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Research Article

Application of crude proteolytic enzyme extract from fresh rhizome ginger to produce coconut oil

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Abstract

Crude extract from fresh rhizome ginger contained crude proteolytic enzyme and phenolic compound as antioxidant. Crude proteolytic enzyme extract from fresh ginger was used to produce coconut oil with high antioxidant activity and low hydrolysis and oxidation susceptibility. The main objective of this work was to produce coconut oil at 60 ^{o}C for 1, 2, 3, 4 and 5 hours by using crude proteolytic enzyme extract from fresh rhizome ginger and determine the total phenolic content, antioxidant activity and stability of coconut oil obtained from this process. Coconut oil was produced by using four different ratios of coconut meat to fresh rhizome of ginger (w/w)–2.5:0.5, 2.5:1.0. Our results indicated that using crude proteolytic enzyme extract from fresh rhizome ginger provided high coconut oil yield of ~17% during 2-3 hours while without protease coconut oil was still not produced. It was found that increasing the fresh rhizome of ginger content resulted in increasing total phenolic content, antioxidant activity and stability of coconut oil. Moreover, coconut oil with low free fatty acid and peroxide value was more inactive to hydrolysis and oxidation reaction. Thus, there is a potential production of coconut oil with high phenolic compounds, antioxidant activity and stabilityfor application in healthy food products or cosmetic.

Keywords: crude extract, rhizome ginger, coconut oil

Introduction

Coconut milk is an oil-water emulsion which is stabilized by the naturally occurring protein such as albumins, globulins as well as phospholipids (Thangsuphoom and Coupland, 2008). This emulsion is destabilized by using extra energy to obtain oil by dry and wet process. In dry process, oil is exposed to high temperature during refining leading to the absence of the natural antioxidant such as vitamin E in oil. While, virgin coconut oil (VCO) obtained from fresh mature coconuts without thermal treatment and refining has more beneficial effect than coconut oil obtained from dry process (Shilhavy, et al., 2004; Marina, et al., 2009a). VCO retains most of the nutritional components, especially antioxidant. VCO is rich medium chain fatty acids and contains 48-53% lauric acid (Marina, et al., 2009b)..The lauric acid in VCO can has function as, antifungal antibacterial and antiviral substances. Moreover, VCO is used in skin care product to prevent wrinkles and sagging. The traditional method for VCO production is fermentation at ambient temperature for 24-46 hr during ths period the oil phase gets separated from aqueous phase. However, the disadvantages of this

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method are low oil recovery, fermented odor and time consume for producing alternative process of VCO production is needed. Using enzymatic destabilization of coconut milk emulsion is non-thermal technique and does not need fermentation process. This method is environmental friendly safety and short time for producing coconut oil with high percentage of yield (McGlon, et al., 1986; Raghavendra & Raghavarao, 2010). Crude extract from fresh rhizome ginger contained crude proteolytic enzyme and phenolic compound as natural antioxidant. Crude proteolytic enzyme extract from fresh ginger could be used to produce coconut oil with high antioxidant activity and low hydrolysis and oxidation susceptibility. The main objective of this work was to produce coconut oil at 60 ^{o}C for 1, 2, 3, 4 and 5 hours by using crude proteolytic enzyme extract from fresh rhizome ginger and determine the total phenolic content, antioxidant activity and stability of coconut oil obtained from this process.

Materials and methods

Materials

Coconut milk was obtained from mature coconuts (10-12 months old). It was bought from the local Thungsong market, Nakhon Si Thammarat Province. All chemicals as analytical grade were procured from J.T. Baker chemicals, USA.

Coconut oil production by using protease from fresh rhizosome of ginger

Coconut oil was produced by using two ratios of coconut meat to fresh rhizome of ginger (w/w)–2.5:0.5 and 2.5:1.0. Fresh rhizome of ginger aged 11-12 months was procured from the local Thungsong market. It was spin by blender to obtain crude enzyme solution and then use it immediately by mixing with coconut milk emulsion. This mixture was then incubated at 60 ^{0}C for 1, 2, 3, 4, 5 h then was centrifuged at 3500 g for 20 min.

Extraction of pheholic compounds

The procedure reported by Kapila (2009) was slightly modified: 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 methanolwater extract was condensed in 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 the coconut oil 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. The absorbance at 725 nm was measured by spectrophotometer (Biochrom S22). Gallic acid was used as standard. Results are expressed as mg of gallic acid per kg of oil.

Radical scavenging activity (RSA) toward DPPH radicals

Radical scavenging activity in coconut oil was 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

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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 mixing was 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 determined from differences in absorbance between control and sample as shown in equation (1).

$$\% \text{ Inhibition} = \frac{(A_{cotrol} - A_{sample}) \times 100}{A_{cotrol}} \quad (1)$$

ABTS method

The antioxidant activity of sample oils was dertermined by the ABTS radical cation ($ABTS^{\cdot+}$) 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 dark at low temperature for 16-18 hours before use. The solution was diluted in ethanol to give an absorbance at 750 nm of 0.800± 0.200 before use. The stock solution of sample oil 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 measure absorbance at 750 nm The solution for determining antioxidant activity were prepared as follows: 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. These solutions were measured absorbance at 750 nm at 30 minutes. The percentage inhibition was calculation by using the following equation: $\%Inhibition=[(Abs\ control_c - Abs\ sample_e)/Abs\ control_c]x100$, when $Abs\ control_c$ is Abs positive – Abs negative, and $Abs\ sample_c$ is Abs sample – Abs bank. The graph between %inhibitions 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.

Peroxide and free fatty acid

Peroxide value and % free fatty acid of coconut oil from different treatments were analyzed according to standard method (Firestone, 1997). Free fatty acid and peroxide value were expressed as percentage of FFA as lauric acid and meq O_2/kg, respectively.

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

Coconut oil yield (%)

The oil yield from different treatments including enzyme from fresh rhizome and without enzyme from fresh rhizome presented in table 1. Destabilization of coconut milk emulsion was achieved by employing enzymes from fresh rhizome, providing high coconut oil yield of ~17.30 – 18.36% during 3 hours. While without protease, no coconut oil was produced. During enzyme treatment, coconut milk emulsion was destabilized by protease in

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resh rhizomethat hydrolyzes peptides bonds at the interior of polypeptide chain to obtain shorter fragment of protein/peptides (Raghavendra & Raghavarao, 2010). These shorter fragments of protein/peptides moved to the aqueous phase and providing high oil yield. This result indicated that protease from fresh rhizome plays an important role in complete destabilization of coconut milk emulsion.

Table 1. Coconut oil yield (%) at various times and ratios of coconut meat to fresh rhizome of ginger

Time (h)	Coconut oil yield (%)		
	2.5:0	2.5:0.5	2.5:1
1	0	15.66	13.14
2	0	17.30	16.02
3	0	17.36	18.36
4	0	15.75	18.18
5	0	12.11	17.64

Figure1. Coconut oil yield (%) by using protease from fresh rhizome

Total phenolic content (PC)

Total phenolic content was calculated from linear regression equation of standard curve ($y = 0.001 + 0.0059x$; $r^2 = 0.9856$). Where x and y are concentration and absorbance of gallic acid as standard solution and absorbance value measured at 725 nm, respectively. We found that total phenolic content was increasing with incubating time in coconut oil at ratios of coconut meat to fresh rhizome of giner as 2.5:0.5. Increasing the fresh rhizome of ginger content resulted in increasing total phenolic content. Total phenolic contentof coconut oil at ratios of coconut meat to fresh rhizome of giner - 2.5:1.0 was higher coconut oil yields than at that at 2.5:0.5. At ratios of coconut meat to fresh rhizome of giner as 2.5:0.5, Total phenolic contentof coconut oil was increased with time. At ratios of coconut meat to fresh rhizome of giner as 2.5:1.0, total phenolic contentof coconut oil was decreased after 2 hr due to phenolic compound decomposed during storage with high temperature (penprapai, 2012).

However, these values were different in comparison to previous reports carried out using different types of extraction processes and solvents (Songsang, 2011; Srivub,2012).

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. In this work, The antioxidant activity was determined DPPH and ABTS assay. %inhibition from DPPH assay and IC_{50} from ABTS as shown in table 3. Aantioxidant activity of coconut oil increased with time. Increasing the fresh rhizome of ginger content resulted in increasing antioxidant activity. Coconut oil at ratios of coconut meat to fresh rhizome of ginger - 2.5:1.0 at 5 hr gave the highest antioxidant activity calculated in %inhibition for DPPH assay as 57.44 and IC_{50} for ABTS assay as 0.7201. However,

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Coconut oil from all treatments were more antioxidant activity than VCO but antioxidant activity of coconut from all treatments was lower than vitamin C.

Table 2. Total phenolic content in coconut oil by using protease from fresh rhizome ginger at various times

	Time (h)	Total phenolic content (mg/1 g oil)
VCO	24	0.77±0.075[a]
2.5:0.5	1	17.13±0.40[b]
	2	19.40±0.12[c]
	3	20.62±0.31[d]
	4	21.61±0.23[d]
	5	25.83±0.46[e]
2.5:1.0	1	34.50±0.18[h]
	2	36.30±0.15[i]
	3	34.44±0.17[h]
	4	32.98±0.47[g]
	5	30.89±0.32[f]

Data points with different superscript within the same rows differ significantly at p≤0.05

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 play important roles on inhibitory effect of ROS and antioxidant agent.

Table 3. %Inhibition (DPPH assay) of coconut oil from two treatments and IC_{50} (ABTS assay)

ratios of coconut meat to fresh rhizome of ginger	Time (h)	% Inhibition	IC_{50} (ABTS)
2.5:0.5	1	30.22±1.40[a]	2.1371±0.0371[e]
	2	31.60±2.39[bc]	2.0769±0.0415[e]
	3	32.89±0.94[bc]	2.0227±0.0423[cd]
	4	28.45±0.46[b]	1.5939±0.0682[c]
	5	36.61±0.66[c]	1.8866±0.0027[cd]
2.5:1.0	1	44.67±2.23[d]	1.1029±0.009[b]
	2	45.73±1.88[d]	0.9776±0.0034[b]
	3	47.93±3.03[d]	0.8334±0.0034[b]
	4	46.47±0.78[d]	0.8556±0.0338[b]
	5	57.44±0.56[e]	0.7201±0.0125[b]
2.5:0	24	6.55±0.89[a]	44.0078±0.3639[f]
Vitamin E			0.0078±0.00004[a]

Data points with different superscript within the same rows differ significantly at p ≤0.05

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Stability of coconut oil

Peroxide value, free fatty acid and induction time of coconut oil obtained from protease enzyme from fresh rhizome of ginger as compared with coconut oil obtained from without protease as shown table 4. Free fatty acid content of coconut oil obtain from all treatment was relatively low and within the limits of Asian and Pacific Coconut Community (APCC) standard (<0.5%). Free fatty acid is formed during hydrolytic rancidity, caused by the hydrolysis of ester by lipase or moisture. Peroxide values of coconut oil obtain from all treatments were low and within the limits of APCC standard (≤ 3 meq peroxide oxygen/kg oil) indicating their high stability against oxidation. Moreover, coconut oil obtained from protease enzyme from fresh rhizome ginger treatment had induction time higher than that without protease enzyme treatment**.** This result indicated that coconut oil obtained from protease from fresh rhizome ginger treatment provied higher oxidation stability than coconut oil without enzyme treatment.

Table 4. Peroxide value, free fatty acid and induction time of coconut oil

Oil sample	Time (h)	Free fatty acid (% as lauric acid)	Peroxide value (meq O_2kg)	Induction time (h)
2.5:0.5	1	.2217±.02259	.5588±.05094	> 72
	2	.2873±.02279	.5591±.05556	
	3	.2481±.02277	.5630±.05895	
	4	.3262±.02299	.5572±.05380	
	5	.3397±.02263	.4277±.05832	
2.5:1.0	1	.2606±.02243	.5229±.05660	
	2	.2735±.00031	.5226±.05196	
	3	.3260±.02259	.5902±.00336	
	4	.3388±.02239	.4223±.05880	
	5	.2869±..02259	.5537±.05503	
VCO		.2089±.02275	.5882±.12969	40
Standard APCC		< 0.5 %	≤ 3 meq peroxide oxygen/kg oil	

Conclusions

Destabilization of coconut milk emulsion was achieved by employing protease enzyme from fresh rhizome ginger with high percent yield coconut oil of ~17-18% after 3 hours at 60 ^{0}C. Without protease no coconut oil was produced. Coconut oil with low free fatty acid and peroxide value was more inhibition hydrolysis and oxidation reaction. Thus, there is a potential production of coconutoil with high phenolic compounds, antioxidant activity and stabilityfor application in healthy food product or cosmetic.

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