Research Article

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Effect of extraction processes on physiochemical properties and antioxidant activity of virgin coconut oil

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Abstract

The present study was to investigate the physiochemical and antioxidant properties of virgin coconut oil (VCO) through different extraction processes including aqueous extraction (AE), aqueous enzymatic extraction (AEE), cold extraction (CE), hotplate heating process (HPH) and microwave extraction (MOH). Physiochemical properties of all the extracted oils were observed to be within the range of Asian and Pacific Coconut Community (APCC) standards with lower iodine, peroxide and free fatty acid values in AE, AEE and CE than those observed in MOH and HPH methods with reference to commercial VCO. However, total phenolic content ranged from 26.4 to 42.1 mg GAE 100 g^{-1} of oil with the effect of extraction processes. As correlation analysis showed significant correlation between total phenolic content and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity, β-carotene bleaching activity and total antioxidant activity, oils extracted by CE, AE and AEE had the highest antioxidant activity corresponding to their highest phenolic content as compared to those of HPH and MOH with reference to commercial VCO. Hence, the present study concluded that CE, AE and AEE could extract VCO with desirable quality and highest antioxidant activity than the other methods of extraction.

Keywords: Antioxident activity, oil extraction, phenolic content, physiochemical properties, virgin coconut oil.

Introduction

Virgin oils have gained the market potential as a functional food with a good amount of antioxidant activity to enhance storage stability of food and beneficial effect to human health against free radicals. During processing and storage, vegetable oil is oxidized resulting in the formation of hydro-peroxides, free radicals and organic compounds which decreases the nutritive and organoleptic value of the products (Richardsa *et al*., 2005). Oxidation also causes rancidity of oil and exhibit reversible or irreversible damages to biological molecules such as DNA, proteins and lipids that leads to arthritis, cancer and heart diseases (Rohman *et al.*, 2011; Goldberg, 2003). Processing of vegetable oil having antioxidant activity has been reported to enhance shelf life maintaining the nutritive value of the product (Bhatnagar *et al*., 2009).

Virgin coconut oil (VCO) has been reported to be an excellent source of antioxidants including phenolic compounds (Marina *et al*., 2009b). VCO supplemented diet has been reported to increase the antioxidant status in rats (Navin and Rajamohan, 2006). Seneviratne and Dissanayake (2008) demonstrated variation in the phenolic fraction of the traditional and commercial coconut oil and reported highest antioxidant activity for hot extracted coconut oil due to its thermally stable phenolic compounds compared to that of cold extracted coconut oil (Seneviratne *et al.,* 2009).

Phenolic compounds have significant influence on the nutritional characteristics, sensory characteristics and stability of food products and prevent deterioration through quenching free radical reactions responsible for lipid oxidation (Marrugat *et al.,* 2004; Ruth *et al.,* 2001). In addition to phenolic compounds, coconut oil

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retains good oxidative resistance because of its high proportion of medium chain saturated fatty acid content (De Leon and Delores, 2005). The health and nutritional attributes of VCO has made it a popular functional edible oil and opens up new research avenues (Marina *et al*., 2009a). VCO with natural coconut aroma provides instant energy to the human body, prevents infection, increases immunity, inverse disease states and cures many types of illness (Enig, 2001).

Virgin coconut oil is extracted from coconut milk of fresh and mature coconut kernel using various methods such as fermentation (Madhavan *et al.,* 2005; Mansor *et al*., 2012), chilling and thawing (Rhagvendra and Raghavarao, 2010), centrifugation (Abdurahman *et al*., 2009) and aqueous enzymatic method (Chen and Diosady, 2003; Rhagvendra and Raghavarao, 2010). Traditionally, coconut oil was extracted by boiling coconut milk which contains significant level of caffeic acid, p-coumaric acid, ferulic acid and catachin (Seneviratne and Dissanayake, 2008).

The antioxidant properties and phenolic content of olive oil has been reported to vary with the method of extraction (Nergiz and Unal,1991; Baldioli, 1996). Several studies have reported the effect of extraction process on phenolic compounds and antioxidant activity of olive oil. The effect of extraction process on antioxidant potential of VCO has not been explored so far. Hence, the present study was formulated to compare the physiochemical properties, total phenolic content and antioxidant activity of coconut oil extracted through various extraction processes and to investigate the extraction processes that could produce VCO with the highest antioxidant activity.

Materials and methods

Fresh and mature (10-12 months old) nuts of East Coast Tall cultivar of coconut (*Cocos nucifera*) were procured from a local garden of Pondicherry, India. Coconut milk was extracted by grinding white coconut kernels with distilled water in the ratio of 1:1 (w/w) in mixer grinder. The ground coconut was filtered to obtain coconut milk which was kept at room temperature for 4 h for the separation of cream to extract oil.

Methods of oil extraction

(i) Aqueous extraction (AE): In this process, the separated cream was incubated at 33 °C for pH 6.5 for 12 -14 h for the separation of oil (Agarwal and Bosco, 2013); (ii) Aqueous enzymatic extraction (AEE): The cream was treated with commercial enzyme (acid protease) to separate oil (Raghvendra and Raghavarao, 2011) with slight modifications; (iii) Cold extraction (CE): Extraction was performed by centrifuging the thawed cream kept in deep freezer at -20 °C for 6 h; (iv) Microwave oven heating (MOH): The cream was heated in microwave oven at constant temperature till the separation of oil and (v) Hot plate heating (HPH): This process extracts the oil by heating the cream on hot plate under controlled temperature and constant stirring. All the extracted oils were stored in stoppered glass bottles at room temperature in dark for analysis with reference to commercial virgin coconut oil (CVCO) provided by Thenarasu Farm Products, Pollachi, Coimbatore.

Physiochemical properties

The virgin coconut oil extracted through various methods were evaluated for specific gravity, refractive index, iodine value (IV), peroxide value (PV), free fatty acid (FFA) as lauric acid and acid value (AV) as per the standard methods (AOAC, 2000).

Extraction and quantification of phenolic content

Phenolic extracts were extracted by centrifuging the mixture of oil sample (10 g) dissolved in n-hexane (50 mL) and 80 per cent (v/v) aqueous methanol at 3000 rpm for 10 min for complete extraction and the pooled supernatant was concentrated in a rotary evaporator under vacuum at 45 °C and methanolic solution of extracts was stored in amber glass bottles in refrigerator for analysis. The total phenolic content (TPC) was determined adding 0.5 mL phenolic extract and made up to 1 mL with methanol and 0.5 mL Folin-Ciocalteu reagent (diluted 1:1 with water) followed by the addition of 1.5 mL of sodium carbonate (20% w/v). The final volume of mixture was made up to 10 mL and incubated at 40 °C for 20 min and the absorbance read at 755 nm (Gutfinger, 1981).

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay

Phenolic extracts (0.5 mL) of oil samples in the concentration of 25, 50, 75, 100 µg mL^{-1} was added to 2 mL of methanolic solution of DPPH (0.2 mM) and incubated in dark at room temperature for 60 min. The absorbance was measured at 517 nm against blank. The percentage inhibition was calculated as $[(A_{control}-A_{sample})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of samples (Brand-Williams *et al*., 1995).

β-carotene linoleate bleaching activity assay

The phenolic extracts (0.2 mL) of oil samples were added to 5 mL of assay reagent which was prepared as described by Hidalgo *et al*., 1994. The mixture was incubated at 50 °C and absorbance at 470 nm was read at 15 min interval for 120 minutes till the disappearance of the color of β-carotene. The antioxidant activity (AA) was expressed as

$AA(\%) = [1-\{(A_o-A_t)/(A^o_o-A^o_t)\}] \times 100$, where A_o and A°_o are the absorbance at the beginning (t= 0 h) for phenolic extract of oil samples and control respectively, A_t and A°_t are the absorbance, after incubation for 120 min, of phenolic extract of oil samples and control respectively.

Total antioxidant activity (TAA) assay

The total antioxidant activity of phenolic extract was estimated by the method of Prieto *et al*., (1999). Phenolic extract (0.1 mL) was mixed with 1 mL of reagent solution (4 mM ammonium molybdate, 28 mM monobasic sodium phosphate, 0.6 M sulfuric acid) and the mixture was incubated at 95 °C for 90 min. The absorbance was read at 695 nm against a blank and the result was expressed as the equivalents of gallic acid.

Reducing power assay

The reducing power of phenolic extract of oil samples was measured as described by Yen and Duh (1995) with modifications. Phenolic extracts (200-1000 µg mL^{-1}) was added with 2.5 mL of 0.2 M phosphate buffer (pH 6.6), 2.5 mL potassium ferricyanide (1% w/v), and incubated at 50 °C for 20 min; 2.5 mL trichloroacetic acid (10% w/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with equal volume of distilled water and 0.5 mL ferric chloride (0.1% w/v). The absorbance was measured at 700 nm against a blank.

Statistical analysis

Results were expressed as mean ± standard deviation of triplicate analysis. The significance of difference between samples was analyzed by one way ANOVA and Duncan multiple range test (DMRT) ($P < 0.05$) using SPSS (Version 16).

Results and discussion

Physiochemical properties

Table 1 shows the physiochemical properties of coconut oil extracted by various processes with

Table 1. Physiochemical properties of virgin coconut oil extracted by different methods

Oil extraction processes*	Specific gravity (at 30 °C)	Refractive index	Iodine value	Free fatty acid (%)	Acid value (as Lauric acid)	Peroxide value meqO_2 kg^{-1}	Color
AE	0.917 ± 0.004	1.447 ± 0.004	4.93 ± 0.15	0.40 ± 0.02	0.80 ± 0.04	0.67 ± 0.06	Water clear
AEE	0.917 ± 0.003	1.443 ± 0.003	5.77 ± 0.15	0.38 ± 0.04	0.76 ± 0.08	0.89 ± 0.28	Pale yellow
HPH	0.916 ± 0.006	1.447 ± 0.004	6.07 ± 0.18	0.63 ± 0.05	1.04 ± 0.07	1.93 ± 0.15	Pale yellow
CE	0.917 ± 0.003	1.442 ± 0.004	4.23 ± 0.16	0.47 ± 0.15	0.94 ± 0.02	0.70 ± 0.26	Water clear
MOH	0.920 ± 0.002	1.448 ± 0.003	6.30 ± 0.26	0.51 ± 0.25	1.06 ± 0.50	1.43 ± 0.41	Pale yellow
CVCO	0.920 ± 0.004	1.444 ± 0.008	5.13 ± 0.20	0.49 ± 0.30	0.98 ± 0.06	0.93 ± 0.21	Water clear
APCC#	0.915-0.920	1.448 -1.449	4.1 – 11.0	0.5	6 max	< 3	Water clear

*AE: Aqueous extraction; AEE: Aqueous enzymatic extraction; HPH: Hot plate heating; CE: Cold extraction; MOH: Microwave oven extraction; Each values are mean of three replicates ± SD (n=3). #APCC: APCC Standard, 2009

reference to those of commercial VCO toned to the Asian and Pacific Coconut Community (APCC, 2009) standards. While iodine value was the lowest in CE, peroxide value (PV) in AE and percentage FFA in AEE, the highest iodine value was in MOH, and both the PV and percentage FFA was observed in HPH. The results showed that there were some differences in the physiochemical properties of the VCO samples produced by different methods, but the differences were not significantly enough to affect the overall quality of the VCO. The results of this study are also in conformity with the findings of Marina *et al*., 2009c; Raghavendra and Raghavrao, 2010 and Mansor, 2012, except PV, which ranged from 0.7-1.9 meq O_2 kg^{-1}. From the color analysis, oils extracted by AE, and CE were water clear as per the specification of APCC (2009) whereas HPH and MOH subject the cream to heat that normally lead to polymerization that will turn oil yellow in color against APCC standards.

Total phenolic content (TPC)

As shown in Fig. 1, significant differences were observed for total phenolic content among coconut oils extracted through different processes supporting that the process of extraction of oil could be a major factor contributing to the variation in phenolic contents in oils (Nevin and Rajamohan, 2006, 2009). TPC was in the range of 26.4 to 43.0 mg gallic acid equivalent (GAE) 100 g^{-1} of oil, similar to the total phenolic content of the VCO produced by different methods, which ranged from 23 to 91 mg caffeic acid equivalent (CAE) kg^{-1} (Dia *et al.,* 2005). Similarly TPC of cold and heat extracted coconut oils were in the range of 6.2 to 6.6 mg and 7.8 to 44.9 mg GAE 100 g^{-1} oil, respectively (Seneviratne *et al*., 2009). While AEE oil had the highest TPC of 42.1 mg GAE 100 g^{-1} of oil, the lowest TPC of 26.4 mg GAE 100 g^{-1} was observed in HPH. Iconomou *et al*., 2010, reported an increase in the phenolic content in the virgin olive oil extracted using enzymes compared to the control oil (without enzyme) supporting the results in present study. AE and AEE have longer contact time between oil phase and aqueous phase to incorporate phenolic compounds in to oil whereas in CE, the aqueous phase at low temperature did not support the incorporation of phenolic compound into oil. Similarly, HPH and MOH processes require their respective heat and high temperature that may destroy or inactivate the phenolic compounds (Marina *et al*., 2009c; Nissiotis and Margari, 2002).

DPPH radical scavenging activity

As shown in Fig. 2, the order of increasing scavenging activity was AE>AEE>CVCO >CE>HPH>MOH with reference to the activity of α tocopherol and BHT, corresponding to the result for TPC in oil samples. Higher the phenolic content, higher free radical scavenging activity was observed in dose dependent manner due to the presence of high phenolic content per unit mass of oil. AE and AEE had the lowest IC_{50} values supporting the theory that antioxidant activity depends on processing conditions (Moure *et al*., 2001). The

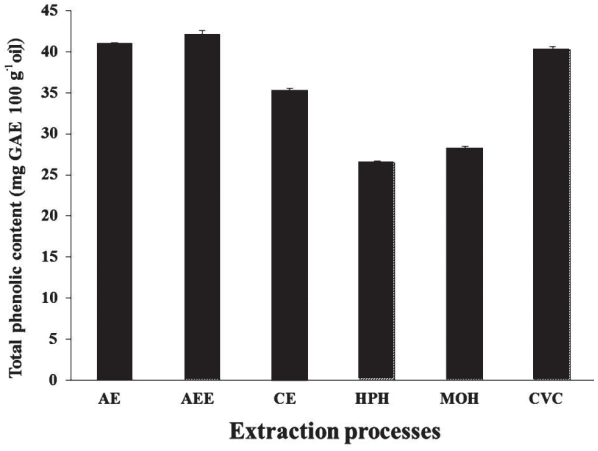

Fig. 1. Total phenolic content of the phenolic extracts of oil samples. Values are mean ± SD (n =3)

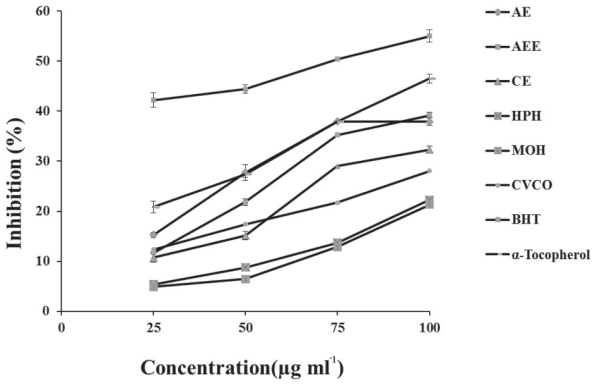

Fig. 2. DPPH radical scavenging activity (%) of the phenolic extracts of oil samples

effect of thermal treatment in HPH and MOH has been reasoned for their reduced DPPH scavenging activity (Henna Lu and Tan, 2009).

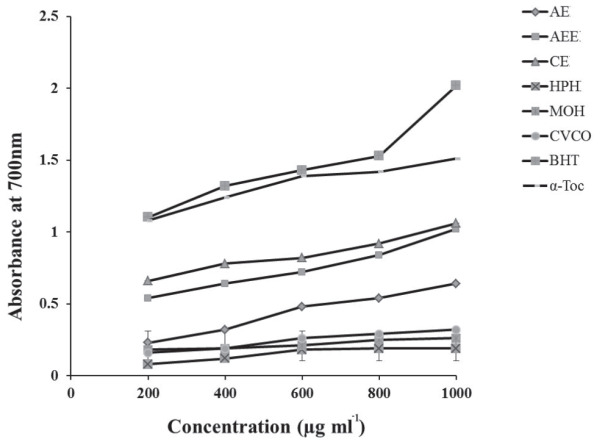

Fig. 3. Percentage inhibition of β-carotene-linoleate of the phenolic extracts of oil samples. Values are mean ± SD(n =3)

Percentage inhibition of β-carotene-linoleate

The inhibition of discoloration of β-carotene in the presence of free radicals in the β-carotene linoleate model system was observed in the increasing order of AEE>AE>CE>CVCO> MOH>HPH as compared to the activity of α tocopherol and BHT in Fig. 3. AEE had the activity significantly higher than that of other extraction processes including CVCO ($P<0.05$). It could be attributed to the enhanced incorporation of minor components (phenols, tocopherol, volatiles, and carotenes) into the oil phase in enzymatic extraction process (Chiacchierini *et al*., 2007).

Reducing power

The reducing power of oil extracts was increased with the increasing concentration of phenolic extracts and the increasing order of reducing power of oil was CE>AEE>AE>CVCO> MOH>CVCO>HPH compared to the activity expressed by α tocopherol and BHT as shown in Fig. 4. CE, AE and AEE were observed for the significant increase in reducing power in dose dependent manner from 500 µg mL^{-1} to 1000 µg mL^{-1} of phenolic extract. The changes in phenolic composition associated with the extraction processes might be attributed to the changes

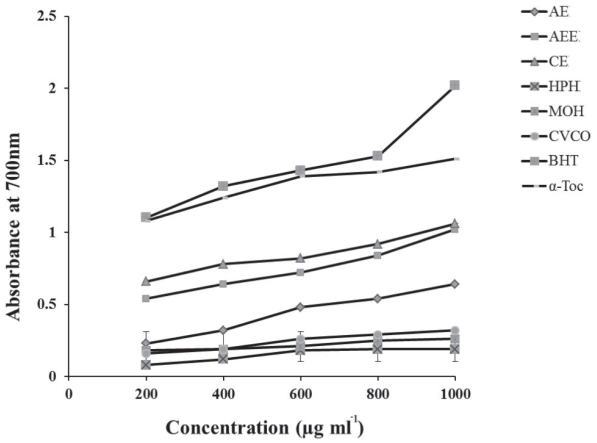

Fig. 4. Reducing power of phenolic extracts of oil samples. Values are mean ± SD (n = 3)

observed in reducing power. The result of this study is supported by the finding of Marina *et al.,* (2009b) for the highest reducing power in chilling extraction process.

Total antioxidant activity (TAA)

Table 2 shows total antioxidant activity in the increasing order of CE>AE>AEE>CVCO> MOH>HPH as noticed in other antioxidant assays in the present study. The range of activity was from 4.1±0.1 in HPH to 6.1±0.3 in CE (mg GAE 100 g^{-1} oil sample) which was comparable only to AE and AEE.

Correlation of total phenolic content with antioxidant activity

The coefficient of correlation (r) between antioxidant tests and total phenolic content of the extracted oils were analysed. Total phenolic content was correlated strongly with the DPPH scavenging activity (r=0.69), β-carotene linoleate bleaching activity (r=0.74) and total antioxidant activity (r=0.84) at $P<0.05$. However, no correlation was observed between total phenolic content and reducing power of oil samples ($r = 0.59$, $P > 0.05$).

From the effect of extraction procedures in CE, AE and AEE, the physiochemical properties and total phenolic content of oils were observed with the slightest changes compared to the other methods of the present study. As correlation analysis showed the positive relation between total phenolic content and antioxidant activity, oils extracted by CE, AE

Table 2. Total antioxidant activity of the phenolic extract of oil samples extracted by different processes

[a]Oil extraction process	[b]Total antioxidant activity (mg GAE 100 g^{-1} oil sample)
AE	6.1 ± 0.34
AEE	5.9 ± 0.21
CE	6.2 ± 0.17
HPH	4.1 ± 0.12
MOH	4.9 ± 0.26
CVCO	5.6 ± 0.19

[a]AE: Aqueous extraction; AEE: Aqueous enzymatic extraction; HPH: Hot plate heating; CE: Cold extraction; MOH: Microwave oven extraction; [b]Each values are mean of three replicates ± SD (n=3).

and AEE were observed with the highest antioxidant activity corresponding to their total phenolic content with reference to CVCO compared to HPH and MOH. Hence the present study concludes that CE, AE and AEE methods could extract VCO with the desirable quality and oxidative stability.

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