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Detoxification of *Jatropha curcas* oil by ultraviolet irradiation combined with ethanol washing

Jh. Xiao[a], X. Mao[b], H. Zhang[b] and L. Niu[a,✉]

[a]Engineering Laboratory for Agro-processing and Safety Control of Jiangxi Development and Reform Commission & School of Food Science and Engineering, Jiangxi Agricultural University, 1101 Zhimin Road, Nanchang 330045, P.R.China
[b]School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, P.R. China

[✉]Corresponding author: nly8483@163.com

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SUMMARY: *Jatropha curcas* oil (JCO) is non-edible due to the content of phorbolesters (PEs) which are very toxic. The aim of this study was to evaluate the potential of JCO, treated by ultraviolet irradiation combined with ethanol washing, as an edible oil. The results showed that PEs can be significantly decreased by 100% (p<0.05), but the treatments produced no significant changes (p<0.05) in the fatty acids composition (FAC) and triacylglycerols (TAGs) in the detoxified *Jatropha curcas* oil (DJCO). In addition, the quality of DJCO was improved with enhanced DPPH radical scavenging. Therefore, DJCO with good quality will become a good resource for edible oil.

KEYWORDS: *Edible oil; Jatropha curcas oil; Phorbolesters; Physiochemical properties*

RESUMEN: ***Detoxificación de aceites de* Jatrofa curcas *mediante irradiación ultravioleta combinado con lavados de etanol*.** El aceite de *Jatropha curcas* (JCO) no es comestible debido a su contenido en esteres de forbol (PES) que son muy tóxicos. El objetivo de este estudio fue evaluar el potencial de JCO como aceite comestible, cuando se trató mediante irradiación ultravioleta en combinación con lavados de etanol. Los resultados mostraron que el contenido de PES puede disminuir significativamente, hasta el 100% (p<0,05), sin embargo, no se detectaron cambios significativos (p<0,05) en la composición de los ácidos grasos (FAC) y de los triglicéridos (TG) en el aceite de *Jatropha curcas* detoxificado (DJCO). Además, las cualidades de DJCO han mejorado teniendo una mayor capacidad de eliminación de radicales DPPH. Por lo tanto, DJCO con estas buenas cualidades se convertirá en un buen recurso de aceite comestible.

PALABRAS CLAVE: *Aceite comestible; Aceite de Jatropha curcas; Phorbolesters; Propiedades fisico-químicas*

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1. INTRODUCTION

Jatropha curcas, a shrub distributed in the subtropical and tropical regions of the world, has received great attention as a source of renewable energy in recent years. It is wellknown as an oilseed in India and China (Abdulla *et al*., 2011; Meher *et al*., 2013; Ye *et al*., 2009). Its content of oil is high, up to 40%–55%, and the total contents of oleic and linoleic acid can be found at up to 80%, which is

similar to olive oil and camellia oil (Devappa *et al*., 2010; Ichihashi *et al*., 2011; Ye *et al*., 2009). The oil has good oxidative stability compared to soybean oil, low viscosity compared to castor oil and a low pour point compared to palm oil. So far, most studies on *Jatropha curcas* oil have been focused on its application in biodiesel production. Few studies have been reported on its physicochemical characteristics for edible oil due to its high level of toxic compounds (Devappa *et al*., 2010; Ichihashi *et al*., 2011; Ye *et al*., 2009).

Phorbolesters (phorbol-12-myristate 13-acetate, PEs) have been identified as the major toxic components in *Jatropha curcas* oils (Devappa *et al*., 2012; Devappa *et al*., 2013; Pradhan *et al*., 2012; Roach *et al*., 2012), and found to be toxic in mice, goats, sheep, rats and fish (Goel *et al*., 2007). Though some genotypes of non-toxic *Jatropha curcas* exist, most of the extensive plantations of *Jatropha curcas* across the world are using toxic genotypes (Martínez-Herrera *et al*., 2006). Given the fatty acid composition and physicochemical characteristics, if the PEs could be removed, *Jatropha curcas* oil should be a good source of edible oil, especially in developing countries.

Recently, various physical, chemical, and biological approaches have become available for the detoxification of *Jatropha curcas*, such as treatment with ozone, air, $NaHCO_3$, ethanol extraction and so on. But those detoxification methods caused significant reductions in its color, taste and nutrients, and even a secondary pollution (Kongmany *et al*., 2013; Makkar and Becker, 2010; Yunping *et al*., 2012). So far no efficient approach has been reported to remove PEs completely.

In the past 40 years, multispecies and multigenerational animal studies have shown that there is no toxic effect from eating irradiated foods (Chen *et al*., 2012). Irradiation, with the advantages of high performance and low secondary pollution, is one possible alternative and additional processing technique for reducing both heat-stable and heat-labile anti-nutrients (Chen *et al*., 2012). Until now, irradiation has been employed as a powerful method to reduce the degrading and transforming of antinutritional compounds and several carcinogenic agents (Urzedo *et al*., 2007; Mir *et al*., 2013). However, there have been few reports about the effect of degradation by irradiation on PEs, with chemical properties which were similar to the previously described carcinogenic agents and antinutritional compounds.

The aim of our study was to explore *Jatropha curcas* oil as an edible oil. The treatment of UV irradiation combined with solvent washing was used. The physicochemical properties of detoxified *Jatropha curcas* oil, such as the content of PEs, FAC, TAGs, saponification value, tocopherols, unsaponifiable matter, phospholipids, antioxidant activity, volatile compounds, and oxidative stability were also characterized.

2. MATERIALS AND METHODS

2.1. Materials

Jatropha curcas seeds were procured from the Si Chuan Province, China. Phorbol- 12-myristate-13-acetate, α-tocopherol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The methanol used for the V_E analysis and the hexane used for the GC-MS analysis were of HPLC grade and purchased from Guoyao Chemical Reagent Co., Ltd. (Chengdu, China). All other chemical solvents used were of analytical grade.

2.2. Extraction of *Jatropha curcas* oil

Jatropha curcas seeds were de-shelled, milled and used for oil extraction. JCO was extracted at 50 °C for 6 h using hexane. Then the hexane was evaporated at 50 °C for 60min by a rotary evaporator in a water bath. And the extracted JCO was stored at 4 °C until needed for further analysis.

2.3. Detoxification of *Jatropha curcas* oil

5 mL of JCO were placed in quartz vessels (Beijing Normal University Photoelectric Instrument Factory, Beijing, China) and irradiated at 25 °C for 40 min under an ultraviolet lamp (UV, Nature Gene Corp., USA). The wavelengths ranged from 220 to 400 nm. The treatment was repeated 10 times, and samples were collected for the subsequent analysis (Xiao *et al*., 2013).

After treatment by UV, the sample was transferred to a separate funnel containing 65% ethanol in a separate funnel oscillator and washed, and then incubated at 50 °C with constant stirring for 1h. After that, the ethanol phase was collected and the same operation was repeated 3 times. Subsequently, the JCO phase was also collected and evaporated at 50 °C for 1.5 h by a rotary evaporator in a water bath to remove the residual ethanol, and then served as the DJCO, which was stored at 4 °C until needed for further analysis (Xiao *et al*., 2013).

2.4. Determination of phorbolesters

Five grams of JCO and DJCO were extracted with methanol and the PE content was determined by HPLC (Agilent Technologies, USA) according to Devappa *et al*., (2010), respectively. The separation was performed at room temperature (28 °C) and the flow rate was 1.3 $mL{\cdot}min^{-1}$ using a gradient elution.

The four PE peaks appeared between 20 and 24.5 min and were determined at 280 nm. The results were expressed as equivalent to a standard (phorbol-12-myristate-13-acetate), which appeared between 25 and 26 min.

2.5. Determination of fatty acids composition (FAC) and triacylglycerols (TAGs)

The FAC was determined on a GC/flame-ionization detector (Shimadzu 2010; fused-silica capillary column: CP-WAX, 30M I.D.0.32 mm) according to the method of GC described by Wang *et al*., (2013).

The TAGs of JCO and DJCO were analyzed according to the method of Nohemí *et al*., (2012) using high performance liquid chromatography equipped with an evaporative light scattering detector (HPLC-ELSD).

2.6. Determination of physicochemical properties

The color of the oils was determined using a lovibond tintometer (Shanghai Technologies, China). Oil density was determined at 20 °C using an Anton-Paar DMA 4500 density meter.

The oxidative stability of the oil was measured with a Rancimat 743 apparatus (Metrohm corp., Switzerland). 5 mL of oil were heated to 120 °C at an air flow of 20 $mL \cdot min^{-1}$. The oxidative stability was expressed as the oxidation induction time (h).

The tocopherol content was determined by HPLC (Agilent Technologies, USA) according to the method described by Eisenmenger *et al*., (2008).

Acid value, iodine value, peroxide value, saponification value, refractive index, unsaponifiable matter, phospholipids and moisture of the oil samples were determined according to the AOCS methods (2000).

2.7. Determination of volatile compounds

The volatile compounds in the oil were analyzed by Headspace solid phase micro-extraction (HS-SPME) according to the method described by Bail *et al*. (2008) with some modifications. The SPME fiber coated with a 75 μm carboxen-polydimethylsiloxane (CAR-PDMS) layer was used. 6.0 g of oil were sealed in vials and then equilibrated for 10 min at room temperature. Then the SPME fiber was exposed to the headspace to extract the volatile compounds at 50 °C for 40 min.

Once the extraction was finished, the SPME fiber was placed immediately into the gas chromatograph-mass spectrometer (GC-MS) instrument (GC-MS-QP2010, Shimadzu, Kyoto, Japan) equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm). The compounds were identified by comparison of their retention indices and mass spectra with the mass spectra library.

2.8. Determination of antioxidant activity

The antioxidant activity of the oil was analyzed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay according to the method described by Jiang *et al*. (2011) with some modifications. A stock solution of DPPH (200 $\mu mol \cdot L^{-1}$) was prepared in methanol and kept cold and in the dark until use. Different volumes of the aliquots of the test samples were mixed with 1.0 mL of DPPH. The mixture was shaken vigorously and immediately placed in the dark for 30 min. The absorbance was monitored at 517 nm using a spectrophotometer (Fullerton, CA, USA). The antioxidant activity of oil was calculated using the following formula:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A_B - A_A}{A_A} \times 100$$

Where: A_B and A_A are the absorbance values of the blank and of the tested samples, respectively.

2.9. Statistical analysis

All the parameters except the content of volatile compounds were expressed as means of triplicate determinations and their standard deviation, and subjected to one way analysis of variance using SPSS (ver. 13.0) software.

3. RESULTS AND DISCUSSION

3.1. Phorbolesters content

The PEs have been reported to be an activator of the protein kinase C (PKC) which regulates different signal transduction pathways. Up to now, PEs have been identified as the major toxic components in JCO and make JCO apt for non-edible purposes. Abud-Aguye *et al*. (1986) had reported that feeding mice with PEs as low as 1 $mg \cdot kg^{-1}$ body weight caused death. However, if PEs were removed completely, JCO may be used as an edible oil.

The results from the biodegradation studies of PEs are given in Figure 1. The content of PEs in the JCO (control) was 3.09 mg/g oil, which was similar to that reported by Aderibigbe *et al*. (2010) (3.15 $mg \cdot g^{-1}$). After treatment by UV irradiation, the content of PEs was degraded to 2.18 $mg \cdot g^{-1}$ oil. By contrast, all the PEs were removed after the 65% ethanol treatment. It is well known that UV irradiation can alter the molecular structure, which is helpful for the changes in the physicochemical properties of compounds (Liu *et al*., 2011). Therefore, all of the degradation products of PEs may be dissolved in the solution of 65% ethanol.

So far, there has been no efficient method reported to remove PEs completely. The method of degradation of UV combined with solvent washing presented in this study is a promising strategy to detoxify JCO and make it acceptable for edible purposes.

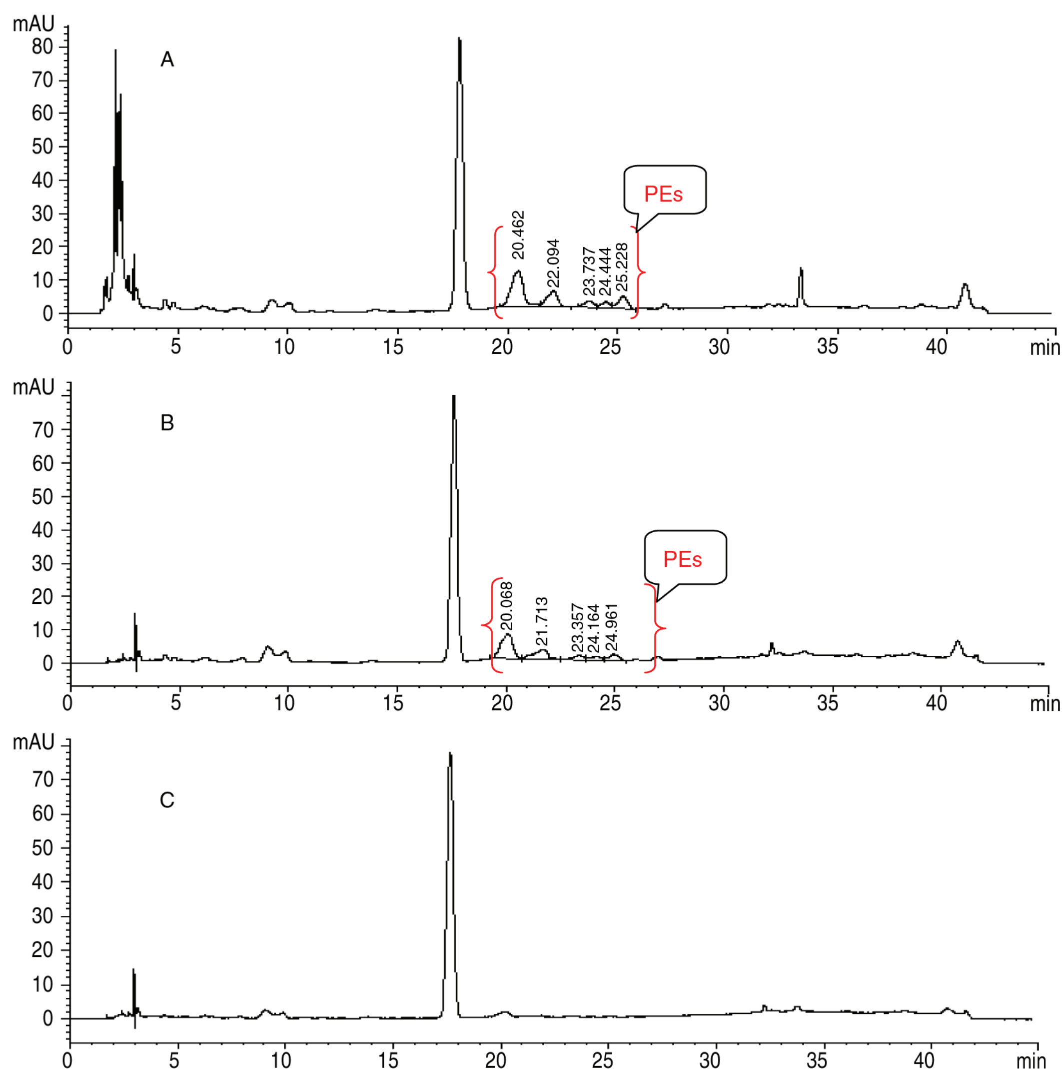

FIGURE 1. HPLC chromatograms from biodegradation studies of PEs.
A: control. B: UV-treated. C: UV-treated combined with ethanol-treated.

3.2. FA and TAGs composition

The FAC is an important indicator for edible oil. Results for the FAC of JCO and DJCO are given in Table 1. JCO and DJCO were rich in unsaturated fatty acid (79.32% and 79.55%, respectively). The data also showed that four major long chain fatty acids were detected in the JCO and DJCO, which were palmitic 13.51% and 13.18%, stearic 6.92% and 6.96%, oleic 43.86% and 44.16%, linoleic 34.63% and 34.59%, respectively. Similar results were reported by Salimon *et al*. (2012). It was more important that the detoxification treatment had no significant effect on the FAC ($p<0.05$), indicating that the quality and nutritional function of JCO were not changed by this method.

TAGs are the main component of plant oils, and analyzing its structure and composition can not only aid in understanding of the nature and purpose of plant oils, but also provide the fingerprint information to identify different plant oils. In addition, many reports showed that the distribution of fatty acid in triglyceride, which was one of the important factors for nutrition, determined the absorption and metabolism of oil.

Results for the TAGs of JCO and DJCO are given in Table 2. The data showed that the TAGs detected in JCO and DJCO were the polysaturated TAGs of PLL, OOL, OLL, POL+SLL, OOO and LLL at high levels (3.96–20.7%), and the monosaturated TAGs of POP, POS and PLL were detected at relatively low levels. The contents of TAGs in the JCO, as well as in the DJCO, were very close to those reported by Salimon and Ahmed (2012), indicating that the detoxification treatment had no effect on the contents of TAGs in the DJCO.

Table 1. Fatty acid composition (%) of JCO and DJCO

	JCO	DJCO
Myristic	0.04±0.00	0.04±0.00
Palmitic	13.51±0.72	13.18±0.50
Palmitoleic	0.62±0.01	0.62±0.01
Stearic	6.92±0.15	6.96±0.15
Oleic	43.86±0.53	44.16±0.85
Linoleic	34.63±0.55	34.59±0.49
Linolenic	0.13±0.02	0.13±0.02
Eicosanoic	0.19±0.02	0.19±0.02
ΣSaturated fatty acid	20.61±0.84	20.31±0.60
ΣUnsaturated fatty acid	79.32±0.03	79.55±0.47

Table 2. TAGs composition (%) of JCO and DJCO

	ENCs	JCO	DJCO
LLL	42	3.96±0.07	3.98±0.07
OLL	44	19.05±0.52	18.92±0.36
POL+SLL	44	8.50±0.18	8.31±0.13
PLL	44	20.74±1.27	20.71±1.19
PPL	46	21.95±0.51	21.98±0.53
OOO	46	1.72±0.17	1.82±0.07
POO	48	8.35±0.48	8.37±0.09
POP	48	9.09±0.06	9.17±0.07
PPP	48	3.31±0.08	3.22±0.11
SOO	50	0.24±0.08	0.26±0.08
POS	50	0.41±0.02	0.45±0.02
Unknown	–	1.85±0.05	1.91±0.06

3.3. Physical and chemical characteristics

Physical and chemical characteristics, such as color, oil density, acidity value, iodine value, peroxide value, saponification value, concentration of V_E and unsaponifiable matter as well as oil stability index, were selected to evaluate the effect of this detoxification treatment on the quality of JCO and DJCO. The results are given in Table 3. The data showed that there was no significant change in iodine value (101.87–102.2), indicating that their quality was as good as commercial vegetable oil with a high iodine value ranging from 97.9–103. The concentration of V_E and oxidative stability were not significantly decreased ($p<0.05$).

Table 3. Physical and chemical characteristics of JCO and DJCO

	JCO	DJCO
Moisture (%)	0.53±0.02	0.52±0.01
Color		
Red index	6.0±1.00	2.67±0.58
Yellow index	20±0.00	20±0.00
Refractive index (n^{20})	1.5±0.06	1.46±0.01
Oil density (d_4^{20})	0.9±0.01	0.88±0.03
Acid value (mgKOH·g^{-1})	2.95±0.15	2.01±0.10
Peroxide value (mmol·kg^{-1})	1.28±0.02	0.56±0.01
Saponification value (mgKOH·g^{-1})	192.87±1.53	183.87±1.53
Iodine value (gI$_2$·100g^{-1})	101.87±1.15	102.2±1.00
Unsaponifiable matter (%)	2.01±0.03	1.08±0.02
Phospholipids (mg·g^{-1})	7.32±0.1	1.15±0.15
α-tocopherol (mg·100g^{-1})	35.44±2.11	31.77±1.35
Oxidative stability	6.53±0.06	6.30±0.10

At the same time, the data also demonstrated that the detoxification treatment significantly decreased the acid value, peroxide value, phospholipids and the color.

Therefore, the detoxification method used is not only effective, but also has no detrimental effects on the quality of DJCO.

3.4. Volatile compounds

The data for the volatile compounds of JCO and DJCO are given in Table 4. Forty-three volatile compounds were identified, which were mainly alcohols, esters, vinyl materials and benzene, ketones, and furans. The major constituents of the volatile compounds were n-pentyl propionate (13.22%, 11.22%), Butane-2,3-diol (11.47%, 13.27%), (+)-Dipentene (10.21%, 10.21%) and beta-Pinene (6.36%, 6.16%) in the JCO and DJCO, respectively.

However, the volatile compounds phenylethylene, 1,2,4-Trimethylbenzene, 3-Octanone, Octadecyl vinyl ether, 3-Etheyl-o-xylene and 1,2-Dimethoxybenzene were discovered in the JCO, but not detected in the DJCO, suggesting that the detoxification method could improve the undesirable flavor of DJCO.

3.5. Antioxidant activity

Results for the antioxidant activities of JCO and DJCO are given in Figure 2. In the range of 1–40 mg/mL, JCO and DJCO exhibited a significant

TABLE 4. Volatile compounds of JCO and DJCO

Code	Retention time	CAS-NO	Name	Area (%)	
				JCO	DJCO
1	7.10	137-32-6	DL-2-Methyl-1-butanol	0.41	0.21
2	8.16	624-54-4	n-Pentyl propionate	13.22	11.22
3	9.03	62484-40-6	2-Chloro-8-methyl-4(3H)-quinazolinone	1.23	1.53
4	9.28	100-41-4	ethylbenzene	3.36	1.06
5	9.45	484-11-7	Neocuproine	0.72	0.12
6	9.73	959236-96-5	8-Fluoro-2,4(1H,3H)-quinazolinedione	3.47	2.47
7	10.15	7785-26-4	(1S)-(-)-alpha-Pinene	2.47	3.47
8	10.46	100-42-5	phenylethylene	0.34	ND
9	10.84	513-85-9	butane-2,3-diol	11.47	13.27
10	11.16	4254-14-2	(S)- 1,2-Propyleneglycol	4.48	5.48
11	11.48	62199-62-6	2,2,4,4,6-Pentamethyl heptane	0.77	1.77
12	11.76	127-91-3	beta-pinene	6.36	6.16
13	12.11	611-14-3	o-Ethyltoluene	0.81	0.31
14	12.31	78-36-4	Linalyl butyrate	2.51	5 .51
15	12.53	80-56-8	alpha-Pinene	4.18	5.55
16	12.66	3777-69-3	2-Pentylfuran	2.04	1.04
17	12.97	95-63-6	1,2,4-Trimethylbenzene	0.89	ND
18	13.11	5989-27-5	(+)-Dipentene	10.21	10.21
19	13.29	2345-26-8	Geranyl isobutyrate	1.52	2.52
20	13.43	22487-87-2	5-Methyl-2-heptene	0.78	1.02
21	13.53	106-68-3	3-Octanone	0.78	ND
22	13.59	83040-92-0	8-Hydroxy-2.2.8-trimethyldeca-5.9-dien-3-one	0.79	1.79
23	13.71	541-73-1	1,3-Dichlorobenzene	3.27	1.27
24	13.86	930-02-9	Octadecyl vinyl ether	0.46	ND
25	14.03	1120-21-4	n-Hendecane	1.17	3.28
26	14.22	933-98-2	3-Etheyl-o-xylene	0.51	ND
27	14.46	1074-43-7	1-Methtl-3-Propylbenene	0.54	0.24
28	14.53	766-97-2	4-Ethynyltoluene	0.81	0.21
29	14.63	96-48-0	Gamma-Butyrolactone	1.81	2.81
30	14.67	104-76-7	2-Ethylhexanol	2.06	1.06
31	14.97	536-60-7	4-Isopropylbenzyl Alcohol	4.42	2.29
32	15.08	2306-91-4	Decanoicacid,3-methylbutylester	0.61	0.21
33	15.36	6228-26-8	1,3-Propanediol, 2,2-methylenebis(oxymethylene)bis2-(hydroxymethyl)-	0.51	0.51
34	15.57	821-55-6	2-Nonanone	0.57	0.87
35	15.66	124-19-6	1-Nonanal	2.21	2.01
36	15.81	98-86-2	Acetophenone	0.44	1.04
37	16.45	1540-36-9	3-N-Butyl-2,4-Pentanedione	0.85	0.85
38	16.50	1072-83-9	2-Acetyl pyrrole	0.42	0.42
39	16.72	464-49-3	D-Camphor	0.41	1.41
40	16.78	91-16-7	1,2-Dimethoxybenzene	0.41	ND
41	16.91	60-12-8	Phenethyl alcohol	0.95	0.25
42	17.10	275-51-4	Azulene	4.28	6.38
43	19.09	56599-40-7	2-Pentadecyl-4-(hexadecyloxy)-1,3-dioxane	0.48	0.18

ND, not detected.

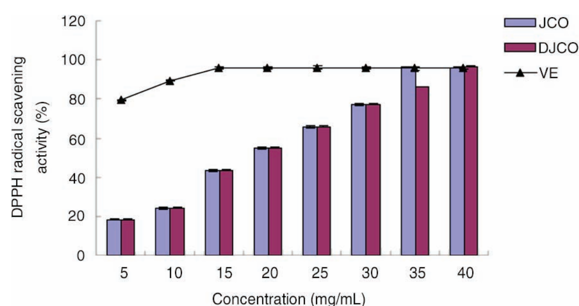

FIGURE 2. Antioxidant activities of JCO and DJCO assessed by DPPH radical scavenging assay.

dose-dependence on the DPPH radical-scavenging activity. When the concentration of JCO and DJCO were higher than 40mg/mL, the DPPH radical-scavenging activity corresponded to V_E with the same concentration. The higher contents of tocopherols in the JCO and DJCO, as well as the higher contents of unsaturated fatty acids, could account for the stronger antioxidant activity. There was no significant difference in the antioxidant activity between JCO and DJCO.

4. CONCLUSIONS

Based on the results of this study, it can be concluded that a combination of UV irradiation and washing with ethanol (65%) can remove PEs from JCO completely, and the quality of DJCO was improved. Though this detoxification process was developed in our laboratory, which was limited by the small quantity of oil used for the experiments 5 (mL), it will pave the way for using DJCO as an edible oil. However, scalingup of the method in order to apply it at industrial scale will require additional research.

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