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Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/111422 since 2016-07-05T08:23:33Z

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which has been published in final form at
EFFECT OF THE CLUSTER HETEROGENEITY ON MECHANICAL PROPERTIES, CHROMATIC INDICES AND CHEMICAL COMPOSITION OF ITALIA TABLE GRAPE BERRIES (VITIS VINIFERA L.) SORTED BY FLOTATION

Running title: Quality characteristics of Italia table grape

Susana Río Sega,¹ Simone Giacosa,¹ Laura de Palma,² Vittorino Novello,³ Fabrizio Torchio,¹ Vincenzo Gerbi¹ & Luca Rolle¹

¹Università degli Studi di Torino, DIVAPRA - Settore di Tecnologie Alimentari, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy.

²Università degli Studi di Foggia, Dipartimento di Scienze Agro-Ambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy.

³Università degli Studi di Torino, Dipartimento di Colture Arboree, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy.

*Author to whom correspondence should be addressed. Telephone: +39 0116708558; fax: +39 0116708549; email: susana.riosegade@unito.it.
The impact of cluster heterogeneity on mechanical properties, chromatic indices and chemical composition of Italia table grape berries sorted by flotation was evaluated in this study. The density sorting at commercial harvest permitted to get berries of different ripeness and relatively different quality attributes.

Individually, some grape chromatic characteristics (yellow/blue colour component and chroma), physical characteristics (weight of the whole berry and berry skin), content and composition of reducing sugars, organic acids and phenolic compounds (flavanols of low molecular mass in berry skins) permitted to characterize and to differentiate berries belonging to different density classes. However, the tested mechanical properties were not related to the berry density. When the variables that significantly contributed to the berry differentiation were globally assessed, the chemical parameters related to sugars (142.3-164.4 g kg\(^{-1}\)), the content of skin flavanols (46.3-137 mg (+)-catechin kg\(^{-1}\)) and the berry skin weight (379-607 mg) were the more representative.

*Keywords*: table grapes, berry density, instrumental texture analysis, chromatic characteristics, phenolic composition.
Introduction

Table grape is one of the most consumed fruits in the world and, according to the International Organization of Vine and Wine statistics, the production for fresh consumption has been increasing in the last years.

The consumer acceptability of table grapes depends on different attributes. Visual characteristics and physical-chemical properties are involved in the sensory and quality evaluation of table grapes (Cliff et al., 1996; Crisosto and Crisosto, 2002; Liu et al., 2006; Peppi et al., 2006; Jayasena and Cameron, 2008, 2009; Piva et al., 2008). Visual attributes such as colour, size and shape of the berry are primary characteristics that consumers observe (Cliff et al., 1996; Zeppa et al., 1999). Furthermore, the texture is an important attribute in consumer acceptance of foods (Tunick, 2011), particularly fresh fruits. Sensory descriptors such as skin friability, skin thickness and flesh firmness have been proposed to characterize commercial table grape cultivars (Cliff et al., 1996; Vargas et al., 2001). Nowadays, the nutritive and functional value of foods is becoming a determining factor in the consumer preference (Valero et al., 2006). Therefore, together with the chemical parameters that define the technological maturity (sugars and organic acids) (Liu et al., 2006; Muñoz-Robredo et al., 2011), phenolic compounds also constitute a key factor in the grape quality because they are relevant contributors for multiple biological effects like antioxidant activity (Baiano and Terracone, 2011). Ripeness grade, soil type, climatic conditions, agronomical practices and growing location are important factors affecting the physical properties and chemical composition of table grapes, but the phenolic composition strongly depends on the table grape variety (Revilla et al., 1995; Baiano and Terracone, 2011).

Many physicochemical parameters are correlated with sensory descriptors and consumer preferences, and can be used as predictors of the consumer acceptability of a product (Abbott, 1999), such as, in table grapes, soluble solid concentration (SSC), total acidity (TA) and SSC/TA ratio (Dokoozlian et al., 1995; Jayasena and Cameron, 2008). Instrumental texture analysis is nowadays a well established analytical
technique for evaluating sensory descriptors that are strongly correlated with the mechanical parameters measured (Sato et al., 1997; Le Moigne et al., 2008). For table grapes, particular attention has been focused on the study of the mechanical properties of the pulp. In fact, the crispness is the most desirable texture for fresh consumption, and the cultivars with a crisp flesh texture are important genetic materials for table grape breeding (Sato et al., 2006). The instrumental assessment of the pulp compactness and berry skin consistency also provides relevant information for customer acceptance of the product (Mencarelli et al., 1994; Uys, 1996; Sato et al., 1997; Sato and Yamada, 2003), and the firmness of the berry is considered as an indicator of its freshness (Vargas et al., 2001). Texture profile analysis (TPA) has been proposed to assess the shelf life of table grapes, and the hardness, springiness, cohesiveness and chewiness were the mechanical parameters used to define the pulp quality (Deng et al., 2005). Moreover, the effect of cultural practices (Sato et al., 2004) and post-harvest conditions (Deng et al., 2005) on the instrumental texture parameters of table grapes, and even the variety effect on the mechanical properties of the whole berry and berry skin have been assessed (Rolle et al., 2011b, 2012a). The use of multivariate analysis is of great importance for the global evaluation of all the parameters studied (Arvanitoyannis et al., 1999; Arvanitoyannis, 2010). However, to our knowledge, there are not published data concerning the influence of the grape ripeness on the berry mechanical properties.

Cv. Italia gives the most popular and common table grape in Italy (65% of the total production) and most of its production is exported to European countries. Very few works have been published on the chemical composition and texture properties of this table grape variety.

The aim of this work was to characterize Italia table grape according to the chemical composition, chromatic attributes, physical characteristics and mechanical properties at harvest. To take into account the bunch heterogeneity, three different ripeness stages were considered sorting the berries by flotation. This knowledge can be of great interest for the “fresh-cut” industry that uses the table grape berries in the production of “ready to eat” fruits salad.
Materials and methods

Grape samples

The study was carried out, in 2010, on the white-berry Italia cultivar (*Vitis vinifera* L.) grown at a commercial vineyard in the North-West of the Bari province (Apulia Region, Southern Italy, 41°9′0″N 16°24′0″E, 230 m a.s.l.). The vines, grafted onto ‘140 Ru’ rootstock, were planted at 2.4 x 2.4 m, and trained to tendone system ‘Puglia’ type. At winter pruning, the vines were cane pruned with four canes of 10/12 buds each one. The grape commercial harvest occurred on 20 September when the sugar content was 16.8 °Brix.

The bunches (n = 25) were randomly sampled from ten plants. Once in the laboratory, all the berries of each cluster were manually separated from the stalk, sorted according to their density by flotation in different saline solutions (from 90 to 140 g L⁻¹ sodium chloride, corresponding to densities comprised between 1060 and 1092 kg m⁻³) as described by Rolle *et al.* (2011a), and the berries belonging to each class were then weighed. The study was carried out separately on the berries belonging to the three predominant density classes that were: A = 1062 kg m⁻³, B = 1067 kg m⁻³ and C = 1069 kg m⁻³, as they represent 15.6±1.6, 55.6±1.1 and 18.8±0.9 % weight percentage, respectively. All remaining classes account for approximately 10% weight percentage. The sorted berries were washed with water and visually inspected before analysis; those with damaged skins were discarded.

According to Rolle *et al.* (2011b), for each density class, three sub-samples of 10 sorted berries were used for the determination of the mechanical properties of the whole berry and of the berry skin. Three sub-samples of 7 sorted berries were used for the determination of the peduncle detachment resistance. Other three sub-samples of 10 sorted berries were used for the determination of the chromatic attributes. Three sub-samples of 10 sorted berries were used for the determination of the phenolic
composition and physical characteristics. The weight of each single berry for each sub-sample of 10 sorted berries, of its skins and of its seeds was determined using a technical balance (Gibertini E1700, Modena, Italy). Finally, the remaining berries (3 replicates) were used for determining standard physicochemical parameters in the grape must obtained by manual crushing and centrifugation.

Instrumental texture analysis

For instrumental texture analysis, a Universal Testing Machine (UTM) TAxT2i Texture Analyzer (SMS-Stable Micro System, Godalming, Surrey, UK) equipped with a HDP/90 platform was used. All the data were acquired at 400 Hz and evaluated using the Texture Expert Exceed software, version 2.54 for Windows 2000. All measurements were performed in the same day as picking to avoid changes. Before each test session, the instrument was calibrated for force and distance, and the berries, arranged as a single layer, were thermally conditioned for 1 hour at 20°C in a thermostatically controlled chamber.

The berry skin hardness was assessed by a puncture test using a SMS P/2N needle probe, a 5 kg load cell and a speed test of 1 mm s⁻¹ (Letaief et al., 2008a). Each one of the 30 berries was individually punctured in the lateral face and three parameters were measured: skin break force (N, as $F_{sk}$), skin break energy (mJ, as $W_{sk}$) and skin Young’s modulus of elasticity (N mm⁻¹, as $E_{sk}$). The first variable corresponds to the skin resistance to the needle probe penetration while the second variable is represented by the area under the force/time curve, which is limited between 0 and $F_{sk}$. The third one is defined as the slope of the stress-strain curve in the linear section and measures the stiffness of the skin to a load applied.

The measurement of the berry skin thickness (μm, as $S_{sk}$) required manual separation of a piece of skin (ca. 0.25 cm²) from the lateral side of each berry with a razor blade, followed by drying with absorbent paper. The test was carried out using a 2-mm SMS P/2 flat cylindrical probe and a speed test of 0.2 mm s⁻¹ (Letaief et al., 2008b). The berry skin thickness is calculated as the distance between the point
corresponding to the probe contact with the berry skin (trigger) and the platform base during the compression test (Río Segade et al., 2011b). Care was taken when removing the pulp from the skin and when positioning the skin sample on the UTM platform to prevent folding (Río Segade et al., 2011b). Furthermore, the insertion of an instrumental trigger threshold equal to 0.05 N enabled the plane surface of the probe to adhere completely to the skin sample before the acquisition began. It allowed a reduction or even elimination of the ‘tail’ effect due to the postponement of the contact point (Letaief et al., 2008b).

For the Texture Profile Analysis (TPA) or double compression test, each one of the 30 whole berries was compressed in the equatorial position with a 35-mm SMS P/35 flat cylindrical probe under 25% deformation, with a waiting time of two seconds between the two bites and a speed test of 1 mm s⁻¹ (Letaief et al., 2008b). Typical berry mechanical parameters that define the whole berry texture characteristics, i.e. hardness (N, as BH), cohesiveness (adimensional, as BCo), gumminess (N, as BG), springiness (mm, as BS), chewiness (mJ, as BCh) and resilience (adimensional, as BR), were calculated by the software (Deng et al., 2005; Letaief et al., 2008b). In this type of compression test, the influence of the pulp and skin properties on the mechanical characteristics of berries is aggregate (Grotte et al., 2001). The berry diameter was calculated as the distance between the whole berry trigger point and the platform base. Since some TPA parameters can be influenced by the berry size, they were also normalized according to the respective berry diameter (norm) expressed in mm (Santini et al., 2011).

The peduncle detachment resistance was determined by a traction test carried out at 1 mm s⁻¹ (Rolle et al., 2009). In this test, the peduncle is anchored to the pliers of the SMS A/PS probe modified with a rigid arm. During the traction, the peduncle passes through the perforated platform of the UTM (hole diameter of 5 mm), while the berry is blocked permitting the determination of the peduncle detachment maximum force (N, as Fped). The peduncle detachment energy (mJ, as Wped) is represented by the area under the force/time curve, which is limited between 0 and Fped.

Colour measurement and indices
Before colour analysis, the bloom was removed from the skin using paper tissue (Rolle and Guidoni, 2007). Three measurements were made around the equatorial belt of each berry. L* (lightness), a* (red/green colour component), b* (yellow/blue colour component), C (chroma) and H (hue angle) were determined (CIE, 1986) with a Minolta CR-410 Chroma Meter (Minolta Corporation, Osaka, Japan) reflectance spectrophotometer. Standard illuminant D65 was used as reference. Two indices were also calculated: CIRG1 (colour index of red grapes) = (180-H) / (L*+C) and CIRG2 = (180-H) / (L*xC) proposed as colorimetric index for table grapes (Carreño et al., 1995).

Chemical analysis

Reagents and standards. Solvents of HPLC-gradient grade and all other chemicals of analytical-reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionised water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Phenol standards (caffeic acid, (+)-catechin, cyanidin chloride) were supplied from Extrasynthèse (Genay, France).

Technological ripeness parameters. Total soluble solids concentration (°Brix, as SSC) was measured with an Atago 0–32 °Brix temperature compensating refractometer (Atago Corporation, Tokyo, Japan), and pH was determined by potentiometry using a Crison electrode (Carpi, Italy). Titratable acidity (TA), expressed as g L⁻¹ tartaric acid, was estimated using the OIV method (2008a). Organic acids (malic acid, tartaric acid and citric acid) and reducing sugars (glucose and fructose) were quantified (as g kg⁻¹ berries) using a P100-AS3000 HPLC system (Thermo Electron Corporation, Waltham, MA, USA), equipped with an UV detector (UV3000) set to 210 nm and a refractive index detector (Waters 2414, Waters Corporation, Milford, MA, USA), respectively. The analyses were performed isocratically at 0.8 mL min⁻¹ flow-rate and
65°C column temperature with a 300 × 7.8 mm i.d. Aminex HPX-87H cation exchange column and a Cation H⁺ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). The mobile phase was 0.0013 mol L⁻¹ H₂SO₄ (Schneider et al., 1987). The data analysis was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA, USA).

Phenol extraction and determination. The berry skins (sk) and seeds (s) were manually removed from the pulp (p) using a laboratory spatula and dried with absorbent paper. The berry skins were quickly immersed in 25 mL of a hydroalcoholic buffer at pH 3.2, containing 5 g L⁻¹ tartaric acid, 2 g L⁻¹ Na₂S₂O₅ and 12% ethanol. The pulp was collected in a beaker containing Na₂S₂O₅ (50 mg). Afterwards, the skins and pulp were separately homogenized with an Ultraturrax T25 (IKA Labortechnik, Staufen, Germany), and centrifuged in a PK 131 centrifuge (ALC International, MI, Italy) for 5 min at 3000 x g at 20°C. The supernatant was then used for analysis (Torchio et al., 2010; Rolle et al., 2011b; Rolle et al., 2012b). The berry seeds, after immersion in 10 mL of the same buffer solution used for skins, were placed in a controlled temperature room at 25ºC for one week (Torchio et al., 2010; Rolle et al., 2012b). Spectrophotometric methods were used to determine total polyphenols (mg (+)-catechin kg⁻¹ grape, as TP) in the berry skin, seeds and pulp, total hydroxycinnamic acids (mg caffeic acid kg⁻¹ grape, as HCTA) in the pulp, and flavanols reactive to vanillin (mg (+)-catechin kg⁻¹ grape, as FRV), total flavonoids (mg (+)-catechin kg⁻¹ grape, as TF) and proanthocyanidins (mg cyanidin chloride kg⁻¹ grape, as PRO) in the skin and seeds (Di Stefano and Cravero, 1991). An UV-1800 spectrophotometer (Shimazu Scientific Instruments Inc., Columbia, MD, USA) was used. The relative standard deviation (RSD) based on repeated analysis (n = 12) of the same sample was 1.58, 1.82, 2.80, 0.93 and 1.74% for TP, HCTA, FRV, TF and PRO, respectively (Torchio et al., 2010; Rolle et al., 2011b).

Statistical analysis
Statistical analyses were performed using the statistical software package SPSS (version 17.0; SPSS Inc., Chicago, IL, USA). The Tukey-b test for $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA). Principal component analysis (PCA) was also used to try the differentiation. The criterion used for the selection of the principal component was higher variance with a confidence level of 95%.

**Results and discussion**

**Instrumental texture analysis**

Instrumental texture parameters of the whole berry and the berry skin, as well as the peduncle detachment resistance, for Italia table grapes picked at commercial harvest and density sorted to get berries of different ripeness grades are shown in Table 1. An increasing tendency was observed in the parameters that define the berry skin hardness ($F_{sk}$ and $W_{sk}$), the skin stiffness ($E_{sk}$) and the peduncle resistance to detachment ($F_{ped}$ and $W_{ped}$) with increasing berries density. The greatest differences were found among berries belonging to the two classes of lower density (A and B), with the exception of $W_{ped}$. On the other hand, the thinnest skins corresponded to the berries of intermediate ripeness level. Furthermore, the highest values of berry hardness, gumminess and chewiness were obtained for these same berries, which also presented the lowest values of the berry resilience. Instead, the berry cohesiveness decreased with density, particularly from the class A to B. Since the berry hardness, gumminess, springiness and chewiness depend on the fruit size, they were normalized according to the respective berry diameter (Santini et al., 2011). A similar behaviour was observed for the berry texture parameters normalized and not normalized (Table 1). However, because of the high dispersion of data, all
these differences in the mechanical properties were not significant. Other works previously published for winegrapes reported that there is no significant change in the instrumental texture parameters of both whole berry and skins with the sugar accumulation (Maury et al., 2009; Torchio et al., 2010; Río Segade et al., 2011a).

Therefore, on the basis of these results, the impact of the cluster heterogeneity on instrumental texture properties was enough limited and only density sorting at 1062 kg m$^{-3}$ could permit a real classification of the berries in two different groups.

Italia table grapes are characterized by harder skins than other table grape varieties analyzed in comparative studies (Rolle et al., 2011b, 2012a). In these works, the average values of F$_{sk}$ ranged between 0.329 N (Black magic) and 0.585 N (Alphonse Lavallée), and the ones of W$_{sk}$ varied between 0.068 mJ (Black Magic) and 0.346 mJ (Regina nera). The skin hardness is a mechanical parameter of great importance for the assessment of the shelf life of table grapes; berries of grape varieties characterized by a higher skin hardness are known to show a slower weight loss (Giacosa et al., 2012), increasing therefore their shelf life. Instead, the skin thickness of the Italia berry was intermediate between the one reported for Matilde (147 µm) and Perlon (305 µm) grapes. The skin thickness is a sensory descriptor proposed to characterize commercial table grape cultivars (Cliff et al., 1996). This characteristic may influence the texture desirability of grapes and, in those varieties with thick skins, if not associated with high skin friability, would limit their commercial acceptance (Cliff et al., 1996). On the other hand, the skin thickness and toughness are factors that contribute to the resistance of table grapes against fungal pathogens (Rosenquist and Morrison, 1988) and to handling injury during harvest, packing, transport and storage (Kök and Çelik, 2004). In the present study, no significant correlations were found between the F$_{sk}$ and S$_{p,sk}$ parameters, in accordance with previous results (Rolle et al., 2011b, 2012a).

Although a high pedicel detachment resistance is not a grape mechanical characteristic as critical in the postharvest preservation of table grapes as during the dehydration process, a higher pedicel detachment facility could cause the irreversible damage of the berry, decreasing its shelf life (Mattheou et
Generally, the detachment force decreases during cold-storage with a halving after 60 days, even if very few data are reported in scientific literature (Deng et al., 2005). At harvest, if compared with Kyoho table grapes (5.7 N), Italia table grapes are characterized by lower values of $F_{ped}$ and this aspect could be particularly favourable to the mechanical removal of the berries from the stalk, the first operation necessary in the use of automatic density sorting equipment (Rolle et al., 2012b).

Instrumental texture parameters of the whole berry evolve during ripening (Vargas et al., 2000) and the changes continue fairly intense throughout postharvest in spite of preservation and packaging (Mencarelli et al., 1994). In particular, the cohesiveness increases during ripening (Le Moigne et al., 2008) and decreases in the postharvest period (Deng et al., 2005). In winegrapes, the double compression parameters seem to be more appropriate than the puncture ones to explain differences in cell tissue mechanics among berries belonging to different density classes. In fact, a decreasing trend was found for all the compression parameters when the sugar content increased (Zouid et al., 2010; Río Segade et al., 2011a; Rolle et al., 2012b), and even the usefulness of the berry cohesiveness as a ripeness predictor has been verified (Río Segade et al., 2011a). However, the mechanical properties of the whole berry did not permit to differentiate Italia table grapes according to the berry density. A possible explanation for the different behaviour of the two types of grapes could be the harder flesh texture of table grapes than that of winegrapes, as previously demonstrated by puncture testing of the pulp (Sato and Yamada, 2003).

If compared the double compression parameters of the whole berry for the Italia variety with those corresponding to different white and coloured table grapes (Rolle et al., 2011b, 2012a), intermediate values of the berry cohesiveness and resilience were obtained for the Italia variety, but higher ones of the berry hardness, gumminess, springiness and chewiness were found with the exception of the berry hardness in coloured Black Magic table grapes. The values of the berry hardness for the Italia variety were higher than those for the Kyoho one (Deng et al., 2005), but the operative conditions used in the TPA tests were different, therefore rendering the comparison of the results difficult. The Italia berries belonging to the density classes B and C showed average values of the berry cohesiveness (~0.520) next to those corresponding to Matilde (0.499) and Sublima seedless (0.500) varieties, which have the lower cohesive
berries among white table grapes analyzed in previous works (Rolle et al., 2011b). The cohesiveness is inversely correlated with sensory quality descriptors such as elasticity, touch resistance and firmness (Le Moigne et al., 2008).

CIEL*a*b* parameters and colour indices

Grape CIEL*a*b* parameters and the colorimetric indices calculated for Italia table grapes sorted at commercial harvest according to density are shown in Table 2. Significantly higher values of the yellow/blue colour component (b*) and chroma (C) were obtained for the berries belonging to the density class B. Unfortunately, this particular behaviour of the chromatic characteristics of the berries sorted by flotation, showing no consecutive changes with the density class, does not permit the berries selection according to colour parameters and indices using only one floating solution.

Italia table grapes belong to the “green-yellow” group according to the OIV code 225 related to the epidermis colour (OIV, 2009), although all the CIELab parameters for the Italia cultivar were different to those found in other white table grapes (Rolle et al., 2011b), and the values of the colour indices disagreed with data reported in the literature for grapes with visual colour “yellow” (Carreño et al., 1995). In particular, the CIRG1 index of the berries subdivided in density classes varied from 1.15 to 1.24, whereas the CIRG2 index varied between 0.06 and 0.08.

In general, the colour indices seem to be varietal dependent and less influenced by the ripening grade, although Mencarelli et al. (1994) have found values of L*, a* and b* different to those reported in Table 2 in Italia grapes harvested at 13 °Brix. However, these differences may partially be imputable to illuminant used in the instrument calibration.

Physical characteristics
Some differences were found in the physical characteristics of sorted Italia table grapes among the three density classes, as it can be seen in Table 3. The weight of the whole berry, that is directly related with that of the skin, increased with increasing the grape density between the density classes A and B. This increase was not significant for the seeds. Furthermore, the results showed that there were no significant weight changes in the whole berry, skin and seeds from the density class B to C.

Chemical analysis

Table 4 shows the parameters that define the grape technological ripeness for Italia table grapes sorted, at commercial harvest, according to density. The values of total soluble solids content (SSC) increased with increasing the berry density, whereas those of titratable acidity (TA) decreased, particularly between the density classes A and B. In winegrapes, a similar behaviour was observed (Rolle et al., 2011a, 2012b) although in this case, unlike the data showed in Table 3, smaller berry sizes were associated with higher values of density.

According to the OIV resolution VITI 1/2008 (OIV, 2008b), white table grapes are considered ripe with values of SSC equal to or higher than 16 °Brix. Jayasena and Cameron (2008), on the Crimson Seedless cultivar, reported that the degree of consumer satisfaction is negatively correlated with the acidity, and that the acceptance increases with increasing SSC from 16 to 20 °Brix. However, this acceptability was not better for SSC values higher than 20 if compared with 20 °Brix. Italia grapes belonging to the density class A reached almost 16 °Brix, whereas the ones included in the density classes B and C can be considered fully ripe.

The SSC/TA ratio was very favourable for the Italia cultivar because its value was higher than 5 for any berry density. It was higher than the one determined in other white and coloured table grapes with
values ranging between 1.34 (Sultanina) and 4.24 (Pizzutello bianco) (Rolle et al., 2011b, 2012a). In fact, the ripening process can also be considered complete for the berries belonging to the density class A because both the SSC average value ranges between 12.5 and 16 °Brix, and the SSC (g L$^{-1}$)/TA (g L$^{-1}$ tartaric acid) ratio is higher than 20 (OIV, 2008b). Some authors reported that the °Brix/acid ratio is a better objective measurement to reflect the consumer acceptability than °Brix or acidity alone, and can be used as a reliable tool to determine the optimum harvesting stage of table grapes (Jayasena and Cameron, 2008). However, the relationship between consumer acceptance and the ratio depends on the TA or SSC range (Crisosto and Crisosto, 2002). Although SSC, TA and SSC/TA ratio are important quality criteria on table grapes consumer acceptance, the consumer sensitivity to them is highly related to ethnic background (Crisosto and Crisosto, 2002) and, therefore, it is not possible to define an universally valid quality standard for table grapes.

Regarding the acid composition, the highest variability among density classes corresponded to the malic acid content that showed lower values for the riper berries. In winegrapes, Fournand et al. (2006) reported that the difference in total sugar content of the berries belonging to two consecutive density classes is ~17 g L$^{-1}$. However, this difference decreased to 6 g kg$^{-1}$ between the density classes B and C.

In terms of the content of reducing sugars, glucose and fructose, an increasing tendency was observed with the berry density, which was more evident between the density classes A and B. These results differed from the slight reductions found in the glucose and fructose contents for Italia grapes in the last stages of their development, probably indicating sensitivity to late harvest (Sabir et al., 2010). The values of the glucose/fructose ratio lower than 1 agreed with the ones usually found at harvest.

The impact of the cluster heterogeneity on technological chemical parameters was quite important and density sorting at 1062 kg m$^{-3}$ and/or at 1069 kg m$^{-3}$ could permit a real classification of the berries into two/three groups with different sensory characteristics.

The phenolic composition of the berry pulp, skin and seeds for Italia sorted berries is shown in Table 5. Very few differences were observed among berries with different densities, and therefore the influence of the cluster heterogeneity was negligible. In general, the content of phenolic compounds
seemed to increase with increasing the berry density between the density classes A and B, with the exception of total flavonoids and proanthocyanidins in skins (TFsk and PROsk, respectively). The higher extractable phenolic content found in seeds for the density class B may be due to the higher number of seeds present in the berries belonging to it, if compared with the remaining density classes A and C. In berry skins, these results agreed with the increase previously reported for red winegrapes in the content of TFsk, PROsk and flavanols reactive to vanillin (FRVsk) when the berry density increased (Torchio et al., 2010; Rolle et al., 2011a, 2012b). However, these changes were only significant in Italia table grapes for the content of FRVsk. In berry seeds, the extractable content of phenolic compounds was not related to the berry density, which disagreed with the results previously published for total flavonoids (TFs) in red winegrapes (Torchio et al., 2010; Rolle et al., 2012b) but agreed with that for proanthocyanidins (PROs) and flavanols reactive to vanillin (FRVs) (Torchio et al., 2010).

The phenolic composition of the Italia cultivar differed from that previously published for the same cultivar grown in the same Italian region, but the hierarchy in the phenolic content was also seeds, skin and pulp (Baiano and Terracone, 2011). These last authors reported that the higher phenolic contents were detected in Italia table grapes, if compared with white ones grown in Apulia region such as Thompson seedless, Baresana and Pizzutello. On the other hand, the concentration of total polyphenols in the berry skin (TPsk) for the Italia cultivar was lower than that corresponding to other white and coloured table grapes (Rolle et al., 2011b, 2012a), which ranged between 612 mg kg⁻¹ (Delizia di Vaprio) and 2052 mg kg⁻¹ (Panse precoce). On the other hand, the contents of total polyphenols and total hydroxycinnamic acids in the berry pulp (TPp and HCTAp, respectively) were intermediate to the ones associated with other table grape varieties (Rolle et al., 2011b, 2012a), which varied from 101 mg kg⁻¹ (Black Magic) to 404 mg kg⁻¹ (Alphonse Lavallée), and from 29 mg kg⁻¹ (Black Magic) to 85 mg kg⁻¹ (Matilde), respectively. The content of HCTAp for the Italia cultivar was close to the highest values published for table grapes (Rolle et al., 2011b, 2012a). Furthermore, the amount of these last phenolic compounds is related to the sugar accumulation in the pulp.
The results indicated that Italia seeds and pulp may be good contributors to the berry antioxidant activity, as already demonstrated in previous studies (Baiano and Terracone, 2011). The extractable phenolic content of the seeds is higher than total phenolic content of the skins, particularly TP, TF and FRV, and the seeds contain high quantities of extractable proanthocyanidins. However, the antioxidant activity may be greatly associated with the pulp juices because of their content in hydroxycinnamyl acids (Sánchez-Moreno et al., 1998). Some authors reported that the berry antioxidant activity depends not only on the concentration of phenolic compounds, but also on the specific chemical structure of each phenol (degree of hydroxylation and extent of conjugation) and on the possible additive or synergistic effect of other compounds (Villaño et al., 2005; Baiano and Terracone, 2011). Baiano and Terracone (2011) found no significant correlations between the phenolic distribution and the antioxidant activity of seeds and pulp.

Principal Component Analysis (PCA) was performed for a global evaluation of the results, and better understanding of the differences found among Italia table grapes sorted at commercial harvest according to density on the basis of the parameters that contributed individually to the berry differentiation. Three principal components explained 94.3% of the variability in the original data. Figure 1 shows the projection of each variable in the two first principal components. Component 1 accounted for 53.8% of the total variance, it being mainly associated with the content and composition of sugars and FRVsk (coefficients ≥ 0.900). Component 2 accounted for 24.6% of the total variance, it being mainly associated with chromatic characteristics such as b*, C and CIRG2 (coefficients ≥ 0.800). Figure 2 shows the distribution of Italia berries, belonging to the three densities studied, in the plane defined by the two first principal components. The first principal component permitted a better separation of the berries belonging to the density class A (located in the left side), whereas the berries belonging to the density classes B and C were closer (located in the right side) corresponding the more positive values to the berries of the density class C. Therefore, the berries belonging to the three density classes can be well differentiated.
Conclusions

The density sorting of Italia table grapes at commercial harvest permitted to get berries of different ripeness level and relatively different quality attributes. In fact, the berries belonging to the same cluster showed a high heterogeneity because of their distribution in seven classes of density, although about 85% of the berries were arranged in only three density classes. The possibility of differentiating the berries based on a physicochemical criterion, such as texture properties, colour characteristics or chemical composition, is of interest in the production of ready-to-use fruits salad. However, the use of automatic berry densimetric sorting machines, proposed for winegrapes, requires the selection of a density value according to objective quality parameters of the berries.

In particular, the mechanical properties of the whole berry and the berry skin, as well as the pedicel detachment resistance, were not related to the berry density. In any way, some chromatic characteristics, such as yellow/blue colour component and chroma, permitted to differentiate density classes. Among phenolic compounds, the content of flavanols in berry skins, assessed with vanillin reaction, permitted to differentiate the density class A from the ones B and C. Multivariate analysis of the variables that significantly contributed to the berry differentiation showed that the content and composition of sugars, the content of skin flavanols with low molecular mass and the weight of the berry skin were the more representative parameters.

Finally, this study permitted to characterize globally the Italia table grapes according to physicochemical attributes. If compared with other white and coloured table grape varieties, the Italia cultivar has quite hard skins and presents high pedicel detachment resistance. These two mechanical characteristics are of great importance for extending the grape shelf life. Cv. Italia has a great potential not only for immediate consumption but also for exportation, as it is well-know in Italy. The high content of total hydroxycinnamic acids in the berry pulp and the phenolic composition of the seeds suggest a strong nutraceutical and functional value for the Italia cultivar.


Table 1. Instrumental texture parameters for Italia table grape berries sorted at commercial harvest according to density.

<table>
<thead>
<tr>
<th>Mechanical parameter</th>
<th>Density class</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>$F_{sk}$ (N)</td>
<td>0.675±0.235</td>
<td>0.785±0.221</td>
</tr>
<tr>
<td>$W_{sk}$ (mJ)</td>
<td>0.584±0.348</td>
<td>0.635±0.234</td>
</tr>
<tr>
<td>$E_{sk}$ (N mm$^{-1}$)</td>
<td>0.388±0.113</td>
<td>0.468±0.151</td>
</tr>
<tr>
<td>$Sp_{sk}$ (μm)</td>
<td>204±57</td>
<td>173±38</td>
</tr>
<tr>
<td>$F_{ped}$ (N)$^a$</td>
<td>4.634±1.697</td>
<td>5.248±1.753</td>
</tr>
<tr>
<td>$W_{ped}$ (mJ)$^a$</td>
<td>4.546±2.402</td>
<td>4.909±2.676</td>
</tr>
<tr>
<td>BH (N)</td>
<td>13.50±3.50</td>
<td>15.18±3.80</td>
</tr>
<tr>
<td>BCo (-)</td>
<td>0.560±0.076</td>
<td>0.523±0.066</td>
</tr>
<tr>
<td>BG (N)</td>
<td>7.44±1.68</td>
<td>7.81±1.59</td>
</tr>
<tr>
<td>BS (mm)</td>
<td>4.40±0.37</td>
<td>4.45±0.39</td>
</tr>
<tr>
<td>BCh (mJ)</td>
<td>32.80±8.46</td>
<td>34.99±8.63</td>
</tr>
<tr>
<td>BR (-)</td>
<td>0.280±0.046</td>
<td>0.262±0.038</td>
</tr>
<tr>
<td>BH$_{norm}$ (N)</td>
<td>0.585±0.158</td>
<td>0.633±0.146</td>
</tr>
<tr>
<td>BG$_{norm}$ (N)</td>
<td>0.322±0.073</td>
<td>0.325±0.058</td>
</tr>
<tr>
<td>BS$_{norm}$ (mm)</td>
<td>0.190±0.009</td>
<td>0.186±0.009</td>
</tr>
<tr>
<td>BCh$_{norm}$ (mJ)</td>
<td>1.414±0.327</td>
<td>1.450±0.291</td>
</tr>
</tbody>
</table>

All data are expressed as average value ± standard deviation. n = 30. $^a$n = 21. Sign: ns indicates not significant differences among the three density classes (Tukey-b test; p < 0.05). $F_{sk}$ = berry skin break force, $W_{sk}$ = berry skin break energy, $E_{sk}$ = berry skin Young’s modulus, $Sp_{sk}$ = berry skin thickness, $F_{ped}$ = peduncle detachment force, $W_{ped}$ = peduncle detachment energy, BH = berry hardness, BCo = berry cohesiveness, BG = berry gumminess, BS = berry springiness, BCh = berry chewiness, BR = berry resilience. norm = normalized according to the respective berry diameter. A = 1062 kg m$^{-3}$, B = 1067 kg m$^{-3}$, C = 1069 kg m$^{-3}$. 
Table 2. CIELab parameters and colour indices for Italia table grape berries sorted at commercial harvest according to density.

<table>
<thead>
<tr>
<th>Colour parameter</th>
<th>Density class</th>
<th>Sign$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>$L^*$</td>
<td>46.05±1.73</td>
<td>45.34±4.69</td>
</tr>
<tr>
<td>$a^*$</td>
<td>-2.08±2.02</td>
<td>-2.08±1.49</td>
</tr>
<tr>
<td>$b^*$</td>
<td>26.88±2.22b</td>
<td>29.96±2.57a</td>
</tr>
<tr>
<td>$H$</td>
<td>94.43±4.17</td>
<td>94.00±2.85</td>
</tr>
<tr>
<td>$C$</td>
<td>27.02±2.24b</td>
<td>30.06±2.56a</td>
</tr>
<tr>
<td>CIRG1</td>
<td>1.17±0.09</td>
<td>1.15±0.11</td>
</tr>
<tr>
<td>CIRG2</td>
<td>0.07±0.01ab</td>
<td>0.06±0.01b</td>
</tr>
</tbody>
</table>

All data are expressed as average value ± standard deviation. n = 30. Different Latin letters within the same row indicate significant differences ($^1$) among the three density classes (Tukey-b test; $p < 0.05$). $^1$: *,**, and ns indicate significance at $p < 0.05$, 0.01 and not significant, respectively. $L^*$ = lightness, $a^*$ = red/green colour component, $b^*$ = yellow/blue colour component, $H$ = hue angle, $C$ = chroma, CIRG1 = $(180-H) / (L^*+C)$, CIRG2 = $(180-H) / (L^*xC)$. $A = 1062$ kg m$^{-3}$, $B = 1067$ kg m$^{-3}$, $C = 1069$ kg m$^{-3}$. 
Table 3. Physical parameters for Italia table grape berries sorted at commercial harvest according to density.

<table>
<thead>
<tr>
<th>Physical parameter</th>
<th>Density class</th>
<th>Sign$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Berry weight (g)</td>
<td>7.83±0.45b</td>
<td>9.77±0.96a</td>
</tr>
<tr>
<td>Berry skin weight (mg)</td>
<td>379±11b</td>
<td>551±88a</td>
</tr>
<tr>
<td>Seeds/5 berries (n°)$^#$</td>
<td>7.33±1.15</td>
<td>10.33±1.53</td>
</tr>
<tr>
<td>Berry seeds weight (mg per berry)</td>
<td>71±15</td>
<td>101±15</td>
</tr>
</tbody>
</table>

All data are expressed as average value ± standard deviation. n = 30. $^\#$n = 6. Different Latin letters within the same row indicate significant differences ($^1$) among the three density classes (Tukey-b test; $p < 0.05$). $^1$: * and ns indicate significance at $p < 0.05$ and not significant, respectively. A = 1062 kg m$^{-3}$, B = 1067 kg m$^{-3}$, C = 1069 kg m$^{-3}$.
Table 4. Technological ripeness parameters for Italia table grape berries sorted at commercial harvest according to density.

<table>
<thead>
<tr>
<th>Ripeness parameter</th>
<th>Density class</th>
<th>Sign¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>SSC (Brix)</td>
<td>15.6±0.2a</td>
<td>16.8±0.1b</td>
</tr>
<tr>
<td>TA (g L⁻¹ tartaric acid)</td>
<td>2.88±0.3b</td>
<td>2.47±0.4a</td>
</tr>
<tr>
<td>SSC/TA</td>
<td>5.42±0.5a</td>
<td>6.80±1.09ab</td>
</tr>
<tr>
<td>pH</td>
<td>3.91±0.02a</td>
<td>4.08±0.03c</td>
</tr>
<tr>
<td>Tartaric acid (g kg⁻¹)</td>
<td>3.62±0.24</td>
<td>3.55±0.30</td>
</tr>
<tr>
<td>Malic acid (g kg⁻¹)</td>
<td>1.60±0.14b</td>
<td>1.57±0.21b</td>
</tr>
<tr>
<td>Citric acid (g kg⁻¹)</td>
<td>0.15±0.01b</td>
<td>0.16±0.03b</td>
</tr>
<tr>
<td>Reducing sugars (g kg⁻¹)</td>
<td>142.3±3.3a</td>
<td>158.1±1.7b</td>
</tr>
<tr>
<td>Glucose (g kg⁻¹)</td>
<td>68.0±1.5a</td>
<td>74.8±0.9b</td>
</tr>
<tr>
<td>Fructose (g kg⁻¹)</td>
<td>74.3±1.8a</td>
<td>83.3±0.8b</td>
</tr>
<tr>
<td>Glucose/ Fructose</td>
<td>0.92±0.01</td>
<td>0.90±0.02</td>
</tr>
</tbody>
</table>

All data are expressed as average value ± standard deviation. n = 3. Different Latin letters within the same row indicate significant differences (¹) among the three density classes (Tukey-b test; p < 0.05). ¹: *, *** and ns indicate significance at p < 0.05, 0.001 and not significant, respectively. SSC = total soluble solids content, TA = titratable acidity. A = 1062 kg m⁻³, B = 1067 kg m⁻³, C = 1069 kg m⁻³.
Table 5. Phenolic composition of Italia table grape berries sorted at commercial harvest according to density.

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Density class</th>
<th>Sign¹</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCTAₚ (mg caffeic acid kg⁻¹)</td>
<td>68.3±13.1</td>
<td>77.8±9.0</td>
<td>80.8±12.3</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TPₚ (mg (+)-catechin kg⁻¹)</td>
<td>146±16</td>
<td>167±35</td>
<td>167±62</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TPₛ (mg (+)-catechin kg⁻¹)</td>
<td>238±16</td>
<td>254±31</td>
<td>225±19</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TFₛ (mg (+)-catechin kg⁻¹)</td>
<td>1069±89</td>
<td>921±101</td>
<td>930±37</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>PROₛ (mg cyanidin chloride kg⁻¹)</td>
<td>480±62</td>
<td>461±12</td>
<td>538±50</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>FRVₛ (mg (+)-catechin kg⁻¹)</td>
<td>46.3±6.6b</td>
<td>137±19a</td>
<td>131±20a</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>TPₛ (mg (+)-catechin kg⁻¹)</td>
<td>330±21</td>
<td>380±39</td>
<td>354±71</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TFₛ (mg (+)-catechin kg⁻¹)</td>
<td>2070±186</td>
<td>2158±270</td>
<td>1957±265</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>PROₛ (mg cyanidin chloride kg⁻¹)</td>
<td>454±62</td>
<td>480±27</td>
<td>410±81</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>FRVₛ (mg (+)-catechin kg⁻¹)</td>
<td>163±19</td>
<td>188±24</td>
<td>174±40</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

All data are expressed as average value ± standard deviation. n = 3. Different Latin letters within the same row indicate significant differences (¹) among the three density classes (Tukey-b test; p < 0.05). ¹: ** and ns indicate significance at p < 0.01 and not significant, respectively. HCTA = total hydroxycinnamic acids, TP = total polyphenols, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin. ᵃ = berry pulp, ᵇ = berry skin, ᵈ = berry seeds. A = 1062 kg m⁻³, B = 1067 kg m⁻³, C = 1069 kg m⁻³.
Figure 1. Projection of each variable in the plane defined by the two first principal components.

BW = berry weight
BskW = berry skin weight
SSCTA = SSC/TA
Figure 2. Projection of Italia table grapes sorted at harvest according to density in the plane defined by the two first principal components.