CIEL*a*b* parameters of white dehydrated grapes as quality markers according to chemical composition, volatile profile and mechanical properties.

CIEL*a*b* PARAMETERS OF WHITE DEHYDRATED GRAPES AS QUALITY MARKERS ACCORDING TO CHEMICAL COMPOSITION, VOLATILE PROFILE AND MECHANICAL PROPERTIES

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Abstract

In the oenological sector, the withering process is of particular importance in the production of dry and sweet dessert wines due to the total or partial use of overripe and/or dehydrated grapes.

This complex process leads to several changes in the chemical-physical characteristics of white grape berries affecting the wine quality and, at the end of the dehydration period, different visual attributes are usually present in the berry skins.

The aim of this work, therefore, was to study the properties of Erbaluce dried grapes of varying external colours, classified into three groups based on reflectance colorimetry (green, gold and blue).

The chemical composition, volatile profile and mechanical attributes were investigated, focusing on establishing relationships between CIEL*a*b* parameters of dehydrated grapes and their chemical-physical characteristics.

The higher values of the glucose-fructose ratio, together with the higher content of sugars, gluconic acid and glycerol, but lower titratable acidity, suggests the presence of *Botrytis cinerea* Pers. infection in blue withered berries, which has been microbiologically confirmed.

Regarding the instrumental mechanical properties, blue dehydrated grapes were characterized by a lower skin hardness and higher skin thickness in agreement with the higher weight loss experienced. Finally, the determination of free and bound volatile compounds showed that some of them were only found in blue withered berries, δ-lactones being considered the main chemical markers of the noble rot infection that are important for the odour character. C-10 alkyl massoia lactone was the most abundant volatile compound in blue botrytized grapes.

Keywords: Dehydrated grapes; Grape colour; Volatile compounds; δ-Lactones; Instrumental mechanical properties; Berry skin hardness.

1. Introduction

Advances in the oenological sector are focused primarily on the enhancement of the sensory characteristics of wine according to cultural and technological practices. The synthesis of secondary metabolites during ripening, which strongly contributes to the aroma, colour and taste, has been widely studied [1,2] and their accumulation in the grape is highly dependent on the cultivar genotype [3]. In recent years, the growing necessity of diversifying oenological products has contributed to the use of postharvest dehydration of wine-grapes to

promote sweet and dry wines with a special aroma like "*Passiti*", Sauternes, Tokaj, Jerez and Amarone wines, as well as many other lesser known wines that are produced in almost all viticultural areas of the world [4,5].

Natural sun-drying is still the most commonly used method for dehydrating wine-grapes in sunny Mediterranean regions [6]. In the production of "icewines" the grape withering is carried out on-vine [7]. Alternatively in the production of numerous "*Passiti*" wines, off-vine dehydration can also be conducted in closed environments, even with environmentally controlled temperature, relative humidity and air-flow [7].

Dehydration is a complex process that induces several changes in the physical-chemical characteristics of grape berries. The overripe fruit is characterized by a loss of firmness and softening of texture, which increase its susceptibility to the mechanical damage and infection by pathogens [8]. In this sense, the dehydration conditions play an active role in determining the texture properties of withered grapes [9]. Conversely, the influence of the skin hardness on the wine-grape dehydration kinetics has also been confirmed [10]. Some authors suggest that berry skin hardness and thickness and peduncle detachment resistance can already be considered efficient indicators of winegrape suitability for on-vine withering [11].

Many studies have been conducted on the modifications in the colour, volatile compounds and phenolic composition induced in wine-grapes by off-vine dehydration [4,12-17]. Since the grape skin is undoubtedly one of the main sources of aroma compounds, fast dehydration promotes the increasing the release of volatile compounds [13]. In white grapes, the dehydration process contributes to carotenoids oxidation which is of great importance for the formation of specific volatiles and can be useful as a stress marker [15]. Carotenoids degradation leads to the production of pleasant aroma compounds like norisoprenoids [18].

These changes which occur during grape withering are induced by grape endogenous metabolism and moulds, both influencing the wine quality [5]. Fungal decay is the major factor affecting the success of the dehydration process or the postharvest deterioration of grapes. *Botrytis cinerea* Pers. at the stage of noble rot has some positive effects on high quality white wines (Sauternes, Tokaj), whereas grey rot may induce negative changes [19]. Economically, gray rot is the most detrimental postharvest disease in grapes due to its ability to develop even at low temperatures and to proliferate by mycelial growth from berry to berry if withering is carried out at high relative humidity.

When grapes are infected by *Botrytis cinerea*, besides the water loss and the increased sugar content that occurs, the release of aroma compounds from the skin into the must is favoured, particularly by terpenes and norisoprenoids [8]. Furthermore, this microorganism oxidizes various monoterpenes [20] altering the varietal aroma of the grape.

In Italy, the production of overripe grapes for sweet wines has a long tradition, Malvasia and Moscato being the main varieties used. Since these wines are produced in limited geographical areas according to traditional winemaking methods, the use of autochthonous grape varieties with special features potentiates the distinctive character of high quality sweet wines. The appellation Caluso *Passito* DOCG (Denomination of Origin Controlled and Guaranteed) protects the wines produced in NW Italy (Piedmont region) from Erbaluce variety of non-aromatic white grapes. This sweet dessert wine was previously characterized in terms of free volatile compounds [21] whereas the volatile profile of Erbaluce grape variety has never been published. Some recent studies on the physical-chemical parameters [22-24] and the influence of the skin hardness on the dehydration kinetics [10] have also been performed for Erbaluce grapes.

The increasing interest of grape producers and winemakers in improving the quality of *Passito* wine requires further effort into understanding the physical attributes and chemical

characteristics of dehydrated grapes. To gain an insight into the postharvest dehydration process, the aim of this work is to study the attributes of dehydrated white wine-grapes with different external visual colours. In this paper, we describe the chemical composition, the volatile profile and the mechanical properties, focusing on a first approach to the establishment of quality markers for dehydrated Erbaluce wine-grapes characterized by different values of CIEL*a*b* parameters.

2. Materials and methods

2.1. Grape, sampling and dehydration process

Grape clusters of Erbaluce white cultivar (*Vitis vinifera* L.) from a vineyard located in Caluso (Piedmont, NW Italy) were carefully harvested when a soluble solid content (SSC) of 18 ± 1 °Brix was reached in the 2009 season. Three 30 kg batches of grapes were placed in perforated boxes (60 × 40 × 15 cm, 6 kg of grapes in each box) in a single layer. For the natural off-vine dehydration process, the boxes were introduced inside a typical room called "*fruttaio*" without temperature, relative humidity and air-flow control (uncontrolled conditions). The dehydration process was carried out for 141 days, from 12 September 2009 to 31 January 2010 (autumn-winter thermohygrometric withering conditions) in accordance with Erbaluce di Caluso DOCG (Denomination of Origin Controlled and Guaranteed) wine production rules.

At the end of the withering process, dehydrated grape berries of each withering batch can be clearly separated into three groups called green, gold and blue, according to the external visual colour (Figure 1). With the aim of assessing the reliability of this classification in the three colour groups identified, the CIEL*a*b* parameters were acquired as described by Rolle and Guidoni [25] using a Minolta CR 410 reflectance colorimeter (Konica Minolta, Tokyo, Japan). The values of lightness (L*), red/green colour component (a*) and yellow/blue

colour component (b*) corresponding to each colour group of Erbaluce dehydrated grapes are reported in Table 1.

For each withering batch and colour group, three sub-samples of 20 berries were randomly selected and weighed to determine the average weight of the grape berry. One sub-sample of 30 berries was used for determining the mechanical properties. Another two sub-samples of 200 berries were used for determining volatile compounds. One sub-sample of 50 berries was used for quantifying *Botrytis cinerea*. The remaining grape berries of each group, subdivided into three replicates, were used for determining the standard physical-chemical parameters in the grape must obtained by manual crushing and filtration.

2.2. Reagents and standards

All chemicals of analytical-reagent grade and standards were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom).

2.3. Botrytis cinerea quantification

The grape sample was crushed and the must obtained was conserved at -20° C. One mL of the must was subjected to the DNA extraction according to the method proposed by Mills et al. [26]. The DNA obtained was subjected to quantitative PCR analysis by using a primer set and specific probe for *Botrytis cinerea*, as described by Cadle-Davidson [27]. The quantification of *Botrytis cinerea* was based on calibration curves prepared from serial decimal dilutions of a culture of a strain of *Botrytis cinerea* in ringer. The initial load of *Botrytis cinerea* used for the performance of the calibration curves was microscopically determined counting the spores in a Burker chamber.

2.4. Standard physical-chemical parameters determination

In the juice obtained, pH and titratable acidity were determined according to International Organization of Vine and Wine (O.I.V.) methods [28]. Gluconic acid was determined using an enzymatic test kit from R-Biopharm Italia (Cerro al Lambro, MI, Italy) and a UV-1601 spectrophotometer (Shimadzu, Japan). Organic acids (malic acid, tartaric acid and citric acid), glycerol and reducing sugars (glucose and fructose) were quantified by HPLC (Thermo Electron Corporation, Waltham, MA, USA) using a UV detector (UV100) at 210 nm and a refractive index detector (RI-150), respectively. The analyses were performed isocratically at 0.8 mL min^{-1} flow-rate and 65° C column temperature with a 300 × 7.8 mm i.d. cation exchange column (Aminex HPX-87H) and a Cation H^+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). The mobile phase was 0.0065 mol L^{-1} H_2SO_4 [29]. The data treatment was carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc., San Jose, CA, USA).

2.5. Instrumental Texture Analysis

A Universal Testing Machine (UTM) TAxT2i Texture Analyzer (SMS-Stable Micro System, Godalming, Surrey, UK) equipped with an HDP/90 platform and a 5 kg load cell was used. All the data acquisitions were made at 400 Hz and data were evaluated using the Texture Expert Exceed software version 2.54 for Windows 2000. For each berry, the skin hardness was assessed by a puncture test using an SMS P/2N needle probe (stainless steel cylinder of 2 mm of diameter with a conical needle bit) and a test speed of 1 mm s^{-1} [11,30]. The berries were placed on the metal plate of the UTM with the pedicel in a horizontal plane in order to be consistently punctured in the lateral face. The hardness of the berry skin is assessed by three parameters: maximum break force (F_{sk}), break energy (W_{sk}) and material resistance to axial deformation (E_{sk}). The first variable corresponds to the resistance of needle

probe penetration while the second variable is represented by the area under the force/time curve, which is limited between 0 and F_{sk} [30]. The third variable is defined as the slope of the stress-strain curve in the linear section and measures the skin stiffness to an applied load [30,31].

Measuring the skin thickness required manually separating a piece of skin (ca. 0.25 cm^2) from the lateral side of each berry with a razor blade. Care was taken in removing the pulp from the skin and in positioning the skin sample (face up) on the UTM platform to prevent folding. The test was performed at 0.2 mm s^{-1} with an SMS P/2 flat probe [32]. Furthermore, it was convenient to insert an instrumental trigger threshold equal to 0.05 N that enabled the plane surface of the probe to adhere completely to the skin sample before the acquisition started. This allowed a reduction or elimination of the "tail" effect due to the postponement of the contact point [30,32]. The berry skin thickness (Sp_{sk}) is given by the distance between the point corresponding to the probe contact with the berry skin (trigger) and the platform base during the compression test [32].

The peduncle detachment resistance was determined by a traction test carried out at 1 mm s^{-1} [11,33]. In this test, the peduncle is anchored to the pliers of the SMS A/PS probe modified with a rigid arm. During traction, the peduncle passes through the perforated platform of the UTM (hole diameter of 5 mm), while the berry is blocked permitting the determination of the peduncle detachment maximum force (F_{ped}). The peduncle detachment energy (W_{ped}) is represented by the area under the force/time curve, which is limited between 0 and F_{ped}. Before each test, the instrument was calibrated for force and distance.

2.6. Free and glycosylated volatile compounds extraction and determination

For each sample, two hundred berries were de-seeded and the pulp was separated from the skin with the addition of $Na_2S_2O_5$ (50 mg). The skins were treated with 20 mL of

methanol for 1 h to release aroma compounds and to inactivate glycosidase enzymes. The pulps and skins were crushed separately under a nitrogen atmosphere with a laboratory blender (Waring Laboratory, Torrington, USA). The skin suspension and pulp homogenate were then combined. The mixture was centrifuged twice (7000 × *g*, 15 min, 4° C), washing the solid residue with tartaric acid buffer (pH 3.2), and the extract (250 mL) was then clarified with a pectolytic enzyme (0.10 mg) without secondary glycosidase activity (Rapidase X-Press, DSM, The Netherlands) at room temperature for 2 h. 1-Heptanol was added as internal standard (200 µL of 44 mg L^{-1} solution in 10 % ethanol) to the samples. Afterwards, an aliquot (100 mL for green and gold grapes or 50 mL for blue grapes) (n=2) was loaded onto a 1 g Sep-Pak tC-18 reversed-phase solid-phase extraction (SPE) cartridge (Waters Corporation, Milford, MA, USA) [34], previously activated with 5 mL of methanol and then with 10 mL of deionized water using a flow-rate of ca. 3 mL min^{-1}. The cartridge was then rinsed with 10 mL of deionized water to eliminate sugars, acids and other low molecular weight polar compounds. The free fraction was then eluted with 12 mL of dichloromethane. The eluate was dried over anhydrous Na_2SO_4 and concentrated to about 200 µL under a stream of nitrogen. The extract containing free volatile compounds was immediately analyzed by gas chromatography/mass spectrometry (GC/MS).

The glycoconjugates were finally eluted from the cartridge with 20 mL of methanol and the eluate was concentrated to dryness using a vacuum rotavapor (Buchi R-210, Switzerland) at 35° C. The dried glycosidic extract was dissolved in 3 mL of citrate-phosphate buffer (0.2 M, pH 5). The enzymatic hydrolysis was carried out using 50 mg of an AR-2000 commercial preparation with glycosidase side activities (DSM Oenology, The Netherlands) and incubated at 40° C for 24 h. After adding 200 µL of 1-heptanol (44 mg L^{-1} solution in 10 % ethanol), glycosylated precursors were then extracted following the SPE method previously described. The dichloromethane extract obtained was dried over anhydrous

Na_2SO_4, concentrated to 200 µL under nitrogen and kept at -20° C until analyzed. All analyses were performed in duplicate.

GC/MS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 µm, J&W Scientific Inc., Folsom, CA, USA). The temperature program started at 35° C for 5 min, and increased at a rate of 2° C min^{-1} to 190° C and 3° C min^{-1} to 230° C for 5 min. The carrier gas (He) flow-rate was 1 mL min^{-1}. Injections of 1 µL were performed in split mode 1:10. The injection port temperature was 250° C, the ion source temperature was 240° C and the interface temperature was 230° C (solvent delay of 6.5 min). 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 30-330. The identification of volatile compounds was confirmed by injection of pure standards and the comparison of their retention indices (a mixture of a homologous series of C5-C28 was used), MS data reported in the literature and in the database (http://webbook.nist.gov/chemistry/). Compounds, for which pure standards were not available, were identified on the basis of mass spectra and retention indices available in the literature. Semiquantitative data (µg L^{-1}) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard.

2.7. Statistical analysis

Statistical analyses were performed using the statistical software package SPSS (version 17.0; SPSS Inc., Chicago, IL, USA). The Tukey-b test for $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Grape colour

During the natural off-vine withering process of Erbaluce grapes, some berries of different clusters changed in colour from initial green-yellow (based on the OIV code 225 colour of skin, as reported in the OIV descriptor list for grape varieties and *Vitis* species) to blue as shown in Figure 1. Generally, these blue berries are uniformly distributed in the cluster, which is characterized by the nearly total presence of green or gold berries. Altogether, about 56-58 % of withered berries belonged to the gold group, 43-46 % to the green group, while only 1-3 % were classified as blue. On average, the weight loss percentage (WL%) at the end of grape withering was 32, 28 and 49 for green, gold and blue berry groups, respectively.

3.2. Botrytis cinerea quantification

The calibration curve obtained by serial dilutions of a *Botrytis cinerea* culture was described by the equation $y = -4.8003x + 65.808$, where x is the count of *Botrytis cinerea* and y is the C_T value. The correlation coefficient of the calibration curve (R^2) was 0.9624 (data not shown). This calibration curve was employed in order to quantify *Botrytis cinerea* in the three types of grapes. Detection and quantification was only possible for blue dehydrated grapes and the *Botrytis cinerea* load determined was 7.94×10^5 spores mL^{-1} of must.

3.3. Standard physical-chemical parameters

The standard physical-chemical parameters obtained for each colour group of Erbaluce dehydrated grape musts are shown in Table 2. In spite of the high variability found for some parameters, particularly in blue dehydrated grapes, the three groups can be significantly different in terms of the glucose content and glucose/fructose ratio. The lower values of

sugars corresponds to green dehydrated grapes whereas, the higher values were associated with blue dehydrated grapes. Glucose/fructose ratios higher than 1 are a little surprising because these decrease during ripening, achieving values lower than 1 at harvest. Furthermore, a progressive reduction of the glucose/fructose ratio was observed during the on-vine dehydration process of Mondeuse grapes [11]. A possible explanation could be the production of β-glucans (glucose polymer) by *Botrytis cinerea* attack [35].

Blue dehydrated grapes showed a significantly lower titratable acidity and citric acid content and higher pH values in relation to green and gold grapes. This lower titratable acidity could be attributed to the concentration of cations caused by higher weight loss in blue berries and, in part, to malic and citric acid depletion under these uncontrolled environmental conditions, as demonstrated in other studies [11,15]. Significant reductions in the content of all the organic acids in blue grapes in respect to green–gold grapes can be better observed when data are expressed in g kg^{-1} grape. In particular, the decrease was of (ca.) 60% and 48% for malic and tartaric acid, respectively.

Blue dehydrated grapes were also shown to contain a high concentration of gluconic acid (1.42 g L^{-1}) and glycerol (15.86 g L^{-1}), confirming the action of *Botrytis cinerea* noble rot on the berries during the withering process. It is known that this microorganism increases the activity of oxidases like laccase and tyrosinase, which also causes colour changes, formation of glycerol and polysaccharides, and, finally, decreases the titratable acidity [36]. In this sense, the gluconic acid concentration is the most commonly used parameter in wineries for monitoring the degree of infection in grapes. Furthermore, the lower berry weight also corresponds to blue dehydrated grapes (~ 28 %) whereas this parameter was similar for green and gold berries. It suggests that the higher weight loss experienced by blue grapes could be due to the attack of *Botrytis cinerea*. In parallel, blue grapes are also characterized by a very low must yield (153 mL kg^{-1} grapes).

3.4. Instrumental Texture Analysis

The instrumental texture parameters of the skin and peduncle determined in the three colour groups of Erbaluce dehydrated grapes are shown in Table 3. The high variability associated with these parameters seems to be related to the inhomogeneous dehydration process experienced by the different berries belonging to the same colour group. In spite of this variability, some significant differences were found among the groups. The skin mechanical properties agreed between green and gold dehydrated grapes, excepting for the break energy, whereas all of the instrumental texture parameters were significantly different for blue grapes. Regarding the texture parameters that characterize the skin hardness (F_{sk} and W_{sk}) along with skin rigidity or stiffness (E_{sk}), much lower values were reported for blue dehydrated grapes (33-88 %). The greater difference corresponds to skin hardness (76-88 %). Instead, skin thickness (Sp_{sk}) results are higher (23 %) for the darker grapes. The greater thickness of the skin detected in blue grapes could be the plant's response to a *B. cinerea* attack. Therefore, the skins of blue dehydrated grapes were softer, springier and thicker than those of green and gold dehydrated grapes. Because the skin hardness increases during the dehydration process under uncontrolled environmental conditions [11], the softer skins of botrytized grapes could be the result of fungal disease which is characterized by the enzymatic attack on the pectins of the skin cell-wall. Further research is necessary on histological and histochemical changes in berry skins during the dehydration process to explain this pattern.

Some authors suggest that the skin break force and the grape variety influences the dehydration kinetics, assessing the correlation between skin hardness of Erbaluce fresh berries and daily weight loss [10]. When the values of both berry weight and skin break force were

compared among the three colour groups of withered grapes, a similar trend was observed for the two parameters.

The peduncle traction parameter values were significantly higher for gold dehydrated grapes whereas they were very low for blue grapes. Although a high resistance to the pedicel detachment is not a berry mechanical characteristic as critical in the postharvest dehydration as during the on-vine withering, blue dehydrated grapes showed a higher facility to the pedicel detachment which could cause the irreversible berry damage (low resistance to shattering).

3.5. Free volatile compounds

A total of 58 free volatile compounds were identified in the three differently coloured Erbaluce dehydrated grapes and were classified into 11 chemical categories (Table 4). The sum of ethyl and methyl esters, terpenoids and norisoprenoids showed a significantly higher content in blue dehydrated grapes. The sum of acids was significantly higher for blue dehydrated grapes than for green. Moreover for this grape variety, the *Botrytis* dehydrating effect, which was only achieved in blue grapes, caused the formation of four important δ-lactones in addition to C-18 unsaturated long-chain esters, one acid (3-furanacetic acid), one benzenoid (methyl benzeneacetate), two terpenoids (*cis*-α-bisabolene and indipone), phenol and glycerol, in accordance with Ravji et al. [37] on the effect of grape molds on the glycerol production.

Among the volatile compounds that showed significant differences in concentration for differently coloured dehydrated grapes, the compounds increased from green to blue grapes except for 1-octanol and C-6 alcohols (1-hexanol, 2-hexen-1-ol and 3-hexen-1-ol isomers) which decreased, probably due to the degradation and/or decrease of the benzenoid class. This is in accordance with the off-vine dehydration effect observed on volatile

compounds in the must from Pedro Ximénez grape variety [38], and from Malvasia and Trebbiano grape varieties [13], where the decrease in aliphatic C-6 volatile compounds during the dehydration process was likely a result of low lipoxygenase activity and a strong reactivity to form other compounds, mainly esters.

In green Erbaluce dehydrated grapes, alcohols were the predominant volatile components representing 54 % w/v of the concentration of all free volatile components. The major varietal compound was 1-hexanol, followed by esters (methyl and ethyl hexadecanoate) (14 %), benzenoids (10 %) such as homovanillic acid, acids (6 %), and terpenoids and ketones (4 % each). Gold dehydrated grapes showed a similar volatile profile even when exhibiting an increase in the acid concentration (22 %), particularly for hexanoic acid, and a relative loss of esters that dropped to 1 %. On the other hand, blue botrytized grapes presented marked changes in volatile fraction, in which δ-lactones were the most abundant volatile compounds, accounting for 67 %, followed by esters (32 %). The esters already present in green and gold dehydrated grapes increased their concentration in blue grapes, but other esters were only detected in high concentrations in blue botrytized grapes such as C-18 unsaturated long-chain esters. δ-Lactones may be considered chemical markers of the noble rot infection as they were not found in green and gold dehydrated grapes. The presence of lactones in wines is already known [39] but generally only in relation to saturated γ-lactone structures [38,40,41]. Among the fragrant volatile δ-lactones detected in blue dehydrated grapes, C-10 alkyl massoia lactone was the most abundant and represented the predominant volatile compound. Its odour descriptors are creamy, fatty, sweet and coconut-like [42]. This molecule was detected in noble rotted grape berries for the production of the Tokaj wine [43] but was not in the majority. It is known as the predominant α,β-unsaturated δ-lactone in massoia bark and heartwood plant oils [44] and in mesquite pods and flour [45]. Furthermore, massoia lactone has recently been detected in Madeira wines [46]. Another quantitatively important δ-lactone

in blue dehydrated grapes is tetrahydro-4-hydroxy-6-pentyl-2H-pyran-2-one, which was also found in noble rotted grape juices for Tokaj wines [47], followed by 5,6-dihydro-4-methyl-2H-pyran-2-one (dehydromevalonic lactone). The latter was also found in Italian traditional balsamic vinegars [48,49] as a likely derivation from Maillard reactions.

A sesquiterpene (indipone) was the main terpenoid compound present in blue dehydrated grapes, followed by a semi-volatile diterpene compound (manoyl oxide). Although the latter is known as a labdane component isolated from several plants with a wide range of biological activities [50], it was never detected in botrytized grapes. In green and gold dehydrated grapes, the major terpenoid was a derivative of the linalool family (8-hydroxy linalool). A significant increase was also observed in a norisoprenoid compound (isocoumarine) from green and gold to blue grapes. To date, this molecule has never previously been detected in grapes.

3.6. Glycosylated volatile compounds

A total of 48 glycosylated volatile compounds, classified into 11 chemical categories, were detected in the three differently coloured Erbaluce dehydrated grapes (Table 5). Unlike free volatile compounds, the glycosylated forms of phenyl propanoids are present whereas those of esters are lacking.

Blue Erbaluce grape berries display a significantly higher content of glycosylated benzenoids, lactones and alcohols that represent 38 %, 27 % and 26 % w/v, respectively, of all bound precursors that were identified. Among benzenoids, homovanillic acid was released in a significantly higher content from blue grapes than from the green and gold, and 2,3-dihydro benzofuran, previously reported in Maria Gomes white variety [51], was only found after botrytizing action. Potentially bound δ-lactones were almost exclusively present in blue grapes with a significant presence of massoia lactone. Alcohols were strongly represented

from C-6 alcohols (1-hexanol, (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol) and aromatic alcohols (phenyl ethanol and benzyl alcohol). Varietal glycosylated terpenoids were hydrolyzed by enzymatic pathway achieving 6 % of glycosylated volatile compounds in blue dehydrated grapes, with linalool derivative compounds being the major terpenoids. The total amount of terpenoids increased in green grapes up to 21 %, displaying an already identified microbial transformation by *Botrytis cinerea* [20,52]. In the three differently coloured Erbaluce withered grapes, the major terpenoid was 8-hydroxy linalool, followed by geraniol in green and gold grapes and linalool oxides in the blue. This is due to the significant decrease in the content of geraniol in botrytized grapes.

In green Erbaluce dehydrated grapes, alcohols were again the predominant volatile components representing 33 % of the concentration of all glycosidically bound precursors. The major varietal compound was 1-hexanol, followed by terpenoids (21 %), norisoprenoids (19 %) such as 3-hydroxy-7,8-dihydro-β-ionol, and benzenoids (18 %) such as homovanillic acid and 3,4,5-trimethoxy benzenemethanol. Gold dehydrated grapes showed a similar volatile profile to green in relation to terpenoids and norisoprenoids whereas the profile was similar to blue grapes in term of alcohols, even if benzenoids, particularly homovanillyl alcohol, were the predominant glycosylated volatile compounds (32 %).

5. Conclusions

Comprehensive assessment of influence of the grape overripeness (withering and *Botrytis cinerea* infection) on the physical-chemical characteristics of Erbaluce dehydrated berries permitts a better understanding of the changes occurring in white grape varieties during the dehydration process, along with the possibility of using the withered berry chromatic characteristics (instrumental and/or visual) as quality markers.

Only blue dehydrated grapes showed a chemical and volatile composition typical of the noble rot infection. Although these berries are present in low percentage (maximum 3%), and are characterized by a low must yield, they can deeply modify the final quality of the *passito* wine, in particular its sweetness and aroma. Some significant differences in the glucose content and volatile composition were also detected between green and gold berries, whose separate vinification can lead to the production of wines with a different sensory profile.

On the basis of the results achieved in this work, a differentiation of the wine characteristics could be possible easily separating the clusters with green dehydrated berries from those with gold berries, and adding different percentages of blue berries. It is of great relevance for wineries where the *passito* wine production is carried out on a small scale and/or the "schiccatura" (manual removal of the berries from stalks) is applied.

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Figure 1. Visual colour of Erbaluce dehydrated grapes: (a) green, (b) gold and (c) blue grapes.

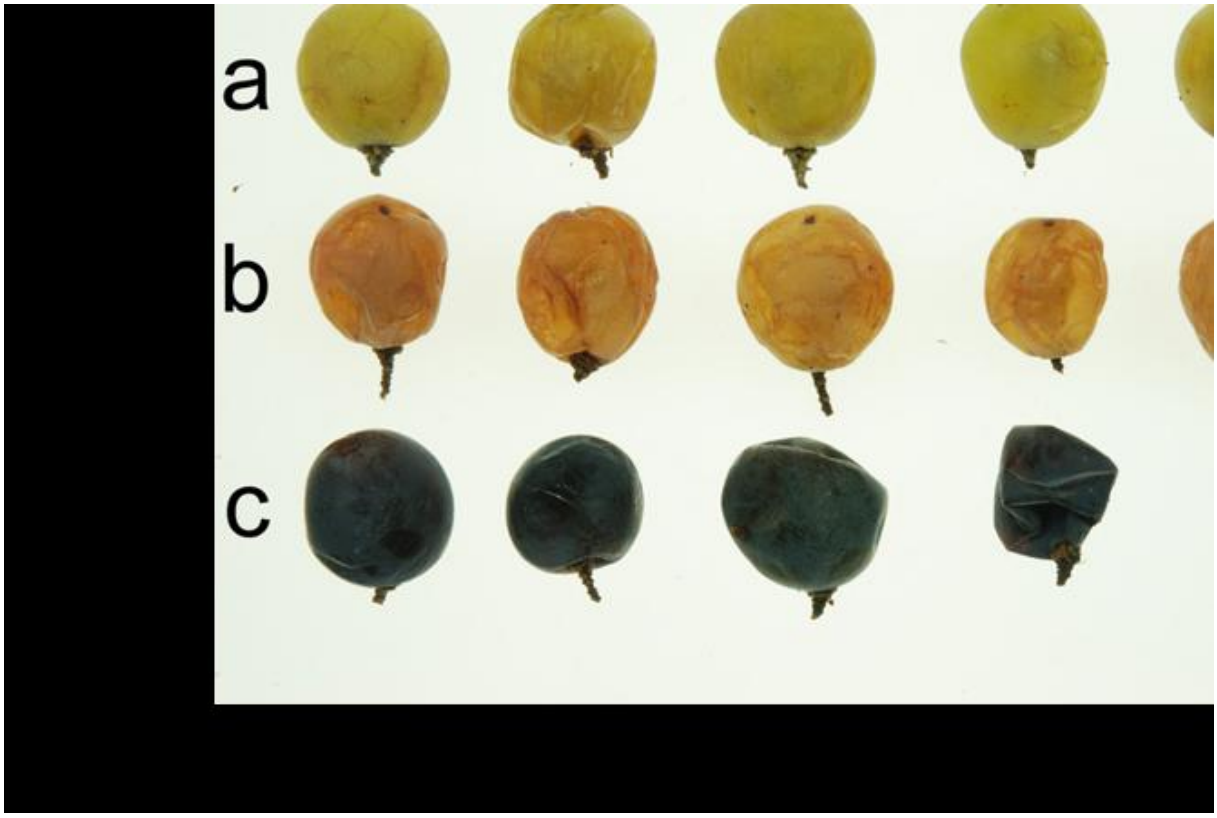

Table 1. CIEL*a*b* parameters for the three differently coloured Erbaluce dehydrated grapes

	Green	Gold	Blue	Sign[a]
L*	40.3±1.9c	37.7±3.6b	28.6±2.7a	***
a*	-0.56±1.51a	5.02±2.23c	0.89±1.13b	***
b*	15.21±2.85b	14.12±3.23b	-0.68±2.48a	***

All data are expressed as average value ± standard deviation (n = 1500). L* = lightness, a* = red/green colour component, b* = yellow/blue colour component. Different Latin letters within the same row indicate significant differences among grape colour groups (*Tukey-b test*; $p < 0.05$). [a]: *** indicate significance at $p < 0.001$.

Table 2. Standard physical-chemical parameters for the three differently coloured Erbaluce dehydrated grape musts

	Green	Gold	Blue	Sign[a]
Berry weight (g)	1.79±0.09b	1.87±0.17b	1.32±0.07a	**
pH	3.46±0.08a	3.62±0.08a	5.38±0.01b	***
Titratable acidity (g L^{-1})	5.70±0.79b	5.16±0.68b	1.80±0.49a	**
Citric acid (g L^{-1})	0.55±0.06b	0.52±0.05b	0.31±0.03a	**
Tartaric acid (g L^{-1})	6.20±0.51	5.31±0.20	7.15±1.68	ns
Malic acid (g L^{-1})	1.87±0.13	1.98±0.15	1.72±0.92	ns
Gluconic acid (g L^{-1})	0.09±0.09a	0.04±0.03a	1.42±0.70b	**
Glycerol (g L^{-1})	0.16±0.22a	0.07±0.10a	15.86±1.06b	***
Glucose (g L^{-1})	201±2a	245±2b	340±42c	**
Fructose (g L^{-1})	206±2a	236±1ab	279±38b	*
Reducing sugars (g L^{-1})	407±4a	480±1a	620±80b	**
Glucose/Fructose	0.98±0.01a	1.04±0.01b	1.22±0.04c	***
Grape must yield (mL kg^{-1} grapes)	376±22b	354±32b	153±25a	***

All data are expressed as average value ± standard deviation (n = 9). Different Latin letters within the same row indicate significant differences among grape colour groups (*Tukey-b test*; $p < 0.05$). [a]: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 3. Instrumental texture parameters for the three differently coloured Erbaluce dehydrated grapes

	Green	Gold	Blue	Sign[a]
F_{sk} (N)	0.83±0.20b	0.89±0.17b	0.20±0.06a	***
W_{sk} (mJ)	1.28±0.32b	1.48±0.39c	0.18±0.12a	***
E_{sk} (N mm^{-1})	0.20±0.06b	0.21±0.05b	0.13±0.08a	***
Sp_{sk} (µm)	245±43a	245±26a	317±64b	***
F_{ped} (N)	1.00±0.51b	1.85±0.63c	0.68±0.50a	***
W_{ped} (mJ)	0.83±0.35a	1.96±1.27b	0.44±0.32a	***

All data are expressed as average value ± standard deviation (n = 90). Different Latin letters within the same row indicate significant differences among grape colour groups (*Tukey-b test*; $p < 0.05$). [a]: *** indicate significance at $p <$, 0.001. F_{sk} = berry skin break force, W_{sk} = berry skin break energy, E_{sk} = berry skin Young´s modulus, Sp_{sk} = berry skin thickness, F_{ped} = peduncle detach force, W_{ped} = peduncle detach energy.

Table 4. Free volatile compounds (μg L^{-1}) for the three differently coloured Erbaluce dehydrated grapes

	Green	Gold	Blue	Sign[a]
Esters				
2-Methyl butanoate	-	3.43±0.04	8.51±0.81	*
Methyl hexanoate	-	0.407±0.094	8.13±3.38	ns
Ethyl (*E,Z*)-2,4-decadienoate	-	-	15.0±4.8	-
Methyl hexadecanoate	70.7±52.8	-	21791±7209	ns
Ethyl hexadecanoate	33.7±20.2	-	869±828	ns
Methyl linoleate	-	-	11183±362	-
Ethyl linoleate	-	-	1215±19	-
Methyl linolenate	-	-	3146±285	-
Ethyl linolenate	-	-	1094±435	-
Σ Esters	104±73a	3.83±0.06a	39330±9135b	**
Alcohols				
Benzyl alcohol	42.9±20.0	35.6±6.6	59.8±10.7	ns
Phenyl ethanol	37.5±3.6a	27.0±1.0a	180±33b	**
2-Phenoxy ethanol	6.54±4.97	8.52±1.41	-	ns
3-Methyl-1-butanol	-	27.8±4.4	136±2	***
(*E*)-3-Penten-2-ol	5.73±3.11a	7.30±0.74a	36.6±5.6b	**
1-Hexanol	251±72b	110±5a	59.8±6.6a	*
(*E*)-2-Hexen-1-ol	44.8±0.0	18.3±1.0	-	***
(*Z*)-2-Hexen-1-ol	3.60±0.00	-	-	-
(*E*)-3-Hexen-1-ol	-	5.18±0.45	-	-
(*Z*)-3-Hexen-1-ol	4.81±0.00	1.52±0.27	-	**
1-Octanol	4.57±0.00	3.39±0.10	-	**

2-Octanol	0.700±0.141	0.398±0.197	-	ns
1,7-Octanediol	5.66±0.00	-	-	-
Glycerol	-	-	698±515	-
Σ Alcohols	408±94	245±18	1170±548	ns
Aldehydes				
Hexanal	1.95±0.09	3.71±1.78	10.0±5.4	ns
(*Z*)-3-Hexenal	-	0.349±0.156	-	-
Σ Aldehydes	1.95±0.09	4.05±1.62	10.0±5.4	ns
Ketones				
3-Hydroxy-3-methyl-2-butanone	2.54±1.92	3.79±0.23	8.76±4.57	ns
2-Octanone	27.1±8.6	-	-	-
Σ Ketones	29.6±10.5	3.79±0.23	8.76±4.57	ns
Acids				
Sorbic acid	8.60±6.60	-	-	-
Hexanoic acid	34.8±18.5	100±0	-	*
3-Furanacetic acid	-	-	127±26	-
Σ Acids	43.4±11.9a	100±0ab	127±26b	*
Benzenoids				
Methyl benzoate	-	0.409±0.370	-	-
Methyl gentisate	9.66±4.80	-	-	-
Methyl benzeneacetate	-	-	7.18±0.72	-
Methyl salicylate	4.13±3.67	9.33±1.34	-	ns
Acetophenone	2.96±0.98	2.10±0.49	-	ns
Vanillin	4.80±2.87	3.47±0.60	-	ns
Homovanillic acid	37.7±19.9	21.3±5.4	-	ns
2,3-Dihydro benzofuran	17.4±3.2	8.22±1.22	-	ns

Σ Benzenoids	76.6±28.1	44.8±4.8	7.18±0.72	ns
Terpenoids				
Terpinen-4-ol	5.21±0.93	-	-	-
Geranic acid	-	2.76±0.74	-	-
Methyl geranate	-	0.780±0.194	-	-
8-Hydroxy linalool	15.0±4.7	10.8±0.7	-	ns
trans-Pyran linalool oxide	2.21±0.57	-	-	-
Nerol	1.41±0.69	1.29±0.51	-	ns
cis-α-Bisabolene	-	-	5.59±2.05	-
Indipone	-	-	121±39	-
Manoyl oxide	9.40±5.46	7.66±0.29	89.8±38.0	ns
Σ Terpenoids	33.2±10.4a	23.4±2.0a	216±79b	*
Phenols				
Phenol	-	-	18.2±10.6	-
m-Tert-Butyl phenol	7.91±7.05	7.44±1.86	-	ns
4-Vinylguaiacol	18.0±5.7	0.650±0.049	-	ns
Σ Phenols	25.9±12.8	8.09±1.91	18.2±10.6	ns
Lactones				
5,6-Dihydro-4-methyl-2H-pyran-2-one (Dehydromevalonic lactone)	-	-	1404±82	-
5,6-Dihydro-6-methyl-2H-pyran-2-one	-	-	548±47	-
5,6-Dihydro-6-pentyl-2H-pyran-2-one (Massoia lactone)	-	-	43145±2839	-
Tetrahydro-4-hydroxy-6-pentyl-2H-pyran-2-one	-	-	38549±10653	-
Σ Lactones	-	-	83645±13362	-
Norisoprenoids				
Dihydroactinidiolide	7.95±8.12	6.18±4.06	-	ns

3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one (Antibiotic ao-2; Isocoumarine)	7.70±4.59a	17.0±5.2a	125±29b	*
Σ Norisoprenoids	15.6±12.7a	23.2±1.2a	125±29b	*
Miscellaneas				
Benzothiazol	4.09±1.44	4.00±1.42	-	ns
Dibutyl formamide	8.04±3.52	4.37±0.50	-	ns
Σ Miscellaneas	12.1±5.0	8.37±1.91	-	ns

All data are expressed as average value ± standard deviation (n = 6). Different Latin letters within the same row indicate significant differences among grape colour groups (*Tukey-b test*; $p < 0.05$). [a]: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 5. Glycosylated volatile compounds (μg L^{-1}) for the three differently coloured Erbaluce dehydrated grapes

	Green	Gold	Blue	Sign[a]
Alcohols				
Benzyl alcohol	61.9±10.0	55.9±12.8	21.1±9.8	ns
3-Methyl benzyl alcohol	2.53±0.85	-	-	-
Phenyl ethanol	96.8±61.1a	70.5±10.5a	357±77b	*
3-Methyl-1-butanol	6.95±5.66	28.8±6.7	115±72	ns
(*E*)-3-Penten-2-ol	16.3±0.3a	16.7±3.7a	90.8±0.1b	***
1-Hexanol	241±9b	140±7a	383±10b	***
(*E*)-2-Hexen-1-ol	23.7±1.8c	11.0±0.7a	17.8±0.5b	**
(*E*)-3-Hexen-1-ol	3.29±0.66	1.66±0.19	-	ns
(*Z*)-3-Hexen-1-ol	3.54±1.91a	2.48±0.18a	11.5±2.7b	*
1-Octanol	12.5±1.3	13.0±1.4	-	ns
2-Octanol	0.447±0.032	-	-	-
2-Octen-1-ol	3.78±0.11	3.83±0.45	-	ns
Σ Alcohols	473±74a	344±7a	996±172b	*
Aldehydes				
Hexanal	13.6±1.3	31.4±14.3	12.3±1.6	ns
(*Z*)-3-Hexenal	5.12±0.40	15.5±7.3	2.20±1.64	ns
Σ Aldehydes	18.7±1.7	47.0±21.6	14.5±3.3	ns
Ketones				
3-Hydroxy-3-methyl-2-butanone	3.34±0.12	-	-	-
Σ Ketones	3.34±0.12	-	-	-
Acids				
Benzoic acid	2.60±1.57	-	-	-

Σ Acids	2.60±1.57	-	-	-
Benzenoids				
Methyl gentisate	56.0±13.0	29.7±19.5	-	ns
Methyl salicylate	15.9±2.5	4.71±0.38	-	*
2-Methyl benzaldehyde	0.897±0.541	-	-	-
Acetophenone	0.552±0.164	-	-	-
Vanillin	2.53±0.90	2.17±0.63	-	ns
Homovanillic acid	91.9±19.0a	53.3±33.1a	1109±148b	**
Homovanillyl alcohol	-	321±80	-	-
3,4,5-Trimethoxy benzenemethanol	91.8±45.6	53.1±12.1	-	ns
2,3-Dihydro benzofuran	-	-	331±299	-
Σ Benzenoids	260±79a	464±15a	1440±447b	*
Terpenoids				
α-Terpineol	3.91±1.21a	4.25±0.82a	12.9±2.3b	*
Geranic acid	29.3±6.6	21.7±9.6	-	ns
Geraniol	63.4±9.9b	57.5±13.8b	15.2±0.5a	*
8-Hydroxy linalool	188±48	155±74	142±37	ns
trans-Pyran linalool oxide	3.33±0.99a	2.19±0.23a	20.0±2.4b	**
trans-Furan linalool oxide	1.17±0.59a	0.883±0.103a	12.8±1.6b	**
cis-Furan linalool oxide	1.91±1.70	-	20.4±5.7	*
Nerol	8.07±0.65	6.41±2.08	-	ns
Exo-2-hydroxycineole	4.36±2.16a	5.36±0.27a	16.0±0.1b	**
Σ Terpenoids	304±72	253±101	239±26	ns
Phenols				
4-Ethyl phenol	-	-	31.6±7.6	-
Σ Phenols	-	-	31.6±7.6	-

Lactones				
5,6-Dihydro-4-methyl-2H-pyran-2-one	-	-	46.2±10.0	-
5,6-Dihydro-6-methyl-2H-pyran-2-one	-	-	10.1±0.3	-
5,6-Dihydro-6-pentyl-2H-pyran-2-one (Massoia lactone)	59.6±32.3	-	955±95	**
Σ Lactones	59.6±32.3	-	1012±106	**
Norisoprenoids				
3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one (Antibiotic ao-2; Isocoumarine)	-	-	17.4±2.3	-
3-Hydroxy-β-damascone	54.5±9.4	31.9±18.3	42.8±5.8	ns
3-Oxo-α-ionol	-	5.82±3.12	-	-
1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-1,3-butanedione	22.5±2.9	25.2±15.8	-	ns
7,8-Dihydro-3-oxo-α-ionol	32.0±6.2	32.9±19.8	-	ns
7,8-Dihydro-3-hydroxy-β-ionol	170±52	199±28	-	ns
Σ Norisoprenoids	279±70	295±85	60.2±8.1	ns
Phenyl propanoids				
Eugenol	4.08±1.19	3.83±1.98	-	ns
Cinnamyl alcohol	21.9±1.7	16.5±9.9	11.0±1.4	ns
Methoxy eugenol	18.9±1.8	13.8±7.5	-	ns
Σ Phenyl propanoids	44.9±4.7	34.1±19.4	11.0±1.4	ns
Miscellaneas				
Benzothiazol	0.678±0.131	-	-	-
Σ Miscellaneas	0.678±0.131	-	-	-

All data are expressed as average value ± standard deviation (n = 6). Different Latin letters within the same row indicate significant differences among grape colour groups (*Tukey-b test*; $p < 0.05$). [a]: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.