



## Characterization of grape seed oil from wines with protected denomination of origin (PDO) from Spain

J.C. Bada<sup>a</sup>, M. León-Camacho<sup>b</sup>, P. Copovi<sup>a</sup> and L. Alonso<sup>a,✉</sup>

<sup>a</sup>Instituto de Productos Lácteos de Asturias-IPLA-CSIC. Paseo Río Linares s/n 33300 Villaviciosa. Asturias, Spain

<sup>b</sup>Instituto de la Grasa-CSIC. Pablo Olavide University Campus- Bldg. 46 41013 Seville, Spain

✉Corresponding author: [lalonso@ipla.csic.es](mailto:lalonso@ipla.csic.es)

Submitted: 09 October 2014; Accepted: 10 February 2015

**SUMMARY:** The aim of this study was to determine the composition and characteristics of red grape seed oils (*Vitis vinifera* L) from wines with protected denomination of origin (PDO) from Spain. Eight representative varieties of grape seed oils from the Spanish wine Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) and Cangas (Mencia, Carrasquín, Albarín and Verdejo) were studied. The oil content of the seeds ranged from 13.89 to 10.18%, and the moisture was similar for all the seeds. Linoleic acid was the most abundant fatty acid in all samples, representing around 78%, followed by oleic acid with a concentration close 16%, the degree of unsaturation in the grape seed oil was over 90%.  $\beta$ -sitosterol and  $\alpha$ -tocopherol were the main sterol and tocopherol, reaching values of 77.31% and 3.82 mg·100 g<sup>-1</sup> of oil, respectively. In relation to the tocotrienols,  $\alpha$ -tocotrienol was the main tocotrienol and accounted for 13.18 mg·100 g<sup>-1</sup> of oil.

**KEYWORDS:** Fatty acids; Grape; Oil; Seed; Sterols; Tocopherol; Tocotrienols

**RESUMEN:** *Caracterización de aceites de semillas de uvas de vinos con denominación de origen protegida (DOP) de España.* El objetivo de este estudio consistió en determinar la composición y características de aceites de semillas de uvas rojas (*Vitis vinifera* L) de vinos con denominación de origen protegida (DOP) de España. Ocho variedades representativas de aceites de semillas de uvas españolas Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) y Cangas (Mencia, Carrasquín, Albarín y Verdejo) fueron estudiadas. Los contenidos en aceite de las semillas oscilaron entre 13.89 y 10.18%, la humedad fue similar para todas las semillas. El contenido en ácido linoléico fue alto en todos los aceites alcanzando un valor del 78%, seguido del ácido oléico con una concentración cercana al 16%, registrando un grado total de insaturación del 90%.  $\beta$ -sitosterol y  $\alpha$ -tocoferol fue el principal esteroles y tocoferol, alcanzado niveles del 77.31% y de un 3.82 mg·100 g<sup>-1</sup> de aceite respectivamente. En relación a los tocotrienoles,  $\alpha$ -tocotrienol fue el mayoritario con un contenido de un 13.18 mg·100 g<sup>-1</sup> de aceite.

**PALABRAS CLAVE:** Aceite; Ácidos grasos; Esteroles; Semillas; Tocoferoles; Tocotrienoles; Uva

**Citation/Cómo citar este artículo:** Bada JC, León-Camacho M, Copovi P, Alonso L. 2015. Characterization of grape seed oil from wines with protected denomination of origin (PDO) from Spain. *Grasas Aceites* 66 (3): e085. doi: <http://dx.doi.org/10.3989/gya.1063142>.

**Copyright:** © 2015 CSIC. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial (by-nc) Spain 3.0 Licence.

## 1. INTRODUCTION

Grapes are one of the fruit crops grown extensively in many areas of the world and 46% of the fresh grapes produced are destined for wine production (Anonymous, 1999). During wine making, large quantities of pomace are produced as a by-product, which is not normally economically utilized except locally. However, the use of pomace in the food industry can create opportunities to lower production costs and to create a new source for human consumption. The pomace accounts for about 20% of the grape and the grape seeds contain about 40–60% w/w pomace. In general, grape seeds are not used to produce oil in Spain. So, grape seeds are often referred to as a significant agricultural and industrial waste (Freitas *et al.*, 2008; Kin *et al.*, 2008; Lutterodt *et al.*, 2011). Thus, finding feasible solutions for treating this residue, including attempts to develop new products, would constitute excellent opportunities.

From the nutritional and therapeutic point of view, grape seed oil has a high linoleic acid content (important for prostaglandin synthesis, which has an influence on platelet aggregation and inflammatory processes), high vitamin E content (helps to reduce the risk of suffering arteriosclerosis) and low values of cholesterol and therefore recognized as beneficial to prevent heart and circulation problems (Oomah *et al.*, 1998; Martinez *et al.*, 1986; Sineiro *et al.*, 1995). Thus, grape seed oils have emerged as a product with potential to be used in pharmaceutical and food applications (Bail *et al.*, 2008; Furiga *et al.*, 2009).

Spain is one of the most prominent countries of the European Union in the production of wine (Agriview, 2008). The production of wine with Protected Denomination of Origin (PDO) in Spain is about 12 million hls per year. The PDO Rioja is the most important region for the production of wine with 2.50 million hls, followed by Cangas (four varieties) with 730 hls, Toro with 0.86 hls, Valencia with 0.645 hls and Ribera de Duero with 0.5 hls. In this work, the seed oils of eight Spanish red wines with PDP were studied. Their contents in oil, protein, moisture, fatty acids, sterols, tocopherols and tocotrienols were characterized.

## 2. MATERIALS AND METHODS

### 2.1. Samples

The seeds from eight wines with PDO produced by five Spanish wineries from the area of Ribera del Duero (Valladolid), Toro (Zamora), Rioja (Logroño), Valencia, and Cangas de Narcea (Asturias) were used in this study (Table 1). The pomace was supplied from those wineries and after drying at 40 °C, the seeds were separated from the pulp and skin

using different sized sieves. The seeds were ground in a Fritsch Pulverisette equipped with a stainless steel rotor and 1 mm sieve ring.

### 2.2. Oil extraction, protein and moisture content

Samples were blanched and ground in an electric grinder. The oil was extracted in a Soxhlet glass apparatus using hexane as solvent (IUPAC, 1987a). Proteins were determined by the method Kjeldahl (Tecator, 2002) using a “Kjeldatherm Block Digestion Systems” (Gerhardt, UK Ltd) “Vapodest Rapad Distillation Systems” (Gerhardt, UK Ltd). Moisture was determined by weight loss after heating in an oven at 100 °C over night in accordance with (IUPAC, 1987b).

### 2.3. Fatty acid analysis

Fatty acid methyl esters (FAME) were analyzed by gas chromatography (GC). FAME were extracted with n-heptane after cold methylation with 2N KOH in methanol (IUPAC, 1987c). FAME analysis was performed on an HP-5890-II apparatus (Hewlett-Packard, Palo Alto, CA) using a fused silica capillary SP-2380 column (60 m×0.25 mm, 0.2 µm film thickness) with a flame ionization detector (FID). The oven temperature was kept at 160 °C for 13 min and was then raised to 190 °C at a rate of 1.5 °C·min<sup>-1</sup> and held isothermally for 20 min. The injector temperature was kept at 225 °C, while the detector temperature was 250 °C. Hydrogen (19 psi inlet pressure) was used as carrier gas, while the make-up gas was nitrogen. Standards of each fatty acid were used to identify the fatty acids. These were purchased from Sigma-Aldrich (St. Louis, MO): palmitic (P=C16:0), palmitoleic (Po=C16:1), stearic (S=C18:0), oleic (O=C18:1 ω9 *cis*), linoleic (L=C18:2) alpha and gamma, linolenic (Lo=C18:3). Fatty acids were identified in the samples by comparing retention times for standards and samples. The area was expressed as percentages of areas of the total fatty acids.

### 2.4. Sterol analysis

The unsaponifiable fraction was extracted as described (European Communities 1991). A 0.5 mL 5-α-cholestanol (Fluka, Buchs, Switzerland) solution in chloroform was added to 5 g of oil as an internal standard. The mixture was saponified for 0.5 hour with 50 mL of 2N ethanolic potassium hydroxide. The solution was then passed to a 500 mL decanting funnel, 100 mL distilled water were added and the mixture was extracted twice with three 80 mL portions of diethyl ether. The diethyl ether extracts were combined in another funnel and were washed several times with 100 mL portions of water, until the wash reached neutral pH.

The diethyl ether solution was dried over anhydrous sodium sulphate and evaporated to dryness in a rotary evaporator at 30 °C under reduced pressure. After purification by thin-layer chromatography (IUPAC, 1987d), the sterol fraction was analyzed and quantified by gas chromatography in an HP 5890-II apparatus equipped with a split-splitless injector and a flame ionization detector. An HP-5 fused silica capillary column (30 m×0.32 mm i.d., 0.25 µm film thickness) was used, with hydrogen (7 psi inlet pressure) as the carrier gas and nitrogen the make-up gas. The oven temperature was held isothermally at 265 °C for 30 min. The injector temperature was 280 °C, while the detector was kept at 300 °C. Previously, the sterol fraction was derived as trimethylsilyl ethers (TMS) according to the method proposed in (European Communities,

1991). Sterols were identified by comparison of the mass spectral data with those of authentic reference compounds and by comparing their retention times with sterols from olive, sunflower and soybean oils (León-Camacho and Morales, 2000).

## 2.5. Tocopherol and tocotrienol analysis

Tocopherols and tocotrienols were quantified by high performance liquid chromatography (HPLC). The HPLC system consisted of a low pressure quaternary pump HP-1050, a Rheodyne injection valve (20 µL loop), a thermostatic furnace and a fluorescence detector RF-235 (Shimadzu, Kyoto, Japan). Separation was performed in a 250×4 mm particle size 5 µm Lichrospher Si-60 (Merck, Darmstadt, Germany) column. The column and detector were

TABLE 1. Geographical parameters of the different varieties of grape seeds

| Origin           | Level of the sea (m) | Latitude       | Length        | Annual rain (mm) | Annual temperature (°C) |
|------------------|----------------------|----------------|---------------|------------------|-------------------------|
| Ribera del Duero | 694                  | 41°39'13"32" N | 4°43'39"78" O | 450              | 2–38                    |
| Toro             | 730                  | 41°31'30"46" N | 5°23'28"49" O | 350–400          | 11–37                   |
| Rioja            | 392                  | 42°27'10"43" N | 2°27'13"57" O | 400              | 5–21                    |
| Valencia         | 17                   | 39°28'12"86" N | 0°22'36"50" O | 586              | 6–25                    |
| Cangas           | 383                  | 43°10'37"41" N | 6°32'56"86" O | 900              | 10–30                   |

TABLE 2. Oil (g·100 g<sup>-1</sup>seed), protein (g·100 g<sup>-1</sup> seed) and moisture (g·100 g<sup>-1</sup> seed) from the different varieties of grape seeds

| Origin   | Variety     | Oil amount              | Protein                 | Moisture                |
|----------|-------------|-------------------------|-------------------------|-------------------------|
| Ribera   | Tempranillo | 12.82±0.40 <sup>a</sup> | 10.18±0.85 <sup>b</sup> | 10.38±0.14 <sup>b</sup> |
| Toro     | Tempranillo | 7.57±0.39 <sup>ab</sup> | 9.53±0.81 <sup>b</sup>  | 11.21±0.13 <sup>a</sup> |
| Rioja    | Garnacha    | 9.57±0.52 <sup>ab</sup> | 10.84±1.11 <sup>b</sup> | 11.36±0.13 <sup>a</sup> |
| Valencia | Tempranillo | 12.09±0.48 <sup>b</sup> | 8.12±0.80 <sup>c</sup>  | 14.06±0.15 <sup>a</sup> |
| Cangas   | Mencia      | 13.29±0.49 <sup>a</sup> | 8.91±0.66 <sup>c</sup>  | 11.18±0.18 <sup>b</sup> |
|          | Carrasquín  | 10.39±0.45 <sup>b</sup> | 9.28±0.56 <sup>ab</sup> | 13.20±0.12 <sup>a</sup> |
|          | Albarin     | 13.89±0.35 <sup>a</sup> | 8.78±0.65 <sup>c</sup>  | 11.16±0.15 <sup>b</sup> |
|          | Verdejo     | 9.48±0.33 <sup>b</sup>  | 9.51±0.50 <sup>b</sup>  | 11.37±0.16 <sup>a</sup> |

<sup>a,b,c</sup>Different letters in the same line mean significant differences ( $p \leq 0.05$ ).

TABLE 3. Fatty acid composition (%) of lipid fractions extracted from grape seed oils

| Origin   | Variety     | C16:0                   | C16:1                   | C18:0                   | C18:1                    | C18:2                   | C18:3                   | C20:0                  | C20:1                  |
|----------|-------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|------------------------|------------------------|
| Ribera   | Tempranillo | 7.15±0.14 <sup>a</sup>  | 0.08±0.01 <sup>a</sup>  | 3.40±0.08 <sup>c</sup>  | 14.03±0.16 <sup>ab</sup> | 73.57±1.12 <sup>a</sup> | 0.43±0.05 <sup>b</sup>  | 0.16±0.02 <sup>b</sup> | 0.16±0.02 <sup>a</sup> |
| Toro     | Tempranillo | 7.58±0.16 <sup>a</sup>  | 0.10±0.02 <sup>a</sup>  | 3.36±0.09 <sup>c</sup>  | 14.20±0.1 <sup>ab</sup>  | 72.13±1.15 <sup>a</sup> | 0.50±0.06 <sup>ab</sup> | 0.18±0.03 <sup>b</sup> | 0.16±0.03 <sup>a</sup> |
| Rioja    | Garnacha    | 6.99±0.13 <sup>ab</sup> | 0.10±0.0 <sup>a</sup>   | 3.61±0.08 <sup>c</sup>  | 15.54±0.18 <sup>a</sup>  | 71.40±1.05 <sup>a</sup> | 0.52±0.04 <sup>ab</sup> | 0.17±0.04 <sup>b</sup> | 0.17±0.02 <sup>a</sup> |
| Valencia | Tempranillo | 7.19±0.18 <sup>a</sup>  | 0.08±0.03 <sup>a</sup>  | 5.61±0.06 <sup>a</sup>  | 15.99±0.16 <sup>a</sup>  | 68.67±1.06 <sup>b</sup> | 0.37±0.04 <sup>c</sup>  | 0.23±0.03 <sup>a</sup> | 0.15±0.01 <sup>a</sup> |
| Cangas   | Mencia      | 5.48±0.12 <sup>b</sup>  | 0.06±0.01 <sup>ab</sup> | 4.64±0.07 <sup>ab</sup> | 12.26±0.12 <sup>ab</sup> | 75.70±1.15 <sup>a</sup> | 0.42±0.06 <sup>b</sup>  | 0.18±0.02 <sup>b</sup> | 0.15±0.04 <sup>a</sup> |
|          | Carrasquín  | 5.76±0.14 <sup>b</sup>  | 0.06±0.01 <sup>ab</sup> | 4.08±0.08 <sup>c</sup>  | 12.37±0.11 <sup>ab</sup> | 78.23±1.19 <sup>a</sup> | 0.46±0.05 <sup>b</sup>  | 0.17±0.03 <sup>b</sup> | 0.14±0.03 <sup>a</sup> |
|          | Albarin     | 5.54±0.11 <sup>b</sup>  | 0.03±0.01 <sup>b</sup>  | 3.81±0.06 <sup>c</sup>  | 13.51±0.14               | 72.58±1.11              | 0.45±0.04 <sup>b</sup>  | 0.15±0.04 <sup>b</sup> | 0.15±0.02 <sup>a</sup> |
|          | Verdejo     | 6.05±0.15 <sup>b</sup>  | 0.08±0.02 <sup>a</sup>  | 4.58±0.07 <sup>a</sup>  | 10.78±0.12 <sup>b</sup>  | 76.51±1.15 <sup>a</sup> | 0.64±0.06 <sup>a</sup>  | 0.20±0.03 <sup>a</sup> | 0.15±0.01 <sup>a</sup> |

<sup>a,b,c</sup>Different letters in the same line mean significant differences ( $p \leq 0.05$ ).

kept at a temperature of 40 °C. The mobile phase was n-hexane/2-propanol 99/1 (v/v). The flow rate was set at 1 mL·min<sup>-1</sup> isocratic elution. Quantification was carried out by a calibration system based on standards (IUPAC, 1987d).

## 2.6. Statistic analysis

Data are presented as mean value ± standard deviation of three samples of each cultivar, analyzed individually in duplicate. The data were treated by analysis of variance (ANOVA) using statistical software SAS (version 8.02, SAS Institute Inc., Cary, NC, USA). Differences among treatments were determined by statistical analysis using a Student *t* test where ( $p < 0.05$ ) was considered statistically significant.

## 3. RESULTS AND DISCUSSION

The oil content, protein and moisture from grape seeds from Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) and Cangas (Mencia, Carrasquín, Albarín and Verdejo) are shown in Table 2. The highest content in oil corresponded to Cangas (Albarín, Mencia) at 13.89±0.35% and 13.29±0.49% followed by Ribera (Tempranillo) at 12.82±0.40% and Rioja at 9.57 (Garnacha)±0.525%. Protein showed the highest content for Rioja (Garnacha) 10.84±1.11% and Ribera (Tempranillo) 10.18±0.85%. Moisture was quite similar for all the seeds showing the highest content for Valencia (Tempranillo) 14±0.15%. The values found are higher for oil content than those values found by Mironeasa *et al.* (2010) in a study of the physical, chemical, structural characteristics and oil content of Rumanian grape seed oils. The fatty acid composition of seeds in Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) and Cangas (Mencia, Carrasquín, Albarín and Verdejo) is shown in Table 3. All the grape seed oils had high amounts of polyunsaturated fatty acids, mainly linoleic acid. Cangas (Carrasquín) seed oil had the highest amount of linoleic acid at 78.23±1.19%, followed by Cangas (Verdejo) at 76.51±1.15%. The lowest value corresponded to Valencia (Tempranillo) seed oil at 68.67±1.06%. These values were similar to those reported by (Baydar *et al.*, 2007; Pardo *et al.*, 2009) in a study of the characterization of grape seeds and pomace oil extract from Turkey of for the characterization of grape seed oil from different grape varieties (*Vitis vinifera*). These results were also similar to those reported by other authors (Tangolar *et al.*, 2009) that referred to linoleic acid as the most abundant fatty acid in grape seed oils with values between 62.5 and 69.24% for seed oils for Alicante Bouschet and Muscat of Hamburg varieties, respectively. The high content of linoleic acid in grape seed oil is particularly interesting since linoleic acid is the major

TABLE 4. Sterol contents (mg·100 g<sup>-1</sup> of oil) of lipid fractions extracted from grape seed oils

| Origen   | Variety     | Cholesterol            | Campesterol             | Stigmasterol             | Cholesterol             | β-Sitosterol             | Sitotanol               | Δ5-Avenasterol          | Δ5,24-Stigmastadienol   | Δ7-Stigmastenol         |
|----------|-------------|------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Ribera   | Tempranillo | 0.34±0.06 <sup>a</sup> | 9.62±0.09 <sup>b</sup>  | 17.65±0.35 <sup>a</sup>  | 0.35±0.07 <sup>a</sup>  | 60.15±1.85 <sup>b</sup>  | 0.72±0.32 <sup>a</sup>  | 1.77±0.15 <sup>a</sup>  | 1.16±0.12 <sup>a</sup>  | 2.79±0.21 <sup>a</sup>  |
| Toro     | Tempranillo | 0.31±0.04 <sup>a</sup> | 9.22±0.08 <sup>b</sup>  | 15.28±0.28 <sup>a</sup>  | 0.40±0.06 <sup>a</sup>  | 63.99±1.96 <sup>b</sup>  | 0.11±0.29 <sup>c</sup>  | 1.44±0.13 <sup>ab</sup> | 0.96±0.08 <sup>a</sup>  | 3.17±0.29 <sup>a</sup>  |
| Rioja    | Garnacha    | 0.17±0.03 <sup>b</sup> | 10.40±1.12 <sup>a</sup> | 14.42±0.31 <sup>a</sup>  | 0.26±0.04 <sup>ab</sup> | 70.54±1.85 <sup>ab</sup> | 0.24±0.21 <sup>c</sup>  | 1.17±0.16 <sup>b</sup>  | 0.70±0.09 <sup>a</sup>  | 2.24±0.18 <sup>ab</sup> |
| Valencia | Tempranillo | 0.27±0.05 <sup>a</sup> | 11.01±0.08 <sup>a</sup> | 11.82±0.24 <sup>ab</sup> | 0.32±0.06 <sup>a</sup>  | 74.15±1.66 <sup>a</sup>  | 0.59±0.14 <sup>a</sup>  | 0.56±0.06 <sup>b</sup>  | 0.48±0.06 <sup>b</sup>  | 1.53±0.22 <sup>c</sup>  |
| Cangas   | Mencia      | 0.15±0.06 <sup>b</sup> | 10.27±0.09 <sup>a</sup> | 12.74±0.26 <sup>a</sup>  | 0.16±0.05 <sup>b</sup>  | 72.64±1.74 <sup>ab</sup> | 0.36±0.19 <sup>ab</sup> | 1.10±0.05 <sup>b</sup>  | 0.62±0.07 <sup>ab</sup> | 2.06±0.13 <sup>ab</sup> |
|          | Carrasquín  | 0.27±0.07 <sup>a</sup> | 10.68±1.12 <sup>a</sup> | 12.91±0.21 <sup>a</sup>  | 0.27±0.06 <sup>ab</sup> | 70.64±1.84 <sup>ab</sup> | 0.42±0.20 <sup>b</sup>  | 1.84±0.06 <sup>a</sup>  | 0.58±0.07 <sup>b</sup>  | 2.38±0.28 <sup>ab</sup> |
|          | Albarín     | 0.24±0.05 <sup>a</sup> | 9.77±0.09 <sup>b</sup>  | 10.58±0.19 <sup>ab</sup> | 0.29±0.05 <sup>ab</sup> | 77.31±1.96 <sup>a</sup>  | 0.31±0.13 <sup>ab</sup> | 0.48±0.03 <sup>c</sup>  | 0.34±0.04 <sup>c</sup>  | 1.11±0.19 <sup>c</sup>  |
|          | Verdejo     | 0.15±0.04 <sup>b</sup> | 10.37±1.10 <sup>a</sup> | 9.60±0.18 <sup>c</sup>   | 0.24±0.06 <sup>ab</sup> | 74.02±1.91 <sup>a</sup>  | 0.48±0.16 <sup>ab</sup> | 1.71±0.07 <sup>a</sup>  | 0.83±0.11 <sup>a</sup>  | 2.68±0.25 <sup>a</sup>  |

<sup>a,b,c</sup>Different letters in the same line mean significant differences ( $p \leq 0.05$ ).



TABLE 5. Tocopherol and tocotrienol contents (mg·100 g<sup>-1</sup> of oil) of lipid fractions extracted from grape sample seed oils

| Origin   | Variety     | $\alpha_{TF}$           | $\beta_{TF}$           | $\gamma_{TF}$          | $\Delta_{TF}$          | $\alpha_{T3}$           | $\beta_{T3}$            | $\gamma_{T3}$           | $\delta_{T3}$          |
|----------|-------------|-------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Ribera   | Tempranillo | 2.38±0.12 <sup>b</sup>  | 0.03±0.01 <sup>c</sup> | 0.34±0.06 <sup>b</sup> | 0.02±0.01 <sup>c</sup> | 13.18±0.06 <sup>a</sup> | 0.13±0.02 <sup>ab</sup> | 10.70±0.35 <sup>a</sup> | 0.22±0.01 <sup>b</sup> |
| Toro     | Tempranillo | 3.82±0.13 <sup>a</sup>  | 0.06±0.01 <sup>b</sup> | 0.59±0.04 <sup>b</sup> | 0.09±0.02 <sup>a</sup> | 8.34±0.10 <sup>b</sup>  | 0.13±0.03 <sup>ab</sup> | 9.8±0.11 <sup>ab</sup>  | 0.20±0.02 <sup>b</sup> |
| Rioja    | Garnacha    | 2.26±0.14 <sup>ab</sup> | 0.05±0.01 <sup>b</sup> | 0.36±0.05 <sup>b</sup> | 0.05±0.01 <sup>b</sup> | 8.53±0.05 <sup>b</sup>  | 0.14±0.04 <sup>ab</sup> | 8.0±0.06 <sup>b</sup>   | 0.17±0.03 <sup>b</sup> |
| Valencia | Tempranillo | 2.15±0.11 <sup>b</sup>  | 0.06±0.02 <sup>b</sup> | 0.41±0.02              | 0.03±0.01 <sup>c</sup> | 3.38±0.01 <sup>c</sup>  | 0.11±0.05 <sup>b</sup>  | 7.0±0.04 <sup>b</sup>   | 0.16±0.04              |
| Cangas   | Mencia      | 3.69±0.13 <sup>a</sup>  | 0.10±0.01 <sup>a</sup> | 1.49±0.04 <sup>a</sup> | 0.09±0.02 <sup>a</sup> | 9.23±0.01 <sup>b</sup>  | 0.21±0.03 <sup>a</sup>  | 10.18±0.06 <sup>a</sup> | 0.39±0.03 <sup>a</sup> |
|          | Carrasquín  | 3.23±0.15 <sup>a</sup>  | 0.05±0.01 <sup>b</sup> | 1.47±0.06 <sup>a</sup> | 0.06±0.01 <sup>c</sup> | 5.46±0.04 <sup>c</sup>  | 0.11±0.06 <sup>b</sup>  | 9.8±0.03 <sup>ab</sup>  | 0.27±0.04 <sup>a</sup> |
|          | Albarín     | 3.16±0.15 <sup>a</sup>  | 0.06±0.02 <sup>b</sup> | 1.59±0.04 <sup>a</sup> | 0.10±0.02 <sup>a</sup> | 7.32±0.11 <sup>ab</sup> | 0.17±0.03 <sup>a</sup>  | 10.16±0.17 <sup>a</sup> | 0.35±0.03 <sup>a</sup> |

<sup>a,b,c</sup>Different letters in the same line mean significant differences ( $p \leq 0.05$ ).

dietary fatty acid that regulates the low density lipoprotein LDL-cholesterol metabolism by means of regulating LDL-cholesterol production and enhancing its clearance (Wijendra and Hayes, 2004). 18:3n6 acid is present in a range of 0.64±0.06% in Cangas (Verdejo) to 0.52±0.04% in Rioja (Garnacha). This fatty acid was detected in the remaining seeds at low levels. Table 4 shows the content in sterols of Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) and Cangas (Mencia, Carrasquín, Albarín and Verdejo) seed oils.  $\beta$ -sitosterol was the major sterol in all the seeds. Cangas (Albarín) seed oil was the one with the highest content at 77.31±1.96% while Ribera (Tempranillo) seed oil was the one that presents the lowest values at 60.15±1.85%. These results are in line with those reported in the literature for a study on grape seed oils (Tiscornia *et al.*, 1973; Beveridge *et al.*, 2005) on the yield and composition of grape seed oils extract by supercritical carbon dioxide and petroleum ether and varietal effects with a content for  $\beta$ -sitosterol of 75±2.19%. The stigmaterol content in Ribera (Tempranillo) was worth noting at 17.65±0.35%, while Cangas (Verdejo) presented a content of 9.60±0.18%. With regard to  $\Delta$ -5 avenasterol, Ribera (Tempranillo) seed oil contained 1.77±0.15%, while Cangas (Albarín) had only 0.48±0.03%. Table 5 shows the content in tocopherols and tocotrienols in Ribera del Duero (Tempranillo), Toro (Tempranillo), Rioja (Garnacha), Valencia (Tempranillo) and Cangas (Mencia, Carrasquín, Albarín and Verdejo) seed oils. Toro (Tempranillo) presented the highest content in  $\alpha$ -tocopherol at 38.22 mg·100 g<sup>-1</sup> of oil and Valencia (Tempranillo) seed oil the lowest content at 21.5 mg·100 g<sup>-1</sup> of oil. The maximum value obtained for  $\alpha$ -tocopherol in the present work was higher than that described by Crew *et al.* (2006) for oil French grape seeds (229 mg·Kg<sup>-1</sup>). Lower amounts were determined by these authors in Italian (160 mg·Kg<sup>-1</sup>) and Spanish (75 mg·Kg<sup>-1</sup>) varieties.  $\gamma$ -tocopherol presented a content of 15.9 mg·100 g<sup>-1</sup> of oil in Cangas (Albarín) and 0.34±0.06 mg·100 g<sup>-1</sup> of oil in Ribera (Tempranillo). With regard to the content in tocotrienols,  $\alpha$ -tocotrienol was the greatest in Ribera

(Tempranillo) seed oil presenting the highest content at 13.18±0.06 mg·100 g<sup>-1</sup> of oil and  $\gamma$ -tocotrienol with 10.70±0.35 mg·100 g<sup>-1</sup> of oil.

In relation to the grapes of the same variety and different geographical origin, which is the case of the variety tempranillo, we observed significant differences for oil, protein, fatty acids, sterols, tocopherols and tocotrienols but we cannot conclude which variety is better than the others because if a variety presented higher levels for certain healthy compounds, the other one presented higher levels for another.

#### 4. CONCLUSIONS

On the basis of the present study, it may be concluded that the results of this work showed that the grape seed oils which are by-products of the pomace obtained after the wine making process can be used as an edible oil source because the nutritional properties possess a high added value. The composition in lipids of these seeds presents a high content in the polyunsaturated fatty acids C18:2 n3, sterols ( $\beta$ -sitosterol) and tocopherols ( $\gamma$ -tocopherol). Those compounds may be used to assess the potential use of the oils from these seeds in foodstuffs and they can also be incorporated into cosmetic preparations.

#### ACKNOWLEDGMENT

The authors wish to thank Dr. M. Prieto from the University of Oviedo, a group associated with the Spanish Scientific Research Council (CSIC) and AGL-2011-261713 project.

#### REFERENCES

- Anonymous. 1999. User's Guide to MSTATC. An analysis of agronomic research experiments. Michigan State University, USA.
- Agriview. 2008. AGV - Wine - General Balance Sheet European Union. Historique des indicateurs les plus utilisés dans le secteur vin, par campagne et par et at membre.
- Bail S, Stuebiger G, Krist S, Unterweger H, Buchbauer G. 2008. Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols. *Food Chem.* **108**, 1122–1132. <http://dx.doi.org/10.1016/j.foodchem.2007.11.063>.

- Baydar NG, Ozkan G, Celtin ES. 2007. Characterization of grape seed and pomace oil extracts. *Grasas Aceites* **58**, 29–33.
- Beveridge THJ, Girard B, Kopp JC, Drover G. 2005. Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects. *J. Agric. Food Chem.* **53**, 1799–1804. <http://dx.doi.org/10.1021/jf040295q>.
- Crew C, Hough P, Godward J, Brereton P, Lees M, Guiet S, Winkelman W. 2006. Quantitation of the main constituents of some authentic grape seed oils of different origins. *J. Agric. Food Chem.* **54**, 6261–6264. <http://dx.doi.org/10.1021/jf060338y>.
- European Union, Official Diary, reglament n°. 479/2008.
- Furiga A, Lonvaud-Funel A, Badet C. 2009. In vitro study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. *Food Chem.* **113**, 1037–1040. <http://dx.doi.org/10.1016/j.foodchem.2008.08.059>.
- Freitas LS, Jacques RA, Richter MF. 2008. Pressurized liquid extraction of vitamin E from Brazilian grape seed oil. *J. Chromatogr. A*, **1200**, 80–83. <http://dx.doi.org/10.1016/j.chroma.2008.02.067>.
- International Union of Pure and Applied Chemistry (IUPAC). 1987a. Standard Methods for the Analysis of Oils, Fats and Derivatives: Method 1122. Determination of oil content (extraction method). 7<sup>th</sup>edn. Eds. C. Paquot, A. Haufenne. Blackwell Scientific Publications, Oxford, UK, 1987d, pp. 14–16.
- International Union of Pure and Applied Chemistry (IUPAC). 1987b. Standard Methods for the Analysis of Oils, Fats and Derivatives: Method 1121. Determination of moisture and volatiles matter content. 7<sup>th</sup>edn. Eds. C. Paquot, A. Haufenne. Blackwell Scientific Publications, Oxford, UK, pp. 13–16.
- International Union of Pure and Applied Chemistry (IUPAC). 1987c. Standards Methods for the Analysis of Oils, Fats and Derivatives. Methods 2301. Preparation of the fatty acid methyl esters. 7<sup>th</sup>edn. Eds. C. Paquot, A. Haufenne. Blackwell Scientific Publications, Oxford, UK, pp. 123–129.
- International Union of Pure and Applied Chemistry (IUPAC). 1987d. Standard Methods for the Analysis of Oils, Fats and Derivatives: Method 2411. Identification and determination of tocopherols. 7<sup>th</sup>edn. Eds. C. Paquot, A. Haufenne. Blackwell Scientific Publications, Oxford, UK, pp. 174–219.
- Kin H, Kim SG, Choi Y, Jeong HS, Lee J. 2008. Changes in tocopherols, tocotrienols, and fatty acids contents. *J. Am. Oil Chem. Soc.* **85**, 487–489. <http://dx.doi.org/10.1007/s11746-008-1214-1>.
- León-Camacho M, Morales MT. 2000. Gas and liquid chromatography: methodology applied to olive oil. In: Harwood JL, Aparicio R (eds). Handbook of olive oil: analysis and properties. Aspen, Gaithersburg, pp. 159–208.
- Luque-Rodríguez JM, Castro MD, Lee J. 2008. Extraction of fatty acids from grape seed by superheated hexane. *Talanta*, **68**, 126–130. <http://dx.doi.org/10.1016/j.talanta.2005.04.054>.
- Lutterodt H, Slavin M, Whent M, Turner E, Yu L. 2011. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chem.* **128**, 391–399. <http://dx.doi.org/10.1016/j.foodchem.2011.03.040>.
- Martinez Massanet G, Montiel JA, Pando E, Rodriguez LF. 1986. Study of agricultural by-products. II. Fatty acid composition of Palomino grapeseed oil. *Grasas Aceites*. **37**, 233–236.
- Mironeasa S, Leahu A, Codina GG, Stroe SG, Mironeasa C. 2010. Grape seed: physico-chemical, structural characteristics and oil content. *J. Agro. Proces. Technol.* **16**, 1–6.
- Oomah BD, Liang J, Godfrey D, Mazza G. 1998. Microwave heating of grapeseed: Effect on oil quality. *J. Agric. Food Chem.* **46**, 4017–4021. <http://dx.doi.org/10.1021/jf980412f>.
- Pardo JE, Fernández E, Rubio M, Alvarruiz A, Alonso GL. 2009. Characterization of grape seed oil from different grape varieties (*Vitis vinifera*). *European J. Lipid Sci. Technol.* **11**, 188–193. <http://dx.doi.org/10.1002/ejlt.200800052>.
- Sineiro J, Dominguez H, Núñez MJ. 1995. Pepitas de uva como fuente de aceite y proteína. *Aliment. Equipos Tecnol.* **14**, 49–56.
- Tangolar SG, Ozugol Y, Tangolar S, Torun A. 2009. Evaluation of fatty acid profiles and mineral content of grape seed oil of some grape genotypes. *Int. J. Sci. Nutri.* **60**, 32–39. <http://dx.doi.org/10.1080/09637480701581551>.
- Tecator. 2002. Application note, A3001. Determination of crude protein (Kjeldahl Nitrogen) in Animal feed, forage (Plant Tissue), grain & oilseeds using block digestion with copper catalyst and steam distillation into boric acid. Foss Tecator, Sweden.
- Tisconia E, Bertini GC. 1973. Composition of sterol fraction of lipids extracted from oilseeds. *Riv. Ital. Sost. Grasse.* **50**, 251–268.
- Wijendran V, Hayes A. 2004. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu. Rev. Nutr.* **24**, 597–615. <http://dx.doi.org/10.1146/annurev.nutr.24.012003.132106>.