Food Research 6 (5) : 161 - 167 (October 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Physicochemical properties of seed oil of the cardinal grape (*Vitis vinifera* L.) originated in Vietnam

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Article history:
Received: 4 August 2021
Received in revised form: 14 September 2021
Accepted: 3 January 2022
Available Online: 25 September 2022

Keywords:
Grape seed oil,
Vitis vinifera L.,
Fatty acid, Linoleic

DOI:
https://doi.org/10.26656/fr.2017.6(5).582

Abstract

Grape (*Vitis vinifera* L.) seed oil was studied for physicochemical characteristics and chemical composition. Through the gas chromatography-flame ionization (GC-FID) process, the fatty acid composition in the oil was analysed, revealing that the oil is very rich in linoleic (65.3%), linolenic (0.43%), and oleic (17.56%) acid. The physicochemical properties of the oil were also examined, including viscosity (87.8±2.75 cP), acid value (2.25±0.75 mg KOH/g), saponification content (185.5±7.45 mg KOH/g) and iodine value (176.4±5.85 g I_2/100 g). The results also indicated that grape seed is a health-beneficial oil due to the high contents of polyunsaturated fatty acids. This research also provided an important base for further investigations on the production of relevant high-value products, such as analysis of other minor nutrients of grape seed oil originated in Vietnam and study of the beneficial effects of grapeseed oil on human health and its application in the cosmetic industry.

1. Introduction

Grape (*Vitis vinifera* L.) is categorized in the Vitaceae family. Grape seeds are by-products of wine, vinegar and molasses production (Yalcin *et al.,* 2017). For many decades, grape seeds were considered agricultural wastes and were mainly burnt and used for feeding cattle. Grape seed is a rich source of many useful components. Grape seed is mainly composed of fibres (40%), complex carbohydrates (29%), oil (16%), proteins (11%), and complex phenols (7%) (Felhi *et al.,* 2016). Some approaches have been made to apply this waste in several other domains such as the production of biodiesel, cosmetic and food purposes to reduce environmental impacts and gain economic benefits. Nowadays, the identification and valorization of new vegetable oils (especially fruit oils) are the trend in the lipid industry (Madawala *et al.,* 2012). According to many studies, grape seed oil can be a potential vegetable oil for the food industry (Dabetic *et al.,* 2020; Shinagawa *et al.,* 2015). Historical records showed that this oil was classified as minor vegetable oil (FAO, 1992), and was used in salad dressings, marinades, baking, and frying.

In general, the grape seed contains between 6 and 22% oil that depending on the grape variety, the maturation degree of the seeds as well as the environmental conditions during ripening and also on the method of extraction (Rubio *et al.,* 2009; Tangolar *et al.,* 2009; Pardo *et al.,* 2011; Fiori *et al.,* 2014; Tociu *et al.,* 2017). The majority of biological activities of grape seed oils are due to the hydrophilic constituents. This fruit oil contains a large amount of phenolic compounds, including phenolic acids (gallic, ferulic, caffeic, vanillin acids), phenolic aldehydes (vanillin), hydroxycinnamic esters (caftaric, coutaric, fertaric acids), monomeric flavanols (catechin, epicatechin and epicatechin-3-O-gallate), flavonols and their glucoside derivatives (kampferol, quercetin, quercetin-3-glucoside, quercetin-3 -rhamnoside, quercetin-3-galactoside) and diverse oligomer procyanidins (procyanidin B1, procyanidin B2) (Karaman *et al.,* 2015; Marci *et al.,* 2015; Duba and Fiori, 2015; Bellili *et al*., 2018). The phenolic content of grape seed oil varies from 24–60 mg gallic acid equivalent/kg depending on the extraction method (Bellili *et al.,* 2018). Moreover, grape seed oil is also rich in lipophilic constituents that often have an important role in the prevention of some cardiovascular diseases and can lower cholesterol levels. Besides vitamin E

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

(tocopherols and tocotrienols) and essential acids, the lipophilic constituents of grape seed oil also include phytosterols, carotenoids, volatile compounds (organic acids, alcohols, esters, aldehydes and ketones of short carbon chain) (Bellili *et al.,* 2018).

Grape seed oil is also among the favourite ingredients of cosmetic products due to its high content of antioxidant compounds that can protect the skin from free radical damage (Garavaglia *et al.,* 2016). Antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic, antimutagenic activities, prevention and delay of cardiovascular diseases are some examples of bioactivities reported for grape seed oil (Garavaglia *et al.,* 2016; Cádiz-Gurrea *et al.,* 2017). Many studies demonstrated the bactericide activity of grape seed extracts against both spoilage and pathogenic bacteria (*Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *E. coli* O157:H7, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella enterica* serovar Typhimurium, *S. aureus* and *Yersinia enterocolitica* (Bellili *et al.,* 2018). The antioxidant activity of grape seed oil is mainly characterized by the presence of unsaturated fatty acid and other lipophilic components including tocopherols, tocotrienols, carotenoids, and chlorophyll because the presence of double bonds in these constituents can trigger oxidation capacity (Felhi *et al.*, 2016; Al Juhaimi *et al.*, 2017; Cádiz-Gurrea *et al.*, 2017; Bellili *et al.*, 2018).

Due to the potential applications of grape seed oil, many studies were conducted to characterize the composition of grape seed oil (Wen *et al.,* 2016; Shinagawa *et al.,* 2018). However, most studies mainly focused on European varieties while the grape seed oil from the Cardinal variety has been rarely studied. Therefore, the study aimed to characterize the composition of seed oil from Cardinal grapes (cultivated in Vietnam).

2. Materials and methods

2.1 Sample

Fresh ripe grapes were collected in Ninh Thuan province, Vietnam and crude seed oil was extracted using a Soxhlet extractor. The grape seeds were dried under air drying at 50°C until the moisture of 15–18% db was obtained. Firstly, the dried grape seeds were ground using a mill and screened through a mesh size of 2 mm. After that, the seeds were subjected to a Soxhlet extractor and extracted with a mixture of hexane and acetone (ratio of 3/2, v/v). The ratio of material/solvent and the extraction time were 1/10 (w/v) and 8 hrs, respectively. The solvent was evaporated under reduced pressure. The separated oil was then filtered and evaporated completely. The extracted oil was transferred into dark bottles and stored in the refrigerator to keep it fresh for further analysis.

2.2 Physicochemical characteristics

The grape seed oil quality was evaluated by testing the principal indices used for edible oil including protein content (AOAC 945.01 - Kjeldahl method), total lipid content (AOAC method (920.39), saponification value (AOAC 920.160–2005), non-saponification value (AOAC 933.08-2005), acid value (ISO 660: 2009), peroxide value (ISO 3960: 2007) and iodine indices (AOAC 920.159-2005). The physical properties were analysed including viscosity at 20°C (Brookfield RVDV-E machine, USA), density (Mai *et al.,* 2013), moisture content (ISO 662:1998), melting point (ISO 6321:2002) and refraction index (AOCS Cc7-25). The colour parameters were evaluated by Minolta Chroma colourimeter and expressed as the CIE value: lightness (L*), redness (a*, red to green axis) and yellowness (b*, yellow to blue axis). These characterizations were performed in triplicate.

2.3 Fatty acid composition

The fatty acid was analysed by using a flame ionization detector (GC-FID). First, the fatty acids were converted to fatty acid methyl esters (FAMEs) by incubating a mixture containing 100 mg grape seed oil and 2 mL of sodium methylate in methanol for 15 mins at 100°C. Following that, 1 mL of boron trifluoride-methanol (20% BF_3) was added and the mixture was heated at 100°C for another 15 mins. Then 1 mL of water and 1 mL of n-hexane were introduced into the mixture, followed by centrifugation. The resulting FAMEs were transferred into a vial and kept at 4°C. The study was carried out by Agilent-6820 gas chromatography. Each unit was analysed in triplicate. Data examination was carried out by using the Agilent Cerity method.

2.4 Analysis of total phenolic contents

Total phenolic content (TPC) was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi (1965). TPC of the oil extracts was isolated from a solution of oil extract in hexane by triple-extraction with water: methanol (60:40, v/v). The concentration of TPC was estimated spectrophotometrically using Folin-Ciocalteu reagent. First, an extract (0.5 mL) was pipetted into a test tube containing 2.5 mL of Folin-Ciocalteu reagent 10% v/v. After 5 mins, 2 mL of Na_2CO_3 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and

incubated for 30 min in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in mg gallic acid equivalents per 100 g of dried weight (mg GAE/100 g DW). The data presented are the average of three measurements.

2.5 Total antioxidant activity determination

To determine the antioxidant activity of the samples, the scavenging free radical using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by Brand-Williams was carried out (Cadiz-Gurea *et al*., 2017). The general principle of the method is based on the ability of antioxidants to scavenge free radicals, in turn causing the change of DPPH solution from colourless to purple. The extract was first diluted to a reasonable concentration. Then 0.5 mL of the diluted sample was taken into a test tube. The control sample was ethanol (99.5%). DPPH solution (1.5 mL; OD 517 nm = 1.1±0.02) was added to the test tube, which was then allowed to stand in the dark for 30 mins. Optical absorbance was measured at 517 nm on a UV-Vis spectrophotometer (Iqbal *et al.,* 2015). Vitamin C (ascorbic acid) was used as the reference standard. The blank was 500 µL solution replaced with EtOH 99.7%. Standard sample: Vitamin C (0.1± 0.01 g) was dissolved EtOH 99.7% into volume flask 100 mL, in the dark (C = 100 µg/mL).

DPPH free radical scavenging activity (IC%) was determined by the following formula:

$$IC\ (\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100$$

Where Abs_C is the optical absorbance of the control sample and Abs_T is the optical absorbance of the sample. The result is reported based on the IC concentration at which the sample removes 50% of DPPH free radicals. Therefore, IC_{50} values are negatively correlated to the antioxidant activity, the lower IC_{50} value means the highest antioxidant activity of the tested sample.

2.6 Statistical analysis

All studies were completed in triplicate. The results were presented as mean value±standard deviation (SD). The study was examined by Statgraphics Centurion XV software.

3. Results

3.1 Physicochemical characteristics

The extraction oil yield was 16.6%, which was slightly higher than those (5.8–13.6%) reported by Beveridge *et al.* (1999) and Cao and Ito (2003). This result is in agreement with that obtained by Ohnishi *et al.* (1990), and Göktürk Baydar and Akkurt (2001). They reported that the oil content of seeds obtained from 9.9 to 20% depends on different grape cultivars. The physicochemical characteristics of grape seed oil were summarized in Table 1. Grape seed oil had a slightly higher density (0.965±0.015 g/cm^3 at 25°C) than that of other fruit oils such as gac oil (0.955±0.012 g/mL) (Mai *et al.,* 2013) and sacha inchi oil (0.90–0.921 g/cm^3) (Mai *et al.*, 2019). The refractive index at 25°C of grape seed oil (1.55) was also slightly comparable to that of gac oil (1.47) (Mai *et al.,* 2013) and sacha inchi oil (1.525) (Mai *et al.*, 2019).

The chemical properties of the grape seed oil include oxidative stability and fatty acid profile. The oxidative level is reflected by iodine value, acid value and saponification value. The viscosity at 20°C and the iodine value of grape seed oil is 87.8±2.75 cP and 176.4±5.85 gI_2/100 g oil, respectively. The high iodine content of the grape seed oil suggested that the oil chains are slightly rich in unsaturated fatty acids. The iodine level of grape seed oil was significantly higher than that of gac oil (76.58±1.90 I_2/100 g) (Mai *et al.,* 2013) and soybean oil (132 g I_2/100 g) and corn oil (116 g I_2/100 g) (Knothe, 2002).

Table 1. Physico-chemical characterization of grape seed oil

	Parameter	Grape seed oil
1	Protein content (%)	0
2	Viscosity at 20°C (cP)	87.8±2.75
3	Refractive index nD at 25°C	1.55±0.002
4	Density (g/mL)	0.965±0.02
5	Acid value (mg KOH/g oil)	2.25±0.75
6	Saponification value (mg KOH/g oil)	185.5±7.45
7	Iodine value (g I_2/100 g oil)	176.4±5.85
8	Peroxide value (meq O_2 per kg of oil)	0.72±0.82
9	Unsaponification matter (g/kg)	2.2±0.15

Values are presented as mean±SD, n = 3.

The acid value of grape seed oil was 2.25±0.75 mg KOH/g oil, lower than that of gac oil (about 2.55±0.57 mg KOH/g oil) (Mai *et al.,* 2013). This is a low acidity value in accordance with Codex Alimentarius Commission, 1999. Similar values have been reported by Navas (2010) for the oil of a red grape variety and by Bosco for the oils of white grape varieties (from 1.9 to 2.5 mg KOH/g oil) (Boso *et al.*, 2018).

The saponification value of grape seed oil, at 185.5±7.45 mg KOH/g, was comparable to that of olive oil of about 185 mg KOH/g and sacha inchi oil (183.5±1.45 mg KOH/g).

3.2 Fatty acid composition

In order to evaluate the nutritional property and stability of grape seed oil, the fatty acid composition was analysed and compared with those reported in different

studies. The fatty acid profile distribution of grape seed oil was presented in Table 2. In general, the fatty acid profile of our grape seed oil is in accordance with that described by other works (Wen *et al.,* 2016; Al Juhaimi and Özcan, 2017; Mai *et al.,* 2019). The results revealed that the total saturated fatty acid composition of grape seed oil (about 14%) was significantly lower than that of other common vegetable oils such as coconut oil (80%) and olive oil (15%). Dominant saturated fatty acids recognized in grape seed oil are palmitic acid (8.91%), stearic acid (4.75), eicosanoic acid (0.15%) and heptadecanoic acid (0.11%).

Table 2. The comparisons of fatty acids in grape seed oils

Fatty acid composition	Grape seed oil
Saturated FA (%)	
Myristic acid (C14:0)	0.05
Palmitic acid (C16:0)	8.91
Pentadecanoic acid (C15:0)	0.04
Heptadecanoic acid (C17:0)	0.11
Stearic acid (C18:0)	4.75
Arachidic acid (C20:0)	0.15
Monounsaturated FA (%)	
Palmitoleic (C16:1)	0.13
Oleic acid (C18:1)	17.50
Eicosenoic acid (C20:1)	0.19
Erucic acid (C22:1)	0.05
Polyunsaturated FA (%)	
Linoleic acid (C18:2)	65.3
Linolenic acid (C18:3)	0.43
Eicosadienoic acid(C20:2)	0.25
Dihomogamma-linoleic acid (20:3)	0.04

The total unsaturated fatty acid content of grape seed oil is more than 80% including both monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The MUFA content in grape seed oil accounted for about 18% of total content and is largely dominated by oleic acid whose content reached 17.5% of total content, higher than that found in coconut oil (7.5%) (Karaman *et al*., 2015) and much lower than in olive oil (70%). For PUFA, its content was 68% and mainly included linoleic acid (65.3%). In general, grape seed oil has more linoleic acid than other vegetable oils such as safflower and sunflower oils (Lampi and Heinonen, 2009). This result is in accordance with those reported in previous studies. For example, the fatty acid profile of grape seed oil from several grape varieties grown in different countries was dominated by 61–75% linoleic acid (Beveridge *et al.,* 1999; Crews *et al.,* 2006). The most abundant fatty acid of different grape seed oils in China was linoleic acid ranging from 63.5 to 76.77%, followed by oleic acid (13.63–22.03%), palmitic acid (6.56–8.55%) and stearic acid (2.06–4.59%) (Wen *et al.,* 2016). A study by Garavaglia *et al*. (2016) also showed that linoleic acid is the most abundant fatty acid in cold-pressed grape seed oils, contributing between 66% and 75% of total fatty acid. Therefore, grape seed oil could be classified in the linoleic acid or the (n-6) polyunsaturated fatty acid class (Dubois *et al.,* 2007). Both oleic acid and linoleic acid have great importance in terms of nutritional implications because they can help to decrease total serum cholesterol and LDL-c (Dhavamani *et al.,* 2014) resulting in inhibiting vascular disorders as well as heart attack (Prado *et al.,* 2011). However, grape seed oil was rather poor in linolenic acid (< 1%) in comparison with other seed oils (Goffman and Galletti, 2001; Castillo *et al.,* 2002; Castillo *et al.,* 2004). For example, the linoleic acid content of black currant seed oil ranged from 43% to 58%. Although linolenic acid has positive effects on cardiovascular health and immune responses, a high level of this fatty acid can produce an unfavourable odour and taste in oil. Linolenic acid is oxidized easily due to having three double bonds on its hydrocarbon chain. The quality of oil rich in linolenic acid during processing and preservation would be unstable and have a short shelf-life (Madawala *et al*., 2012).

The fatty acids composition of edible oils differs in the variety, environmental cultivation and extraction methods (Al Juhaimi and Özcan, 2017). The fatty acid composition of the seeds remained relatively constant during the harvesting period, but the origin of berries had a significant effect on the fatty acid composition and the level (Yang and Kallio, 2002; St George and Cenkowski, 2007). In comparison with Brazilan cold pressed grape seed oil, Vietnamese grape seed oil exhibited higher contents of monosaturated oleic (C18:1 n-9) (17.5% versus 14.8%) and palmitic acid (C16:0) (8.91% versus 6.26%). However, the linoleic acid (C18:2 n-6) of Vietnamese grape seed oil was lower than that of Brazilian grape seed oil (65.3% versus 74.15%) (Shinagawa *et al.,* 2018). The portion of saturated fatty acid and unsaturated fatty acid in grape seed oil from Vietnam was about 15% and 85%, respectively, which was equivalent to those of the grape seed oil from Brazil. In comparison with grape seed oils from China, which were characterized by high contents of unsaturated fatty acids (86.41–91.08%) including 63.88% to 77.12% of polyunsaturated fatty acids (Wen *et al.,* 2016), Vietnamese grape seed oil demonstrated an equivalent content of total unsaturated fatty acid (85%).

3.3 Total phenolic content and antioxidant activity of grape seeds oil

Phenolic compound plays a vital role in plants due to their scavenging ability. The previous result illustrated that the total phenolic content (TPC) of the grape seed oil ranged from 50.51±3.21 mg/100 g to 560.21±6.22 mg/100 g. Our study showed that the TPC value of

Vietnamese grape seed oil was 609.08±5.82 mg GAE/g. Figure 1 illustrates shows the DPPH radical scavenging activity of the grape seed oil from Vietnam and that of vitamin C. The free radical scavenging capacities of the grape seed oil were presented using an IC_{50} value of 25 µg/mL while vitamin C had activities with IC_{50} values of 4.48 µg/mL. The smaller the reading value of IC-, the stronger the catching ability of free radicals. The results showed that grape seed oil has 5 times higher catching ability of free radicals than in that vitamin C. Oxidation resistance also depends on the characteristics and extraction conditions of each type of material (Madawala *et al.*, 2012). Nevertheless, this result also shows great potential in exploiting the antioxidant capacity of grape seed oil from Vietnam.

4. Conclusion

Grape seed oil is a by-product of the winemaking industry which is rich in essential, high phenolic compounds and high antioxidant activity, with good benefits to human health. Other minor nutrients of grape seed oil from Vietnam need to be studied in future research. Further research is also needed on the beneficial effects of grape seed oil on human health and its application in the cosmetic industry. The presence of total phenolic compounds and high antioxidant activity might emphasize the role of grape seed oil as an antioxidant and as an important ingredient to facilitate oxidative balance.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by grants from Nong Lam University, Thu Duc city, Ho Chi Minh City, Vietnam.

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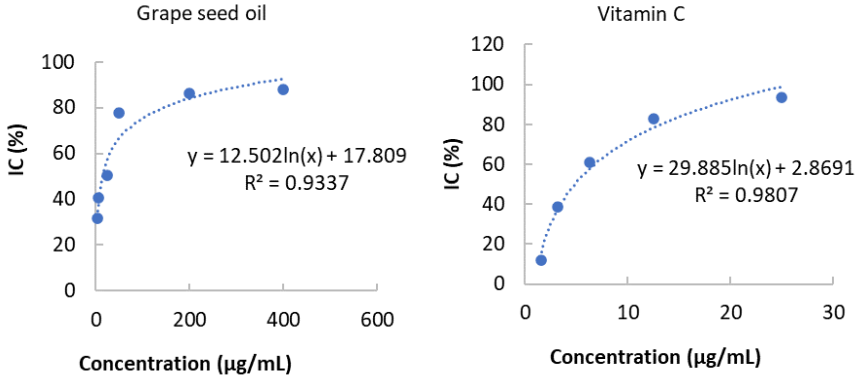

Figure. 1. DPPH radical scavenging activity

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eISSN: 2550-2166