BioTechnologia vol. 100 (4) • pp. 349–358 • 2019
Journal of Biotechnology, Computational Biology and Bionanotechnology RESEARCH PAPERS
http://doi.org/10.5114/bta.2019.90236

Antioxidant property and GCMS profile of oil extracted from *Cocos nucifera* using a fermentation method

ALABI A. ADENIKE, PETER ADEGBOLA *, OLUMIDE S. FADAHUNSI
Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Abstract

Cocos nucifera L. is known as a tree of life because of its economic, domestic, and nutritional usefulness. Coconut oil (CO), which is derived from *Cocos nucifera* L., has received considerable attention because of its reported folkloric, nutritional, biological, and pharmacological properties. We previously reported the outstanding physicochemical properties of CO; therefore, we analyzed its antioxidant activity and chemical composition in this study. CO was extracted using a fermentation method with and without applying heat. Its antioxidant activity was investigated using the DPPH free radical scavenging method, the metal chelation capacity, the reduction of antioxidant power, and the nitric oxide scavenging capacity index. The compounds were identified using GC-MS. The data were expressed as a mean ± SEM and analyzed by one-way analysis of variance (ANOVA) using SPSS 21.0 with data being considered significant at $P < 0.05$. The results showed that CO demonstrated a concentration-dependent DPPH radical scavenging activity and nitric oxide scavenging capacity index, with the highest activity in the heat-extracted virgin CO (HEVCO). The ferric-reducing antioxidant power and metal chelation capacity significantly varied ($P < 0.05$) between the HEVCO and the cold extracted virgin CO (CEVCO). The GCMS analysis of virgin CO identified important active compounds. The results revealed a higher content of phenolic compounds in HEVCO compared to CEVCO. In conclusion, applying heat favored incorporating phenolic compounds into CO and consequently improved the antioxidant potential of HEVCO compared to CEVCO.

Key words: Coconut oil; antioxidant; heat; phenolics; extraction; fermentation

Introduction

Coconut (*Cocos nucifera* L.) is a member of the Aracaceae (*palm*) family and is extensively grown in the world's tropical countries (Assa and Konan, 2010). In some parts, coconut tree is referred to as a "tree of life" or a "tree of thousand uses", thus alluding to its economic, domestic, and nutritional usefulness, as well as the significance and importance of all the morphological parts of the tree (Bruce, 2004; Gervajio, 2007). Among the plant's edible products, coconut oil (CO) has gained considerable attention because of its reported folkloric, nutritional, biological, and pharmacological properties (Prades et al., 2012; Shankar et al., 2013). CO is a colorless to pale brownish-yellow oil having a melting point of 23–26°C (Bezard et al., 1971; Thampan, 1988). CO is derived by drying and pressing the dry pulp (copra) of coconut at a low temperature; however, there are other methods for extracting CO that have been previously described in the literature (Aliwalas, 1970; Bernardini, 1970; Cancel et al., 1976; Leonard, 1983). CO is composed of fatty acids of some 12 or fewer carbons, which are known and classified as medium chain fatty acids (MCFA) (Babayan, 1988; Heydnger and Nakhasi, 1996; Conrado, 2003). In addition, there is a significant presence of lauric acid in CO, which prevents the deposition of fats in blood vessels and consequently, prevents the risk of atherosclerosis (Conrado, 2003; Popova, 2011; Moigradean et al., 2013; Orsavova et al., 2015). Furthermore, CO is reported to be highly resistant to peroxidation, a considerably low cholesterol content, as well as to peroxide and iodine value compared to other oils (groundnut, melon, sunflower, and soybean) (Adegbola et al., 2018). CO has been reported to demonstrate significant beneficial and therapeutic activities such as anti-tumor

* Corresponding author: Ladoke Akintola University of Technology, Ogbomoso, Nigeria; e-mail: useablevesselofgod@gmail.com

(Kopeć et al., 2011), hypocholesterolemic and anti-aging (Adam et al., 2007), anti-stress (Nevin and Rajamohan, 2006; Arunima and Rajamohan, 2013; Yeap et al., 2015), hepato-protective (Hanaa et al., 2013; Otuechere et al., 2014), anti-inflammatory and anti-pyretic (Intophuak et al., 2010); wound healing (Nevin and Rajamohan, 2010), blood sugar control (Garfinkel et al. 1992; Kasai et al., 2003) and sight-enhancing (Haliza et al., 2015) activities. These biological potentials are primarily influenced by and attributed to presence of polyphenols, vitamin E, and lauric acid (Adam, 2007; Marina et al., 2009; Arlee et al., 2013 and Yousefi et al., 2013). CO's physicochemical properties were established in our previous study (Adegbola et al., 2018); however, this study evaluated the antioxidant activity and chemical composition of CO, which was extracted by different methods.

Materials and methods

Materials

Coconut fruits were purchased from a local market in Esa-Oke, Osun, and all other chemicals that were used were of analytical grade.

Extraction of coconut oil

Matured coconuts were first crushed to obtain the coconut fruits. Then, the back of the fruits was removed and washed, and the fruits were ground and sieved such that we could obtain some coconut milk. Virgin CO (VCO) was then extracted from the coconut milk using a fermentation method. Subsequently, coconut milk was extracted from freshly harvested coconuts and fermented by airborne lactic acid bacteria for 72 h (Srivastava et al., 2016), after which the oil phase was separated from the aqueous phase using a syringe. The resultant wet oil was heated at a low temperature for a short time period to remove the moisture and then finally filtered. For extracting the oil without heat, after separating into the oil and aqueous phase, the resultant oil in the upper phase was slowly dispensed using a syringe and then finally filtered (Marina et al., 2009).

DPPH radical scavenging activity of CO

The DPPH scavenging activity was determined using Brand-Williams et al.'s (1995) with slight modification. For preparing the stock solution, 40 mg was dissolved in methanol (100 ml) missing 3.5 ml of the stock solution with methanol, the absorbance was obtained using a UV spectrophotometer at a wavelength of 517 nm. Approximately 100 µl of the oil sample with 1 ml methanol DPPH solution was prepared and maintained in the dark for 2 h to allow the scavenging reaction to occur. The percentage of DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100$$

where A is the absorbance.

Ferric Reducing Antioxidant Power of CO

The ferric reducing antioxidant power (FRAP) of CO was determined using Benzie and Strain's (1996) method as described by Ishtiaq et al. (2014). The FRAP reagent was prepared fresh using a 300 mM acetate buffer at pH 3.6 (3.1 g sodium acetatetrihydrate, 16 ml glacial acid made up to 1 : 1 with distilled water), 10 mM (2,4,6-tris (2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10 : 1 : 1 to afford the working reagent. Approximately 100 µl of the extracted sample was added to 1 ml FRAP reagent and the absorbance was measured at 595 nm after 30 min. Trolox's calibration curve was set up to estimate the activity capacity of the samples. The result was expressed as milligram of Trolox equivalents per 100 gram of the sample (mgTE/100 g of FW).

Determination of metal chelating activity of CO

Chelation of Metal Ions – Cu^{2+}

A violet-colored pyrocatechol reagent was used for determining Cu^{2+} chelating activity, as described by Saiga et al. (2003). A mixture of 1.0 ml of sodium acetate buffer (100 mM, pH 4.9), 100 ml of Cu (II) standard solution (1.0 mg/ml), and 100 ml of the sample (200 µg) was prepared in a test tube and allowed to react for 5 min at room temperature. Then, to the mixture, 25 ml of a violet-colored pyrocatechol solution (4.0 mM) was added and the absorbance was obtained at 632 nm. The chelating activity was extrapolated using the following formula:

$$\text{Chelating activity (\%)} = (1 - \text{sample A632/control A632}) \times 100$$

Chelation of Metal Ions – Fe^{2+}

The method reported by Carter (1971) was used for determining Fe^{2+} chelating activity. A mixture of 1.0 ml

of sodium acetate buffer (100 mM, pH 4.9), 100 ml of Fe(II) standard solution (1.0 mg/ml), and 100 ml of the sample (200 μg) was prepared in a test tube and allowed to react for 5 min at room temperature. Then, 50 ml of a ferrozine solution (40 mM) was added and the Fe^{2+} chelating activity was determined by measuring the formation of the Fe^{2+}-ferrozine complex at 562 nm. The following formula was used to extrapolate the

$$Fe^{2+} \text{ chelating activity (\%)} = (1 - \text{sample A562/control A562}) \times 100$$

where control A562 is the absorbance of control reaction (without sample) and sample A562 is the absorbance in the presence of a sample.

Nitric oxide radical scavenging activity of CO

To determine the nitric oxide radical scavenging activity, reaction mixtures containing 2.0 ml of 10 mM NaN_3 in a phosphate-buffered saline (pH = 7.4) and 1.0 ml of various concentrations (20–80 μg/ml) of the oil were incubated at 25°C for 150 min. Next, 1.0 ml of 0.33% sulfanilic acid in 20% glacial CH_3COOH was added to 0.3 ml of the incubated solution and allowed to stand for 5 min. Then, 0.5 ml of 0.1% (w/v) napthylethylenediamine dihydrochloride was added to the mixture and incubated at 25°C for 30 min (Kuate et al., 2010; Kumar et al., 2010). The absorbance was measured at λ_{max} = 540 nm using quercetin as a blank. Then, the nitric oxide scavenging capacity index (NOSCI) of the oil sample was calculated as follows:

$$\text{NOSCI \%} = (1 - \text{absorbance of test}) / \text{absorbance of blank}) \times 100$$

The NOSCI% was expressed as scavenging capacity index (SCI_{50}), which is defined as the concentration (μg/ml) of the extract required to scavenge 50% of NO^-.

GCMS analysis of CO

The detection of compounds in VCO was performed on a GC-MS system (Agilent Technologies 789OA coupled with MSD VL5975C) with HP5MS column (30 × 0.0320 mm × 0.25 μm) we used He as the carrier gas at a constant flow rate of 2 ml/min. The sample injection volume was 1 ml, and the oven temperature was programmed to 80°C for 2 min at a rate of 10°C/min to 240°C with a holding time of 6 min. The samples were then run at a mass spectral scan range of 35–550 (m/z). The compounds were then identified by comparing the spectrum of the separated components with that of MS library 2014, the National Institute of Standards and Technology (NIST), Maryland, USA.

Statistical analysis

All data were expressed as mean ± SEM. The values were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 21.0. The difference between the means was analyzed by Duncan's multiple range test (DMRT) at $P < 0.05$.

Results and discussion

DPPH radical scavenging activity

The DPPH radical reaction has an advantage that the reaction process is not interrupted by certain side reactions such as metal-ion chelation and enzyme inhibition because of various additives. Moreover, the DPPH test is important because it recognizes free-radical scavenging effects and not pro-oxidant activity (Ruiz et al., 2015). The method is based on the ability of antioxidants to donate protons and thus neutralize the free radical character of the DPPH and produce the non-radical DPPH (Saiqa et al., 2014). Figure 1 shows the DPPH radical scavenging activity of VCO extracted with and without heat. A significant ($P < 0.05$) increase was observed in the activity of VCO in a concentration-dependent manner. Furthermore, the activity in the HEVCO significantly increased ($P < 0.05$) compared with CEVCO. The highest activity of 10.6% and 8% was observed at 80 μg/ml for HEVCO and CEVCO, respectively. The observation made in this study supported the antioxidant activity previously described by Arlee et al. (2013) for VCO extracted using the fermentation method. The oil extracted by the fermentation process goes through various processing steps such as heating during fermentation and drying off water from the oil. Because phenolics are polar, they are easily dissolved in the aqueous phase of coconut milk; thus, the removal of water by heat might have incorporated more phenolic compounds into the HEVCO. Therefore, the removal of water might be responsible for the higher anti-oxidant activity observed in HEVCO.

Nitric oxide radical scavenging activity

Adegbola et al. (2017) previously reviewed the role of NO radicals in the pathogenesis of diseases such as cardiovascular diseases. They reported that NO plays

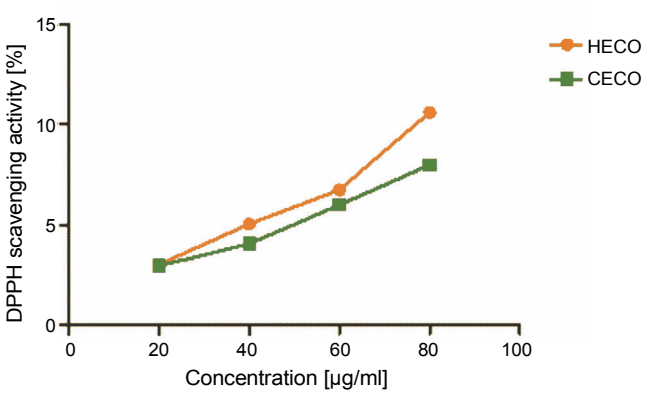

Fig. 1. DPPH radical Scavenging activity of CO extracted by the cold and hot method; the values are expressed as mean ± SEM and considered significant at $P < 0.05$; CEVCO – cold extracted virgin CO, HEVCO – hot extracted virgin CO

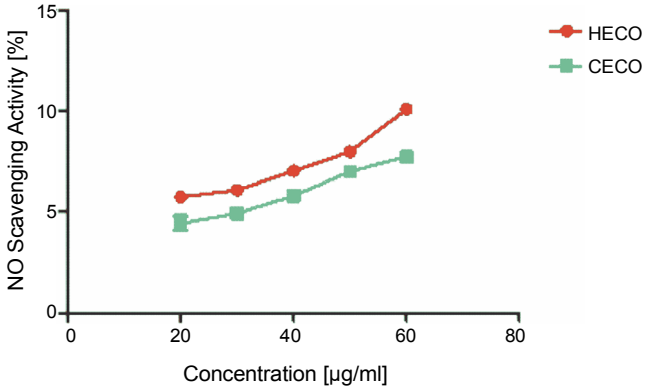

Fig. 2. Nitric oxide radical scavenging activity of CO extracted by the cold and hot method; the values are expressed as mean ± SEM and considered significant at $P < 0.05$; CEVCO – cold extracted virgin CO, HEVCO – hot extracted virgin CO

a significant role in various inflammatory processes. A persistent increase in the concentration of NO radical in the vascular endothelium causes direct toxicity to tissues and contributes to vascular collapse, which is associated with septic shock (Tylor et al., 1997). Furthermore, the chronic expression of NO radical is associated with various carcinoma and inflammatory conditions such as juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis (Tylor et al., 1997). The peroxynitrite anion (ONOO.) was formed when NO reacted with superoxide radical and had a greater toxicity. Moreover, its formation has been connected with reduced NO bioavailability in the vascular endothelium (Adegbola et al., 2017). The NO radical scavenging activity of VCO was measured as the nitric oxide scavenging capacity index increased in a concentration-dependent manner. Figure 2 shows a significant ($P < 0.5$) difference in the NO scavenging activity of the oil: it was higher in HEVCO than in CEVCO. The observations showed that VCO might possess some ability to prevent NO radical formation and be able to counteract its accompanying role in cardiovascular diseases.

Antioxidant and metal ion chelating activity of CO

The FRAP assay measures the total antioxidant capacity in a sample by considering the oxidation-reduction potential (Ishtiaq et al., 2014). The interaction of antioxidants with the ferric tripyridyltriazine complex favors the reduction of the complex to produce ferrous tripyridyltriazine with an intense blue color (Gülçin, 2012). In this study, the ferric-reducing antioxidant power was measured as the equivalent of Trolox. As listed in Table 1, HEVCO showed the highest ferric-reducing potential of 93.15 ± 1.20 mg TE / 100 g. This ferric-reducing property is by breaking a free radical chain by donating a hydrogen atom (Duh et al., 1999). The metal ion chelating capacity is an important mechanism of antioxidant activity. Transition metals, especially ferrous ions, are potent catalysts that are capable of initiating lipid peroxidation via a Fenton reaction in the cellular membrane (Moguel-Ordóñez et al., 2015). Moreover, ferrous ions can accelerate the peroxidation by the decomposition of lipid hydroperoxides into both alkoxyl and peroxyl radicals that are capable of abstracting hydrogen and initiating the chain reaction of lipid peroxidation (Halliwell, 1991; Chang et al., 2002). The chelation of metal ions is a key strategy to avoid the generation of free radicals that are associated with redox-active metal catalysis (Moguel-Ordóñez et al., 2015). The significant metal ion chelation observed with VCO, the highest in the HEVCO, is an indication of its potential to break the chain of lipid peroxidation (Table 1). Among the various transition metals, iron is known as the most important lipid oxidation pro-oxidant because of its high reactivity (Halliwell and Gutterridge, 1984; Kuate et al., 2010). As observed in this study, VCO is a better Fe^{2+} chelator with 36.3 ± 0.14% and 15.65 ± 0.49% activity than Cu^{2+} with 31.9 ± 0.42% and 14.55 ± 0.21% activity for HEVCO and CEVCO, respectively. Therefore, VCO might be important for limiting lipid peroxidation and consequently prevent associated diseases. The overall antioxidant activity observed in this study supports Srivastava et al. (2016),

Table 1. Antioxidant activity of CO extracted by the hot and cold method

Sample	FRAP [mgTE/100 g]	Cu^{2+} chelating activity [%]	Fe^{2+} chelating activity [%]
HEVCO	93.15 ± 1.20^{a}	15.65 ± 0.49^{a}	36.3 ± 0.14^{a}
CEVCO	90.49 ± 0.81^{b}	14.55 ± 0.21^{b}	31.9 ± 0.42^{b}

The values are expressed as mean ± SEM and considered significant at $P < 0.05$; the value with different superscripts varied significantly; CEVCO – cold extracted virgin CO; HEVCO – hot extracted virgin CO

Table 2. Compounds identified in VCO extracted with cold and heat

CEVCO	HEVCO
Hexadecanoic acid, methyl ester	Hexadecanoic acid, methyl ester
10,13–octadecadienoic acid, methyl ester	10,13–octadecadienoic acid, methyl ester
9, 12–octadecadienoic acid (Z,Z), methyl ester	9, 12–octadecadienoic acid (Z,Z), methyl ester
Methyl stearate	Methyl–9–cis,11–trans–octadecadienoate
Methyl–18–methylnonadecanoate	11–octadecenoic acid, methyl ester
Docosanoic acid, methyl ester	Methyl stearate
Tetracosanoic acid, methyl ester	Methyl 9–cis, 11, trans, 13, trans–octadecatrienoate
	Methyl–18–methylnonadecanoate
	Tricyclo–5.2.1.0(2,6) decane, 4–methyl
	Docosanoic acid, methyl ester
	Tetracosanoic acid, methyl ester
	Dodecanoic acid, 1,2,3–propanetriyl ester
	2–Hydroxycarbazole

CEVCO – cold extracted virgin CO; HEVCO – hot extracted virgin CO

study on HEVCO. As previously reported, heat favored the incorporation of additional phenolics into the VCO sample extracted with heat. Therefore, a higher phenolic content of HEVCO could contribute to the observed antioxidant activity.

GC-MS profiles of compounds in CO

The constituents in VCO were identified using GCMS. The identified compounds in the HEVCO and CEVCO included certain fatty acids, as shown in Table 2. The fatty acid content included palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and PUFAs such as linoleic acid (C18:2) and linolenic acid (C18:3). Figure 3 and Figure 4 show the spectra and structure of the identified compounds. Some of these identified compounds have been associated with several biological functions such as antioxidant, anti-inflammatory, antimicrobial, anticancer, and hypocholesterolemic activities (Yu et al., 2005; Intahphuak et al., 2010; Chandrasekaran et al., 2011; Jegadeeswari et al., 2012; Upgade and Anusha, 2013; Parthipan et al., 2015).

For instance, hexadecanoic acid methyl ester (palmitate ester) was described to be toxic towards bacteria and fungi (Chandrasekaran et al., 2011). Moreover, it was also reported to possess antioxidant and hypocholesterolemic activities (Jegadeeswari et al., 2012; Upgade and Anusha, 2013). The selective cytotoxicity effects of 9,12–octadecadienoic acid (Z,Z), methyl ester (linolate methyl ester), and palmitate ester against cancerous cells established their anti-tumor activity (Harada et al., 2002; Maria et al., 2011; Ravi and Krishnan, 2017). The anti-inflammatory activity of 9,12–octadecadienoic acid (Z,Z) and methyl ester found in the leaves and stem bark of *Pleiospermium alatum* and seed of *Croton tiglium* has been previously reported (Mangunwidjaja et al., 2006). Methyl–9–cis,11–trans-octadecadienoate (rume-

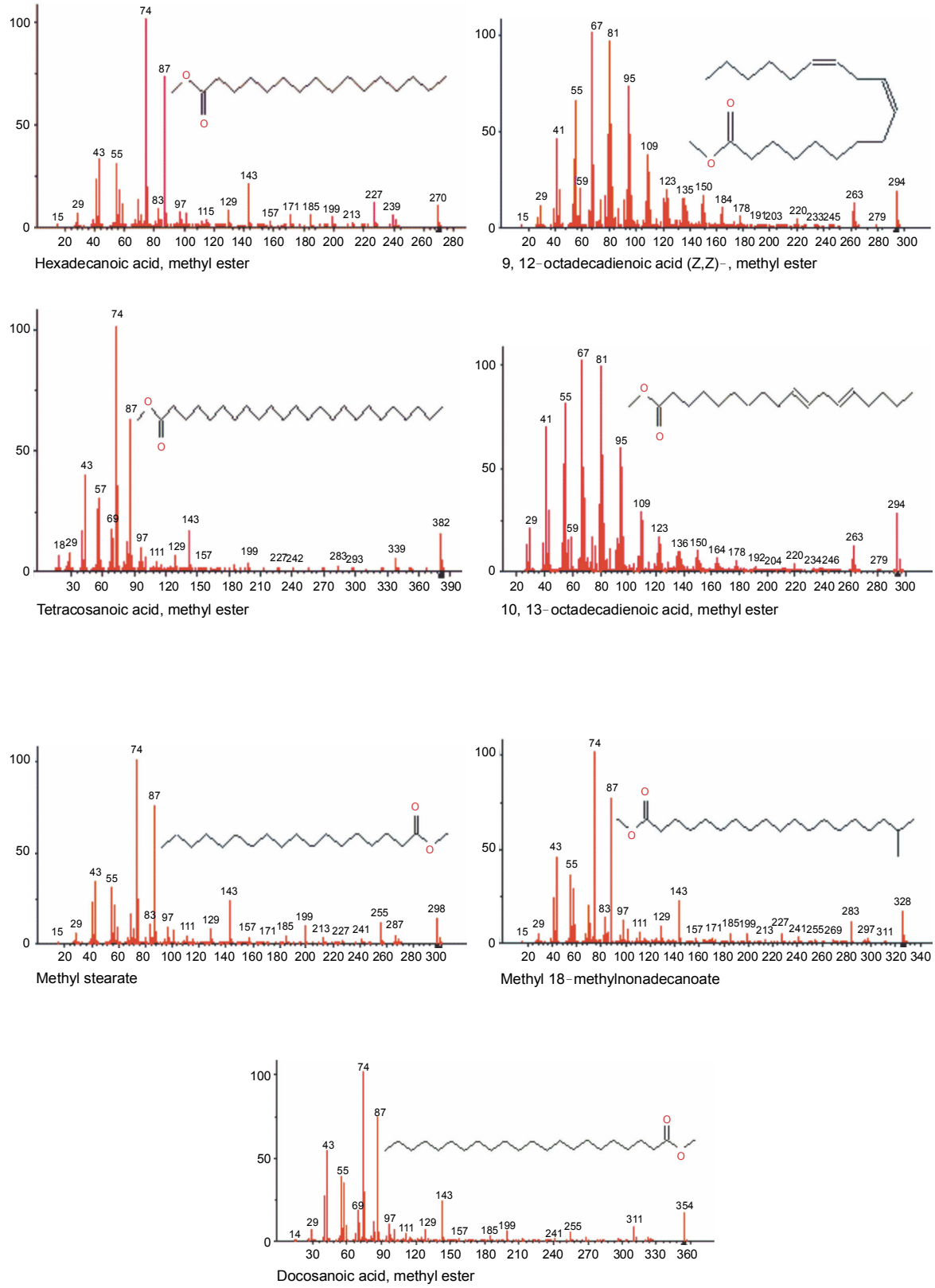

Fig. 3. Spectra and structures of some compounds identified in CO extracted by the cold method

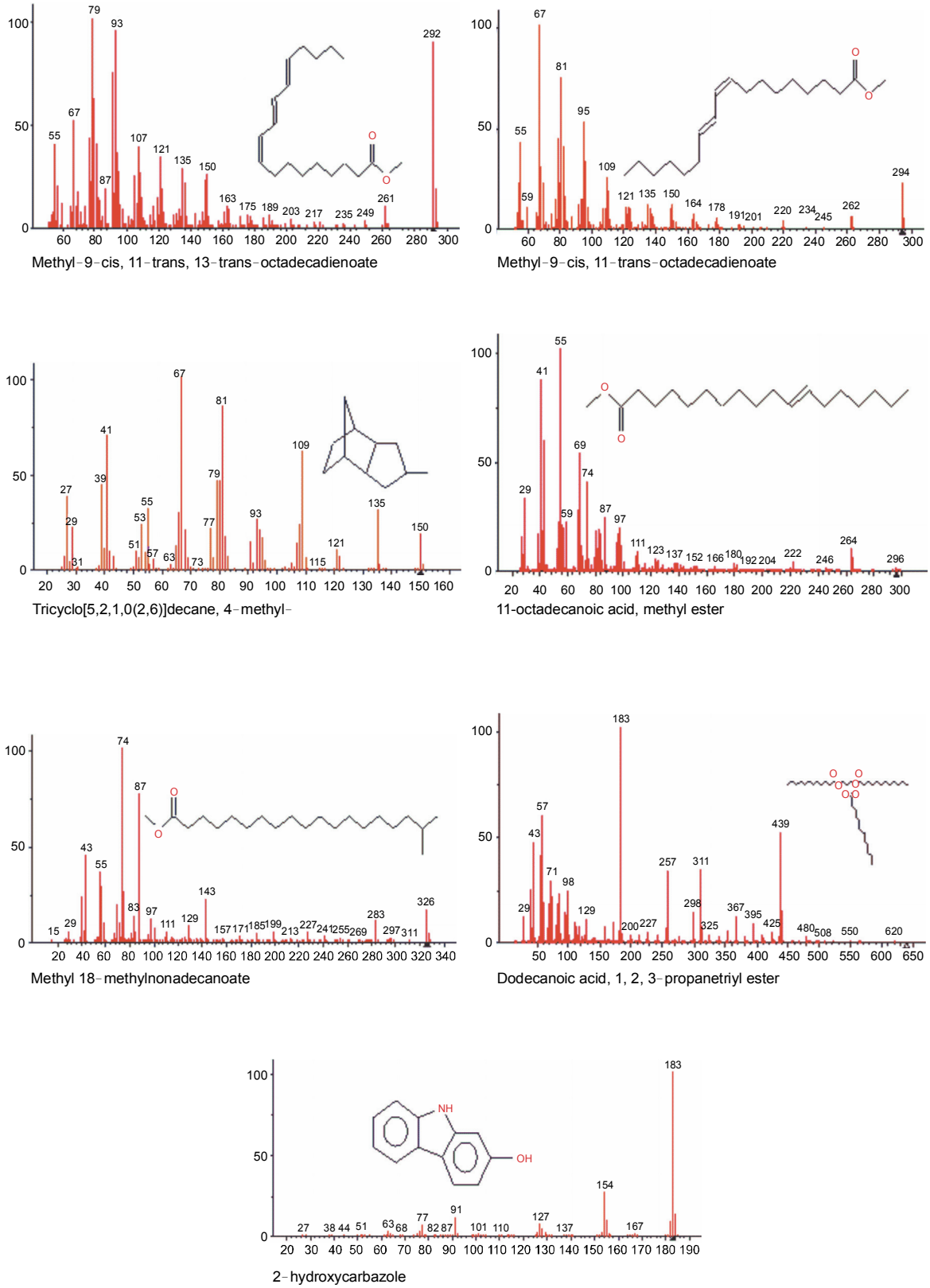

Fig. 4. Spectra and structures of some compounds identified in CO extracted by the hot method

nic acid) is an isomer of conjugated linoleic acid (CLA) found in natural products (Kramer et al., 1998) that was reported to possess anti-inflammatory activity (O'Shea et al., 2004). 2–hydroxycarbazole is a phenolic alkaloid belonging to a wide group of compounds called carbazole, which have diverse biological activities such as antioxidative, anti-inflammatory, antibacterial, and cytotoxic activities (Zhang et al., 2010; Bandgar et al., 2012; Biamonte et al., 2013; Peng et al., 2013). The presence of 2–hydroxycarbazole in the HEVCO might have influenced its antioxidant activity via the carbazole N–H and hydroxyl moiety. Lauric acid (dodecanoic acid) is one of the most abundant and important fatty acids found in CO (Ghani et al., 2018). Note that it was not detected by GC-MS analysis. Moreover, studies have reported that lauric content of CO varies with location, the variety of crop (Laureles et al., 2000), age of nuts (Balleza and Sierra, 1976), and time of harvest (Carandang, 2008).

Depending on the extraction method, compounds identified in the VCO varied among the samples, and they were present in higher amounts in HEVCO than in CEVCO. Previously, studies have reported that a higher temperature favors the incorporation of more phenolic compounds into oil (Marina et al., 2009; Srivastava et al., 2016). This might explain the reason for the number of compounds identified in the HEVCO. Because phenolics are polar and are easily dissolved in the aqueous layer of coconut milk, compared to the oil layer during fermentation, some phenolic in CEVCO might have been lost during collection (Arlee et al., 2013).

Conclusions

This study confirms that CO has some antioxidant activity, which is responsible for its medicinal and nutritional benefits. In this study, the extraction methods influenced the activity and constituents of CO. Moreover, it is evident that applying heat favored the incorporation of phenolics into CO and consequently improved the antioxidant activity of HEVCO compared to CEVCO.

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