

# Distribution of major sugars, acids and total phenols in juice of five grapevine (*Vitis* spp.) cultivars at different stages of berry development

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## Abstract

The juices of five grapevine cultivars cultivated in a typical Mediterranean climate were analyzed for sugars, organic acids, and phenols at four distinct stages of berry development. When the unripe berries were almost in full size, the glucose and fructose contents, based on HPLC detection, ranged from 13.3 to 30.7 g L<sup>-1</sup> and from 8.3 to 23.7 g L<sup>-1</sup> for 'Muscat of Alexandria' and 'Muscat of Hamburg', respectively. At this stage, tartaric acid concentration was between 10.3 ('Italia') and 12.3 g L<sup>-1</sup> ('Muscat of Alexandria'), while the level of total phenols was low. Up to véraison, there were slight reductions in organic acids, while sugar content increased slightly. However, dramatic changes in all genotypes were apparent after véraison. Slight reductions were determined in the glucose and fructose levels of 'Italia' prior to final analysis, possibly indicating this cultivar's sensitivity to late harvest. In the final analysis, glucose and fructose content varied from 86.4 ('Italia') to 107.0 g L<sup>-1</sup> ('Muscat of Hamburg'), and from 73.1 ('Italia') to 94.1 g L<sup>-1</sup> ('Alphonse Lavallée'), respectively. Tartaric acid content of ripe berries was between 3.8 ('Alphonse Lavallée') and 5.2 g L<sup>-1</sup> ('Isabella') with a mean value of 4.6 g L<sup>-1</sup>, and phenol content of mature berries ranged from 2,253 to 2,847 mg L<sup>-1</sup>. This study provides valuable information for further understanding the sugar, acid and total phenol changes that occur in some grape cultivars during berry maturation. Therefore, these results will be useful for future research on the biochemistry of the grape berry.

**Additional key words:** components of grape juice, HPLC, maturation stages.

## Resumen

### Distribución de los principales azúcares, ácidos y fenoles totales en el zumo de cinco cultivares de vid (*Vitis* spp.) en diferentes etapas de desarrollo de las uvas

Se analizaron los azúcares, ácidos orgánicos y fenoles del zumo de cinco cultivares de vid, cultivados en un clima típico mediterráneo, en cuatro etapas distintas del desarrollo de las uvas. Cuando las uvas sin madurar estaban en su tamaño casi definitivo, los contenidos de glucosa y fructosa, detectados por HPLC, oscilaron de 13,3 a 30,7 g L<sup>-1</sup> y de 8,3 a 23,7 g L<sup>-1</sup> para 'Moscatel de Alejandría' y 'Moscatel de Hamburgo', respectivamente. En esta etapa, la concentración de ácido tartárico se situó entre 10,3 ('Italia') y 12,3 g L<sup>-1</sup> ('Moscatel de Alejandría'), mientras que el nivel de fenoles totales fue bajo. Hasta el envero hubo ligeras reducciones en ácidos orgánicos, mientras que el contenido de azúcar aumentó ligeramente. Sin embargo, después de envero se evidenciaron cambios dramáticos en todos los genotipos. Se observaron ligeras reducciones en los niveles de glucosa y de fructosa en 'Italia' antes del análisis final, lo que posiblemente indica la sensibilidad de esta variedad a una cosecha tardía. En el análisis final, el contenido de glucosa y de fructosa varió de 86,4 ('Italia') a 107,0 g L<sup>-1</sup> ('Moscatel de Hamburgo'), y de 73,1 ('Italia') a 94,1 g L<sup>-1</sup> ('Alphonse Lavallée'), respectivamente. El contenido de ácido tartárico de las uvas maduras varió entre 3,8 ('Alphonse Lavallée') y 5,2 g L<sup>-1</sup> ('Isabella'), con un valor medio de 4,6 g L<sup>-1</sup>, y el contenido de fenol de uvas maduras fue de 2.253 a 2.847 mg L<sup>-1</sup>. Este estudio permite comprender mejor los cambios que se producen en los azúcares, ácidos y fenoles totales en algunas variedades de vid durante la maduración de la uva. Por lo tanto, estos resultados serán de utilidad para futuras investigaciones sobre la bioquímica de la uva.

**Palabras clave adicionales:** componentes de zumo de uva, etapas de maduración, HPLC.

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Abbreviations used: AL (*cv.* Alphonse Lavallée), GAE (gallic acid equivalents), HPLC (high pressure liquid chromatography), IS (*cv.* Isabella), IT (*cv.* Italia), MA (*cv.* Muscat of Alexandria), MH (*cv.* Muscat of Hamburg), MSD (minimum significant differences), RID (radio interference detection), UV (ultra violet).

## Introduction

The common grape (*Vitis vinifera* L.), with an annual production of roughly 3.9 million tons (FAO, 2007), contributes to the Turkish national income thanks to a wide range of uses such as table grapes, raisins, juice or wine production, marmalades, pies and blends with other commodities (Sabir *et al.*, 2008; Uzun and Bayir, 2008). With regard to human health, recent findings revealed that phenol-rich foods like grapes play a significant role in the maintenance of health and in the protection against inflammation, cardiovascular disease, cancer and age-related disorders (Weston, 2005). For this reason, the growth and maturation processes of grape berries have received remarkable scientific scrutiny due to the importance of this fruit as a significant component of the human diet and wine industry (Conde *et al.*, 2007). During the ripening stage, grape berries will gain the characteristic properties of their cultivar, including the features that determine their suitability for different uses. Hence, estimation of the optimum maturity stage of a certain cultivar depends on the final commodities that will be produced from that grape. For example, raisin grapes in Turkey are usually harvested at 22-24°Brix, while table grapes are mostly harvested between 14-20°Brix. Approximately 37% of the fresh grapes produced in Turkey are processed for grape juice and other local consumption products such as sausage, kofter, pekmez (boiled concentrate juice); 3% are used in wine production; 27% are sold as table grapes; and 33% are dried as seedless or seeded raisin (Uzun and Bayir, 2008). Although, in Turkey, a significant quantity of the grapes is processed for juice, the estimation of grape maturity is based mostly on sugar concentration values and visual or sensory detections.

From a biochemical point of view, the maturity stage at which grapes can be harvested is determined by certain quality indicators such as sugar accumulation, acid content, sugar to acid ratio, and phenolic compounds that contribute to the quality of the produce and its derivatives. Achievement of the optimum concentrations of these components is governed by viticulture strategies that can be applied to optimize ripening (Coombe and McCarthy, 2000; Carmona *et al.*, 2008). An understanding of the critical accumulation stages of such components during berry maturation is needed to adjust grape growing practices and thus modify wine typology (Conde *et al.*, 2007).

The aim of this work was to analyze the proportional changes of predominant sugars, acids, and total phenols

in five grape cultivars grown in Mediterranean ecological conditions (with typical rainy and cold winters, and dry and hot summers) during four stages of berry development.

## Material and methods

Twenty-five year-old vines of the red cultivars 'Alphonse Lavallée' (AL) ('Ribier'), 'Muscat of Hamburg' (MH, Schiava grossa × Muscat Alexandria) and 'Isabella' (IS) (*V. labrusca* L.), and of white cultivars 'Italia' (IT) and 'Muscat of Alexandria' (MA) (*V. vinifera* L.) were selected on the basis of unity in vine growth and cultivation conditions in vineyard. The vines were planted at a row spacing of 2 × 3 m (within and between rows) in the same orientation, and pruning was done according to the Guyot system using cordons. They were cultivated under the same agricultural conditions, with an annual average rainfall of 488 mm, most of which took place in the period between dormancy and full bloom (from December to April) (Anonymous, 2007). The cultivars used in the study are highly regarded by consumers as well as growers in Turkey because of their good quality and/or tolerance to certain diseases. They usually reach maturity in mid-season, and under the same ecology, harvest time occurs at a similar period (Sabir, 2008).

Grape berry samples were harvested from the viticulture research area of the Agriculture Faculty at Cukurova University at four different stages of berry maturation, as performed in previous similar studies (Possner and Kliewer, 1985; Coombe, 1987): (1) when the berries were unripe and almost full size (unripe stage), (2) onset of maturity (defined by the French word *véraison*), (3) three weeks after *véraison* (early ripe stage), and (4) three weeks after third sampling date (ripe stage). The experiment used a randomized complete block design with three replicates consisting of 15 vines for each cultivar in total. For berry sampling, approximately 20 clusters, representing five vines of each replicate, were used.

The mixture of about 100 berries taken from each replicate was squeezed in cheesecloth. To prevent deterioration of grapes and interconversions between compounds, the juice samples were then freeze-dried and stored at -80°C for later analysis.

Solvents, chromatography standards and reagents were purchased from Sigma. Ultrapure water was obtained using a Millipore system.

## Extraction of sugars and acids

Before analysis, the frozen juice samples were thawed at 25°C and 1 mL of the thawed must was transferred to a screw cap Eppendorf tube with 20 mL of aqueous ethanol (80%, v/v).

The mixture was moved to an ultrasonic bath and sonicated for 15 min at 80°C. The mixture was then filtered and the extraction procedure was repeated twice more. All the filtered extracts were combined and evaporated to dryness on a boiling water bath. The remaining residue was dissolved with 2 mL of distilled water and filtered through a 0.45 µm membrane filter (Millipore Millex-HN nylon) before HPLC analysis (Miron and Schaffer, 1991). The injection volume was 20 µL. Sugar and organic acid content was expressed as g L<sup>-1</sup> of juice.

Sugars and acids were analyzed using a liquid chromatographic apparatus (Agilent 1100 series) consisting of an in-line degasser, pump, and controller coupled to a RID (refractive index detector) and a UV detector (Agilent 1100 series), equipped with an automatic injector (20 µL injection volume) and interfaced to a PC running Class VP chromatography manager software (Agilent, USA). Sugar separations were performed on a 250 × 4.6 mm *i.d.*, 5 µm, reverse-phase NH<sub>2</sub> analytical column (Beckman), operating at 40°C column temperatures with a flow rate of 1 mL min<sup>-1</sup>. Elution was isocratic, acetonitrile:water (3:1). Components were identified by comparison of their retention times to those of authentic standards under analysis conditions. A 20 min equilibrium time was allowed between injections. As for the acids, separations were carried out on a 250 × 4.6 mm *i.d.*, 5 mm, reverse-phase ultrasphere ODS analytical column (Beckman) at room temperature with a course rate of 1 mL min<sup>-1</sup>. Detection was obtained with a sensitivity of 0.1 AUFS (absorbance unit full scale) between 210 nm wavelengths. Elution was isocratic, with 0.5% aqueous meta-phosphoric acid. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions. Between injections, a 10 min equilibrium time was allowed.

## Quantitative analysis

Samples were injected to the reverse phase chromatography column. For the stock solution of organic acid standards, tartaric, malic, and citric acid were dissolved

in methanol at a concentration of 1 mg mL<sup>-1</sup>, while sugar standards were dissolved in water at a concentration of 1 mg mL<sup>-1</sup>. Samples and standards were injected three times and average values were calculated.

Total phenol content was detected by UV spectrophotometry (Perkin Elmer Lambda 25), estimated as gallic acid equivalents (GAE), and defined as mg<sub>gallic acid</sub> L<sup>-1</sup> (Singleton *et al.*, 1999). To ca. 6.0 mL H<sub>2</sub>O, a 100 µL sample was transferred to a 10 mL volumetric flask to which 500 µL of undiluted Folin-Ciocalteu reagent were subsequently added. After 1 min, 1.5 mL 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> were supplemented and the volume was made up to 10 mL with H<sub>2</sub>O. After 2 h incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Data were presented as the average of triplicate analysis.

Data were statistically evaluated by one way analysis of variance (ANOVA). Between the maturation periods of cultivars, statistical differences with *p* values under 0.05 were considered significant and column means were compared by Tukey's MSD (Minimum Significant Differences) test at 5% level, using SPSS program version 13.0 (SPSS Inc., Chicago, IL).

## Results

### Distribution of reducing sugars during berry maturity

The bud break, full bloom, and berry sampling dates of the cultivars analyzed in 2007 are shown in Table 1. The results for glucose, fructose, total sugar content, and the glucose/fructose (G/F) ratio for the cultivars at different stages of berry maturation are presented in Table 2. At the unripe stage, the concentration of glucose in the juice of the different cultivars was very low, ranging from 13.3 (MA) to 30.7 g L<sup>-1</sup> (MH), with a mean value of 24.6 g L<sup>-1</sup>. The accumulation of glucose in cultivars was quite slow up to véraison, varying between 32.8 (AL) and 43.9 g L<sup>-1</sup> (IT) during this stage, whereas glucose synthesis underwent a significant increase after véraison. During the early ripe stage, the AL and IT cultivars almost reached their maximum levels, with values of 90.0 and 90.2 g L<sup>-1</sup>. In the final analysis, glucose concentration of the cultivars varied between 86.4 (IT) and 107.0 (MH), with an average of 98.5 g L<sup>-1</sup>.

The fructose content at the unripe stage was in the range of 8.3 (MA) and 23.7 (MH) g L<sup>-1</sup>, similar to that of glucose. According to the mean values, although

**Table 1.** Bud break, full bloom, and berry sampling dates of cultivars in 2007 (day/month)

| Cultivars <sup>1</sup> | Bud break | Full bloom | Berry sampling dates |         |         |           |
|------------------------|-----------|------------|----------------------|---------|---------|-----------|
|                        |           |            | Fist                 | Second  | Third   | Fourth    |
| AL                     | 24 March  | 06 May     | 15 June              | 28 June | 18 July | 07 August |
| MA                     | 27 March  | 06 May     | 15 June              | 26 June | 15 July | 04 August |
| IS                     | 29 March  | 10 May     | 18 June              | 30 June | 20 July | 10 August |
| IT                     | 24 March  | 07 May     | 15 June              | 30 June | 20 July | 10 August |
| MH                     | 27 March  | 05 May     | 12 June              | 26 June | 15 July | 04 August |

<sup>1</sup> AL: 'Alphonse Lavallée'. MA: 'Muscat of Alexandria'. IS: 'Isabella'. IT: 'Italia'. MH: 'Muscat of Hamburg'.

fructose synthesis increased slightly between the unripe and véraison stages, the highest amount of fructose accumulated between véraison (37.2 g L<sup>-1</sup>) and the early ripe stage (77.3 g L<sup>-1</sup>). After the early ripe stage, however, fructose accumulated rather slowly, in a similar manner to glucose. Among the cultivars, AL stood out with a rapid increase in fructose between the unripe (19.3 g L<sup>-1</sup>) and the early ripe stages (93.1 g L<sup>-1</sup>). At the ripe stage, the highest fructose content was

detected in this cultivar (94.1 g L<sup>-1</sup>), followed closely by MH (91.9 g L<sup>-1</sup>). Conversely, IT had the lowest fructose content at the final stage (73.1 g L<sup>-1</sup>).

Considering the sum of the amounts of individual sugars, total sugar content of cultivars at the unripe stage was between 22.5 (MA) and 54.4 g L<sup>-1</sup> (MH). After a slow accumulation rate up to véraison, total sugar accumulation rapidly increased between véraison and early ripe stage in all cultivars. At the ripe stage, the

**Table 2.** Average and standard deviations (in triplicate) of glucose, fructose, total sugar content (g L<sup>-1</sup>), glucose/fructose (G/F) ratio, tartaric, malic, citric, and total acid content (g L<sup>-1</sup>) of grape cultivars at different stages of berry maturation

|            | Cultivars | Glucose                 | Fructose               | Total sugar             | G/F                     | Tartaric              | Malic                  | Citric                | Total                  |
|------------|-----------|-------------------------|------------------------|-------------------------|-------------------------|-----------------------|------------------------|-----------------------|------------------------|
| Unripe     | AL        | 25.7±3.6 <sup>b</sup>   | 19.3±1.4 <sup>b</sup>  | 45.0±4.9 <sup>b</sup>   | 1.33±0.08 <sup>bc</sup> | 10.9±0.7              | 15.1±0.8 <sup>a</sup>  | 4.4±0.6 <sup>ab</sup> | 30.4±1.9 <sup>a</sup>  |
|            | MH        | 30.7±6.9 <sup>a</sup>   | 23.7±1.0 <sup>a</sup>  | 54.4±7.3 <sup>a</sup>   | 1.29±0.21 <sup>c</sup>  | 10.4±0.6              | 9.1±0.1 <sup>b</sup>   | 2.3±0.2 <sup>c</sup>  | 21.8±1.2 <sup>b</sup>  |
|            | IS        | 29.3±2.6 <sup>ab</sup>  | 17.6±0.6 <sup>b</sup>  | 46.9±3.0 <sup>b</sup>   | 1.66±0.11 <sup>a</sup>  | 11.5±1.4              | 12.3±3.8 <sup>ab</sup> | 4.1±0.2 <sup>bc</sup> | 27.9±2.2 <sup>ab</sup> |
|            | IT        | 24.2±1.0 <sup>b</sup>   | 18.1±0.8 <sup>b</sup>  | 44.3±4.2 <sup>b</sup>   | 1.34±0.07 <sup>bc</sup> | 10.3±1.1              | 14.1±0.6 <sup>a</sup>  | 4.5±0.7 <sup>a</sup>  | 29.0±0.6 <sup>a</sup>  |
|            | MA        | 13.3±1.2 <sup>c</sup>   | 8.3±0.7 <sup>c</sup>   | 22.5±2.9 <sup>c</sup>   | 1.62±0.04 <sup>ab</sup> | 12.3±0.3              | 14.2±3.4 <sup>a</sup>  | 4.2±0.3 <sup>ab</sup> | 30.7±1.4 <sup>a</sup>  |
|            | Mean      | 24.6±0.9 <sup>d</sup>   | 17.4±0.8 <sup>d</sup>  | 42.6±5.7 <sup>d</sup>   | 1.44±0.04 <sup>a</sup>  | 11.0±1.4 <sup>a</sup> | 12.9±0.8 <sup>a</sup>  | 3.9±0.4 <sup>a</sup>  | 27.9±2.3 <sup>a</sup>  |
| Véraison   | AL        | 32.8±2.9                | 28.8±2.1 <sup>d</sup>  | 61.7±5.0                | 1.14±0.02               | 8.4±0.8               | 13.8±0.7 <sup>a</sup>  | 3.0±0.2 <sup>bc</sup> | 25.2±1.2 <sup>a</sup>  |
|            | MH        | 34.6±5.4                | 33.0±3.5 <sup>cd</sup> | 67.7±7.5                | 1.11±0.06               | 7.0±0.6               | 8.9±0.2 <sup>b</sup>   | 1.9±0.1 <sup>c</sup>  | 17.8±1.8 <sup>b</sup>  |
|            | IS        | 38.5±2.3                | 37.8±1.5 <sup>bc</sup> | 76.3±2.8                | 1.02±0.02               | 8.0±0.6               | 9.7±0.7 <sup>ab</sup>  | 2.8±0.0 <sup>bc</sup> | 20.5±0.2 <sup>ab</sup> |
|            | IT        | 43.9±2.6                | 44.0±1.9 <sup>a</sup>  | 87.9±9.5                | 1.11±0.06               | 8.5±0.4               | 11.6±1.5 <sup>ab</sup> | 5.1±0.2 <sup>a</sup>  | 25.2±0.7 <sup>a</sup>  |
|            | MA        | 42.6±3.5                | 42.2±3.0 <sup>ab</sup> | 84.8±3.4                | 1.12±0.09               | 11.0±0.7              | 10.1±2.2 <sup>ab</sup> | 4.3±0.1 <sup>ab</sup> | 25.4±1.5 <sup>a</sup>  |
|            | Mean      | 38.5±1.3 <sup>c</sup>   | 37.2±6.1 <sup>c</sup>  | 75.9±8.6 <sup>c</sup>   | 1.10±0.08 <sup>b</sup>  | 8.6±0.8 <sup>b</sup>  | 10.8±1.5 <sup>b</sup>  | 3.4±0.1 <sup>ab</sup> | 22.8±2.8 <sup>b</sup>  |
| Early ripe | AL        | 90.0±5.1 <sup>a</sup>   | 93.1±6.4 <sup>a</sup>  | 183.1±6.4 <sup>a</sup>  | 0.97±0.07 <sup>b</sup>  | 6.3±0.7               | 5.2±0.5 <sup>a</sup>   | 0.5±0.3 <sup>b</sup>  | 11.9±0.8               |
|            | MH        | 76.6±6.4 <sup>b</sup>   | 72.7±3.4 <sup>b</sup>  | 149.3±4.9 <sup>b</sup>  | 1.05±0.01 <sup>b</sup>  | 5.7±0.4               | 4.5±0.0 <sup>b</sup>   | 0.4±0.2 <sup>b</sup>  | 10.6±0.5               |
|            | IS        | 71.2±3.8 <sup>c</sup>   | 70.1±5.2 <sup>bc</sup> | 141.2±3.9 <sup>bc</sup> | 1.02±0.05 <sup>b</sup>  | 6.4±0.5               | 4.9±0.6 <sup>ab</sup>  | 0.9±0.2 <sup>a</sup>  | 12.2±0.7               |
|            | IT        | 90.2±1.1 <sup>a</sup>   | 85.9±1.8 <sup>a</sup>  | 176.2±5.1 <sup>a</sup>  | 1.05±0.04 <sup>b</sup>  | 6.1±0.3               | 4.3±0.5 <sup>b</sup>   | 0.6±0.4 <sup>b</sup>  | 11.1±0.7               |
|            | MA        | 75.5±3.7 <sup>bc</sup>  | 64.9±2.9 <sup>c</sup>  | 140.4±5.5 <sup>c</sup>  | 1.16±0.02 <sup>a</sup>  | 6.0±0.4               | 4.5±0.3 <sup>b</sup>   | 0.6±0.0 <sup>b</sup>  | 11.0±0.4               |
|            | Mean      | 80.7±8.4 <sup>b</sup>   | 77.3±8.1 <sup>b</sup>  | 158.1±8.2 <sup>b</sup>  | 1.05±0.07 <sup>b</sup>  | 6.1±0.4 <sup>c</sup>  | 4.7±0.6 <sup>c</sup>   | 0.6±0.1 <sup>c</sup>  | 11.4±1.3 <sup>c</sup>  |
| Ripe       | AL        | 93.6±7.1 <sup>ab</sup>  | 94.1±3.5 <sup>a</sup>  | 187.7±7.6 <sup>bc</sup> | 0.99±0.06 <sup>b</sup>  | 3.8±0.3 <sup>b</sup>  | 3.6±0.4                | 0.4±0.1               | 7.8±0.6 <sup>ab</sup>  |
|            | MH        | 107.0±3.3 <sup>a</sup>  | 91.9±2.7 <sup>a</sup>  | 198.9±4.1 <sup>a</sup>  | 1.10±0.04 <sup>ab</sup> | 4.2±0.2 <sup>b</sup>  | 2.8±0.1                | 0.3±0.0               | 7.3±0.5 <sup>b</sup>   |
|            | IS        | 100.8±6.1 <sup>ab</sup> | 80.4±4.1 <sup>b</sup>  | 181.2±5.0 <sup>c</sup>  | 1.19±0.03 <sup>a</sup>  | 5.2±0.5 <sup>a</sup>  | 3.4±0.3                | 0.3±0.0               | 8.9±0.7 <sup>a</sup>   |
|            | IT        | 86.4±1.8 <sup>b</sup>   | 73.1±3.5 <sup>b</sup>  | 163.5±8.2 <sup>d</sup>  | 1.12±0.09 <sup>ab</sup> | 4.8±0.5 <sup>a</sup>  | 3.1±0.5                | 0.2±0.0               | 8.1±1.1 <sup>a</sup>   |
|            | MA        | 104.9±2.3 <sup>a</sup>  | 88.5±5.3 <sup>a</sup>  | 197.8±13.3 <sup>a</sup> | 1.09±0.09 <sup>ab</sup> | 5.0±0.6 <sup>a</sup>  | 3.0±0.4                | 0.3±0.2               | 8.3±1.2 <sup>a</sup>   |
|            | Mean      | 98.5±9.1 <sup>a</sup>   | 85.6±8.5 <sup>a</sup>  | 185.9±7.4 <sup>a</sup>  | 1.98±0.11 <sup>b</sup>  | 4.6±0.3 <sup>d</sup>  | 3.2±0.4 <sup>d</sup>   | 0.3±0.0 <sup>c</sup>  | 8.1±0.6 <sup>d</sup>   |

AL: 'Alphonse Lavallée'. MH: 'Muscat of Hamburg'. IS: 'Isabella'. IT: 'Italia'. MA: 'Muscat of Alexandria'. Cultivar means on columns of each stage with distinct letters were significantly different.

highest total sugar content was detected in MH (198.9 g L<sup>-1</sup>), and the lowest in IT (163.5 g L<sup>-1</sup>). At the unripe stage of berry maturation the glucose/fructose (G/F) ratio ranged from 1.29 (MH) to 1.66 (IS). Remarkable declines in G/F ratios were observed up to véraison. During this period, the highest reduction was observed in IS (*V. labrusca*), which decreased from 1.66 to 1.02, surprisingly. At the early ripe stage, there was a near-unity G/F ratio, with a general mean of 1.05. However, slight increases were observed between the early ripe and the ripe stages, varying from 0.99 (AL) to 1.19 (IS), with the mean value of 1.09 between cultivars. The highest G/F ratio was obtained in the IS cultivar, at both the unripe and ripe stages.

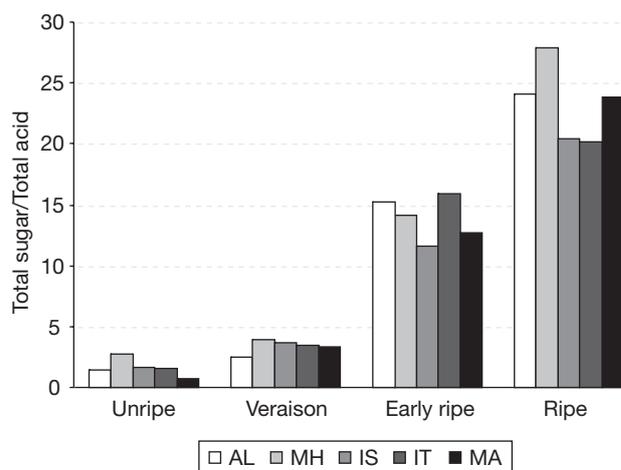
### Distribution of organic acids during berry maturation

Tartaric, malic, citric, and total acid content of cultivars at different stages of berry maturation are also shown in Table 2. The tartaric acid values of unripe samples were very similar, ranging from 10.3 (IT) to 12.3 g L<sup>-1</sup> (MA), although the differences are statistically insignificant. Tartaric acid content of all cultivars decreased gradually throughout the maturation period, inversely to sugar content. No significant difference was seen in terms of tartaric acid up to maturity. Nevertheless, the tartaric acid content between cultivars was significantly different at the ripe stage, varying from 3.8 (AL) to 5.2 g L<sup>-1</sup> (IS), with a mean value of 4.6 g L<sup>-1</sup>.

At the unripe stage, there were significant differences in the malic acid content between cultivars, varying from 9.1 (MH) and 15.1 g L<sup>-1</sup> (AL). After véraison, malic acid content decreased sharply. The highest malic acid content overall was detected in AL, with a final value of 3.6 g L<sup>-1</sup>. With regard to citric acid, at the unripe stage, MH varied from the other cultivars with a relatively lower content (2.3 g L<sup>-1</sup>), while in the rest of the cultivars the citric acid content varied between 4.1 and 4.5 g L<sup>-1</sup>. The most remarkable decrease occurred in IT, whose citric acid content changed from 4.5 (unripe stage) to 0.2 g L<sup>-1</sup> (ripe stage).

At the unripe stage, total acid content of the cultivars ranged from 21.8 (MH) to 30.7 g L<sup>-1</sup> (MA). Total acid content of the cultivars, however, was fairly similar at the final analysis, ranging from 7.3 (MH) to 8.9 g L<sup>-1</sup> (IS).

The changes in total sugar/total acid ratio during berry maturation are shown in Figure 1. Little increase

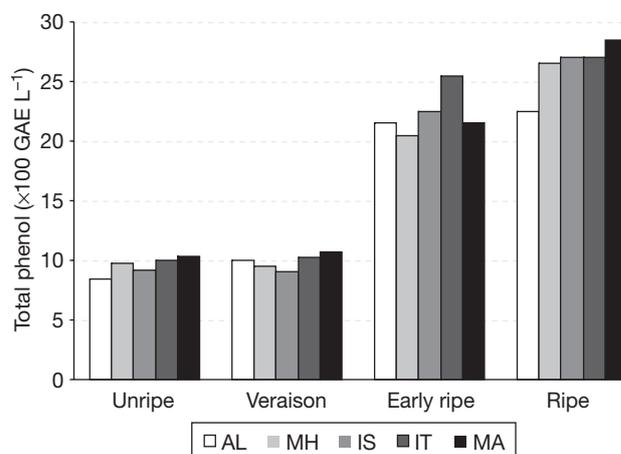


**Figure 1.** Total sugar/total acid ratio of cultivars at different stages of berry maturation.

was observed in the overall total sugar/total acid ratio between the unripe stage and véraison, while a sharp acceleration took place between véraison and the early ripe stage. At the ripe stage, the total sugar/total acid ratio was significantly different between the cultivars, varying from 20.2 (IT) to 27.9 (MH).

### Distribution of total phenol during berry maturity

At the unripe stage, total phenol content revealed by the Folin-Ciocalteu assay was lower than 1,000 mg L<sup>-1</sup> (GAE L<sup>-1</sup>), and little changes occurred up to véraison (Fig. 2). However, total phenol content of cultivars accelerated after véraison, exceeding the 2,000 mg L<sup>-1</sup>



**Figure 2.** Total phenol content of cultivars at different stages of berry maturation.

level. At the early ripe stage, IT stood out with a significantly higher phenol content. At the ripe stage, phenol content ranged from 2,253 (AL) to 2,847 mg L<sup>-1</sup> (MA).

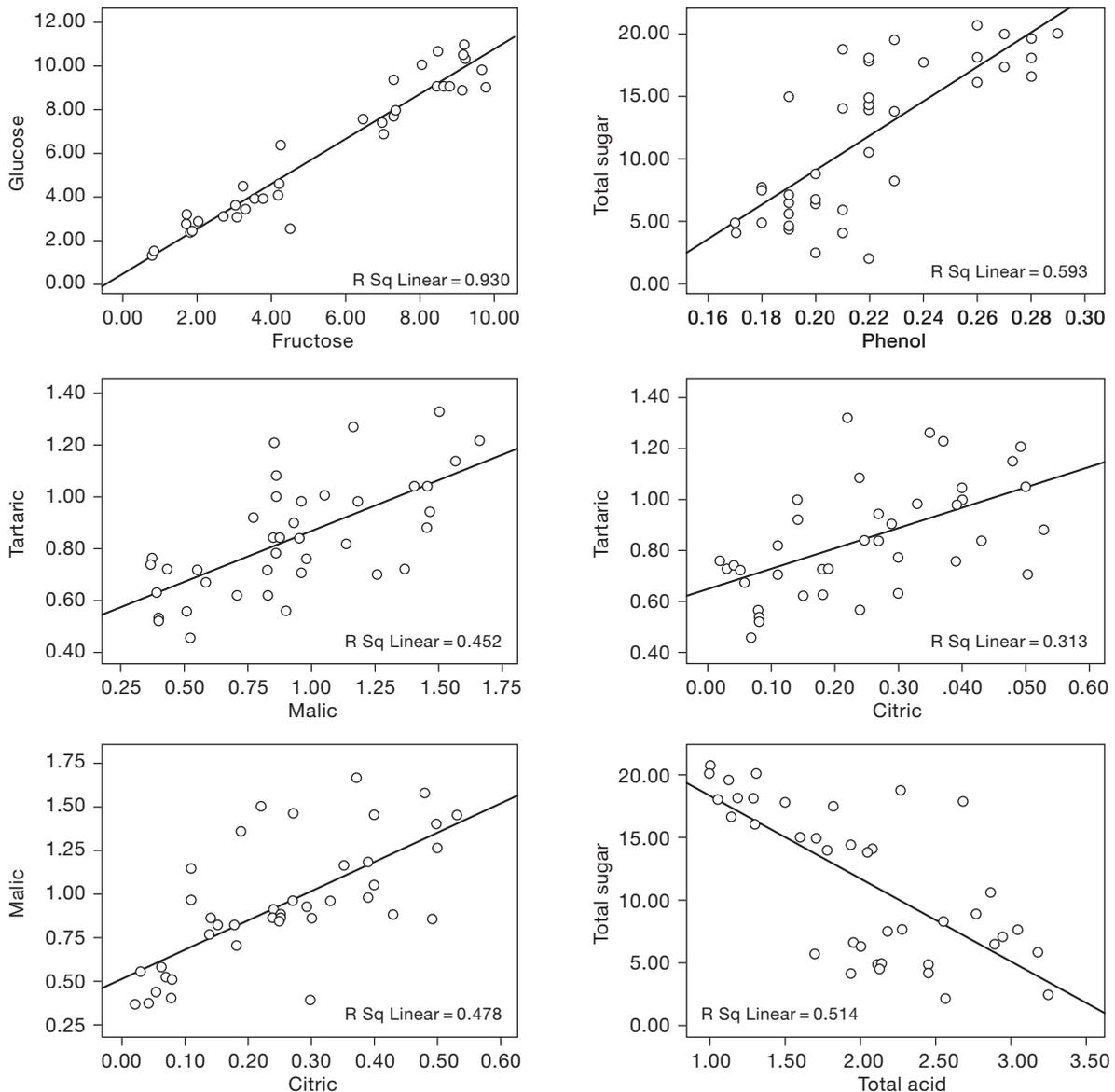
### Correlation between sugars, acids and phenols

According to the regression analysis shown in Figure 3, there was a high correlation between the glucose and fructose values ( $r^2 = 0.930$ ). There was also a significant correlation between total sugar and total phenol content ( $r^2 = 0.593$ ). Among the organic acids,

the highest correlation was observed between malic and citric acid ( $r^2 = 0.478$ ), followed by the correlation of tartaric and malic acid ( $r^2 = 0.452$ ), whereas a weak linear correlation was found between tartaric and citric acid ( $r^2 = 0.313$ ), at a significance level of  $p < 0.01$ . On the other hand, there was a negative correlation between total sugar and total acid content ( $r^2 = 0.514$  at  $p < 0.01$ ).

### Discussion

Grape berries exhibit a double sigmoid growth pattern (Coombe, 1992). As previously reported, the most



**Figure 3.** Regression analyses between the sugars, phenols and acids of the grape cultivars.

dramatic changes in the chemical composition of berries take place at the beginning of véraison (Coombe, 1992; Jackson and Lombard, 1993; Artés-Hernández *et al.*, 2003; Rusjan *et al.*, 2008). With regard to the period of massive sugar accumulation, grapevine cultivars displayed differences in their response aptitudes to the same ecological factors. For instance, AL displayed very little increase in glucose accumulation after the early ripe stage, while there was a slight loss of glucose content in IT. This is indicative of IT's sensitivity to delays in harvest time. On the other hand, glucose synthesis of the remaining cultivars underwent significant increases after this stage. A similar case was demonstrated by Liu *et al.* (2006), who emphasized differences in biochemical reactions among cultivars when analyzing the sugar and acid contents of 98 grape cultivars. The fructose accumulation processes of the cultivars were very similar to those of glucose. It should be emphasized that a fructose reduction was also detected in IT after early ripe stage analysis, coinciding with the results for glucose in this cultivar. Special attention is therefore required in the determination of the optimum harvest stage for IT.

Artés-Hernández *et al.* (2003) investigated the total sugar content of 'Autumn Seedless' at harvest time in Spain for two consecutive years, obtaining values of 201 and 213 g L<sup>-1</sup>, respectively. Similarly, Rusjan *et al.* (2008) previously reported an average value of 154 g L<sup>-1</sup> in red grapes grown in Slovenia. The results of the present study are close to those of Artés-Hernández *et al.* (2003), possibly due to similar growing conditions of the Mediterranean region. But they considerably differed from those of Rusjan *et al.* (2008), most likely the result of cultivation conditions as well as varietal differences. On the other hand, there were no significant differences between the red and white grapes analyzed in this study with regard to sugar accumulation, a result which is in contrast to the assertion set forth by Rusjan *et al.* (2008) who obtained a higher amount of total sugar in red cultivars.

The literature consulted revealed a great variation between studies on G/F value. For example, Soulis and Avgerinos (2006) reported that the G/F ratio varied from 1.95 (at unripe stage of the berry) to 1.55 (over-ripe stage), while Kliewer (1967) and Artés-Hernández *et al.* (2003) found variations between 0.74-1.05 and 0.98-1.05, respectively. The final values of the present study were closer to those of the two latter studies, while the findings of the former work were far higher. Such disparities confirm that growing conditions as

well as genotypic differences have a great impact on the glucose and fructose proportions of grape cultivars. Physiologically, the differences seen in glucose and fructose accumulation between cultivars could be associated to the level of activity of the enzymes involved in the sugar metabolism of certain genotypes, such as invertase, and sucrose phosphate synthesis as previously indicated by Conde *et al.* (2007). Sucrose moves from the phloem to the berries where it is hydrolyzed to glucose and fructose by invertase, but glucose could also have its origin in starch (Takayanagi and Yokotsuka, 1997). Therefore, specific responses of certain cultivars to distinct ecological conditions should be scrutinized in order to obtain a favorable grape quality with respect to utility purposes.

The evaluation of organic acid content in grapes is one of the most significant quality criteria as it indicates the suitability of a cultivar for certain uses and it also reflects the berries' metabolic activities during growth (Lamikanra *et al.*, 1995). Tartaric and malic acid decrease during berry maturation is generally attributed to an increase in membrane permeability allowing acids stored in cell vacuoles to be respired (Kliewer, 1967), and the transformation of acids to sugars (Winkler *et al.*, 1974), apart from many other physiological processes that take place inside the cells. The final tartaric acid values of this study are quite similar to those of Liu *et al.* (2006) who reported mean tartaric acid values between 3.8 and 4.3 g L<sup>-1</sup> for two consecutive years, analyzing wider genotypes of *Vitis* (*V. vinifera*, *V. labrusca*, and hybrids with *V. thunbergii* and *V. amurensis*). In the juice of the analyzed cultivars, malic acid content was predominant, although its amount decreased during berry development proportionally to the increases in sugar content. These relationships are in accordance with the assertion set forth by Coombe (1987) who analyzed the changes of different chemical components in grape berries at four stages of development. At the unripe stage, malic acid content was higher than tartaric acid content overall. But the decrease rate of malic acid was greater than that of tartaric acid, in agreement with several reports (Ruffner *et al.*, 1983; Possner and Kliewer, 1985). The decrease of organic acid content that begins at the onset of ripening is mainly associated with a sudden induction of malate oxidation (Coombe, 1992). In this study, malic acid content of the cultivars in the ripe stage was significantly lower than that of tartaric acid. Malate is mostly used as an energy source in the berry during maturation; therefore its levels decrease faster than

that of tartrate (Jackson and Lombard, 1993). On the other hand, tartaric and malic acids are synthesized and degraded by different biochemical processes, despite their structural similarities (Liu *et al.*, 2006; Conde *et al.*, 2007).

Although citric acid is defined as one of the minor constituents of the grape berry, it significantly contributes to the acidity of must (Mato *et al.*, 2005). Decreases in citric acid content were not consistent between genotypes. For instance, the highest citric acid amount at the unripe ripe stage was detected in IT (4.5 g L<sup>-1</sup>), although the same cultivar had the lowest value at the ripe stage. The values observed in the ripe stage were lower than the results obtained by Kanellis and Roubelakis-Angelakis (1993), and were adjusted with those of Soyer *et al.* (2003) and Baydar (2006).

Analyzing a total of 259 accessions of different *Vitis* spp., Shiraishi (1995) found wide differences in total acid contents, ranging from 0.36 to 3.95 g/100 mL, while Liu *et al.* (2006) found variations between 4.92-7.19 and 6.54-9.11 g/100 mL in a total of 98 cultivars of *V. vinifera*, *V. labrusca* and hybrids of *V. thunbergii* and *V. amurensis*. The total acid values of the present study are similar to those Liu *et al.* (2006), while the findings of Shiraishi (1995) are far lower. Such a wide variation would be related to the differences among the genotypes, environments, and cultural practices (Jackson and Lombard, 1993), as well as principles of the instrumental analysis methods employed.

The ratio of sugars to organic acids and the amount of phenolic compounds are proven as valuable parameters in determining the quality of table grapes (Soulis and Avgerinos, 2006). In this study, changes in the total sugar to total acid ratio exhibited a similar pattern to those of total phenol content up to the ripe stages of cultivars. Physiologically, Conde *et al.* (2007) explained that the formation of flavors in the ripening grape berry is the result of the balance of the sugar to acid ratio as well as synthesis of flavor and aromatic compounds. The present case indicates the accompanying effects of flavor components and sugar to acid ratio on berry quality.

Immediate increments of total phenols detected after véraison verify the findings of Ramos *et al.* (1999) on the changes of phenols during maturation. At the ripe stage, the lowest amount of total phenol was found in the neutral cultivar IT, while aromatic cultivars displayed similar contents. This simply reflects a natural quantitative difference between cultivars of neutral and flavored juices (Coombe and McCarthy, 2000). In a

recent study, phenol content at maturity varied from 1,757 to 1,429 mg L<sup>-1</sup> in IS, and from 2,873 to 2,588 mg L<sup>-1</sup> in 'Concorde' juice (Gollücke *et al.*, 2009). The results of the present study on total phenols are in partial agreement with the aforementioned research. Nevertheless, it must be underlined that phenols are characterized by a high chemical reactivity that complicates their analysis (Robards *et al.*, 1999; De Beer *et al.*, 2004). Therefore, the findings relevant to phenol content may significantly vary between different studies under separate conditions.

Certain aspects of grape berry growth and ripening processes, particularly the rapid changes in acid and sugar levels during ripening, have been studied in the past. Overall consideration of the results with literature indicates that each cultivar requires specific judgment according to the array of criteria relevant to usage of grapes and their ultimate produces. The results of the present study provided considerable information to understand the sugar, acid and total phenol dynamics of five commercially important grape cultivars throughout the berry maturation period. Therefore, the findings are anticipated to aid grape growers worldwide, as well as provide relevant data for future studies on the biochemistry of grape berries. The results will also be useful for future research on the analysis of the chemical composition of grape juices.

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