activity calculated in IC_{50}, 0.35 mg/ml and 2.66 for ABTS and DPPH radical scavenging assay, respectively. Based on the Folin-Ciocalteu method, the total phenolic content was found in the range of 2.95 to 132.42 mg/g oil. Coconut oil with extracted *Curcuma Zediaria* Roscoe had the highest total phenolic content (132.42 mg/g oil) among other species.

Keywords: coconut oil, total phenolic content, antioxidant activity, curcuma species

Introduction

Some species of Curcuma in Thailand have been widely used as food and in cosmetic products. Its extracts have been tested against bacteria and fungal, antioxidant, anticancer, and antiinflame activity (Jarikasem et al., 2005). Some species of curcuma contain phenolic compounds such as curcumin with high antioxidant activity. Phenolic compounds are natural antioxidants such as curcumin to replace other antioxidants was used for food, cosmetic and other application (Martinez-Correa et al., 2017). The conventional method, curcumin from some species of curcuma was extracted with organic solvent which was dangerous to environment and experimentee. To solve this problem, coconut milk was used as a solvent to extract phenolic compound from some species of cucuma to obtain coconut oil enriched with phenolic compounds as called coconut oil with some extracted from curcuma species.

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Coconut oil is one of vegetable oil which is rich in saturated fatty acids (93%) (Marina et al., 2009). It contains medium chain fatty acids (60%), especially lauric acid (C12:0) (45%), which are burnt for energy rather than stored in the body. Lauric acid, found in coconut oil in major amounts, is known for their unique antiviral, antibacterial and antiprotozoal properties. Moreover, coconut oil lead to a normalization of body lipids, protect against alcohol damage to the liver and improves the immune sytem anti-inflammatory response. The objectives of this work were to produce coconut oil with extracted from some curcuma species including *Cucuma aeruginosa* Roxb*, Cucuma parviflora* Wall, *Curcuma Zediaria* Roscoe*, Curcuma aurantiaca* Van Zijip*.,* and to determine the antioxidant activity and the total phenolic content of coconut oil obtained.

Materials and methods

Materials

Coconut milk was extracted from mature coconuts (10-12 months old) . It was purchased from local markets in Thongsong, Nakron Si Thammarat, Thailand. All chemicals as analytical grade were procured from J.T. Baker chemicals, USA.

Preparation of coconut oil with some extracted

Five kilograms of fresh rhisozome of some curcuma species including *Cucuma aeruginosa* Roxb*, Cucuma parviflora* Wall, *Curcuma Zediaria* Roscoe*, Curcuma aurantiaca* Van Zijip*.,* and 500 mL of water were mixed and blended in blender. This mixture slurry was filtered though a cheesecloth to obtain solution from fresh rhisozome of some curcuma. Then the this solution was added into a flask (10 L) containing coconut milk (5 kg of coconut endosperm: 5L of water). This mixture was stored at room temperature for 24 hr to allow some compounds of fresh rhisozome to be extracted into the oil layer. The oil layer was separated from the cream and water. Seven oil samples including coconut oil with extracted *Curcuma aromatica* Salisb (CAS), coconut oil with extracted *Curcuma longa* L. (CLL), coconut oil with extracted *Curcuma xanthorrhiza* Roxb. (CXR), coconut oil with extracted *Curcuma Zediaria* Roscoe (CZR), coconut oil with extracted *Cucuma aeruginosa* Roxb (CAR), coconut oil with extracted *Curcuma aurantiaca* Van Zijip (CAV), and coconut oil with extracted *Cucuma parviflora* Wall H. (CPW), were obtained from this experiment. These oil samples were kept in brown flask.

Extraction of phenolic compounds

Five grams of oil sample was weighed, dissolved in 25 ml hexane and transferred to a separatory funnel. Twenty–five milliliters of the methanol-water mixture (80:10 v/v) was added. After 2 min of shaking the lower methanol-water layer was removed. The extraction was repeated twice and the methanol-water phase was combined. The methanol-water extract was condensed in a rotary evaporator under vacuum at 40 ^{o}C. The dry residue was then diluted in 1 ml of methanol.

Determination of total phenolic content

The content of total phenolic compounds in oil sample was determined by Folin-Ciocalteau reagent. The reaction mixture contained 1 ml of methanol-water extract, 1 ml of freshly prepared diluted Folin-Ciocalteau reagent and 8 ml of sodium carbonate solution. The mixture was kept in the dark at ambient conditions for 30 min to complete reaction.

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The absorbance at 725 nm was measured by spectrophotometer (Biochrom S22). Gallic acid was used as a standard. Results were expressed as mg of gallic acid per kg of oil.

Radical scavenging activity (RSA) toward DPPH radicals

Radical scavenging activity in seven oil samples including coconut oil with extracted *Curcuma aromatica* Salisb (CAS), coconut oil with extracted *Curcuma longa* L. (CLL), coconut oil with extracted *Curcuma xanthorrhiza* Roxb. (CXR), coconut oil with extracted *Curcuma Zediaria* Roscoe (CZR), coconut oil with extracted *Cucuma aeruginosa* Roxb (CAR), coconut oil with extracted *Curcuma aurantiaca* Van Zijip (CAV), and coconut oil with extracted *Cucuma parviflora* Wall H. (CPW), were examined by reduction of DPPH radical in ethyl acetate. One gram of oil (exactly weighted) was dissolved in ethyl acetate in 10 ml volumetric flask, then 1 ml of this solution was transferred into the second 10 ml volumetric flask containing DPPH radical solution, which was freshly prepared in ethyl acetate at a concentration of 10^{-4} M. Reaction flask was shaken for 10 s in vortex apparatus and it was allowed to stand in the dark for 30 minutes. The absorption of this mixturewas measured by spectrophotometer (Biochrom S22) in a 1 cm quartz cell at 30 min against a blank of pure ethyl acetate without DPPH radicals. After that %Inhibition was determimed from differences in absorbance of DPPH solution with or without sample (control).

$$\% \text{ Inhibition} = \frac{(A_{cotrol} - A_{sample}) \times 100}{A_{cotrol}}$$

The graph between %inhibitions vs. concentration of sample or standard antioxidant was plotted to obtain linear equation. The concentration of an sample or standard antioxidant at with 50% inhibition of free radical activity was determined.

ABTS method

The antioxidant activity of sample oils was dertermined by the ABTS radical cation ($ABTS^{\cdot+}$) delocourisation assay. The $ABTS^{\cdot+}$ working solution was prepared from ABTS (7 mM) 8 ml and potassium persulfate (2.45 mM) 12 ml and allowing the mixture to stand in the dark at low temperature (4 ^{o}C) for 16-18 hours before used. The solution was diluted in ethanol to give an absorbance at 750 nm of 0.800± 0.200 before used. The stock solution of oil sample and standard antioxidant were prepared. In the test reaction, $ABTS^{\cdot+}$ working solution and sample were mixed. The mixture was shaken vigorously and left at 30 minutes to be measured at 750 nm The solution for determining antioxidant activity was prepared as following: 1) sample solution was the mixture between the oil sample and ABTS working solution, 2) blank solution was the mixture between the oil sample and ethanol, 3) positive solution was the mixture between ethanol and ABTS working solution, 4) negative solution was the ethanol. Absorbance of these solution were measured at 750 nm at 30 minutes. The percentage inhibition was calculation by using the following equation:

$$\%\text{Inhibition} = [(\text{Abs control}_c - \text{Abs sample}_e)/\text{Abs control}_c] \times 100,$$

where Abs control_c is Abs positive – Abs negative, and Abs sample_c is Abs sample – Abs bank. The graph between %inhibition vs. concentration of sample or standard antioxidant was plotted to obtain linear equation. The concentration of a sample or standard antioxidant at with 50% inhibition of free radical activity was determined.

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Statistical analysis

All experiments were carried out in triplicate and expressed as mean ±standard deviation (SD). SPSS (Version 16.0) statistical softwere was used to verify significant differences by one-way-analysis of variance (ANOVA) followed by scheffe's honest significant difference test at $p \leq 0.05$ to identify differences among groups.

Results and discussion

Oil yield of coconut oil with extracted some curcuma species

Coconut milk is an oil-in-water emulsion of coconut oil droplets stabilized by coconut proteins such as cocosin, globulins and albumins act as emulsifier and phospholipids (Monera & del Rosario, 1982). If Coconut milk is not stable it can separate into cream (coconut cream) and serum (coconut skim milk) layers (Seow & Gwee, 1997). In our previous work, we investigated production of coconut oil by using crude proteolytic enzyme extract from fresh rhizome ginger (Penprapai S., et al., 2016) Protease can hydrolyzes peptides bonds at the interior of polypeptide chain to obtained shorter fragment of protein. Then shorter fragments of protein can move to aqueous phase and after that coconut oil was obtained (Raghavendra & Raghavarao, 2010). In this work, coconut oil with some extracted is produced by fermentation method, where coconut milk expelled from freshly harvested coconuts is fermented for 24 h during this period, the oil phase gets separated from aqueous phase.

The yield of coconut oil with some extracted curcuma species including CAS, CLL, CXR, CZR, CAR, CAV, CPW as compared with virgin coconut oil (VCO) was presented in table 1. Oil yield of CLL, CXR and CZR was slightly different as compared with vergin coconut oil but they were significantly different ($p \leq 0.05$) as compared with CAV and CPW. We showed the added some extracted curcuma species such as CAV and CPW stabilize coconut milk emulsion while the added some extracted curcuma species such as CLL, CXR and CZR destabilize the emulsion coconut milk.

Table 1. oil yield of coconut oil with some extracted curcuma species

Oil sample	%yield
CAS	12.57 ± 0.89^{abcd}
CLL	14.76 ± 0.13^{cde}
CXR	15.30 ± 0.42^{de}
CZR	14.40 ± 0.51^{cde}
CAR	11.52 ± 1.02^{abc}
CAV	11.22 ± 0.55^{ab}
CPW	9.75 ± 1.06^{a}
VCO	17.12 ± 0.43^{e}

Data points with different superscript within the same rows differ significantly at $p \leq 0.05$

Total phenolic content (PC)

Total phenolic content was calculated from linear regression equation of standard curve ($y = 0.001 + 0.0549x$; $r^2 = 0.9856$). The total phenolic content in coconut with some extracted curcuma species was found in the range of 2.95 to 132.42 mg/g oil. However, the total phenolic content significantly varied among the studied species. The uppermost amounts were

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observed for CZR had the highest total phenolic content (132.42 mg/g oil) among other species (Table 2) whereas minimum ones were noticed for CPW (2.95 mg/g oil) while total phenolic content in CAS and CLL was the second highest. The total phenolic content decreased in the following order: CZR> CAS > CLL > CXR > CAV > CAR >CPW. However, these values were different in comparison to previous reports carried out using different types of extraction processes and solvents (Songsang, 2011). Coconut oil enriched with phenolic compounds can be used for healthy food and cosmetic.

Table 2. Total phenolic content of with some extracted curcuma species

Oil sample	Total phenolic content (mg/1 g oil)
CAS	118.94 ± 7.13^{d}
CLL	112.02 ± 1.41^{d}
CXR	38.39 ± 0.85^{c}
CZR	132.42 ± 4.30^{e}
CAR	7.05 ± 0.140^{ab}
CAV	15.66 ± 0.69^{b}
CPW	2.95 ± 0.78^{a}
VCO	0.77 ± 0.08^{a}

Values are mean±SD of three independent experiments.
Data points with different superscript within the same rows differ significantly at $p \leq 0.05$

Antioxidant activity

Approaches can be used to study radical scavenging activity (RSA) of compounds as DPPH and ABTS. Scavenging of DPPH radicals is mechanized by the donation of hydrogen atom to the unpaired electron of nitrogen bridge causing the purple color turn to yellowish. Meanwhile, the ABTS radical cation undergoes the reduction process by hydrogen donating antioxidant and can be spectrophotometrically measured. It is well accepted that the phenolic content significantly influence the antioxidant activities (Othman, et al. 2014; Qader, et al., 2011, Wong, et al., 2006). In agreement, our findings showed that radical scavenging of DPPH and ABTS were positively correlated to the phenolic content. Phenolic compound such as curcumin, curcumin derivative play important roles on inhibitory effect of ROS and antioxidant agent. Phenolic compound such as curcumin and derivative of cucumin in coconut oil wtth some extracted cucuma species is an antioxidant agent (Kostova, et al., 2011).

In this work, the antioxidant activity of resulting coconut oil was assayed with DPPH (2,2-diphenyl-1-prohydrozyl), and ABTS and was shown in table 3. Antioxidant activity with ABTS and DPPH calculated in IC_{50}. IC_{50} values at 30 min were found in the range of 0.3594 to 13.5833 mg/ml and 2.6630 to 179.4004 for ABTS and DPPH, respectively. The results showed that the coconut oil with extracted *Curcuma Zediaria* Roscoe had the highest antioxidant activity calculated in IC_{50}, 0.3594 mg/ml and 2.6630 for ABTS and DPPH radical scavenging assay, respectively. CZR, CAS and CLL had the highest antioxidant activity with low IC_{50} for both of DPPH and ABTS assay. However, all of coconut with extracted some curcuma species were more antioxidant activity than VCO but antioxidant activity of all coconut oil with some extracted curcuma species was lower than vitamin C.

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Table 3. IC_{50} of oil sample at 30 min by ABTS and DPPH assay

Oil sample	IC50, mg/ml (ABTS)	IC50, mg/ml (DPPH)
CAS	0.5001±0.0011^{bc}	10.3081±0.5843^{a}
CLL	0.5329±0.01512^{bc}	3.7803±0.2614^{a}
CXR	0.9103±0.0085 ^{c}	4.3236±0.1979 ^{a}
CZR	0.3594±0.0123^{ab}	2.6630±0.1723^{a}
CAR	11.5675±0.0413^{e}	179.4004±10.1823^{e}
CAV	1.3634±0.0455^{d}	44.8033±1.0831^{b}
CPW	13.5833±0.1036^{f}	116.0057±2.8563^{c}
VCO	44.0078±0.3640^{g}	182.0554±3.0816^{e}
Vitamin C	0.0024±0.0000^{a}	0.0054±0.0.0020^{a}

Values are mean±SD of three independent experiments.
Data points with different superscript within the same rows differ significantly at p ≤0.05

Conclusion

Total phenolic content of coconut oil with some extracted curcuma species was found in the range of 2.95 to 132.42 mg/g oil. Coconut oil with extracted *Curcuma Zediaria* Roscoe had the highest total phenolic content (132.42 mg/g oil) among other species. Total phenolic content in CAS and CLL was second highest. Total phenolic content correlated antioxidant activity of coconut oil with some extracted curcuma species. The coconut oil with extracted *Curcuma Zediaria* Roscoe had the highest antioxidant activity calculated in IC_{50}, 0.3594 and 2.6630 mg/ml for ABTS and DPPH radical scavenging assay, respectively. All coconut oil with some extracted some curcuma species had higher more antioxidant activity than VCO

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References

Jarikasem, S., Thubthimthed, S., Chawananoraseth, K. & Suntorntanasat, T. (2003, February). Essential oils from three Curcuma species collected in Thailand. In *III WOCMAP Congress on Medicinal and Aromatic Plants-Volume 1: Bioprospecting and Ethnopharmacology 675* .37-40.

Kostova, I., Bhatia, S., Grigorov, P., Balkansky, S., S Parmar, V., K Prasad, A. & Saso, L. (2011). Coumarins as antioxidants. *Current Medicinal Chemistry*, *18*(25), 3929-3951.

Marina, A. M., Man, Y. C., Nazimah, S. A. H. & Amin, I. (2009). Chemical properties of virgin coconut oil. *Journal of the American Oil Chemists' Society*, *86*(4), 301-307

Martinez-Correa, H. A., Paula, J. T., Kayano, A. C. A., Queiroga, C. L., Magalhães, P. M., Costa, F. T. & Cabral, F. A. (2017). Composition and antimalarial activity of extracts of Curcuma longa L. obtained by a combination of extraction processes using supercritical CO 2, ethanol and water as solvents. *The Journal of Supercritical Fluids*, *119*, 122-129.

Monera, O. D. & Del Rosario, E. J. (1982). Physico-chemical evaluation of the natural stability of coconut milk emulsion. *Annals of tropical research: technical journal of the Visayas State College of Agriculture*.

Othman, A., Mukhtar, N. J., Ismail, N. S. & Chang, S. K. (2014). Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants. *International Food Research Journal*, *21*(2).

Penprapai P., Naksir J. & Saiyang A. (2017). Thai Patent, Request number 1703000072, Bangkok: Department of Intellectual Property Ministry of Commerce.

Qader, S. W., Abdulla, M. A., Chua, L. S., Najim, N., Zain, M. M. & Hamdan, S. (2011). Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. *Molecules*, *16*(4), 3433-3443.

Raghavendra, S. N. & Raghavarao, K. S. M. S. (2010). Effect of different treatments for the destabilization of coconut milk emulsion. *Journal of Food Engineering*, *97*,341–347.

Seow, C. C. & Gwee, C. N. (1997). Coconut milk: chemistry and technology. *International journal of food science & technology*, *32*(3), 189-201.

Songsang, J. (2011). Antioxidant activity and active compounds of some *Curcuma* species in Thailand. Doctoral dissertation, Department of Horticulture, Faculty of Agriculture, Kasetsart University.

Tangsuphoom, N. & Coupland, J. N. (2009). Effect of surface-active stabilizers on the surface properties of coconut milk emulsions. *Food Hydrocolloids*, *23*(7), 1801-1809.

Wong, S. P., Leong, L. P. & Koh, J. H. W. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food chemistry*, *99*(4), 775-783.