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Comparison of the phenolic-dependent antioxidant properties of coconut oil extracted under cold and hot conditions

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ABSTRACT

The antioxidant activities of coconut oil extracted under hot and cold conditions were compared. The coconut oil extracted under hot conditions (HECO) contained more phenolic substances than the coconut oil extracted under cold conditions (CECO). The antioxidant potential of HECO was higher than that of CECO as demonstrated by DPPH assay, deoxyribose assay and *in vivo* assay of serum antioxidant capacity. It is the common belief that virgin coconut oil extracted under cold conditions preserves several thermally unstable antioxidants and, as a result, better beneficial qualities can be expected for virgin coconut oil. However, high temperatures used in the hot extraction of coconut oil favour the incorporation of more thermally stable phenolic antioxidants into coconut oil. Therefore, the consumption of HECO may result in the better improvement of antioxidant related health benefits compared with the consumption of CECO.

1. Introduction

Wet extraction of coconut oil from coconut milk (emulsion obtained by pressing scraped coconut kernel) is used to extract high-quality coconut oil under cold and hot conditions. In the cold extraction method, coconut milk is chilled to about 10 °C and the solidified lipid layer is separated. This method is used to extract virgin coconut oil (VCO). In the hot extraction method, coconut milk is heated and the water is evaporated from the emulsion. The remaining coconut oil can be siphoned out or separated by decantation. To extract coconut oil by this method, the coconut milk emulsion is heated to 100–120 °C. Common methods of oil extraction include pressing of dried seed kernels and solvent extraction. The extraction of oils from the emulsions of seed lipids under cold and hot conditions is less common for the extraction of vegetable oils and more usual for the extraction of coconut oil.

The phenolic substances are mainly responsible for the antioxidant properties of olive oil (Baldioli, Servili, Perretti, & Montedoro, 1996). Phenolic content of olive oil varies with the method of extraction. Solvent-extracted oils contain more phenolic substances than virgin oils (Gutfinger, 1981).

Both positive and negative health effects of coconut oil have been reported. Formation of some pro mutagenic DNA adducts is reported to be lower in rats fed with a coconut oil supplemented diet, compared with the formation of such adducts in rats fed with linoleic acid-rich diets (Eder et al., 2006). Total serum cholesterol levels in rats fed with coconut oil were higher than those in rats fed with more unsaturated oils (Mohamed, Hussein, Bhathena, & Hafez, 2002). The health effects of coconut oil in most of the studies have been attributed to the lipid portion of coconut oil. Antioxidant properties of the phenolic fraction of olive oil are well documented (Gutiérrez, Amaud, & Garrido, 2001; Tsimidou, Papadopoulos, & Dimitrios, 1992). The beneficial qualities of CECO or VCO have been reported (Nevin & Rajamohan, 2004). However, the detailed studies of the antioxidant activity of the phenolic fraction of coconut oil have not been conducted. In addition, there are no reports on any studies of the health effects or antioxidant properties of HECO to the best of our knowledge.

There is a remarkable difference between coconuts and other seeds that are used to extract edible oils. Coconut endosperm contains a liquid portion, white coconut kernel and a thin brown outer skin of coconut kernel known as coconut testa. In addition to the method of extraction, the components of the endosperm may also play an important role in determining the final phenol content of coconut oil.

Cold extraction conditions that are used in the extraction of virgin coconut oil may preserve thermally unstable antioxidant compounds. However, low temperatures may prevent the efficient incorporation of phenolic substances into coconut oil from coconut milk emulsion. If phenolic substances with antioxidant properties are inactivated or destroyed at the high temperatures that are used

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in the extraction, relatively lower phenolic-dependent antioxidant capacities can be expected for HECO, compared with CECO. On the other hand, the solubility of polar phenolic substances in non-polar coconut oil is certainly improved at high temperatures, and more phenolic substances that are present in coconut emulsion will be dissolved in coconut oil during the hot extraction of coconut oil. If more phenolic substances are incorporated into coconut oil during hot extraction, better antioxidant properties can be expected for HECO, compared with CECO. Therefore, it is important to compare total phenol contents and antioxidant properties of HECO with those of CECO in order to understand the effect of extraction temperature on the antioxidant potential of coconut oils. In the present study, phenolic-dependent antioxidant properties of CECO and HECO were determined, using chemical and biological systems. The effect of the composition of the endosperm on the total phenol content was also investigated.

2. Materials and methods

2.1. Chemicals

Authentic standards for HPLC analysis, 1,1-diphenyl-2-picrylhydrazyl (DPPH), diammonium salt of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO). Deoxyribose and authentic standards for GC analysis were purchased from Fluka (Buchs, Switzerland).

2.2. Preparation of coconut milk

Coconuts of 12–14 months' maturity from 'ordinary tall' coconut cultivars were used for the oil extraction. Coconut milk was prepared by hand pressing scraped coconut endosperm. The viscous slurry resulting from the hand pressing of coconut endosperm was squeezed through a cheesecloth to obtain coconut milk. Different components of coconut endosperm, namely white coconut kernel (WK), white coconut kernel and coconut testa (WK + CT), white coconut kernel and coconut water (liquid endosperm) (WK + CW), and white coconut kernel, coconut testa and coconut water (WK + CT + CW) were used for the preparation of coconut milk. The endosperm components used in this study were from the coconuts that were used for the oil extraction, and additional amounts of any component were not added in excess from other coconuts.

2.3. Oil extraction

2.3.1. Cold extraction

Coconut milk was chilled at 10 °C for 10 h to solidify the lipids. Then the aqueous layer was discarded and the lipid block was allowed to stand at 30 °C until it dissolved completely. Then the mixture was centrifuged and the oil layer was separated.

2.3.2. Hot extraction

Coconut milk emulsion was heated to 100–120 °C and the water in the emulsion was evaporated. Oil was decanted from the deposits at the bottom of the container.

2.4. Determination of total phenol content

Methanol/water (80:20 v/v, 1 ml) was mixed with coconut oil (5.0 g), and the phenolic substances were extracted using a vortex at 40 Hz for 2 min (twice). The mixture was centrifuged (1080 g, 10 min) and the resultant clear solution was separated. This procedure was repeated four times for the same oil sample with fresh 1 ml portions of the solvent system. The resultant extracts were collected, diluted to 5.0 ml and stored in a dark brown vial under nitrogen until further experiments were carried out.

Total phenol contents of the phenolic extracts were determined by the Folin–Denis method. The Folin–Denis reagent was prepared by the method of Perry, Burgess, and Glennie (2001). The absorption measurements were performed on a UV–visible spectrophotometer (Optima SP-3000 plus, Tokyo, Japan) at 745 nm. The same procedure was followed to analyse total phenols in WK and CT using 1.0 g of each of the component for the extraction of phenolic substances. Total phenols in CW were directly determined by the same colorimetric method.

2.5. HPLC studies

The phenolic extracts of CECO and HECO were purified by the procedure given by Montedoro, Servili, Baldioli, and Miniati (1992). HPLC experiments were performed using an Agilent 1100 liquid chromatographic system (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 1200 diode array detector. A Zorbax SB-C18 column (250 mm × 4.6 mm × 5 μm particle size) maintained at room temperature was used for this purpose. The mobile phases consisted of 2% acetic acid in deionized water (**A**) and methanol (**B**). The total running time was 30 min with a flow rate of 0.5 ml/min. The elution gradient began using 85% **A** and 15% **B**. From 2 to 10 min, solvent **B** was increased to 30%. Then solvent **B** was increased to 45% from 10 to 20 min, to 48% from 20 to 25 min and to 50% from 25 min to 30 min. Phenolic compounds were detected at a wavelength of 280 nm. Phenolic compounds were quantified by a previously reported method (Seneviratne & Dissanayake, 2008). Standard curves for quantitative analysis were plotted using the known concentrations of the authentic standards of identified phenolic compounds. Data were stored and processed with Agilent Chemstation software.

2.6. In vitro assay of antioxidant activity

2.6.1. Measurement of DPPH radical-scavenging activity

DPPH radical-scavenging activity of the phenolic extracts of coconut oil was measured according to the method reported by Hatano, Kagawa, Yasuhara, Tasuhara, and Okuda (1988). Phenolic substances were extracted by the method explained under the determination of total phenol content from CECO (WK + CT + CW) and HECO (WK + CT + CW) samples. Total phenol contents of the phenolic extracts measured using Folin–Denis method were adjusted to the required concentrations by suitable dilutions with the same solvent system used for the extraction of phenolic substances. Each phenolic extract (300 μl) of various concentrations (25, 50, 75 and 100 mg/l) from CECO and HECO was added to a methanolic solution of DPPH (0.3 ml, 0.8 mM) and the resultant mixture was vortexed at 40 Hz for 5 min. After 30 min of incubation at room temperature in the dark, the absorbance of each reaction mixture was measured at 517 nm. The DPPH radical-scavenging activity was measured similarly for a series of 25–100 mg/l solutions of BHT. The inhibitory effect of DPPH radical was calculated according to the following formula:

Inhibition (%) = $[(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the reaction mixture with solvent system instead of phenolic extract or BHT and A_1 is the absorbance of the reaction mixture with phenolic extract or BHT.

2.6.2. Effect of phenolic extracts on the oxidation of deoxyribose

The method of Halliwell, Gutteridge, and Aruoma (1987) was used to evaluate the inhibition of deoxyribose degradation. Phenolic substances were extracted, by the method explained under

the determination of total phenol content, from the CECO and HECO samples. Total phenol contents of the phenolic extracts measured using Folin–Denis method were adjusted to the required concentrations, by suitable dilutions with the same solvent system used for the extraction of phenolic substances. The reaction mixture (1 ml) contained deoxyribose (6 mM) dissolved in KH_2PO_4–K_2HPO_4 buffer (100 μl, 20 mM, pH 7.4), each phenolic extract (500 μl) at various concentrations (20, 40, 60 and 80 mg/l) from CECO and HECO, $FeCl_3$ (100 μl, 200 μM), EDTA (100 μl, 1.0 mM), H_2O_2 (100 μl, 1.0 mM) and ascorbic acid (100 μl, 1.0 mM). After an incubation period of 1 h at 37 °C, the extent of deoxyribose degradation was measured by the reaction of formed malonaldehyde (MDA) with thiobarbituric acid (TBA). TBA (1.0 ml, 1% in 50 mM NaOH) and 2.8% trichloroacetic acid (TCA) (1.0 ml) were added to the reaction mixture and heated at 100 °C for 20 min. After cooling the reaction mixture, the absorbance was measured at 532 nm, against a blank containing only buffer and deoxyribose. The assay was performed similarly for a series of 20–80 mg/l solutions of BHT. The inhibitory effect of deoxyribose degradation was calculated according to the following formula:

Inhibition (%) = $[A_0 - A_1/A_1] \times 100$, where A_0 is the absorbance of the reaction mixture with solvent system instead of phenolic extract or BHT, and A_1 is the absorbance of the reaction mixture with phenolic extract or BHT.

2.7. Animals

Male weaning Wistar rats (weighing 100–110 g) were randomly collected from the Medical Research Institute, Colombo. They were placed individually in cages and housed in a room with a temperature range of 25 ± 2 °C with a 12 h light-darkness cycle. Prior to the initiation of the experiment, the rats were acclimatised to the basal diet for 6 days. Then the rats were randomly assigned to treatment groups (5 rats/group) and fed diets containing tested oils. The diet and water were given for a period of 86 days. They had free access to food and water. The food intake was monitored daily and the growth of animals was monitored weekly. The experimental protocol was approved by the ethical committee of University of Sri Jayawardenapura, Sri Lanka. The rats were anaesthetised humanely under light diethyl ether and blood was collected through the tail vein of each Wistar rat.

2.8. Antioxidant capacity in serum

The $ABTS^{\cdot+}$ radical cation (Wolfenden & Willson, 1982) was produced by the method of Re et al. (1999). A solution of ABTS (2.27 ml, 8 mM) was mixed with a solution of potassium persulfate (11.5 ml, 3 mM). The mixture was protected from light and stored at room temperature for 12–16 h. Formation of $ABTS^{\cdot+}$ was checked by its absorbance at 734 nm. The $ABTS^{\cdot+}$ solution was diluted with water to an absorbance of 0.70 (±0.02) at 734 nm. The blood samples were centrifuged promptly (3000 g, 15 min) at room temperature. Separated top layer of serum from each sample was used to carry out the ABTS assay. Blood serum (20 μl) was mixed with $ABTS^{\cdot+}$ solution (2.9 ml) and phosphate buffer saline (80 μl, 5 mM, pH = 7.4). Reduction of absorbance was measured at 734 nm after 5 min.

Trolox was used as the standard substance for the comparison of antioxidant activity as Trolox equivalent antioxidant capacity (TEAC). A calibration curve describing the percentage inhibition of the absorbance as a function of Trolox concentration was prepared as reported (Re et al., 1999; van den Berg, Haenen, van den Berg, & Bast, 1999). The percentage inhibition was plotted against the respective Trolox concentrations. A calibration curve in a concentration range between 0 and 62.5 μM was prepared.

2.9. Determination of the acid value and peroxide value

Acid value and peroxide value of the oil samples were determined by the reported methods (Kirk & Sawyer, 1991).

2.10. Determination of fatty acid composition

Methylation of fatty acids was carried out by the method of Garcés and Mancha (1993). The fatty acids were identified by comparison of the retention times of the signals with those of authentic fatty acid methyl ester standards. A gas chromatograph (Finnigan Trace; Thermo Fisher Scientific Inc., Waltham, MA) equipped with capillary column Rtx^R- WAX (Crossbond with PEG, 30 m × 0.32 mm × 0.25 μm; Thames Restek UK Ltd, Saunderton, UK) and flame ionisation detector (FID) was used for this analysis. The injector and detector temperatures were 230 °C and 250 °C, respectively. The carrier gas was helium at 30 kPa in constant pressure mode, and analyses were performed in split mode (split ratio 100:1). The sample (0.4 μl) was injected into the GC system. A temperature programme of 130 °C (3 min), 130–210 °C at 45 °C/min, and 210 °C (12 min) was used.

2.11. Statistical analysis

All analyses were run in triplicate unless otherwise indicated. Tukey's pairwise test was carried out after ANOVA for the determination of significant differences ($p < 0.05$) between the means. Data were analysed using Minitab (version 13.30 for Windows, Minitab, State College, PA).

3. Results and discussion

3.1. Total phenol contents

Table 1 shows the total phenol contents of coconut oils extracted under cold and hot conditions. The results indicate that HECO is richer in phenolic substances, compared with CECO. The coconut milk emulsion used in the cold and hot extraction methods contains an aqueous phase and an organic (oil) phase that make the emulsion milky. Phenolic compounds are partitioned preferably in the aqueous phase because of the polar nature of phenolic substances. In the cold extraction method, the phenolic substances are not properly incorporated into coconut oil due to the mild temperature conditions that are used in the cold extraction. However, in the hot extraction method the temperature of the coconut milk emulsion reaches above 100 °C. The concentration of the phenolic substances increases when the water in the emulsion evaporates during the hot extraction process. Higher phenolic concentrations and higher temperatures favour the incorporation of more phenolic substances into coconut oil.

Phenolic contents also vary with the composition of endosperm, and this variation is significant only in the hot extraction method, indicating that even when phenolic substances are present at high concentrations in the aqueous phase, they are not efficiently incorporated into coconut oil at low temperatures. The phenolic con-

Table 1
Total phenolic contents of coconut oils.

Components of endosperm	Total phenol content mg/kg oil	
	CECO	HECO
1. WK	62 ± 2[a]	78 ± 2
2. WK + CT	64 ± 2	250 ± 4
3. WK + CW	65 ± 3	358 ± 4
4. WK + CT + CW	66 ± 3	449 ± 4

[a] Each data point represents the mean of six replicates ± standard deviation.

tents of the WK, CT, and CW are 55 ± 8 mg /kg, 3976 ± 28 mg/kg and 130 ± 3 mg/kg, respectively. The approximate weight ratio WK:CT:CW present in a coconut is 55:1:25 and the same proportion was used to prepare coconut milk. Even though CT contains the highest amount of phenolic substances, the phenolic content of Entry 2 is smaller than that of Entry 3 in Table 1. This is due to the relatively small amount of CT present in coconut.

There are suggestions about the possible inactivation or destruction of some antioxidants at high temperatures. α-Tocopherol was destroyed during the processing of raw corn and other selected foods (Wyatt, Carballido, & Mendez, 1998). Hydroxytyrosol derivatives present in virgin olive oil were also destroyed during thermal oxidation (Nissiotis & Margari, 2002). Even though high temperatures may destroy or inactivate certain phenolic compounds, the results in Table 1 indicate that hot extraction is a better method than cold extraction to incorporate more phenolic substances into coconut oil.

In order to compare the composition of phenolic substances, HPLC experiments were carried out for the phenolic extracts of CECO and HECO. The attention was focused on the comparison of the two phenolic profiles of CECO (WK + CT + CW) and HECO (WK + CT + CW). The chromatograms of the phenolic extracts of CECO and HECO are given in Figs. 1 and 2, respectively. Fig. 1 shows that there are three main compounds in the phenolic region of the chromatogram of the phenolic extracts of CECO (WK + CT + CW) and Fig. 2 shows that there are several compounds in the chromatogram of the phenolic extracts of HECO (WK + CT + CW). Eight major free phenolic compounds were identified in HECO. Most of the unidentified signals in Fig. 2 may be due to oxidised phenolic compounds and/or bound forms of phenolic compounds that are incorporated into coconut oil under high temperature conditions.

3.2. Antioxidant activity

Even if some phenolic substances are destroyed or inactivated during the hot extraction of coconut oil, there is a possibility that the inactivated or oxidised phenolic compounds contribute to the overall absorption during the colorimetric quantification of total phenols. The inactivated or oxidised phenolic substances may also appear in the phenolic region of the HPLC chromatograms. If this happens, the colorimetric and chromatographic phenolic contents may not reflect the antioxidant potentials of the phenolic extracts. Therefore, both *in vitro* and *in vivo* antioxidant assays were carried out to determine the antioxidant potentials of the phenolic extracts of CECO and HECO.

3.3. DPPH radical-scavenging activity and inhibition of deoxyribose degradation

The antioxidant properties of phenolic substances vary significantly depending on their functional groups (Rice-Evans, Miller, & Paganga, 1996). Therefore, when the antioxidant activity of a mixture of phenolic substances is evaluated it is important to compare the effect of concentration as well as the quality of phenolic substances on the antioxidant activity. The antioxidant activity of the phenolic extracts of CECO and HECO was determined by DPPH radical-scavenging activity assay and deoxyribose assay. The DPPH radical-scavenging activity and the inhibition of deoxyribose degradation are shown in Figs. 3 and 4, respectively. Both concentration effect and the quality of phenolic substances on the antioxidant activity are illustrated in Figs. 3 and 4. To determine the direct dependence of antioxidant activity on the phenolic contents, the colorimetrically determined total phenolic contents of CECO and HECO were adjusted to the known concentrations by suitable dilutions. BHT was used as the control to compare the antioxidant activity. According to Figs. 3 and 4, both DPPH radical-scavenging activity and the inhibition of deoxyribose degradation increase with the concentration of phenolic substances. Therefore, due to the presence of higher phenolic content per unit mass of oil, higher phenolic-dependent antioxidant capacities can be expected for HECO, compared with CECO. Studies have shown that the DPPH radical-scavenging activity and the inhibition of deoxyribose degradation rapidly increase with the total phenol concentration or antioxidant concentration initially and then slow down when the antioxidant activities are close to maximum values (Chung, Chang, Chao, Lin, & Chou, 2002; Kaur, Arora, & Singh, 2008). In Fig. 3, the CECO response reaches close to the maximum value at 50 mg/l and thereafter, further increase of the total phenol concentration improves the CECO response only moderately. Similarly, the HECO response in Fig. 4 reaches close to the maximum value around 25 mg/ml and the increase of the total phenol con-

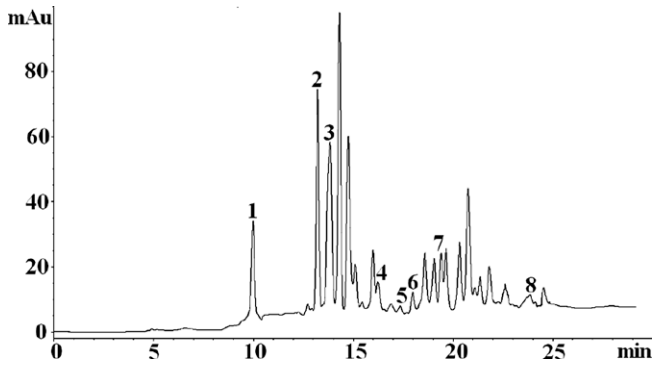

Fig. 2. HPLC chromatogram of the phenolic fraction of HECO: (1) gallic acid (20.2 ± 10.1), (2) (−)–epigallocatechin (26.7 ± 1.7), (3) (+)–catechin (81.7 ± 22.7), (4) *p*-hydroxybenzoic acid (4.8 ± 1.0), (5) (+)–epicatechin (1.4 ± 0.6), (6) caffeic acid 4.6 ± 1.5) and (7) syringic acid 4.1 ± 0.9 and ferulic acid 22.1 ± 8.9 (means of five samples ± standard deviation, mg/kg oil).

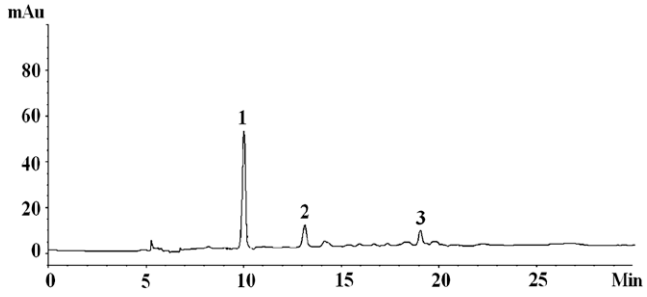

Fig. 1. HPLC chromatogram of the phenolic fraction of CECO: (1) gallic acid (28.1 ± 10.5), (2) (−)–epigallocatechin (26.7 ± 1.7) and (3) syringic acid (1.4 ± 0.1) (means of five samples ± standard deviation, mg/kg oil).

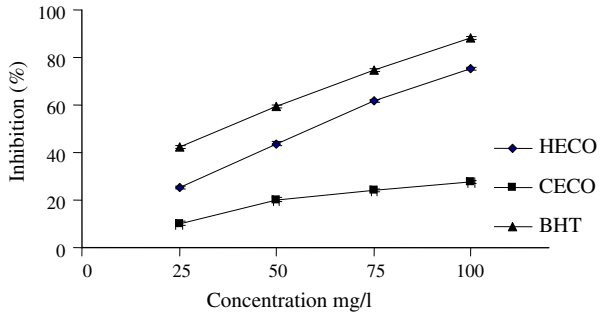

Fig. 3. DPPH radical-scavenging activity of the phenolic extracts of CECO and HECO.

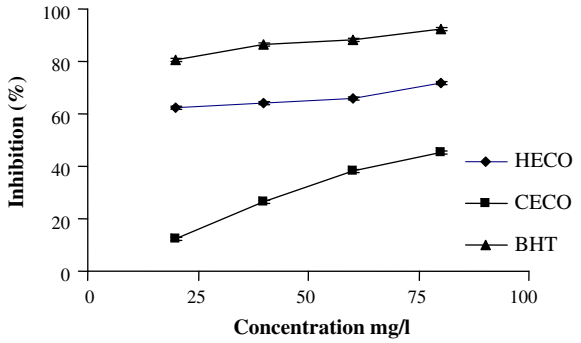

Fig. 4. Inhibition of deoxyribose degradation by phenolic extracts of CECO and HECO.

centration beyond this value improves the HECO response only moderately.

Figs. 3 and 4 also show that the antioxidant activity of the phenolic extracts of HECO is superior to that of CECO at all tested phenolic concentrations. This means that the phenolic extracts of HECO display higher antioxidant capacities than those of CECO at any given total phenol concentration. This higher antioxidant capacity of the phenolic extracts of HECO should be associated with the more complex phenolic composition of HECO.

3.4. Serum TEAC

The TEAC values of the samples of blood serum collected from the rats fed with diets containing CECO and HECO were obtained from the capacity of the two oils to reduce the $ABTS^{\cdot+}$ radical cation to ABTS. During this reduction process, the blue–green $ABTS^{\cdot+}$ radical is decolorised. The extent of inhibition of the absorbance of $ABTS^{\cdot+}$ was measured with the concentration of Trolox, in order to evaluate the TEAC. The regression coefficient (r^2) calculated from the calibration curve was 0.9964.

It was observed by plotting the percentage inhibition with time that 5 min is the appropriate time to measure the absorbance after mixing $ABTS^{\cdot+}$ with Trolox or phenolic extracts.

Table 2 shows the variation of TEAC of the blood serum of rats fed with CECO and HECO. There is a better improvement of TEAC in the blood serum of rats fed with HECO, compared with those fed with CECO. Studies have shown that VCO, with more vitamin E and polyphenols than copra oil, exhibits increased levels of antioxidant enzymes in rats fed with diets containing these coconut oils for 45 days (Nevin & Rajamohan, 2006). The present study, involving the monitoring of TEAC for 84 days, indicates that HECO is always superior to CECO (VCO) in improving the antioxidant status in blood. According to Table 2, the baseline serum TEAC of the HECO-fed rats was maintained for 28 days. Then the TEAC increased further after 28 days. The maximum TEAC was maintained during the 56–84 day period. However, the baseline TEAC of the CECO-fed rats decreased during the first 28 days. Feeding of rats for a period longer than 28 days caused the improvement of TEAC back to the baseline reading. The overall results of this experiment after 84 days indicate that the serum TEAC increased significantly ($p < 0.05$) in HECO-fed rats and did not increase in CECO-fed rats.

Table 2
Variation of TEAC values within the period of 84 days.

Sample name	Baseline	28 days	56 days	84 days
	Trolox equivalent (μmol/l)			
CECO	39.8 ± 1.8^{ax}	34.8 ± 0.2^{bx}	36.1 ± 0.9^{bcx}	37.5 ± 1.2^{ax}
HECO	41.9 ± 0.7^{ax}	41.1 ± 0.2^{ay}	47.1 ± 0.8^{by}	47.4 ± 0.3^{by}

Values are means of five rats ± standard deviation. Letters a, b, and c were used to compare statistical significance in rows ($p < 0.05$).
[b]Statistically significant compared to baseline reading.
[c]Statistically significant compared to 28 days reading. Letters x and y were used to compare statistical significance in columns ($p < 0.05$).
[y]Statistically significant compared to CECO.

Table 3
Fatty acid composition, acid value and peroxide value of CECO and HECO.

Property	CECO[a]	HECO
Saturated fatty acids (%)	90.7 ± 3.1	89.5 ± 3.8
Monounsaturated fatty acids (%)	4.9 ± 0.3	5.2 ± 0.7
Polyunsaturated fatty acids (%)	1.1 ± 0.2	1.2 ± 0.3
Acid value (mg KOH/g oil)	0.6 ± 0.1	1.0 ± 0.5
Peroxide value (absorbance at 500 nm)	0.482 ± 0.007	0.482 ± 0.006

[a] Each data point represents the mean of six replicates ± standard deviation.

Dietary fat affects the antioxidant defence systems in the body (Pulla Reddy & Lokesh, 1994). However, the results in Table 3 show that there is no significant difference in fatty acid compositions between CECO and HECO, suggesting that the improvement of TEAC is clearly due to the higher phenolic content of HECO, compared with CECO.

The major fraction of an edible oil is the saponifiable fraction, which contains triglycerides and free fatty acids. Attempts to improve the quality of the non-saponifiable fraction may affect the quality of the saponifiable fraction. Therefore, it is important to investigate how the modifications to the methods and conditions of oil extraction to improve the quality of the non-saponifiable fraction affect the quality of the saponifiable fraction. The fatty acid composition, acid value and peroxide value of the CECO and HECO were determined in order to estimate the effect of cold and hot conditions of oil extraction on the quality of the saponifiable fraction. According to the results in Table 3, there was no significant difference between the fatty acid contents, acid values and peroxide values of CECO and HECO, indicating that temperature-assisted quality improvements can be made to coconut oil without affecting the quality of the saponifiable fraction. Due to a high percentage of saturated fatty acids, coconut oil has much better thermal stability and oxidative stability, compared with several other edible oils (Jayadas & Nair, 2006).

Even though the hot extraction method is an ideal method for the incorporation of better nutritional qualities to coconut oil, due to the thermal stability and oxidative stability of the saponifiable fraction of coconut oil, such high temperature extraction methods may affect the quality of saponifiable fraction of oils that contain higher percentages of unsaturated fatty acids. Further research work is needed to understand the limitations of the use of hot extraction conditions to extract oils containing high percentages of polyunsaturated fatty acids.

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References

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. F. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists' Society, 73*, 1589–1593.

Chung, Y. C., Chang, C. T., Chao, W. W., Lin, C. F., & Chou, S. T. (2002). Antioxidant activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *Journal of Agricultural and Food Chemistry, 50*, 2454–2458.

Eder, E., Wacker, M., Lutz, U., Nair, J., Fang, X., Bartsch, H., et al. (2006). Oxidative stress related DNA adducts in the liver of female rats fed with sunflower-,

rapeseed-, olive- or coconut oil-supplemented diets. *Chemico-Biological Interactions, 159*, 81–89.
Garcés, R., & Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry, 211*, 139–143.
Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists' Society, 68*, 966–968.
Gutiérrez, F., Amaud, T., & Garrido, A. (2001). Contribution of polyphenols to the oxidative stability of virgin olive oil. *Journal of the Science of Food and Agriculture, 81*, 1463–1470.
Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.
Hatano, T., Kagawa, H., Yasuhara, T., Tasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical-scavenging effects. *Chemical and Pharmaceutical Bulletin, 36*, 2090–2097.
Jayadas, N. H., & Nair, K. P. (2006). Coconut oil as base oil for industrial lubricants-evaluation and modification of thermal, oxidative and low temperature properties. *Tribology International, 39*, 873–878.
Kaur, R., Arora, S., & Singh, B. (2008). Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss. *Bioresource Technology, 99*, 7692–7698.
Kirk, R., & Sawyer, R. (1991). *Pearson's composition and analysis of foods*. Longman: Essex.
Mohamed, A. I., Hussein, A. S., Bhathena, S. J., & Hafez, Y. S. (2002). The effect of dietary menhaden, olive, and coconut oil fed with three levels of vitamin E on plasma and liver and plasma fatty acid composition in rats. *Journal of Nutritional Biochemistry, 13*, 435–441.
Montedoro, G. F., Servili, M., Baldioli, M., & Miniati, E. (1992). Simple and hydrolyzable phenolic compounds in virgin olive oil.1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry, 40*, 1571–1576.
Nevin, K. G., & Rajamohan, T. (2004). Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clinical Biochemistry, 37*, 830–835.
Nevin, K. G., & Rajamohan, T. (2006). Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chemistry, 99*, 260–266.
Nissiotis, M., & Margari, M. T. (2002). Changes in antioxidant concentration of virgin olive oil during thermal oxidation. *Food Chemistry, 77*, 371–376.
Perry, N. B., Burgess, E. J., & Glennie, V. I. (2001). *Echinacea* standardization: Analytical methods for phenolic compounds and typical levels in medicinal species. *Journal of Agricultural and Food Chemistry, 49*, 1702–1706.
Pulla Reddy, A. C., & Lokesh, B. R. (1994). Alterations in lipid peroxides in rat liver by dietary *n*-3 fatty acids: Modulation of antioxidant enzymes by curcumin, eugenol and vitamin E. *Journal of Nutritional Biochemistry, 5*, 181–188.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.
Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.
Seneviratne, K. N., & Dissanayake, D. M. S. (2008). Variation of phenolic content in coconut oil extracted by two conventional methods. *International Journal of Food Science and Technology, 43*, 597–602.
Tsimidou, M., Papadopoulos, G., & Dimitrios, B. (1992). Phenolic compounds and stability of virgin olive oil-part 1. *Food Chemistry, 45*, 141–144.
van den Berg, R., Haenen, G. R. M. M., van den Berg, H., & Bast, A. (1999). Applicability of an improved TEAC assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry, 66*, 511–517.
Wolfenden, B. S., & Willson, R. L. (1982). Radical-cations as reference chromogens in kinetic studies of one-electron transfer reactions: Pulse radiolysis studies of 2, 2 -azinobis-(3-ethylbenzthiazoline-6-sulphonate). *Journal of the Chemical Society-Perkin Transactions, 2*, 805–812.
Wyatt, C. J., Carballido, S. P., & Mendez, R. O. (1998). Tocopherol content of selected foods in Maxican diet. *Journal of Agricultural and Food Chemistry, 46*, 4657–4661.