JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Oil Obtained from Grape Seeds Collected during Berry Development

MANUELA RUBIO, MANUEL ALVAREZ-ORTÍ, ANDRÉS ALVARRUIZ, ENRIQUE FERNÁNDEZ, AND JOSE E. PARDO*

Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071-Albacete, Spain

The surpluses of the wine industry that originate from wine production in the European countries must be reduced. Green harvesting, consisting in collecting the grapes when they are still green, could contribute to the reduction of the yield of vineyards. The green grapes are not suitable for wine production, but they can be used for seed oil extraction. Grape seed oil is a linoleic acid rich oil that has been suggested as an alternative to traditional vegetable edible oils. In this work, grape samples were collected at different stages during berry development for seed oil extraction. The grapes collected at a very early stage showed a very low oil extraction yield compared with that of the samples collected at later stages. The oil from the grapes collected at an early stage had considerably higher sterols content and had a significantly different fatty acid composition compared with those of the oil extracted from grapes collected at later stages. However, the oil samples from grapes collected before veraison did not show significant differences with samples collected after veraison as regards oil extraction yield, fatty acids composition, and total sterol content and composition. Our data suggest that grapes collected from green harvesting near veraison could be suitable for seed oil extraction, with characteristics similar to those of the oil extracted from the seeds of mature grapes.

KEYWORDS: Berry development; grape seed oil; veraison

INTRODUCTION

The wine sector in the European Union is immersed in a reform period dealing with the large production of wine in the European countries, which originates a surplus that is economically detrimental. One of the key objectives of the E.U. regulations on the common organization of the market in wine is to rebalance the market by reducing the wine production (*1*). The measures proposed by the E.U. include the grubbing up of vines in order to reduce the wine surplus. But European wines are often associated with the beatiful landscapes where they are grown, which form part of the cultural heritage of the wine producing regions. In addition, we cannot forget the benefit of a vegetal cover for the environment. In some particularly vulnerable regions, grubbing up vineyards can pose a threat to the environment, and should therefore be limited or even excluded.

An alternative to grubbing up for reducing wine production is green harvesting. Green harvesting is a modern technique that consists of collecting the grapes when they are still green in order to reduce the yield of the vineyards. In addition, this technique allows the plant to focus its nutrients on the remaining grape bunches, which results in better quality wine production.

Grape berry development is divided into three major phases (*2*). Phase I is characterized by cell division and subsequent cell expansion, which originates a sigmoidal curve of growth. Phase II is defined as a lag phase in which cell expansion ceases and sugar begins to accumulate. Finally, veraison marks the beginning of the third major phase (phase III) in which berries undergo a second period of sigmoidal growth due to additional mesocarp cell expansion. In this phase, accumulation of anthocyanin pigments for berry color, accumulation of volatile compounds for aroma, softening, peak accumulation of sugars, and a decline in organic acid accumulation is produced (*3*).

The green grapes are not suitable for wine production, but a benefit can be obtained by the extraction of oil from the grape seeds. They contain 6−20 wt % of oil, which is usually extracted with solvents from the grape pomace produced after pressing in the wine producing process (*2*−*8*). Actually, grape seed oil is produced as a specialty byproduct of wine manufacture. The virgin grape seed oil is characterized by a pleasant vinous and fruity aroma, which also reminds one of raisins if high quality raw material is used for the production, and high digestibility and a slight increase in viscosity when used for batch frying (*9, 10*).The high-quality virgin grape seed oil has been considered a valuable addition to the market of virgin edible vegetable oils (*9*).

Grape seed oil is rich in unsaturated fatty acids and vitamin E compounds, and has low values of cholesterol, which make

* To whom correspondence should be addressed. E-mail: Jose.PGonzalez@uclm.es.

10.1021/jf803627t CCC: $40.75
Published on Web 03/03/2009

it an alternative to traditionally used edible vegetable oils for its health benefits (*5*, *11*).

To our knowledge, there are no data related to the characteristics of oil extracted from seeds of immature grapes. In this work, the characteristics of the oil from seeds collected at different ripening times (before and after veraison) are evaluated in order to check if seeds from green grapes collected before veraison are suitable to produce a good quality oil.

MATERIAL AND METHODS

Plant Material and Experimental Design. The variety used was the Tempranillo or Cencibel (*V. vinifera* L.) grafted on 41 B rootstock. This is the most used red variety in Castilla-La Mancha (Spain). The grapes come from an experimental field in the Esuela Tecnica Superior de Ingenieros Agrónomos de Albacete, where a wide collection of national and foreign varieties is maintained.

Harvesting and Seed Collection. Harvesting was done in 2007, at four different times. The first one was before the beginning of ripening, with the grapes totally green (4 weeks after anthesis), the second was in the moments previous to veraison, with the grapes still green (8 weeks after anthesis), and the other two during the ripening process after veraison (12 and 16 weeks after anthesis). Four replicates were made for each sample. Harvesting was done manually, randomly collecting grape bunches from different vines. One hundred grapes were selected from the different samples to determine the weight and diameter. Seed collection was performed manually. Collection was done to obtain 5 kg, necessary to make the analytical determinations.

Extraction of Grape Seed Oil. The grape seeds were dried at room temperature (20 °C for 7 days). They were extended over a blotting paper and turned regularly to obtain uniform drying. Once dried, the seeds were ground with a hammermill to get a granulometry of 2 mm. Then they were mixed and homogenized. At this point, the moisture was measured according to UNE 55-031-73, using a dissication oven with forced ventilation.

The total fat content was measured in a Soxhlet extractor, using hexane as solvent (Panreac). In a first step, the seeds were extracted for 6 h. After that, the seeds were ground once more and subjected to another 6 h extraction cycle, to ensure complete oil extraction. The yield of the oil extraction was estimated in percent of dry weight.

Analytical Determinations. The analytical determinations made were acidity, fatty acid composition, and aliphatic alcohol and sterol composition. Determination of the regulated physicochemical quality parameters was carried out, following the analytical methods described by Regulation EEC/2568/1991 and EEC/1989/2003 of the Commission of the European Union (*12*, *13*). Free acidity, given as % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide ethanolic solution.

In order to determine fatty acid composition, a trans-esterification method with cold methanolic solution of potassium hydroxide was used (*14*). Methyl-esters were formed by vigorous shaking at room temperature for a few seconds of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potassium hydroxide solution. The methyl-esters were then analyzed by GC with a Hewlett-Packard (HP 6890) chromatograph equipped with a FID Detector. A fused silica column (50 m length × 0.25 mm i.d.), coated with SGL-1000 phase with 0.25 μm thickness (J & W Scientific Inc., Folsom, CA, USA), was used. Helium was employed as a carrier gas with a flow through the column of 1 mL/min. The temperatures of the injector and detector were set at 250 °C with an oven temperature of 210 °C. An injection volume of 1 μL was used (Regulation EEC 2568/1991, corresponding to AOCS method Ch 2−91) (*12*, *15*).

The sterol extract was prepared by saponification with potassium hydroxide in ethanolic solution, followed by extraction with diethyl ether and separation by chromatography on a basic silica gel plate according to the Methods of Analysis of the International Olive Oil Council (*16*). Sterols (%) were determined with a Hewlett-Packard (HP 6890) gas chromatograph with a capillary column (25 m length × 0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor). Helium was used as a carrier gas with a flow through the column of 1.2 mL/min. The temperature of the injector and detector were set at 280 and 290 °C, respectively, with an oven temperature of 260 °C. An injection volume of 1 μL was used (Regulation EEC 2568/1991, corresponding to AOCS method Ch 6−91) (*12*, *15*). Aliphatic alcohols were determined using the same methodology used for sterols. Analytical tests were performed at least in duplicate.

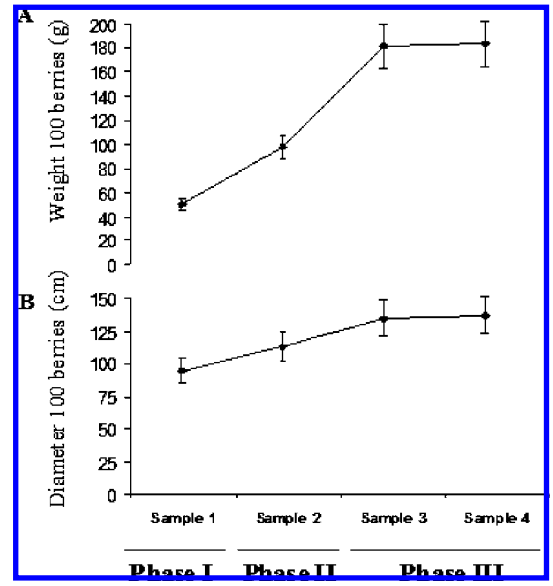

Figure 1. Evolution of berry weight (**A**) and berry diameter (**B**) during grape berry development. Diameter 100 berries and weight 100 berries refer to the sum of the measurement of 100 berries.

Statistical Analysis. Significant differences among samples were determined by an analysis of variance, which applied a Duncan test with a 95% significant level ($P < 0.05$), using the SPSS program, release 11.5 for Windows.

RESULTS AND DISCUSSION

Evolution of the Physicochemical Characteristics of the Grape Berry. Our grape samples were collected during berry development. Sample 1 (S1) was collected in phase I of development, during the first growth period. Sample 2 (S2) was collected at the end of phase II, a few days before veraison. Samples 3 and 4 (S3 and S4) were collected at different times in phase III, during the second period of sigmoidal growth. The evolution of diameter and weight of the berry is shown in **Figure 1A**, and **B**. A gradual increase in grape diameter and weight is produced during berry development until the end of ripening, when a stop in growth is observed. These data agree with the results obtained by other researchers about berry development in other grape varieties: Cabernet franc (*17*), Cabernet Sauvignon (*3*), and Chardonnay (*18*). Seed development follows a different pattern than berry development. Growth is linked to the increase in berry mass during phase I. The maximum seed weight is reached 1 week before veraison and then declines by about 16% until harvest (*17*).

Moisture and Oil Extraction Yield of the Grape Seed (Figure 2). The moisture of the grape seed decreased during the different developmental stages of the grape. At the beginning of development, when the grapes are still green, the seed moisture reached 11.0%. This percentage decreased over the development period until the final sample, collected when the grapes are totally mature, when the seed moisture values were 7.3% (**Figure 2**).

The oil extraction yield followed the opposite trend. In the first sample, it was very low (4.4%). The second sample, with the grapes in phase II of development previous to veraison, the oil extraction yield was considerably higher (15.1%) and

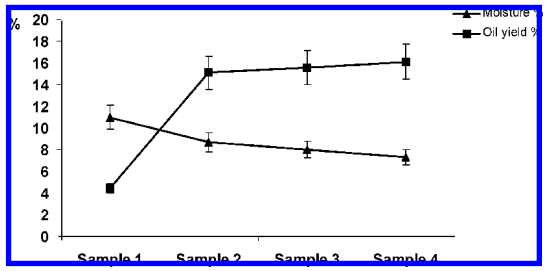

Figure 2. Evolution of moisture and oil yield of grape seed during grape berry development.

Table 1. Free Acidity (%)[a] and Fatty Acid (%) Composition of the Grape Seed Oil Samples[a]

	sample 1 (15-07-07)	sample 2 (06-08-07)	sample 3 (03-09-07)	sample 4 (26-09-07)
free acidity (%)	9.71 a	1.52 b	0.97 b	0.93 b
myristic $C_{14:0}$	0.06 a	0.04 b	0.04 b	0.04 b
palmitic $C_{16:0}$	9.33 a	8.52 b	8.49 b	8.36 b
palmitoleic $C_{16:1}$	0.12 a	0.11 ab	0.10 b	0.12 a
margaric $C_{17:0}$	0.09 a	0.07 b	0.07 b	0.07 b
margaroleic $C_{17:1}$	0.05 a	0.04 b	0.04 b	0.04 b
stearic $C_{18:0}$	3.26 b	4.18 a	4.11 a	4.36 a
oleic $C_{18:1}$	12.28 b	18.22 a	18.73 a	18.28 a
linoleic $C_{18:2}$	72.98 a	68.03 b	67.61 b	67.69 b
linolenic $C_{18:3}$	0.97 a	0.36 b	0.35 b	0.35 b
arachidic $C_{20:0}$	0.33 a	0.18 b	0.19 b	0.20 b
gadoleic $C_{20:1}$	0.13 b	0.15 a	0.16 a	0.16 a
behenic $C_{22:0}$	0.06 a	0.02 b	0.02 b	0.03 b
erucic $C_{22:1}$	0.13 a	0.02 b	0.02 b	0.02 b
lignoceric $C_{24:0}$	0.02 a	0.01 b	0.01 b	0.01 b

[a] Free acidity refers to the free fatty acids content, expressed as % of oleic acid. Different letters in the same line mean significant differences ($p < 0.05$) using the Duncan test.

remained high with no significant differences for the rest of the samples collected at different ripening stages. Our data suggest that the complete maturity of the berry is not necessary to obtain a good oil extraction yield, similar to the data obtained by other researchers (*5, 6, 11, 19*−*21*). Grapes collected from green harvesting are suitable to get an oil extraction yield similar to that collected when they are completely mature.

Analytical Determinations of Grape Seed Oil. Table 1 shows the free acidity and fatty acid composition of the grape seed oil samples. The free acidity decreased during the development of the grape, with very high values at the beginning (9.71% in S1). In S2, prepared from seed grapes collected prior to veraison, the free acidity decreased to a value of 1.52%, with no significant differences in the rest of the samples prepared from grapes collected in phase III of development. The free acidity values were all above the maximum level allowed by the Spanish health regulation for edible vegetable oils (*22*); nevertheless, this regulation applies to refined oils and not to crude oils as is our case.

The values for the different fatty acid contents were within the range required by Spanish regulations (*22, 23*) for grape seed oils (myristic ≤ 0,1%; palmitic = 5−10%; palmitoleic ≤ 1,2%; stearic = 3−5%; oleic = 12−26%; linoleic = 58−77%; arachidic ≤ 0,1%; linolenic ≤ 1%; gadoleic ≤ 1%) for all of the samples studied, except for arachidic acid, which was slightly higher in all samples, especially in S1. There are no data about margaric, margaroleic, behenic, erucic, and lignoceric acids.

Grape seed oil is rich in unsaturated fatty acids, which can amount to about 90% (*9*). The most abundant fatty acid is linoleic acid, with a percentage ranging from 72.98% in oils prepared from the seeds of green grapes (S1) to 67.61% in the oil samples prepared from the seeds of mature grapes. These values agree with those found in other research papers (*4*−*6, 9*).

Table 2. Sterol (%) and Aliphatic Alcohol (%) Composition of the Grape Seed Oil Samples[a]

	sample 1 (15-07-07)	sample 2 (06-08-07)	sample 3 (03-09-07)	sample 4 (26-09-07)
cholesterol	0.29 a	0.17 c	0.21 b	0.23 b
24-methylencholesterol	0.06 b	0.06 b	0.06 b	0.10 a
campesterol	10.79 b	9.41 b	9.43 b	9.28 b
campestanol	0.22 b	0.37 a	0.41 a	0.43 a
stigmasterol	11.87 b	15.65 a	16.11 a	16.03 a
Δ7-campesterol	0.38 c	1.54 a	0.58 b	1.11 a
clerosterol	1.02 b	1.14 ab	1.22 a	1.04 b
β-sitosterol	69.80 a	62.73 b	61.30 b	61.54 b
sitostanol	3.47 c	4.48 a	4.31 a	3.97 b
Δ5-avenasterol	1.12 c	1.86 a	1.17 c	1.56 b
Δ5,24-stigmastadienol	0.63 ab	0.56 b	0.78 a	0.66 ab
Δ7-stigmastenol	0.23 c	1.37 b	2.74 a	2.88 a
Δ7-avenasterol	0.09 c	0.65 b	1.13 a	1.14 a
apparent β-sitosterol[b]	76.04 a	70.77 b	68.78 b	68.77 b
total sterols ($mg \cdot kg^{-1}$)	18530 a	4052 b	3497 b	3686 b
aliphatic alcohols	185 a	45 c	81 b	82 b

[a] Different letters in the same line mean significant differences ($p < 0.05$) using the Duncan test. [b] Apparent β-sitosterol = Δ5,23-stigmastadienol + Clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol.

There were no significant differences in fatty acid composition in S2, S3, and S4, which means that fatty acid composition in seed oil prepared from grapes collected prior to veraison is similar to that in oils prepared from complete mature grapes.

Although differences were found between oils from S1 and the rest, the pattern of fatty acids of the oils from S1 ranged within the values obtained in other oils prepared from mature grapes (*4*−*6*). The only exception was a slightly lower percentage of oleic acid.

Other interesting compounds of vegetable oils are phytosterols. They are a group of steroid alcohols naturally occurring in plants. Their presence in plant oils is of increasing interest because of their role in the control of cholesterol level and, by implication, reduction of arteriosclerosis (*5*).

Phytosterols have been found in a broad range in grape seed oil ranging from 2,580 to 11,250 mg/kg (*6, 9, 10*). **Table 2** shows the sterol composition in the different grape seed oil samples studied. In S1, the total sterol composition reached very high levels (18,530 mg/kg). In S2, the sterol composition ranged in values similar to that obtained by other researchers, and it is maintained with no significant differences in S3 and S4 prepared from grapes collected during the ripening process.

Although the information about sterols in grape seed oil is scarce, campesterol, stigmasterol, β-sitosterol, and Δ^5-avenasterol are common findings. The β-sitosterol has been reported to be the most abundant phytosterol (*5*). In the samples of grape seed oil studied, the main sterols were β-sitosterol, stigmasterol, and campesterol, which did not show significant differences in S2, S3, and S4. Other sterols appeared in small quantities (**Table 2**). The levels of cholesterol were very low in all samples, below the 0.5% level allowed by the regulations (*22*).

In conclusion, in the samples of oil studied, no significant differences were found in fatty acid composition or phytosterol total content and composition when oil was extracted from seeds of grapes collected before veraison and during phase III of grape development (S2, S3, and S4). In addition, the oil extraction yield was very similar in these samples. Our data suggest that seeds from grapes collected before veraison can be suitable for oil extraction, with similar characteristics compared to those obtained from mature grapes.

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Received for review November 20, 2008. Revised manuscript received February 5, 2009. Accepted February 13, 2009.

JF803627T