20th GiESCO International Meeting
Mendoza, Argentina
November 5th – 10th 2017

20ma Reunión Internacional de GiESCO
Mendoza, Argentina
5 -10 de noviembre de 2017

Book of Full Manuscripts /
Libro de Manuscritos Completos

Organized by / Organizada por
COMBINED EFFECT OF BERRY DENSITY AND HARVEST DATE ON THE ACCUMULATION OF PHENOLIC COMPOUNDS DURING RIPENING OF “ITALIA” TABLE GRAPE CULTIVAR

EFFECTO COMBINADO DE LA DENSIDAD DE BAYA Y FECHA DE VENDIMIA SOBRE LA ACUMULACIÓN DE COMPUERTOS FENÓLICOS DURANTE LA MADURACIÓN DEL CULTIVAR DE UVA DE MESA “ITALIA”

BELVISI, Simonetta; TORCHIO, Fabrizio; NOVELLO, Vittorino; GIACOSA, Simone; DE PALMA, Laura; RIO SEGADE, Susanna; GERBI, Vincenzo; ROLLE, Luca

1Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo Paolo Braccini 2, 10125 Grugliasco (TO), Italy; 2Università Cattolica del Sacro Cuore, Istituto di Enologia e Ingegneria Agro-Alimentare, Via Emilia Piamonte 84, 29122 Piacenza, Italy; 3Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, degli Alimenti e dell’Ambiente, Via Napoli 25, 71100 Foggia, Italy

*These authors contributed equally to the study.

*Corresponding author: susanna.riosegade@unito.it

Abstract

In the last years, consumers are increasingly demanding for healthy foods. Table grapes are considered an important source of bioactive compounds and therefore their availability in the market should last as long as possible while preserving the phenolic quality. The aim of this study was to determine the influence of berry heterogeneity in the vineyard and harvest date on the phenolic profile of Vitis vinifera L. cv. “Italia” table grapes during ripening. Grapes were sampled for five consecutive weeks and the single berries were densitometrically sorted. The results showed that this cultivar is rich in phenolic compounds with health-promoting properties, particularly at early harvest stage (341 and 178 mg/kg berries of total phenols in skin and pulp, respectively). Caffeic acid and p-Coumaroyl-glucose were the most abundant compounds in the skin (28.95 – 51.93 mg/kg) and pulp (6.39 – 17.18 mg/kg), respectively. The evolution of phenolic compounds during berry ripening was modelled using response surface methodology according to the combined effect of sampling date and berry density. The regression models were highly significant for catechin and trans-resveratrol in the skin, and total hydroxycinnamoyltartaric acids in the pulp (R ≥ 0.80). These models could be a valuable tool for better exploitation of maximum accumulation of phenolic compounds in the vineyard. An adapted sampling strategy could be implemented to increase the content of specific bioactive phenolic compounds, thus promoting the nutraceutical quality of fresh table grapes and ready-to-eat fruit salads.

Keywords: Table grapes, Vitis vinifera, phenolic compounds, ripening, berry density

Resumen

En los últimos años, los consumidores demandan cada vez más alimentos saludables. Las uvas de mesa son un fuente importante de compuestos bioactivos y, por lo tanto, su disponibilidad en el mercado debe durar el mayor tiempo posible, preservando a su vez la calidad fenólica. El objetivo de este estudio fue determinar la influencia de la heterogeneidad de las bayas en el viñedo y la fecha de vendimia sobre el perfil fenólico durante la maduración de la uva de mesa “Italia” (Vitis vinifera). Las uvas se muestrearon durante cinco semanas consecutivas y las bayas individuales se clasificaron densitometricamente. Los resultados mostraron que este cultivar es rico en compuestos fenólicos con propiedades promotoras de la salud, particularmente en vendimia temprana (341 y 178 mg/kg bayas de fenoles totales en hollejo y pulpa, respectivamente). El ácido cáustico y p-coumaricil-glucosa fueron los compuestos más abundantes en el hollejo (28.95 – 51.93 mg/kg) y la pulpa (6.39 – 17.18 mg/kg), respectivamente. La evolución de los compuestos fenólicos durante la maduración de las bayas se ajustó a modelos utilizando la metodología de superficie de respuesta, teniendo en cuenta el efecto combinado de la fecha de vendimia y la densidad de las bayas. Los modelos de regresión fueron fuertemente significativos para catequina y trans-resveratrol en el hollejo, y para los ácidos hidroxicinnamónol tartárico totales en la pulpa (R ≥ 0.80). Estos modelos podrían representar una valiosa herramienta para una mejor explotación de la máxima acumulación de compuestos fenólicos en el viñedo. De este modo, sería posible implementar una estrategia de muestreo adaptada para aumentar el contenido de compuestos fenólicos bioactivos específicos, promoviendo así la calidad nutraceutática de las uvas de mesa para consumo fresco y en ensaladas de frutas.

GiESCO Mendoza 2017 - 534
**Palabras clave:** Uva de mesa, *Vitis vinifera*, compuestos fenólicos, maduración, densidad de bayas

**Introduction**

A healthy diet includes the consumption of fresh fruits and, among them, table grapes are a valuable source of phenolic compounds. These compounds are probably responsible for most of the health-promoting effects of grapes as a consequence of their key role in the protection against chronic diseases. In table grapes, the main classes of phenolic compounds are phenolic acids, anthocyanins, proanthocyanidins and stilbenes, which are differently distributed within the grape tissues.

Some authors pointed out that the maturity stage of berries at harvest can greatly affect the overall quality of table grapes in terms of texture, colour and chemical composition (Bainano and Terracane, 2011; Rolle et al., 2015). Particularly, the content of phenolic compounds changes during grape ripening. In the vineyard, each berry can ripen at different rates depending on the position, environmental factors and management of cultural practices. Therefore, the in-field berry variability greatly influences all the quality traits and the consumer acceptance of table grapes (Rolle et al., 2015). Recently, berry densimetric sorting and size have facilitated the separation of berries with different chromatic characteristics, texture parameters, aromatic profiles and phenolic composition (Rolle et al., 2015; Rio Segade et al., 2013).

Table grapes can be consumed fresh, processed as juice or added to salads, drinks and desserts. Hence their presence in the market lasts as long as possible while preserving the quality attributes. In particular, the nutraceutical properties of table grapes play an important role in determining their overall quality and increasingly influence the consumer choice. Therefore, the aim of this work was to study the combined effect of harvest time and berry density on the content of phenolic compounds during ripening of ‘Italia’ seeded white table grape cultivar, and to develop a model using response surface methodology (RSM) that allows the selection of the most suitable harvest date and berry density to achieve the highest accumulation of bioactive phenolic compounds.

**Materials and Methods**

*Grape sampling and selection*

*Vitis vinifera* L. cultivar Italia table grapes were harvested in 2012 from a vineyard located in Puglia region (Southern Italy) for five consecutive weeks. Randomly-sampled single berries with attached short pedicels were densimetrically sorted by flotation in saline solutions ranging from 190 to 80 g/L NaCl (Rolle et al., 2011). In order to evaluate the in-field grape variability and to model the compositional differences, the most represented (according to weight percentage) five density classes for each harvest date were used separately for all subsequent analyses.

*Technological ripeness determination*

For each sample, two sets of 50 berries each were taken and the respective juices were obtained by manual crushing and centrifugation. The juices were used for determining titratable acidity, organic acids and reducing sugars (Rolle et al., 2015). The SSC/TA ratio was calculated as the ratio between the sugar content (as sum of glucose and fructose) and titratable acidity values, both expressed in g/L.

*Extraction and determination of phenolic compounds from berry skin and pulp*

For each sample, three sets of ten berries each were taken, weighed and peeled using a laboratory spatula. The pulp was collected in a flask containing 100 mg of NaNO$_2$, diluted 9:1 (v/v) with 5 mol/L H$_2$SO$_4$, homogenized using a UltraTurrax at 6000 rpm and centrifuged (Rolle et al., 2015). The resulting solution was used for pulp analysis. The skins were immersed into a hydroalcoholic buffer solution (12 % v/v ethanol, 5 g/L tartaric acid, 2 g/L NaNO$_2$, and adjusted to pH 3.2). The skins were homogenized using a UltraTurrax at 8000 rpm and then centrifuged (Rolle et al., 2015). The supernatant was used for skin analysis. Skin and pulp extracts were stored at -20 °C until analysis.

*Spectrophotometric determinations*

For each of the three replicates per sample, all the spectrophotometric determinations were done with a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Total phenol index was evaluated, after sample purification through Sep-Pak C$_{18}$ cartridges, using the Folin-Ciocalteau assay (Singleton and Rossi, 1965; Di
Stefano and Cravero, 1991). The hydroxycinnamoyl tartaric acid (HCTA) index of the pulp extracts was determined following the method proposed by Di Stefano and Cravero (1991).

**HPLC-PDA – ESI-MS/MS determinations**

For each of the three replicates per sample, the preparation of the skin extracts for HPLC analysis consisted in the 2 % addition of 1 mol/L H3PO4. The pulp extracts were purified using Sep-Pak C18 cartridges and methanol as eluent. The methanolic extract was then evaporated to dryness under a N2 stream at 0.5 mL/min flow-rate and 35°C, and the solid residue was re-dissolved in 1 mL of 0.1 % v/v formic acid in water.

The HPLC-PDA-ESI-MS/MS equipment and experimental conditions were reported in the literature (Belviso et al., 2014). The separation was performed on a Kinetex 5 μm Phenyl-Hexyl 100A 150 × 4.6 mm analytical column equipped with a SecurityGuard™ cartridge (Phenomenex, Italy). The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (methanol). PDA spectra were recorded in full scan mode in the λ range from 220 to 600 nm. Masses were recorded in the m/z range of 100-700 amu using the enhanced mass spectrum (EMS) scan experiment.

**Response surface methodology experimental design**

A two-factor experimental design was carried out to model the evolution of the chemical composition during ripening, where the first factor consisted of the five sampling dates and the second factor of the selected five density classes (as juice sugar content). For each parameter studied, the average results of each sampling date-density class combination were considered, and then analysed to fit the second-order polynomial model. The equations were calculated and expressed as surface plots using RSM.

**Results and Discussion**

The second-order polynomial equations were obtained for the different chemical parameters studied. The two independent variables (sugar content and sampling time) had almost exclusively linear effects on the predicted responses, and the interaction effect was very small. Figures 1 and 2 represent the surface plots obtained using RSM for the most significant regressions according to the correlation coefficient (R ≥ 0.80). Figure 1 shows that the SSC/TA ratio can be significantly predicted by the RSM model (R = 0.98). The greatest richness in organic acids corresponded to the berries with the lowest sugar contents that were sampled at the beginning of the ripening process. Consequently, the highest values of SSC/TA ratio were associated with a combination of the highest sugar contents and the latest sampling dates, the sugar content effect being stronger than the sampling date effect. Similar trend was found with the sugar amount in a previous study on Italia berries (Rio Segade et al., 2013).

A total of 14 phenolic compounds were found in Italia table grapes: protocatechuic acid (skin), caftaric acid (skin and pulp), procyanidin B1 (skin), catechin (skin), coumaric acid (skin and pulp), caffeic acid (pulp), trans-ferratic acid (pulp), procyanidin B3 (skin), epicatechin (skin and pulp), p-cumaroyl glucose (pulp), ferulic acid (pulp), rutin (skin), isoquercetin (skin) and trans-resveratrol (skin). Figure 2 shows the modelling of the evolution of total phenol index, contents of catechin and trans-resveratrol in the skin, as well as of the HCTA index in the pulp because of goodness-of-fit. In Italia table grapes, the total phenol index was higher in the skin than in the pulp. The maximum contents of total phenols in the skins were obtained either from the berries rich in sugars at the beginning of the ripening process or from the berries with the lowest contents in sugars at the latest harvest dates (Figure 2a).

Among flavan-3-ols, procyanidin B1 (2.92-6.46 mg/kg, berries) and catechin (Figure 2b) were the most abundant compounds in the skin, followed by procyanidin B2 (0.15-0.97 mg/kg) and epicatechin (0.10-0.24 mg/kg). Crupi et al. (2015) showed that Italia grapes contain relatively high amounts of catechin with respect to other table grape cultivars. trans-Resveratrol was certainly the most studied phenolic compound for its health benefits but, to our knowledge, it is the first time that resveratrol was determined in Italia table grapes (Figure 2c). In the present work, the greatest richness in catechin and trans-resveratrol was achieved in berries with the highest sugar content that were sampled at the beginning of the ripening process. Although catechin contents generally decreased when decreasing the sugar content or delaying the sampling date, relatively high contents were also found in berries with the lowest sugar content at any sampling date. Instead, the positive effect of the sugar content was more accentuated on the trans-resveratrol content than the effect of sampling date. A study revealed a progressive accumulation of trans-resveratrol in several grape varieties (Gatto et al., 2008).
The highest values of HCTA index in the pulp corresponded to the first sampling dates, particularly for sugar contents between 110 and 220 g/L, whereas those lowest were associated with a combination of the latest sampling dates and the highest sugar contents (Figure 2d). At any sampling date, an increasing trend of HCTA index was observed with the increase in the sugar content up to 160 g/L, and then it decreased. In general, the value of HCTA index decreased with delaying the sampling date. Another study performed on Italia berry pulp showed that the values of HCTA index increased when increasing the berry density, but the differences were not significant (Río Segade et al., 2013). However, the increase was significant for Muscat Hamburg table grapes (Rolle et al., 2015).

Italian berries were particularly rich in isoquercitrin, and therefore they could be taken into consideration as an interesting source of this bioactive compound. Isoquercitrin prevailed among flavonols (3.52–15.41 mg/kg berries), displaying the highest content in the skin on sampling day 21. The contents of isoquercitrin found in this study were in the range reported in literature for the skin of the same cultivar (Capriotti et al., 2012). However, the results observed were not adequately fitted into second-order polynomial models for the flavonols detected.

Regarding hydroxyxannamic acids, caftaric acid (> 37 mg/kg berries) was the most abundant compound in both skin and pulp, followed by caftaric acid (> 2 mg/kg), although they were mainly located in the skin. The compound identified as p-coumaroyl-glucose was found in the pulp at contents (6.39–17.18 mg/kg) comparable to those of caftaric acid. The RSM model cannot significantly predict the content of these compounds.

Once reached the minimum ripeness threshold established by the OIV resolution V111 1/2008 for table grapes (SSC/TA > 20), the maximum contents of total phenols, catechin and trans-resveratrol in Italia berry skins can be obtained from the berries rich in sugars at the beginning of the ripening process. Instead, the maximum contents of HCTA in the pulp can be reached at early harvest by selecting berries with sugar contents ranging from 110 to 220 g/L.

Conclusion

The knowledge of grape phenolic profile during ripening permits to determine the best harvest date to exploit the maximum accumulation in the vineyard of specific bioactive compounds. In this sense, modelling the evolution of chemical composition of table grapes during ripening, taking into account the simultaneous effects of sugar content and harvest date, allows a better selection of the berries according to real quality objectives, such as health-promoting properties. Depending on which compounds to potentiate, it is possible to implement certain sampling strategies that contribute to the valorisation of the Italia cultivar.

References


CRUPI P., BERGAMINI C., PERNOTA L., DIPALMO T., CLODOVEO M.L., ANTONACCI D., 2015. A chemometric approach to identify the grape cultivar employed to produce nutraceautical fruit juice. European Food Research and Technology, 241, 487–496.


Figure 1. Modelling SSC/TA ratio of Italia table grapes with different sugar content during ripening

Figure 2. Modelling total phenol index (a), catechin (b), trans-resveratrol (c) in the skin and HCTA index (d) in the pulp of Italia table grapes with different sugar content during ripening