# Effect of Kaolin/Defoliation Combined with Dry Ice on Lambrusco Red Wine Production to Constrain the Effects of Climate Change

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**Since viticulture is affected considerably by climate change, it is imperative to encourage research on new strategies in order to constrain these critical effects on the composition of berries and the quality of wines. A multi-strategy approach composed of (i) kaolin application on foliage, (ii) late tree defoliation and (iii) cryomaceration of grapes with dry ice was evaluated in the production of Lambrusco Salamino wines. Physical, chemical and sensory analyses were carried out on the sample set, including the control wines. In general, cryomaceration with dry ice proved to be a winning choice to lower alcoholic strength (roughly 5%). In addition, the wines showed an increase in anthocyanin content by approximately 17%, while the content of catechins, flavanols and hydroxycinnamic acids decreased. Consistent with the increase in the anthocyanin content, an increase in colour indices and sensory colour intensity scores was observed. As for the aromatic profile, 2-phenylethanol showed an increase of approximately 18% in the treated wines while, in parallel, a lower content of $C_6$ alcohols and volatile fatty acids was observed. The multiple adaptation strategies put in place in the present study show an alternative way to mitigate the severe effects of climate change on wine production, and to face changing consumer demands.**

## INTRODUCTION

In recent decades, climate change has affected agriculture significantly by worryingly modifying the composition of crops, harvest times and production areas. As for viticulture, the increase in the sugar concentrations due to higher average summer temperatures yields more alcoholic wines with lower acidity. Such wines are appreciated less by consumers, who find them too alcoholic and hard to combine with food. The partial dealcoholisation of wines (EC, 2009) is a feasible solution, even if it affects the cost of production and partially modifies the volatile profile. However, such a high concentration of sugars, as well as the ethanol that develops, alters the activity of microorganisms during the fermentation process, thus affecting their biosynthesis of volatile compounds (De Orduna *et al*., 2010). Indeed, the concentrations of specific amino acids, which are metabolic precursors of higher alcohols, can be altered by sunlight and exposure to high temperatures (Gregan *et al*., 2012; Friedel *et al*., 2015).

Higher average temperatures also affect the secondary metabolism of grapes, with marked effects on the colour and flavour of the wines. Moreover, a misalignment between technological and phenolic maturity is considered a major problem (De Orduna *et al*., 2010; Mozell & Tach, 2014; Pons *et al*., 2017). This phenological phenomenon accelerates the accumulation of sugar, which reaches an optimal range that is no longer associated with suitable phenolic maturity due to this occurring later. The result is a domino effect that forces winemakers to postpone the harvest, thus further increasing the sugar content.

Although Lambrusco grapes are not particularly aromatic cultivars, the biosynthesis of varietal aroma are strongly influenced by environmental factors, such as light, temperature and water availability (Rapp & Mandery, 1986; Vasile Simone *et al*., 2018). An increase in average summer temperatures can also lead to greater dispersal of the volatile compounds, resulting in the reduction of the aromatic notes in grapes (Rapp & Mandery, 1986).

In order to reduce these critical effects, the wine industry has adopted a series of agronomic and technological strategies over time, including late defoliation, i.e. a pruning applied during the vegetative time of the plant, and the application of kaolin to foliage. In particular, the late defoliation causes

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a delay in the phenological phases of the grapes through a decrease in photosynthetic activity, thus lowering the sugar concentrations and maintaining a good level of acidity (Van Leeuwen & Seguin, 2006; Lereboullet *et al*., 2013; Parker *et al*., 2013; Teixeira *et al*., 2013). In addition, a less dense canopy allows better air circulation, which prevents moisture stagnation, thus preventing plant diseases and obtaining higher-quality grapes. A further technique that is already used widely in organic and biodynamic viticulture for pest control is the application of kaolin to foliage. This is done to reflect solar radiation, thus reducing losses due to evapotranspiration and damage caused by thermal stress on the grapes (Conde *et al*., 2016).

As for technological procedures, cryomaceration (CM) using dry ice (DI) represents an innovative pre-fermentative practice. Originally approved by regional regulations in organic winemaking (Reg. EU 203/2012) to reduce spontaneous fermentations and inhibit the activity of oxidase enzymes (Carillo *et al*., 2011), it has recently also been used in standard winemaking. The advantage of this procedure is twofold: while the $CO_2$ layer, derived from sublimation, provides a protective barrier for the grapes against oxygen and other environmental factors (Mencarelli & Bellincontro, 2018), DI increases the phenolic extraction by breaking the cell vacuoles in the peel (Sevcech *et al*., 2015).

The general aim of this project was the assessment of a multi-strategy approach to tackle climate change in viticulture in Northern Italy. The DI application, in particular, has several advantages when applied to grapes during harvest and in subsequent winemaking. The cold temperatures reduce the yeast metabolism (spontaneous fermentations); however, when DI is used in CM, it favours lees sedimentation, thus allowing a regular fermentation process by selected yeasts. In addition, DI enhances the cracking of the grape peel, thus facilitating the extraction of anthocyanins and varietal aromas. In this general framework, the present study was aimed at evaluating the effectiveness of the kaolin/defoliation application combined with DI on Lambrusco Salamino red wines and the effect on their chemical and sensory quality.

## MATERIALS AND METHODS

### Chemicals

Pure reference compounds, including 2-octanol used as an internal standard (IS), as well as glycerol, Folin-Ciocâlteu reagent, potassium and sodium hydroxide (KOH, NaOH), tartaric acid and potassium metabisulphite were supplied by Merck-Sigma-Aldrich (Milan, Italy). Sulphuric acid and HPLC-grade solvents, methanol, ethanol and dichloromethane, were purchased from VWR Srl (Milan, Italy). Isolute SPE $C_{18}$ (EC) cartridges (5 g) were obtained from Biotage (Uppsala, Sweden). Deionised water was obtained using the Elix$^{3UV}$ system (Merck-Millipore, Milan, Italy).

### Description of the experiments and sampling

Vine row 1 of Lambrusco Salamino (LS) (Fig. 1), situated in Mandria di Correggio (Gelosini company, Reggio Emilia, Northern Italy; GPS coordinates 44°82'99'' N, 10°71'09'' E), was treated with pure water and used as control (lot 1, called $LS_C$). Two agronomic treatments, (i) kaolin application to foliage and (ii) kaolin application to foliage combined with late defoliation, were applied on all the LS plants of vine rows 2 and 3, respectively. Kaolin (4 kg 100 $L^{-1}$ water containing 100 mL surface-active agent) was applied as aqueous solution to all vines of the two adjacent vine rows 2 and 3 (lot 2, called $LSK_C$, and lot 3) at the end of August, by means of a sprayer and using a total volume of 200 L suspension. In addition, late defoliation was carried out manually for every vine of row 3 to remove the four leaves present on the branch just above each grape cluster (lot 3 only, called $LSKD_C$). Finally, in September, before the harvest, an anti-*Botrytis* treatment was carried out on all the rows (4 kg $ha^{-1}$).

Three hundred kilograms of LS grapes from each of the three lots were selected based on their health and hygiene conditions, and harvested manually when they had reached technological maturity (18.4°Brix to 19.0°Brix). Immediately before the transfer to the Astra experimental winery, Innovazione e Sviluppo, based in Tebano (Emilia-Romagna, Italy) and where the fermentations were carried out, a sub-group of each of the three lots (called $LS_{DI}$, $LSK_{DI}$ and $LSKD_{DI}$) was sprinkled with DI (see the diagram in Fig. 1). Destemmed and crushed grapes of all three sub-groups were subjected to CM. The DI used guaranteed a decrease in temperature to 10°C to 15°C for 36 h. In contrast, the control lots ($LS_C$, $LSK_C$ and $LSKD_C$) were immediately subjected to the fermentation process.

In short, grapes with the addition of potassium metabisulphite (10 g 100 $kg^{-1}$ grapes) were pressed and destemmed. Selected yeasts (20 g $hL^{-1}$, Zymaflor F15, Laffort Italia S.r.l., Greve in Chianti, Italy) and biological activator VitaDrive®F3 (10 g $hL^{-1}$, Erbsloh, Geisenheim GmbH, Geisenheim, Germany), composed of inactive yeast, yeast cell walls (14%), diammonium hydrogen phosphate (1%) and thiamine (0.13%), were added to each grape must. The fermentation temperature was set at 20°C to 22°C, and punch-down was carried out daily for four consecutive days. Once the first fermentation phase had finished, the grape pomaces were pressed, and the fermentation process was completed at 18°C in 10 days. Sulphur dioxide was added to the wine up to a level of 30 mg $L^{-1}$, hence the samples were racked and collected in bins and finally stored at -4°C for stabilisation.

After two months, the sulphur dioxide concentration was increased to 80 mg $L^{-1}$, and the samples were then filtered and poured into glass bottles, closed with crown caps and stored at 4°C until the analysis. All analyses were carried out in duplicate using aliquots of each sample coming from different bottles pooled together and mixed thoroughly.

### Chemical analysis

#### *Physical and chemical analysis*

The evaluation of the main standard parameters, i.e. alcohol by volume (ABV), °Brix, residual sugars (RS), dry matter (DM), pH, titratable acidity (TA) and volatile acidity (VA), was carried out using standard methods (EU Official Gazette, 1990; OIV, 2019). Yeast assimilable nitrogen (YAN) was determined using the method described by Gump *et al*. (2002).

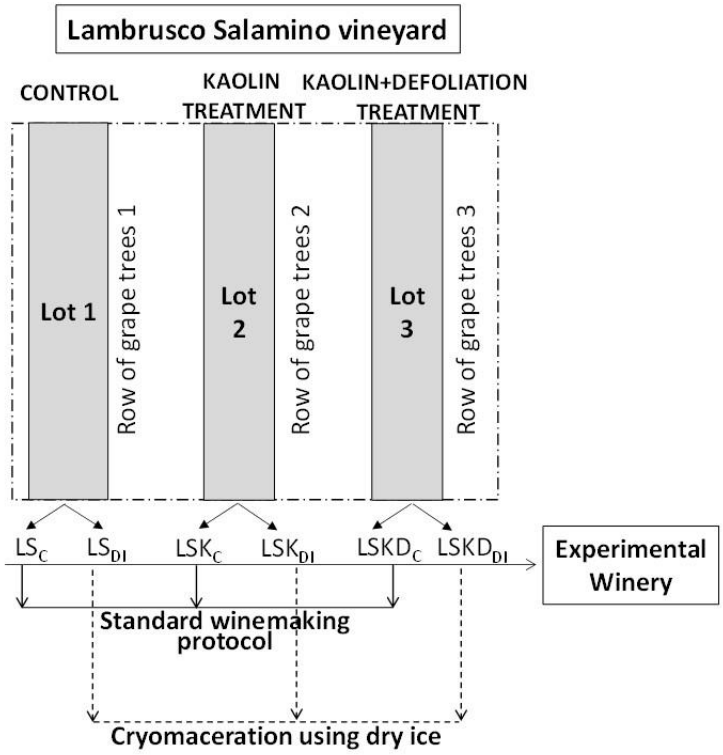


FIGURE 1

Diagram of the viticultural and winemaking processes and sample set obtained.

LSC, Lambrusco Salamino control; LSKC, Lambrusco Salamino with kaolin; LSKDC, Lambrusco Salamino with kaolin and defoliation; LSDI, Lambrusco Salamino control collected and macerated in dry ice; LSKDI, Lambrusco Salamino with kaolin collected and macerated in dry ice; LSKDDI Lambrusco Salamino with kaolin and defoliation collected and macerated in dry ice.

***HPLC determination of organic acids, glycerol, anthocyanins and phenolic compounds***

Organic acids and glycerol were determined using the HPLC method described by Montevecchi *et al*. (2012), while anthocyanins were quantified using the HPLC method described by Vasile Simone *et al*. (2013) and, finally, polyphenols (hydroxybenzoic and hydroxycinnamic acids, and flavan-3-ols) were determined using the HPLC method described by Ricci *et al*. (2019).

Peaks were identified by comparing the retention times of pure standards, while the quantification was performed through external standard calibration curves.

***Spectrophotometric and colorimetric determinations***

Colour absorbance and total phenolic content (TPC) were determined using a UV-VIS spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, USA). The absorbance of each sample was read at 420, 520 and 620 nm using 1 mm optical path analytical glass cuvettes. The values obtained were used to calculate the intensity of colour (IC) (Glories, 1984; Hunt & Pointer, 2011):

$$IC = A_{420nm} + A_{520nm} + A_{620nm}$$

TPC was determined using Folin-Ciocâlteu reagent and expressed in mg equivalents of gallic acid (GAE) using a linear calibration curve (Singleton & Rossi, 1965).

CIELab coordinates (L*, a* and b*) were measured using a tristimulus colorimeter (Chroma Meter CR-400, Konica Minolta, Milan, Italy), with the standard illuminant D65 and 10° standard observer (CIE, 1976).

***Determination of volatile compounds***

Stock reference standard solutions for each pure compound (10 000 mg $L^{-1}$), as well as for IS, were dissolved in absolute ethanol and used for the identification of volatile compounds and for calibration. To simulate the wine medium, an aliquot of each stock solution was used to prepare a 100 mg $L^{-1}$ standard mixture using a 12% ethanol solution, with the addition of 2.5 g $L^{-1}$ glycerol and 5 g $L^{-1}$ tartaric acid. The resulting synthetic wine was adjusted to pH 3.2 with a KOH aqueous diluted solution. This was subjected to a solid-phase extraction procedure (Vasile Simone *et al*. 2018) in order to determine the recovery percentage of each analyte and its specific detective response. The same extraction and concentration protocol was applied to the real samples, and all analyses were carried out in duplicate.

**Descriptive sensory evaluation**

Sensory evaluation was carried out on the sample wines (Meilgaard *et al*., 1999). Twenty judges (nine men and eleven women, aged between 24 and 55) took part to the panel test sessions as volunteers. The judges were selected based on general guidelines (ISO 8586-1, 1993) and because

of their interest in wine consumption. All of them had already attended several previous panel sessions on wine and, for this reason, they were regarded as "sufficiently trained" to carry out this test.

The sensory evaluation consisted of a descriptive analysis for the purpose of evaluating the intensity of different attributes, using a 0 ÷ 10 numeric intensity scale (Meilgaard *et al*., 1999). Seven attributes were chosen, namely colour intensity, alcoholic aroma, flavour, overall aroma aftertaste, overall taste and flavour persistence. At the end of the sessions, a judgment based on the samples' drinking pleasure was asked of each panellist.

**Statistical analysis**

The differences among the samples were assessed via an analysis of variance (two-way ANOVA) based on two replicates for each sample, and considering the dry ice treatment (DIT) and agronomic treatments (KDT) as factors, as well as their interaction (DIT × KDT). When a significant effect (at least $p \leq 0.05$) was evident, comparative analyses were carried out by post hoc Tukey's test. All tests were performed with Statistica v. 8.0 software (Stat Soft Inc., Tulsa, USA).

## RESULTS AND DISCUSSION

**Chemical and physical analysis of grape musts**

The °Brix, pH, TA, YAN, and the concentration of tartaric, malic and citric acid in the LS grape musts are reported in Table 1 as mean values of two determinations. The data obtained were consistent with that reported in the literature and did not show significant differences among the treatments, except for TA, tartaric acid and YAN. The TA also was higher when kaolin and defoliation practices were applied (Table 1), in contrast to what has been reported in some literature (Coniberti *et al*., 2013).

On the other hand, YAN showed significant differences among the agronomic treatments (Table 1). Moreover, there was a significant interaction among the factors, which would indicate a delay in the metabolism of the wild yeast due to the low temperature brought about by the protective action of kaolin, enhanced by the cooling action of DI, with a consequent saving of nitrogen.

**Chemical and physical analysis of LS wines**

In the wine samples, CM with DI caused a significant decrease in ABV (5.17% on average), while no effects were observed when the agronomic treatments were taken into consideration. In parallel, the glycerol content, as well as DM, increased in the samples treated with DI. These trends could be explained by the enhancement of the metabolic pathways of the yeasts compared to alcoholic fermentation.

The concentrations of TA and of each organic acid (tartaric, malic and citric) increased in the samples obtained using CM with DI, thus confirming the results already observed in their corresponding grape musts (Table 1). In addition, a greater inhibition of the activity of lactic acid bacteria in cryomacerated samples was another natural consequence observed.

As for the anthocyanins, CM with DI caused a significantly higher extraction of the majority of 3-*O*-glucosidic forms, as well as of total anthocyanins (Table 2). The reduced temperature stress on the grapes due to the agronomic treatments and to DI did not cause a significant variation in 3-*O*-acyl glucoside derivatives in the treated samples, as already observed by Tarara *et al*. (2008).

In general, TPC increased when DI treatment was applied to the wines; nonetheless, an even higher increase was found in CM samples obtained via agronomic treatments in comparison with their corresponding control samples (+12% and +17% in $LSK_C$ vs. $LSK_{DI}$ and $LSKD_C$ vs. $LSKD_{DI}$, respectively) (data not shown). These results were also consistent with the profiles shown by hydroxybenzoic acids (Table 2).

In contrast, flavan-3-ols (+)-chatechin and (-)-epichatechin decreased, while caffeic acid, ferulic acids (hydroxycinnamic acids), as well as their tartaric derivatives (i.e. caftaric acid and fertaric acid) did not show a constant trend (Table 2). Only when DI was combined with kaolin did the total content of hydroxycinnamic acids increase.

Used in CM, DI causes a rapid cooling of grape must, which helps inhibit polyphenol oxidase enzyme activity (Heredia *et al*., 2010). In addition, the formation of ice crystals breaks the grape pomace cell, thus enhancing the release of anthocyanins and aromatic compounds (Parenti *et al*., 2004). On the other hand, DI reduces the extraction of flavan-3-ols. Despite a notable release of phenolic pigments, the sublimating carbon dioxide replaces the air around the grape mass, thus decreasing polyphenol oxidation. For all these reasons, the grape colour is affected positively. Indeed, the present study highlighted a significant increase in the content of anthocyanin 3-*O*-glycosides in the treated samples (1449 to 1621 mg $L^{-1}$) compared with the untreated ones (1141 to 1276 mg $L^{-1}$) (Table 2). This clearly indicates that the low temperatures have an enhancing effect on the extraction of red colour from the grape skin during CM.

However, results obtained in similar studies have not reached unanimous agreement in terms of an improvement in pigment concentration for wines treated with DI in comparison with the control wine (Pérez-Lamela *et al*., 2007; Soto-Vázquez *et al*., 2010). These inconsistencies could be due to the different varieties of grapes used, with each of them having a different phenolic composition and a different thickness of the berry skin (Hortega-Heras *et al*., 2012), thus leading to a major or minor marked release of phenolic compounds.

Significant differences among the samples in terms of colour indexes (Table 3) showed a trend consistent with the anthocyanin content in the wines. All wines treated with DI showed significantly higher values of colour intensity. pH strongly affects the colour of wine: at pH 3, only 42% of anthocyanins are coloured (flavylium cation-quinoidal base), while this percentage falls to 20% at pH 4 (Riberau-Gayon *et al*., 2006). In the present study, the pH of wines treated with DI was lower, on average, than the values of the controls (3.32 vs. 3.46, respectively), and this could have contributed to the resulting higher IC in the treated samples.

The differences shown in other classes of polyphenols could be associated with a delay of phenolic maturation due to the partial shadowing of leaves due to the kaolin application; indeed, this could lead to a decrease in

TABLE 1.

Chemical and physical analysis carried out on the must and wine samples. DM: dry matter; RS: reducing sugars; TA: titratable acidity; VA: volatile acidity; YAN: yeast assimilable nitrogen (evaluated as formol index). The data are expressed as a mean value of two determinations, while *sd* (standard deviations) are reported alongside. LS: Lambrusco Salamino; the letter C as subscript by the sample name: control; letters DI as subscript close to the sample name: dry ice treatment; K: treatment in the vineyard with kaolin; D: treatment with vine defoliation. Results of two-way ANOVA (DIT: dry ice treatment; KTD: agronomic treatments; DIT × KTD: dry ice treatment × agronomic treatments; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s.: not significant); Tukey's test results are reported (just for significant $F_{value}$). Different letters identify factors that are significantly different ($p \leq 0.05$).

| | | | | | | | | | | | | | ***Two-way ANOVA*** | | | ***DIT factor*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MUST** | **$LS_C$** | *sd* | **$LS_{DI}$** | *sd* | **$LSK_C$** | *sd* | **$LSK_{DI}$** | *sd* | **$LSKD_C$** | *sd* | **$LSKD_{DI}$** | *sd* | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| °Brix | 19.20 ± | *0.28* | 18.95 ± | *0.78* | 19.00 ± | *0.14* | 19.00 ± | *0.42* | 18.95 ± | *0.92* | 19.00 ± | *0.00* | n.s. | n.s. | n.s. | | |
| pH | 3.15 ± | *0.21* | 3.10 ± | *0.14* | 3.05 ± | *0.21* | 3.05 ± | *0.35* | 3.20 ± | *0.28* | 3.05 ± | *0.35* | n.s. | n.s. | n.s. | | |
| TA g $L^{-1}$ | $7.05^{b}$ ± | *0.07* | $8.75^{a}$ ± | *0.35* | $8.55^{a}$ ± | *0.07* | $9.55^{a}$ ± | *0.07* | $7.30^{b}$ ± | *0.42* | $9.00^{a}$ ± | *0.28* | *** | | * | a | b |
| | | | a | | | | b | | | | a | | | ** | | | |
| YAN Nmg $L^{-1}$ | $141^{bc}$ ± | *0.99* | $160^{ab}$ ± | *7.92* | $128^{c}$ ± | *4.95* | $144^{bc}$ ± | *5.94* | $174^{a}$ ± | *2.97* | $169^{a}$ ± | *1.98* | n.s. | | ** | a | b |
| | | | b | | | | a | | | | c | | | *** | | | |
| Tartaric ac. g $L^{-1}$ | $4.11^{b}$ ± | *0.02* | $5.21^{a}$ ± | *0.29* | $4.51^{ab}$ ± | *0.44* | $5.10^{a}$ ± | *0.14* | $4.57^{ab}$ ± | *0.04* | $5.12^{a}$ ± | *0.17* | *** | n.s. | * | a | b |
| Malic ac. g $L^{-1}$ | 3.45 ± | *0.15* | 3.76 ± | *0.27* | 4.29 ± | *0.99* | 4.30 ± | *0.78* | 3.95 ± | *0.62* | 4.31 ± | *0.59* | n.s. | n.s. | n.s. | | |
| Citric ac. g $L^{-1}$ | 0.58 ± | *0.17* | 0.40 ± | *0.10* | 0.73 ± | *0.24* | 0.57 ± | *0.04* | 0.64 ± | *0.06* | 0.52 ± | *0.13* | n.s. | n.s. | n.s. | | |
| **WINE** | | | | | | | | | | | | | | | | | |
| ABV v $v^{-1}$% | 11.70 ± | *0.14* | 10.95 ± | *0.07* | 11.40 ± | *0.14* | 11.05 ± | *0.07* | 11.70 ± | *0.14* | 11.00 ± | *0.00* | *** | n.s. | n.s. | b | a |
| pH | 3.49 ± | *0.01* | 3.33 ± | *0.18* | 3.41 ± | *0.01* | 3.27 ± | *0.23* | 3.48 ± | *0.04* | 3.35 ± | *0.07* | n.s. | n.s. | n.s. | | |
| TA g $L^{-1}$ | $7.00^{a}$ ± | *0.14* | $8.55^{b}$ ± | *0.35* | $7.45^{a}$ ± | *0.21* | $8.60^{b}$ ± | *0.28* | $7.40^{a}$ ± | *0.00* | $8.85^{b}$ ± | *0.21* | *** | n.s. | * | a | b |
| VA g $L^{-1}$ | 0.24 ± | *0.01* | 0.33 ± | *0.04* | 0.22 ± | *0.03* | 0.29 ± | *0.01* | 0.28 ± | *0.03* | 0.33 ± | *0.04* | ** | n.s. | n.s. | a | b |
| RS g $L^{-1}$ | 1.55 ± | *0.07* | 1.45 ± | *0.07* | 1.73 ± | *0.04* | 1.55 ± | *0.07* | 1.65 ± | *0.07* | 1.40 ± | *0.14* | * | n.s. | n.s. | b | a |
| DM g $L^{-1}$ | $27.10^{b}$ ± | *0.14* | $27.95^{b}$ ± | *0.07* | $25.95^{a}$ ± | *0.07* | $27.30^{b}$ ± | *0.42* | $26.15^{a}$ ± | *0.21* | $27.90^{b}$ ± | *0.14* | *** | | * | a | b |
| | | | b | | | | a | | | | a | | | ** | | | |
| Glycerol g $L^{-1}$ | $7.15^{ab}$ ± | *0.49* | $7.90^{b}$ ± | *0.14* | $6.50^{a}$ ± | *0.28* | $7.15^{ab}$ ± | *0.21* | $6.70^{a}$ ± | *0.00* | $7.10^{ab}$ ± | *0.14* | ** | | * | a | b |
| | | | b | | | | a | | | | a | | | * | | | |
| Tartaric ac. g $L^{-1}$ | 2.05 ± | *0.07* | 2.75 ± | *0.35* | 1.95 ± | *0.07* | 2.75 ± | *0.35* | 1.95 ± | *0.07* | 2.75 ± | *0.35* | ** | n.s. | n.s. | a | b |
| Malic ac. g $L^{-1}$ | $4.30^{a}$ ± | *0.14* | $5.10^{ab}$ ± | *0.28* | $4.65^{ab}$ ± | *0.07* | $5.05^{ab}$ ± | *0.07* | $4.70^{ab}$ ± | *0.42* | $5.60^{b}$ ± | *0.57* | ** | n.s. | * | a | b |
| Citric ac. g $L^{-1}$ | 0.30 ± | *0.14* | 1.30 ± | *0.28* | 0.55 ± | *0.07* | 1.30 ± | *0.42* | 0.75 ± | *0.21* | 1.40 ± | *0.57* | ** | n.s. | n.s. | a | b |
| Lactic ac. g $L^{-1}$ | 0.30 ± | *0.00* | 0.23 ± | *0.04* | 0.25 ± | *0.07* | 0.25 ± | *0.07* | 0.35 ± | *0.07* | 0.20 ± | *0.00* | ** | n.s. | n.s. | b | a |

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TABLE 2

Profile of anthocyanins [Dp-glc, Cy-glc, Pt-glc, Pn-glc, Mv-glc: delphinidin-, cyanidin-, petunidin-, peonidin-, and malvidin-3-*O*-glucoside; Pt-Glc-(epi)cat: petunidin-3-*O*-epicathechin; Mv-Ac-glc: malvidin-(6-*O*-acetyl)-3-*O*-glucoside; Pt-Coum-glc, Pn-Coum-glc, Mv-Coum-glc: petunidin-, peonidin-, malvidin-(6-*O*-*p*-coumaroyl)-3-*O*-glucoside]. Determination of polyphenols expressed by total amount (anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids and flavan-3-ols) and total phenol content (TPC). The data are expressed as a mean value of two determinations, while *sd* (standard deviations) are reported alongside. LS: Lambrusco Salamino; the letter C as subscript by the sample name: control; letters DI as subscript close to sample name: dry ice treatment; K: treatment in the vineyard with kaolin; D: treatment of vine defoliation; GAE; gallic acid equivalents. Results of two-way ANOVA (DIT: dry ice treatment; KTD: agronomical treatments; DIT × KTD: dry ice treatment × agronomical treatments; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s.: not significant); Tukey's test results are reported (just for significant $F_{value}$). Different letters identify factors that are significantly different ($p \leq 0.05$).

| | | | | | | | | | | | | | ***Two-way ANOVA*** | | | ***DIT factor*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Anthocyanins mg L$^{-1}$** | **LS$_C$** | ± *sd* | **LS$_{DI}$** | ± *sd* | **LSK$_C$** | ± *sd* | **LSK$_{DI}$** | ± *sd* | **LSKD$_C$** | ± *sd* | **LSKD$_{DI}$** | ± *sd* | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| Dp-Glc | 59.50$^{a}$ | *2.12* | 88.50$^{abc}$ | *12.02* | 58.00$^{a}$ | *2.83* | 96.00$^{bc}$ | *12.73* | 64.00$^{ab}$ | *1.41* | 110.50$^{c}$ | 9.19 | *** | n.s. | * | a | b |
| Cy-Glc | 13.75$^{a}$ | *1.06* | 19.90$^{ab}$ | *0.14* | 17.15$^{ab}$ | *4.03* | 20.35$^{ab}$ | *2.62* | 15.85$^{ab}$ | *0.21* | 22.95$^{b}$ | 0.07 | ** | n.s. | * | a | b |
| Pt-Glc | 22.50 | *0.71* | 30.50 | *12.02* | 27.00 | *5.66* | 26.50 | *2.12* | 24.00 | *2.83* | 33.00 | 4.24 | n.s. | n.s. | n.s. | - | - |
| Pn-Glc | 24.50$^{b}$ | *0.71* | 54.00$^{ab}$ | *1.41* | 59.00$^{a}$ | *16.97* | 50.50$^{ab}$ | *7.78* | 33.00$^{ab}$ | *1.41* | 60.00$^{a}$ | 4.24 | ** | n.s. | * | a | b |
| Mv-Glc | 1 156$^{abc}$ | *22.63* | 1 279$^{cd}$ | *63.64* | 980$^{a}$ | *18.74* | 1 256$^{bcd}$ | *86.27* | 1 031$^{ab}$ | *26.16* | 1 394$^{d}$ | 78.49 | *** | n.s. | * | a | b |
| Pt-Glc-(epi)cat | 31.00$^{a}$ | *2.83* | 57.00$^{bc}$ | *2.83* | 25.50$^{a}$ | *0.71* | 47.00$^{b}$ | *2.83* | 28.50$^{a}$ | *2.12* | 58.50$^{c}$ | 3.54 | *** | | * | a | b |
| | | | a | | | | a | | | | b | | | ** | | | |
| Mv-Ac-Glc | 41.60 | *5.09* | 50.55 | *12.09* | 41.95 | *7.00* | 44.45 | *2.05* | 36.50 | *3.54* | 39.00 | 11.31 | n.s. | n.s. | n.s. | - | - |
| Pt-Coum-Glc | 56.00 | *11.31* | 71.50 | *10.61* | 48.50 | *13.44* | 68.00 | *9.90* | 55.00 | *11.31* | 68.00 | 15.56 | n.s. | n.s. | n.s. | - | - |
| Pn-Coum-Glc | 34.50 | *6.36* | 33.00 | *1.41* | 31.00 | *1.41* | 31.50 | *3.54* | 30.50 | *0.71* | 33.00 | 5.66 | n.s. | n.s. | n.s. | - | - |
| Mv-Coum-Glc | 421.50$^{b}$ | *68.59* | 316.00$^{ab}$ | *36.77* | 258.00$^{a}$ | *16.97* | 322.50$^{ab}$ | *3.54* | 268.00$^{a}$ | *12.02* | 302.00$^{ab}$ | 36.77 | n.s. | | * | - | - |
| | | | b | | | | ab | | | | a | | | * | | | |
| ∑ **Anthocyanins** | 1 861$^{ab}$ | *40.09* | 2 000$^{a}$ | *101.61* | 1 546$^{b}$ | *49.57* | 1 963$^{a}$ | *113.70* | 1 587$^{b}$ | *7.57* | 2 121$^{a}$ | 3.61 | *** | | * | a | b |
| | | | b | | | | a | | | | ab | | | * | | | |
| **Polyphenols mg L$^{-1}$** | | | | | | | | | | | | | | | | | |
| Hydroxybenzoic acids | 17.55$^{ab}$ | *1.20* | 20.75$^{c}$ | *0.92* | 13.10$^{d}$ | *0.14* | 18.20$^{abc}$ | *0.42* | 16.20$^{a}$ | *0.42* | 19.25$^{bc}$ | 0.07 | *** | | *. | a | b |
| | | | a | | | | b | | | | a | | | *** | | | |
| Flavan-3-ols | 32.35$^{d}$ | *0.78* | 28.25$^{c}$ | *0.92* | 25.30$^{bc}$ | *0.42* | 21.35$^{a}$ | *0.78* | 24.70$^{b}$ | *0.71* | 21.65$^{a}$ | 0.78 | *** | | *. | b | a |
| | | | b | | | | a | | | | a | | | *** | | | |
| Hydroxycinnamic acids | 57.40$^{cd}$ | *2.55* | 50.45$^{abc}$ | *2.05* | 47.50$^{ab}$ | *2.83* | 59.95$^{d}$ | *1.63* | 52.95$^{bcd}$ | *0.64* | 45.20$^{a}$ | 0.28 | n.s. | | *** | - | - |
| | | | b | | | | b | | | | a | | | * | | | |
| TPC mg GAE L$^{-1}$ | 1 328$^{ab}$ | *22.63* | 1 402$^{ab}$ | *112.43* | 1 221$^{a}$ | *37.48* | 1 426$^{ab}$ | *50.91* | 1 299$^{ab}$ | *14.85* | 1 457$^{b}$ | 56.57 | ** | n.s. | *. | a | b |

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TABLE 3

Intensity of colour (IC), lightness (L*), redness index (a*), and yellowness index (b*) values, hue and chroma expressed as the mean values of two replicated ± *sd* (standard deviation). ΔE (colour distance) of each pair: "control vs. DI". LS: Lambrusco Salamino; the letter C as subscript by the sample name: control; letters DI as subscripts close to the sample name: dry ice treatment; K: treatment in the vineyard with kaolin; D: treatment with vine defoliation. Results of two-way ANOVA (DIT: dry ice treatment; KTD: agronomical treatments; DIT × KTD: dry ice treatment × agronomical treatments; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s.: not significant); Tukey's test results are reported (just for significant $F_{value}$). Different letters identify factors that are significantly different ($p \leq 0.05$).

| | IC | | L* | | a* | | b* | | Hue[1] | | Chroma[2] | | ΔE[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | *sd* | **Mean** | *sd* | **Mean** | *sd* | **Mean** | *sd* | **Mean** | *sd* | **Mean** | *sd* | |
| **$LS_C$** | $6.01^{a}$ | ± 0.91 | $30.4^{d}$ | ± 0.6 | $58.7^{c}$ | ± 1.1 | $34.5^{a}$ | ± 0.9 | $1.04^{b}$ | ± 0.02 | $68.0^{ab}$ | ± 0.4 | 15.1 |
| **$LS_{DI}$** | $10.31^{b}$ | ± 0.78 | $18.7^{a}$ | ± 0.4 | $50.3^{ab}$ | ± 0.4 | $39.6^{ab}$ | ± 0.8 | $0.92^{a}$ | ± 0.01 | $63.4^{a}$ | ± 0.8 | |
| **$LSK_C$** | $6.37^{a}$ | ± 0.83 | $24.6^{bc}$ | ± 0.5 | $56.6^{bc}$ | ± 1.5 | $43.0^{ab}$ | ± 1.5 | $0.92^{a}$ | ± 0.03 | $71.1^{ab}$ | ± 0.7 | 8.7 |
| **$LSK_{DI}$** | $9.40^{b}$ | ± 0,.8 | $20.5^{ab}$ | ± 2.2 | $49.6^{a}$ | ± 0.8 | $40.1^{ab}$ | ± 0.8 | $0.89^{a}$ | ± 0.03 | $64.0^{a}$ | ± 1.3 | |
| **$LSKD_C$** | $6.24^{a}$ | ± 0.41 | $29.1^{cd}$ | ± 1.3 | $59.8^{c}$ | ± 2.1 | $45.6^{b}$ | ± 2.1 | $0.92^{a}$ | ± 0.01 | $75.1^{b}$ | ± 2.6 | 13.6 |
| **$LSKD_{DI}$** | $10.14^{b}$ | ± 0.37 | $19.0^{a}$ | ± 1.4 | $51.5^{ab}$ | ± 2.1 | $41.7^{ab}$ | ± 4.6 | $0.89^{a}$ | ± 0.03 | $66.3^{ab}$ | ± 4.5 | |

| | | IC | L* | a* | b* | Hue[1] | Chroma[2] |
|---|---|---|---|---|---|---|---|
| **$F_{value}$ DIT** | | 102.65*** | 146.6*** | 62.5*** | n.s. | 18.8** | 27.8** |
| **DIT factor** | C | a | b | b | - | b | b |
| | DI | b | a | a | - | a | a |
| **$F_{value}$ KDT** | | n.s. | n.s. | n.s. | 11.3** | 12.1** | 5.04* |
| **$F_{value}$ DIT × KDT** | | 10.15* | 10.2** | 21.1** | 4.77* | 4.67* | 8.50* |

[1] Hue = arctang (a*/b*); [2] Chroma = $(a^{*2} + b^{*2})^{1/2}$;

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polyphenols biosynthesis (Bernardo *et al*., 2018).

All the CIELab coordinates (L*, a* and b*) were influenced by the DI treatment, which inevitably caused them to decrease. The behaviour of L* was the result of a lower light energy reflected back by the samples treated with DI, and this was due to their higher anthocyanin content, while a* and b* appeared to be affected more by the anthocyanin type. Indeed, increasing the number of substituents, such as methoxy groups, in the B ring of the flavylium cation (viz. Delphinidin-Glc and Malvidin-Glc) causes a colour shift from orange to red-violet and purple hues, with a consequent decrease in a* and of the hue (Heredia *et al*., 1998). A reduction in b* leads to a fainter yellow-orange hue.

Colour distances calculated for each pair of control samples vs. the samples treated with DI were expressed with ΔE values (CIE, 1976). As for the three pairs, all ΔE values were higher than 8. The highest colour distances were observed in LSc vs. $LS_{DI}$ (ΔE = 15.07), and in $LSKD_C$ vs. $LSKD_{DI}$ (ΔE = 13.6) (Table 3), thus highlighting a colour difference among the samples that was perceivable by the naked eye.

**Volatile compound profile**

The volatile concentrations are shown in Table 4. Due to the neutral profile of the grapes used, mainly volatile compounds of fermentative origin were investigated. Fifty-six aromatic compounds were identified and quantified.

The effects of DI on the wines' aromatic compounds are reported in the literature (Parenti *et al*., 2004; Coniberti *et al*., 2013). Some studies have shown that an increase in the carbonic gas pressure can modify the production of higher alcohols during alcoholic fermentation (Baumes, 1998; Couasnon, 1999) as a consequence of a higher amino acid extraction (Bayonove, 1999). In the present study (Table 4), this phenomenon was confirmed for 2-phenylethanol (from phenylalanine), 3-methyl-thio-1-propanol (from methionine) and γ-butyrolactone (from glutamic acid), which were actually found in higher concentrations in the samples treated with DI (Carrau *et al*., 2008; Montevecchi *et al*., 2011), although kaolin/defoliation also proved to have a significant effect.

The isoamyl alcohol content did not change after DI treatment. In addition, according to Cai *et al*. (2014), concentrations of isobutyl alcohol are also constant, although in this study they were quite low. In fact, isobutyl alcohol is partially water soluble (80 mg $L^{-1}$), and this characteristic could be the cause of its lower recovery during extraction.

In general, CM time affects the wines' aromatic content in various ways (Mihnea *et al*., 2015) and, since the duration of the process in the present study was rather short (36 h), this could have had an effect on the aromatic content.

The short-term cryomaceration may not have a great influence, thus offering the possibility of different treatments in order to modulate the aromatic profile of the wines, even if it is not yet possible to fully understand which is the best combination of the various factors. This, in turn, could contribute to an improvement in the quality and greater complexity of the wines produced using CM techniques combined with the use of DI. However, it is necessary to modulate the parameters appropriately to avoid an excessive accumulation of higher alcohols, whose notes can become pungent and therefore not appreciated by consumers (Montevecchi *et al*., 2015).

Petrozziello *et al*. (2011) described a CM combined with DI on Nebbiolo grapes and did not report any effects on the volatile compounds in the treated wine when compared to the control wines. The concentration of the volatile compounds depends mainly on the origin of the grapes and on the vintage, as well as on the conditions of cold maceration using DI in the winemaking process. In contrast, the volatile compounds in the present study had a tendency to decrease, except for some of the ethyl esters (viz. ethyl succinate and diethyl succinate). In addition, a significant decrease in $C_6$ alcohols (hexanol, *trans*-hexen-3-ol, *trans*-hexen-2-ol and *cis*-hexen-3-ol) was observed for the CM wines as a natural consequence of the $CO_2$ scavenging effect, and of the lower enzymatic activity linked to a lower temperature (Franco *et al*., 2004; Lukić *et al*., 2016).

Statistically significant differences in both hexanol and *cis*-hexen-3-ol concentrations were found between $LS_C$ and $LS_{DI}$, while no effect was detected when DI was combined with kaolin or defoliation.

**Sensory analysis**

As for sensory analysis, the judges evaluated the samples treated with DI as sweet-smelling wines, and as lacking any perception of an aftertaste in relation to bitterness and astringency. In addition, no difference was highlighted in the alcohol perception (Fig. 2A, 2B and 2C). In contrast, the judges noticed a considerable difference in the colour intensity among the wines; specifically, they indicated that wines treated with DI looked darker, thus confirming the chemical results.

Finally, with regard to the evaluation of pleasantness, smell, taste and brightness were the most appreciated characteristics.

## CONCLUSIONS

Whether it is true or not that climate change might produce positive effects for some regions of the world, but negative for most others, it is undoubtedly essential to keep looking for adaptation strategies with the purpose of mitigating any negative effects. Bearing this in mind, it should not be forgotten, however, that winemaking has already survived a thousand-year history, which at times has been influenced by strong environmental changes.

The results of this research study have helped widen scientific knowledge on the effects of agronomic and oenological strategies to combat the effects of climate change on the wine sector. As an example, the application of cryomaceration combined with dry ice has led to the development of wine into ethanol being limited.

It should also be noted that, despite the much less marked effect of agronomic treatments on winemaking as compared to cryomaceration, their invaluable contribution is evident in maintaining a good level of grape must acidity, which otherwise would be affected negatively by a high imbalance in sugar content. On the other hand, cryomaceration proved to be a winning choice in the extraction of wine colour without affecting the wines' aromatic profile. There does,

TABLE 4

Concentrations of volatile compounds (mg $L^{-1}$) in the wine expressed as the mean values of three replications ± *sd* (standard deviation).

LS: Lambrusco Salamino; the letter C a subscript by the sample name: control; letters DI as subscript close to the sample name: dry ice treatment; K: treatment in the vineyard with kaolin; D: treatment with vine defoliation. Results of two-way ANOVA (DIT: dry ice treatment; KTD: agronomical treatments; DIT × KTD: dry ice treatment × agronomical treatments; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s.: not significant); Tukey's test results are reported (just for significant $F_{value}$). Different letters identify factors that are significantly different ($p \leq 0.05$).

| | | | | | | | | | | | | | *Two-way ANOVA* | | | *DIT factor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Alcohols** | **$LS_C$** | ± *sd* | **$LS_{DI}$** | ± *sd* | **$LSK_C$** | ± *sd* | **$LSK_{DI}$** | ± *sd* | **$LSKD_C$** | ± *sd* | **$LSKD_{DI}$** | ± *sd* | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| Isobutyl alcohol | 0.18 | 0.03 | 0.19 | 0.01 | 0.17 | 0.01 | 0.19 | 0.05 | 0.14 | 0.01 | 0.20 | 0.05 | n.s. | n.s. | n.s. | | |
| Isoamyl alcohols | 133.89 | 7.66 | 126.19 | 13.19 | 147.35 | 35.73 | 123.49 | 6.94 | 118.68 | 5.36 | 121.12 | 7.78 | n.s. | n.s. | n.s. | | |
| 1-Butanol | 0.29 | 0.02 | 0.22 | 0.01 | 0.27 | 0.01 | 0.29 | 0.06 | 0.20 | 0.01 | 0.29 | 0.08 | n.s. | n.s. | n.s. | | |
| 4-Methyl-1-pentanol | $0.49_b$ | 0.02 | $0.15_a$ | 0.00 | $0.49_b$ | 0.03 | $0.21_a$ | 0.01 | $0.40_c$ | 0.01 | $0.16_a$ | 0.01 | *** | | * | b | a |
| | | | a | | | | a | | | | b | | | ** | | | |
| *n*-Heptanol | $0.04_a$ | 0.00 | $0.06_{abc}$ | 0.002 | $0.05_{ab}$ | 0.01 | $0.07_{bc}$ | 0.00 | $0.05_{ab}$ | 0.00 | $0.07_b$ | 0.00 | ** | n.s. | * | a | b |
| 3-Methyl-1-pentanol | $1.33_c$ | 0.04 | $0.68_a$ | 0.04 | $1.12_b$ | 0.03 | $0.83_a$ | 0.06 | $1.16_b$ | 0.01 | $0.75_a$ | 0.06 | *** | n.s. | ** | b | a |
| 3-Ethoxy-1-propanol | 0.55 | 0.05 | 0.54 | 0.06 | 0.56 | 0.06 | 0.75 | 0.11 | 0.65 | 0.00 | 0.64 | 0.11 | n.s. | n.s. | n.s. | | |
| 1-Octen-3-ol | 0.34 | 0.06 | n.d. | | 0.30 | | n.d. | | 0.66 | 0.09 | 0.40 | 0.15 | *** | | n.s. | b | a |
| | | | a | | | | a | | | | b | | | *** | | | |
| 2-Heptanol | $0.10_c$ | 0.01 | $0.07_{ac}$ | 0.02 | $0.01_b$ | 0.00 | $0.06_{ac}$ | 0.01 | $0.03_{ab}$ | 0.01 | $0.03_{ab}$ | 0.02 | n.s. | | * | - | - |
| | | | b | | | | a | | | | a | | | ** | | | |
| *n*-Octanol | $0.04_b$ | 0.00 | $0.03_a$ | 0.00 | $0.04_{ab}$ | 0.00 | $0.03_{ab}$ | 0.00 | $0.04_{ab}$ | 0.00 | $0.03_{ab}$ | 0.00 | * | n.s. | * | b | a |
| 3-(Methylthio)-1-propanol | $0.36_{bc}$ | 0.00 | $0.52_a$ | 0.01 | $0.33_b$ | 0.09 | $0.49_{bc}$ | 0.01 | $0.44_{abc}$ | 0.01 | $0.51_a$ | 0.02 | ** | n.s. | * | a | b |
| Benzyl alcohol | $0.12_b$ | 0.03 | $0.16_{ab}$ | 0.01 | $0.18_a$ | 0.00 | $0.17_a$ | 0.01 | $0.18_a$ | 0.00 | $0.20_a$ | 0.00 | n.s. | | n.s. | - | - |
| | | | b | | | | a | | | | a | | | ** | | | |
| 2-Phenylethanol | $26.60_{ab}$ | 1.44 | $37.61_d$ | 0.79 | $22.98_a$ | 0.32 | $27.92_{ab}$ | 0.58 | $25.76_{ab}$ | 0.70 | $30.36_c$ | 0.36 | *** | | ** | a | b |
| | | | c | | | | a | | | | b | | | *** | | | |
| **∑ Alcohols** | **164.33** | | **166.44** | | **173.84** | | **154.49** | | **148.38** | | **154.82** | | | | | | |
| **$C_6$ Alcohols** | | | | | | | | | | | | | | | | | |
| *n*-Hexanol | $10.66_c$ | 0.44 | $9.13_{abc}$ | 0.10 | $10.11_{bc}$ | 1.06 | $8.21_{ab}$ | 0.35 | $8.35_{ab}$ | 0.14 | $7.59_a$ | 0.22 | ** | | * | b | a |
| | | | a | | | | a | | | | b | | | ** | | | |



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TABLE 4 (CONTINUED)

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | *Two-way ANOVA* | | | *DIT factor* | | |
| **Alcohols** | **$LS_C$** | ± *sd* | | **$LS_{DI}$** | ± *sd* | | **$LSK_C$** | ± *sd* | | **$LSK_{DI}$** | ± *sd* | | **$LSKD_C$** | ± *sd* | | **$LSKD_{DI}$** | ± *sd* | | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| *trans*-3-Hexen-1-ol | $0.07_a$ | 0.017 | a | $0.046_a$ | 0.007 | | $0.145_b$ | 0.030 | b | $0.057_a$ | 0.017 | | $0.054_a$ | 0.011 | a | $0.034_a$ | 0.014 | | ** | ** | * | b | a |
| *cis*-3-Hexen-1-ol | $0.38_b$ | 0.01 | c | $0.30_a$ | 0.01 | | $0.319_a$ | 0.01 | b | $0.29_a$ | 0.02 | | $0.27_a$ | 0.00 | s | $0.26_a$ | 0.02 | | *** | *** | ** | b | a |
| *trans*-2-Hexen-1-ol | 1.28 | 0.11 | a | 1.21 | 0.22 | | 1.36 | 0.27 | ab | 1.62 | 0.36 | | 1.66 | 0.04 | b | 1.56 | 0.01 | | n.s. | ** | n.s. | - | - |
| **∑ $C_6$ Alcohols** | **12.39** | | | **10.68** | | | **11.92** | | | **10.18** | | | **10.33** | | | **9.44** | | | | | | | |
| **Esters of fatty acids** | | | | | | | | | | | | | | | | | | | | | | | |
| Ethyl isovalerate | 0.22 | 0.04 | | 0.11 | 0.02 | | 0.10 | 0.02 | | 0.29 | 0.13 | | 0.16 | 0.02 | | 0.27 | 0.06 | | n.s. | n.s. | n.s. | | |
| Ethyl-4-OH-butanoate | 0.23 | 0.01 | | 0.26 | 0.03 | | 0.20 | 0.03 | | 0.26 | 0.01 | | 0.26 | 0.002 | | 0.23 | 0.02 | | n.s. | n.s. | n.s. | | |
| Ethyl-3-OH-butanoate | $0.29_b$ | 0.054 | ab | $0.09_a$ | 0.02 | | $0.30_b$ | 0.09 | b | $0.16_{ab}$ | 0.01 | | $0.05_a$ | 0.00 | a | $0.15_{ab}$ | 0.00 | | * | * | * | b | a |
| Ethyl hexanoate | $1.63_b$ | 0.07 | b | $0.50_a$ | 0.07 | | $1.77_b$ | 0.21 | a | $0.61_a$ | 0.08 | | $1.07_c$ | 0.00 | a | $0.50^a$ | 0.03 | | *** | ** | ** | b | a |
| Ethyl octanoate | $4.60_b$ | 0.49 | a | $1.17_a$ | 0.22 | | $5.30_b$ | 0.51 | a | $1.50_a$ | 0.050 | | $3.29^c$ | 0.00 | b | $1.14^a$ | 0.10 | | *** | ** | * | b | a |
| Ethyl decanoate | $0.82_b$ | 0.09 | b | $0.26_a$ | 0.04 | | $0.99_b$ | 0.01 | c | $0.29_a$ | 0.01 | | $0.61^c$ | 0.01 | a | $0.22^a$ | 0.02 | | *** | ** | ** | b | a |
| **∑ Esters of fatty acids** | **7.80** | | | **2.39** | | | **8.65** | | | **3.11** | | | **5.45** | | | **2.52** | | | | | | | |
| **Acetates** | | | | | | | | | | | | | | | | | | | | | | | |
| Isoamyl acetate | $1.38^{bc}$ | 0.06 | ab | $0.54^a$ | 0.08 | | $1.98^c$ | 0.45 | b | $0.62^{ab}$ | 0.06 | | $0.85^{ab}$ | 0.00 | a | $0.60^a$ | 0.07 | | ** | * | * | a | b |
| Hexyl acetate | $0.10^{bc}$ | 0.01 | ab | $0.02^a$ | 0.01 | | $0.14^c$ | 0.05 | b | $0.02^a$ | 0.00 | | $0.04^{ab}$ | 0.00 | a | $0.01^a$ | 0.00 | | ** | * | * | b | a |
| Ethyl phenylacetate | $1.55^b$ | 0.07 | a | $0.94^a$ | 0.14 | | $1.60^b$ | 0.05 | a | $0.76^a$ | 0.012 | | $0.94^a$ | 0.01 | b | $0.77^a$ | 0.04 | | *** | *** | ** | a | b |



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TABLE 4 (CONTINUED)

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | *Two-way ANOVA* | | | *DIT factor* | | |
| **Alcohols** | **$LS_C$** | ± *sd* | **$LS_{DI}$** | ± *sd* | **$LSK_C$** | ± *sd* | **$LSK_{DI}$** | ± *sd* | **$LSKD_C$** | ± *sd* | **$LSKD_{DI}$** | ± *sd* | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| **∑ Acetates** | **3.03** | | **1.50** | | **3.72** | | **1.40** | | **1.82** | | **1.38** | | | | | | |
| **Volatile acids** | | | | | | | | | | | | | | | | | |
| 3-Hydroxybutanoic acid | $0.02_b$ | 0.00 | $0.01^a$ | 0.00 | $0.02^b$ | 0.00 | $0.01^a$ | 0.00 | 0.02ab | 0.00 | 0.01a | 0.00 | ** | n.s. | n.s. | b | a |
| Isobutyric acid | 0.26 | 0.01 | 0.25 | 0.09 | 0.22 | 0.07 | 0.33 | 0.02 | 0.23 | 0.00 | 0.34 | 0.06 | n.s. | n.s. | n.s. | | |
| 3-Methylbutanoic acid | 1.24 | 0.13 | 1.40 | 0.35 | 1.18 | 0.28 | 1.79 | 0.02 | 1.48 | 0.01 | 1.87 | 0.15 | n.s. | n.s. | ** | a | b |
| Hexanoic acid | $27.01^b$ | 1.11 | $9.90^a$ | 0.19 | $30.04^b$ | 1.64 | $10.72_a$ | 0.16 | 21.43 | 0.11 | $9.80_a$ | 0.29 | *** | | ** | a | b |
| | | | b | | | | a | | | | c | | | *** | | | |
| *trans*-2-Hexenoic acid | $0.19^a$ | 0.00 | $0.20^{ab}$ | 0.02 | $0.16^a$ | 0.02 | $0.25_{bc}$ | 0.01 | $0.18_c$ | 0.01 | $0.29_c$ | 0.018 | *** | | ** | a | b |
| | | | a | | | | ab | | | | b | | | * | | | |
| Octanoic acid | $13.43^c$ | 0.50 | $4.75^a$ | 0.07 | $15.75^d$ | 1.071 | $4.96_a$ | 0.03 | $10.62_a$ | 0.06 | $4.21_a$ | 0.28 | *** | | ** | b | a |
| | | | b | | | | c | | | | a | | | *** | | | |
| Decanoic acid | $2.19^c$ | 0.02 | $0.78^a$ | 0.05 | $2.63^d$ | 0.119 | $0.78_d$ | 0.02 | $1.73_b$ | 0.07 | $0.64_a$ | 0.01 | *** | | *** | b | a |
| | | | b | | | | c | | | | a | | | *** | | | |
| Hydroxybenzoic acid | $0.16^a$ | 0.00 | $0.15^a$ | 0.01 | $0.17^{ab}$ | 0.01 | $0.20_b$ | 0.01 | $0.18_{ab}$ | 0.02 | $0.20_b$ | 0.01 | * | | * | a | b |
| | | | b | | | | a | | | | a | | | ** | | | |
| Phenylacetic acid | 1.05 | 0.03 | 1.11 | 0.32 | 0.70 | 0.26 | 1.14 | 0.07 | 0.69 | 0.04 | 1.17 | 0.07 | * | n.s. | n.s. | a | b |
| **∑ Volatile acids** | **45.54** | | **18.56** | | **50.88** | | **20.19** | | **36.56** | | **18.53** | | | | | | |
| **Esters of other acids** | | | | | | | | | | | | | | | | | |
| Ethyl lactate | 2.13 | 0.16 | 1.80 | 0.61 | 2.11 | 0.41 | 2.08 | 0.14 | 1.94 | 0.02 | 2.18 | 0.22 | n.s. | n.s. | n.s. | | |
| Diethyl succinate | 8.72 | 0.31 | 10.05 | 1.66 | 8.37 | 2.02 | 10.86 | 0.52 | 9.39 | 0.02 | 11.53 | 0.09 | * | n.s. | n.s. | a | b |
| Ethyl succinate | 117.89 | 6.46 | 150.95 | 41.76 | 95.29 | 10.25 | 160.80 | 2.96 | 121.23 | 1.02 | 163.22 | 1.66 | **. | n.s. | n.s. | a | b |
| Diethyl malate | 11.07 | 0.25 | 11.58 | 3.61 | 11.81 | 2.23 | 14.48 | 0.49 | 12.37 | 0.10 | 14.96 | 0.13 | n.s. | n.s. | n.s. | | |
| Ethyl phenyl lactate | 0.56 | 0.01 | 0.73 | 0.08 | 0.73 | 0.08 | 0.62 | 0.00 | 0.64 | 0.03 | 0.63 | 0.01 | n.s. | n.s. | n.s | | |
| Ethyl laurate | 0.63 | 0.02 | 0.69 | 0.10 | 0.55 | 0.07 | 0.73 | 0.00 | 0.73 | 0.01 | 0.62 | 0.05 | n.s. | n.s. | n.s | | |
| **∑ Esters of other acids** | **141.00** | | **175.80** | | **118.87** | | **189.57** | | **146.31** | | **193.15** | | | | | | |

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TABLE 4 (CONTINUED)

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | *Two-way ANOVA* | | | *DIT factor* | | |
| **Alcohols** | **$LS_C$** | ± *sd* | | **$LS_{DI}$** | ± *sd* | | **$LSK_C$** | ± *sd* | | **$LSK_{DI}$** | ± *sd* | | **$LSKD_C$** | ± *sd* | | **$LSKD_{DI}$** | ± *sd* | | **DIT** | **KDT** | **DIT × KDT** | **C** | **DI** |
| **Lactones** | | | | | | | | | | | | | | | | | | | | | | | |
| γ-Butyrolactone | $0.29_{ab}$ | 0.00 | b | $0.34_{a}$ | 0.00 | | $0.18_{c}$ | 0.01 | a | $0.36_{a}$ | 0.04 | | $0.25_{b}$ | 0.00 | ab | $0.36_{a}$ | 0.00 | | *** | * | ** | a | b |
| γ-Nonalactone | $0.20_{ab}$ | 0.00 | b | $0.24_{a}$ | 0.01 | | $0.16_{b}$ | 0.01 | a | $0.22_{a}$ | 0.008 | | $0.20_{ab}$ | 0.00 | ab | $0.21_{ab}$ | 0.03 | | *** | ** | * | a | b |
| **∑ Lactones** | **0.49** | | | **0.58** | | | **0.35** | | | **0.58** | | | **0.45** | | | **0.57** | | | | | | | |
| **Volatile phenols** | | | | | | | | | | | | | | | | | | | | | | | |
| 2-Methoxy-4-vinylphenol | 0.23 | 0.03 | | 0.20 | 0.01 | | 0.08 | 0.02 | | 0.20 | 0.08 | | 0.21 | 0.02 | | 0.20 | 0.00 | | .n.s | n.s. | n.s. | | |
| 4-Ethylphenol | $0.001_{a}$ | 0.000 | a | $0.019_{d}$ | 0.000 | | $0.001_{a}$ | 0.000 | b | $0.010_{bc}$ | 0.002 | | $0.007_{b}$ | 0.000 | a | $0.014_{c}$ | 0.003 | | *** | * | *. | a | b |
| 4-Vinylphenol | 0.59 | 0.05 | | 0.44 | 0.18 | | 0.19 | 0.04 | | 0.54 | 0.12 | | 0.47 | 0.03 | | 0.51 | 0.15 | | n.s. | n.s. | n.s. | | |
| **∑ Volatile phenols** | **0.83** | | | **0.66** | | | **0.27** | | | **0.75** | | | **0.69** | | | **0.73** | | | | | | | |
| **Miscellaneous** | | | | | | | | | | | | | | | | | | | | | | | |
| N-(3-Methyl-butyl)acetamide | 0.35 | 0.03 | | 0.24 | 0.08 | | 0.44 | 0.21 | | 0.31 | 0.12 | | 0.26 | 0.01 | | 0.25 | 0.04 | | n.s. | n.s. | n.s. | | |
| N-(2-Phenyl)acetamide | $0.73_{ab}$ | 0.01 | | $0.94_{bc}$ | 0.10 | | $0.44_{a}$ | 0.06 | | $1.33_{d}$ | 0.19 | | $0.42_{a}$ | 0.05 | | $1.40_{d}$ | 0.12 | | *** | n.s. | *** | a | b |
| Acetamidoethyl acetate | $0.10_{ab}$ | 0.00 | | $0.16_{a}$ | 0.03 | | $0.07_{b}$ | 0.02 | | $0.18_{a}$ | 0.03 | | $0.12_{ab}$ | 0.00 | | $0.17_{a}$ | 0.02 | | * | n.s. | * | b | a |
| 2,3-Butandiol | $0.12_{ab}$ | 0.035 | | $0.09_{b}$ | 0.016 | | $0.09_{b}$ | 0.009 | | $0.23_{a}$ | 0.10 | | 0.17ab | 0.00 | | $0.25_{a}$ | 0.07 | | * | n.s. | n.s. | a | b |
| Ethyl 4-OH-3-methoxybenzoate | $0.41_{ab}$ | 0.001 | | $0.48_{ab}$ | 0.078 | | $0.54_{b}$ | 0.034 | | $0.39_{ab}$ | 0.035 | | $0.47_{ab}$ | 0.022 | | $0.35_{a}$ | 0.007 | | * | n.s. | ** | a | b |
| 4-OH-3-methoxy-2-butanone | 0.60 | 0.05 | | 0.74 | 0.14 | | 0.47 | 0.21 | | 0.72 | 0.06 | | 0.84 | 0.05 | | 0.80 | 0.02 | | n.s. | n.s. | n.s. | | |
| 4-OH-benzeneethanol | $20.83_{ab}$ | 1.81 | a | $20.21_{ab}$ | 3.82 | | $12.75_{a}$ | 2.19 | a | $28.22_{ab}$ | 5.207 | | $28.60_{ab}$ | 2.40 | b | $29.95_{b}$ | 6.36 | | n.s. | * | * | | |
| 1H-Indol-3-ethanol acetate | $0.39_{ab}$ | 0.15 | | $0.51_{a}$ | 0.01 | | $0.07_{b}$ | 0.02 | | $0.58_{a}$ | 0.01 | | $0.30_{ab}$ | 0.03 | | $0.59_{a}$ | 0.20 | | * | n.s. | * | a | b |
| **∑ Miscellaneous** | **23.52** | | | **23.38** | | | **14.87** | | | **32.02** | | | **31.15** | | | **33.76** | | | | | | | |

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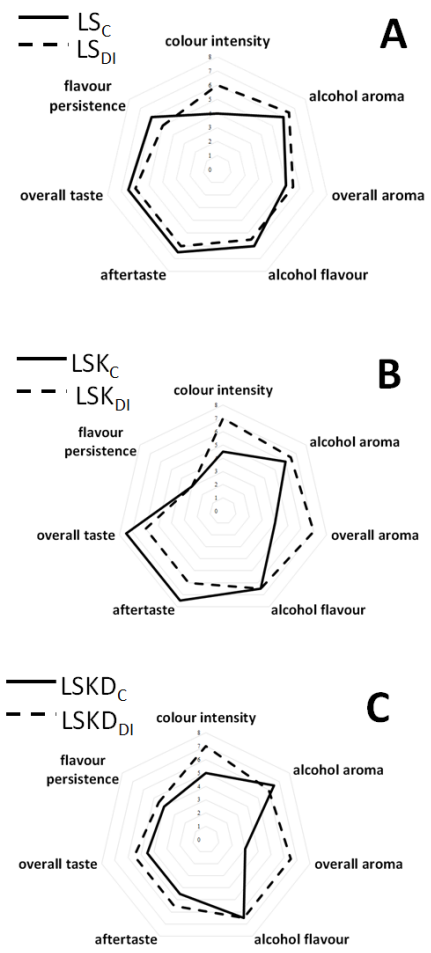


FIGURE 2

Radar graphs of sensory analysis (A, LSC vs. LSDI; B, LSKC vs. LSKDI; C, LSKDC vs. LSKDDI). LSC, Lambrusco Salamino control; LSKC, Lambrusco Salamino with kaolin; LSKDC, Lambrusco Salamino with kaolin and defoliation; LSDI, Lambrusco Salamino control collected and macerated in dry ice; LSKDI, Lambrusco Salamino with kaolin collected and macerated in dry ice; LSKDDI Lambrusco Salamino with kaolin and defoliation collected and macerated in dry ice.

however, seem to be further scope for the modulation of the sensory qualities of the wine by acting on certain factors such as temperature and time, as well as the use of standardised protocols for the partial preventive inoculation of selected and recommended yeast strains in cold maceration.

Finally, future research work should include the design and implementation of appropriate policies on adaptation measures with regard to the protection or recalibration of origin regimes. In this context, the typicality of wines and the terroir in which grapes are grown, as well as other local peculiarities, could be enhanced. This implies working on product innovation, or rather on its reinterpretation, with an eye to the production, for example, of low-alcohol wines with a content similar to that of beer. To this end, the methods of communication among scientific researchers, stakeholders and consumers should be improved for the purpose of strengthening the capacity framework and transfer of knowledge to the wine sector, as well as increasing consumer acceptance of all the necessary changes.

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