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Influence of different berry thermal treatment conditions, grape anthocyanin profile, and skin hardness on the extraction of anthocyanin compounds in the colored grape juice production

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ABSTRACT

To reduce the production surplus of grapes in the wine industry and, at the same time, to meet the growing demand of nutritious foods by health-conscious consumers, alternative processes with better sustainability are needed. In the production of colored grape juices for direct consumption, the application of heat on crushed grapes is a possible technology to increase extraction of anthocyanins and other phenolic compounds. To achieve this aim, three different thermal treatments were performed on crushed grapes of two different red varieties with peonidin or malvidin prevalence in the anthocyanin profile. Qualitative and quantitative differences in the chemical composition and color characteristics of the juices were evaluated to pinpoint the time-temperature combination that allowed the highest extraction of anthocyanins. Furthermore, the modification of skin mechanical properties during thermal treatment and impact of the peonidin/malvidin ratio on juice color were investigated. The use of high temperatures for short times was the most profitable time-temperature combination based on the extraction efficacy, color stability and energetic requirements.

Keywords: grape juice, thermomaceration, chromatic characteristics, anthocyanin profile, skin hardness.

Introduction

Due to the growing concern about the production surplus affecting the wine industry of some European production areas, in the last years new solutions are demanded to reduce the negative consequences for the market value of the products and, at the same time, to minimize the environmental impact, as requested by the European Commission in 2001. Since the effort is to put on an appropriate usage of wastes, and the production of bioethanol from excess grapes is not energetically efficient, alternative managements are needed in order to reduce the gravity of the problem.

Nowadays, many consumers are increasingly including functional foods in their diet. Therefore, the production of grape juices is one possibility to avert the grape surplus from wine production and, at the same time, to meet the growing demand of nutritious foods by health-conscious consumers (Cabrera, Jang, Kim, Lee, Lee, Chung, et al., 2009; Chiusano, Cravero, Borsa, Tsolakis, Zeppa, & Gerbi, 2015). In fact, in addition to the nutritional value, grapes are a major source of health promoting bioactive compounds with anti-inflammatory action, antiplatelet activity and antioxidant properties (Carrieri, Milella, Incampo, Crupi, Antonacci, Semeraro, et al., 2013; Lutz, Jorquera, Cancino, Ruby, & Henriquez, 2011).

When concerning the transformation of red grapes in juice, the efficient extraction of phenolic compounds from skins is of paramount importance to assure a high quality level of the product. Since phenolics are extremely important for their strong influence on color and antioxidant capacity but also on astringency and bitterness (Kelebek, Canbas, & Selli, 2009), the extraction from the grape skin is fundamental for the chemical composition and sensory attributes of red grape juices. The extraction of these components relies upon different factors: grape variety, grape maturity level, concentrations in the berry, pectolitic enzymes activity, and, in pre-maceration and during maceration, temperature and skin contact time (Girard, Yuskel, Cliff, Delaquis, & Reynolds, 2001; Gómez-Plaza, Gil-Muñoz, López-Roca,

Martínez-Cutillas, & Fernandez-Fernandez, 2001; Heatherbell, Dicey, Goldsworthy, & Vanhanen, 1996; Vrhovšek, Vanzo, & Nemanic, 2002). Particularly, the extraction of anthocyanins in an aqueous medium, as grape juices cannot rely on the slight ethanol effect that is limitedly present during a juice-wine maceration with fermentation, is affected by the grape crushing and the subsequent contact time, by the presence of variable quantities of sulphur dioxide (SO_2), and by the hydrophilicity of the single molecules (González-Neves, Gil, & Barreiro, 2008). Skin mechanical properties are also important in the extraction of anthocyanins. A strong relationship between skin hardness and release capacity of winegrapes for anthocyanins has been observed in previous works (Rolle, Torchio, Ferrandino, & Guidoni, 2012a; Zouid, Siret, Jourjon, Mehinagic, & Rolle, 2013; Battista, Tomasi, Porro, Caicci, Giacosa, & Rolle, 2015).

In order to reduce the enzymatic activity due to the presence of indigenous microorganisms such as *Botrytis cinerea* and acetic bacteria, in the wine production the application of a rapid heat treatment on grapes (i.e. thermovinification) has been proposed as pre-fermentative treatment. Beyond the microbiological impact, the heating of intact or crushed grapes has other important consequences, enhancing the release of polyphenols as a result of both the increased mass transfer (Corrales, García, Butz, & Tauscher, 2009) and the higher solubility of cell components (El Darra, Grimi, Maroun, Louka, & Vorobiev, 2013a). The heat damages the cell membranes (Boulton, Singleton, & Bisson, 1996; Clarke and Bakker, 2008) and inhibits oxidizing enzymes, such as laccase and polyphenol oxydase (Clarke and Bakker, 2008). Moreover, heating reduces enzymatic activities responsible for the production of methanol (as a result of the activity of pectolitic enzymes). Therefore, shorter maceration times are required and lesser quantities of SO_2 can be added. Usually the temperatures involved in the process are not lower than 60°C (Sacchi, Bisson, & Adams, 2005; El Darra, Grimi, Vorobiev, Louka, & Maroun, 2013b) for variable times according to

the method. Some industrial plants consist in flash-heating the uncrushed grapes to induce the release of anthocyanins during maceration, whereas other methods make use of heat exchangers to heat the must (Sacchi et al., 2005) or even the crushed grapes are heated with constant pumping of the liquid during juice processing (Lima, Dutra, Toaldo, Corrêa, Pereira, de Oliveira, et al., 2015). Some experiments on grapes reported that time/temperature combinations influence differently the yield of extraction, according to the type of molecules considered: while at 55°C tannin extraction is favored over red pigment extraction, at 63°C the maximum extraction of anthocyanins occurs after 20 min (Celotti and Rebecca, 1998). For higher temperatures, the treatments are shorter because the release of phenolics in the must is faster. However, heating for long times deteriorates thermo-sensitive compounds, such as antioxidants, and results in a higher consumption of energy (El Darra et al., 2013b). Given the importance of anthocyanin extraction for grape juice production, the right combination of skin contact time and treatment temperature during maceration is important to achieve good chromatic characteristics of the produced juices.

Therefore, the purpose of this work was to study the effect of temperature and time on the release of grape anthocyanins to determine the best thermal treatment for the production of grape juices. The impact on winegrape varieties with different anthocyanin profile (i.e. ratios between di- and tri-substituted forms) and skin hardness was also evaluated because of the different trend of these two anthocyanin forms to be released at the beginning of maceration and of the relationship between skin mechanical properties and the release capacity for anthocyanins.

Materials and Methods

Grape samples

Red winegrapes of two different *Vitis vinifera* L. cultivars were harvested in vineyards of Piedmont (northwest Italy) in 2014. These cultivars were selected on the basis of their recognized different anthocyanin profiles: Malvidin Prevalent Variety (MPV, cv Barbera) and Peonidin Prevalent Variety (PPV, cv Nebbiolo). The first variety was chosen as already used in fruit juice production with a base consisting mainly of grape must (Chiusano et al., 2015), and the latter due to their characteristic profile and to their availability in the same production area, since it is used for the wine production. In order to use berries at the same level of maturity, flotation in different saline solutions was conducted as reported by Rolle, Río Segade, Torchio, Giacosa, Cagnasso, Marengo, et al. (2011a). All the berries belonging to the density class of 1100 kg m^{-3} were selected. For each variety, different sets of 30 sorted berries were randomly selected, and then weighed and crushed. To uniform the berries crushing process, it was performed using an Universal Testing Machine TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with a P/75 cylindrical probe, which permitted the accurate control of the crushing process until 3 mm of sample height at 1 mm s^{-1} speed. The crushing was done in a 80-mm i.d. Petri dish (10 berries at once), and the resulting crushed berry components were placed in 100-mL amber glass bottles with the addition of 50 mg kg^{-1} $Na_2S_2O_5$.

Another subsample of 230 sorted berries was used for the determination of technological ripeness variables, total anthocyanin concentration, anthocyanin profile and skin texture properties of fresh berries.

Technological ripeness variables

Two replicates of 100 fresh berries were used for determining the standard physicochemical variables in the grape juice obtained by manual crushing and centrifugation of fresh berries. Soluble solids concentration (Brix) was measured using an Atago 0−32 Brix temperature compensating refractometer (Atago Corporation, Tokyo, Japan), pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany), and titratable acidity (g L^{-1} tartaric acid) was determined using the OIV method (OIV, 2008). Organic acids were determined by HPLC using the chromatographic conditions reported by Giordano et al. (2013).

Thermal treatment conditions

Three conditions of thermal treatments for the production of red juices were experimented in triplicate (three sets of 30 crushed berries for each treatment): 80°C for 3 min (treatment A), 70°C for 20 min (treatment B) and 60°C for 60 min (treatment C). At the end of the thermal treatment, the samples were cooled to room temperature (20±1°C). The control sample (rosé juice), also in triplicate (three sets of 30 crushed berries), was not thermally treated and the skins were left in contact with the juice for 60 min at room temperature. To uniform the pressing process, this was performed only on the skins using the TA.XTplus texture analyzer previously described, equipped with the P/75 probe and, for this application, with a 50-kg load cell. The skins were placed in a polyethylene (PE) container and three pressing cycles (until 500 N instrumentally measured) were applied. The resulting juice was joined with the free-run juice. The residual skins and the juice were then analyzed. The chromatic characteristics of the juice, texture properties of the skin, and total anthocyanin concentration and anthocyanin profile of both juice and macerated skins were determined.

Skin mechanical properties

The Universal Testing Machine TA.XTplus texture analyzer, equipped with a HDP/90 platform, a P/2N needle probe and a 5-kg load cell, was used for the evaluation of skin mechanical properties in fresh berries and macerated berry skins, performing a puncture test at 1 mm s^{-1} test speed until 3 mm penetration depth (Letaief, Rolle, Zeppa, & Gerbi, 2008). Thirty intact fresh berries were tested, while 30 macerated berry skins were punctured for each replicate. The intact fresh berries were individually punctured on the equatorial side, whereas macerated berry skins were individually punctured on a cylindrical plastic adapter (15 mm diameter and 10 mm height) with a 3-mm hole that was placed on the platform. This adapter permitted to avoid the shift of the sample during the test, thus not touching the probe and not interfering with the puncture test (Giacosa, Marengo, Guidoni, Rolle, & Hunter, 2015).

The maximum force opposed by the berry skin until penetration was expressed in N and indicated as berry skin break force (F_{sk} or F_{msk} for intact or macerated berry skins, respectively). Using the force-distance curve, berry skin break energy (W_{sk} or W_{msk} for intact or macerated berry skins, respectively) was calculated as the area under the force-distance curve from the test start until the registered maximum force peak. Finally, the Young's modulus of elasticity (E_{sk} or E_{msk} for intact or macerated berry skins, respectively) was defined as the slope of the stress-strain curve in the linear section and measures the stiffness of the skin to an applied load.

Anthocyanin extraction and determination

Once the intact fresh berries were punctured, they were subdivided in three replicates of 10 berries and the berry skins were manually removed from the pulp using a laboratory spatula. For each replicate, intact or macerated skins were quickly immersed into a wine-like

hydroalcoholic buffer solution at pH 3.20 containing 2 g L^{-1} $Na_2S_2O_5$, 5 g L^{-1} tartaric acid and 12% v/v ethanol in order to prevent oxidation (Rolle and Guidoni, 2007). Afterwards, the skins were homogenized at 8000 rpm for 1 min with an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) and centrifuged for 15 min at 3000×g at 20°C. The supernatant was used for anthocyanin determination.

The total anthocyanin concentration was determined by a spectrophotometric method based on the dilution of the skin extract and the juice with an ethanol/water/37% HCl 70:30:1 (v/v) solution and the measurement of absorbance at 540 nm using an UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan). The anthocyanin concentration was expressed as mg of malvidin-3-O-glucoside chloride (Extrasynthèse, Genay, France) per kg of grape when referring to the skins or per L when referring to the juice. The relative standard deviation of this determination, based on repeated analyses (n = 20) of ten skin extracts, was 1.14 (Torchio, Río Segade, Gerbi, Cagnasso & Rolle, 2011).

The determination of the anthocyanin profile was performed after the berry skin extract and the juice 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. The HPLC-DAD system, chromatographic conditions and peak identification were previously reported in the literature (Rolle and Guidoni, 2007). 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 as follows: A= formic acid/water (10:90, v/v); B= formic acid/methanol/water (10:50:40, v/v). Individual anthocyanins were expressed as percentage. All of the analyses were performed in duplicate.

Juice color determination

The juice color was assessed by color intensity ($A_{420} + A_{520} + A_{620}$) and tonality ($A_{420} / A_{520}$) and by the CIEL*a*b* coordinates (OIV, 2006). The spectrophotometric measurements were carried out using a 2 mm optical path length. The CIEL*a*b* variables determined were clarity (L*), red/green color component (a*) and yellow/blue color component (b*), from which the variables correlated with the color perception were obtained, such as chroma (C*) and hue angle (H*). The CIEL*a*b* color difference variable (ΔE*) was calculated as: $\Delta E^* = (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{0.5}$ (OIV, 2006).

Statistical analysis

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

Results and Discussion

Chemical composition and skin mechanical properties of fresh berries

The composition and skin mechanical properties of MPV (i.e. Barbera) and PPV (i.e. Nebbiolo) fresh grape berries under study are presented in Table 1. Despite of using sorted berries by flotation density, some small differences were found in technological ripeness variables between varieties, in particular in Brix, titratable acidity, and malic acid concentration. This confirmed that densimetric sorting is able to select homogeneous subsamples from the berries of the same sample, but is not useful to select berries with the same sugar concentration and acidity when belonging to different varieties or to different

growing locations (Rolle et al., 2011a; Rolle, Torchio, Giacosa, Río Segade, Cagnasso, & Gerbi, 2012b).

The skin mechanical properties of Nebbiolo grapes showed similar stiffness (E_{sk}) to Barbera skins but lower hardness (F_{sk} and W_{sk}) in agreement with previously published results (Rolle, Gerbi, Schneider, Spanna, & Río Segade, 2011b). As expected, the total anthocyanin concentration of Barbera grapes was higher (about 900 mg kg^{-1}) with a profile characterized by a prevalence of malvidin (ratio between di- and tri-substituted anthocyanidins of about 0.5), whereas Nebbiolo grapes contained a low amount of total anthocyanins (about 550 mg kg^{-1}) with a prevalence of peonidin (ratio between di- and tri-substituted anthocyanidins of about 1.5) (Table 1). In the two varieties, simple glucosides prevailed but Nebbiolo was less rich in acetyl and cinnamoyl glucosides than Barbera. These results agreed with previous data reported in scientific literature (Cagnasso, Rolle, Caudana, & Gerbi, 2008; Rolle and Guidoni, 2007). The results confirmed that Barbera and Nebbiolo grapes used in the present work have different anthocyanin profiles and skin hardness. Therefore, it is possible to evaluate the effect of temperature and time of thermal treatments on winegrape varieties with different anthocyanin composition and skin mechanical properties.

MPV juice

MPV red juices were not different in CIEL*a*b* coordinates when the treatments were compared among each other (Table 2), but the control sample, being a rosé juice, was different to thermally treated samples for every variable considered. The values of L* (clarity) and H* (hue) for the control sample were higher than those for the treated samples. The control sample hue was shifted towards yellow. The application of heating treatments resulted in juices whose color was shifted toward reddish hue. In spite of the lack of differences in CIEL*a*b* coordinates among the red juices, there were differences in color over the

perceptibility threshold according to the ΔE* variable (Gonnet, 2001). The total color difference was observed especially for the red juice from the treatment C when compared to the red juices from treatments A and B (ΔE*= 7.0 and 6.8, respectively), whereas these last two juices were more similar among each other (ΔE*=1.2). In spite of the absence of differences in CIEL*a*b* variables, the juices from treatments A and B can be however discerned only after accurate observation (ΔE*>1). The high value of ΔE* variable for the red juice from the treatment C suggests that the heat exposure time was more important than the heat intensity, resulting in small but detectable differences in the color of the final juice.

Also the analysis of color according to tonality and intensity, calculated from absorbance at 420, 520, and 620 nm, did not show differences among the treated samples but highlighted differences between control and treated samples. In particular, the rosé juice showed high values of color tonality, which were comparable to those commonly observed for aged wines, whereas those for the treated samples agreed with the values found for young wines (Ribéreau-Gayon, Glories, Majuean, & Dubourdieu, 2006). The absorbance at 520 nm was very low in the control sample, confirming the lower value found for a* (red/green color component).

The data concerning the extraction of anthocyanins for MPV berries (Table 3) indicated that the extraction yield of anthocyanins from the skins agreed for all of the tested thermal treatments. However, a slightly lower value of the extraction yield for the treatment C resulted in a lower release of anthocyanins in the juice. An increase in the temperature of the maceration process contributed to an increase in anthocyanins of grape juices (Cabrera et al., 2009; Lima et al., 2015). Nevertheless, some authors indicated that no increase in anthocyanins was observed using higher temperatures (Iyer, Sacks, & Padilla-Zakour, 2010). Therefore, the contact time is also a critical factor. The control sample showed a lower

amount of pigments in the juice and a high concentration of non- extracted anthocyanins in the skins when compared to the treated samples.

Regarding the anthocyanin profile (Table 4), the rosé juice from the control sample was composed, among simple glucosides, almost exclusively of malvidin. Delphinidin and petunidin were extracted in traces (<1%), while cyanidin and peonidin were present in higher relative amounts, confirming the early extraction of these two last anthocyanin compounds as already supported by other authors (González-Neves et al., 2008).

The effect of heat seemed evident on the relative amounts of delphinidin and petunidin, with twenty-fold percentage increases in the red juices with respect to the rosé juice. The extraction kinetics of delphinidin and petunidin is known to be generally slow (Cagnasso et al., 2008), but the effect of high temperatures on the properties of the medium, such as the increase in mass transfer (Corrales et al., 2009) and the higher solubilization of cell components (El Darra et al., 2013a), could improve the release kinetics of these components allowing higher yields. Therefore, the treated samples presented high quantities of slowly-released anthocyanins in the juices and, in comparison, lower quantities of rapidly-released compounds. This means that the anthocyanin composition of the treated juices showed a strong similarity with that of the fresh grapes (Table 1), a condition resembling that of long-run macerated musts. Malvidin was predominant in all cases, particularly in the control juice. Being malvidin not easily oxidized by enzymes, the presence of high quantities of malvidin is positive for color stability. Furthermore, acylated anthocyanins are very important because they participate in intramolecular copigmentation processes, protecting the flavylium cation (Gil-Muñoz, Moreno-Pérez, Vila-López, Fernández-Fernández, Martínez-Cutillas, & Gómez-Plaza, 2009). Higher percentages of acylated anthocyanins were found in the juices from the treated samples in relation to those from the control sample.

As occurred for the total anthocyanin concentration of the red juices from thermally treated samples (Table 3), different percentages of delphinidin, petunidin, and malvidin were found in the MPV juice from the treatment C (lower relative amounts of delphinidin and petunidin but higher ones of malvidin) when compared to the juices from treatments A and B (Table 4). Instead, considering the highest extraction yield of anthocyanins in the red juice from the treatment A (Table 3), this juice showed the higher amount of malvidin. However, the ratios of the three classes of glucosides were not greatly affected by the treatment conditions. In the residual skins, very few differences were observed in the anthocyanin profiles of the treated and control samples (Table 4), which in turn were similar to those of fresh berries (Table 1). The impact of the thermal treatment on any skin anthocyanin did not depend on temperature and contact time.

Along with the relatively high amount of malvidin, the effect of temperature on color stability can be assured by the denaturation temperatures of the enzymes responsible for the oxidation of anthocyanins. The optimum temperature for these enzymes in grapes is near 40°C (De Aguiar Cipriano, Ekici, Barnes, Gomes, & Talcott, 2015). The depletion of polyphenol oxidase is faster with higher temperatures. About a 50% reduction of grape polyphenol oxidase was observed after 20 min at 65°C and complete inactivation at 75°C after 15 min (Yoruk & Marshall, 2003).

The application of high temperatures for short times is usual in the food industry as a mean of chemical and microbiological stabilization. The high temperature/short time combination allows also the reduction of the energetic requirements and low environmental impacts. MPV grapes did not show thermal damages in terms of pigment losses when the most severe treatment was applied. Since the reduced time (3 min) for the treatment A needs a small amount of energy and allows more cycles per equipment, it is the most economically sustainable solution among the tested treatments. Therefore, the treatment A was the best

thermal treatment of choice for the production of grape juices due to the results obtained for the yield and profile of the extracted anthocyanins and to reasons related to color stability, energy consumption and economical sustainability.

Conversely to what was expected, the mechanical properties of Barbera skins were not affected by any tested treatment (Table 5), as also compared to those of fresh grapes (Table 1), although a trend toward hardening was observed with temperature. In this sense, higher values of F_{msk} and W_{msk} variables in the skins from treatments A and B, with respect to C, could help to explain the higher extraction of anthocyanins (7% and 9% for treatments B and A, respectively). In fact, as demonstrated in a previous study, harder skins show generally a higher capability to release anthocyanins in a wine-like solution (Rolle et al., 2012a).

PPV juice

Colorimetric data of PPV juices (Table 2) showed a different pattern to those of MPV juices. In this case, differences emerged not just between treated and untreated samples but also, and more importantly, among the different treatments. The visual differences among Nebbiolo red juices were confirmed by the total color difference (ΔE*). The greatest difference (ΔE*=14.6) was observed between treatments A and B, while the greatest similarity (ΔE*=6.0) was observed between B and C. In the latter case, the entity of the difference was similar to the greatest difference observed among the red juices from Barbera (ΔE*=7.0 and 6.8 when the treatment C was compared to treatments A and B, respectively). Regarding Nebbiolo red juices, therefore, there was a great difference between short and long treatments. The reason is found in the values of CIEL*a*b* coordinates: treatments B and C resulted in juices with lighter (L*) and more yellowish color (b*) than those from the treatment A and, notwithstanding the values of L* and b* coordinates were not different from each other (treatments B and C).

The red juice from the treatment A also showed higher values of color tonality and intensity when compared to those from treatments B and C (Table 2). The intermediate values of color intensity reported for treatment C were similar to those for treatment B.

On the whole, Nebbiolo treated grape skins released lower amounts of anthocyanins than Barbera due to a lower amount of these compounds in the fresh grapes (Tables 1 and 3) but, when yields were considered, higher values than Barbera were found in almost all the cases (Table 3). Nonetheless, the extraction yields of Nebbiolo grapes were not as high as those previously found (Río Segade, Torchio, Giacosa, Ricauda Aimonino, Gay, Lambri, et al., 2014). In treated skins, the lowest release of anthocyanins for Nebbiolo was observed in the juice from the treatment B (-13%), in contrast with the pattern observed for Barbera. These results could explain the lower absorbance observed at 520 nm (Table 2). As occurred for Barbera, the lack of differences in the residual quantities of anthocyanins found in the treated skins (Table 3) strongly suggests an inconsistent importance of the type of treatment (time/temperature combinations) on the extraction of pigments. Therefore, the lower amounts of extracted anthocyanins in the juice (Nebbiolo grapes under the treatment B and Barbera grapes under the treatment C) could be ascribed to the post-extraction loss of the pigments.

The anthocyanin profile of the rosé juice was characterized by low relative amounts of delphinidin and petunidin, whereas cyanidin and peonidin accounted for 73% of extracted total simple glucosides (Table 4), as a consequence of their different extraction kinetics. As already reported for Barbera, the heat effect caused an increase in the proportions of tri-substituted anthocyanidins to the detriment of di-substituted compounds in the red juices in relation to the rosé juice. In Nebbiolo, the profiles of the juices obtained with the treatments of thermomaceration (Table 4), instead, were different due to small variations in the relative amounts of peonidin and malvidin simple glucosides, and acetyl glucosides. Although substantially the ratios of the three classes of glucosides were not greatly affected by the

treatment conditions, the application of high temperatures caused a major release of the most abundant anthocyanin form in the fresh grapes, i.e. peonidin. In particular, considering the equal extraction yield of anthocyanins in the red juices from treatments A and C (Table 3), it is possible to state that the red juice from the treatment A showed a higher amount of peonidin than that from the treatment C (+1.6%) and a lower amount of malvidin (-2.2%). The relative amounts of the other anthocyanins were not different. Also for Nebbiolo, the thermal treatments resulted in profiles of red juices that were very similar to the profile of fresh grapes, whereas the control sample was richer in simple glucosides (particularly di-substituted forms) but poorer in acetyl and cinnamoyl glucosides. The profiles of the residual anthocyanins in the skins were very similar for all untreated and treated samples (Table 4), and in turn similar to the profile for the fresh grapes (Table 1).

In contrast with the results of Barbera skins, the mechanical properties of Nebbiolo grape skins were modified during the treatments (Table 5). Thermally treated Nebbiolo grapes resulted in harder skins than the control sample, as previously observed (Río Segade et al., 2014). Since the final values of the skin break force (F_{msk}) were the same for the three treatments, the application of heat could be the main factor affecting the F_{msk} values, while a combination of time and temperature could be responsible for the different values of the skin Young's modulus of elasticity (E_{msk}) and of the skin break energy (W_{msk}) (Table 5). The treatment B induced the lowest increase in E_{msk} and this resulted in the highest energy needed for the rupture of the skins. In the specific case of the treatment A, there was no change in the W_{msk} value when compared to the control sample. The heat supplied by the treatment A modified the mechanical properties of the skins towards a type of glassy behavior, while the treatment B resulted in rubbery skins. Further research is needed in order to determine the causes of these modifications and the evolution of the mechanical properties of the skins exposed to different temperatures for different times.

Conclusions

The performances of three thermomaceration conditions were studied on two red winegrape varieties with different anthocyanin profiles and skin hardness, and compared to those of traditional maceration. Although the efficacy of high temperatures in the extraction of anthocyanins and other phenolic compounds from grapes is largely supported by the scientific literature, it is not possible to establish general conditions of thermal treatment that are acceptable for every cultivar due to the high varietal variability in the grape composition. The results suggested that MPV and PPV behaved differently under the same conditions. The application of high temperatures resulted in the same extraction yield of anthocyanins from the skins, but in different amounts of pigments in the juices. Small losses of anthocyanins were observed in the MPV juice produced at the lowest temperature and longest time (treatment C), while significant losses were found in the PPV juice from the treated sample at the intermediate temperature and time (treatment B).

The treatment A gave better outcomes for MPV samples, whereas treatments A and C produced red juices with the best characteristics from PPV grapes. For an optimized thermal treatment with the aim of increasing the extraction yield of anthocyanins to be implemented, low energy requirements should be also pursued. The hotter and faster treatment (treatment A) may be selected as the most profitable time/temperature combination for both MPV and PPV grapes not only based on the extraction efficacy and the inactivation of the oxidative enzymes, but also on considerations of energetic and economic feasibility.

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Table 1. Chemical composition and skin mechanical properties of Barbera and Nebbiolo fresh berries used for the experimental thermal treatment.

	MPV (Barbera)	**PPV (Nebbiolo)**	**Sign**
Brix[1]	22.6±0.2	23.5 ±0.1	*
pH[1]	3.18±0.01	3.26 ±0.06	ns
Titratable acidity (g L^{-1} tartaric acid)[1]	9.34±0.37	7.18 ±0.03	*
Tartaric acid (g L^{-1})[1]	5.62±0.08	5.40 ±0.06	ns
Malic acid (g L^{-1})[1]	2.75±0.04	1.56 ±0.03	**
F_{sk} (N)[2]	0.776±0.152	0.577±0.116	***
W_{sk} (mJ)[2]	0.761±0.216	0.395±0.131	***
E_{sk} (N mm^{-1})[2]	0.360±0.058	0.386±0.047	ns
Total anthocyanins (mg kg^{-1} malvidin-3-glucoside chloride)[3]	909±31	553±5	***
Delphinidin-G (%)[3]	10.2±0.4	6.3±0.4	***
Cyanidin-G (%)[3]	2.8± 0.1	16.5±1.5	***
Petunidin-G (%)[3]	11.2±0.3	4.6±0.3	***
Peonidin-G (%)[3]	4.0±0.5	40.5±2.4	***
Malvidin-G (%)[3]	45.3±0.6	19.1±2.3	***
∑ simple G (%)[3]	73.5±0.8	87.1±0.9	***
∑ acetyl G (%)[3]	13.7±0.8	3.9±0.2	***
∑ cinnamoyl G (%)[3]	12.8±0.5	9.0±0.7	***

All data are expressed as average value ± standard deviation. [1] n=2; [2] n= 30; [3] n=3. Sign: ***, **, * and ns indicate significance at $p < 0.001$, 0.01, 0.05 and not significant, respectively. MPV: Malvidin Prevalent Variety, PPV: Peonidin Prevalent Variety. F_{sk}: berry skin break force,W_{sk}: berry skin break energy, E_{sk}: Young's modulus of elasticity. G: 3-glucoside.

Table 2. Colorimetric data of Barbera and Nebbiolo juices from thermally treated berries under different conditions and untreated berries.

Cultivar	Treatment	L*	a*	b*	C*	H*	Color Tonality	Color Intensity	A_{420}	A_{520}	A_{620}
MPV (Barbera)	A	6.31±1.64	47.00±9.4	13.65±7.3	49.60±6.7	0.30±0.21	0.37±0.01	19.95±2.3	4.97±0.52	13.37±1.57	1.62±0.18
	B	6.75±1.99	47.90±8.8	14.40±8.1	50.75±5.8	0.31±0.22	0.37±0.01	19.61±2.2	4.87±0.50	13.15±1.48	1.58±0.19
	C	5.85±0.99	53.04±2.3	10.08±1.4	53.99±2.5	0.19±0.02	0.38±0.01	19.22±0.8	4.88±0.20	12.72±0.52	1.62±0.08
	sign[1]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	T	39.86±0.60	24.25±1.4	36.85±1.1	44.12±1.2	0.99±0.03	1.52±0.05	3.77±0.1	1.91±0.05	1.26±0.03	0.60±0.01
	sign[2]	***,***,***	**,*,***	**,**,***	ns,ns,**	**,**,***	***,***,***	***,***,***	*,*,*	*,*,*	*,*,*
PPV (Nebbiolo)	A	8.24±1.17a	42.95±10.7	18.90±6.3a	47.59±7.8	0.44±0.2	0.69±0.03b	10.03±0.5b	3.56±0.18c	5.85±0.34b	0.91±0.06b
	B	17.12±1.51b	47.02±2.1	30.18±1.1b	55.88±2.3	0.57±0.01	0.63±0.02a	7.21±0.3a	2.45±0.07a	4.46±0.21a	0.57±0.06a
	C	13.36±2.32b	43.11±2.6	27.74±1.8b	51.26±3.1	0.57±0.01	0.63±0.02a	8.54±0.9a	2.89±0.33b	5.28±0.54b	0.68±0.08a
	sign[1]	**	ns	*	ns	ns	*	**	*	*	*
	T	54.58±0.82	40.71±0.3	20.92±2.1	45.8±0.8	0.47±0.04	1.08±0.04	2.3±0.1	1.08±0.06	1.02±0.01	0.17±0.03
	sign[2]	***,***,***	ns,**,ns	ns,**,*	ns,**,*	ns,*,**	***,***,***	**,***,***	*,*,*	*,*,*	*,*,*

All data are expressed as average value ± standard deviation (n = 3). [1,2] Sign: ***, **, * and ns indicate significance at $p < 0.001$, 0.01, 0.05 and not significant, respectively, for the differences ([1]) among time-temperature conditions of thermal treatment and ([2]) among each treatment and the control. Different letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). MPV: Malvidin Prevalent Variety, PPV: Peonidin Prevalent Variety. L*: clarity, a*: red/green color component, b*: yellow/blue color component, C*: chroma, H*: hue angle, A_{420}, A_{520}, A_{620}: absorbance measured at 420, 520 and 620 nm, respectively. A: 80°C for 3 min, B: 70°C for 20 min, C: 60°C for 60 min, T: control.

Table 3. Total anthocyanin concentration in juice and skin from thermally treated berries under different conditions and untreated berries of Barbera and Nebbiolo winegrapes.

Cultivar	Treatment	TA juice (mg kg^{-1} grape)	TA treated skin (mg kg^{-1} grape)	% Anthocyanin extracted
MPV (Barbera)	A	562 ± 42b	345 ± 17	62 ± 5
	B	542 ± 49b	336 ± 16	60 ± 5
	C	480 ± 12a	376 ± 25	53 ± 1
	sign[1]	*	ns	ns
	T	50 ± 2	859 ± 29	5 ± 2
	sign[2]	***,***,***	***,***,***	***,***,***
PPV (Nebbiolo)	A	387 ± 12b	167 ± 7	70 ± 2b
	B	317 ± 14a	172 ± 4	57 ± 2a
	C	387 ± 9b	170 ± 2	70 ± 2b
	sign[1]	**	ns	**
	T	74 ± 6	479 ± 1	15 ± 1
	sign[2]	***,***,***	***,***,***	***,***,***

All data are expressed as average value ± standard deviation (n = 3). [1,2] Sign: ***, **, * and ns indicate significance at $p < 0.001$, 0.01, 0.05 and not significant, respectively, for the differences ([1]) among time-temperature conditions of thermal treatment and ([2]) among each treatment and the control. Different letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). MPV: Malvidin Prevalent Variety, PPV: Peonidin Prevalent Variety. TA: total anthocyanins. A: 80°C for 3 min, B: 70°C for 20 min, C: 60°C for 60 min, T: control.

Table 4. Anthocyanin profile (%) in juice and skin from thermally treated berries under different conditions and untreated berries of Barbera and Nebbiolo winegrapes.

Cultivar		Treatment	Delphinidin-G	Cyanidin-G	Petunidin-G	Peonidin-G	Malvidin-G	∑ simple G	∑ acetyl G	∑ cinnamoyl G
MPV (Barbera)	Juice	A	9.2 ± 0.4b	3.4 ± 0.2	10.4 ± 0.4ab	5.1 ± 0.3	46.1 ± 0.4b	74.3 ± 0.2b	15.5 ± 0.1	10.3 ± 0.2b
		B	9.4 ± 0.1b	3.7 ± 0.2	10.6 ± 0.1b	4.9 ± 0.1	44.5 ± 0.5a	73.1 ± 0.4a	16.1 ± 0.4	10.8 ± 0.2c
		C	8.0 ± 0.5a	3.7 ± 0.2	9.8 ± 0.1a	5.1 ± 0.2	47.6 ± 0.8c	74.3 ± 0.2b	16.1 ± 0.1	9.6 ± 0.2a
		sign[1]	**	ns	*	ns	**	**	*	***
		T	0.5 ± 0.2	4.1 ± 0.4	0.7 ± 0.4	8.0 ± 0.9	69.3 ± 1.3	82.5 ± 0.6	12.3 ± 0.7	5.2 ± 0.4
		sign[2]	***,***,***	ns,ns,ns	***,***,***	**,**,**	***,***,***	***,***,***	**,**,***	***,***,***
	Skin	A	11.8 ± 0.1	3.1 ± 0.4	12.5 ± 0.3	4.0 ± 0.4	40.3 ± 0.4	71.7 ± 0.7	13.6 ± 0.4	14.7 ± 0.3
		B	13.0 ± 0.8	3.7 ± 0.6	13.7 ± 1.1	4.2 ± 0.5	42.2 ± 5.0	76.8 ± 7.9	11.2 ± 3.2	12.0 ± 4.7
		C	11.6 ± 1.0	3.4 ± 0.3	13.0 ± 0.7	4.4 ± 0.4	43.1 ± 1.5	75.5 ± 3.7	10.3 ± 3.6	14.1 ± 0.1
		sign[1]	ns	ns	ns	ns	ns	ns	ns	ns
		T	10.8 ± 0.4	2.7 ± 0.1	11.8 ± 0.3	3.7 ± 0.5	43.9 ± 0.6	73.0 ± 0.8	13.8 ± 0.8	13.2 ± 0.5
		sign[2]	*,*,ns	ns,ns,*	*,ns,*	ns,ns,ns	***,ns,ns	ns,ns,ns	ns,ns,ns	*,ns,ns
PPV (Nebbiolo)	Juice	A	6.3 ± 0.1	19.2 ± 0.3	4.3 ± 0.2	42.0 ± 0.4b	18.2 ± 0.3a	90.0 ± 0.3b	3.9 ± 0.1a	6.1 ± 0.2
		B	6.1 ± 0.1	18.0 ± 0.7	4.4 ± 0.1	41.3 ± 0.6ab	19.2 ± 0.9ab	89.0 ± 0.2a	4.3 ± 0.1b	6.6 ± 0.1
		C	6.3 ± 0.2	17.6 ± 0.9	4.7 ± 0.1	40.4 ± 0.7a	20.4 ± 1.1b	89.4 ± 0.4ab	4.2 ± 0.2ab	6.4 ± 0.3
		sign[1]	ns	ns	ns	*	*	*	*	ns
		T	3.6 ± 0.3	25.7 ± 0.5	2.6 ± 0.1	47.4 ± 0.4	15.8 ± 0.1	95.0 ± 0.3	2.7 ± 0.2	2.3 ± 0.3
		sign[2]	***,***,***	***,***,***	***,***,***	***,***,***	***,**,**	***,***,***	***,***,***	***,***,***
	Skin	A	6.7 ± 0.2	17.0 ± 0.7	4.9 ± 0.2	39.4 ± 1.0	17.8 ± 0.5	85.8 ± 0.9	3.6 ± 0.3	10.6 ± 0.6
		B	6.5 ± 0.3	16.9 ± 1.1	4.8 ± 0.2	39.7 ± 0.5	17.7 ± 1.3	85.5 ± 0.4	3.5 ± 0.4	10.9 ± 0.4
		C	6.7 ± 0.3	16.6 ± 0.6	5.0 ± 0.1	39.4 ± 1.0	18.6 ± 0.6	86.3 ± 0.7	3.5 ± 0.4	10.2 ± 0.4
		sign[1]	ns	ns	ns	ns	ns	ns	ns	ns

	T	6.5 ± 0.4	16.0 ± 1.6	4.7 ± 0.3	40.1 ± 2.5	19.3 ± 2.4	86.6 ± 0.9	4.0 ± 0.2	9.4 ± 0.7
	sign[2]	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,ns,ns	ns,*,ns

All data are expressed as average value ± standard deviation (n = 3). [1,2] Sign: ***, **, * and ns indicate significance at $p < 0.001$, 0.01, 0.05 and not significant, respectively, for the differences ([1]) among time-temperature conditions of thermal treatment and ([2]) among each treatment and the control. Different letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). MPV: Malvidin Prevalent Variety, PPV: Peonidin Prevalent Variety. A: 80°C for 3 min, B: 70°C for 20 min, C: 60°C for 60 min, T: control. G: 3-glucoside.

Table 5. Skin mechanical properties from thermally treated berries under different conditions and untreated berries of Barbera and Nebbiolo winegrapes.

Cultivar	Treatment	F_{msk} (N)	W_{msk} (mJ)	E_{msk} (N mm^{-1})
MPV (Barbera)	A	0.823 ± 0.177	0.550 ± 0.187	0.520 ± 0.146
	B	0.825 ± 0.180	0.529 ± 0.163	0.527 ± 0.108
	C	0.789 ± 0.184	0.487 ± 0.151	0.536 ± 0.133
	sign[1]	ns	ns	ns
	T	0.760 ± 0.152	0.472 ± 0.148	0.495 ± 0.102
	sign[2]	ns,ns,ns	ns,ns,ns	ns,ns,ns
PPV (Nebbiolo)	A	0.734 ± 0.166	0.373 ± 0.110a	0.582 ± 0.150c
	B	0.788 ± 0.147	0.608 ± 0.200c	0.422 ± 0.061a
	C	0.751 ± 0.130	0.492 ± 0.140b	0.481 ± 0.067b
	sign[1]	ns	***	***
	T	0.557 ± 0.109	0.354 ± 0.101	0.374 ± 0.059
	sign[2]	***,***,***	ns,***,***	***,**,***

All data are expressed as average value ± standard deviation (n = 30). [1,2] Sign: ***, ** and ns indicate significance at $p < 0.001$, 0.01 and not significant, respectively, for the differences ([1]) among time-temperature conditions of thermal treatment and ([2]) among each treatment and the control. Different letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). MPV: Malvidin Prevalent Variety, PPV: Peonidin Prevalent Variety. F_{msk}, W_{msk}, E_{msk}: break force, break energy and Young's modulus of elasticity, respectively, for macerated berry skins. A: 80°C for 3 min, B: 70°C for 20 min, C: 60°C for 60 min, T: control.