Review

Received: 8 September 2017 Revised: 10 January 2018 Accepted article published: 25 January 2018 Published online in Wiley Online Library: 26 February 2018

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8910

Recent advances in postharvest technology of the wine grape to improve the wine aroma

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Abstract

Postharvest techniques are widely used for the handling and storage of fresh horticultural crops. Some of these techniques are interesting for use with wine grapes to improve the quality of wine. In this review, we consider the postharvest techniques that are already commercially used in the wine sector and others that may be significant in inducing or extracting the aroma from grapes to produce high-quality wines. Precooling consists of rapidly lowering the grape temperature, which allows the preservation/increase of volatile organic compounds (VOCs). We also discuss sustainability. Partial dehydration consists of the partial removal of water from grapes, and if a suitable environment is adopted it can be used to produce and extract berry VOCs. As a solid, carbon dioxide is used in wine processing for the rapid cooling of grapes and, as a gas, it is used for carbonic maceration. Ozone has been used for sanitation purposes in wineries for a long time, but more recently it has been used to produce wine without sulfite addition and to increase the aromatic quality of wine grapes. Ethylene application is not used commercially for wine grapes, but promising results in terms of phenolic extraction and aromatic changes in grapes are discussed. A comparison among the proposed techniques is reported in terms of grape aromatic quality and process features. The proposed techniques could help a winemaker to maintain or induce aromatic compounds in grape berries before the vinification process. The choice depends on the desired wine and economic consistency.

Keywords: wine grape; cooling; withering; dehydration; ozone; ethylene

INTRODUCTION

Postharvest technology is mainly adopted for fresh fruit and vegetables and includes the techniques of handling, storage, packing and shipping. The main purpose of postharvest technology is to preserve the harvest quality of a product as whole or chopped, sliced or diced (minimally processed or fresh cut), while keeping it alive. A slight weight loss is tolerable and physiological, but changes in colour, texture and aroma are frequent. Processing techniques to produce jam and juice or the freezing process must not be included in postharvest technology. In this review, we address the main postharvest techniques that can be used on wine grapes to increase the aromatic quality of the wine.

NODS TO METABOLISM OF PLANT AROMA

'Many of us like to relax with a nice glass of wine, but have you ever considered the complex chemistry at play on your nose and palette when you first raise the glass?' This is the beginning of the article 'The molecular basis for wine grape quality – a volatile subject aroma' by Lund and Bohlmann,[1] published in *Science* magazine in 2006. They argued that 'the consistent production of high-quality grapes for winemaking has traditionally been more art than science'. There is a great confusion over the terms used to identify the complex interaction between odour-active compounds (we prefer to use the term *volatile organic compounds*, VOCs, because while all volatile molecules are active to nose and mouth, human sensitivity depends on the receptors) and the nose and mouth. Aroma usually indicates a strong, pleasant smell from food and drink (Cambridge Dictionary). However, in English, the terms *odour* and *flavour* are also used to identify, aromatically speaking, a food. Multiple sensory interactions occur in the perception of flavour, including olfactory, gustatory and trigeminal sensations,[2] and flavour perception takes place when odour-active molecules interact with mouth and nose receptors, producing an electrophysiological stimulation to the brain for the flavour perception.[3] The human olfactory epithelium accommodates millions of olfactory sensory neurons that are attached to olfactory receptors, each of which is capable of detecting multiple compounds with common functional groups. At the same time, multiple different receptors can recognize the same odour compound if multiple functional groups are present.[4,5] Therefore, when we smell a glass of wine, our perception is directly nose dependent, but when we taste it the nose response is mouth mediated. In both cases, nose receptors must be activated by VOCs, whose production in the grape berry is the result of different and complex metabolisms.

More scientific papers have been published on wine aroma (2825; Scopus) than on grape aroma (1481, or approximately 50% less), but to characterize a grape variety the grape VOCs, not those of wine, should be analysed. When a vinification process occurs, yeasts play an important role in modifying the aroma panorama. Monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, methoxypyrazines and volatile sulfur compounds

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contribute grape-derived aroma compounds.[6,7] An exhaustive review of the class of VOCs found in grapes and wine was given by Robinson *et al.*[8]

However, although there is knowledge about the various classes of compounds, little information is available on why the grape produces hundreds of VOCs from fruit set to full ripening. This question is the second point of criticism related to the above sentence of Lund and Bohlmann.[1] Indeed, several studies have reported on how vine management (pruning, leaf removal, irrigation etc.) can affect the aromatic metabolism[9–16] but, as Lund and Bohlmann[1] wrote, 'The limitation is that such research has been focused on cause and effect but the mechanisms underlying these processes remain largely unknown and unexplored.'

VOCs are typically lipophilic, with high vapour pressures and low solubility in water, and with very high partition coefficients (the ratio of the weight of the component in a unit volume in the air phase to that weight in the same unit volume in the water phase). The production of VOCs depends on the inherent hydrophobicity of the volatile compound as a consequence of the ratio of the number and size of nonpolar to polar groups and their positioning in the particular molecule.[17] The partition coefficient is strictly conditioned by the temperature of the aqueous solution (cell sap and intercellular space meniscus), the presence of other constituents, the concentration of the volatile component in the aqueous solution and the presence of other volatile substances.[17] In plant cells, the non-conjugated VOCs can cross membranes freely and evaporate into the atmosphere when there are no barriers to diffusion.[18] In fruits such as grape that are for wine production, VOCs must diffuse from the exocarp, where they are mostly concentrated, to the must before or during fermentation. As the main objective of the winemaker is to obtain a grape berry that is fully rich in VOCs, it is thus essential to reduce the loss of these compounds on the vine during berry ripening. The berry surface has few stomata and lenticels,[19] so the main route for water vapour and volatile molecules to escape is by diffusion through the cuticle. A study on grape berry permeability to water vapour was performed by Becker and Knoche,[20] who showed that the cuticle covering the berry surface is the primary barrier to water uptake and transpiration. The permeability for water uptake is, on average, 2.9 times higher than that for transpiration, and diffusion is the primary mechanism in the case of transpiration. VOCs are released from leaves, flowers and fruits into the atmosphere and from roots into the soil. The main functions of VOCs are to defend plants against herbivores and pathogens, to attract pollinators and seed dispersers, and to serve as signals in plant–plant communication[21] and sometimes as wound healers.[22] Because of this defence function, VOCs are synthesized in living fruit cells, mesocarp and exocarp, but the greatest biosynthesis is in the peel cells, overall for messenger compounds, e.g., terpenes. However, biosynthesis can occur also in the mesocarp cell: in the 'Cesanese' wine grape[23] and the 'Aglianico' and 'Uva di Troia' wine grapes,[24] free and bound volatile organic compounds are more concentrated in the flesh juice than they are in the peel.

As many of the constitutive defence volatile molecules may be toxic at high concentrations to the plant itself, the plant must be able to generate and to store such substances. The obvious strategy to overcome this problem is to store VOCs in extracellular compartments, as is the case for glandular trichomes, or as inactive precursors, e.g. glycosides.[25] In the grape berry, over 240 putative glycosyltransferases have been identified in *Vitis vinifera*,[26] which is the enzyme responsible for the addition of an activated sugar moiety to aglycone (glycosylation). The addition of the sugar moiety increases the solubility of the compound (i.e. linalool has a predicted log octanol water coefficient of 3.38, but when linalool is glycosylated the log octanol water coefficient is 2.33),[27] preventing diffusion across cellular membranes and thus providing a convenient storage form.[28] Thus this chemical reaction permits the transfer of the lipophilic aroma molecules into the cell vacuole and their storage. In grapes, the aglycone moiety of glycosides is often dominated by monoterpenes, C_{13}-norisoprenoids and benzene derivatives.[29] In the wine world, the glycosylated compounds are known as 'bound', though not in odour-active form. However, upon the hydrolysis of a glycoside by means of yeast action, the aglycone moiety may be volatilized, becoming, potentially, an odour-active molecule. In both the grape and the wine, a large proportion of the volatile aroma compounds is found in the 'bound' form.

The knowledge of the glycosylation mechanism is important to program vine management (e.g. light exposure, water stress, green pruning) to stimulate or to protect the 'bound' forms; in addition, postharvest technology can be used: (i) to stimulate the production of VOCs or their chemical changes; (ii) to facilitate the translocation of the VOCs from epidermic cells to flesh or vice versa; (iii) to favour glycosylation to protect berry VOCs from loss before the fermentation process; and (iv) to favour solubilization by avoiding evaporation.

Below, a series of adoptable postharvest techniques to improve the aromatic quality of wines by working on grape extraction and/or on synthesis of VOCs and favouring their glycosylation is presented.

POSTHARVEST TECHNIQUES FOR THE WINE GRAPE

Grape precooling

In 2017, a long period of drought and exceptionally high temperatures in late spring and summer compromised and significantly reduced wine grape production in Mediterranean countries. Without vineyard irrigation, in some areas where no rain occurred for 4 months and after a scarce rainy season during the winter, the basal leaves of the vines became yellow early, and grape ripening occurred 15–20 days earlier than usual. Beyond a significantly reduced yield, ripening was not excellent because malic acid was consumed and polyphenols were not synthesized sufficiently. In such a situation, postharvest cooling is essential to reduce the berry temperature as quickly as possible to save aromatic compounds and, depending on the cooling time, permit a new polyphenol synthesis.

Cooling and chilling are used interchangeably to indicate the adoption of low temperatures above 0 °C to store food. There is no maximum limit because the constraint is the use of low temperatures, e.g. a specific practice or technique to reduce the environmental temperature. Cooling is used to maintain quality and safety in food storage. In fruit and vegetables, cooling is used to maintain the quality and freshness of the product; in particular, rapid cooling, also known as precooling, encompasses any cooling treatment that is administered to the product before shipping, storage or processing. A stricter definition of precooling would include only those cooling methods that cool the product rapidly, and certainly within 24 h of harvest before storage or shipping.[30] Thus, in the case of wine production, grape cooling must be considered preprocessing cooling. Why do we need to precool the wine grape? The main reason is to remove heat from the berries. For most fruits and vegetables, the objective of heat removal is to reduce the respiration rate, but for the wine grape the reason for heat removal is to avoid the escape of VOCs. By reducing bunch temperatures, field heat (sun-exposed bunches have higher field heat than shaded bunches do; in the same latitude and in the same growing ambience, grape bunches in a goblet keep more heat than in the pergola training system because they are closer to the soil and less ventilated), latent and sensible heat are removed from the grape, all of which affect the respiration rate based on the Van't Hoff equation. Every milligram of CO_2 produced by respiration causes 2.55 cal to be released (1 cal is equal to 4.187 J). Wine grapes generally have a respiration rate between 20 and 40 mg CO_2 kg^{-1} h^{-1} at room temperature, so 1 ton of grapes produces between 51 and 102 kcal in 1 h at 20 °C; at 30 °C, these values can increase by 30%. This increase is just the heat from respiration rate, which, in bulk conditions such as wine grapes in a bin, can represent 50% of the total heat produced by the grapes. Thus rapidly lowering the temperature will slow the metabolic processes and, at the same time, reduce the boiling points of VOCs. In addition, the partition coefficient will change, and VOCs will become more soluble. An accurate calculation of heat load to build a cold room or a tunnel for precooling is needed to avoid the oversizing of cooling capacity, with a consequent high energy consumption, or downsizing, resulting in an inefficient cooling and long period for cooling. To cool produce, the specific heat, weight and difference in temperature between the produce at harvest and the final desired temperature must be known.

An important parameter for evaluating the efficiency of a precooling plant is the energy coefficient (E_c):

$$E_c = M \times c_p \times \left(T_i - T_f\right) / (E \times C)$$

where M = mass of product, c_p = specific heat, T_i and T_f = initial and final temperature, E = electricity needed, C = 3413 Btu kW h^{-1}.[31] Based on an Australian Government report (http://www.winesa.asn.au/_r5829/media/system/attrib/file/1194/A%20guide%20to%20energy%20efficiency%20innovation%20in%20Australian%20wineries – energy%20efficiency%20best%20practice%202003.pdf), 40–60% of the energy used in a winery is due to the refrigeration system, and it has been calculated that it is possible to save up to 30% of the energy if the energy efficiency is well managed. The highest energy consumption occurs during harvest time and the vinification process, when the daily kW h increases three- to fourfold. Prefermentative cold maceration, also known as cold soaking or cryomaceration, is increasingly being used by oenologists to improve some important quality characteristics of wine, such as colour and aroma. During cold soaking, the must before fermentation is kept at a low temperature, usually 10–15 °C, for several days,[32] but Heredia *et al.*[33] reported that 'This technique consists in maintaining the crushed grapes at low temperatures (5–10 °C) for a variable period from one to several weeks.' In this review, precooling refers to treatments applied to whole grape bunches, not crushed grapes. Heredia *et al.*[33] showed that maintaining grapes in a cold-storage room (below 4 °C) for 24 h prior to crushing and successive cold maceration at 3–8 °C provide wine with more intense and stable colours, higher chroma values and more red-bluish hues than does direct maceration with dry ice. To rapidly lower the grape temperature, large-capacity refrigerator groups can be used or cryogenic gas (CO_2 or N_2). Carillo *et al.*[34] showed the efficiency of a cooling tunnel with a liquid CO_2 injection system, chilling 100 kg grapes from 25 to 10 °C in 8 s with a consumption of 15 kg CO_2. Considering that a cooling plant is used during the vinification process, the same cooling plant can also be fitted for precooling by using, for example, a forced-air cooler such as the one used for precooling fresh fruit and vegetables. An accurate computation of heat loads is important, especially because 60% (total energy consume is 54 kW h ton^{-1}) of the total electricity consumption comes from fans, lights and walls. The advantages of a precooling technique that uses a modern, sustainable cooling plant include accurate temperature maintenance and an easy temperature change. A recent study[35] that kept 'Falanghina' white wine grapes at 4 °C for 24 h or at 4 and 20 °C, alternately every 6 h for 24 h of treatment in a tunnel cold room, showed a significant increase in pectinmethylesterase activity during the alternating temperature treatment, with a consequent lowering of peel resistance and a significant increase in nor-isoprenoids and terpenes in the wines (Table 1).

Table 1. The main class of VOCs (μg L^{-1}) as the sum of single analysed compounds in Falanghina wines from grapes cooled at 4 °C for 24 h or at 4 and 20 °C for 24 h, with an alternate step of 6 h at each temperature

	Control	4 °C	4 + 20 °C
Esters	14 854 ± 1254a	14 920 ± 1674a	14 717 ± 1111a
Alcohols	54 222 ± 2345	51 463 ± 2280a	51 381 ± 2289a
Acids	10 724 ± 789a	7773 ± 699b	8202 ± 890b
Phenols and vanillins	4676 ± 579a	5466 ± 676a	4449 ± 489a
Terpenes	107 ± 12c	139 ± 16b	157 ± 11a
Nor-isoprenoids	69 ± 9b	64 ± 7b	85 ± 12a
Others (mainly cumaran)	113 ± 8a	82 ± 8b	51 ± 10c

Data are the mean (± SD) of three GC–MS analyses from three different lots of berries. Different letters in each row indicate a significant difference per $P < 0.05$.[35]

In Fig. 1, a new tunnel for grape precooling is observed: the tunnel can cool 24 pallets (approximately 14 tons of grape bunches) from 30 to 10 °C in 8–10 hours, at an 80 kW cooling capacity.

Partial dehydration

Withering, dehydration, raisining and drying are a confusion of terms to indicate approximately the same process based on a physical event: water loss. Mencarelli and Tonutti[36] wrote a glossary defining: (i) dehydration as the dynamic process of water loss from the berries occurring on-vine (overripening, late harvest, icing, *Botrytis*) or after harvest under more or less controlled environmental conditions; (ii) drying as the process of intense water loss of the berries after harvest carried out under open-air conditions; and (iii) withering as the consequence of a long dehydration process in grape berries that combines water loss and berry senescence stresses.

The water loss of a grape berry is a physical process that has been used for thousands of years, mainly to make raisins for food. However, water loss has also been used to make sweet wines that were much appreciated in ancient times.[37] In the past and, in some cases, today, especially in the Mediterranean islands (Santorini, Cyprus, Aeolian Islands, Pantelleria Island) or the southern mainlands of Europe (Alentejo, Andalusia, Peloponnesus), the water loss process is still favoured by leaving the grapes under the sun, preferably in a ventilated environment, on most of the islands. Where the ambient condition does not permit a secure and efficient water loss, closed facilities are used. In Italy, Amarone and Sfurzat as dry wines or numerous sweet wines are produced in closed facilities called 'fruttaie' or 'appassitoi'. One old practice, which is used in

Figure 1. New tunnel for the rapid cooling of wine grapes.

Tuscany to improve the aromatic and structural quality of Chianti wine, is 'governo', where withered grapes are used to referment the wine produced immediately after harvest. A similar practice is used also in Pantelleria Island (Italy) to make Passito di Pantelleria and is known as 'passolata'. In the Valpolicella–Verona area, another wine called 'Ripasso' is made by letting a dry wine rest on the exhausted peels of Amarone wine.[38]

An 'aromatic' question arises if we taste two sweet wines made in Italy from the same grape variety, 'Muscat of Alexandria': why is the sweet wine produced in northern Italy, e.g. in the Piedmont, recognizable as Muscat, whereas it is not recognizable if the wine is made in southern Italy, Pantelleria Island, where the 'Muscat of Alexandria' is called Zibibbo? The reason for this significant aromatic difference is related to temperature. Returning to the glossary,[36] the grapes exposed to sun are subjected to a drying process because the irradiation is very high, so the temperature rises greatly; thus, under the windy conditions of the island, water loss is hastened. In this case, varietal aromatic compounds are oxidized, and only the aroma of dried figs, dried apricots and honey is perceived in these sweet wines. Franco *et al.*,[39] comparing wine from sun-dried 'Pedro Ximenez' grape with fully ripe grapes, found higher concentrations of acetoin, γ-butyrolactone, ethyl acetate, isoamyl alcohols, isobutanol, 2-phenylethanol and isobutanoic acid in the former wine; the significant increase in the higher alcohols could be ascribed to a rapid activation of amino acid catabolism, which indicates rapid cell death. Costantini *et al.*[40] showed that anaerobic fermentation occurs during off-vine dehydration under controlled environmental conditions in a long-term dehydration where the berry cell is still alive and that amino acid catabolism occurs later, which is the opposite of what occurs in sun drying.

Ruiz *et al.*[41] compared 'Pedro Ximenez' grapes sun dried at temperatures above 40 °C and nocturnal values infrequently below 18 °C, relative humidity (RH) of 45%, with grapes dried for 5 days in a chamber at 40 °C and RH of 30%. No difference existed in terms of aroma between the must samples; acetoin was also the major odorant, followed by ethyl acetate, phenethyl alcohol, isobutanol and isoamyl alcohol. The authors concluded that 'caramelized was the greatest aroma contributor in all musts, its concentration increasing throughout the drying process and its OAV exceeding 150 at the end'. In this case, the temperatures of both experimental situations were very high, and the relative humidity was very low, so the weight loss was very rapid. Changes in the VOCs in grapes of var. 'Garnacha Tintorera' during raisining without controlled conditions, with a total water loss of approximately 62% in the berries at the end of the process, have been reported[42]; a high concentration of VOCs was found, and bound volatiles occurred in higher concentration than did free volatiles, suggesting that glycosylation occurred during raisining. The article also reported a comparison between the VOCs of raisined Garnacha and those found in Pedro Ximenez by Ruiz *et al.*,[41] reporting that in both wines the caramelized and floral nuances marked the aromatic profile. De Torres *et al.*[43] used a temperature of 60 °C to dry grapes of 'Carmenere' and 'Cabernet Sauvignon' and found a large decrease in terpenes, sesquiterpenes, acids, alcohols, esters, benzene derivatives and C_6 compounds, and an increase in norisoprenoids and derivative compounds of furan, pyran and lactones from the browning reactions. These identified aromatic compounds are all the result of very stressful metabolisms and chemical reactions occurring in the grape berry due to high temperature and rapid water loss, including oxidation, amino acid catabolism and the Maillard reaction. As a consequence, the aromatic taste is buffered, and hundreds of aromatic compounds go into a funnel, where our nose and mouth perceive very few nuances, the main one of which is caramel.

One question arises: Why use different varieties if the final aromatic nuances are similar and very few? It is well known that using a low temperature is the best way to maintain the aroma in food and in the wine process. In the above paragraph, the positive effect of rapidly cooling the wine grape has been discussed. The same concept drove our research group to study different temperatures to dehydrate wine grapes, thanks to the availability of a new dehydration tunnel.[44] Few papers have been published on this aspect. In 2008, using a dehydration tunnel (air speed 1.0–1.8 m s^{-1}, 38% RH and 22 °C), more terpenes (guaiacol, citronellol, geraniol and eugenol) and norisoprenoids (β-ionone and β-damascenone) were found in Pinot Noir grapes than in wine made from harvest grapes.[45] 'Aleatico' grapes in a dehydration tunnel at 12 °C and 60% RH reached a reduction of 31% of weight and an increase in aroma of 35% and 5%, respectively, per berry weight or per berry.[46] In the same experiment, the dehydration of grape bunches in shaded open air (temperature 16–37 °C, mean of 22 °C; RH 30–65%, mean of 52%) of the same 'Aleatico' grapes reduced the aroma concentration by 15% and 35%, per berry weight and per berry, respectively. Thus temperature makes a difference. Similar results were obtained in the Aeolian Islands, in 'Malvasia' grapes kept in the open air, shaded or sun exposed, where the shaded grapes were 67% richer in free

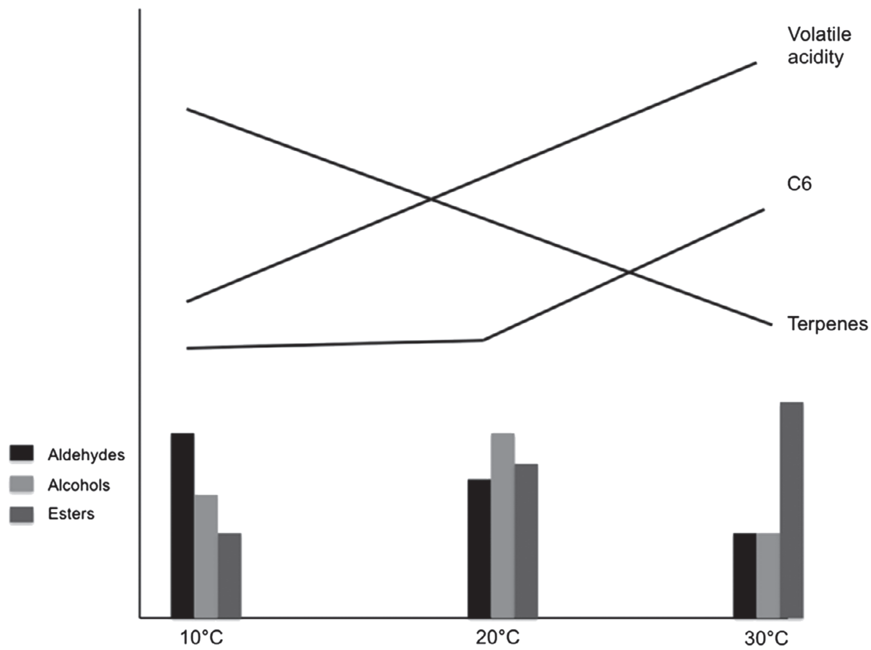

Figure 2. Potential changes (no unit of measurement is reported because the interest is the line trends) of the main classes of aromatic compounds of wine grapes during postharvest partial dehydration at different temperatures.

volatiles and 20% more in terpenoids.[47] The reason for this difference was not the shadow but the lower temperature reached in the shadow (7 °C). To confirm the importance of working at low temperatures (with RH not too low and air flow not too high) during dehydration, by guaranteeing a slow water loss process, Bellincontro *et al.*[48] compared different temperatures and different air flows for dehydration (10 and 20 °C, 60% RH and 1.5 or 2.5 m s^{-1}), and found that the lowest temperature and lowest air flow mitigated the water stress and its effects on the physical, chemical and biochemical characteristics (aerobic fermentation). This result allowed the preservation of the varietal aroma and the VOCs that are more oxidizable.[49,50] In a comparison of postharvest dehydration of 'Aleatico' wine grapes at three temperatures – 10, 20 and 30 °C – with 60% RH and 1.5 m s^{-1} air flow, polyphenol oxidase activity increased significantly and rapidly at 20 and 30 °C, indicating a rapid oxidation process.[51]

The aim of using low temperatures is not only to preserve VOCs (higher solubility, higher partition coefficient) but also to delay berry water stress and cell death. This is why, in the Legal Rules of Amarone wine production (Disciplinare),[38] vinification is not permitted before 1 December (unless some anomalous climactic condition occurs), even though the grape harvest is at the end of September. In these 2–3 months of water loss, the temperature is low, RH is generally high and ventilation is continuous. Thus slow water loss is the key to reaching the Amarone sweet spot.[52] If low temperature is between 0 and 10 °C but the RH is very low (below 50%) and a high air flow is maintained around the grapes (above 2 m s^{-1} among grape clusters), the response of the berries could be similar to a process that uses a higher temperature or fast air flow, driving the berry to a rapid water loss and cell death.[48] This consideration was confirmed by Panceri *et al.*[53]: using 7 °C, 35% RH and a very high air delivery capacity (12 m^3 s^{-1}) to dehydrate 'Cabernet Sauvignon' and 'Merlot' produced a wine with higher content of aldehydes, vanillin derivatives and fatty acids, and lower content of higher alcohols, esters and lactones compared with the control wine, which was obtained immediately at harvest. Confirmation that the process was very fast is provided by the measured high concentration of furfural, which the authors explain as 'the Maillard reaction during the dehydration process because furfural is a typical product of chemical browning reaction during grape dehydration, and its concentration increases with drying time and temperature'. Notwithstanding the use of low temperatures, the very low RH and the high flow rate have created the conditions for strong oxidation.

Zenoni *et al.*,[54] in a very complete molecular paper on postharvest dehydration at low temperature of different varieties, reported: 'Only a few metabolites were depleted (in particular, *n*-hexanal, 2-hexenal, and eugenol), whereas many compounds accumulated after harvest, following diverse trends' to demonstrate the efficiency of low-temperature withering, with an increase in sesquiterpenes and balsamic monoterpenes contributing to the final aroma of Amarone wine.[55] In Fig. 2, the potential changes of the main classes of grape aroma, following postharvest dehydration at different temperatures, are reported, and in Fig. 3 the concentration changes in the classes of VOCs during grape dehydration at 10 °C are given. In general, the temperature change from 10 to 30 °C provokes varietal VOC loss and increases VOC oxidation, but aroma complexity is reached at 20 °C. Temperatures of 30 °C and above lead to strong oxidation and tend to homologate the aromatic features of all the varieties. A low dehydration temperature is a benefit for aromatic varieties because it permits the preservation of the varietal VOCs. The low aromatic varieties ('Trebbiano', 'Falanghina', 'Fiano', 'Cesanese', 'Sagrantino') have the advantage of low temperature to preserve the low levels of VOCs, but they need to develop these VOCs. In this case, the work in the vineyard is essential, but the correct management of dehydration becomes increasingly important.

Ethylene

Ethylene, or ethene in chemistry, is a colourless, flammable gas that consists of two carbon atoms joined by a double bond and has the formula C_2H_4. Ethylene was the first hormone to

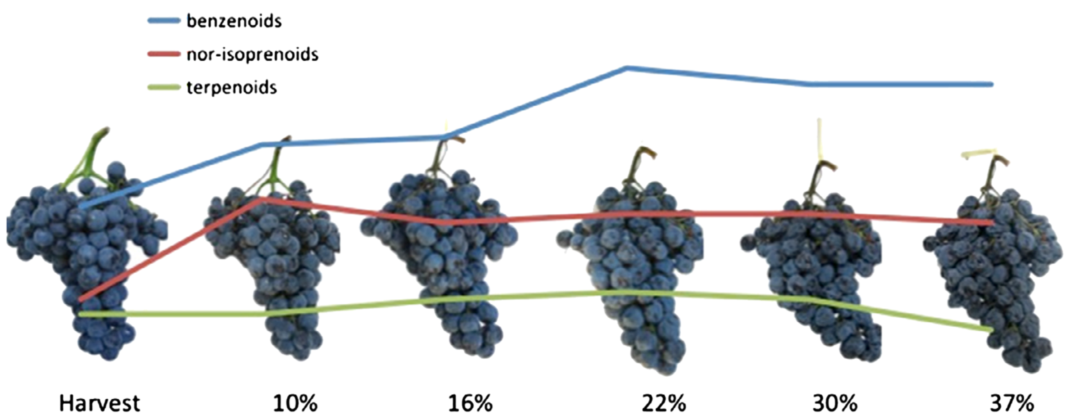

Figure 3. Changes in the main class of VOCs during dehydration at 10 °C of Cesanese grapes at different percentages of mass loss.[23]

be identified in a plant. Commercially, ethylene is used to ripen bananas because its main role in the plant cell is to trigger fruit ripening, but it can regulate seed germination and adjust seedling growth to soil conditions, the rate and extent of leaf expansion and the timing of vegetative senescence and abscission.[56] In addition, ethylene may mediate the responses to environmental challenges such as wounding, pathogen invasion and water stress. In 1970, Hale *et al.*[57] applied ethylene gas or CEPA (2-chloroethylphosphonic acid), an ethylene-releasing compound, in the vineyard of 'Doradillo' table grapes and 'Shiraz' wine grapes and found an increase in the ripening rate; the response was dependent on the application time. Other papers were subsequently published on ethylene and field ripening.[58,59] However, it was only in 2003 that applications of CEPA or ethylene, at veraison, showed increases in anthocyanins[60] and berry size[61] upon affecting endogenous ethylene biosynthesis. In postharvest, ethylene application has been tested on table grapes to ascertain the effect on decay[62] or to study its involvement in rachis browning.[63] In the 'Aleatico' wine grape, postharvest ethylene treatment (500 μg L^{-1} for 15 h) increased the terpenols, alcohols and C_6 compounds,[64] and this effect was related to the increase in cell wall enzyme activities.[65] Postharvest treatment with gaseous ethylene (1000 ppm for 36 h) on 'Sangiovese' wine grapes induced changes in berry metabolism and, consequently, in the wine aroma compound (C_{13}, esters, phenols) concentration and its ratio of free/glycosylated.[66] Beyond the positive effect on polyphenol metabolism (which is not the topic of this review), ethylene postharvest treatment may be a worthwhile treatment to increase the aromatic fraction of wine grapes.

Carbon dioxide

As a gas, carbon dioxide (CO_2) is widely used in fruit postharvest for controlled atmosphere storage or the preshipping of strawberries and other berries sensitive to *Botrytis cinerea*. In the wine grape, carbon dioxide is used for two main reasons: carbonic maceration (CM) for Beaujolais wine and rapid cooling of grape or must. In addition to cooling, solid carbon dioxide (dry ice) releases, at room temperature, CO_2 gas, which can remove oxygen. The cooling effect of carbon dioxide is well known but is usually exploited for must because it has a positive effect on the wine aroma.[67,68] The cost of dry ice and the application are the limiting factors. For CM the main reason for its use is the strong effect on wine aroma.[69] Due to the development of anaerobic fermentation in the absence of oxygen and saturation with CO_2, the VOC profile changes significantly. In 'Monastrell' grapes, CM resulted in the formation of almost all esters and fatty acids before alcoholic fermentation started.[70] Applying CM in the 'Castelao' variety and analysing the resulting wine, lower concentrations of 1-hexanol, 2-phenylethanol, diethyl succinate and diethyl malate, and higher concentrations of benzyl alcohol and ethyl lactate than in the skin fermentation/maceration wine were found.[71] In 'Baco Noir' and 'Gamay' wines from grapes kept in carbonic maceration, secondary metabolites of glyceropyruvic fermentation and low concentrations of higher alcohols were measured.[72] More recently, in a sensorial survey of wines from different macerations, CM wines showed a higher dominance of woody, spicy, pungent and acid sensations, and a lower dominance of red/black berry aroma and astringency.[73] This technique is complex, and it is not widely used, even though it could have interesting results in terms of aroma. There are some steps that must be followed accurately and other steps that have not been clarified: (i) The ripening stage at harvest should be studied; the response in terms of VOCs could change completely during carbonic maceration. (ii) Absolute sound bunches and berries are needed. (iii) Bunches must be placed in perforated boxes in only one layer. (iv) An airtight cold room is needed. (v) The best temperature for the carbonic maceration should be studied because the main and secondary metabolisms can change in response to temperature and CO_2. (vi) How long must the grapes rest with 100% CO_2 in the room? (vii) Nitrogen together with carbon dioxide could help in aroma development.

Ozone

Ozone, one of the strongest oxidizing agents, has been used by the fresh produce industry[74] as an antimicrobial agent for years, and in 2001 it was generally recognized as safe (GRAS) (US FDA, 2001; http://www.fda.gov/).[75,76] In contrast, no specific legislation for ozone treatment on fresh produce in the EU exists, but if the active substance was already on the market on 1 September 2013 then companies using it had to submit applications for the approval of the active substance by 1 September 2016. Products that were supported before September 2016 can be used until the active substance is approved.[76] Ozone has been used in winery as a sanitizing agent, and its use has been proposed at different stages of wine production to reduce the spoilage microbiota in grapes and to sanitize barrels, tanks, hoses and bottles.[77–80] Postharvest ozone treatment has been used on Petit Verdot grapes to produce wine without sulfite addition, and this treatment increased the fruity flavour.[81] Ozone has always been viewed negatively for food aroma, which is true if we consider processed food; however, if we

Table 2. Advantages (+) and disadvantages (–) of the proposed technologies

	Precooling[a]	Partial dehydration[b]	CO_2 solid	CO_2 gas[c]	Ethylene[c]	Ozone[d]
Equipment cost	–	– –	+	+ –	+ –	+
Energy cost	– –	– –	–	+	+	+
Process speed	+	– –	+ +	+ –	+ –	+ –
Process ease	+	+ –	+ +	+	+	+
Process duration[e]	+	– –	++	+–	+	+
Berry aroma modification		++		++	+	+
Berry aroma maintenance	+		++			

[a] Cold room, perforated boxes, accurate stacking.
[b] Specific room with cooling, dehumidification and ventilation, specific perforated boxes, accurate stacking, absolute sound berries.
[c] Airtight room, perforated boxes.
[d] Cold room, perforated boxes.
[e] + means a few hours; + – means more than 24 h; – – means several days.

consider living plant cells that can respond to stressors, ozone, if it is accurately managed, can have a positive effect on aroma. Ozone as a pollutant has been studied for a long time on different plant tissues, and the toxicity is mainly due to an enhanced generation of reactive oxygen species (ROS) in plants due to disruption of cellular homeostasis.[82] Plant response to ozone (0.15–0.30 mg L^{-1} in air) produces isoprene, monoterpenes and C_6 compounds.[83] Ozonolysis of isoprene produces methyl vinyl ketone and methacrolein, and the first reaction with phenol produces raspberry ketone, with a wild hyacinth aromatic nuance.[84] In addition, one of the first recognized ozone effects is lipid oxidation in cellular membranes. Thus volatiles that are associated with lipid peroxidation are also emitted in ozone-stressed leaves. Compounds such as C_6, methanol and methyl salicylate are markers of ozone damage,[83] but isoprene and monoterpenes are also emitted in response to acute and heavy doses of ozone (150–300 µg L^{-1}).[85] In addition, acyltransferases, which catalyse the transfer of an acetyl group from acetyl-CoA to an alcohol for the formation of esters, are considered modifying enzymes in the formation of volatile compounds emitted by plant cells under stress conditions.[18,86] Collecting this information, the potential role of ozone to stimulate the synthesis of some metabolic pathways involved in the generation of aromatic compounds is evident.

In wine, ester formation with a fruity odour occurs especially during fermentation because of yeast activity,[87] but esters can also be generated during berry ripening and overripening due to the generation of ethanol and cell membrane lipid oxidation metabolites.[88,89] Ozone has been seen to change the aromatic profile of wine grapes, favouring glycosylation,[90] but ozone also induces the formation of small but significant amounts of compounds of sugar oxidation such as furaldehyde (sweet, brown, woody, bready, caramellic and with a slight phenolic nuance), hydroxymethylfurfural (fatty, buttery, musty, waxy and caramellic) and 3-hydroxy-2,3-dihydromaltol (sweet, caramellic, cotton candy, jammy fruity and burnt with bready nuances), in white wine grapes treated with ozone at low temperature.[91] These compounds are generally the result of sugar dehydration and are indicative of maderization of must and heat-treated wine, i.e. a strong oxidation process.[92,93] The use of ozone gas during the postharvest dehydration of wine grapes was demonstrated to increase the content of total VOCs. Among terpenes, which are responsible for floral and fruity aromas, linalool, geraniol and nerol were the major aromatic markers of Moscato Bianco grapes, and at the molecular level postharvest dehydration and ozone exposure induced the biosynthesis of monoterpenes via the methylerythritol phosphate (MEP) pathway and of aldehydes from the lipoxygenase–hydroperoxide lyase (LOX-HPL) pathway.[94] In other fruits, the effect of ozone on aroma is contradictory: ozone reduced ester production in strawberries stored for 2 days at 2 °C with 350 ppb of gaseous ozone[95] and in tomato fruit treated with ozone gas at 4 ppm for 30 min every 3 h,[96] but it did not affect the sensorial quality of cantaloupe melon aroma (10 000 ppm for 30 min),[97] grapes (continuously exposed to ozone at 100 ppb for 60 days)[98] or papaya treated with ozone at 1.5–5.0 ppm for 4 days.[99] The reason for these contradictory results is the sensitivity of species, the variety, the ripening stage, the concentration used, the application time and the temperature used for treatment. Finally, ozone could affect compound extractability, which means that the ozone could modify the cell wall and cell membrane, thereby increasing their permeability. This effect could facilitate VOC extractability more than generation and not only VOCs but also polyphenol and acids. 'Nebbiolo' shows that ozone increased polyphenol extractability but did not do so in Barbera.[100]

Advantages and disadvantages of the proposed technology

With the objective of helping operators determine the best technology for their own purposes, Table 2 presents a comparison of the cited postharvest techniques for some general characteristics. Precooling of wine grapes is already used, although not very frequently, but its great effect on primary aroma maintenance in grape must during partial maceration is known, and only specific temperatures and times of application should be elucidated to make the treatment more economically and environmentally sustainable. Precooling requires a cold room to store grape bunches harvested in perforated boxes; thus an accurate computing of cooling power should be done to avoid over- or undersizing the cooling capacity. Moreover, accurate stacking is needed. Partial dehydration is a postharvest technique that can be adopted to modify the aroma profile of wine grapes and provide aromatic nuances of oxidation. These nuances depend on the temperature of dehydration used as well as the rate and intensity of water loss. Amarone, Ripasso and Governo are different dry wines produced in Italy with the partial dehydration technique. To run an accurate partial dehydration process, an insulated building is needed with a cooling and dehumidifying plant. Dehumidification especially requires abundant electricity. In addition to temperature and relative humidity, air flow is needed, so the ventilation must be accurate to invest grapes in perforated boxes that are stacked logically in the dehydration building. The solid carbon dioxide postharvest technique is widely used because of its easy application, but its cost is the limiting factor. Approximately 5–7 kg are needed to cool 100 kg grape bunches at 10 °C in 1 h, with a cost of 2–2.5 € kg^{-1} in Italy. CO_2 gas is used only for the carbonic maceration of whole grape bunches. Gas is less expensive than solid, but in this case an airtight room is needed to prevent oxygen from leaking into the room. Ozone has fewer prospects for use in affecting the aroma unless it is adopted for sanitizing grapes. The same goes for ethylene, which can be better applied to affect the polyphenol content than to affect the aroma.

CONCLUSIONS

In wine, the continuous appreciation by the consumer of its aroma requires specific practices and techniques to be used both in the vineyard, to produce grapes with high concentrations of odour-active compounds or their precursors, and in the winery, to maintain or express these odour-active compounds. Today, postharvest techniques adapted to wine grapes can be very useful to help the winemaker preserve or improve the grape aroma.

ACKNOWLEDGEMENTS

We thank the following: ARSIAL (Agenzia Regionale per lo Sviluppo e l'Innovazione nel Lazio) of Regione Lazio (Latium Region Government) for financially supporting part of the experimental work reported here; Rinaldo Botondi, University of Tuscia, Viterbo, for assistance in the MS thesis (ref. 35); Roberto Forniti, University of Tuscia, Viterbo, for technical assistance in our experimental work carried out during these years; and Claudio D'Onofrio, University of Pisa, for GC–MS analysis.

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