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# Sugars and Total Phenolic Contents in Different Fractions of Autochthonous Grape Varieties Grown in Tunisia

**Mounira Harbi[1#] • Imen Tlili[1#*] • Rym Bouhlal[1] • Sami Fattouch[2]**

[1] Laboratory of Horticulture, National Agricultural Research Institute of Tunisia, Tunis, Rue Hédi Karray 2049 Ariana, Tunisia
[2] Biotechnology and Chemistry Laboratory, National Institute of Applied Sciences and Technology (INSAT), Tunis, Tunisia, Centre Urbain Nord, BP n° 676 -1080 Tunis Cedex. Tunisia
# These authors contributed equally to the work.
*Corresponding author*: * **imen_tlili2008@yahoo.fr**

## ABSTRACT

Grapes (*Vitis vinifera* L.) is one of the economically important fruit in the world and the important quality determining parameters of it are sweetness related compounds and antioxidants. In this experiment, reducing, sugar, total sugar and phenolic contents were quantified in different parts (peel, pulp, and seeds) of 15 grape varieties: ('Muscat de RafRaf', 'Rafrafi', 'Boukhasla', 'Farrani', 'Bith El H'mem', 'Hammémi', 'Kohli', 'Chaâraoui', 'Vieux Beldi', 'Razzégui', 'Farranah', 'Bith El H'mem Rose', 'Essifi', 'Marsaoui' and 'Bézoul El Khadem'). Significant differences were found between grape varieties within different parts in total sugar, reducing sugar and phenolic contents. 'Muscat de RafRaf' variety showed the highest amount of total sugar (12.28 g 100 $g^{-1}$ FW), reducing sugar (7.43 g 100 $g^{-1}$ FW) and phenolic contents (28.33 mg EAG $g^{-1}$ FW). The pulp of grapes showed high reducing and total sugar contents. The total phenolic content varied between 0.47 and 80.93 mg EAG $g^{-1}$ FW. Seeds had a greater phenolic compound content, between 18.09 and 80.93 mg EAG $g^{-1}$ FW, which was higher than those of other fruit parts.

**Keywords:** phenolics, quality, reducing sugar, total sugar, *Vitis vinifera*

## INTRODUCTION

Grapes are one of the most widely grown fruit crops throughout the world. It is mainly processed to juice, wine or raisins and cultivated largely for the wine industry. Consumer preference for this fruit is determined largely by its sweetness (i.e., sugar content), flavor or aroma, and more recently as a rich source of phytonutrients (Fuleki and Ricardo-da-Silva 2003; Conde *et al*. 2007; Duchêne *et al.* 2012). In addition to its superior consumer preference, the grape is becoming increasingly popular as an extremely healthy food choice, and is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components (Subramani *et al*. 2002; Fuleki and Ricardo-da-Silva 2003). Therefore, the content of different classes of antioxidants and the antioxidant activity are important parameters in the qualitative evaluation of fruits and vegetables (Lenucci *et al*. 2006; Ilahy *et al*. 2011; Tlili *et al*. 2011).

In recent years, natural compounds, particularly phenolics, have received a great interest because of their antioxidant activity against free radicals, suggesting protective roles in reducing risk of chronic diseases, such as cancer and cardiovascular disease (Rice-Evans *et al*. 1996; Giovanucci 1999; Agarwal and Rao 2000).

According to many authors, antioxidant activity of fruits results mainly from phenolics, particularly flavonoids. The antioxidant activity of fresh grapes is thus attributable to different types of phenolic constituents, and the antioxidant effectiveness on low density lipoprotein (LDL) lipid peroxidation is correlated to distinct types of phenolics and their relative concentrations in various samples (Teissedre and Landrault 2000; Nardini *et al.* 2006; Jacob *et al*. 2008). In fact, it has been already reported that grape juice compounds can prevent platelet aggregation, LDL oxidation and oxidative damage to DNA, coronary diseases and atherosclerosis (Frankel *et al*. 1998; Singletary *et al*. 2003; Nandakumar *et al*. 2008).

Grapes contain a large amount of different phenolic compounds in skin, pulp and seeds (Santos *et al*. 2011) and these are the main compounds responsible of colour, taste, mouth feel, oxidation and other chemical reactions in wine (Roggero *et al*. 1986). According to many authors (Macheix *et al*. 1990; Soares *et al*. 2008; Santos *et al*. 2011), grapes are among the fruits containing the highest content of phenolic substances.

Within the same fruit type, the growing season, variety, environmental and climatic conditions, plant disease, soil type, geographic locations, and maturity seem to influence the amount of phenolic compounds (Subramani *et al*. 2002; Montealegre *et al*. 2006; Obreque-Slier *et al.* 2010).

In addition, sweetness is an important quality attribute for consumer preference. Sugar content and composition are the major criteria used in judging the quality of the fruit of grapes.

However, little is known about the genetic variability in sugar contents among different grape varieties commonly grown and consumed in Tunisia. In fact, it has been reported that there is a large genetic variability among grape varieties in terms of sugar content (Huglin and Schneider 1998, Mercado-Martin *et al*. 2006, Dai *et al*. 2011). Nevertheless, these parameters are generally measured at harvest in the berry pulp, and they depend mainly on harvest dates and climatic conditions (Jackson and Lombard 1993; Dai *et al*. 2011).

Many studies have demonstrated that grapes contain a wide array of phytochemicals (Fuleki and Ricardo-da-Silva 2003; Santos *et al*. 2011; Du *et al*. 2012), but in Tunisia, many grapes species and cultivars have not been analyzed for these important compounds. In this context, the aim of this study was to assess the phenolic content of peel, pulp, and seed of fifteen grape varieties, with a view to exploiting its potential as a source of natural antioxidant.

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## MATERIALS AND METHODS

### Grape samples

Fifteen grape cultivars ('Muscat de RafRaf', 'Rafrafi', 'Boukhasla', 'Farrani', 'Bith El H'mem', 'Hammémi', 'Kohli', 'Chaâraoui', 'Vieux Beldi', 'Razzégui', 'Farranah', 'Bith El H'mem Rose', 'Essifi', 'Marsaoui' and 'Bézoul El Khadem'), grown in RafRaf, Bizerte (latitude 30° 11′ 27″ N, longitude 10° 11′ 00″ E), Tunisia, were obtained during growing period 2008 (August-November). Grape samples were collected during the last month of ripening coincided with the technological maturity of the grapes. Firstly grape berries were removed from each bunch. After that all fruits were first flushed with tap water and then washed in distilled water for three times before the peel, pulp and seed fractions were carefully separated. The seeds were dried at ambient temperature (25°C). The different parts were homogenized in an ice cooled blender (Waring 32BL 79, Massachusetts, USA) and stored at −18°C until analysis.

### Sugar content determination

Total sugar and reducing sugar contents were extracted as described by Jain *et al*. (2002) on triplicate aliquots of homogeneous suspension (0.5 g). Reducing sugar assay was performed following the DNS method and total sugar assay by anthrone-sulfuric acid method as described by Yemm and Wills (1954).

### Total phenolic content determination

Total phenols were extracted as described by Martinez-Valverde *et al*. (2002) on triplicate aliquots of homogeneous suspension (0.3 g). The total phenol assay was performed by using the Folin-Ciocalteu reagent (F9252, Sigma-Aldrich) as described by Spanos and Wrolstad (1990) on triplicate 50 mL aliquots of the supernatant. The absorbance was read at 750 nm using a spectrophotometer (Beckman DU 650, Beckman Coulter Inc., CA, USA). Results were expressed in mg gallic acid equivalent (GAE)/kg fresh weight (FW).

### Statistical analysis

Effects of variety on the nutritional properties of cultivars were assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using the least significant difference (LSD) test ($P < 0.05$). All statistical comparisons were performed using SAS Version 6.1 software (SAS Institute, Cary, NC, USA).

**Table 1** Reducing sugar, total sugar and phenolic contents of the different studied grape varieties within different parts of table grape.

| Parts | Varieties | Reducing sugar (g 100 g$^{-1}$ FW) | Total sugar (g 100 g$^{-1}$ FW) | Total phenolic (mg GAE kg$^{-1}$ FW) |
|---|---|---|---|---|
| **Seeds** | Muscat de RafRaf | 5.89 ± 0.68 bc | 8.05 ± 0.20 de | 80.93 ± 0.68 a |
| | Rafrafi | 3.95 ± 0.59 f | 4.35 ± 0.45 g | 50.37 ± 0.59 de |
| | Boukhasla | 4.89 ± 0.96 d | 9.28 ± 0.07 c | 48.18 ± 0.96 e |
| | Farrani | 7.15 ± 0.45 a | 9.23 ± 0.45 c | 40.23 ± 0.45 f |
| | Bith El H'mem | 1.89 ± 0.28 g | 8.66 ± 0.74 cd | 53.46 ± 0.28 cde |
| | Hammémi | 3.98 ± 0.67 f | 4.56 ± 0.16 g | 54.17 ± 1.25 cd |
| | Kohli | 1.80 ± 0.46 g | 5.70 ± 0.04 f | 56.07 ± 0.46 c |
| | Chaâraoui | 4.79 ± 0.22 d | 5.12 ± 0.25 fg | 55.35 ± 1.95 cd |
| | Vieux Beldi | 6.02 ± 0.17 b | 7.05 ± 0.33 e | 18.09 ± 0.17 h |
| | Razzégui | 6.20 ± 0.03 b | 11.73 ± 0.20 b | 30.45 ± 0.03 g |
| | Farranah | 0.47 ± 0.05 h | 1.60 ± 0.01 h | 72.79 ± 0.63 b |
| | Bith El H'mem Rose | 5.61 ± 1.15 c | 7.14 ± 0.53 e | 58.68 ± 1.72 c |
| | Essifi | 4.10 ± 0.97 ef | 15.55 ± 0.56 a | 37.28 ± 0.67 f |
| | Marsaoui | 3.89 ± 1.06 f | 4.77 ± 0.10 fg | 30.03 ± 1.64 g |
| | Bézoul El Khadem | 4.38 ± 0.86 e | 4.64 ± 0.52 fg | 36.78 ± 0.86 f |
| **Peels** | Muscat de RafRaf | 7.32 ± 0.19 bc | 14.67 ± 0.30 a | 2.42 ± 0.12 i |
| | Rafrafi | 7.95 ± 0.26 ab | 10.56 ± 0.26 d | 3.82 ± 0.06 h |
| | Boukhasla | 6.93 ± 0.19 cd | 9.62 ± 0.26 e | 6.90 ± 0.21 e |
| | Farrani | 6.68 ± 0.33 d | 8.57 ± 0.17 ghi | 9.70 ± 0.20 ab |
| | Bith El H'mem | 5.83 ± 0.24 e | 11.27 ± 0.11 c | 5.09 ± 0.02 g |
| | Hammémi | 4.31 ± 0.19 f | 13.48 ± 0.17 b | 5.86 ± 0.12 f |
| | Kohli | 6.78 ± 0.24 cd | 8.19 ± 0.46 hij | 3.73 ± 0.26 h |
| | Chaâraoui | 7.95 ± 0.41 ab | 8.93 ± 0.14 fg | 7.49 ± 0.04 d |
| | Vieux Beldi | 7.97 ± 0.16 a | 9.42 ± 0.05 ef | 6.74 ± 0.27 e |
| | Razzégui | 6.71 ± 0.07 cd | 8.86 ± 0.24 fgh | 9.86 ± 0.15 a |
| | Farranah | 5.83 ± 0.16 e | 7.88 ± 0.17 jk | 8.41 ± 0.14 c |
| | Bith El H'mem Rose | 7.67 ± 0.10 ab | 8.02 ± 0.32 ij | 6.55 ± 0.22 e |
| | Essifi | 2.66 ± 0.08 g | 6.70 ± 0.30 l | 9.26 ± 0.10 l |
| | Marsaoui | 4.83 ± 0.12 f | 7.16 ± 0.16 l | 6.98 ± 0.14 e |
| | Bézoul El Khadem | 5.76 ± 0.22 e | 7.26 ± 0.01 kl | 5.18 ± 0.19 g |
| **Pulp** | Muscat de RafRaf | 9.09 ± 0.35 a | 14.12 ± 0.14 c | 1.66 ± 0.07 b |
| | Rafrafi | 7.35 ± 0.20 cd | 12.21 ± 0.20 d | 0.73 ± 0.01 h |
| | Boukhasla | 4.44 ± 0.23 g | 16.52 ± 0.23 a | 0.84 ± 0.02 gh |
| | Farrani | 6.26 ± 0.06 e | 14.49 ± 0.24 bc | 1.22 ± 0.02 cd |
| | Bith El H'mem | 4.80 ± 0.06 fg | 10.62 ± 0.17 e | 1.03 ± 0.06 ef |
| | Hammémi | 4.64 ± 0.13 g | 15.07 ± 0.25 b | 0.47 ± 0.01 i |
| | Kohli | 7.11 ± 0.34 d | 8.89 ± 0.14 f | 2.02 ± 0.02 a |
| | Chaâraoui | 7.13 ± 0.27 d | 10.46 ± 0.34 e | 0.94 ± 0.02 fg |
| | Vieux Beldi | 7.44 ± 0.33 cd | 8.54 ± 0.26 f | 2.04 ± 0.01 a |
| | Razzégui | 8.27 ± 0.12 b | 12.63 ± 0.31 d | 1.67 ± 0.03 b |
| | Farranah | 3.72 ± 0.17 h | 7.65 ± 0.06 g | 0.97 ± 0.01 fg |
| | Bith El H'mem Rose | 5.42 ± 0.28 f | 7.19 ± 0.07 g | 1.56 ± 0.08 b |
| | Essifi | 4.20 ± 0.12 gh | 7.42 ± 0.28 g | 1.62 ± 0.01 b |
| | Marsaoui | 5.35 ± 0.09 f | 6.39 ± 0.27 h | 1.13 ± 0.06 de |
| | Bézoul El Khadem | 7.89 ± 0.03 bc | 10.32 ± 0.28 e | 1.29 ± 0.05 c |

Probability level of 1%; ns: not significant. Values in the same column followed by the same letters do not differ significantly (LSD test, $P<0.05$).

## RESULTS AND DISCUSSION

### Sugar content

The reducing and total sugar content values of the different studied grape cultivars within different parts are listed in **Table 1**.

With regards to total sugar content, the data show statistically significant differences ($P < 0.01$) among the grape cultivars. When averaged across parts, the total sugar varied between 5.71 and 12.28 g 100 $g^{-1}$ FW. The average total sugar content reached the highest values in 'Muscat de RafRaf' (12.28 g 100 $g^{-1}$ FW) and 'Boukhasla' (11.81 g 100 $g^{-1}$ FW), whose means did not differ significantly. 'Farranah' (5.71 g 100 $g^{-1}$ FW) showed the lowest average content of total sugar.

Our results are close to the range reported by Jain *et al*. (2002) ranging from 4.46 to 16.08 g 100 $g^{-1}$ FW, while studying the variation in the sugar accumulation pattern of grape varieties.

The results also showed that total sugar content varied significantly between studied parts within all varieties ($P < 0.01$). Values ranged between 1.60 and 16.52 g 100 $g^{-1}$ FW. Therefore, for all varieties, the mean values of total sugar content was highest in pulp followed by peel and the lowest values were obtained in seeds (**Fig. 1A**).

The total sugar content in pulp ranged from 6.39 to 16.52 g 100 $g^{-1}$ FW in 'Marsaoui' and 'Boukhasla', respectively. In peels values ranged from 6.70 to 14.67 g 100 $g^{-1}$ FW in 'Essifi' and 'Muscat de RafRaf', respectively. However total sugar content in seeds ranged from 1.60 to 15.55 g 100 $g^{-1}$ FW in 'Farranah' and 'Essifi', respectively.

For reducing sugar, the obtained data showed that values varied significantly between studied grape varieties ($P < 0.01$). When averaged across parts, the reducing sugar varied between 3.34 and 7.43 g 100 $g^{-1}$ FW. The highest values were obtained for 'Muscat de RafRaf' and 'Vieux Beldi' with 7.43 and 7.14 g 100 $g^{-1}$ FW, respectively. The lowest value was obtained for 'Farranah'. To our knowledge, this is the first report on reducing sugar in grape varieties.

Reducing sugar content varied significantly between studied parts within all cultivars ($P < 0.01$) (**Fig. 1B**). Values ranged between 0.47 and 9.09 g 100 $g^{-1}$ FW. When averaged across varieties, the highest values were obtained for Pulp (3.72 - 9.09 g 100 $g^{-1}$ FW) and peel (2.66-7.97 g 100 $g^{-1}$ FW) parts, whose means did not differ significantly. The pulp of 'Muscat de RafRaf' revealed the highest value of reducing sugar content however the peel of 'Vieux Beldi' revealed the highest value. Reducing sugar content in seeds ranged from 0.47 to 7.15 g 100 $g^{-1}$ FW in 'Farranah' and 'Farrani', respectively, which was lower than those of other fruit parts.

To our knowledge, these are the first results describing distribution of reducing and total sugar in different fractions of grape fruit.

### Total phenolic content

The amounts of total phenolics of the investigated grape cultivars within different parts are shown in **Table 1**. The data, expressed on a fresh weight (FW) basis, show statistically significant differences ($P < 0.01$) among the grape cultivars.

When averaged across different parts, total phenolic content reached the highest value in 'Muscat de RafRaf' (28.33 mg EAG $g^{-1}$ FW) and 'Farranah' (27.39 mg EAG $g^{-1}$ FW), whose means did not differ significantly, and the lowest in 'Vieux Beldi' (8.96 mg EAG $g^{-1}$ FW). The obtained values were considerably higher compared to those reported by Du *et al*. (2012) ranging from 103.1 to 257 mg GAE 100 $g^{-1}$ FW in 'Milk grape' and 'Cabernet Gernischt' grape varieties, while studying the phenolic content and antioxidant activity of wine grapes and table grapes. Considerably higher values ranging between 33.50 and 150.69 mg EAG $g^{-1}$ FW were obtained by Qusti *et al.* (2008) in white and red grape varieties. These divergent results were probably due to variety or environmental differences. In fact, Qusti *et al.* (2008) reported that red grape ranking the first and white grape ranking the ninth among fourteen fruits that provide high levels of phenolics. These data proved that grape berries can constitute a good source of phenolics in Tunisian diet because of its availability and high consumption.

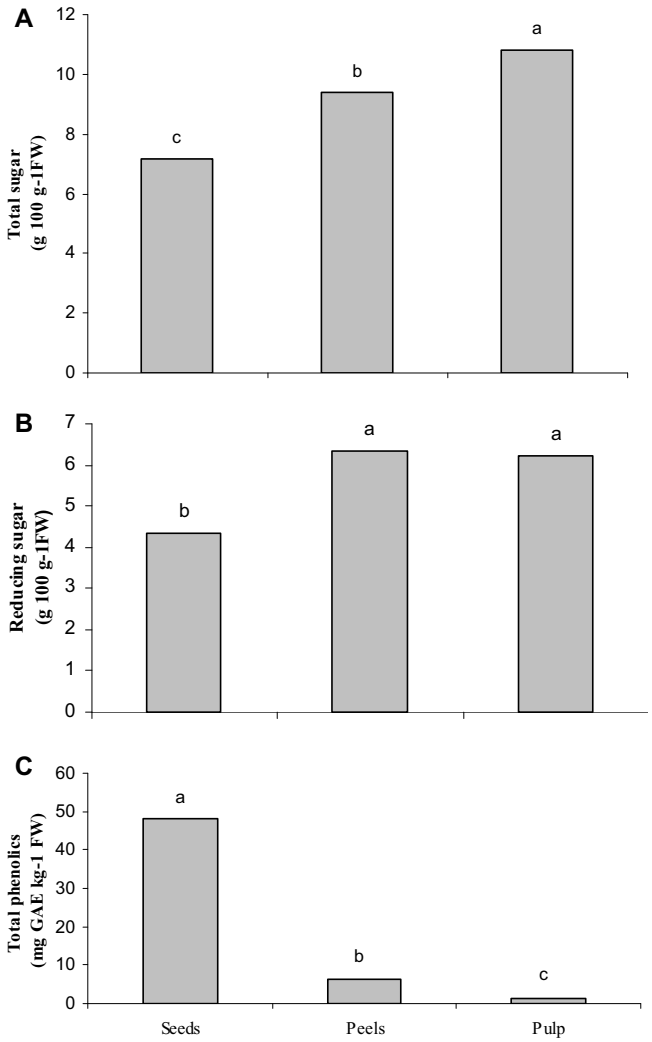


**Fig. 1 Total sugar (A), reducing sugar (B), and total phenolics (C) for all grape varieties within the different parts.** Values for each sampling area with the same letters are not significantly different (LSD test, $P<0.05$).

Total phenolic content varied significantly between studied parts within all cultivars ($P < 0.01$) (**Fig. 1C**). Values ranged between 0.47 and 80.93 mg EAG $g^{-1}$ FW. Seeds had a greater phenolic compound content, between 18.09 and 80.93 mg EAG $g^{-1}$ FW, which was higher than those of other fruit parts, particularly in the 'Muscat de RafRaf' and 'Essifi'. These values are lower to those obtained by Santos *et al*. (2011) in seeds of four grape varieties, which had polyphenol contents between 89.83 to 122.35 mg EAG $g^{-1}$ FW. Considerably higher values ranging between 79.2 and 154.6 mg EAG $g^{-1}$ FW in seeds of 'Senso' and 'Papaz karasi' grapes varieties were obtained by Bozan *et al*. (2008).

The phenolic compound content in peels ranged from 1.43 to 9.86 mg EAG $g^{-1}$ FW. The peel of 'Farrani' variety revealed the highest value of total phenolic content. The obtained values were considerably higher compared to those reported by many authors (Soares *et al*. 2008; Santos *et al*. 2011) in peels of grape varieties ranging from 1.43 to 2.46 mg EAG $g^{-1}$ FW.

In pulp, the amount of phenolic compounds (in mg EAG $g^{-1}$) was 0.47–2.04. The pulp of 'Vieux Beldi' and 'Kohli' revealed the highest values of total phenolic content. The results are much higher to those obtained by Santos *et al*. (2011) ranging between 0.04–0.11 mg EAG $g^{-1}$ in 'Benitaka' and 'Isabel grape varieties', while studying the phenolic compounds in different parts of four grape varieties. In studies by Xu *et al*. (2009), the amounts of polyphenols in pulp of 'Redglobe' grape were close to 0.08 mg EAG $g^{-1}$.

These differences in phenolic composition may be attributed to the influence of varietal differences and many external factors such as soil composition, geographical location, climatic conditions, and light intensity (Garrido and Borges 2011).

## CONCLUSIONS

Our results demonstrated that grapes are potential source of phenolics and has confirmed the important role played by genetic in determining antioxidant components of grapes varieties. Moreover, this study demonstrated that total phenolic contents were higher in seeds, followed by the peel and pulp. In fact, the different parts or extracts can be used as antioxidant sources by the industry, utilized as nutraceutical, besides providing important information to wine-making industry. Therefore variability detected among the grape varieties emphasized the need to evaluate *Vitis vinifera* biodiversity in order to improve its nutritional value.

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