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Variations in Physico-Chemical and Antioxidant Attributes of Grape Seed Oil as Function of Extraction Techniques

(Variasi dalam Atribut Fiziko-Kimia dan Antioksidan Minyak Biji Anggur sebagai Fungsi Teknik Pengekstrakan)

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ABSTRACT

The aim of the current research work was to assess and compare the impact of two extraction techniques on the physico-chemical, and antioxidant parameters of grape seed oil (GSO). The GSO extracted by Soxhlet and Folch methods indicated a notable variation in the oil yield (8.58 % and 10.19%) and saponification value (196.35 and 189.33 mg of KOH g^{-1}), respectively. However, no significant (p<0.05) variation was detected for density, acid value, refractive index, iodine no, unsaponifiable matter, and free fatty acids between the tested two oils. Meanwhile, the oil produced by Soxhlet method exhibited relatively a higher extent of unsaturated dienes, trienes, peroxide value, *p*-anisidine value, and thus poor oxidation state. A notable variation in the content of principal fatty acid (linoleic acid) was recorded between Folch extracted oil (70.11%) and Soxhlet extracted oil (66.57%). The contents of total tocopherols were noted to be considerably higher for Folch extracted oil (105.55 mg kg^{-1}) than the Soxhlet extracted oil (73.70 mg kg^{-1}). Among the individual phenolics analyzed by HPLC, gallic acid (14.02 mg kg^{-1}) and caffeic acid (5.20 mg kg^{-1}) were detected as major component in Folch extracted oil and Soxhlet extracted oil, respectively. The results of the present comparative study support that Folch method is relatively a good choice for the extraction of GSO with promising nutritive quality in terms of oxidation parameters, contents of linoleic acid and antioxidant phenolics.

Keywords: Antioxidants; grape seed oil; HPLC; phenolic acids; physico-chemical parameters

ABSTRAK

Matlamat penyelidikan ini adalah untuk menilai dan membandingkan kesan dua teknik pengekstrakan ke atas parameter fiziko-kimia dan antioksidan minyak biji anggur (GSO). GSO yang diekstrak oleh kaedah Soxhlet dan Folch menunjukkan variasi ketara masing-masing dalam hasil minyak (8.58 % dan 10.19%) dan nilai saponifikasi (196.35 dan 189.33 mg KOH g^{-1}). Walau bagaimanapun, tiada variasi ketara (p<0.05) dikesan untuk ketumpatan, nilai asid, indeks biasan, nombor iodin, bahan tidak boleh tercemar dan asid lemak bebas antara dua minyak yang diuji. Sementara itu, minyak yang dihasilkan melalui kaedah Soxhlet mempamerkan tahap diena tak tepu, triena, nilai peroksida, nilai p-anisidin, yang menunjukkan pengoksidaan yang lemah. Satu variasi ketara dalam kandungan asid lemak utama (asid linoleik) telah direkodkan antara minyak yang diekstrak Folch (70.11%) dan minyak yang diekstrak Soxhlet (66.57%). Kandungan jumlah tokoferol didapati jauh lebih tinggi untuk minyak yang diekstrak Folch (105.55 mg kg^{-1}) daripada minyak yang diekstrak Soxhlet (73.70 mg kg^{-1}). Antara fenol individu yang dianalisis oleh HPLC, asid galik (14.02 mg kg^{-1}) dan asid kafeik (5.20 mg kg^{-1}) dikesan sebagai komponen utama masing-masing dalam minyak ekstrak Folch dan minyak ekstrak Soxhlet. Keputusan kajian perbandingan menyokong bahawa kaedah Folch secara relatifnya merupakan pilihan yang baik untuk pengekstrakan GSO dengan kualiti nutrien yang menggalakkan dari segi parameter pengoksidaan, kandungan asid linoleik dan fenol antioksidan.

Kata kunci: Antioksidan; asid fenol; HPLC; minyak biji anggur; parameter fiziko-kimia

INTRODUCTION

Grape (*Vitis vinifera* L.), belonging to *Vitaceae* family, is one of the earliest and world's biggest fruit crops (Fernandez-Marin et al. 2013). About 80% of the total crop is generally utilized in wine-making (Mironeasa et al. 2010). Grape cultivation is reported world over in more than 90 countries with total production of sixty-nine (69) million tons annually (Fernandez-Marin et al. 2013). Globally, the countries such as Italy, Spain, United States of America, China, France, and Turkey are considered as the main grape producers (Fernandez-Marin et al. 2013; Riaz et al. 2007). Grape seed is 1-4 % of the fruit weight (Mironeasa et al. 2010) and has 10-20% oil yield depending upon the varieties (Ahmadi et al. 2011). Approximately 3 million tons grape seeds are produced annually by the food and wine processing industries and usually these are discarded as an agro-waste (Fernandez et al. 2013).

The interesting feature of GSO is its high degree of un-saturation (88%) with 72-76% contribution of linoleic acid (Fernandez et al. 2010). This fatty acid promotes cardiovascular health by lowering LDL cholesterol level (Peralbo-Molina et al. 2013). Daily intake of 45 g grape seed oil has been reported to increase good lipoprotein called as HDL-C level by 13% and reduced LDL-C level by 7% in the duration of three weeks (Cerchiara et al. 2010). The conjugated linoleic acid (CLA) can be best prepared by the linoleic acid rich oil of this plant, which is a positional and configurational isomers of linoleic acid. Recent clinical data proved that CLA can be possibly used as anti-cancer, anti-atherogenic and hypoglycemic agents (Cao & Ito 2003).

Several pharmaceutical activities such as antithrombic, cholesterol lowering (Cao & Ito 2003), antibacterial (Fernandes et al. 2013), chemoprotective and cytoprotective properties have been associated with GSO composition (Beveridge et al. 2005). Presence of vitamin E in grape seed oil has neuroprotective, anti-aging and antitumoral properties (Abd El-Rahim et al. 2009; Fernandes et al. 2013).

Various extraction methods and solvents, varying in their efficiencies and specificities (Jones et al. 2012), have a significant impact on yield (Goli et al. 2004) and basic bioactive composition of seed oils (Bhatnagar & Gopala Krishna 2014). For example, Soxhlet extraction method, based on solid-liquid extraction, gives higher oil yield when compared to supercritical CO_2 extraction (Da Porto et al. 2012) and cold pressing (Ixtaina et al. 2011). Folch method is a classical and more reliable method for the extraction of low, intermediate, higher lipid contents (Fiori et al. 2013). Folch extraction method, over the last half century, has become a selective and effective method for lipid extraction employing a combination of $CHCl_3$/CH_3OH (2:1 v/v) solvents (Abbott et al. 2013).

As such, no detailed study is available in the literature investigating the effects of different extraction techniques on yield, and composition of GSO. Therefore, the purpose of the current research work was to evaluate and compare the effects of two different extraction techniques on the yield, physicochemical, and antioxidant attributes of grape seed oil.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

The standards of phenolic acids, and tocopherols (α, ß, δ, and γ-tocopherols) were taken from Sigma Chemical Co. (St. Louis, MO, USA). Reagents and few chemicals of analytical grade was also used in the present experiments, were similarly purchased from Sigma Chemicals (St. Louis, MO, USA).

GRAPE SEED PREPARATION

Fruits of grapes (*Vitis vinifera* L.) of a local variety namely Manakka were purchased from the local market in Faisalabad, Pakistan. Manually seeds were removed from the fruit and then washed with water to remove any pulp residues. The seeds were dried at 40 °C by placing in a hot air-oven and crushed for extraction purposes using a domestic grinder.

OIL EXTRACTION

Two extraction techniques namely, Soxhlet method and Folch method were employed for grape seed oil extraction. In Soxhlet method, grape seed powder (80 g) filled in a paper thimble, was placed in a Soxhlet assembly. The extraction was performed using n-hexane for 6 h. After extraction, excess n-hexane was recovered under reduced pressure using a rotary evaporator (Eyela, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan).

The Folch extraction was done by the previous method described by Folch et al. (1957). Briefly, ground grape seed material (40 g) was extracted by homogenizing with 400 mL mixture of chloroform, and methanol (2:1 v/v) for 5 min using a homogenizer (Leerlaufdrehzahl, Germany) set at run speed of 13500 rpm. The extract was filtered through a filter paper and then poured into a separating funnel. Then, deionized water (100 mL) was added into the separating funnel,

gently mixed, after the mixing, the mixture was then allowed to separate into two layers. The lipid containing lower layer of chloroform was collected in a separate beaker. The excess chloroform was concentrated by using a rotary evaporator to obtain crude oil.

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF OIL

Physicochemical properties like density, acid value, refractive index, free fatty acid value, peroxide value, saponification value, un-saponifiable matter and iodine value of extracted oils were determined according to American Oil Chemist's Society (AOCS) methods (AOCS 1997). Colour in terms of red and yellow units was noted by a Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom) using 1-inch cell. Oxidation parameters including unsaturated dienes, and trienes, in terms of specific extinctions at 232 and 268 nm, respectively, and *p*-anisidine value were determined using standard IUPAC methods (Paquot & Hautfenne 1987).

ANALYSIS OF FATTY ACID COMPOSITION USING GAS LIQUID CHROMATOGRAPHY

The fatty acids present in GSO were transesterified into methyl esters (FAMEs) using the procedure described by Christie (1993). The FAMEs were analyzed by a Shimadzu-Gas Chromatography (GC, model 17-A) equipped with a flame ionization detector (FID). A Supelco SP-2330 polar capillary column (Supelco Inc. Supelco Park Bellefonte, PA 16823-0048 USA) with dimension (30 m × 0.32 mm; 0.2 µm methyl lignocerate coated film) was used for the separation of individual fatty acids. Nitrogen was used as a mobile phase at a flow rate of 3.5 mL/min. The initial column temperature was set at 180 °C and gradually increased by the rate of 5 °C min^{-1} to a final temperature of 220 °C. The initial and final holds up temperature were adjusted for 2 and 10 min, respectively. The injector and detector temperatures were fixed at 230 and 250 °C, respectively. The identification of unknown fatty acids was based upon comparing their retention times with standards and quantified based upon calculation of relative percent area (normalization process). The fatty acid composition was expressed as gram of fatty acid per100 gram of total fatty acids.

ANALYSIS OF TOCOPHEROLS BY HPLC

Individual tocopherols component in GSO were analyzed by high performance liquid chromatography (HPLC) following an earlier described procedure (Wrolstad 2003) with some modifications. Briefly, 0.1 g oil sample and 0.05 g ascorbic acid (vitamin C) were placed in a test tube (16 × 125 mm) and then mixed with 5 mL of ethanol (90%) and 0.5 mL of aqueous potassium hydroxide (80%). The mixture was vortexed for 30 s. After flushing the test tube with nitrogen, it was incubated in water bath (72 °C) for 30 min with periodical vortexing. After incubation, the tube was cooled in an ice bath for 5 min. Then, 3 mL of distilled water and 5 mL of n-hexane were added to dissolve the contents of test tube. The contents of the test tube were shaked for 30 s, causing a separation of two layers.

The upper layer of n-hexane was taken in a new test tube, while the remaining aqueous layer was again washed with n-hexane. The recovered two n-hexane layers were collected and allowed to dry under nitrogen streaming. Then, 1 mL of mobile phase was added in this test tube to dissolve the extract residue by vortexing prior to transferring into sample vials. A 20 µL extract solution was injected into a LC-Si column with dimension 250 × 4.6 mm (Supelco Inc. Supelco Park, Bellefonte, KY, USA) fitted with HPLC instrument (Prostar 325 is Detector LC Detector Series, Varian), using an isocratic elution mode. A combination of ethyl acetate/acetic acid/ hexane, 1/1/98 (% v/v), was used as a mobile phase at a flow rate of 1.5 mL/min. The eluent bands were detected at 295 nm using a UV-Vis detector. For identification of individual tocopherols, relative retention times of the unknown were compared with those of known standards and quantified by external standard calibration method using a computer-based data software.

EXTRACTION AND ANALYSIS OF PHENOLICS BY HPLC

The naturally occurring bioactive antioxidant components such as phenolics in GSO were extracted and analyzed by HPLC system (Parry et al. 2005). Concisely, 1 g of GSO was taken into a test tube followed by addition of 3 mL of 80% aqueous methanol. The test tube, after vigorous shaking, was centrifuged at 6000 rpm for 4-6 min and the supernatant was collected. The residue was re-extracted for three times and the extracts recovered were combined. The excess solvent was removed under nitrogen streaming and the final extract volume was made up to 10 mL with extraction solvent. A hypersil ODS (C18) reverse phase column (250 × 4.6 mm), fitted with an HPLC machine (Prostar 325 UV-Vis Detector LC Detector Series, Varian) was used for separation purposes. An S-1122 dual piston solvent delivery system and UV/Vis diode array detector (Sykam GmbH, Kleinostheim, Germany) were fitted with HPLC. Mobile

phase (absolute methanol + 2.5% glacial acetic acid, 99:1 v/v) was employed at a flow rate of 1.5 mL min^{-1} following the gradient mode of elution. The eluent bioactives compounds bands/peaks were noticed at 250 and 320 nm and the compounds identified by matching their retention times with those of pure standards. The targeted bioactives (mostly phenolic acids) were quantified using external calibration method.

Results and Discussion

Oil yield

The yield of Soxhlet extracted grape seed oil (GSO) was 8.58% while that of Folch extracted oil 10.19% (Table 1), showing considerable variations between the two extraction methods. A higher oil yield by Folch method may be attributed to effective extraction in due part to the compatibility and solubilization power of organic solvents mixture employed for extraction of grape seed lipids. In Soxhelt method, n-hexane, due to its non-polar nature, mostly non-polar lipids and rarely polar lipids are extracted but chloroform/methanol mixture employed in Folch method can extract both non-polar and polar lipids along with a variety of antioxidants (Bhatnagar & Gopala Krishna 2014; Ramadan & Moersel 2002).

TABLE 1. Yield and physicochemical parameters of Soxhlet and Folch extracted grape seed oil

Parameters	Soxhlet extracted oil	Folch extracted oil
Oil yield (%)	8.58 ± 0.40	10.19±0.50
Density (30 °C, mg mL^{-1})	0.92 ± 0.04	0.93 ± 0.07
Refractive index (40 °C)	1.4700 ± 0.01	1.4800 ± 0.01
Color index Red units	3.2 ± 0.80	3.6 ± 0.50
Color index Yellow units	60 ± 1.00	70 ± 1.50
Acid value (mg KOH per g of oil)	2.07 ± 0.10	2.26 ± 0.12
Free fatty acid value (% as oleic acid)	1.03 ± 0.06	1.13 ± 0.05
Unsaponifiable matter (% w/w)	1.03 ± 0.06	1.19 ± 0.08
Saponification value (mg of KOH per g of oil)	196.35 ± 1.60	189.33 ± 1.50
Iodine value (g of I per100g of oil)	138.32 ± 1.50	139.59 ±1.25

Values are means ± standard deviations for triplicate determinations

Physicochemical characterization of extracted GSO

No significant difference was observed for density, refractive index, free fatty acid value, acid value, unsaponifiable matter, and iodine no. of GSO in relation to two extraction methods. However, these methods significantly (p<0.05) influenced the saponification value and colour (yellow) of the oils extracted. Saponification value for Soxhlet extracted oil (196.35) was higher than that of GSO extracted by Folch method (189.33). Colour measurement is an important parameter to check the purity and grade of vegetable oils. Colour of GSO in terms of yellow units was found to be 60 and 70 yellow units for Soxhlet and Folch extracted oils, respectively. The colour of vegetable oils is mainly due to the existence of various coloring compounds/pigments like chlorophyll

and carotenoids (Anwar et al. 2008). Colour of Folch extracted GSO was darker than Soxhlet extracted GSO and this difference may be due to higher content of coloring components/pigments in the former oil.

OXIDATION PARAMETERS

Peroxide value (PV) for Soxhlet and Folch extracted oils were 2.98 and 2.85 meq. O_2 per kg of oil, respectively (Table 2). The low PV of Folch extracted GSO can be attributed to the non-thermal mode of Folch method, as well as extraction of relatively higher levels of antioxidants components such as tocopherols, phenolics and carotenoids by the methanol: chloroform mixture. The New Zealand Food Regulation (1984) suggested the maximum limit of PV up to 10 meq. of O_2 per kg of oil/fat while the Codex Alimentarius Commission (1999) stipulated this value up to 15 meq.of O_2 per kg of oil for virgin or cold-pressed oils (Teh & Birch 2013). Overall, GSO produced by both the extraction techniques exhibited reasonable good oxidation state due to lower PV. Fernandez et al. (2013) reported somewhat lower peroxide value (2.19) for GSO produced from wine industry (Ciudad Real) of Spain. A lower *p*-anisidine value for Folch extracted GSO can also be referred to good oxidation state as compared to Soxhlet extracted GSO. Similarly, Folch extracted GSO exhibited lower levels of conjugated diene and conjugated triene values (0.39, 0.09) as compared to Soxhlet extracted GSO (0.49, 0.11). The measurement of conjugated dienes and conjugated trienes is an important indicator to evaluate the quantity of secondary oxidation products in oils (Anwar et al. 2008).

TABLE 2. Comparison of oxidation status of Soxhlet and Folch extracted grape seed oil

Parameters	Soxhlet extracted oil	Folch extracted oil
Peroxide value (mEq. O_2 per kg)	2.98 ± 0.08	2.85 ± 0.07
p-anisidine value	6.59 ± 0.50	3.84 ± 1.00
Conjugated diene value $\varepsilon^{1\%}$ 1cm (λ 232)	0.49 ± 0.08	0.39 ± 0.07
Conjugated triene value $\varepsilon^{1\%}$ 1cm (λ 268)	0.11 ± 0.01	0.09 ± 0.01

Values are means ± standard deviations for triplicate determinations

FATTY ACID COMPOSITION

Quantification of individual fatty acids is an important parameter for the assessment of nutritional value and industrial uses of a vegetable oil or fat. The physicochemical and nutritional characteristics of vegetable oils are strongly influenced by the types and proportions of fatty acids (Nehdi 2013). As demonstrated from the data in Table 3, linoleic acid was found to be the major fatty acid among the fatty acids identified in both the Soxhlet and Folch extracted GSO with contribution of 66.57 and 70.11%, respectively (comparison of FAs is depicted in Figure 1). Linoleic acid content was slightly higher in case of Folch extraction. According to some investigations, extraction methods considerably influence the fatty acid composition of GSO as described by Beveridge et al. (2005). Beveridge et al. (2005) described that the linoleic acid values ranging from 66.8-73.6% in seed oils of few other grape varieties extracted by supercritical carbon dioxide, and petroleum ether. Similarly, Rubio et al. (2009) reported values of linoleic acid ranging from 67.61-72.98% for Soxhlet extracted GSO. Lutterodt et al. (2011) characterized cold pressed seed oils of four different grape varieties and found linoleic acid content as high as 66% (Ruby red) and 75% (concord). Fernandez et al. (2013) analyzed contents of linoleic acid in the range of 63.00-73.10% for Soxhlet extracted (using petroleum ether) GSO from ten Portuguese grape varieties.

Oleic acid was the second key fatty acid found in both the tested oils and was quantified as high as 24.40 and 23.74% for Folch and Soxhlet extracted GSO, respectively. In the present study, oleic acid was

comparable for GSO extracted by both the techniques but a small concentration of linolenic acid and arachidic acid were only detected in Soxhlet extracted GSO. Degree of total unsaturation was noted to be 91.14 and 93.88% for Soxhlet and Folch extracted GSO, respectively, with slight difference.

TABLE 3. Comparison of fatty acids composition of Soxhlet and Folch extracted grape seed oil

Fatty acids (g/100 g of oil)	Soxhlet extracted oil	Folch extracted oil
Palmitic acid ($C_{16:0}$)	6.15 ± 0.01	8.06 ± 0.03
Oleic acid ($C_{18:1}$)	23.74 ± 0.60	24.40 ± 1.09
Linoleic acid ($C_{18:2}$)	66.57 ± 2.49	70.11 ± 0.72
Linolenic acid ($C_{18:3}$)	0.17 ± 0.01	ND
Arachidic acid ($C_{20:0}$)	0.80 ± 0.06	ND
SFAs	6.15± 0.16	8.86± 0.21
MUFAs	23.74± 0.23	24.40± 0.16
PUFAs	66.74± 0.36	70.11± 0.19

Values are means ± standard deviations for triplicate determinations

SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, ND: not detected

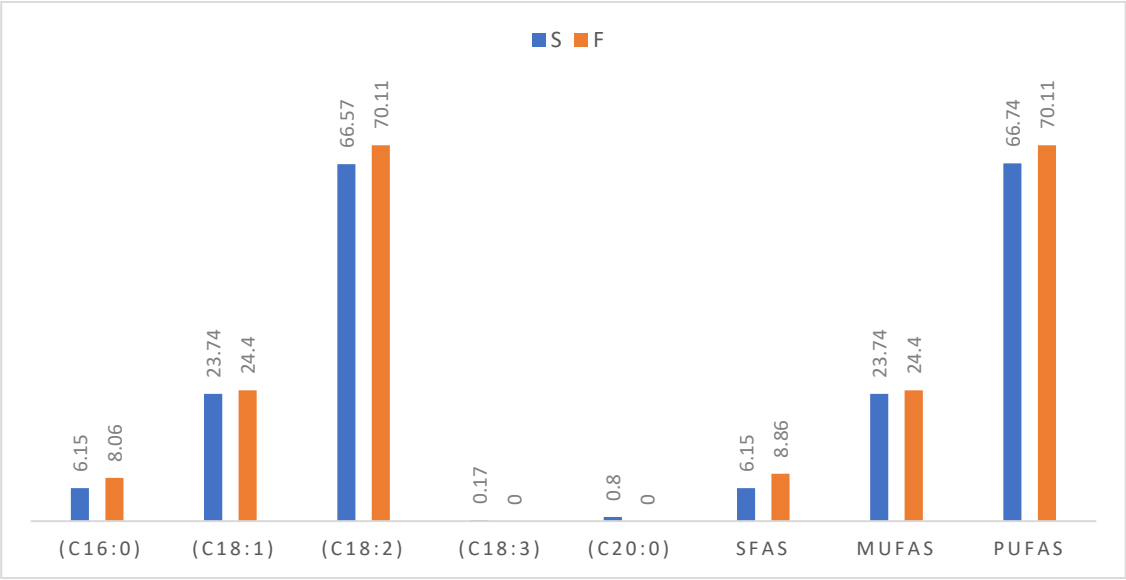

FIGURE 1. Comparison of fatty acids composition in GSO using Soxhlet and Folch methods

TOCOPHEROLS

From the results in Table 4, it can be seen that α-tocopherol (84.75 mg kg^{-1}) is the major component detected in Folch extracted oil as well as in Soxhlet extracted oil (56.60 mg kg^{-1}). It can be guessed that Folch extraction technique is more effective for the recovery of α-tocopherol from grape seed oil. The γ-tocopherol content was low and comparable in both the oils. Whereas, β-tocopherol was not detected in both the tested oils. As expected, total tocopherols content was found to be higher in case of Folch extraction (105.55 mg kg^{-1}) as compared to Soxhlet extraction (73.70 mg kg^{-1}) (Figures 2 & 3).

TABLE 4. Comparison of tocopherols in Soxhlet and Folch extracted grape seed oil

Tocopherols (mg kg^{-1})	Soxhlet extracted oil	Folch extracted oil
α-tocopherol	56.60 ±1.27	84.75 ± 1.39
γ-tocopherol	17.10 ± 0.68	20.80 ± 1.20
Total contents	73.70 ± 1.95	105.55 ± 2.59

Values are means ± standard deviations for triplicate determinations

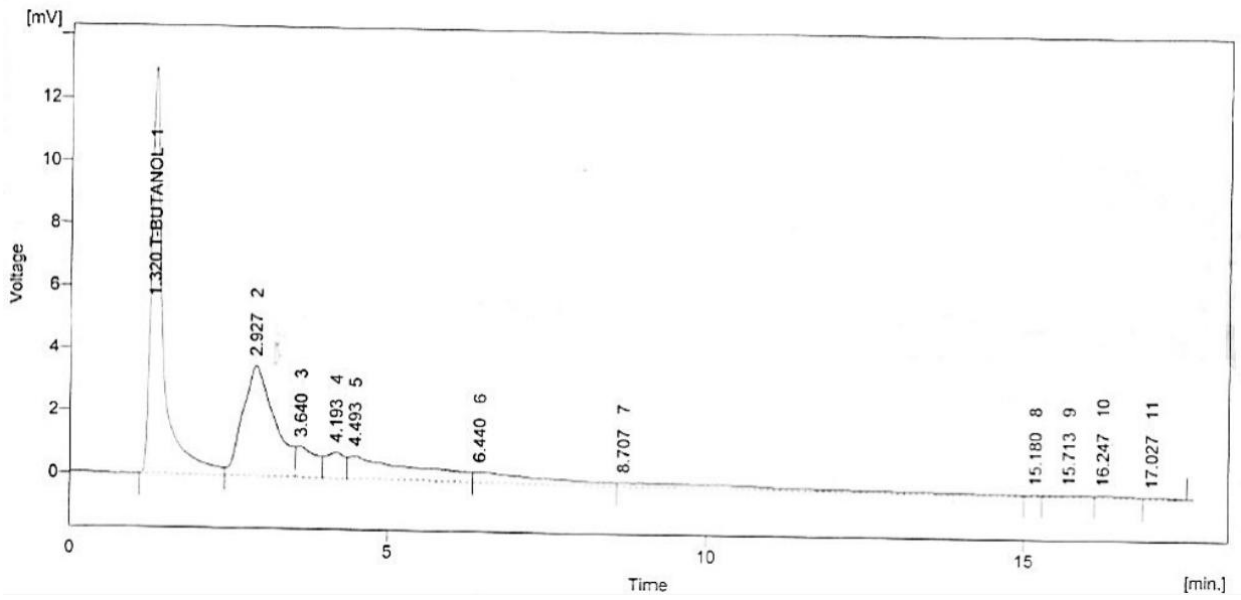

FIGURE 2. HPLC chromatogram showing the separation of tochopherols in Folch extracted GSO

PHENOLIC ACIDS

The comparison of main bioactive phenolics identified in GSO by HPLC is depicted in Figure 4. The phenolics analysis made by HPLC can be seen by a typical chromatogram (Figure 5). As can be noted from data of Table 5, gallic acid (14.02 mg kg^{-1}) was found to be the major component followed by caffeic acid (8.42 mg kg^{-1}) in case of Folch extracted GSO. Whereas, Soxhlet extracted GSO showed the presence of caffeic acid (5.02 mg kg^{-1}) as major phenolic acid followed by gallic acid (3.70 mg kg^{-1}). Protocatechuic acid, 3,5 di-hydroxybenzoic, gentisoc and ellagic acids were also

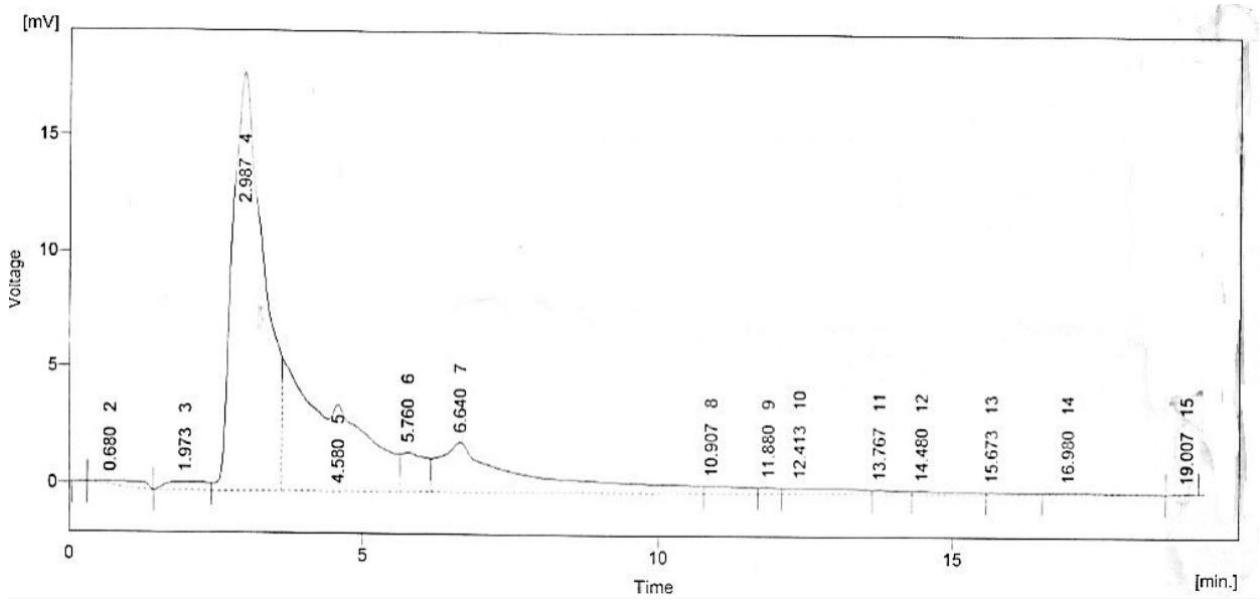

FIGURE 3. HPLC chromatogram showing the separation of tochopherols in Soxhlet extracted GSO

detected in higher amount in case of Folch extraction method. On the other side, Folch extracted GSO did not show the presence of chlorogenic acid and 4-hydroxy benzoic acid. Overall, considerable variations can be noted in the phenolics profiling of GSO as function of two extraction methods employed in this study.

These quantitative variations may be due to the effect of extraction solvents and techniques. Functional compounds present in seed oils may be decomposed due to accelerated and prolonged heating during Soxhelt extraction (Bozan & Temelli 2002). On the contrary, Folch extraction technique is a solvent based cold technique involving no accelerated temperature. Nature of the solvent also affects the quantity of antioxidants e.g., methanol is universally accepted organic solvent especially for the extraction of antioxidants. In Folch extraction method, a combination of methanol and chloroform was employed as extraction solvent, therefore, this method provides/extracted higher quantity of phenolics as compared to Soxhlet technique. The data in Table 5 showed that the Folch technique is obviously more appropriate extraction technique as compared to Soxhlet extraction especially for extraction of antioxidants enriched oil.

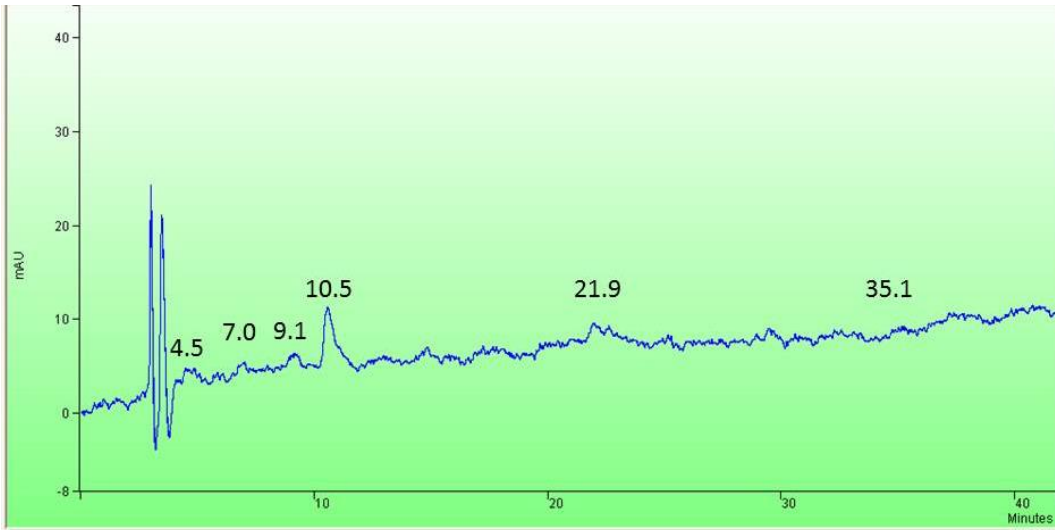

FIGURE 4. A typical HPLC chromatogram showing the separation of phenolics in Soxhlet extracted GSO

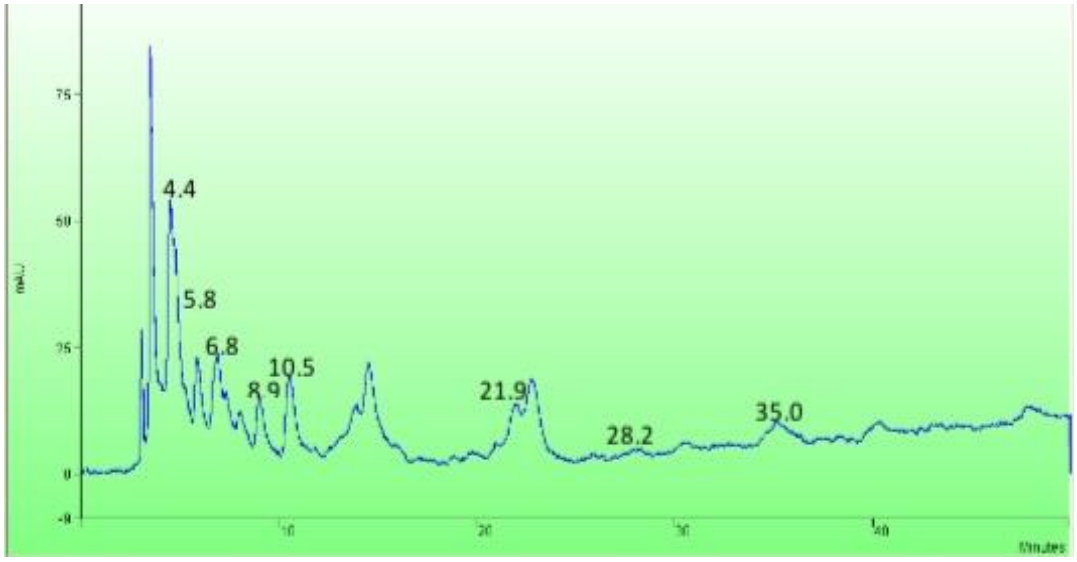

FIGURE 5. A typical HPLC chromatogram showing the separation of phenolics in Folch extracted GSO

TABLE 5. Comparison of phenolic acid composition between Soxhlet and Folch extracted grape seed oil

Phenolic acids (mg kg^{-1})	Soxhlet extracted oil	Folch extracted oil
Gallic acid	3.70 ± 0.15	14.02 ± 0.75
Chlorogenic acid	1.10 ± 0.03	ND
4-Hydroxy benzoic acid	2.20 ± 0.11	ND
Caffeic acid	5.20 ± 0.24	8.42 ± 0.65
Ellagic acid	1.50 ± 0.05	3.50 ± 0.17
Protocatechuic acid	ND	6.61 ± 0.25
Gentisic acid	ND	4.7 ± 0.18
3,5 di-hydroxybenzoic acid	ND	6.12 ± 0.35

Values are means ± standard deviations for triplicate determinations
ND: not detected

CONCLUSION

The results of this comparative study demonstrated that Folch method is relatively more suitable for extraction of higher yield and better quality of GSO in terms of oxidation state, contents of other valuable nutrients such as antioxidants (phenolics and tocopherols) and linoleic acid. Consequently, this method can be recommended for extraction of good quality oil from under-utilized grape seeds.

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