Qualitative effects of the addition of withered grapes to a freshly produced red wine: the traditional *governo all'uso toscano* practice

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Abstract

Background and Aims: Governo all'uso toscano (GUT) is a traditional winemaking practice where withered grapes are added to a freshly fermented wine. This results in a second alcoholic fermentation and a distinctive wine, in which there is renewed interest. Grape withering is problematic for winemakers, however, both in terms of cost and risks. It is therefore important to determine the minimum amount of withered grapes needed to typify the wine in order to minimise risks and costs.

Methods and Results: During the trials, Sangiovese grapes were withered for 2 months in a dedicated room, a 'fruttaio', with a resulting mass loss of about 40%. Afterwards, withered grapes (5–20% berry mass/wine mass) were added to a Control wine to reproduce the GUT procedure. The GUT technique significantly changed the chemical profile of the wines. Ethanol, colour intensity and hue increased, while acidity decreased. Malolactic fermentation occurred simultaneously with the second alcoholic fermentation in all GUT wines, but not in the Control wines. The GUT practice significantly changed the volatile profile, including the concentration of several primary grape compounds, such as aromatic alcohols, C6 compounds and terpenoids, and of several fermentation compounds.

Conclusion: Under the experimental conditions, the optimal amount of withered grapes added to the base wine was 5%.

Significance of the Study: The study described the oenological effects of GUT and the optimisation of the amount of withered grapes required to make a wine with this characteristic winemaking practice clearly recognisable.

Keywords: *Sangiovese, traditional winemaking practices, volatile compounds, withering, young wines*

Introduction

Oenology reveals a rich picture of little-known, distinctive winemaking practices (Mencarelli and Tonutti 2013). These unusual ways of making wine are often related to both specific environmental conditions and the cultural heritage of the winemaking area. Furthermore, these lesser-known and little-used practices result in a clearly recognisable wine. Although such practices have received little attention in the scientific literature, their description and understanding has several benefits for both winemakers and the scientific community. First, it preserves winemaking cultural heritage by explaining why a particular solution was developed in a particular place at a specific time (and suggests simple solutions that can be applied to solve similar problems). Second, it is an opportunity for winemakers to differentiate their product in the global market and, finally, it contributes to the body of winemaking knowledge.

In Italy, governo all'uso toscano (GUT) is a production process that consists of adding partially dried grapes to a freshly produced wine, which triggers an additional fermentation. More formally, it has been described as the result of a first alcoholic fermentation combined with a grape-withering technique, which is used in Central Italy to improve the aromatic and structural quality of wines (Mencarelli and Bellincontro 2018). The practice is recognised under European law and is recorded in the European Commission regulation 607/2009 (European Commission 2009). Grapes are harvested between September and October and stored in rooms called 'fruttai' or 'appassitoi', where berries are partially dried. They are pressed in November, and the juice obtained is added to wine that has recently completed fermentation, triggering further fermentation. The Oxford Companion to Wine (Robinson and Harding 2016) reports that wine produced in this way has a slight increase in ethanol concentration and has higher dissolved carbon dioxide. The result is a young wine ready to be consumed in the following spring. Two Protected Designation of Origin wines are allowed to use the designation, namely, Chianti and Chianti Classico, as are wines from the Colli della Toscana Centrale, a Protected Geographical Indication. The technique is found in the Marche and Umbria regions of Italy, where it is used on Verdicchio white wines. Production peaked in the 1970s, before virtually disappearing due to the transformation of Chiantis from young to aged wines. The last decade, however, has seen a resurgence of interest in GUT wines. Similar practices can be found in all parts of Italy, from the north (Ripasso della Valpolicella) to the south (Passolata di Pantelleria).

During withering, grape berries undergo several physical and chemical changes, notably water loss. The driving forces for drying are widely reported in the literature: environmental parameters, such as temperature, moisture and air speed over the berries, play a key role (Cirilli et al. 2012). As water evaporates, the concentration of sugars and other soluble solids increases; however, other phenomena occurring during the withering contribute to the particular chemical composition of a withered berry. Several other metabolic

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changes, notably gene expression and enzyme activity, have been linked to withering (Chkaiban et al. 2007, Cirilli et al. 2012). Furthermore, grape acid composition changes (Rösti et al. 2018), as do phenolic substances (Toffali et al. 2011, Figueiredo-González et al. 2013), protein profile (Di Carli et al. 2011) and volatiles related to primary (i.e. directly related to grapes) and secondary (i.e. linked to microbial activity) wine aroma (Mencarelli and Bellincontro 2018). These chemical and physical changes have been widely reported in the literature, as well as the resulting effects on the final wines.

Something different, however, occurs in the GUT process. Here, withered grapes are added to a medium with high ethanol concentration (over 10%). Under these conditions, the solubility and solubilisation kinetics of grape compounds are completely different to that normally found in unfermented grape juice or during cold soaking. Furthermore, grape skins' cell wall and membrane permeability and/or integrity are modified at this alcohol concentration, another phenomenon that is not usually found in grape juice (Ribéreau-Gayon et al. 2006, Río Segade et al. 2016, Gao et al. 2019). In addition, the high sugar concentration of withered grapes is diluted into a finished wine with no residual sugars, and microorganisms on berries (Salvetti et al. 2016) have to compete during the second fermentation with a yeast population residual from the first alcoholic fermentation and with other microbial species eventually found in the winemaking environment. These characteristics, together with the importance of preserving oenological know-how and tradition, makes the GUT technique an interesting case study.

Our work aimed to evaluate the qualitative effects of the GUT and to understand its role in modern oenology. From an agro-industrial perspective, grape withering and the subsequent second alcoholic fermentation represent both a cost and a risk for companies (Lorenzini et al. 2013), and thus, a key challenge is to optimise the amount of withered grapes to be added to the freshly produced base wine. Hence, this paper reports an experiment where different amounts of grapes were added and describes the effect on the wine produced.

Materials and methods

Sangiovese grapes, from the Chianti Colli Fiorentini Denominazione di Origine Controllata e Garantita (DOCG) area, were manually harvested during the 2018 vintage (15 September) from the Cantina Sociale Colli Fiorentini winery (Montespertoli, Firenze, Italy). Grapes were divided into two batches; the first was used to produce the base wine, while the second was stored in a fruttaio for withering.

Base Chianti winemaking protocol

The batch of base Chianti wine used in the trials was produced at the industrial scale, resulting in a final volume of 2000 hL of wine, as follows. First, grapes were destemmed and pressed to obtain a must with a final sugar concentration of 175 g/L. Sulfur dioxide was added, using potassium metabisulfite, and the must was inoculated with 20 g/hL of commercial dry yeast (ENARTIS Ferm, San Martino, Novara, Italy). Fermentation lasted roughly 1 week, when the base wine was obtained. After this period, it was separated from berry skins and stored in a stainless-steel tank until its use in the GUT process. At the end of the fermentation, the resulting wine had the following composition: ethanol 12.02%, residual sugar <1 g/L, TA 7.08 g/L, malic acid 1.30 g/L, total sulfur dioxide 42 mg/L and free sulfur dioxide 5 mg/L.

Grape withering

Withering was conducted in a dedicated fruttaio. The total volume of the fruttaio was 594 m^3 (6.0 $\times$ 18.0 $\times$ 5.5 m). The fruttaio was filled with roughly 26 000 kg of Sangiovese grapes. Grapes were stored in a single layer in perforated plastic boxes. Each box (0.8 $\times$ 0.6 $\times$ 0.15 m) contained approximately 15 kg of grapes at the beginning of the withering. The height of the box stacks was 4 m (27 boxes each). This configuration ensured that all berries were equally exposed to the air. Withering lasted 2 months; during this time, berry mass and sugar concentration were measured once per week, and they were visually inspected to ensure the absence of rot. The fruttaio was equipped with three fans to homogenise the temperature and the environmental humidity (Hept 63, Sodeca, Italy). Three thermo-hygrometers (EBI 300, Ebro, Germany) continuously monitored temperature and RH.

The governo all'uso toscano winemaking protocol

We tested the effect of the addition of four sets of withered grapes expressed as a proportion at the laboratory scale. First, the withered grapes were destemmed and manually crushed. Five stainless-steel tanks of a nominal capacity of 5 L were filled with 3.5 L of the base Chianti wine. Then, different amounts of withered grapes were added: 175 g (GUT5%), 350 g (GUT10%), 525 g (GUT15%) and 700 g (GUT20%). No withered grapes were added to the base wine in the fifth tank, which was used as a control (GUT0%). The content of all tanks was inoculated with 1.5 g of the same yeast strain (ENARTIS) used to produce the base Chianti (which used 30 g/hL, as recommended by the yeast producer) after initial rehydration in 20 mL of water. During GUT fermentation, berry caps above the wine were manually punched down once per day. Fermentation trends were monitored once per day for density variations. All the alcoholic fermentations had ended after 3 weeks [residual sugars <1 g/L (Table 1)]. Trials were performed in three replicates; hence, three fermentations occurred for each proportion of withered grapes for a total of 12 GUT and three Controls.

Chemical analyses

Ethanol concentration, TA, tartaric acid, malic acid, citric acid, lactic acid, A_{420}, A_{520}, A_{620}, colour intensity and hue were measured with a near-infrared spectrometer (NIRS) (wine scan flex, FOSS, Hilerød, Denmark). Many studies have been published regarding NIR calibration in winemaking and analyses of other liquid foods (Wang et al. 2017).

The volatile profile was measured by a combination of headspace solid-phase microextraction (HS-SPME) and GC–MS using the multiple internal standard approach described in Guerrini et al. (2016). An Agilent 7820 gas chromatograph was used, coupled to a 5977 mass spectrometer with electron ionisation (Agilent Technologies, Palo Alto, CA, USA). A three-phase (Carboxen/PDMS/DVB) 75-μm, 1 cm-long fibre (Supelco, Bellefonte, PA, USA) was used. After 5 min of equilibration, the fibre was exposed in the headspace for 5 min at 60°C for volatile compound sampling. Consistent SPME extraction conditions were ensured by a Gerstel MPS2 XL autosampler (Gerstel, Mülheim an der

Table 1. Effect of addition of partially dried grapes to newly fermented wine on the composition of governo all'uso toscano wines.

Parameter	GUT0%	GUT5%	GUT10%	GUT15%	GUT20%
Ethanol (% v/v)	12.00 ± 0.10a	12.00 ± 0.23a	12.18 ± 0.24a	12.58 ± 0.09b	12.80 ± 0.13c
Residual sugar (g/L)	<1	<1	<1	<1	<1
TA (g/L)	7.1 ± 0.2a	6.0 ± 0.2b	6.0 ± 0.1b	6.0 ± 0.1b	6.2 ± 0.1b
Tartaric acid (g/L)	4.05 ± 0.03a	3.24 ± 0.02b	3.20 ± 0.02b	3.17 ± 0.03bc	3.14 ± 0.03c
Malic acid (g/L)	1.28 ± 0.32a	0.19 ± 0.06b	0.17 ± 0.02b	0.14 ± 0.02b	0.17 ± 0.02b
Lactic acid (g/L)	0.18 ± 0.04a	0.95 ± 0.01b	0.93 ± 0.01b	0.93 ± 0.03b	0.92 ± 0.03b
Ethyl lactate (mg/kg)	2.4 ± 0.1a	19.3 ± 4.1b	31.0 ± 9.8b	26.4 ± 8.8b	33.4 ± 20.6b
Citric acid (g/L)	0.21 ± 0.01a	0.11 ± 0.01b	0.12 ± 0.01b	0.13 ± 0.01b	0.14 ± 0.02b
A_{420}	2.643 ± 0.049a	3.025 ± 0.034b	3.105 ± 0.017b	3.126 ± 0.012b	3.110 ± 0.045b
A_{520}	4.914 ± 0.145a	4.997 ± 0.079a	5.168 ± 0.033b	5.230 ± 0.040b	5.217 ± 0.112b
A_{620}	0.716 ± 0.002a	0.940 ± 0.022b	0.958 ± 0.004b	0.954 ± 0.006b	0.924 ± 0.015b
Colour intensity	8.573 ± 0.131a	8.930 ± 0.131b	9.230 ± 0.046c	9.310 ± 0.053c	9.253 ± 0.168c
Hue	0.512 ± 0.004a	0.609 ± 0.003b	0.601 ± 0.002b	0.598 ± 0.003b	0.596 ± 0.005b

The letters a, b and c represent a statistically significant difference identified with the Tukey HSD post hoc test ($P < 0.05$). GUT, governo all'uso toscano.

Ruhr, Germany), equipped with a temperature-controlled agitated tray. The column was a J&W INNOWax [Agilent Technologies (50 m, 0.2 mm and i.d. 0.4 μm DF)]. The injection temperature was 250°C in splitless mode. The initial oven temperature was 40°C and was maintained for 1 min before being increased to 60°C at 2°C/min, 3°C/min to 150°C, 10°C/min to 200°C and 25°C/min to 260°C. The final temperature was maintained for 6.6 min. In order to normalise the analyte area, the following internal standards were added to samples and calibrations: ethyl acetate D8, butanol D10, ethyl hexanoate D11, acetic acid D3, hexanoic acid D11, 3,4-dimethyl phenol and 5-methyl hexanol. The choice of the internal standard for each analyte was made as follows: when the isotope analogue of an analyte was present among the internal standard list, it was selected for that compound; then, compounds similar by chemical class and chromatographic retention time were selected; finally, a trial-and-error approach was used for all the available internal standards until the best line fitting was obtained in the calibration.

Statistical analyses

The grape-withering addition was tested in three replicates. Thus, each proportion of withered grapes was added to three masses of base wine, for a total of 15 wines. A one-way ANOVA was performed in order to understand the effect of the GUT proportion on each of the wine variables tested. Statistical significance was set at $P < 0.05$, and a Tukey honest significant difference (HSD) post hoc test was applied to identify differences among the proportion of GUT added.

A one-way multivariate analysis of variance (MANOVA) was run to test the effect on overall wine composition of the proportion of GUT added. The following dependent variables were chosen: alcohol, TA, volatile acidity, malic acid, A_{420}, A_{520}, primary aroma compounds and secondary aroma compounds (primary and secondary aroma compounds are the sum of several volatile compounds; see the Results and discussion). Differences among groups were tested using a linear discriminant analysis (LDA).

Results and discussion

Berry withering

During withering, the temperature, expressed as the mean of all three probes, was 16.2 ± 2.2°C (Figure 1a). Relative humidity was 79.4 ± 2.6% during the same period (Figure 1b). Berries lost about 40% of their initial mass, and consistently, TSS concentration increased about 1.7-fold (Rösti et al. 2018). Thus, under the withering conditions, the increase in sugar was lower than that usually reported in literature. Evaporation was the fastest at the beginning of withering (an average of 1.8% per day during the first 2 weeks) and decreased towards the end of storage (an average of 0.2% per day during the last 2 weeks). As the temperature in the fruttaio was not controlled, the withering temperature is representative of environmental conditions for the same period in the Chianti zone (Figure 1a reports the environmental temperature in Montespertoli during the same period), and mimic conditions were found in traditional fruttaio that are not equipped with temperature controls. Similar to other withering techniques that rely upon a closed facility and a cool climate (e.g. to produce Amarone or Sfurzat), traditionally, the GUT procedure is carried out at low temperature. Theoretically, the technique delays berry catabolism reactions (Costantini et al. 2006), leading to an increase in grape primary aroma (Mencarelli and Tonutti 2013). At the end of the withering, no rots were detected on visual inspection.

Ethanol concentration, acidity and colour changes in GUT wines

The GUT technique significantly changed the chemical profile of wines (Table 1). Differences were found in almost all of the measured parameters between the base Chianti and the GUT wines. Among the GUT wines, several trends were found, which were consistent with the proportion of withered grape added.

Ethanol concentration slightly increased in all GUT wines. Both the base wine and GUT5% contained 12.00 ± 0.10% (v/v) ethanol. A linear increase was found for GUT10%, GUT15% and GUT20% wines, with GUT 20% wines having 12.80 ± 0.13% at the end of the two fermentations. Sugar concentration was under the detection threshold of 1 g/L in all samples (i.e. no significant difference was found).

Both TA and acid composition changed in the GUT wines. Total acidity decreased from 7.1 ± 0.2 g/L (in the base Chianti) to 6.0 ± 0.1 g/L in the GUT wines, but no difference was found among GUT wines. In contrast, a decrease in tartaric acid (roughly 0.8 g/L in GUT samples compared to the base Chianti) was consistent with the proportion of dried grapes added. Specifically, GUT5% wines contained 3.24 ± 0.02 g/L, while GUT20% wines contained

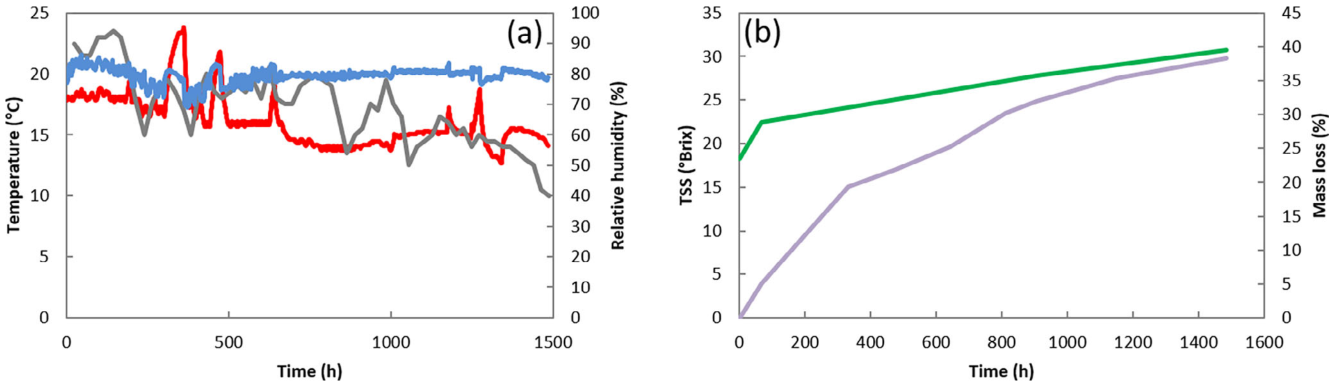

Figure 1. (a) Average temperature from three probes (——) and RH (——) inside the fruttaio and the environmental temperature in Montespertoli during the same period (——). (b) Effect of withering on the increase in sugars (——) and the loss of berry mass (——).

3.14 ± 0.03 g/L. This small decrease is consistent with the findings of Rösti et al. (2018). Rösti and co-workers explained the tartaric acid decrease as an enhanced potassium tartrate precipitation inside the berry. Furthermore, the reduction of the structural integrity of the berries due to dehydration (Rolle et al. 2013) could favour the precipitation. The subject is still controversial, but our results appear to be consistent with the hypothesis of Rösti and co-workers. The change in wine acidity, however, can also be ascribed to MLF. The base Chianti contained, on average, 1.28 ± 0.32 g/L of malic acid and 0.18 ± 0.04 g/L of lactic acid, while GUT wines contained 0.17 ± 0.02 g/L and 0.93 ± 0.03 g/L of malic and lactic acids, respectively, with no difference among the GUT wines. Further differences were found for citric acid, and a decrease in citric acid is considered to be another effect of MLF (Ribéreau-Gayon et al. 2006).

A decrease in TA due to MLF has been widely reported (Celik et al. 2019), while the induction of MLF was, historically, one of the drivers of the diffusion of the GUT technique (Robinson and Harding 2016). Traditionally, environmental conditions in Tuscany at the end of alcoholic fermentation (usually the month of October) did not support the starting of spontaneous MLF, which was often carried out in the following spring when temperature began to rise. As Ribéreau-Gayon et al. (2006) note, however, a temperature below 18°C can result in malolactic delay and the risk of microbial spoilage. The second alcoholic fermentation, triggered by the addition of withered grapes, provided the temperature required to complete MLF. This was seen in our trials, where Control wines had roughly 1 g/L of malic acid, while GUT wines had around the same amount of lactic acid.

Consistently, we observed a higher concentration of ethyl lactate (the ester formed from lactic acid and ethanol) in GUT wines. This again suggests that spontaneous MLF occurred along with the second alcoholic fermentation. The importance of a reliable method to induce MLF has recently been underlined by the development of several technological strategies (Capozzi et al. 2010, Sumby et al. 2014, Bartowsky et al. 2015). Another work points out the importance of indigenous bacterial microflora in enhancing regional differences in wines (Capozzi et al. 2010, Capozzi and Spano 2011, Bartowsky et al. 2015, González-Arenzana et al. 2015). In our experimental conditions, all 12 GUT micro-fermentations involved MLF, while all three Controls did not. Although the GUT technique raises operating costs and increases risks during the grape withering, it supports MLF driven by indigenous bacteria.

The GUT procedure also significantly affected wine colour; the A_{420}, A_{520} and A_{620} indices all increased. Hence, the addition of withered grapes to a wine enhances the yellow, red and blue colours. Colour intensity followed the same behaviour for A_{420} and A_{520} and reflected the increase in wine made from dried grapes (Figueiredo-González et al. 2013). Hue values were higher in GUT wines than in the base Chianti. Maximum values (0.609 ± 0.003) were found for GUT5% wines, while they were the lowest (0.596 ± 0.005) in GUT20% wines. Thus, the GUT practice shifts the wine colour to a yellowish tone.

GUT-related changes in volatile profile

Several changes were observed for the resulting wine volatile profile (Table 2). These changes affected both primary and secondary aroma. The change in primary aroma related to the three main classes of compounds responsible for primary aroma in Sangiovese, namely, terpenoids, aromatic alcohols and C6 alcohols/aldehydes (Bellincontro et al. 2004, Mencarelli and Tonutti 2013, Mencarelli and Bellincontro 2018).

Three terpenoids (*p*-cymene, α-terpineol and β-ionone) significantly increased as a function of GUT proportion. *p*-Cymene is reported to have a balsamic-like aroma (Slaghenaufi and Ugliano 2018). High amounts have been found in Amarone wines, another product that is typically made with withered grapes (Mencarelli and Tonutti 2013). α-Terpineol is a cyclic monoterpenoid that brings a floral note to wines (Capone et al. 2013); similar to *p*-cymene, it is found in Amarone wines (Slaghenaufi and Ugliano 2018, Zapparoli et al. 2018). Finally, β-ionone is a norisoprenoid compound derived from the metabolism of carotenoids in grapes; it has a characteristic note of violet (Kotseridis et al. 1999). During a withering trial on Pinot Noir grapes, β-ionone was found to increase as result of grape dehydration (Moreno et al. 2008). The concentration of terpenoids in wines made from withered grapes has been found to be related to withering conditions. In particular, they increase in mild conditions (low temperature and high humidity) and decrease at higher temperature (Mencarelli and Bellincontro 2018). Hence, it is unsurprising that terpenoids were higher in GUT wines dehydrated at 16.2°C and 80% RH and that wines with a higher proportion of withered grapes had higher terpenoid concentration.

Table 2. Effect of addition of partially dried grapes to newly fermented wine on the volatile composition of governo all'uso toscana wines.

Parameter	GUT0%	GUT5%	GUT10%	GUT15%	GUT20%
Primary metabolites (ng/kg)					
p-Cymene	24 ± 7a	61 ± 7b	68 ± 7b	73 ± 15bc	91 ± 6c
α-Terpineol	35 ± a	41 ± 2bc	44 ± 3bc	45 ± 1bc	47 ± 2c
β-Ionone	277 ± 56a	301 ± 28a	362 ± 56ab	498 ± 60b	730 ± 100c
Benzyl alcohol	170 ± 40a	187 ± 36a	294 ± 44b	267 ± 49b	303 ± 34b
2-Phenylethanol	41 ± 7a	45 ± 5ab	48 ± 6ab	47 ± 6ab	56 ± 7b
1-Hexanol	512 ± 40a	815 ± 35b	880 ± 88b	737 ± 75b	792 ± 75b
Z-3-Hexen-1-ol	89 ± 4a	94 ± 7ab	110 ± 6b	102 ± 6ab	110 ± 7b
Secondary metabolites (mg/kg)					
Acetaldehyde	0.55 ± 0.14a	0.30 ± 0.06b	0.18 ± 0.13c	0.03 ± 0.03d	0.18 ± 0.05c
Acetic acid	74.1 ± 2.83a	105.4 ± 15.7bc	92.2 ± 1.8b	97.5 ± 0.8b	114.1 ± 4.6c
Ethyl acetate	18.6 ± 2.5a	35.7 ± 1.2d	31.1 ± 2.2c	26.6 ± 1.6b	37.5 ± 9.1bcd
Diethyl succinate	0.56 ± 0.14a	12.07 ± 2.42c	12.39 ± 1.49c	10.62 ± 2.05c	8.00 ± 0.13b
Diacetyl	0.34 ± 0.06a	4.05 ± 1.12b	4.87 ± 1.05d	4.45 ± 0.37c	5.41 ± 1.13e
Acetoin	0.52 ± 0.20a	1.56 ± 0.48b	2.27 ± 0.32c	5.25 ± 2.09d	10.17 ± 4.10e
1-Butanol	1.67 ± 0.09a	1.98 ± 0.33ab	1.82 ± 0.06b	1.77 ± 0.05ab	1.86 ± 0.14b
3-Methyl-1-butanol	208 ± 17a	224 ± 10ab	220 ± 13ab	213 ± 8a	235 ± 15b
Isobutyric acid	0.92 ± 0.06a	1.06 ± 0.10b	1.28 ± 0.06d	1.18 ± 0.04c	1.25 ± 0.12d
4-Ethyl guaiacol	0.00 ± 0.00a	0.08 ± 0.02b	0.06 ± 0.01b	0.06 ± 0.01b	0.05 ± 0.02b
Ethyl octanoate	0.25 ± 0.01a	0.10 ± 0.01c	0.09 ± 0.03c	0.10 ± 0.01c	0.19 ± 0.05b
Ethyl decanoate	17.6 ± 1.4a	7.1 ± 2.4b	4.2 ± 2.4c	4.6 ± 0.6c	8.4 ± 6.0bc

The letters a, b, c and d represent a statistically significant difference identified by the Tukey HSD post hoc test ($P < 0.05$). GUT, governo all'uso toscano.

Two aromatic alcohols (benzyl alcohol and 2-phenylethanol) increased in GUT wines as a function of the proportion of withered grapes added. Benzyl alcohol is usually found in grape juice before alcoholic fermentation (Dennis et al. 2012); it is described as 'floral, rose, phenolic and balsamic', and it increases during berry ripening (Guerrini et al. 2018). It has been found at higher concentration in healthy grapes used for other withered wines (e.g. Amarone), while there are reports that it decreases following rot attack (Zapparoli et al. 2018). It has been observed to increase in sweet, sun-dried Pedro Ximénez grapes (Lopez De Lerma et al. 2012) and during the production of sweet Fiano wines (Genovese et al. 2007). 2-Phenylethanol has a characteristic, floral rose flavour. It has also been observed to increase in sun-dried grapes used to make Pedro Ximénez (Franco et al. 2004) and Malvasia delle Lipari wines (Piombino et al. 2010).

Finally, two C6 grape compounds (1-hexanol, and Z-3-hexen-1-ol) were found to increase with the addition of withered grapes. These two compounds are considered to be the most pronounced aliphatic alcohols in wines (Mencarelli and Tonutti 2013); they are a lipoxygenase-related product in wines *(*Costantini et al. 2006) and in other vegetable matrices (Guerrini et al. 2017). In wine, both are related to the greenery tone (Pisarnitskii 2001) and are not considered to be particularly positive. 1-Hexanol is described as 'ethereal, fusel oil, fruity, alcoholic, sweet and green' and, in Sangiovese wines, has been reported at a concentration ranging from <1 mg/kg (Guerrini et al. 2018) to >10 mg/kg (Guerrini et al. 2019). Z-3-Hexen-1-ol is a 'green leaf' volatile (Jaeger et al. 2010). Both have been found to increase in Pedro Ximénez sun-dried grapes (Lopez De Lerma et al. 2012), sweet Fiano wines (Genovese et al. 2007) and Amarone wines made with healthy, withered grapes (Zapparoli et al. 2018). In our experiment, 1-hexenol was found at higher concentration in GUT wines than in base wines, while the amount of withered grapes made no difference. Similarly, Z-3-hexen-1-ol was found at a higher concentration in GUT10, -15 and -20% wines, while no difference was found between GUT0% and GUT5%.

Table 2 summarises changes in compounds related to the Sangiovese primary aroma. These changes could be ascribed to withering conditions. Consistent with the literature (Mencarelli and Bellincontro 2018), mild conditions (16°C and 80% RH) lead to a moderate increase in C6 aliphatic compounds and a significant increase in terpenoids and grape aromatic alcohols.

The change in secondary aroma is the combined result of withering, alcoholic fermentation and MLF. For compounds related to ethanol oxidation, a significant change was found for acetaldehyde, acetic acid and ethyl acetate. The former decreased in wines made with withered grapes, while acetic acid and ethyl acetate increased. These results are consistent with those reported for grapes dehydrated at low temperature for acetic acid and ethyl acetate (Chkaiban et al. 2007, Santonico et al. 2010, Cirilli et al. 2012), although, in contrast, an increase in acetaldehyde has been reported (Costantini et al. 2006). Most studies of the changes in volatile compounds due to MLF find a decrease in acetaldehyde (Lasik 2013). This is probably why we found a decrease, rather than an increase, under similar withering conditions. In contrast, the literature reports a decrease in ethyl acetate in wines where alcoholic fermentation and MLF occurred together (Abrahamse and Bartowsky 2012, Rossouw et al. 2012). As we noticed an ethyl acetate increase in our trial, it appears that withering had a greater impact than MLF on the final concentration of ethyl acetate.

Succinic acid is a compound produced by yeasts during alcoholic fermentation under osmotic stress. It has been found at higher concentrations in sweet wines than in base wines (García-Martínez et al. 2011, López de Lerma and Peinado 2011). In this study, we did not measure it directly; instead, we report on its diethyl ester (i.e. diethyl succinate). Diethyl succinate was found at a higher concentration in GUT wines. The combined effect of MLF and osmotic stress due to the addition of withered grapes can also be seen in the increase in diacetyl and acetoin. A significant difference between the control (GUT0%) and all GUT samples was found, while a linear increase from GUT0% to GUT20% was found for acetoin. These compounds are related to each

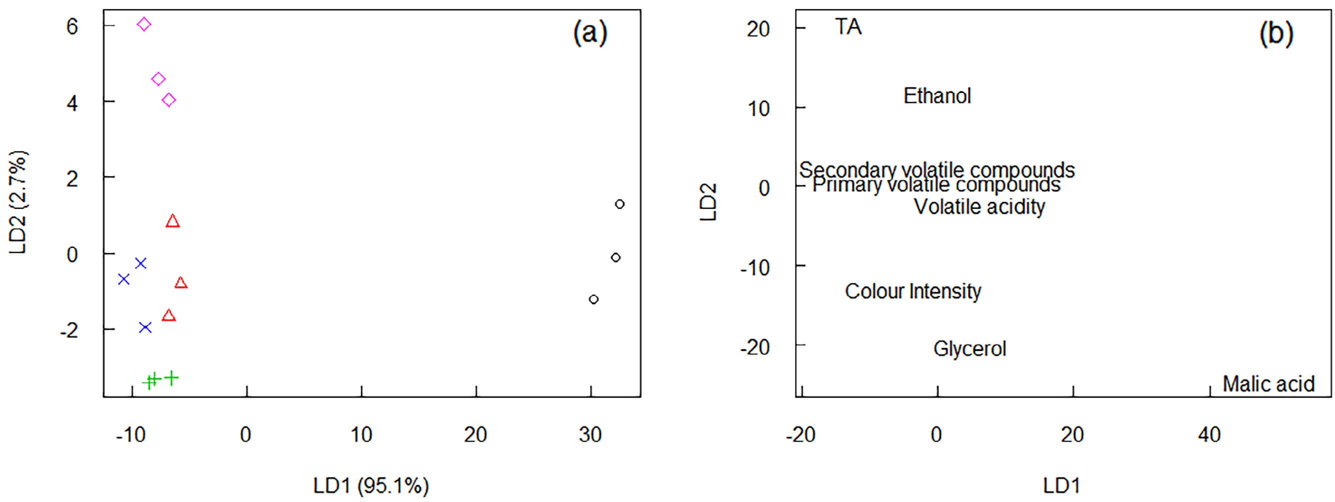

Figure 2. Linear discriminant analysis (LDA) plots showing (a) scores for governo all'uso Toscana wines (GUT)0% (○), GUT5% (△), GUT10% (+), GUT15% (×) and GUT20% (◇)and (b) loadings for the chemical composition of the GUT wines.

other, as acetoin is the reduction of diacetyl, and together they are related to the 'buttery' attribute of wines due to *Oenococcus oeni* metabolism (Bartowsky and Henschke 2004). Furthermore, both acetoin and diacetyl have been found to be related to osmotic stress during sweet wine production (García-Martínez et al. 2011, López de Lerma and Peinado 2011).

Significant changes were found for nine other volatile compounds, with an impact on wine aroma. Amounts increased for two esters, two alcohols, one acid and one volatile phenol, while a decrease was observed for two esters and one alcohol. A significant difference was found between GUT0% and GUT5–20% wines, but not among GUT levels. The concentration of 1-butanol, 3-methyl-1-butanol, isobutyric acid and 4-ethyl guaiacol increased, while ethyl octanoate and ethyl decanoate decreased. The change in the concentration of five of these six compounds could characterise the wines produced, improving the recognition of GUT wines, while 4-ethyl guaiacol deserves different consideration. Despite the grapes appearing to be in good condition on visual inspection, this volatile phenol can be related to microbial spoilage (Jensen et al. 2009), and the relationship between GUT and this compound highlights the risks related to this oenological practice.

Withered grape optimisation

The data presented above show that the biggest difference was found between GUT0% (i.e. the base or Control wine) and all GUT wines with addition of withered grapes. Consistently, the MANOVA analysis found that GUT% was statistically significant ($P = 0.003$). Differences between GUT% were evaluated using an LDA. The results (Figure 2a) show a clear separation (proportion of trace of 0.951) between GUT0% and GUT5–20% wines. The separation among GUT5–20% is provided by LD2 (proportion of trace of 0.027). As the LDA analysis correctly identified the GUT5–20% groups using both LD1 and LD2 (100% correct recognition), we decided to focus on the largest LD1 differences. This identified a clear distinction between GUT5% and GUT0% and almost no difference between GUT10–20% and GUT5% wines. Thus, our study suggests that GUT5% is the optimal compromise as it clearly changes wine composition while requiring the least amount of withered grapes. The addition of GUT5% both ensures a distinctive wine and minimises operational costs and risks.

Conclusions

Traditional practices are a rich source of inspiration for oenology. Although developed at a specific time, under particular conditions, they can still contribute to our understanding of several, modern oenological challenges. Governo all'uso toscano is one example of a traditional winemaking practice that has recently been rediscovered. Our experiment highlighted that it is able to change the chemical profile of wine, notably by increasing ethanol and acidity and enhancing its colour. The procedure supported MLF by indigenous bacteria that would not otherwise occur in our (cool) conditions. It also enhanced Sangiovese aromas that are usually found at low concentration in wines made with this grape cultivar. In our experimental conditions, a minimum of 5% withered grape addition was able to significantly distinguish the wine produced, while further addition (up to 20%) resulted in a less-pronounced change.

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