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BERRY DENSITY AND SIZE AS FACTORS RELATED TO THE
PHYSICOCHEMICAL CHARACTERISTICS OF MUSCAT HAMBURG TABLE
GRAPES (*Vitis vinifera* L.)

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Running title: Berry density and size affecting the characteristics of table grapes

Abstract

The aim of this work was to determine the impact of using independently berry density and diameter as sorting methodologies on important physicochemical parameters affecting the grape quality, such as mechanical properties, phenolic composition and aromatic profile. Muscat Hamburg berries were classified according to the density by flotation in different salt solutions and to the diameter. The three most representative density (1081, 1088 and 1094 kg/m³) and diameter (16-17, 18-19 and 20-21 mm) classes were selected. The results showed that there were relationships of both density and diameter with the mechanical properties and chemical composition of the berries. Densimetric sorting is a more promising methodology to separate grape berries with different quality attributes, particularly skin hardness, berry cohesiveness and resilience, total hydroxycinnamic acids, anthocyanins and rose oxide, than diameter sorting. This knowledge can be of great interest for the ‘fresh-cut’ industry in the production of ‘ready-to-eat’ fruits salad.

Keywords: berry density, berry size, mechanical properties, phenolic composition, monoterpenes, table grapes

1. Introduction

The world production of grapes for fresh consumption, intended for table use, has registered a notable upward trend during the last decade (OIV, 2013). Furthermore, the world human consumption of fresh grapes has increased significantly year-on-year, which attracts market interest. The production of table grape cultivars with sensory characteristics highly appreciated by consumers constitutes a major concern for breeding, and it is essential for the highly competitive market. In fresh fruits, texture is a key attribute for the assessment of freshness and enjoyment of eating (Fillion & Kilcast, 2002; Péneau, Hoehn, Roth, Escher, & Nuessli, 2006). The sensory quality of table grapes also depends greatly on the content and composition of numerous chemical constituents, including sugars, organic acids, phenolic compounds and volatile compounds. Important sensory traits of table grapes, such as skin color, astringency and bitterness, are directly related to the phenolic composition (Liang, Owens, Zhong, & Cheng, 2011). The aroma perceived during grape tasting is a quality factor of great importance and it is a result of the volatile composition (Ruiz-García, Hellín, Flores, & Fenoll, 2014).

Nowadays, many consumers are increasingly including functional foods in the diet. In addition to the nutritional value, table grapes are a major source of health promoting bioactive compounds with antioxidant properties, anti-inflammatory action, antiplatelet activity and regulating potential of the endothelial function (Baiano & Terracone, 2011; Carrieri et al., 2013; Lutz, Jorquera, Cancino, Ruby, & Henriquez, 2011). Particularly, colored grapes are the most active because of their richness in phenolic compounds with multiple biological effects and potential health benefits (Carrieri et al., 2013; Crupi et al., 2012). Phenolic compounds,

including flavonoids (anthocyanins, flavanols and flavonols), phenolic acids and stilbenes, are distributed differently among the various parts of the berry (skin, pulp and seeds), thus each part contributing to the antioxidant activity of the berry in a different measure (Baiano & Terracone, 2011; Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011; Lutz et al., 2011). Anthocyanins are the most abundant phenolic compounds in colored grapes (Crupi et al., 2012; Lago-Vanzela et al., 2011).

Regarding the volatile composition, Muscat aroma is greatly appreciated in grapes destined for fresh consumption (Ruiz-García et al., 2014), and it is directly related to monoterpenes, such as linalool, rose oxide, citral, geraniol, nerol and citronellol (Fenoll, Manso, Hellín, Ruiz, & Flores, 2009). Rose oxide is a useful indicator for the identification and selection of table grape cultivars according to Muscat aroma (Ruiz-García et al., 2014). Furthermore, the qualitative and quantitative composition of aromatic and phenolic compounds depends on numerous factors, such as cultivar, ripening stage, environmental conditions and agronomical practices (Baiano & Terracone, 2011; Crupi et al., 2012; Fenoll et al., 2009; Lago-Vanzela et al., 2011; Liang et al., 2011; Lutz et al., 2011; Sonego, Lurie, Zuthi, Kaplonov, Ben-Arie, & Kosto, 2002; Yang, Wang, Wu, Fang, & Li, 2011).

The grapes undergo many physical and biochemical changes during the ripening process. Therefore, optimum grape quality requires harvesting at the appropriate stage of maturity. The existence of temporal asynchrony in growth and maturity among berries differently positioned within a bunch, and among bunches within the vine, makes it necessary the implementation of classification methods to minimize berry heterogeneity, to exploit these variations to make different wine types and to provide possible trends in the evolution of major compounds and texture properties during grape ripening (Garcia de Cortazar-Atauri,

Brisson, Ollat, Jacquet, & Payan, 2009; Rolle, Río Segade et al., 2011). In various studies on winegrapes, some classifications of grape berries have already been performed according to the diameter and density (Rolle, Río Segade et al., 2011; Rolle, Torchio, Giacosa, Río Segade, Cagnasso, & Gerbi, 2012; Šuklje et al., 2012). In table grapes, densimetric sorting can be easily automated and has been used successfully to separate berries with different chromatic and texture parameters (Río Segade, Giacosa, de Palma et al., 2013; Río Segade, Giacosa, Torchio et al., 2013; Sonogo et al., 2002).

Berry size constitutes an important quality trait of winegrapes because it is related to the skin-to-pulp ratio of the berry. Generally, smaller berries contain a higher concentration of many skin-located compounds (aromatic and phenolic compounds) that play a key role in the wine quality (Barbagallo, Guidoni, & Hunter, 2011; Roby, Harbertson, Adams, & Matthews, 2004). In fact, image analysis can accurately assess the berry size of winegrapes and other fruits, and it may be easily implemented on sorting tables to automate the selection of high quality fruits (Cubero, Diago, Blasco, Tardáguila, Millán, & Aleixos, 2014; Li, Wang, & Zhu, 2012). Berry size may also be an important quality attribute of table grapes.

There are fewer works published on table grapes than on winegrape varieties, whose texture attributes and chemical constituents have a relevant role in determining the overall quality, and therefore the market value. Furthermore, the scientific literature on table grape classification is even more limited. The aim of this work was to better understand the differences in the berry quality for table grapes and to select berries with desirable quality characteristics on the basis of berry diameter and berry density. The grape berries were classified according to either their size or their density at harvest, and the relationships of these two physical parameters with objective quality parameters, such as the mechanical

properties, the phenolic composition and the aromatic profile, were determined. The study was performed on the Muscat Hamburg table grape cultivar, grown in many parts of Europe, which is highly appreciated for its black berries and pleasant Muscat aroma. This knowledge can be important for the ‘fresh-cut’ industry that uses table grape berries in the production of ‘ready-to-eat’ fruits salad.

2. Materials and methods

2.1. Grape samples

The study was carried out, in 2012, on a 30 kg commercial sample consisting of whole bunches of the Muscat Hamburg red table grape cultivar (*Vitis vinifera* L.). Once in the laboratory, all berries (about 6000) were manually separated from the stalk with attached short pedicels. The berries were randomly subdivided into four sample sets with approximately the same weight. Two sample sets of berries were sorted according to their density by flotation in saline solutions of different concentrations (from 100 to 170 g/L sodium chloride, corresponding to densities comprised between 1069 and 1115 kg/m³) as described by Rolle, Río Segade et al. (2011). The difference in density of two consecutive saline solutions was 6 kg/m³. The berries were separated in eight density classes starting from the most to the least dense solution, and those belonging to each density class were then weighed. The other two sample sets were classified according to berry diameter using Perspex plates with holes of diameters from 13 to 22 mm, increasing at 1 mm intervals. The berries were individually sorted from the largest to the smallest diameter obtaining ten different size classes. The berries in each diameter class were also weighed. The distribution percentages

were established on the basis of berry density and diameter. The three most representative density ($A=1081 \text{ kg/m}^3$, $B=1088 \text{ kg/m}^3$, $C=1094 \text{ kg/m}^3$) and diameter ($S=16\text{-}17 \text{ mm}$, $M=18\text{-}19 \text{ mm}$, $L=20\text{-}21 \text{ mm}$) classes were selected for further studies. The sorted berries were visually inspected before analysis; those with damaged skins were discarded.

For each sample set and class, one subsample of 20 sorted berries was used for the determination of the mechanical properties of the whole berry and the berry skin. Other two subsamples of 10 sorted berries were used for the determination of the phenolic composition. Two subsamples of 200 sorted berries were used for the determination of the volatile composition. Finally, the remaining berries in each sample set and class, subdivided into two replicates, were used for determining the technological ripeness parameters in the grape must obtained by manual crushing and centrifugation.

2.2. Chemical analysis

2.2.1. 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 deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, UK). Standards were supplied from Extrasynthèse (Genay, France) and Sigma (Milan, Italy).

2.2.2. Technological ripeness parameters

Soluble solids concentration (°Brix, as SSC) was measured using an Atago 0-32 °Brix temperature compensating refractometer (Atago Corporation, Tokyo, Japan), pH was determined by potentiometry using an InoLab 730 pHmeter (WTW, Weilheim, DE), and titratable acidity (g/L tartaric acid, as TA) was estimated using the OIV method (OIV, 2008a). Reducing sugars (glucose and fructose) and organic acids (citric acid, tartaric acid and malic acid) (g/L) were determined using a HPLC system equipped with a refractive index detector and a diode array detector (DAD) set to 210 nm (Río Segade, Giacosa, Torchio et al., 2013).

2.2.3. Extraction and determination of phenolic compounds

The berry skins were manually removed from the pulp using a laboratory spatula. The pulp was introduced into a tube containing 100 mg sodium metabisulphite, weighed and subsequently diluted (9:1, m/m) with 5 mol/L sulphuric acid (Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa, Gerbi, & Novello, 2011; Rolle, Giacosa, Gerbi, Bertolino, & Novello, 2013). Afterwards, the pulp was homogenized at 9500 rpm for 30 s with an Ultraturrax T10 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) and centrifuged in a PK 131 centrifuge (ALC International, MI, Italy) for 15 min at 3000×g at 20 °C. The resulting solution was then used for pulp analysis. The skins were weighed and quickly immersed into 25 mL of a hydroalcoholic buffer (pH 3.2) containing 5 g/L tartaric acid, 2 g/L sodium metabisulphite and 12% v/v ethanol (Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al., 2013). Afterwards, the skins were homogenized at 8000 rpm for 1 min with an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik) and centrifuged for 15 min at 3000×g at 20 °C. The supernatant was then used for skin analysis. Spectrophotometric methods were used to determine total polyphenols (mg (+)-catechin/kg grape, as TP) in the berry skin and pulp, total hydroxycinnamic acids (mg caffeic

acid/kg grape, as HCA) in the pulp, and total anthocyanins (mg malvidin-3-O-glucoside chloride/kg grape, as TA), flavanols reactive to vanillin (mg (+)-catechin/kg grape, as FRV), total flavonoids (mg (+)-catechin/kg grape, as TF) and proanthocyanidins (mg cyanidin chloride/kg grape, as PRO) in the skin. A UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used. The relative standard deviation (RSD) based on repeated analysis (n = 12) of the same sample was 1.58, 1.82, 1.14, 2.80, 0.93 and 1.74% for TP, HCA, TA, FRV, TF and PRO, respectively (Río Segade, Giacosa, de Palma et al., 2013; Rolle et al., 2013).

The determination of the anthocyanin profile was performed after the berry skin extract had been submitted to reverse-phase solid-phase extraction (RP-SPE) using a 1 g Sep-Pak C-18 cartridge (Waters Corporation, Milford, MA, USA) with methanol as the eluent (Rolle et al., 2013). The HPLC-DAD system and chromatographic conditions were previously reported in the literature (Rolle et al., 2013). A LiChroCART analytical column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrospher 100 RP-18 (5 µm) particles supplied by Alltech (Deerfield, IL, USA), was used. The mobile phases were: A = formic acid/water (10:90, v/v); B = formic acid/methanol/water (10:50:40, v/v). All analyses were performed in duplicate.

2.2.4. Extraction and determination of free volatile compounds

The berries were crushed under a nitrogen atmosphere with a laboratory blender (Waring Laboratory, Torrington, USA) for 1 min and centrifuged twice (7000×g, 15 min, 4 °C). An 5 ml-aliquot of the supernatant, diluted with equal volume of water, was adjusted at pH 5 and transferred to a 20 ml glass headspace sampling vial containing 2 g of sodium

chloride and 1-heptanol as internal standard (200 μ L of 1.55 mg/L solution in 10% v/v ethanol). Silicone septa from Supelco (Bellefonte, PA, USA) were used with 18 mm diameter screw caps to seal the vials. The sealed vials were carefully shaken to dissolve sodium chloride.

A 50/30 μ m DVB/CAR/PDMS fibre from Supelco was used (Sánchez-Palomo, Díaz-Maroto, & Pérez-Coello, 2005). The fibre was exposed to the headspace of the capped vial for 20 min at 40 °C. SPME injections were performed in the splitless mode at 250 °C for 5 min during which time thermal desorption of analytes from the fiber occurred.

The GC-MS system consisted of an Agilent 7890C gas chromatograph (Little Falls, DE, USA) equipped with a Gerstel MPS2 XL autosampler (Gerstel, Baltimore, MD, USA) and coupled to an Agilent 5975 mass selective detector. The software used was Agilent G1701-90057 MSD ChemStation. The chromatographic conditions were previously reported by Sánchez-Palomo et al. (2005) with some modifications. A DB-WAXETR capillary column (30 m \times 0.25 mm, 0.25 μ m, J&W Scientific Inc., Folsom, CA, USA) was used. The temperature program started at 40 °C for 5 min, and increased at a rate of 2 °C/min to 200 °C for 10 min and 5 °C/min to 220 °C. The oven was then held at 220 °C for 5 min before returning to the initial temperature. The carrier gas (He) flow-rate was 1 mL/min. The injection port temperature was 250 °C, the ion source temperature was 150 °C, and the interface temperature was 280 °C. The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode, using an ionization energy of 70 eV. The mass acquisition range was m/z 35-350. Volatile compounds were identified on the basis of their mass spectra and retention indices by comparing with either those of pure standards when available, or those reported in the literature and in the database

(<http://webbook.nist.gov/chemistry/>). Semiquantitative data ($\mu\text{g/L}$) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard. All analyses were performed in duplicate.

2.3. Instrumental texture analysis

A Universal Testing Machine (UTM) TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with a HDP/90 platform and a 5 kg load cell, was used for grape texture analysis. All data acquisitions were made at 500 points per second. Typical deformation curves for the different tests performed on table grapes were shown in a work previously published (Rolle, Giacosa et al., 2011).

The mechanical properties of the whole berry were evaluated by a Texture Profile Analysis (TPA) test. Each whole berry was individually compressed in the equatorial position using a 35 mm SMS P/35 flat cylindrical probe (Stable Micro Systems) under 25% deformation, with a waiting time of 2 s between the two bites and a test speed of 1 mm/s (Rolle, Giacosa et al., 2011; Rolle et al., 2013). The following typical TPA parameters were determined: 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) (Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al., 2013). The berry diameter was calculated as the distance between the berry trigger point and the platform base. Because some TPA parameters can be influenced by berry size, they were also normalized according to the respective berry diameter (norm), expressed in mm (Río Segade, Giacosa, de Palma et al., 2013).

The berry skin hardness was assessed by a puncture test using a SMS P/2N needle probe (Stable Micro Systems), a test speed of 1 mm/s and a penetration applied of 3 mm (Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al., 2013). Each berry was individually punctured in the lateral face, and the following three parameters were measured: skin break force (N, as F_{sk}), skin break energy (mJ, as W_{sk}) and skin resistance to the axial deformation (N/mm, as E_{sk}).

The measurement of the berry skin thickness (μm , as Sp_{sk}) by a compression test required the manual separation of a piece of skin (ca. 0.25 cm^2) from the lateral side of each berry with a razor blade. The test was carried out using a 2 mm SMS P/2 flat cylindrical probe and a test speed of 0.2 mm/s (Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa et al., 2011).

2.4. Statistical analysis

Statistical analyses were performed using the SPSS Statistics software package version 19.0 (IBM Corporation, Armonk, NY, USA). The Tukey-b test at $p < 0.05$ was used to establish significant differences by one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Berry distribution

The distribution percentage of the berries in different density and diameter classes for the Muscat Hamburg cultivar, at commercial harvest, is shown in Figure 1. Gaussian bell-shaped distributions are attributable to in-field grape variability as a consequence of physical and environmental factors (exposure, soil, topography, microclimate). Therefore, heterogeneous ripening is one of the major factors in typifying the grape variety features. A total of 91.7% w/w of the berries were grouped in five density classes (1075, 1081, 1088, 1094 and 1100 kg/m³). The three most representative density classes were 1081 kg/m³ (A), 1088 kg/m³ (B) and 1094 kg/m³ (C), which accounted for 70.2% w/w of the berries (17.0, 26.7 and 26.5% w/w, respectively). Regarding the contribution of each diameter class, six classes (16, 17, 18, 19, 20 and 21 mm) represented more than 94% w/w of the berries. With the aim of obtaining enough berries for subsequent analysis, the six classes were regrouped in the three following diameter classes 16-17 mm (S), 18-19 mm (M) and 20-21 mm (L) with a relative berry weight of 23.5, 51.6 and 19.2% w/w, respectively. Therefore, the most representative size class corresponded to the berries of diameters comprised between 18 and 19 mm.

On the other hand, no significant correlation was found between the diameter and density determined in each berry ($n = 300$, $R^2 = 0.0891$). Contrary to the results reported for winegrapes (Rolle et al., 2012), smaller/larger table grape berries were not always associated with lower/higher values of berry density. Therefore, the berries of the same diameter had different density and viceversa, which could have an impact on secondary metabolites (Šuklje et al., 2012).

3.2. Technological ripeness parameters

Table 1 shows the parameters that define the technological ripeness of Muscat Hamburg table grapes sorted, at commercial harvest, according to berry density and diameter. As expected, the value of SSC and the amount of reducing sugars increased significantly with increasing berry density. Furthermore, the reducing sugars/TA ratio increased regularly with increasing berry density. These changes agreed with those reported in other table grapes (Jayasena & Cameron, 2008; Río Segade, Giacosa, de Palma et al., 2013; Río Segade, Giacosa, Torchio et al., 2013). Differences lower than 0.8 °Brix, 7 g/L and 1.1 were found in the value of SSC, the content of reducing sugars and the reducing sugars/TA ratio, respectively, for the berries belonging to different diameter classes (Table 1). In a previous study, a close correlation between berry diameter and SSC was observed during ripening until 20 °Brix. However, the berries of the same diameter had different values of SSC because of the functional link between sugar accumulation, transpiration and water accumulation (Šuklje et al., 2012). Other researchers also reported a weak relationship between °Brix and berry fresh mass in winegrapes (Roby et al., 2004). In the present work, no trend was observed for the glucose/fructose ratio with berry density and diameter. The value of TA and the acid profile were not significantly related to berry density and diameter.

According to the OIV resolution VITI 1/2008 (OIV, 2008b), table grapes are considered ripe with SSC values ≥ 16 °Brix, or when the reducing sugars (expressed as g/L)/TA (expressed as g/L tartaric acid) ratio is > 20 . As reported in Table 1, Muscat Hamburg berries reached the OIV maturity requirements for any berry density and diameter evaluated. Other researchers found similar values of technological ripeness parameters of Muscat Hamburg grapes to our findings (Fenoll et al., 2009; Orak, 2007). From the perspective of a consumer, the sensory quality of table grapes depends primarily on the sugar content, titratable acidity, the organic acid composition and the balance between these factors

(Muñoz-Robredo, Robledo, Manríquez, Molina, & Defilippi, 2011; Sonego et al., 2002). However, it is difficult to define universally valid quality chemical standards because they can differ between cultivars, regions and seasons (Sonego et al., 2002). In the present work, densimetric sorting permitted the classification of the berries according to real chemical characteristics (SSC, reducing sugars/TA and reducing sugars), but other important quality criteria for the consumer acceptance have to also be considered, such as skin external color, aroma and texture attributes.

3.3. Phenolic compounds

Total contents of phenolic compounds and of the different classes of phenolics in the berry pulp and skin, and the anthocyanin profile were determined in Muscat Hamburg table grapes sorted according to berry density and diameter. The results obtained are shown in Table 2. Berry density significantly influenced the contents of HCA_p, TP_p, TF_{sk} and TA_{sk}. In fact, the berries belonging to the density class C were richer in these compounds in relation to those of the classes A and B. In Italia table grapes, the content of phenolic compounds was not significantly related to berry density, with the exception of FRV_{sk} (Río Segade, Giacosa, de Palma et al., 2013). Instead, the results obtained in the present work better agreed with those reported for Nebbiolo red winegrapes, for which a significant increase was observed in the contents of TA_{sk} and TF_{sk} with increasing berry density, while the contents of PRO_{sk} and FRV_{sk} were independent on the density (Rolle, Río Segade et al., 2011; Rolle et al., 2012). However, the significance of these relationships was zone dependent (Rolle et al., 2012).

The impact of berry diameter on the phenolic composition was not significant, with the exception of the contents of TF_{sk} and PRO_{sk} because they were significantly more

abundant in the smaller berries (class S, Table 2). In Syrah and Cabernet Sauvignon red winegrapes, the larger berries had lower quality characteristics because the concentration of total polyphenols and anthocyanins decreased with increasing berry weight/size (Barbagallo et al., 2011; Roby et al., 2004). Although similar decreasing trends were observed in the Muscat Hamburg cultivar, the phenolic composition seems to be more insensitive to berry size in table grapes than in winegrapes. Other authors also showed that the relationship of berry diameter with the content of HCA_p was not significant in Sauvignon Blanc winegrapes (Šuklje et al., 2012).

Although the general anthocyanin profile was quite similar in all classes (Table 2), the berries of the density class A showed significantly lower percentages of cyanidin and peonidin derivatives in favor of malvidin forms of anthocyanins when compared with those of the density classes B and C. The relative abundance of unacylated forms of anthocyanins was also significantly lower in the berries belonging to the density class A in favor of acetylated and cinnamoylated forms. The influence of berry diameter on the anthocyanin profile was not significant.

Densimetric sorting permitted the classification of Muscat Hamburg berries according to the phenolic composition (HCA_p, TP_p, TF_{sk} and TA_{sk}). Therefore, useful grape densimetric separation could be performed at 1088 kg/m³ to obtain pulps with stronger antioxidant activity because it is greatly associated with the content of polyphenols, particularly HCA (Baiano & Terracone, 2011), and to get more colored skins. By selecting this density (density class C), the highest percentages of cyanidin and peonidin derivatives were also obtained, particularly unacylated forms, which are considered nutraceuticals because of their strong antioxidant effects (Fukumoto & Mazza, 2000). On the other hand, the selection of the

smallest berries (16-17 mm) would permit to increase the content of PRO_{sk} (high molecular weight flavanols), which usually leads to a softening effect on the mouthfeel (lower astringency perception) and to stronger antioxidant activity (Baiano & Terracone, 2011).

Muscat Hamburg showed a medium content of phenolic compounds when compared with other white and red table grape cultivars (Crupi et al., 2012; Liang et al., 2011; Río Segade, Giacosa, de Palma et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al., 2013). Despite some small differences attributable to the grape maturity, agronomic factors, growing zone and seasonal variability, the phenolic composition of Muscat Hamburg grapes agreed with that previously published for the same cultivar. Concretely, average contents of 123 and 254 mg/kg were found for HCA_p and TA_{sk}, respectively (Liang et al., 2011), and 385 mg/kg for TA_{sk} (Orak, 2007). Muscat Hamburg grapes were characterized by an anthocyanin profile made up mainly of molecules tri-substituted in the B-ring (56.5-62.4%) with a prevalence of malvidin derivatives. Among di-substituted anthocyanins, peonidin-3-glucoside was the most abundant red pigment. The percentage of acetylated forms was less than 1.3%. Liang et al. (2011) reported that peonidin and malvidin derivatives are the main anthocyanin forms in colored table grapes, and that most of malvidin derivatives are unacylated and cinnamoylated forms.

3.4. Free volatile compounds

The contents of different free volatile compounds detected in Muscat Hamburg table grape berries sorted according to the density and diameter are shown in Table 3. The relationship of both berry density and diameter with the contents of linalool, nerol and geraniol was significant. This finding agreed with the significant correlations previously

observed by other researchers between geraniol and linalool concentrations, and between geraniol and nerol (Fenoll et al., 2009). Linalool and nerol are considered to be biosynthesized from geraniol (Hidalgo Togores, 2002). Therefore, higher contents of nerol with increasing berry density and of linalool with increasing berry diameter could be related to lower contents of geraniol. Furthermore, berry density significantly influenced the contents of rose oxide. The two isomers (*cis* and *trans*) of rose oxide were more abundant in the berries of the density class C, although the difference in the content of *c*-rose oxide was not significant among berries of the density classes B and C. The contents of citral, citronellol and geranic acid were independent on berry density and diameter. The variations found in the contents of free volatile compounds among berries belonging to different density classes agreed with those reported for the same cultivar growing in other country, with the exception of linalool, nerol and citral (Fenoll et al., 2009). The differences in the evolution of these three compounds during ripening could be attributed to the grape maturity, agronomic factors, growing zone and seasonal variability (Fenoll et al., 2009).

The odor activity values (OAVs) were calculated to estimate the sensory contribution of each monoterpene to the general aroma of Muscat Hamburg grapes (Table 4). OAVs were calculated as the ratio of the content of each compound in the grape samples and the odor threshold value of the compound in water. Only those compounds with OAVs > 1 contribute potentially to the aroma. In all berry classes, linalool was the most abundant free monoterpene detected in the Muscat Hamburg cultivar, followed by geraniol, nerol and geranic acid. Previous works identified linalool and geraniol as the main free monoterpeneols at the last maturity stage (19.4-19.7 °Brix) of Muscat Hamburg grapes with average contents ranging from 186.7 to 201.4 µg/kg for linalool and from 66.2 to 94.0 µg/kg for geraniol (Fenoll et al., 2009). High concentrations of linalool, geraniol and nerol were also identified in other Muscat

cultivars such as Moscatuel and Bimeijia (Fenoll, Martinez, Hellin, & Flores, 2012; Yang et al., 2011). In the present work, linalool was also generally the most active odorant, followed by rose oxide, geraniol and geranic acid. Rose oxide, linalool and geraniol were also detected in several Muscat cultivars at levels above their odor threshold values (Fenoll et al., 2009, 2012; Ruiz et al., 2014). However, the contribution of *t*-rose oxide to the potential aroma was higher than that of linalool in Muscat Hamburg berries belonging to the density class C (Table 4), although the concentration of *t*-rose oxide was almost ten times lower than that of linalool (Table 3). The contribution of rose oxide to the aroma occurred at low concentrations because of its low odor threshold value in water (0.5 µg/L). Rose oxide has been proposed as a useful indicator for the identification and selection of table grape cultivars according to Muscat aroma because the presence of rose oxide and Muscat flavor are highly correlated (Ruiz et al., 2014). On the contrary, in spite of the richness of Muscat Hamburg grapes in nerol and geranic acid, only geranic acid contributed actively to the global aroma in the smaller berries (diameter class S) at any berry density (Table 4). Finally, nerol, citronellol and citral were not responsible for Muscat aroma. According to Fenoll et al. (2009), when free and glycosidically-bound volatile compounds are considered, citral, nerol and citronellol also contribute potentially to the final aroma of Muscat Hamburg grapes, although to a lesser extent than linalool, rose oxide and geraniol. Ruiz et al. (2014) also pointed that nerol and citronellol are not active odorants in various Muscat cultivars.

Densimetric sorting at 1081 kg/m³ would permit to potentiate the contribution of either both linalool and geraniol (density class A) or rose oxide (density classes B and C) to the global aroma of Muscat Hamburg berries. On the contrary, the classification of the berries according to the diameter did not provide important aromatic advantages.

3.5. Instrumental texture analysis

Instrumental texture parameters of the whole berry and berry skin for Muscat Hamburg table grapes sorted according to the density and diameter are shown in Table 5. The berries belonging to the density class A had significantly lower values of some mechanical properties measured in the skin (F_{sk} , E_{sk}) and in the whole berry (BH, BG, BCh), but significantly higher ones of other texture parameters also measured in the whole berry (BCo, BR), than those of the density classes B and C. Therefore, the less dense berries (density class A) were characterized by a lower force necessary to attain a given deformation (lower hardness) and to disintegrate the berry until it is ready for swallowing (lower gumminess), a lower energy necessary to chew the berry until it is ready for swallowing (lower chewiness), a higher strength of the internal bonds comprising the body of the berry (higher cohesiveness), a better fight to regain the original position (higher resilience), as well as by their softer and springier skins (lower F_{sk} , W_{sk} and E_{sk}). After normalization, the mechanical parameters BH, BG, BCh and BR showed similar trends with berry density in relation to the respective parameters without normalization. The normalization by berry diameter amplified the density effect (higher significant differences in the values among density classes) on BCh and BS, and it reduced the effect on BCo and BR. Therefore, at equal diameters, the berries belonging to the density class A were characterized by a higher ability to recover the initial form (higher springiness) when compared with those of the density classes B and C. On the basis of these results, the impact of in-field grape variability on the instrumental texture properties was enough to classify the berries in two different groups by densimetric sorting at 1081 kg/m³. The skin thickness (Sp_{sk}) was independent on berry density.

The relationship of diameter was particularly evident with the mechanical properties measured in the whole berry. In fact, the three diameter classes were well differentiated according to the parameters BH, BS, BG and BCh. The larger berries were significantly harder, gummier, springier and chewier. Therefore, three groups of berries with different mechanical properties could be separated by diameter sorting at 17 and 19 mm. As expected, the normalization by berry diameter reduced the size effect on the two first texture parameters while maintaining the same trend. However, after normalization, the diameter effect remained similar on BG and BCh in relation to the respective parameters without normalization. On the other hand, the berries belonging to the diameter class S had significantly springier skin tissues than those of the diameter classes M and L. No significant differences were found in the values of F_{sk} , W_{sk} , Sp_{sk} , BCo and BR among diameter classes.

For Italia table grape berries densimetrically sorted at harvest, Río Segade, Giacosa, de Palma et al. (2013) reported that there were no significant changes in the instrumental texture parameters of the whole berry and the berry skin with variations in berry density, as a consequence of the high dispersion of data. However, an increasing tendency was also observed in the parameters that define the skin hardness (F_{sk} and W_{sk}) and the skin stiffness (E_{sk}) with increasing berry density. This agreed with the results obtained in the present work for the Muscat Hamburg cultivar.

TPA parameters respond to variations in cell tissue mechanics due to berry density. In fact, berry cohesiveness has been proposed for monitoring the ripening process of table grapes (Río Segade, Giacosa, Torchio et al., 2013). Although the influence of the pulp and skin characteristics on the berry mechanical properties is aggregated during a compression test, the changes in the cell-wall composition are particularly notable at the cellular level of the pulp

(Vargas, Pérez, Zoffoli, & Pérez, 2001). Other previously published works showed that the relationship of density with the mechanical properties of the whole berry was variety dependent in relation to the significance and trend of the variations (Giacosa et al., 2014; Río Segade, Giacosa, Torchio et al., 2013). In the present work, the differences in the cohesiveness were significant among berries belonging to different density classes, but they were not related with the diameter. However, the changes among berries of different density classes were not evident when the cohesiveness was normalized by the diameter berry to berry.

The skin hardness can be considered an influential parameter in handling injury during harvest, packing, transport and storage (Kök & Çelik, 2004), and therefore in the shelf-life of table grapes. The skin thickness is a sensory descriptor proposed to characterize commercial table grape cultivars (Cliff, Dever, & Reynolds, 1996). In thick-skinned varieties, this characteristic may influence the texture desirability of the grapes and, if the thickness is not associated with high skin friability, would limit their commercial acceptance (Cliff et al., 1996). In comparative studies, the average values of F_{sk} , W_{sk} and Sp_{sk} ranged from 0.329 N (Black magic) to 0.787 N (Italia), from 0.068 mJ (Black Magic) to 0.635 mJ (Italia) and from 147 μm (Matilde) to 313 μm (Red Globe), respectively (Río Segade, Giacosa, de Palma et al., 2013; Río Segade, Giacosa, Torchio et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al., 2013). In the present work, Muscat Hamburg berries of all density and diameter classes tested displayed intermediate values of these three skin texture parameters.

When compared the texture parameters of the whole berry for the Muscat Hamburg cultivar with those of other white and red table grapes (Río Segade, Giacosa, de Palma et al., 2013; Río Segade, Giacosa, Torchio et al., 2013; Rolle, Giacosa et al., 2011; Rolle et al.,

2013), intermediate values were generally obtained. Nevertheless, the values of BH, BCo and BG were next to those found in Regina Nera berries (characterized by low hardness and gumminess but high cohesiveness). The cohesiveness is inversely correlated with sensory quality descriptors such as elasticity, touch resistance and firmness (Le Moigne, Maury, Bertrand, & Jourjon, 2008).

Regarding the mechanical properties, the berries belonging to the density classes B and C could be selected by densimetric separation at 1088 kg/m^3 to extend their shelf-life and to obtain a better sensory quality.

4. Conclusions

In-field grape variability had a strong impact on important quality characteristics of Muscat Hamburg table grapes. Our results suggested advantages of exploiting this variability for classification purposes. By sorting the berries according to their density and diameter, significant relationships were observed between the density or diameter and objective physicochemical parameters, which could influence the consumer acceptance of 'ready-to-eat' fruits salad. Particularly, differences in mechanical properties, phenolic composition and aromatic profile were found among berries belonging to different density or diameter classes, even though the variations were generally greater with the density. Muscat Hamburg berries having densities $> 1088 \text{ kg/m}^3$ could be advantageous from the nutraceutical and sensory point of view. Further investigations of the relationship of berry density and diameter with the texture parameters and chemical composition of other table grape cultivars will be of great importance in better understanding the sorting efficiency to obtain berries with desirable

quality features and to diversify the offer according to consumer preferences. The possibility of using the same density or diameter for different vineyards and even years should be also evaluated, at least, as a tool to homogenize the chemical composition of table grape berries at harvest. The automatic classification of grape berries according to density and size can be nondestructively performed at an industrial scale because there are winery machines for this purpose.

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Fig. 1. Distribution percentage of Muscat Hamburg table grape berries in different density (a) and diameter (b) classes at commercial harvest.

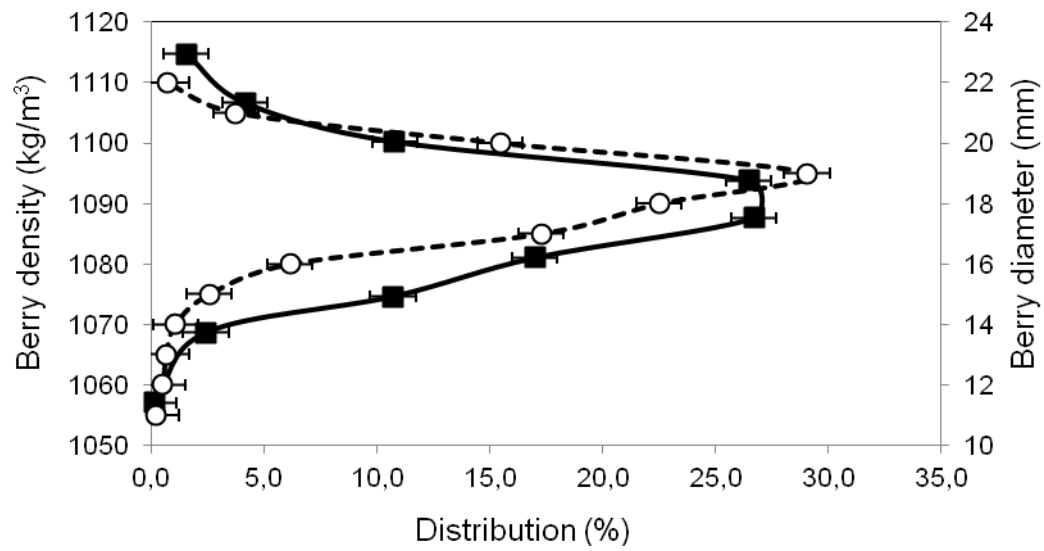


Table 1. Technological ripeness parameters of Muscat Hamburg table grape berries sorted at commercial harvest according to density and diameter.

Ripeness parameter	Density class			Sign ^a	Diameter class			Sign ^b
	A	B	C		S	M	L	
SSC (°Brix)	19.2±0.4a	20.7±0.0b	22.2±0.1c	**	21.1±1.1	21.0±0.2	20.4±0.1	ns
pH	3.66±0.01a	3.72±0.01b	3.73±0.00b	*	3.69±0.04	3.70±0.03	3.73±0.01	ns
TA (g/L tartaric acid)	4.35±0.11	4.26±0.03	4.11±0.19	ns	4.39±0.05	4.41±0.19	4.20±0.05	ns
Reducing sugars/TA	42.86±1.79a	48.24±0.62ab	54.29±3.11b	*	48.22±3.66	47.67±3.20	48.74±1.61	ns
Tartaric acid (g/L)	4.42±0.11b	4.14±0.02a	4.21±0.01ab	*	4.62±0.08	4.47±0.04	4.37±0.12	ns
Malic acid (g/L)	2.58±0.13	2.64±0.10	2.52±0.11	ns	2.42±0.23	2.56±0.13	2.42±0.03	ns
Citric acid (g/L)	0.27±0.01	0.27±0.01	0.28±0.00	ns	0.29±0.04	0.29±0.01	0.27±0.00	ns
Reducing sugars (g/L)	186.3±3.3a	205.3±3.9b	222.6±2.7c	**	211.5±13.5	209.7±5.2	204.7±4.2	ns
Glucose/ Fructose	0.97±0.00	0.97±0.01	0.97±0.00	ns	0.95±0.00	0.95±0.01	0.95±0.00	ns

All data are expressed as average value ± standard deviation (n = 4). Different Latin letters within the same row indicate significant differences (^a) among the three density classes and (^b) among the three diameter classes (*Tukey-b test*; $p < 0.05$). ^{a,b}Sign: *, ** and ns indicate significance at $p < 0.05$, 0.01 and not significant, respectively. SSC = soluble solids content, TA = titratable acidity, Reducing sugars/TA = Reducing sugars (expressed as g/L)/TA (expressed as g/L tartaric acid). A = 1081 kg/m³, B = 1088 kg/m³, C = 1094 kg/m³. S = 16-17 mm, M = 18-19 mm, L = 20-21 mm.

Table 2. Phenolic composition of Muscat Hamburg table grape berries sorted at commercial harvest according to density and diameter.

Phenolic compounds	Density class			Sign ^a	Diameter class			Sign ^b
	A	B	C		S	M	L	
HCA _p (mg caffeic acid/kg)	94.8±4.5a	97.2±2.2a	111.8±7.7b	**	105.6±7.3	103.3±8.6	105.4±6.6	ns
TP _p (mg (+)-catechin/kg)	169.6±5.6a	173.3±14.9a	198.1±15.6b	*	169.7±37.1	136.1±53.9	127.1±35.2	ns
TP _{sk} (mg (+)-catechin/kg)	689.5±65.2	708.4±58.5	760.8±106.2	ns	837.6±114.8	729.5±71.2	712.7±84.1	ns
TF _{sk} (mg (+)-catechin/kg)	1407.1±79.5a	1464.7±71.2a	1588.8±22.8b	**	1720.5±109.6b	1525.6±47.7a	1392.9±57.0a	***
PRO _{sk} (mg cyanidin chloride/kg)	1647.6±79.5	1502.8±29.5	1652.0±146.6	ns	1935.3±88.1c	1697.6±58.4b	1476.6±174.6a	**
FRV _{sk} (mg (+)-catechin/kg)	525.8±16.2	526.5±57.9	587.4±122.4	ns	524.1±86.5	500.5±37.4	483.1±79.0	ns
TA _{sk} (mg malvidin glucoside chloride/kg)	336.6±36.6a	367.6±5.2a	410.7±14.8b	**	396.3±34.5	372.9±33.3	348.1±7.9	ns
Anthocyanin profile								
Σ Delphinidin derivatives (%)	10.39±1.24	9.65±0.57	9.92±1.39	ns	8.88±0.36	9.56±0.46	9.72±0.53	ns
Σ Cyanidin derivatives (%)	7.89±0.43a	8.50±0.42ab	8.97±0.56b	*	8.63±0.84	7.91±0.40	8.25±0.44	ns
Σ Petunidin derivatives (%)	8.67±0.56	8.48±0.24	8.61±0.76	ns	7.83±0.12	8.34±0.43	8.50±0.45	ns
Σ Peonidin derivatives (%)	29.72±1.65a	33.18±1.13b	34.52±2.19b	**	34.81±2.08	33.00±1.72	33.05±1.36	ns
Σ Malvidin derivatives (%)	43.32±1.73b	40.19±0.96a	37.97±1.00a	***	39.85±2.78	41.19±2.01	40.48±1.51	ns

Σ <u>Non-acylated</u> glucosides (%)	92.57±0.33a	93.80±0.35b	93.85±0.19b	***	92.91±0.74	93.08±0.44	93.26±0.31	ns
Σ Acetyl glucosides (%)	1.20±0.02b	1.04±0.06a	1.16±0.05b	**	1.14±0.08	1.14±0.04	1.18±0.04	ns
Σ Cinnamoyl glucosides (%)	6.23±0.32b	5.16±0.29a	4.99±0.18a	***	5.95±0.67	5.79±0.43	5.56±0.28	ns

All data are expressed as average value \pm standard deviation ($n = 4$). Different Latin letters within the same row indicate significant differences (^a) among the three density classes and (^b) among the three diameter classes (*Tukey-b test*; $p < 0.05$). ^{a,b}Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. HCA = total hydroxycinnamic acids, TP = total polyphenols, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, TA = total anthocyanins. _p = berry pulp, _{sk} = berry skin. A = 1081 kg/m³, B = 1088 kg/m³, C = 1094 kg/m³. S = 16-17 mm, M = 18-19 mm, L = 20-21 mm.

Table 3. Free volatile composition of Muscat Hamburg table grape berries sorted at commercial harvest according to density and diameter.

Volatile compounds ($\mu\text{g/kg}$)	Density class			Sign ^a	Diameter class			Sign ^b
	A	B	C		S	M	L	
<i>t</i> -Rose oxide	10.42 \pm 2.14a	14.12 \pm 2.64b	18.74 \pm 0.45c	***	14.58 \pm 1.56	15.32 \pm 4.05	15.59 \pm 4.49	ns
<i>c</i> -Rose oxide	2.82 \pm 0.73a	4.03 \pm 0.73b	5.09 \pm 0.21b	**	4.21 \pm 0.59	4.47 \pm 1.00	4.30 \pm 1.18	ns
Linalool	300.47 \pm 12.96b	186.30 \pm 6.19a	174.99 \pm 30.54a	***	317.30 \pm 25.48b	205.91 \pm 62.14a	376.20 \pm 67.71b	**
Citral	4.80 \pm 0.25	5.28 \pm 0.78	5.31 \pm 0.43	ns	6.10 \pm 0.48	5.31 \pm 0.78	5.13 \pm 1.04	ns
Citronellol	14.72 \pm 1.50	17.54 \pm 5.01	21.48 \pm 5.49	ns	21.75 \pm 5.16	17.23 \pm 4.79	17.44 \pm 5.86	ns
Nerol	54.25 \pm 3.39a	64.14 \pm 3.87b	76.58 \pm 7.79c	***	76.76 \pm 8.59b	65.82 \pm 7.69b	47.92 \pm 7.76a	**
Geraniol	94.79 \pm 2.50b	90.56 \pm 5.12ab	84.45 \pm 6.08a	*	90.12 \pm 16.12b	70.24 \pm 8.17ab	65.06 \pm 11.58a	*
Geranic acid	60.13 \pm 9.29	47.66 \pm 13.23	44.95 \pm 3.34	ns	45.06 \pm 12.85	33.62 \pm 9.49	33.29 \pm 6.83	ns

All data are expressed as average value \pm standard deviation ($n = 4$). Different Latin letters within the same row indicate significant differences (^a) among the three density classes and (^b) among the three diameter classes (*Tukey-b test*; $p < 0.05$). ^{a,b}Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. A = 1081 kg/m³, B = 1088 kg/m³, C = 1094 kg/m³. S = 16-17 mm, M = 18-19 mm, L = 20-21 mm.

Table 4. Odor activity values (OAVs) of free volatile compounds of Muscat Hamburg table grape berries sorted at commercial harvest according to density and diameter.

Volatile compounds	Odor threshold ($\mu\text{g/L}$) ^a	Density class			Diameter class		
		A	B	C	S	M	L
<i>t</i> -Rose oxide	0.5	22.45	30.63	40.88	31.58	33.23	33.83
<i>c</i> -Rose oxide	0.5	6.07	8.74	11.10	9.13	9.70	9.33
Linalool	6	53.96	33.66	31.80	57.26	37.22	68.12
Citral	32	0.16	0.18	0.18	0.21	0.18	0.17
Citronellol	40	0.40	0.48	0.59	0.59	0.47	0.47
Nerol	300	0.19	0.23	0.28	0.28	0.24	0.17
Geraniol	40	2.55	2.45	2.30	2.44	1.91	1.77
Geranic acid	40	1.62	1.29	1.23	1.22	0.91	0.90

^aOdor threshold values taken from Fenoll et al. (2009) and Noguerol-Pato et al. (2012). All data are expressed as average value ($n = 4$). A = 1081 kg/m^3 , B = 1088 kg/m^3 , C = 1094 kg/m^3 . S = 16-17 mm, M = 18-19 mm, L = 20-21 mm.

Table 5. Instrumental texture parameters of Muscat Hamburg table grape berries sorted at commercial harvest according to density and diameter.

Mechanical parameter	Density class			Sign ^a	Diameter class			Sign ^b
	A	B	C		S	M	L	
F _{sk} (N)	0.458±0.083a	0.527±0.115b	0.514±0.083b	**	0.452±0.111	0.457±0.089	0.447±0.077	ns
W _{sk} (mJ)	0.386±0.116a	0.463±0.165b	0.445±0.133ab	*	0.408±0.166	0.392±0.123	0.363±0.112	ns
E _{sk} (N/mm)	0.254±0.033a	0.282±0.031b	0.278±0.028b	***	0.231±0.031a	0.249±0.027b	0.261±0.028b	***
Sp _{sk} (µm)	191±34	190±41	189±40	ns	197±42	188±41	186±32	ns
BH (N)	4.817±0.790a	5.495±0.666b	5.524±0.686b	***	4.428±0.688a	5.098±0.683b	5.799±0.686c	***
BCo (-)	0.703±0.026b	0.689±0.023a	0.682±0.030a	**	0.682±0.050	0.698±0.025	0.684±0.031	ns
BG (N)	3.379±0.523a	3.780±0.439b	3.757±0.388b	***	3.008±0.434a	3.550±0.415b	3.961±0.421c	***
BS (mm)	3.982±0.239	4.007±0.240	3.896±0.232	ns	3.550±0.127a	3.916±0.133b	4.212±0.127c	***
BCh (mJ)	13.525±2.588a	15.204±2.349b	14.688±2.111b	**	10.683±1.605a	13.898±1.666b	16.694±1.952c	***
BR (-)	0.372±0.023b	0.356±0.019a	0.351±0.024a	***	0.348±0.031	0.352±0.025	0.347±0.024	ns
BH _{norm} (N/mm)	0.256±0.035a	0.288±0.029b	0.297±0.030b	***	0.262±0.042a	0.276±0.037ab	0.291±0.033b	**
BCo _{norm} (1/mm)	0.038±0.003	0.036±0.003	0.037±0.003	ns	0.040±0.003	0.038±0.004	0.034±0.002	ns
BG _{norm} (N/mm)	0.179±0.023a	0.198±0.019b	0.202±0.016b	***	0.178±0.026a	0.192±0.023b	0.199±0.021b	***

BS _{norm} (-)	0.212±0.003b	0.210±0.002a	0.210±0.003a	***	0.210±0.004a	0.212±0.003b	0.211±0.002ab	*
BCh _{norm} (N)	0.715±0.107a	0.793±0.090b	0.789±0.080b	***	0.632±0.094a	0.752±0.085b	0.837±0.089c	***
BR _{norm} (1/mm)	0.020±0.002b	0.019±0.002a	0.019±0.002ab	*	0.021±0.002	0.019±0.002	0.017±0.003	ns

All data are expressed as average value \pm standard deviation (n = 40). Different Latin letters within the same row indicate significant differences (^a) among the three density classes and (^b) among the three diameter classes (*Tukey-b test*; $p < 0.05$). ^{a,b}Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. F_{sk} = berry skin break force, W_{sk} = berry skin break energy, E_{sk} = berry skin resistance to the axial deformation, Sp_{sk} = berry skin thickness, 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 (mm). A = 1081 kg/m³, B = 1088 kg/m³, C = 1094 kg/m³. S = 16-17 mm, M = 18-19 mm, L = 20-21 mm.