Grasas y Aceites 75 (4)
October-December 2024, 2173
ISSN-L: 0017-3495, eISSN: 1988-4214
https://doi.org/10.3989/gya.1200232.2173

# Evaluation of different green technological approaches for the extraction of oil from grape seeds: A comparative study

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*Submitted: 30 April 2024; Accepted: 21 January 2025; Published: 15 April 2025*

**SUMMARY:** The present study aimed to assess the impact of eco-friendly approaches, including supercritical $CO_2$ (SCO), supercritical $CO_2$+ethanol (SCE), *para*-cymene (PC), and the cold-press (CP) method on the quality characteristics of grape seed oil (GSO). The results revealed that the PC approach produced the maximum oil yield of 19.46 %, followed by SCE (18.61 %), Hexane (HX) (18.03 %), SCO (16.47 %), and CP (11.01 %). The fatty acid profile and antioxidant activity of SCE oil were superior to all other tested approaches. The total phenolic content (TPC) in the extracted oils ranged from 109.77 mg GAE·kg$^{-1}$ to 132.01 mg GAE·kg$^{-1}$, with SCE having the highest TPC and HX having the lowest TPC. PC, SCO, SCE, and CP extracted oils exhibited high total tocopherol contents (TTC) of 233.61, 257.01, 264.26 and 251.89 mg·kg$^{-1}$ as compared to 214.13 mg·kg$^{-1}$ in hexane, respectively. The overall findings demonstrate that adopting environmentally sustainable solvents and extraction methods could serve as a preferable alternative to conventional approaches without compromising the quality characteristics of the extracted grape seed oil.

***KEYWORDS****: Fatty acid; Grape seed oil;* Para-*cymene; Supercritical carbon-dioxide.*

**RESUMEN:** ***Evaluación de diferentes enfoques tecnológicos verdes para la extracción de aceite de semillas de uva: Un estudio comparativo***. El presente estudio tuvo como objetivo evaluar el impacto de los enfoques ecológicos, incluidos el $CO_2$ supercrítico (SCO), el $CO_2$ supercrítico + etanol (SCE), el *para*-cimeno (PC) y el prensado en frío (CP) sobre las características de calidad del aceite de semilla de uva (GSO). Los resultados revelaron que el enfoque PC produjo el máximo de aceite (19,46 %), seguido por el SCE (18,61 %), el hexano (HX) (18,03 %), el SCO (16,47 %) y el CP (11,01 %). El perfil de ácidos grasos y la actividad antioxidante del aceite SCE fueron superiores a todos los demás enfoques probados. Además, el contenido fenólico total (TPC) en los aceites extraídos varió de 109,77 mg GAE·kg$^{-1}$ a 132,01 mg GAE·kg$^{-1}$, siendo el SCE el que tuvo el TPC más alto y el HX el que tuvo el TPC más bajo. Los aceites extraídos de PC, SCO, SCE y CP exhibieron un contenido elevado de tocoferol total (TTC) de 233,61, 257,01, 264,26 y 251,89 mg·kg$^{-1}$ en comparación con 214,13 mg·kg$^{1}$ en hexano, respectivamente. Los resultados generales demuestran que la adopción de solventes y métodos de extracción ambientalmente sostenibles podría servir como una alternativa preferible a los enfoques convencionales sin comprometer las características de calidad del aceite de semilla de uva extraído.

***PALABRAS CLAVE****: Aceite de semilla de uva; Ácido graso; Dióxido de carbono supercrítico;* Para-*cimeno.*

**Citation/Cómo citar este artículo**: Ubaid M, Saini CS. 2024. Evaluation of different green technological approaches for the extraction of oil from grape seeds: A Comparative Study. *Grasas Aceites* **75** (4), 2173. https://doi.org/10.3989/gya.1200232.2173

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## 1. INTRODUCTION

Grapes (*Vitis vinifera*) are one of the world's most widely grown fruit crops. They are cultivated in temperate regions with typical climatic patterns of warm summers and mild winters. Grape seeds are commonly obtained as waste from processed fruit products. However, apart from being a highly valuable waste resource, generally, they exacerbate already existing serious disposal problems. Grape seeds have become increasingly popular in recent years because of their potential benefit as a source of several other nutrients. Grape seeds contain a high concentration of non-digestible carbohydrates (60-70 %), proteins (11 %), fatty acids (13-19 %) and antioxidants (Yu and Ahmedna, 2013). The dried grape seed contains about 10-15 % oil. Grape seed oil (GSO) has good nutritional value because of its high content of

unsaturated fatty acids. Palmitoleic acid, oleic acid, linoleic acid and linolenic acid are considered unsaturated fatty acids (Ahamad *et al*., 2024). Unsaturated fatty acids are necessary in order to stay healthy and avoid chronic diseases. For example, GSO is composed of linoleic acid, an omega-6 fatty acid which is well-known for its ability to reduce LDL cholesterol and promote heart health. The omega-9 fatty acid, i.e. oleic acid, is known to provide anti-inflammatory and possibly cardiovascular health benefits (Elisia *et al*., 2024). Grape seed oil also contains biologically important bioactive compounds such as tocopherols, tocotrienols, phytosterols, phenolic acids, and flavonoids (Rombaut *et al.,* 2014). Grape seed oil can be extracted with bioactive compounds along with other nutritional components by using various oil extraction methods (conventional and non-conventional) like cold pressing, solvent extraction (through petroleum-based solvents or green solvents), and the supercritical fluid extraction technique.

Cold pressing does not require heat or chemical treatments. It is environmentally friendly, and requires little energy (Al Juhaimi *et al.,* 2018). Because of these characteristics, consumers prefer cold-pressed oils for natural and safe food products. In solvent-extracted oil, the residual solvent may be present in the extracted oil as a result of inadequate solvent removal. Alternative oil extraction methods are being evaluated in order to address these issues. *n*-hexane derived from petroleum is a common solvent used in the food industry, which, in fact, is a non-renewable resource. In order to avoid environmental concerns, there have been various efforts to discover substitute solvents in place of hexane for both extraction and analysis purposes (Kumar *et al.,* 2017). In general, 'green' solvents are those which are derived from renewable resources, eco-friendly, biocompatible, nontoxic, non-flammable and inert solvents (Chemat and Vian, 2014). *Para*-cymene (PC) is structurally an aromatic hydrocarbon found in abundance in tree leaf oils. PC has a relatively high flash point in comparison to n-hexane. As a result, it has less flammability and, hence, is less dangerous. On the other hand, supercritical fluid extraction (SFE) has received a great deal of attention in past years as a good potential substitute for conventional extraction methods because of the production of products with high quality, and it has been regarded as green technology (Duba and Fiori, 2019). Therefore, the present study aimed to investigate the effect of using green approaches for the oil extraction from grape seeds and characterize the oil in terms of fatty acid composition, tocopherol content, total phenolic content, antioxidant activity, and oxidative stability.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All chemicals and solvents used in this study were of analytical grade. Pyrogallol, ethanol, methanol, n-hexane and KOH, Folin-Ciocalteu reagent, gallic acid, formic acid, reference standards for tocopherol and for total phenolic content (Gallic acid as a standard) as well as other solvents and reagents used in the research were procured from Hi–Media, Mumbai, India.

### 2.2. Sample preparation

The grape seeds (Bangalore Blue variety) used for the extraction of oil were procured from Elite Vintage Winery, Karnataka, India. The obtained grape seeds were ground into finely powdered form with the aid of a grinder (Sujata, 401 AI, Powermatic, India). The ground sample was sieved through a 150 μm particle size sieve. The ground grape seed powder samples were kept in sealed plastic bags under refrigerated conditions until extraction.

### 2.3. Soxhlet extraction

The ground grape seed powder (100 g) was put inside a thimble for extraction. The extraction of grape seed oil (GSO) was performed using *n*-hexane as solvent under reflux for 8 h. The time of 8 h for the soxhlet extraction method using hexane is the most efficient for achieving the highest yield. With this duration, maximum oil content was extracted from the grape seeds. A duration of less than 8 hours would not extract the oil completely from the grape seeds and the oil yield would be less than normal. Several studies performed on the extraction of grape seed oil employed an extraction time of up to 8 h with hexane as solvent (Di Stefano *et al*., 2021; Wada *et al*., 2018) and the evaporation of the remaining solvent present in the oil was done using a rotary vacuum evaporator (BUCHI Switzerland, Rotavapor R-100).

### 2.4. Extraction with para-cymene (*p*-cymene)

The extraction of grape seed oil by *p*-cymene (terpene) as solvent was completed in two steps. The first step was the extraction of grape seed oil from grape seed powder with *p*-cymene using the soxhlet extraction method. In order to ensure ideal circumstances for the extraction, the temperature was chosen based on its boiling point (i.e. 172 °C). The second step was the separation of the grape seed oil from the solvent (*p*-cymene). In the second step, a round-bottom flask containing the mixture (oil + solvent + distilled water) was subjected to heating at 95 °C in the rotary vacuum evaporator (BUCHI Switzerland, Rotavapor R-100). Both the distilled water and the solvent were evaporated from the round-bottom flask, passed through a vertical condenser, and collected in the receiving flask. In the end, only the oil remained inside the round-bottom flask. The oil was stored for further analysis.

### 2.5. Cold-press extraction

The dried and cleaned grape seed samples were subjected to screw pressing (Organik, India) for the extraction of oil run by a semi-automatic machine with a capacity of 2 kg in a small-size facility for cold-pressed oils. The obtained grape seed oil was kept at room temperature for 48 hours for insoluble impurities to settle down. Finally, after decantation, a clear grape seed oil sample was obtained, which was used for further analysis.

### 2.6. Supercritical fluid extraction

Grape seed oil was extracted with supercritical $CO_2$ using the methodology followed by Coelho *et al.* (2020). As far as supercritical fluid extraction with ethanol as a co-solvent is concerned, ethanol was used in a ratio of 10% (w/w). The supercritical fluid extraction with and without ethanol was performed at 40 °C and 300 bar. Supercritical fluid extraction equipment (Deven Supercritical Private Limited, Daman, India) with a capacity of 1 L was used for the above purpose. The quantity of extracted lipids recovered from U-tubes using an organic solvent was measured gravimetrically and used for further analysis.

### 2.7. Oil extraction yield

For the determination of the GSO yield (%), the following equation was used:

$$\text{Yield (\%)} = \frac{w_e}{w_t} \times 100 \quad (1)$$

where,
$w_e$ = the mass of obtained GSO from the sample,
$w_t$ = the mass of the sample before extraction

### 2.8. Refractive index

For the determination of the refractive index of oil samples, an Abbe Refractometer (Reichert AR 700) was used at a temperature of 20 °C. All the experiments were done in triplicate, and the values obtained were averaged.

### 2.9. Color

The color parameters *L*, a*,* and *b** of all GSOs were measured with a Tintometer® Colorimeter (Model F 14118, The Tintometer Limited, UK).

### 2.10. Peroxide value (PV)

About 5 g of grape seed oil were mixed with an acetic acid: chloroform mixture of 30 mL (3:2, v/v). Then, 0.5 mL saturated solution of potassium iodide was added to the mixture and the mixture was shaken by hand swiftly. The mixture was kept in the dark for 5 min. Then, 30 mL of distilled water were added followed by the addition of 2 mL of starch indicator (1%). Sodium thiosulfate (0.1 M) was used for the titration of the mixture until the color turned white. The peroxide value was determined using the equation below:

$$\text{PV (meq } O_2\text{/ kg)} = (V - V_0) \times C \times 1000 / m \quad (2)$$

where,
V = volume of sodium thiosulfate taken by the sample (mL),
$V_0$ = volume of sodium thiosulfate taken by the blank (mL),
M = weight of oil (g), and
C = sodium thiosulfate concentration (M)

### 2.11. Conjugated diene (CD 232) and conjugated triene (CT 268)

The determination of conjugated diene (K232) and conjugated triene (K268) was conducted as followed by Dabrowski *et al.* (2016). In this modified method, a 0.03 g GSO sample was dissolved in hexane (50 mL), where K is related to the specific absorption value with respect to each wavelength.

### 2.12. Total phenolic content (TPC)

The determination of the TPC was performed following the Folin–Ciocalteu spectrophotometric method according to Haiyan *et al.* (2007) with slight modifications. The grape seed oil sample (3 g) was dissolved in 15 mL hexane and extracted with methanol by continuous shaking for 3 min for each extraction. The oil sample was kept standing overnight. The washing of methanolic extract was done with 25 mL of hexane. After this, an aliquot (1 mL) was poured into a volumetric flask (10 mL), and then 0.5 mL Folin-Ciocalteu reagent was added. The solution was vigorously shaken and kept standing for 3 min before the addition of 1 mL of saturated sodium carbonate solution, and the volume was made up with water. After 1 h, absorbance at 725 nm against a reagent blank was measured using a spectrophotometer. The calibration curve was prepared by using gallic acid. TPC was expressed in mg of gallic acid equivalent per kilogram of oil (mg GAE·kg$^{-1}$ of oil).

### 2.13. Antioxidant activity

#### *2.13.1. DPPH radical-scavenging activity*

The DPPH method was used to determine radical-scavenging capacity. Grape seed oil of about 0.5 g was diluted into ethyl ether (3 mL). 1 mL aliquot of DPPH methanolic solution (0.02 %) was added to it. The mixture was vigorously shaken and allowed to stand for 30 min in the dark for incubation. At 517 nm, the solution absorbance was measured against the blank once the 30 min incubation time was completed. The calculation of the antioxidant activity was done according to the following equation (Lee *et al.,* 2002):

$$\text{DPPH Radical scavenging activity} = \frac{A_0 - A_s}{A_0} \quad (3)$$

where,
$A_S$ = absorbance value of sample,
$A_0$ = absorbance value of blank

#### *2.13.2. Ferric reducing antioxidant power (FRAP) assay*

This test was carried out in accordance with Benzie and Strain (1996) with slight modification. 0.2 g of grape seed oil was added to methanol (1 mL) and shaken vigorously for 1 min. The mixtures were then vortexed. The sample mixture was centrifuged (DS-RC-AR, Dinesh Scientific, India) at a rate of 6000 rpm for 10 min. For further analysis, supernatant was used immediately. A FRAP solution was prepared by combining a TPTZ solution (10 mmol·L$^{-1}$) in hydrochloric acid (40 mmol·L$^{-1}$), and 25 mL of an acetate buffer (0.3 mol·L$^{-1}$, pH 3.6), and 2.5 mL of a $FeCl_3$ solution (20 mmol·L$^{-1}$). Previously prepared methanol extract (100 µL) was put in the volumetric flask (5 mL). Then, earlier prepared FRAP reagent (3 mL) was poured into the flask and the mixture was allowed to react under an incubation period of about 40 min in the dark before the absorbance was measured at 593 nm. As a standard, trolox was used. The results were expressed in terms of µM Trolox equivalent·100 g$^{-1}$ of grape seed oil.

### 2.14. Fatty acid composition

The conversion of fatty acids into volatile methyl esters was performed as mentioned by Ichihara and Fukubayashi (2010). About 1.5 mL methanol and 0.3 ml HCl (8 %) were mixed into a grape seed oil sample. Then the mixture was subjected to heating at 100 °C in the water bath for 1 h. 1 mL water was added after cooling and 1 mL hexane was added for extraction. A GC system was used to perform the analyses. A GC Column CP-Sil 88 with dimensions of (100 m × 0.25 mm × 0.2 µm) was used for separation. Helium was used as the carrier gas at a flow rate of 1 mL·min$^{-1}$ . The injector and detector temperatures were 250 and 270 °C, respectively. The starting temperature of the oven was 80 °C, which was increased to 220 °C ( 4 °C a min$^{-1}$), held for about 5 min, and then increased once again to 240 °C, where it was again held for about 10 minutes. The identification of compounds was made by comparing their retention times with

respect to standard peaks and the results were presented as percentage of FAME (total fatty acid methyl esters).

### 2.15. Calculated oxidizability value (COX)

Calculated oxidizability values (COX) were determined on the basis of UFA ($C_{18}$) percentages according to the following equation (Fatemi and Hammond, 1980):

$$\text{COX value} = \\ = [\text{C18:1}(\%) + 10.3 \times \text{C18:2}(\%) + 21.6 \times \text{C18:3}(\%)]/100 \quad (4)$$

### 2.16. Total tocopherol content

The determination of tocopherol content was made in accordance with Spika *et al.* (2015). 250 mg grape seed oil were mixed with n-heptane (25 mL). 20 µL of this mixture were injected into the HPLC column (25 cm × 4.6 mm ID, Merck, Germany) a flow rate of 1.3 mL/min. Tocopherol determination was made using a Shimadzu-HPLC system loaded with a PDA detector along with a LiChroCART Silica 60 column (5 µm, 4.6 mm x 250 mm). Tocopherol standard solutions for α-, β-, γ- and δ-tocopherol were prepared in the concentrations of 0 to 100 mg/L. All the analyses were done in triplicate.

### 2.17. Oxidative stability

For the determination of oxidative stability, a Rancimat apparatus (Metrohm, Switzerland) was used. About 2.50 g oil sample were put in the tube. The oil samples were subjected to the same conditions of temperature and constant air flow at 120 °C and 20 L/h, respectively. The rancimat apparatus showed induction times (h) automatically with an accuracy rate of 0.005. In the rancimat test analysis, the production of primary oxidation products with the application of heating and aeration was analyzed.

### 2.18. Statistical analysis

The results are presented as the mean of three observations and their standard deviation (±). The data analysis was conducted using IBM's statistical tool, SPSS Statistics (Version 26). The Duncan's Multiple Range Test (DMRT) was used to assess significant variations among the average values. Significance was assessed as $p < 0.05$ at 95% confidence level.

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical composition of grape seed powder

The moisture content of grape seed powder was 8.74%, while the ash content was about 2.89. As far as oil content was concerned, grape seed powder contained high amounts of oil, about 19.47%. Baydar and Akkurt (2001) reported the oil content of 18 grape seed cultivars and their values varied from 11.6 to 19.6%, which in terms of oil content was comparable to the present study. Rababah *et al.* (2008) examined five grape seed cultivars and found the oil content to range from 10.92 to 14.52 g·100 $g^{-1}$ seed. The chemical composition of grape seeds is dependent on several variables, such as cultivar, variety type, genotype, etc.

### 3.2. Oil yield and color

The extraction of oil with the aid of the soxhlet apparatus is typically conducted with solvents which are non-polar in nature like hexane. In the present study, apart from hexane, different alternative green approaches such as the cold-press method (CP), *p*-cymene (PC), supercritical $CO_2$ (SCO), supercritical $CO_2$ + ethanol (SCE) have been used. The GSO obtained by standard soxhlet method using hexane (HX) resulted in oil yield of 18.03% (Table 1). Grape seed oil obtained by the cold-press method produced an oil yield of 11.01% approximately, which was less than oil extracted with hexane. The SCO extraction gave 16.47% oil yield at 40 °C and 300 bar. Moreover, when ethanol was added as a polar entrainer to supercritical $CO_2$, it acted as a volatility intermediate between the two, the component which was to be extracted and the said supercritical fluid, which resulted in increased solvent power of $CO_2$ as well as the extraction of polar compounds such as free fatty acids, glycolipids, phospholipids, etc. When ethanol (10%) was introduced to supercritical $CO_2$, the oil yield from grape seeds raised to 18.61%; whereas 16.47% oil yield was obtained by using only pure supercritical $CO_2$ under the same conditions of temperature and pressure (40 °C and 300 bar). Da Silva *et al.* (2008) extracted oil from Victoria and red globe grape seeds through supercritical $CO_2$ and supercritical $CO_2$ + ethanol and observed an oil yield of 4.4 and 2.0% without ethanol and 14.7 and 11.8% with ethanol, respectively.

**Table 1**. A comparison between oil yield, refractive index, oil color, extraction method, and solvent type under the different conditions used for grape seed oil extraction.

| Extraction Method | Solvent | Time | Conditions | Oil yield (%) | Refractive index | *L** | *a** | *b** |
|---|---|---|---|---|---|---|---|---|
| Soxhlet | Hexane | 8 h | Soxhlet extraction through thermal cycle | 18.03 ± 0.14$^{c}$ | 1.472 ± 0.001$^{a}$ | 79.31 ± 0.13$^{e}$ | –0.2 ± 0.01$^{e}$ | 40.78 ± 0.06$^{a}$ |
| Soxhlet | *p*-cymene | 6 h | Soxhlet extraction through thermal cycle | 19.46 ± 0.52$^{a}$ | 1.469 ± 0.001$^{a}$ | 86.50 ± 0.11$^{b}$ | –19.48 ± 0.04$^{c}$ | 35.72 ± 0.09$^{b}$ |
| Supercritical Fluid Extraction | $CO_2$ | 4 h | Temperature 40 °C; Pressure 300 bar | 16.47 ± 0.21$^{d}$ | 1.477 ± 0.001$^{a}$ | 84.86 ± 0.08$^{c}$ | –21.99 ± 0.03$^{a}$ | 30.06 ± 0.10$^{d}$ |
| Supercritical F luid Extraction | $CO_2$ + Ethanol (10%) | 4 h | Temperature 40 °C; Pressure 300 bar | 18.61 ± 0.15$^{b}$ | 1.475 ± 0.001$^{a}$ | 83.91 ± 0.12$^{d}$ | –20.53 ± 0.02$^{b}$ | 28.64 ± 0.07$^{e}$ |
| Cold-Press Extraction | Nil | 0.5 h | Temperature Initial 90 and Final 40 °C | 11.01 ± 0.61$^{e}$ | 1.476 ± 0.001$^{a}$ | 88.23 ± 0.15$^{a}$ | –18.51 ± 0.04$^{d}$ | 31.09 ± 0.12$^{c}$ |

Means (±) standard deviations values (n=3); different letters in the same column show significantly different values ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT) by SPSS Statistics Software.

In comparison to pure SCO, improved solvent power was shown by SCE, which might be the reason for having higher lipid yields by this method. GSO extracted by p-cymene (PC) showed a yield of around 19.46% in the present study. In the present study, greater oil yield was achieved with p-cymene solvent in comparison to SCE and SCO. This might be due to the capability of terpene solvents to penetrate into the cell wall up to the globule level. A high boiling temperature may indicate terpenes' greater ability to dissolve lipids, as a result of which, the solvent's viscosity was reduced, hence resulting in increased oil yield.

### 3.3. Refractive index (RI)

As far as the analysis of refractive index is concerned, no significant difference was observed. Grape seed oils' optical properties are reflected in their refractive index (RI), which ranges from 1.469 to 1.477 and is influenced by both their composition and extraction method (Table 1). The highest value (1.477) was obtained for the grape seed oil extracted with supercritical $CO_2$ (SCO); while the lowest value (1.469) was obtained for oil extracted with *p*-cymene (PC). The refractive index of grape seed oils generally ranges between 1.462 and 1.478. This range is typical of vegetable oils, suggesting that the quality is consistent, regardless of the extraction technique. Similar results were reported by Vieira *et al*. (2015) for grape seed oils from 1.462 to 1.478, respectively. RI values in the present findings are greater than those reported earlier for grape seed oil obtained from Turkish wine grape cultivars such as Narince, Emir and Hasandede, which were 1.462, 1.466 and 1.460, respectively (Baydar, 2007).

### 3.4. Peroxide value (PV)

Peroxide value (PV) is the amount of all primary oxidation products found in the edible oils (Anusuya *et al.,* 2013). The PV of grape seed oil extracted by different extraction methods ranged from 2.60 to 6.50 meq $O_2 \cdot kg^{-1}$ (Table 2). GSO by SCE had the lowest peroxide values. The resulting PV for all the GSOs analyed was < 10 meq $O_2 \cdot kg^{-1}$, which is the recommended maximum PV for commercial oils. All extracted GSOs had at least 62.94 to 69.55% PUFA and 16.27 to 21.70% MUFA in their fatty acid profiles. The PV of SCO and CP extracted oil were 2.90 and 3.70 meq $O_2 \cdot kg^{-1}$; while for HX and PC extracted oils, the PV value was 6.50 and 3.10 meq $O_2 \cdot kg^{-1}$, respectively. High temperatures might have favored lipid oxidation during the hexane-assisted extraction of grape seed oils;, whereas, in the case of SCO and SCE extraction, the samples were not exposed to oxygen, and as a result, the initiation of the lipid oxidation process was limited.

### 3.5. Conjugated diene ($CD_{232}$) and conjugated triene ($CT_{268}$)

The hydroperoxides which indicate the early stage of oxidation were characterized by showing maximum absorbance at 232 nm and 268 nm and were related to the formation of conjugated diene

**Table 2.** Antioxidant activity, conjugated diene, conjugated triene, peroxide value, phenolic compounds and oxidation induction time of grape seed oils extracted by different methods.

| | Grape seed oils obtained by different extraction methods | | | | |
|---|---|---|---|---|---|
| | **CP** | **HX** | **SCO** | **SCE** | **PC** |
| DPPH radical scavenging effect (%) | 26.78 ± 0.30$^{d}$ | 24.25 ± 0.36$^{e}$ | 33.43 ± 0.32$^{b}$ | 36.80 ± 0.51$^{a}$ | 30.01 ± 0.90$^{c}$ |
| FRAP (μM TE· 100 $g^{-1}$) | 492.8 ± 5.3$^{d}$ | 481.4 ± 3.1$^{e}$ | 511.1 ± 11.2$^{b}$ | 522.6 ± 8.3$^{a}$ | 505.8 ± 9.2$^{c}$ |
| Conjugated diene ($CD_{232}$) | 2.405$^{a}$ | 2.533$^{a}$ | 2.447$^{a}$ | 2.428$^{a}$ | 2.410$^{a}$ |
| Conjugated triene ($CT_{268}$) | 0.326$^{d}$ | 0. 247$^{e}$ | 0.536$^{b}$ | 0.889$^{a}$ | 0.462$^{c}$ |
| PV (meq $O_2 \cdot kg^{-1}$) | 3.70$^{b}$ | 6.50$^{a}$ | 2.90$^{d}$ | 2.60$^{e}$ | 3.10$^{c}$ |
| Total phenolic compounds (mg GAE·$kg^{-1}$) | 117.54$^{d}$ | 109.77$^{e}$ | 127.18$^{b}$ | 132.01$^{a}$ | 123.53$^{c}$ |
| Rancimat induction time (h) | 1.8± 0.06$^{c}$ | 1.2± 0.07$^{e}$ | 1.9± 0.02$^{b}$ | 2.2± 0.2$^{a}$ | 1.5± 0.1$^{d}$ |

Means (±) standard deviations values (n=3); different letters in the same row show significantly different values ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT) by SPSS Statistics Software.
CP: cold-pressed grape seed oil; HX: grape seed oil extracted with hexane; SCO: grape seed oil extracted with supercritical $CO_2$; SCE: grape seed oil extracted with supercritical $CO_2$ + ethanol (10%); PC: grape seed oil extracted with p-cymene.

and conjugated triene, respectively. The CD and CT values may be used to figure out the level of oxidation of edible oils. When unsaturated fatty acids undergo oxidation, it results in the formation of conjugated dienes and gives stable free radicals. The conjugated diene value for cold-pressed (CP) grape seed oil was 2.405; while for SCO extraction and SCE extraction, the values were 2.447 and 2.428 (Table 2). The conjugated diene values for HX and PC extracted GSO were 2.533 and 2.410, respectively. A higher rate of unsaturation of fatty acids in oils leads to the production of a higher quantity of conjugated dienes. Maghsoudlou *et al.* (2017) found a similar increase in the conjugated diene value in canola oil samples. They likened their findings to conjugated compound polymerization and Diles-Alder reactions. The SCO had the lowest conjugated diene value.

### 3.6. Total phenolic content (TPC)

According to Downey *et. al.* (2003) the seeds contain approximately 60% of the overall phenolic compounds present in the grapes, apart from the stem, which contains approximately 20%, and about 15 to 20% of these compounds are found in the scarfskin. The TPC ranged from 109.77 (mg GAE·$kg^{-1}$) in HX to 132.01 (mg GAE·$kg^{-1}$) in SCE extracted grape seed oil (Table 2). The total phenolic contents found in SCO, CP and PC extracted GSOs were 127.18, 117.54 and 123.53 (mg GAE·$kg^{-1}$), respectively. Bail *et. al.* (2008) estimated that the total phenolic contents in nine samples of GSO extracted from various grape varieties via cold pressing to be in the range of 59.0 ± 0.02 to 115.5 ± 0.005 (mg GAE·$kg^{-1}$). Lower TPC in seed oils can be attributed to the low solubilization of lower molecular-weight phenolic compounds in oils, as well as some phenolic acids bound to the seeds.

### 3.7. Antioxidant activity

The GSO extracted by HX had the lowest DPPH radical-scavenging activity (24.25%), while the GSO obtained by SCE showed the highest value for DPPH radical-scavenging activity (36.80%) (Table 2). GSO extracted by using SCO, CP extraction, and PC extraction showed antioxidant activities of 33.43, 26.78 and 30.01%, respectively. Li *et al.* (2020) showed relative DPPH free radical-scavenging activity as 26.4 and 13.0 μmol TEAC·$g^{-1}$ grape seed oil for $CO_2$ expanded ethanol and hexane. Fernandes *et al.* (2013) found DPPH radical-scavenging potential in the range of 38.68 to 69.89% for Portuguese seed oils from wine grape cultivars (*Trincadeira preta* and *Cornifesto*), respectively. The oil extracted by SCE showed the highest antioxidant activity of about 522.6 μM TE·100 $g^{-1}$ followed by SCO, PC and CP extractions. The lowest value for antioxidant activity was found in GSO extracted by HX (481.4 μM TE·100 $g^{-1}$).

### 3.8. Fatty acid composition

In GSO samples, ten different types of fatty acids were detected and quantified (Table 3). All the obtained GSO samples had more than 80% unsaturated fatty acids (UFA). The dominant fatty acid in all the GSO samples was linoleic acid, which contributed to between 62.03 and 69.11% of the total fatty acids. GSO is congregated as a polyunsaturated fatty acid (PUFA) in the linoleic acid (LA) subgroup, along with safflower, paprika seed, melon seed and evening primrose oils (Dubois *et al.,* 2007). All of the GSOs mentioned contained well over 60% linoleic acid. The GSOs were also found to have large levels of oleic acid varying from 16.18 to 21.46%, palmitic acid ranged between 8.26 and 9.56%, and stearic acid varied from 3.54 to 5.14%. Minor amounts of myristic acid, palmitoleic acid, linolenic acid, and arachidic acid were detected. Behenic acid was only detected in CP in a minor quantity of about 0.05%; whereas lignoceric acid was detected in small quantities in all the extracted oils except SCE. The highest percentage of linoleic acid was determined in the SCE followed by the PC extraction, CP extraction, and SCO; while HX was the poorest source of it. Among all the extracted GSOs, oleic acid was the second largest fatty acid in terms of quantity. In GSO extracted by PC (21.46%), oleic acid was present in s higher amount compared to GSOs obtained through other methods. On the other hand, the percentage of palmitic acid, which is a main saturated fatty acid (SFA), varied between 8.26 and 9.56% FAME. According to Sabir *et al.* (2012), COX values are derived to determine the oxidative stability of edible oils. The GSO extracted by hexane had the lowest COX value (Table 2). As a result, this finding is suggestive of oxidative stability in oil extracted by hexane. The analyzed GSO samples had a desirable PUFA/SFA ratio which ranged between 4.37 for HX and 5.24 for PC extraction. Da Silva *et al.* (2008)

**Table 3.** Fatty acid profile of grape seed oils obtained by different extraction methods.

| | Grape seed oil obtained by different extraction methods | | | | |
|---|---|---|---|---|---|
| | **CP** | **HX** | **SCO** | **SCE** | **PC** |
| *Fatty acids (% of total FAME)* | | | | | |
| Myristic acid ($C_{14:0}$) | 0.14±0.01$^{a}$ | 0.12±0.01$^{a}$ | 0.11±0.0$^{a}$ | 0.05±0.01$^{b}$ | 0.12±0.02$^{a}$ |
| Palmitic acid ($C_{16:0}$) | 9.56±0.02$^{a}$ | 9.40±0.02$^{b}$ | 8.73±0.01$^{d}$ | 8.26±0.01$^{e}$ | 9.13±0.04$^{c}$ |
| Palmitoleic acid ($C_{16:1}$) | 0.27±0.01$^{a}$ | 0.24±0.01$^{a}$ | 0.23±0.00$^{a}$ | 0.09±0.00$^{b}$ | 0.24±0.0$^{a}$ |
| Stearic acid ($C_{18:0}$) | 3.62±0.03$^{d}$ | 4.58±0.03$^{b}$ | 4.28±0.04$^{c}$ | 5.14±0.06$^{a}$ | 3.54±0.01$^{d}$ |
| Oleic acid ($C_{18:1}$) | 19.31±0.01$^{c}$ | 21.46±0.04$^{a}$ | 20.21±0.05$^{b}$ | 16.18±0.01$^{e}$ | 18.82±0.02$^{d}$ |
| Linoleic acid ($C_{18:2}$) | 65.91±0.01$^{c}$ | 62.03±0.01$^{e}$ | 64.93±0.08$^{d}$ | 69.11±0.07$^{a}$ | 67.25±0.07$^{b}$ |
| Linolenic acid ($C_{18:3}$) | 0.52±0.01$^{c}$ | 0.91±0.01$^{a}$ | 0.64±0.01$^{b}$ | 0.44±0.01$^{d}$ | 0.64±0.01$^{b}$ |
| Arachidic acid ($C_{20:0}$) | 0.14±0.01$^{a}$ | 0.21±0.00$^{a}$ | 0.19±0.00$^{a}$ | 0.19±0.00$^{a}$ | 0.12±0.00$^{a}$ |
| Behenic acid ($C_{22:0}$) | 0.05±0.00$^{a}$ | ND | ND | ND | ND |
| Lignoceric acid ($C_{24:0}$) | 0.06±0.00$^{a}$ | 0.08±0.00$^{a}$ | 0.07±0.00$^{a}$ | ND | 0.04±0.00$^{a}$ |
| SFA | 13.57±0.01$^{b}$ | 14.39±0.01$^{a}$ | 13.38±0.01$^{c}$ | 13.64±0.01$^{b}$ | 12.95±0.02$^{d}$ |
| MUFA | 19.58±0.02$^{c}$ | 21.70±0.08$^{a}$ | 20.44±0.01$^{b}$ | 16.27±0.03$^{e}$ | 19.06±0.1$^{d}$ |
| PUFA | 66.43±0.01$^{c}$ | 62.94±0.08$^{e}$ | 65.57±0.10$^{d}$ | 69.55±0.02$^{a}$ | 67.89±0.01$^{b}$ |
| UFA | 86.01±0.01$^{b}$ | 81.64±0.08$^{d}$ | 86.01±0.02$^{b}$ | 85.82±0.02$^{c}$ | 86.95±0.01$^{a}$ |
| PUFA/SFA | 4.89$^{c}$ | 4.37$^{d}$ | 4.90$^{c}$ | 5.09$^{a}$ | 5.24$^{b}$ |
| COX values | 7.09$^{c}$ | 6.80$^{d}$ | 7.02$^{c}$ | 7.37$^{a}$ | 7.25$^{b}$ |

Means ± standard deviation values (n = 3) followed by different superscript letters in the same row are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT) by SPSS Statistics Software.
CP: cold-pressed grape seed oil; HX: grape seed oil extracted with hexane; SCO: grape seed oil extracted with supercritical $CO_2$; SCE: grape seed oil extracted with supercritical $CO_2$ + ethanol (10%); PC: grape seed oil extracted with p-cymene; FAME: fatty acid methyl esters; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; ND: not detectede; COX: calculated oxidizability value.

extracted oil from Victoria and red globe grape seeds through supercritical $CO_2$ and supercritical $CO_2$ + ethanol. Their fatty acid profile showed PUFA in the range of 66-71%. Li *et al.* (2020) showed a grape seed oil yield of 13.6% with recyclable $CO_2$ expanded ethanol and 12.7% with hexane as solvent. Apart from yield, they found unsaturated fatty acids at 87.4% for $CO_2$ expanded ethanol and 83.6% for hexane.

### 3.9. Tocopherol composition

The α-tocopherol content in the analyzed GSO samples was found in the range of 194.99 to 239.88 mg·kg$^{-1}$; whereas δ-tocopherol ranged from 0.53 to 0.83 mg·kg$^{-1}$; while the β and γ-tocopherol contents combined varied from 18.61 to 23.55 mg·kg$^{-1}$. The results obtained show that the α-tocopherol content is quite dominant in comparison to β, γ and δ -tocopherols in GSO (Table 4). The total tocopherol content in the analyzed GSO samples ranged from 214.13 mg·kg$^{-1}$ (HX extracted) to 264.26 mg·kg$^{-1}$ (SCE extracted). The total tocopherol content of GSO decreased for solvent extraction methods which require some amount of heat. In comparison to the GSO obtained through SCE, the oil obtained by SCO, CP, PC and HX extraction methods showed reductions in tocopherol contents by 2.49, 4.35, 11.43, and 18.71%, respectively. This can be attributed to the fact that grape seeds are exposed to higher temperatures during the solvent extraction process, which results in an increase in thermal oxidation processes which further cause a reduction in tocopherol content. Beveridge *et al.* (2005) extracted oil from grape seeds and found α-tocopherol contents between 7.67 and 30.9 and 3.58 and 27.2 mg·100 g$^{-1}$ of oil for supercritical $CO_2$ and petroleum ether.

### 3.10. Rancimat test

The rancimat method is used to determine the oxidative stability of oil by measuring its induction period (IP). Oil with a longer induction period has better oxidation stability. The oil obtained by SCE had the longest induction time among all the GSOs tested. In the case of SCE grape seed oil, wherein the peroxide value (PV) was 2.60 meq $O_2$·kg$^{-1}$, the induction time was approximately 2.2 h (Table 2). It was found that oils extracted by SCO and CP were somewhat similar as far as induction times are concerned; whereas oil extracted with PC showed an induction time of 1.5 h. SCE extracted oil showed the lowest and HX extracted oil indicated the highest PV value among all the GSOs tested (2.60 and 6.50 meq $O_2$·kg$^{-1}$, respectively). The time it took PV to reach the acceptable value of 10 meq $O_2$/kg was determined as an important aspect of the rancimat test. As the polyunsaturated fatty acid (PUFA) amount in seed oils increases, so does the primary oxidation products' formation rate. PUFAs were present in the range of 62 to 69% and resulted in the fastest oxidation of all obtained GSOs. Antioxidants like tocotrienols and tocopherols also influence the oxidative stability of obtained oils. In the case of oil extracted with HX with a PV value of 6.5 meq $O_2$·kg$^{-1}$, the induction time was roughly one half (1.2 h) that of the SCE extracted oil. Based on the rancimat test results, it was found that oil extracted with HX presented a short time because of higher its initial oxidation state. According to Redondo-Cuevas *et al.* (2018) and Maszewska *et al.* (2018), rapeseed oil induction times at 120°C were 4.7 h and 4.3 h, respectively. Green solvents seem preferable to solvents derived from petroleum because of their bio-based nature. Furthermore, green solvents such

**Table 4.** Total tocopherol content of grape seed oils extracted with different methods.

| Tocopherols (mg·kg$^{-1}$) | Grape seed oil obtained by different extraction methods | | | | |
|---|---|---|---|---|---|
| | CP | HX | SCO | SCE | PC |
| α | 229.44$^{c}$ | 194.99$^{e}$ | 233.89$^{b}$ | 239.88$^{a}$ | 212.45$^{d}$ |
| β + γ | 21.68$^{c}$ | 18.61$^{e}$ | 22.31$^{b}$ | 23.55$^{a}$ | 20.41$^{d}$ |
| δ | 0.77$^{a}$ | 0.53$^{b}$ | 0.81$^{a}$ | 0.83$^{a}$ | 0.75$^{a}$ |
| Total tocopherols | 251.89$^{c}$ | 214.13$^{e}$ | 257.01$^{b}$ | 264.26$^{a}$ | 233.61$^{d}$ |

Means (±) standard deviations values (n=3); different letters in the same row show significantly different values ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT) by SPSS Statistics Software.
CP: cold-pressed grape seed oil; HX: grape seed oil extracted with hexane; SCO: grape seed oil extracted with supercritical $CO_2$; SCE: grape seed oil extracted with supercritical $CO_2$ + ethanol (10%); PC: grape seed oil extracted with p-cymene.

as para cymene, supercritical $CO_2$ and ethanol have the potential to substitute traditional solvent systems in many ways.

## 4. CONCLUSIONS

The extraction of oil from grape seeds obtained from winery waste was investigated and a comparison was drawn between green approaches and hexane. The GSO obtained using p-cymene yielded the maximum oil output, at around 19.46 %. The oil yield of GSO extracted using SCO, CP, and HX was about 16.47, 11.01, and 18.03 % respectively; while the combination of the supercritical $CO_2$ + ethanol extraction (SCE) technique yielded an approximate oil yield of 18.61 %. In addition, compared to the hexane-aided oil extraction method, the oil production from p-cymene (PC), supercritical $CO_2$ + ethanol (SCE) was higher by 7.93 and 3.21 %, respectively. In terms of PUFA content, the fatty acid profile of SCE was superior, followed by PC, CP, SCO and HX. As far as the antioxidant activity of grape seed oil extracted using different extraction approaches is concerned, it showed the following order SCE > SCO > PC > CP > HX. These extracted oils possess potential health benefits due to the presence of total tocopherols, PUFA as well as various phenolic compounds which improve the nutritional value of the final product. The present investigation is indeed a valuable and effective approach for the extraction of grape seed oil without having detrimental effects on the environment and human health.

## 5. ACKNOWLEDGEMENTS

The authors are thankful to the Central Research Laboratory, Sant Longowal Institute of Engineering and Technology (SLIET), Longowal, for providing the necessary infrastructure and research facilities.

## 6. DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

## 7. AUTHORSHIP CONTRIBUTION STATEMENT

**M. Ubaid:** Investigation, Formal analysis, Data curation, Data analysis, Manuscript writing.

**C. S. Saini:** Conceptualization, Resources, Methodology, Supervision, Manuscript editing and review.

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