



## Virgin Coconut Oil: Extraction, Physicochemical Properties, Biological Activities and Its Authentication Analysis

Abdul Rohman<sup>a,b</sup>, Irnawati<sup>a</sup>, Yuny Erwanto<sup>c</sup>, Endang Lukitaningsih<sup>a</sup>, Muhamad Rafi<sup>c</sup>, Nurulhidayah A. Fadzilah<sup>d</sup>, Anjar Windarsih<sup>e</sup>, Ainin Sulaiman<sup>b</sup>, and Zalina Zakaria<sup>b</sup>

<sup>a</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia; <sup>b</sup>University of Malaya Halal Research Centre (UMHRC), Universiti Malaya, Kuala Lumpur, Malaysia; <sup>c</sup>Institute for Halal Industry and Systems, Universitas Gadjah Mada, Yogyakarta, Indonesia; <sup>d</sup>International Institute for Halal Research and Training, Gombak, Malaysia; <sup>e</sup>Research Division for Natural Product Technology, Indonesian Institute of Sciences (LIPI), Yogyakarta, Indonesia

### ABSTRACT

Virgin coconut oil (VCO) has emerged as functional food oil due to its capability to provide some biological activities which are beneficial to human health. This is due to the fact that some minor components like tocopherols and phenolics compounds are retained. VCO is prepared from fresh, mature kernel of the coconuts by mechanical or natural means, with or without the use of heat, but specifically without any chemical refining, bleaching and deodorizing (RBD). As a consequence, VCO has slight difference in terms of some physico-chemical properties with RBD coconut oil. Due to phenolics compounds contained, VCO exhibited several pharmacological activities including antioxidant, anti-inflammatory and immunomodulatory, anti-hyperlipidemia, anticancer, antidiabetic, anti-bacterial and neuroprotective activities. VCO has commanded high price value in the fats and oils industry, hence, VCO can be target of adulteration with low priced oils. Fourier transform infrared (FTIR) spectroscopy and chromatographic techniques combined with multivariate analysis has been successfully reported for analysis of adulteration practice involving the substitution or replacement VCO with other oils. This review highlights some techniques for VCO extraction, physicochemical (characterization), biological activities and authentication analysis of VCO.

### KEYWORDS

Virgin coconut oil; authentication analysis; functional oil; physicochemical properties; health benefits

## Introduction

Coconut oil, known as *minyak kelapa* in Indonesia and Malaysia, is one of edible oils obtained from the extraction of coconut kernel in the mature stage using either mechanical or thermal processing. Because of the high saturated fatty acid and fat contents, coconut oil is resistant to oxidative modifications, which make them suitable for cooking.<sup>[1]</sup> Coconut is a widespread plantation and is grown in more than 80 countries. The world production of coconut is estimated at around 55 million tons annually. Coconut oil has significant use in the toiletry, food and various industrial applications.<sup>[2]</sup>

Some different types of oils prepared from coconut include coconut testa oil (CTO), virgin coconut oil (VCO) and copra oil (CO).<sup>[3]</sup> Data from Research and Market reported

**CONTACT** Abdul Rohman  [abdulkimfar@gmail.com](mailto:abdulkimfar@gmail.com)  Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta

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that the global market of VCO in the market is USD 2.7 billion in 2018 and is forecasted that by 2024 the VCO market grow over 9% to reach USD 4.7 billion.<sup>[2]</sup> The difference in coconut oil's preparation itself causes changes in the physicochemical properties and the biological activities.<sup>[3]</sup> CO is an oil collected with mechanical milling from copra (the coconut kernel dried with direct sunlight or with the oven to reduce the contents of water). CTO is the emerging form of coconut oil obtained by extracting the coconut testa using isopropyl alcohol. Zhang et al.<sup>[4]</sup> reported that the optimum yield of CTO having up to 63–76% was extracted using the extraction condition of temperature of 60°C, period of extraction of 3 h and the ratio of substrate: isopropyl alcohol (1:4). Due to the involvement of organic solvents during CTO extraction, this oil has not been widely applied yet for the edible purposes.

VCO is obtained by extracting the fresh coconut kernel using natural means and does not undergo any kinds of chemical treatments such as refining, bleaching and deodorizing to produce refined-bleached-deodorized (RBD) oil.<sup>[5]</sup> In other words, VCO is produced through wet method, namely via coconut milk.<sup>[6]</sup> Philippine National Standard together with Bureau of Agriculture and Fisheries Product Standards<sup>[7]</sup> and Srivastava et al.<sup>[8]</sup> has defined VCO as the oil obtained from fresh, mature kernel of the coconuts by mechanical or natural means, with or without the use of heat, but specifically without chemical RBD which does not lead to the alteration of the natural content of the oil. Since VCO is produced differently from RBD coconut oil, the oil obtained is slightly different in terms of its sensory characteristics. VCO is nearly colorless, with a slightly detectable acid aroma, sweet and salty taste, and is perceptible nutty aroma and flavor. On other hand, RBD coconut oil is distinctively yellow, slightly salty, and has no perceptible aroma and flavor.<sup>[9]</sup>

VCO is considered as saturated fat because the contents of saturated fatty acids are more than 90%. From epidemiological study, the consumption of high amounts of cholesterol and saturated fat contributed to high blood cholesterol, as a consequence, the perception on coconut oil is bad.<sup>[10]</sup> In the past few years, however, the pre-clinical and clinical trials have been carried out on oil-derived coconut, and the reported results showed that VCO exhibited positive outcomes to human health which might counter those arguments.<sup>[6]</sup> The main fatty acids composed TG are considered as medium chain triglyceride (MCT) which are more easily hydrolyzed and more absorbable than long chain TG by some lipases in the gastrointestinal tract of humans. VCO is rich in lauric acid, therefore, the hydrolysis of MCT yields mono-lauryl glycerol such as monolaurin which is considered as pharmacologically active compound.<sup>[11]</sup> Having rich in MCT, the consumption of VCO is associated with the increased levels of serum TG, but the lipid profile may be improved due to the incorporation of structured lipid. Prior et al.<sup>[12]</sup> reported that Polynesian populations consumed VCO regularly are not associated with coronary heart diseases. Therefore, the campaign that VCO and other coconut oils contributed to the bad cholesterol is a myth. VCO may have more beneficial effects than CO, since it retains most of the unsaponifiable components. VCO exhibits some important biological activities such as antiviral, antifungal, antiparasitic, antibacterial, cardioprotective, hepatoprotective, antidiabetic, hypolipidemic, and antioxidants which are beneficial to human health.<sup>[13]</sup> These effects may be attributed to the large amount of short-chain fatty acids like caproic, caprylic, and capric acids.<sup>[9]</sup> Nevin and Rajamohan<sup>[14]</sup> reported that VCO can lower triacylglycerols, phospholipids, total

cholesterol, low density lipoprotein (LDL), and very low-density lipoprotein (VLDL) cholesterol levels. VCO also increase high density lipoprotein (HDL) in tissues and serum. Phenolics contents of VCO were able to prevent LDL oxidation *in vitro* with the formation of reduced level of carbonyl compounds.

The objective of this review was to discuss some extraction techniques to obtain VCO along with its physicochemical characteristics and its biological activities. VCO commands high price value which can be subjected to the adulteration with low quality edible oils, hence, the authentication analysis of VCO was also addressed. During performing this review, we explored several databases such as Science Citation index, PubMed, Medline, Scopus, and Google Scholar to identify and to download the abstracts, reports, review articles, and research papers related to the extraction, physicochemical properties, biological activities of VCO. The keywords used during searching of information were: extraction + physicochemical + antioxidant (or anti-inflammation, anti-hyperlipidemia, anti-bacteria) activities + authentication + VCO.

## The extraction of VCO

The terms of “virgin” in VCO could be understood that VCO was prepared without any RBD process, as a consequence, there was any alteration in the nature of oil.<sup>[6]</sup> VCO can be extracted in a straightforward manner from coconut under ambient temperature; therefore, the loss of minor components like pro-vitamin A, tocopherol, and phenolics compounds due to solar UV irradiation during coconut drying can be avoided.<sup>[15]</sup> Based on the mode of preparation, several types of VCO are available, namely cold extraction, hot extraction, fermentation technique, and enzymatic extraction.

Cold extraction (CE) or aqueous extraction method is used to extract VCO directly from coconut milk without the aid of heat. This method involves the chilling of coconut milk at temperature of 2–8°C overnight and the separated oil is collected by centrifugation, filtered, and stored. This is a simpler and cheapest method available for VCO preparation. CE method eliminates the use of solvents because of the absence of RBD processes, therefore this method is low cost and is environmentally friendly.<sup>[8]</sup> The disadvantage of CE method is that the oil yield is relatively low which limits the application in commercial industry.<sup>[6]</sup> CE is rather difficult due to the involvement of the emulsion breaking having high stability in coconut milk. Onsaard et al.<sup>[16]</sup> have proposed three stages to break the emulsion (creaming, flocculation, and coalescence). The creaming stage occurred through gravitational force which resulted into two phases, namely aqueous phase with higher specific gravity (in the down phase) and oil phase with lower specific gravity (the top phase). The flocculation stage involved the oil phase moves as a form of group which does not involve the rupture of the interfacial film, consequently the original globule does not change. Finally, the coalescence step, the most critical destabilization phase of emulsion. In this step, the interfacial area is broken and the globules are joined together thus reducing the interfacial area. The emulsion of coconut milk can be also broken by pH adjustment at pH 3.0–5.6, and then inoculated with bacteria cultures.<sup>[17]</sup> The coconut cream can be destabilized using acetic acid treatment. Che Man et al.<sup>[18]</sup> have reported that acetic acid 25% vol/vol at different levels (0.1%, 0.2%, 0.3%, and 0.4% vol/vol) in coconut cream for reaction time of 10–14 h at ambient temperature could improve the yield of VCO extracted, with oil recovery up to 60%. Some techniques have been also reported to break the coconut milk emulsion including heat and salts treatment, action of

enzymes, refrigeration, and the use of short waves.<sup>[19]</sup> All techniques were possible because milk proteins are easily precipitated at acidic pH (below 4).

Hamid et al.<sup>[20]</sup> have made innovation of VCO preparation through integrated wet process. In this study, the meat of fresh coconut was pressed using mechanical pressing to obtain coconut milk. In order to break emulsion, the coconut milk was chilled at temperature of 10°C, so that the water and coconut butter are separated. The coconut butter was transferred and then heated to 45°C and subjected to centrifugation for separating oil (VCO) from non-oil fraction. Finally, VCO was filtered for removing any solid materials. VCO produced is colorless retaining fresh aroma and sweet taste. The authors reported that this process can maximize yield of approximately of 30–40%, which is 10–20% higher than conventional technique with minimum cost, time, and energy.

Hot extraction (HE) which involved heat treatment is another technique used for VCO extraction from coconut milk. This technique was traditionally used in Southern India and VCO obtained was conventionally used in the Ayurvedic medicinal system for treatments of children's skin ailments. In HE system, the coconut milk is subjected to temperature of 100°C for 60 min or more until the oil was separated completely from the milk. Finally, the oil formed is collected using filtration. It is reported that the use of heat can help increasing the oil yield and releasing the bound phenolic acids.<sup>[11]</sup>

The fermentation technique for VCO extraction involves the uses of bacterial activity to generate VCO. There are two types of fermentation, namely natural fermentation and induced fermentation. In natural fermentation technique, the fresh-grated coconut kernel is extracted using its water to collect the coconut milk and then is allowed at room temperature (or until 45°C) for 24–48 h to allow fermentation and separation of oil layer. The oil obtained is then scooped out, filtered, and stored.<sup>[14]</sup> In induced fermentation technique, some bacteria are used to extract VCO from coconut milk. The induced fermentation method is less popular than natural fermentation. The induced fermentation using bacteria of *Saccharomyces cerevisiae*, *Lactobacillus plantarum* (strain 1041 IAM), and *Lactobacillus delbrueckii* has been reported for the extraction of VCO from coconut milk.<sup>[21]</sup> Previously, Che Man et al.<sup>[22]</sup> have also used induced fermentation using pure culture of *Lactobacillus plantarum* strain 1041 IAM to extract coconut oil and reported that this technique able to extract as much as 95% of VCO.

VCO can be also obtained from enzymatic technique in aqueous extraction system. In this technique, a mixture of enzymes is used to release the oil portion from the coconut milk. The enzymes consisted of  $\alpha$ -amylase to produce simpler carbohydrates from starch, protease to remove plant proteins as well as polygalacturonase and cellulase to remove cell wall components.<sup>[3]</sup> These enzymes are needed because plant cell wall consists of complex carbohydrate molecules (hemicellulose, cellulose, arabinogalactans, mannans, galactomannans, and pectin) and protein.<sup>[6]</sup> Che Man et al.<sup>[18]</sup> have successfully extracted coconut oil with 1% enzyme mixture of cellulase, alfa-amylase, polygalacturonase, and protease with an oil yield of 74%.

## Physicochemical properties of VCO

Physicochemical properties of VCO were evaluated by determining some constants related to edible fats and oils such as acid number, saponification number, etc., analysis of fatty acid and triglyceride compositions, and identification and quantitative analysis of minor components like tocopherols and phenolics contents. Marina et al.<sup>[6]</sup> have reported that

**Table 1.** Some physicochemical properties of virgin coconut oil extracted using different techniques.

Analysis	Extraction techniques <sup>[23]</sup>					<sup>b</sup> Indonesian Standard <sup>[24]</sup>
	Chilling	Fermentation	Fresh-dry	Enzyme	<sup>a</sup> APCC <sup>[7]</sup>	
Iodine value	4.13	4.3	4.18	4.26	4.10–11.00	4.10–11.00
Free fatty acid Saponification value	0.31	0.29	0.46	0.35	Maximal 0.5	Maximal 0.2
Moisture content (% wt)	258.23	256.73	258.42	262.72	Min. 250–260	Not available
Viscosity (Pa.s)	0.11	0.06	0.04	0.11	0.1–0.5	Maximal 0.2
	48.93	48.73	50.93	48.93	Not available	Not available

<sup>a</sup>APCC = Asian and Pacific Coconut Community; <sup>b</sup>Standard Nasional Indonesia (National Standard of Indonesia) SNI 7381: 2008.<sup>[22]</sup>

VCO had iodine value of 4.47–8.55 g I<sub>2</sub>/100 g, Saponification value of 252.45–260.67 mg KOH/g, acid value of 0.13–0.27 mg KOH/g, peroxide value of 0.21–0.63 mEq/kg and anisidine value of 0.16–0.49. Mansor et al.<sup>[23]</sup> have compared the physicochemical properties of VCO prepared from fresh-dry (grated coconut route), chilling and thawing, enzymatic and fermentation method and the results showed that the physicochemical parameters of studied VCOs were in accordance with those established by Codex Alimentarius Commission and the Asian and Pacific Coconut Community (APCC), as shown in Table 1. However, the moisture contents of produced VCO exceed the maximum limit set by Indonesian Standard SNI 7381 (2008).<sup>[24]</sup>

Dayrit et al.<sup>[25]</sup> have compared the physicochemical properties of VCO and RBD coconut oil (RBD-CO). VCO had higher moisture, volatile matter, and free fatty acids (FFAs) and lower peroxide value than RBD-CO, but the value range overlapped and there was no single parameter could be used for differentiation of VCO from RBD-CO. In addition, VCO and RBD-CO could be distinguished by the total amount of diglycerides (DGs) in which VCO had average DGs content of 1.55% wt/wt, whereas RBD-CO gave DGs of 4.10% wt/wt. Edible fats and oils are basically composed from TG, an ester of glycerol with three fatty acids. Each edible oil has different fatty acid compositions in terms of types and amount, therefore fatty acids can be used to characterize edible fats and oils. Fatty acid (FA) compositions of VCO from several standards namely Indonesian Standard, Codex Alimentarius Standard, Asian and Pacific Coconut Community (APCC) along with those reported by some authors are presented in Table 2. Basically, the composition of fatty acids in different types of coconut oil (VCO, RBD oil) is not

**Table 2.** Fatty acid composition of virgin coconut oil (VCO) and refined, bleached and deodorized (RBD) coconut oil from various sources.

Fatty acid composition (%)	<sup>a</sup> Codex <sup>[26]</sup>	<sup>b</sup> APCC <sup>[7]</sup>	<sup>c</sup> Indonesia Standard <sup>[24]</sup>	<sup>d</sup> MS for VCO <sup>[27]</sup>	Marina et al. <sup>[28]</sup>	Dia et al. <sup>[29]</sup>
C6 (caproic acid)	nd-0.70	0.40–0.60	ND-0.7	0.80–0.95	0.52–0.69	nd-0.60
C8 (caprylic acid)	4.60–10.0	5.00–10.00	4.6–10.0	8.00–9.00	7.19–8.81	5.98–10.44
C10 (capric acid)	5.0–8.0	4.50–8.00	5.0–8.0	5.00–7.00	5.65–6.59	5.37–6.60
C12 (lauric acid)	45.10–53.20	43.00–53.00	45.1–53.2	47.00–50.00	46.89–48.03	47.63–52.55
C14 (myristic acid)	16.80–21.00	16.00–21.00	16.8–21.0	17.00–18.50	16.23–18.90	16.79–20.08
C16 (palmitic acid)	7.50–10.20	7.50–10.00	7.5–10.2	7.50–9.50	7.41–9.55	6.38–10.17
C18:0 (stearic acid)	2.00–4.00	2.00–4.00	2.0–4.0	2.50–3.50	2.81–3.57	7.45–10.73
C18:1 (oleic acid)	5.00–10.00	5.00–10.00	5.0–10.0	4.50–6.00	5.72–6.72	5.15–6.03
C18:2 (linoleic acid)	1.00–2.50	1.00–2.50	1.0–2.5	0.70–1.50	0.90–1.60	nd-0.12
C18:3 (linolenic acid)	nd-0.20	<0.5	Nd-0.2	nd	nd	nd

<sup>a</sup>standard for RBD coconut oil; <sup>b</sup>APCC = Asian and Pacific Coconut Community; <sup>c</sup>Indonesian Standard No. 7381 (2008); <sup>d</sup>MS = Malaysian Standard.

**Table 3.** Triglyceride (TG) composition in VCO obtained from different method calculated using normalization technique of peak area of liquid chromatogram.<sup>[23]</sup>

TG composition	Extraction method <sup>†‡</sup>			
	Chilling	Enzyme	Fermentation	Fresh-dry
CClCl	–	0.31	0	0.44
CpCpLa	0.54	0.79	0.41	0.95
CpCLa	4.11	3.86	3.81	4.07
CCLa	13.55	13.36	13.82	12.71
CLaLa	17.05	17.34	18.9	21.1
LaLaLa	22.62	23.94	22.21	21.63
LaLaM	19.83	17.94	19.51	18.89
LaLaO	1.84	1.88	1.72	1.88
LaMM	11.07	10.8	12.17	10.77
LaMO	1.07	1.24	1.04	1
LaMP	6.62	5.94	5.01	4.72
LaOO	0.61	0.74	0.57	0.58
LaPP	1.19	1.64	0.83	1.08
MOO	0.27	0.22	0	0.19

Cp, caproic; Cl, caprylic; C, capric; La, lauric; M, myristic; P, palmitic; O, oleic.

significantly different. But, the main difference of VCO and RBD coconut oil is related to the composition of active compounds such as tocopherol and phenolics which are retained in VCO.<sup>[6]</sup> The main fatty acids composed VCO is lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids which are classified as medium chain fatty acids. Lauric acid (C12:0) was the most dominant of fatty acids present in VCO. In addition, VCO contains less mono- and polyunsaturated FAs.

Another chemical property used for the characterization of VCO is triglyceride (TG) compositions, usually determined by liquid chromatography equipped with general detectors like refractive index, evaporative light scattering detector, and mass spectrometer detector.<sup>[30]</sup> Marina et al.<sup>[28]</sup> have reported that the major TG in VCO samples consisted of LaLaLa accounting of 22–25%, CCLa of 14–16%, CLaLa of 19–21%, LaLaM of 13–15%, and LaMM of 7–9%. The authors also compared TG in VCO obtained from Malaysia and Indonesia, and the results showed that Malaysian VCO had relatively higher contents of CpCpLa, CpCCpLa, and LaOO while Indonesian VCO had more of LaMP (Cp = caproic; La = lauric, C = capric, M = myristic, P = palmitic, O = oleic acids). Mansor et al.<sup>[23]</sup> have evaluated TG of VCO prepared from fresh-dry, chilling and thawing, enzymatic and fermentation methods, calculated using normalization technique of peak area of liquid chromatogram, as shown in Table 3.

## Pharmacological activities of VCO

Several pharmacological effects have been reported in VCO which include antioxidants, anti-inflammation, anti-hyperlipidemia and antibacterial activities.<sup>[1]</sup> VCO prepared by cold and fermentation techniques were extensively studied due to the active compounds such as phenolics and tocopherols contained. Different researchers also studied the pharmacological effects of VCO prepared from different extraction methods.

### Antioxidant activities of VCO

Phenolics compounds are group of bioactive compounds present in edible oils capable of exerting the antioxidant activities through several mechanisms, mainly hydrogen transfer

and reducing power. Several epidemiological studies showed that there is relationship between antioxidant activity and diet containing phenolics compounds. The clinical studies also revealed that VCO rich in polyphenol exhibit beneficial effects against cardiovascular disease in recent randomized control trials.<sup>[31,32]</sup> Illam et al.<sup>[33]</sup> reported that coconut oils contained some phenolic compounds namely ferulic acid, p-coumaric acid, caffeic acid, quercetin, and catechins, and the these phenolics acids are found to be higher in VCO compared to RBD-coconut oil (RBD-CO). The common phenolics compounds present in VCO and commercial coconut oil are compiled in Table 4.

Marina et al.<sup>[35]</sup> have evaluated the antioxidant activities of VCO produced by chilling and fermentation and compared its activity with RBD coconut oil using three *in vitro* antioxidant methods, namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, beta-carotene-linoleate bleaching, and reducing power. In general, VCO revealed higher antioxidant capacities than those of RBD-CO. This is based on the fact that some minor components such as tocopherols and phenolics components are retained in VCO.<sup>[14]</sup> The phenolic acids in VCO which correlated with these antioxidant activities were ferulic acid and p-coumaric acid. These phenolics compounds were highly correlated with DPPH radical scavenging activity, reducing power, and beta-carotene bleaching with coefficient of correlation (r) values of 0.91, 0.96, and 0.83, respectively. The study confirmed that phenolics compounds present in coconut oil contributed significantly to the antioxidant capacities.<sup>[36]</sup>

VCO extracted from fresh coconut meat at temperature of 50°C was evaluated for *in vivo* antioxidant activities through the measurement of antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione content (GSH), and lipid peroxidation levels in male Sprague–Dawley rats, compared to CO and groundnut oil (GO) as control for 45 days. The results showed that polyphenol fractions in VCO increased the antioxidant enzymes and reduced the lipid peroxide content.<sup>[37]</sup> In addition, VCO polyphenols (tentatively identified as flavanones or dihydroflavonols like compounds) able to inhibit lipid peroxidation than polyphenols from CO and GO. VCO extracted from fresh coconut meat having high content of active components is superior in antioxidant property than coconut oil extracted by dry process.<sup>[14]</sup> Rahim et al.<sup>[38]</sup> also reported that male Wistar rats administrated with VCO could increase 8% SOD activity, 54% CAT activity, 20% GSH content, and 12% GPx activity than those in control rats. In separate study, Illam et al.<sup>[33]</sup> reported that VCO increased the levels of intracellular GSH which involved actively during phase II detoxification system through GSH conjugation either in cell culture or in animal models and in diabetic rats.<sup>[1]</sup> Furthermore, male inbred BALB/c mice treated

**Table 4.** Phenolic present in commercial coconut oil and in virgin coconut oil.

Phenolic acid (mg/Kg)	Commercial coconut oil	Virgin coconut oil
	(mean ± standard error) <sup>[34]</sup>	(mean ± standard deviation) <sup>[35]</sup>
Total polyphenols	91 ± 11	250.9 ± 22.6
Catechin	0.87 ± 0.1	not reported
Vanillic acid	not reported	2.08
Syringic acid	not reported	0.45 ± 0.3
p-Coumaric acid	0.34 ± 0.01	0.75 ± 0.1
Caffeic acid	0.13 ± 0.06	0.12 ± 0.1
Ferulic acid	0.31 ± 0.20	5.09 ± 2.3

with VCO exhibited higher levels of brain antioxidants than mice control. VCO could reduce lipid peroxidation and increase SOD activity in the serum subjected to the forced swim test.<sup>[39,40]</sup>

Arunima and Rajamohan<sup>[41]</sup> compared the endogenous antioxidant status *in vivo* of VCO CO, olive oil (OO), and sunflower oil (SFO) in Male Sprague-Dawley rats. The dietary VCO improved the antioxidant status compared to CO, OO, and SFO ( $P < .05$ ), as indicated by the increased levels of antioxidant enzymes. Concentration of reduced glutathione was also found to be increased significantly in liver, heart, and kidney due to administration of VCO compared to those given by CO, OO, and SFO ( $P < .05$ ). VCO administration could prevent the oxidative stress, which is indicated by the decreased formation of lipid peroxidation such as hydroperoxides, malondialdehyde, conjugated dienes and by decreased protein oxidation products including protein carbonyls in serum and tissues compared to rats fed with other oils ( $P < .05$ ). The authors concluded that VCO has a beneficial role in improving antioxidant status and hence preventing lipid oxidation.

### ***Anti-inflammatory and immunomodulatory effects of VCO***

Several studies on the anti-inflammatory and immunomodulatory effects of VCO have been reported in various experimental designs. Intahphuak et al.<sup>[42]</sup> have evaluated cold-extracted VCO in acute and chronic inflammation models using rats as animal model. VCO exhibited the protective effect toward granuloma formation in ear and paw oedemic models induced by ethyl phenylpropionate, carrageenan, and arachidonic acid. VCO also showed the inhibitory activity on chronic inflammation by reducing granuloma formation, the transudative weight, and serum alkaline phosphatase. VCO prepared by fermentation technique has been reported to reduce arthritis in rats induced by adjuvant, through the mechanism of downregulating the expressions of cyclooxygenase, an enzyme responsible for inflammatory action, inducible by nitric oxide synthase and tumor necrosis factor (TNF- $\alpha$ ).<sup>[43]</sup> Zakaria et al.<sup>[44]</sup> reported that fermentation-prepared VCO could reduce acute inflammation efficiently, but it revealed less effective inflammation in chronic models.

The immunomodulatory effect of VCO has been investigated on skin inflammation induced by lipopolysaccharide (LPS). LPS could alter the immune system when it enters the body. LPS induced the stimulation of proinflammatory cytokine expression. Administration of VCO showed beneficial effect to suppress proinflammatory cytokine in monocytic leukemia both in protein and gene expression level.<sup>[45]</sup> VCO could suppress the levels of proinflammatory cytokines of TNF- $\alpha$  and interleukins (IL) in protein and gene expression responsible for inflammation, tissue damage, fever, and cell death. These studies suggested that anti-inflammatory effects of VCO were achieved by suppressing the inflammatory markers which include TNF- $\alpha$ , IL, and interferon (IFN- $\gamma$ ).

VCO showed the immunomodulatory effect in mice administered with diet containing high refined carbohydrate.<sup>[46]</sup> High refined carbohydrate induced inflammatory disorder in mice as well as altered the immune system by stimulating the high expression of proinflammatory cytokines and leukocytes. Administration of VCO showed beneficial effects on suppressing proinflammatory cytokines. VCO decreased several proinflammatory cytokines concentration including TNF- $\alpha$  and IL-6. VCO also reduced the number of leukocytes as well as mononuclear and polynuclear circulating cells.

The phenolic fraction of VCO showed immunomodulatory effects on human peripheral blood mononuclear induced by oxidized low-density lipoprotein (LDL). The oxidized LDL could have several negative impacts on human body including stimulation of inflammation signaling pathway through NF- $\kappa$ B, stimulation of toll like receptor (TLR), and stimulation of proinflammatory cytokines such as IL-6 and TNF- $\alpha$ . Phenolic fraction of VCO inhibited either the expression or translocation of NF- $\kappa$ B, therefore the inflammatory signal is inhibited. Moreover, phenolic fraction of VCO decreased the proinflammatory cytokines expression of TNF- $\alpha$  and IL-6 as well as decreased the production of inflammatory mediators, especially prostaglandin (PGE2) and nitric oxide (NO).<sup>[47]</sup>

### ***Anticancer and amelioration of toxicity induced by chemotherapy***

Lauric acid (LA) present dominantly in VCO has been studied for anticancer activities. LA is known to exhibit anticancer activity by reducing glutathione (GSH) level in cells. GSH is believed to protect cancer cells from the increased oxidative stress inducing apoptosis.<sup>[48]</sup> LA has been reported to induce apoptotic changes in numerous colorectal cancer cells mediated by several reactive oxygen species and nitrogen reactive species (ROS/NOS).<sup>[49]</sup> LA also could induce the cell cycle arrest in G0/G1 phase and G2/M phase and to activate Rho associated kinase-mediated pathway and p21-dependent apoptosis.<sup>[50]</sup>

Craig-Schmidt et al.<sup>[51]</sup> reported that mice treated with the combination of coconut oil rich in LC and menhaden oil exhibited the significant reduction of mammary tumorigenesis induced by carcinogenic of dimethylbenzanthracene (DMBA). In another study, the diet rich in VCO could reduce efficiently the incidence of colon cancer model induced by azoxymethane-dextran sodium sulfate by increasing the levels of intestinal protein Mucin 2. This protein involved in the proper maintenance of intestinal barrier integrity.<sup>[52]</sup> VCO exhibited the cytotoxicity effects on human hepatocarcinoma cells (HepG2) by stimulating hydroxyl radicals ( $\bullet$ OH) to damage cancer cells promoting cell death.<sup>[53]</sup>

Due to its activity as antioxidant, the supplementation of VCO has been reported to have hepatoprotective activity toward hepatotoxicity and oxidative damage which correlated to the amelioration of toxicity-induced chemotherapy agents.<sup>[54]</sup> VCO supplementation could attenuate chemotherapy using anticancer drug of methotrexate through inhibition of oxidative stress in rats. VCO supplementation could enhance the resistance of biochemical alterations in rats induced by methotrexate.<sup>[55]</sup> VCO also demonstrated hepatoprotective and chemotherapy using antibiotics of sulfamethoxazole-trimethoprim<sup>[56]</sup> and anticancer drug of cyclophosphamide.<sup>[57]</sup>

### ***Anti-diabetic activities***

Diabetes mellitus is a metabolic disorder characterized by the increased levels of blood glucose. Several studies have been carried out to evaluate the antidiabetic activities of VCO. Maidin and Ahmad<sup>[58]</sup> have reported that VCO prepared from fermentation (F-VCO) method could reduce the glucose levels of diabetic rats induced by alloxan. The similar study on antidiabetic activity of VCO prepared from cold extraction method was also conducted by Iranloye et al.<sup>[59]</sup> in which rats were treated with alloxan to induce diabetic. The results showed that VCO had hypoglycemic action, enhance the insulin secretion and also ameliorate the oxidative stress induced in type I diabetic male rats. El-Shemy<sup>[60]</sup> also

found that VCO prepared from cold extraction method and administered orally at 10 mL/kg body weight of rats, daily for 4 weeks was very effective against deleterious hyperlipidemic on rats induced by alloxan. Besides, VCO also revealed the similar results of antidiabetic activity on rats treated with streptozotocin.<sup>[61]</sup>

Siddalingswamy et al.<sup>[62]</sup> have compared the antidiabetic activities of VCO extracted from hot method (H-VCO) and cold-extracted VCO (C-VCO) on diabetic rats induced by streptozotocin. The results showed that H-VCO had higher hypoglycemic activity and insulin-sensitizing agent than C-VCO. These effects may be attributed to increased polyphenolic and other antioxidants compounds present in H-VCO. F-VCO also efficiently prevents the development of insulin resistance in high fructose-fed rats<sup>[3]</sup> and exerts the protective effects on renal dysfunction in diabetic rats. It is believed that the phenolic compounds play an important role in its antidiabetic activity and protective effects on renal dysfunction by inhibiting reactive oxygen species such as hydroxyl radicals responsible for the death of the beta cells.<sup>[62,63,64]</sup>

### ***Anti-hyperlipidemia activities of VCO***

VCO contain high levels of saturated fatty acids which are correlated with high levels of blood cholesterol,<sup>[65]</sup> however, several studies revealed that VCO had the positive outcomes to lipid profiles, mainly due to the presence of medium chain triacylglycerol (MCT) contained in VCO. Srivastava et al.<sup>[66]</sup> have compared the hypolipidemic effects of VCO prepared by cold extraction (CE), hot extraction (HE) and that commercially available (C-VCO) in India using male Wistar rats. The cholesterol and LDL of in blood plasma of rats treated with VCOs have been decreased significantly by on average of 25% and 40%, respectively, while high-density lipoprotein (HDL) was significantly increased ( $p < .05$ ) by 21% compared to control rats.

The effects of cold-prepared VCO on some lipid parameters in comparison with CO have been evaluated by Nevin and Rajamohan.<sup>[67]</sup> The results revealed that VCO had lower triglycerides in serum and tissues than those in CO and control animals. HDL cholesterol in rats treated with VCO was increased, while LDL cholesterol level was decreased significantly compared to CO. This finding also suggested that polyphenol fractions extracted from VCO was found to be more effective than those extracted from CO, as indicated by preventing the copper-induced oxidation of LDL as indicated by the low thiobarbituric acid reactive substance (TBARS) and reduced carbonyl formation. In the subsequent study, the same authors<sup>[14,15]</sup> also reported that the inhibitory effects on microsomal lipid peroxidation were increased in rats treated with VCO, as indicated by reduced malondialdehyde (MDA) and conjugate diene content in the tissues. The levels of lipid peroxide were also significantly decreased in the heart, liver and kidney of VCO fed rats compared to CO. These findings indicated the potential benefits of VCO in maintaining lipid metabolism.<sup>[5]</sup>

The clinical studies on the effects of daily VCO on plasma lipoproteins levels involving 35 healthy Thai volunteers using randomized, controlled, crossover trial approach have been performed by Chinwong et al.<sup>[68]</sup> The results showed that daily VCO intake significantly ( $p < .001$ ) increased HDL cholesterol by 5.72 mg/dL compared to the control regimen (2% carboxymethylcellulose solution). The daily consumption of 30 mL VCO in young healthy adults significantly increased HDL cholesterol. There were no safety issues reported during the daily consumption of VCO for 8 weeks.

### **Antibacterial activities**

VCO has been also reported to have antibacterial activity. Ogbolu et al.<sup>[69]</sup> reported the antibacterial activities of fermentation-prepared VCO on *Candida* species. They found that the anticandidal effects of VCO diluted 1:4 were comparable with those of fluconazole diluted 1:2. VCO also revealed the antibacterial activity toward *Staphylococcus aureus*.<sup>[70]</sup> VCO obtained from the Philippines could reduce the mortality of Nile Tilapia fish (*Oreochromis niloticus*) subjected to *Streptococcus iniae* infection.<sup>[71]</sup>

VCO could reduce plaque-related gingivitis, an oral disease induced by bacterial infection.<sup>[72]</sup> Lauric acid, a major fatty acid present in VCO, is reported to be the possible compound responsible for antimicrobial activities.<sup>[73]</sup> In addition, Manohar et al.<sup>[74]</sup> found that monolaurin compounds, a major metabolite of VCO, contributed to antimicrobial activities of VCO.

### **Neuroprotective effects of VCO**

The studies on the effect of VCO with respect to neuroprotective activities have been carried out. Nafar and Mearow<sup>[75]</sup> reported the possible beneficial effects of cold pressed VCO in the prevention of neurodegenerative disorders. The treatment of VCO on cortical neuronal cells could improve cell survival by reducing mitochondrial alterations. VCO has several protective effects on cortical neurons disturbed by  $\beta$ -amyloid by enhancing the signaling pathway in cortical neurons and inhibiting the oxidative reactions of some oxidative markers of cellular stress, namely Caspase3 and reactive oxygen species (ROS). The neuroprotective effect of VCO is also shown by activation of Akt and Erk signaling pathways.<sup>[76]</sup> Fernando et al.<sup>[77]</sup> reported the possibilities of VCO as a potential therapeutic agent against neurological disorders including Alzheimer's disease.

Rahim et al.<sup>[38]</sup> have reported that VCO could enhance Wistar rats' memory. The improvement in memory function is associated with cholinergic activity by enhancing the cognitive functions of the brain. VCO inhibits acetylcholinesterase activity, as a result of the increased levels of acetylcholine, an important neurotransmitter for cognitive functions in the brain. VCO has a good neuroprotective effect by stimulating the expression of *p*-tyrosine hydrolase and nerve growth factors. The neuroprotective effect of VCO is also correlated with the antioxidant activity of VCO through the inhibition of lipid peroxidation reactions. Furthermore, the improvement of the nervous system was also increased as indicated by the presence of intracellular signaling molecules in the mesenteric lymph node and thymus.<sup>[78]</sup> VCO showed activity in normalizing NLRP3 (NOD-like receptor family pyrin domain) in rats treated with  $\beta$ -amyloid and high fat diet. Either  $\beta$ -amyloid or high fat diet significantly enhanced NLRP3, causing the impairment of memory and learning. Administration of VCO at concentrations of 8% and 10% exerted a good effect on normalizing NLRP3 levels; as a consequence, memory and learning functions were repaired.<sup>[79]</sup>

### **Authentication analysis of VCO**

VCO has emerged as a functional oil in the fats and oils industry due to its capability to provide positive outcomes from the evaluations of biological activity *in vitro* and *in vivo*. As a consequence, VCO has a higher price value than common vegetable oils like

palm, corn, and soybean oils. Unethical players may take advantage by replacing or diluting VCO with low priced oils to get economical profits. Thus, the adulteration issue may be raised due to the price difference, and analytical methods based on physicochemical analysis have been developed, validated and used for detection of edible fats and oils adulteration including VCO' such as spectroscopy, differential scanning calorimetry, chromatography, and electronic nose.<sup>[80,81]</sup> Table 5 compiled the analytical methods used for authentication analysis of VCO from other edible oils such as palm oil and animal fats such as lard.

Fourier transform infrared (FTIR) spectroscopy combined with the chemometrics of multivariate calibration for quantitative analysis and discriminant analysis (DA) for classification between VCO and VCO adulterated with other oils and animal fats appeared as the most reported method for the authentication analysis of VCO. This is based on the fact that FTIR spectra of fats and oils are fingerprint in nature, therefore, it is convenient to search the specific peaks which are characteristics to VCO and VCO's adulterant.<sup>[92]</sup> The general procedure for authentication analysis of edible oils using FTIR spectroscopy combined with chemometrics is as follows: (1) preparation of calibration and validation samples (standards) or well characterized samples, (2) the acquisition of FTIR spectra using certain FTIR spectral condition, (3) optimization of FTIR spectra condition capable of providing the desired results which included spectral pre-processing and selection of wavenumbers, (4) selection of calibration and validation sets, (5) calibration modeling from calibration datasets, (5) validation the calibration models, and (6) evaluation of the developed models in terms of its validation features namely accuracy, precision, sensitivity, and its application to predict unknown samples.<sup>[93]</sup>

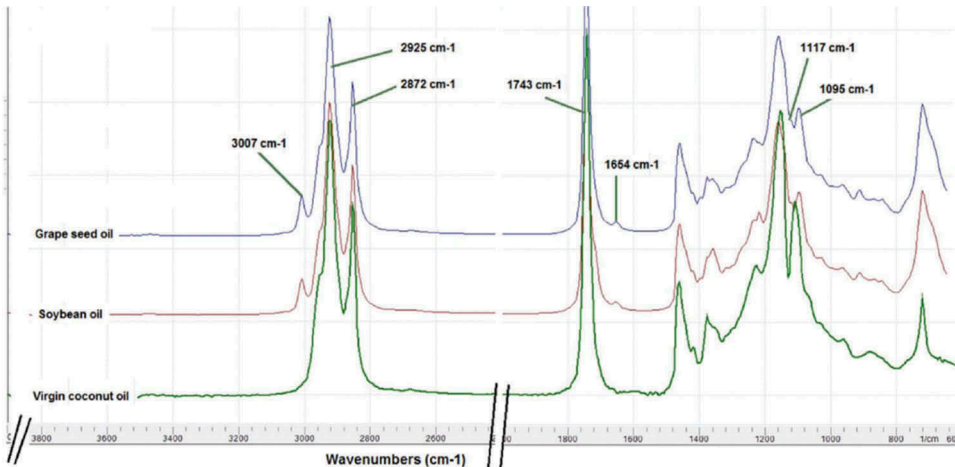
Currently, Rohman et al.<sup>[94]</sup> have developed FTIR spectroscopy combined with multivariate calibration of partial least square calibration (PLSR) and discriminant analysis (DA) for the authentication of VCO from grape seed oil (GSO) and soybean oil (SO). FTIR spectra of VCO, GSO, SO and its binary mixture of VCO-SO, and VCO-GSO were scanned at mid infrared region ( $4000\text{--}650\text{ cm}^{-1}$ ) using attenuated total reflectance (ATR) technique and subjected to FTIR spectral treatments. For quantitative analysis purpose, the wavenumbers ( $1/\lambda$ ) was selected based on its capability to provide the best prediction models in terms of highest  $R^2$  and lowest root mean square error for calibration (RMSEC) and root mean square error for prediction/validation (RMSEP). For the classification,  $1/\lambda$  was selected based on its capability to classify authentic VCO and adulterated VCO. Fig. 1 showed FTIR spectra of VCO, GSO, and SO at mid infrared region ( $4000\text{--}650\text{ cm}^{-1}$ ). Each bands/peaks and shoulders are characteristics for FTIR spectra of triglyceride (TG). There is a bit difference in terms of bands and shoulders intensity between VCO and two other oils, mainly at  $1/\lambda$  of about  $3007$  and  $1654\text{ cm}^{-1}$ . Bands at  $1/\lambda$  of  $3007$  and  $1654\text{ cm}^{-1}$  were absent in FTIR spectrum of VCO. These bands, corresponding to stretching vibration of unsaturation degree ( $=\text{CH}$  vinyl and  $\text{C}=\text{C}$ ), were observed in FTIR spectra of GSO and SO. The difference was also observed at  $1/\lambda$  of  $1120\text{--}1095\text{ cm}^{-1}$ , corresponding to ether ( $\text{C-O}$ ) vibration. VCO showed one peak at  $1117\text{ cm}^{-1}$ , while GSO and SO revealed two peaks at  $1117$  and  $1097\text{ cm}^{-1}$ , respectively. These differences were used as basis for the authentication analysis of VCO.

PLSR using absorbance values at combined  $1/\lambda$  of  $1200\text{--}900$  and  $3027\text{--}2985\text{ cm}^{-1}$  revealed reliable method for the quantification of GSO in VCO, as indicated by high value of  $R^2$  ( $>0.99$ ) and low value of RMSEC ( $0.007\%$  vol/vol) and RMSEP ( $1.32\%$  vol/vol). In addition, PLSR using FTIR spectra at the combined  $1/\lambda$  of  $1200\text{--}1000$  and  $3025\text{--}2995\text{ cm}^{-1}$  was preferred for



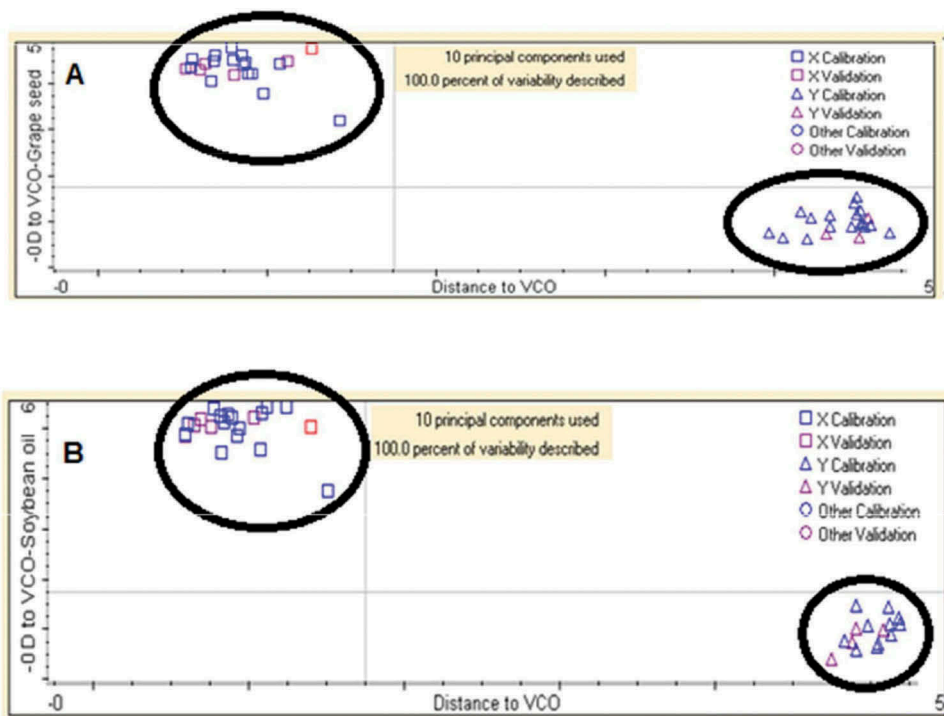
**Table 5.** The different analytical techniques used for authentication of virgin coconut oil reported by authors.

Methods	Oil adulterant	Analytical results	References
FTIR-ATR spectroscopy	Palm kernel oil (PKO)	Using whole mid IR at wavenumbers (1/λ) 4000–650 cm <sup>-1</sup> combined with PLSR using 10 Principle components, PKO at 1% could be detected. Discriminant analysis could classify VCO and VCO mixed with other vegetable oils (walnut, extra virgin olive, soybean, sunflower, grapeseed, sesame, canola and corn oils).	[82]
FTIR-ATR spectroscopy	Palm oil (PO)	PLSR at combined 1/λ of 3010–3000, 1660–1650 and 1120–1105 cm <sup>-1</sup> exhibited a good relationship between actual and FTIR-predicted values of PO with coefficient of determination (R <sup>2</sup> ) of 0.999 and standard error of calibration of 0.533. Discriminant analysis using 7 factors could classify pure VCO and that adulterated with PO.	[83]
FTIR-ATR spectroscopy	Palm oil (PO) in ternary systems with VCO and olive oil	PLSR at combined wavenumbers of 1120–1105 and 965–960 cm <sup>-1</sup> is successfully applied for quantification of VCO adulterated with PO with high R <sup>2</sup> (0.9996) and low RMSEC (0.494)	[84]
FTIR-ATR spectroscopy	Corn oil (CO) and sunflower oil (SFO)	PLSR using variable of absorbance values at combined wavenumbers of 858–705, 943–863, 1392–983, and 3027–2983 cm <sup>-1</sup> was successfully used for prediction the levels of CO in VCO, and at 1685–686, 2946–1887, and 3027–2983 cm <sup>-1</sup> was used for quantification of SFO in VCO. The R <sup>2</sup> > 0.999 for both CO and SFO. The RMSEC values of CO	[85]
FTIR-ATR spectroscopy	Lard (LD)	and SFO in VCO were 0.866% and 0.374% (v/v), respectively. The combined 1/λ of 3020–3000 cm <sup>-1</sup> and 1120–1000 cm <sup>-1</sup> using PLSR able to predict LD contents in VCO with R <sup>2</sup> value of 0.9990 for the relation between actual value of LD and FTIR predicted value.	[86]
Nuclear magnetic resonance spectroscopy ( <sup>31</sup> P NMR)	Refined coconut oil (RCO)	RMSEC = 0.722%; RMSECV = 1.54%. DA at the same 1/λ could classify VCO and VCO adulterated with LD. Phosphorus-containing dioxaphospholane derivatives of monoglycerides (MGs), diglycerides (DGs), sterols and free fatty acids (FFA) were analyzed by 31P NMR. VCO had 40% higher of 1-MG content than RCO and lower DG content (1.5%) than RCO (4.1%). VCO had sterol contents of 0.096%, lower than RCO (0.032%). VCO had FFA contents 8 times higher than RCO.	[87]
Differential scanning calorimetry (DSC)	Soybean oil (SO), sunflower oil (SFO) and palm kernel oil (PKO)	The heating curves of VCO adulterated with SFO and SO exhibited that adulteration peak appeared at the lower temperature region from 10% adulteration level. SMLR was used to predict the percent adulterant with R <sup>2</sup> of 0.9390 for SFO and 0.9490 for SO. For PKO adulteration, no adulteration peak was observed, but there is good relationship between peak height of PKO and adulteration levels.	[88]
DSC	Lard (LD)	DSC provides unique thermal profiling for VCO and VCO adulterated with LD. In the heating thermogram, one major endothermic peak (called with peak A) gradually smoothed out to the major peak with the increased levels of LD. In the cooling thermogram, there are two major exothermic peaks, peak C which increased as LD% increased and peak D which decreased in size as the LD% increased. SMLR able to predict LD% adulteration in VCO with R <sup>2</sup> (adjusted) of 95.82.	[89]
Electronic nose- SAW detector	Palm kernel oil (PKO)	Electronic nose using zNose equipped with surface acoustic wave (SAW) detector has been used for detection of VCO adulteration with PKO. PCA using adulterant peaks was applied successfully to classify VCO and VCO adulterated with PKO with 74% and 17% of the variations accounted for PC1 and PC2, respectively.	[90]
Electronic nose-SAW detector	Lard (LD)	Binary admixtures of LD in VCO in various percentage concentrations ranging from 1% to 50% (v/v) have been successfully assayed using electronic nose-SAW system. Ten different chromatogram peaks were identified as the adulterant peaks. One peak (peak J) was found to have the best relationship, with R2 value of 0.9344.	[86]
Two-dimensional gas chromatography coupled with time of flight mass spectrometry (GC x GC-TOF-MS) (MT)	Animal fats of lard (LD), chicken fat (CF), beef tallow (BF), and mutton tallow (MT)	The Changes in the cholesterol levels due to the addition of animal fats with VCO were used for detection of VCO adulteration. The increased cholesterol levels in VCO are valid parameter that could be used to detect the adulteration of VCO with animal fats at a level as low as 0.25%.	[91]



**Figure 1.** FTIR spectra of virgin coconut oil, grape seed oil, and soybean oil scanned at wavenumbers of 4000–650  $\text{cm}^{-1}$  using attenuated total reflectance as sampling technique. Taken from Rohman et al.<sup>[94]</sup> with permission from publisher.

quantitative analysis of SO in VCO. Discriminant analysis, one of the supervised pattern recognitions, was also successfully used for the discrimination between VCO and VCO added with adulterants of GSO and SO using the same wavenumbers used for quantitative analysis, as shown in Fig. 2.



**Figure 2.** The Coomans plot of virgin coconut oil (VCO) and adulterants: (□) VCO; (△) VCO containing adulterants of canola oil (A); grape seed oil (C); and Soybean oil (F). Taken from Rohman et al.<sup>[94]</sup> with permission from publisher.

Che Man and Rohman<sup>[95]</sup> have applied FTIR spectroscopy combined with PLSR and DA for quantification and classification of VCO adulteration with canola oil (CaO). The studied oils (pure VCO, pure Ca-O, and the mixture of VCO-CaO) were scanned using FTIR-spectrophotometer at wavenumbers of 4000–650  $\text{cm}^{-1}$  using sampling technique of horizontal-attenuated total reflectance (HATR). The authors have applied several pre-processing techniques including derivative spectra to obtain the best prediction model. FTIR normal spectra at combined  $1/\lambda$  of 1200–900 and 3027–2985  $\text{cm}^{-1}$  were suitable for the quantitative analysis of CaO due to their capabilities to provide the high  $R^2$  values and low RMSEC and RMSEP values. DA using the same wavenumbers used for quantitative analysis was able to discriminate VCO and VCO adulterated with CaO without any misclassification reported.

## Conclusion

VCO was prepared from wet methods without any RBD treatment, hence, the active components such as phenolics and tocopherols are retained in VCO. These compounds are responsible for biological activities including antioxidant, anti-inflammatory and immunomodulatory, anti-hyperlipidemia, anticancer, antidiabetic, antibacterial and neuroprotective as proved from *in vitro* and *in vivo* studies. VCO commanded high price in fats and oils industry which can be target of adulteration, therefore reliable analytical techniques such as FTIR spectroscopy and chromatography has been successfully applied for the authentication analysis. From this review, VCO is considered as functional food oils which are potential to be used as component in food products.

## Acknowledgments

The authors acknowledged the Ministry of Research and Higher Education, the Republic of Indonesia for financial support during preparing this review article through the scheme “World Class Research 2019” with contract number of 1973/UN1.DITLIT/DIT-LIT/LT/2019 awarded to Prof. Dr Abdul Rohman and the program development of Twin center Universitas Gadjah Mada, awarded to Dr Yuny Erwanto.

## Funding

This work was supported by The ministry of Research and higher education [1973/UN1.DITLIT/DIT-LIT/ LT/2019] through the scheme of World Class Research.

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