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Differences in chemical components and antioxidant-related substances in virgin coconut oil from coconut hybrids and their parents

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Abstract

Chumphon Horticultural Research Centre (CHRC) is Thailand's main coconut research unit. CHRC has developed three coconut cultivars: Sawi Hybrid No. 1 (Malayan Yellow Dwarf x West African Tall: MYD x WAT), Chumphon Hybrid No. 60 (Thai Tall: THT x WAT) and Chumphon Hybrid No. 2 (MYD x THT). This study compared some chemical components in virgin coconut oil (VCO) from coconut hybrids with their parents. The VCO was extracted by cold pressing and fermentation methods, and was analyzed for fatty acid profiles, triacylglycerol profile, acid value, tocopherol content, total phenolic content, and antioxidant activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals. The findings showed that hybrids contained lauric acid ranging from 46.63 to 48.34% of total fatty acid. Chumphon 60 had the highest lauric acid content, 48.34% of total fatty acids, which was not significantly different ($p > 0.05$) from that of the parents. In contrast, the cultivars from MYD, Sawi 1 and Chumphon 2, had significantly greater lauric acid content than the parent MYD ($p \leq 0.05$). Cold pressing and fermentation provided an oil extraction yield of 25 and 20%, respectively. The proportions of lauric acid in VCO from these two methods were not significantly different ($p > 0.05$), but the cold pressing method resulted in higher tocopherol content ($p \leq 0.05$). The VCO of Chumphon 60 from the cold pressed method had tocopherol content close to that of the parent WAT ($p > 0.05$) but significantly higher than that of the other parent THT ($p \leq 0.05$). In addition, it contained the highest total phenolic contents among the three cultivars, 57.89 mg GAE/100 g oil, leading to antioxidant activity with a low EC_{50} of 0.53 mg GAE/ml. Overall, the hybrid of WAT x THT, Chumphon 60, was outstanding among the cultivars; it had the highest levels of lauric acid, total phenolic compounds, and antioxidant activity.

Introduction

Coconut is an economically important crop, and Thailand was ranked the world's sixth largest coconut producer, 1.3 million tons, in 2010 (FAO, 2012). In general, coconut are classified as tall, dwarf and hybrid. The hybrids are mostly produced via pollination to develop certain qualities, i.e. greater precociousness and vigor, higher nut yield, larger nut size, higher oil yield, and pest and disease resistance.

A primary component of coconut oil is lauric acid, which has been found to have potent antiviral activity (Hornung *et al*., 1994), antibacteria (Enig, 1997), reduces total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol and increases high density lipoprotein (HDL) cholesterol in serum and tissues (Nevin and Rajamohan, 2004; Ravnskov, 1995). Lauric acid levels in coconut oil are regulated by genetics and the environment (Rosell *et al*., 1985; Laureles *et al.*, 2002; Akpan *et al*., 2006; Brou, 2010). The most important enzymes for lauric acid synthesis are laurate-CoA-preferring lysophosphatidic acid acyltransferase (Padley *et al*., 1994), lauroyl-ACP thioesterase (Voelker *et al*., 1996), malate dehydrogenase, and isocitrate lyase (Eccleston and Ohlrogge, 1998). Environmental effects also play a major role: for example, coconut oil from West African Tall variety grown in the Philippines, Papua New Guinea, Vanuatu, North Sulawesi and Sri Lanka has varying lauric acid content, ranging from 45 to 52.6% (Rossell *et al*., 1985; Laureles *et al.*, 2002).

Three well-known coconut hybrids in Thailand have been developed by the Chumphon Horticultural Research Centre (CHRC) in Chumphon Province, Thailand: Sawi Hybrid No. 1, Chumphon Hybrid No. 60 and Chumphon Hybrid No. 2. Fatty acid profiles of these three hybrids have been previously reported, but not the values of some of the other components and the resulting antioxidant activity.

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Therefore, this study aimed to statistically compare the oil composition (lauric acid, fatty acid profile, and triacylglycerol), antioxidant-related compounds (tocopherol content and total phenolic content) and antioxidant activity (EC_{50}) of these hybrids with their parents. In addition, the results of using two methods of extracting virgin coconut oil were compared in order to obtain useful information for further study and application.

Materials and Methods

Coconut oil

Three coconut varieties and three hybrids in this experiment were collected from the CHRC. The coconuts consisted of mature fruits aged 9–12 months. The varieties were the West African Tall (WAT), Malayan Yellow Dwarf (MYD) and Thai Tall (THT), and the three cultivars were Sawi Hybrid No. 1 (WAT x MYD), Chumphon Hybrid No. 2 (MYD x THT) and Chumphon Hybrid No. 60 (WAT x THT). Virgin coconut oil (VCO) was extracted from all varieties and cultivars by two methods: fermentation and cold pressing.

Fermentation method

VCO was produced from coconut milk, using a 1:1 ratio of coconut meat:water. Coconut milk from the extraction was placed into a fermentation container controlled the temperature at 70-80°C and allowed to sit for 16 to 24 h for natural fermentation which generally 3 groups of cultures; lactic acid bacteria, yeast and mold were play a role of hydrolysis and breaking coconut milk emulsion. After fermenting, the oil was separated, filtered through an eight-layer filter cloth bag and then the water in oil was dried out under low heat at 65°C. The clear VCO was kept in a dry container at −20°C.

Cold pressing method

Dehusked coconut nuts were grated using a motorized grater; the coconut meat was then dried at 60°C in a hot-air drier until the moisture content was reduced to 10–12%. VCO was extracted by a pressurized stainless steel expeller; the oil was then filtered through a three-layer filter cloth bag. After drying under low heat (65°C), the clear VCO was kept in a dry container at −20°C.

Fatty acid analysis

The fatty acid methyl esters (FAMEs) of the coconut oil were prepared using the method of Durmaz *et al.* (2007) with some modifications. Coconut oil (30 mg) and acetyl chloride/methanol (1:19 v/v, 5 mL) were mixed for 2 min in a vortex mixer, and then heated at 80°C for 1 h. After the reaction, the test tube was cooled; then 2 mL of hexane and 1 mL of distilled water were added, followed by centrifugation at 3,000 × g for 15 min at 20°C. The hexane layer containing FAMEs was transferred to a vial and analyzed using a gas chromatograph (model 6890N; Agilent Technologies, USA) fitted with a flame ionization detector. The column used was a 100 m × 0.25 mm fused silica capillary column (model SP™-2560; Sigma-Aldrich, USA). The initial column temperature was 60°C, which was held for 1 min. This was then increased to 170°C at a rate of 10°C /min, held for 10 min at 170°C, then increased at a rate of 4°C /min to 224°C and held for 15 min at 224°C. Injector temperature was set at 280°C, and flame ionization detector temperature was set at 250°C. Fatty acids were identified by comparing retention times with fatty acid methyl ester standards (Supelco® 37 Component FAME Mix; Sigma-Aldrich Singapore). Each fatty acid was calculated as a percentage of total fatty acids.

Triacylglycerol composition

Triacylglycerol (TAG) composition was obtained by gas chromatography, based on the methods of Ulberth and Gaberning (1997), with some modifications. Coconut oil (10 mg) was dissolved in 1 mL of heptane, mixed for 5 min and transferred to a vial, followed by subsequent analysis using a gas chromatograph fitted with a flame ionization detector (model 6890N; Agilent Technologies). The column used was an Agilent DB-1 (25 m length, 0.32 mm internal diameter and 12 μm film thickness). The initial column temperature was 250°C, which was held for 1 min; this was then increased at a rate of 3°C/min to 320°C and held for 15 min at 320°C. Injector temperature was set at 320°C and flame ionization detector temperature was set at 320°C. Triacylglycerol peaks (or groups of peaks) were identified by their carbon number and based on the retention time of saturated TAG standards tricaprylin, tricaprin, trilaurin, trimyristin and tripalmitin. TAG data was calculated as peak area percentages.

Acid value

Acid value was determined according to AOCS methods (2003). Ten g of oil was mixed into isopropyl alcohol:toluene (1:1). The solution was then titrated with 0.1 N KOH; phenolphthalein was used as an indicator.

Tocopherol content

One ml of sample was mixed with 2 ml of isopropyl alcohol and incubated at 37°C for 30 min with vigorous shaking; 0.4 ml was then pipetted

into 1 ml of reagent solution (0.4 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 37°C for 30 min with vigorous shaking. Absorbance of the aqueous phase at 695 nm was measured against a blank using UV-VIS spectrophotometer (Model Genesys 10S UV/Vis; Thermo Fisher Scientific, UK). The tocopherol content was expressed as mg equivalents of tocopherol per 100 g oil, as given by the formula:

$$\text{Tocopherol content (mg/100 g oil)} = [(A - A_o) \times V \times 1{,}000] / (v \times m)$$

where A and A_o are the measured absorbance values of the sample and blank solution, respectively; V and v are the volume in ml of the solvent and in µl of the extracted sample, respectively; and m is the mass, in g, of the test portion (Prieto *et al.*, 1999).

Preparation of polyphenol extract

Ten g of oil sample was dissolved in 50 ml hexane and extracted three times with 20 mL portions of 60% ethanol. The three extracts were pooled and then evaporated to dryness (40ºC) using a rotary evaporator (model R-210; Buchi, Flawil, Switzerland), then dissolved in a known volume of ethanol for further analysis.

Total phenolic content

The total phenol content was estimated according to the method of Marina *et al.* (2009) with some modification. An aliquot of test sample (1 mg/mL) was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted tenfold with deionized water); 0.8 mL of 7.5% of sodium carbonate solution was added, and the mixture was then kept at room temperature for 30 min. Absorbance of the aqueous phase was measured at 725 nm using a UV-Vis spectrophotometer (Model Genesys 10S UV/Vis; Thermo Fisher Scientific, UK). Gallic acid (0.1–0.5 mg/mL) was used as the standard for the calibration curve. Total phenolic content of the extract was expressed as gallic acid equivalent (GAE) per 100 g oil.

Antioxidant activity

The antioxidant activity of the coconut oil was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method (Marina *et al*., 2008). Each oil extract (0.1–3.0 mg/ml) in ethanol was mixed with 1 ml of ethanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm (Model Genesys 10S UV/Vis; Thermo Fisher Scientific, UK). The inhibition percentage was expressed using the following equation:

$$\text{Inhibition\%} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution in ethanol without sample, and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH solution in ethanol with sample (after 30 min of incubation). The EC_{50} (mg GAE/ml) value was calculated based on the amount of coconut oil extract necessary to decrease the initial DPPH radical concentration by 50%.

Statistical analysis

All data were subjected to analysis of variance (ANOVA). Significant differences between the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

Results and Discussion

Fatty acid and triacylglycerol profiles

VCO from three varieties, WAT, MYD and THT, and three hybrids, Sawi 1 (MYD x WAT), Chumphon 2 (THT x MYD) and Chumphon 60 (THT x WAT), were prepared by two methods, cold pressing and fermentation. Oil extraction yield of all three varieties and three cultivars prepared by cold pressing was 25% – higher than from the fermentation method, which yielded 20%. The array of fatty acids from those varieties and cultivars comprised caproic (C6), caprylic (C8), capric (C10), lauric (C12), myristic (C14), palmitic (C16), stearic (C18) oleic (C18:1) and linoleic (C18:2) acids (Table 1). The VCO contained significant amounts of saturated fatty acids, particularly lauric acid. The proportion of lauric acid in the VCO was not different ($p > 0.05$) based on oil extraction methods, but there were differences between parents and hybrids. The findings showed that cultivars had lauric acid content ranging from 46.63 to 48.34% of total fatty acids. The cultivars from MYD (Sawi 1 and Chumphon 2) had higher lauric acid content than the parent MYD ($p \leq 0.05$). Chumphon 60, a cultivars of THT and WAT, had the highest lauric acid content, 48.34% of total fatty acids, which was not significantly different ($p > 0.05$) from the parents. This clearly indicated that the lauric acid content was controlled via genetics, where the regulating enzymes were laurate-CoA-preferring lysophosphatidic acid acyltransferase (Padley *et al*., 1994), lauroyl-ACP thioesterase (Voelker *et al*., 1996), malate dehydrogenase, and isocitrate lyase (Eccleston and Ohlrogge, 1998). According to oil extraction yield and lauric acid content, the Chumphon 60 prepared by cold pressing method was

Table 1. Fatty acid profile of virgin coconut oil (%wt)* from different coconut cultivars and oil extraction methods

Variety of coconut cultivars	Oil extraction method	Fatty acids (% of total fatty acids)								
		C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
WAT	Fermentation	0.38^{ab}	6.70^{b}	5.57^{bcd}	48.23^{a}	20.20^{cd}	8.85^{cd}	2.88^{cd}	5.86^{ef}	1.27^{a}
	Cold pressing	0.37^{ab}	6.67^{b}	5.58^{bcd}	48.18^{a}	20.40^{bcd}	8.78^{cd}	3.71^{ab}	5.75^{ef}	1.10^{b}
MYD	Fermentation	0.38^{ab}	5.43^{d}	4.76^{f}	44.86^{d}	21.00^{a}	11.00^{a}	3.92^{a}	7.12^{a}	1.37^{a}
	Cold pressing	0.40^{ab}	5.70^{cd}	4.91^{ef}	45.26^{d}	20.86^{ab}	10.77^{a}	3.84^{ab}	6.80^{ab}	1.33^{a}
THT	Fermentation	0.35^{b}	6.26^{bc}	5.92^{ab}	48.25^{a}	19.15^{e}	9.18^{c}	3.39^{abcd}	6.20^{de}	1.29^{a}
	Cold pressing	0.40^{ab}	6.36^{bc}	5.76^{abc}	48.09^{a}	19.09^{e}	9.11^{c}	3.48^{abc}	6.23^{cde}	1.27^{a}
Sawi 1	Fermentation	0.41^{ab}	6.95^{ab}	5.22^{de}	47.31^{b}	20.61^{abc}	9.16^{c}	2.93^{cd}	6.35^{bcd}	0.95^{cd}
	Cold pressing	0.40^{ab}	6.49^{bc}	5.37^{cd}	47.35^{b}	20.62^{abc}	9.17^{c}	2.97^{cd}	6.50^{bcd}	1.05^{bc}
Chumphon 2	Fermentation	0.43^{a}	6.43^{bc}	5.29^{de}	46.63^{c}	20.38^{abcd}	9.74^{b}	3.23^{bcd}	6.69^{abc}	1.05^{bc}
	Cold pressing	0.45^{a}	6.67^{b}	5.51^{bcd}	46.93^{bc}	20.50^{bcd}	9.74^{b}	3.20^{bcd}	6.04^{de}	0.88^{d}
Chumphon 60	Fermentation	0.40^{ab}	7.01^{ab}	5.78^{abc}	48.05^{a}	20.01^{d}	8.76^{cd}	2.94^{cd}	5.80^{ef}	1.16^{b}
	Cold pressing	0.43^{a}	7.61^{a}	6.16^{a}	48.34^{a}	19.43^{e}	8.49^{cd}	2.81^{cd}	5.54^{f}	1.15^{b}

*Values within each column with different superscripts are significantly different $p \leq 0.05$.
WAT: West African Tall; MYD: Malayan Yellow Dwarf; THT: Thai Tall.
Sawi 1: WAT x MYD; Chumphon 2: THT x MYD; Chumphon 60: WAT x THT.

Table 2. Triacylglycerol profile of virgin coconut oil (%wt)* from different coconut cultivars and oil extraction methods

Variety of coconut cultivars	Oil extraction method	Triacylglycerol carbon number							
		30	32	34	36	38	40	42	44
WAT	Fermentation	4.13^{e}	17.22^{d}	23.59^{bc}	24.28^{f}	15.11^{f}	10.00^{d}	4.09^{hi}	1.58^{e}
	Cold pressing	4.10^{f}	17.23^{d}	23.71^{b}	24.56^{e}	15.10^{f}	9.91^{d}	4.02^{j}	1.38^{g}
MYD	Fermentation	2.37^{k}	10.99^{i}	18.07^{i}	26.08^{a}	19.25^{a}	14.28^{a}	6.433^{a}	2.53^{a}
	Cold pressing	2.43^{j}	11.26^{h}	18.41^{h}	26.10^{a}	19.04^{b}	14.02^{a}	6.26^{b}	2.48^{a}
THT	Fermentation	4.97^{a}	19.10^{a}	24.57^{a}	23.04^{j}	14.01^{h}	9.13^{f}	3.74^{k}	1.44^{fg}
	Cold pressing	4.42^{b}	17.95^{b}	23.64^{b}	23.76^{i}	14.67^{g}	9.85^{d}	4.18^{gh}	1.53^{ef}
Sawi 1	Fermentation	3.15^{i}	13.66^{g}	20.54^{e}	25.33^{b}	17.62^{cd}	12.27^{b}	5.36^{e}	2.06^{bc}
	Cold pressing	3.40^{g}	14.80^{e}	21.37^{d}	25.20^{c}	16.76^{e}	11.60^{c}	4.96^{f}	1.90^{d}
Chumphon 2	Fermentation	4.22^{d}	17.31^{d}	23.45^{c}	24.14^{g}	15.21^{f}	9.95^{d}	4.26^{g}	1.46^{fg}
	Cold pressing	4.29^{c}	17.48^{c}	23.45^{c}	24.03^{h}	15.06^{f}	10.02^{d}	4.20^{gh}	1.47^{fg}
Chumphon 60	Fermentation	3.19^{h}	13.82^{g}	20.29^{f}	25.03^{d}	17.54^{d}	12.48^{b}	5.53^{d}	2.13^{b}
	Cold pressing	3.15^{i}	13.59^{f}	20.09^{g}	25.14^{c}	17.75^{c}	12.60^{b}	5.65^{c}	2.03^{c}

*Values within each column with different superscripts are significantly different $p \leq 0.05$.
WAT: West African Tall; MYD: Malayan Yellow Dwarf; THT: Thai Tall.
Sawi 1: WAT x MYD; Chumphon 2: THT x MYD; Chumphon 60: WAT x THT.

suggested to produce the VCO commercially.

The chromatogram of triacylglycerols (TAGs) of coconut oil showed eight major peaks differing in carbon number (CN): 30, 32, 34, 36, 38, 40, 42 and 44 (Table 2). The chromatogram corresponds well with that of coconut oil TAGs reported by Laureles et al. (2002). TAG of CN 36 was the highest (23.04–26.10%) in all varieties and cultivars analyzed, followed by CN 34 and CN 38 (18.07–24.57% and 14.01–19.25%, respectively). TAGs composition varied significantly among varieties and cultivars, similar to the report by Laureles *et al.* (2002), due to the proportion of individual fatty acids and the product processing history (Reske *et al.*, 1997).

Acid value

The acid value (AV) is a measure of the free fatty acids (FFA) present in the fat or oil, a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides (due to moisture, temperature or enzymes), and was determined by titrating the oil against standard alkali in alcoholic medium. The acid values obtained from all six experiments were relatively low, indicating that the samples were highly stable against oxidation (Figure 1).

Table 3. Tocopherol content, total phenolic content and antioxidant activity (EC_{50})

Variety of coconut cultivars	Oil extraction method	Tocopherol content (mg/100 g oil)	Total phenolic content (mg GAE/100 g oil)	EC_{50} (mg GAE/ml)
WAT	Fermentation	4.15 ± 0.12^{ef}	53.71 ± 0.26^{c}	0.81 ± 0.02^{de}
	Cold pressing	5.40 ± 0.06^{ab}	57.77 ± 0.36^{a}	0.48 ± 0.01^{g}
MYD	Fermentation	2.14 ± 0.32^{j}	51.72 ± 0.15^{d}	0.87 ± 0.01^{d}
	Cold pressing	4.67 ± 0.24^{cd}	53.15 ± 0.58^{cd}	0.74 ± 0.09^{e}
THT	Fermentation	2.66 ± 0.22^{i}	52.10 ± 1.56^{d}	0.79 ± 0.03^{de}
	Cold pressing	3.67 ± 0.18^{fg}	55.45 ± 1.61^{b}	0.62 ± 0.03^{f}
Sawi 1	Fermentation	3.27 ± 0.05^{gh}	52.91 ± 1.10^{cd}	1.15 ± 0.06^{b}
	Cold pressing	4.45 ± 0.69^{de}	56.66 ± 0.80^{ab}	1.01 ± 0.15^{c}
Chumphon 2	Fermentation	4.03 ± 0.25^{ef}	48.17 ± 0.74^{e}	1.27 ± 0.04^{a}
	Cold pressing	5.78 ± 0.18^{a}	48.60 ± 0.80^{e}	1.20 ± 0.02^{ab}
Chumphon 60	Fermentation	2.99 ± 0.36^{hi}	55.38 ± 0.40^{b}	0.80 ± 0.09^{de}
	Cold pressing	5.04 ± 0.07^{bc}	57.89 ± 0.57^{a}	0.53 ± 0.0^{fg}

* Values within each column with different superscripts are significantly different $p \leq 0.05$.
WAT: West African Tall; MYD: Malayan Yellow Dwarf; THT: Thai Tall.
Sawi 1: WAT x MYD; Chumphon 2: THT x MYD; Chumphon 60: WAT x THT.

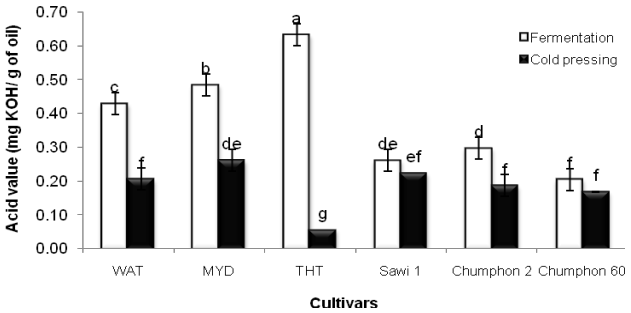

Figure 1. Acid value of virgin coconut oils from different coconut cultivars and oil extraction methods

Values are means ± standard deviation from three measurements. Superscripts are significantly different ($p \leq 0.05$).
WAT: West African Tall, MYD: Malayan Yellow Dwarf, THT: Thai Tall.
Sawi 1: WAT x MYD, Chumphon 2: THT x MYD, Chumphon 60: WAT x THT.

According to the Thailand Ministry of Public Health (1981), the acid value for VCO should be less than 4.00 mg KOH/g oil. The acid values of the studied oils ranged from 0.06 to 0.63 mg KOH/g oil (Figure 1), which was far below the maximum limit. The difference in the acid value of the VCO samples could arise from the different methods of processing. The VCO of THT obtained by the fermentation method contained the highest acid value (0.63 mg KOH/g oil). According to Lawson (1985) and Che Man *et al.* (1997), hydrolysis is accelerated by high temperatures and excessive amounts of water. Thus, VCO produced through the fermentation method would have high free fatty acid content due to the action of lipolytic enzymes, which was enhanced by the addition of water (Lalas and Tsaknis, 2002).

Tocopherol and total phenolic compounds

Tocopherols are natural lipophilic antioxidants found in vegetable oils. Table 3 shows the α-tocopherol content of the three varieties and three cultivars of coconut oils, which was affected by the processing method. It was found that VCO extracted by the cold pressing method had higher tocopherol content compared with the fermentation method ($p \leq 0.05$). This might because of 3 effects; polar/non polar compatible, dilution effect and oxidation effect. In the fermentation process, scraped coconut kernels were mixed with water to obtain coconut milk and then left to ferment overnight. Tocopherol in coconut kernels was diluted with water and could be oxidized during the process. Therefore, α-tocopherol content from fermentation method was significantly lower than those prepared by cold pressing.

Phenolic compounds are potential natural antioxidants found in foods (Parr and Bolwell, 2000). VCO is also a source of phenolic compounds, and this study found that the contents varied based on coconut varieties and oil extraction processes (Table 3). Total phenolic contents of the VCO were 48.17–57.89 mg GAE/100 g oil. The results indicated that VCO extracted by the cold pressing method was richer in phenolic substances compared with the fermentation method ($p \leq 0.05$). This was because during the fermentation process coconut milk was left to ferment overnight; the oil was released and settled on the top layer, separating from the aqueous phase. Phenolic compounds are polar compounds that are easily dissolved in the aqueous phase of coconut milk and thus are subsequently lost during oil collection. Therefore, the total phenolic content in VCO using the fermentation method was less than when the cold pressing method was used.

Among varieties, WAT had the highest total phenolic content (57.77 mg GAE/100 g oil) while MYD had the lowest (53.15 mg GAE/100 g oil) ($p \leq 0.05$). The cultivar from WAT and MYD, Sawi 1, had a total phenolic content between the two parents, 55.45 mg GAE/100 g oil, ($p \leq 0.05$). Similar to Sawi 1, WAT characteristics were transferred to the cultivar Chumphon 60, giving it the highest phenolic content of 57.89 mg GAE/100 g oil.

Antioxidant activity

The antioxidant properties of phenolic substances vary significantly, depending on their functional groups (Rice-Evans *et al*., 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. For VCO, the phenolic substances were protocatechuic, vanillic, caffeic, syringic, ferulic and *p*-coumaric acids (Marina *et al*., 2008)

The antioxidant activity of the phenolic extracts of VCO was determined by DPPH radical scavenging activity assay. The oil extracts were able to reduce the stable radical DPPH to a yellow color, meaning that the oil possessed hydrogen-donating capabilities and acted as an antioxidant. The antioxidant activity of VCO samples ranged from the EC_{50} of 0.48 to 1.27 mg GAE/ml. The highest antioxidant activity was observed in WAT and its hybrid Chumphon 60 (cold pressing method) ($p \leq 0.05$), which might be because they had a high content of phenolic compounds, as previously mentioned. Compared with cold pressed VCO, fermented VCO had gone through more processing steps during sample preparation, such as heat during fermentation and drying off water from the oil.

Conclusion

This study was undertaken to compare the chemical composition and antioxidant activity of the VCO obtained from three varieties and three hybrids prepared by cold pressing and fermentation methods. Different oil extraction methods resulted in differences in oil extraction yield, acid value, tocopherol content, total phenolic compounds and EC_{50}, but not fatty acid profile. The desirable characteristics of WAT were transferred to its hybrid Chumphon 60. Therefore, the VCO of Chumphon 60 not only had a similar fatty acid profile to WAT but it was also found to have similar tocopherol content, total phenolic compounds and EC_{50}. Considered together with other information from the CHRC, its precociousness, vigor, nut size, high oil yield, and pest and disease resistance made Chumphon 60 remarkable among hybrids grown in Thailand.

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