



# Flint glass bottles cause white wine aroma identity degradation

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Due to marketing recommendations, white wines are often bottled in flint glass to improve aesthetics and showcase wine color. Although this practice is known to cause a wine fault, the influence of light on the fruity and flowery aromatic profile of wine is unknown. The aim of this study was to investigate the changes to the white wine volatile under typical supermarket shelf conditions, using 1,052 bottles of 24 white wines. After only 7 d of shelf life in flint glass bottles, a dramatic loss in terpenes (10 to 30%) and norisoprenoids (30 to 70%) was recorded, whereas colored glass bottles did not evidence such behavior even after 50 d, and darkness preserved the wine's fruity and flowery aromatic integrity. We also proposed an alternative mechanism for the insurgence of the lightstrike off-odor, which takes the varietal aroma loss into account. In light of this understanding of the flint glass negative impact on white wine aroma identity and sensorial character, this packaging should be strongly discouraged. The same findings should be valid for a wide range of several daily consumed foodstuff where transparent packaging is used.

metabolomics | wine faults | off-odor | GCxGC-ToF-MS | lightstrike

According to the International Organisation of Vine and Wine (OIV), wine packaging containers can be made of glass, plastic, or plastic-lined paper or metal products (1). However, glass bottles are the most common and popular packaging, especially due to reasons linked with tradition and culture and because of the possibility of recycling. Traditional bottle colors are green or amber, but lately, there are environmental pressures and marketing/commercial demands on the wine industry to use lighter/thinner and flint glass bottles. This trend toward lighter bottles is driven by the need to lower energy, transport, and recycling costs, while the flint bottles are addressing the request to showcase the color of rosé or white wines and to optimize the aesthetics of the packaging. On the other hand, it has been demonstrated that light exposure can damage the quality of foodstuffs, shorten their lifetime, harm their nutritional value, and cause serious and irreversible sensorial problems (2–6). In 1977, Haye et al. (7) reported a wine fault called gout de lumière (lightstrike) caused to Champagne wines by the light that passes through bottle glass and tried to explain it. Today, we know that flint glass bottles do not filter the ultraviolet-visible (UV-Vis) radiation enough, and it results in white wines with lightstrike, with descriptors like boiled cabbage, corn nuts, wet dog, and soy/marmite; whereas amber or dark-colored glass bottles are able to protect wines from this phenomenon (3, 4, 8–11). However, there are still debates about the mechanisms that promote the appearance of lightstrike because no clear correlation between the off-odor and volatile compounds has been demonstrated in wine.

Wine is one of the beverages whose quality and commercial value can be improved during maturation and aging, under optimal storage conditions. The aromatic profile of the wine, which includes alcohols, esters, ethers, terpenes, sulfur compounds, norisoprenoids, and volatile phenols, is strongly influenced by the storage conditions. Many researchers demonstrated that excessive exposure to oxygen and high temperatures leads to a loss of the fruity and flowery aromas (12) and the appearance of, for example, unpleasant notes such as honey-like, farm-feed, hay, and rotten food. Even though white wines are commonly bottled in flint glass bottles and stored in supermarket shelves for weeks or months, the literature lacks studies about the influence of light exposure on their aromatic profile. We should underline that for white wines the aroma character is the most important parameter of quality, as opposed to red wines, where taste is also crucial.

Dozon and Noble (8) described the sensorial changes caused in sparkling and still white wines by light irradiation, mentioning the loss of citrus aroma and an increase of the lightstrike fault already after a few hours. Mattivi et al. (9) obtained similar results in their study based on 85 white wines (e.g., Chardonnay, Pinot gris), which proved the important role of riboflavin. A study on Sauvignon blanc wine light irradiation showed that flint glass resulted in wines with more vegetable aroma and less citrus (13). As far as the chemical analysis of the aroma profile is concerned, a few studies showed that red and white wine esters were negatively influenced by artificial light (12, 14, 15), but there

## Significance

Transparent packaging is increasingly used for foodstuffs, including wine, milk, beer, and juice. This choice is based on the marketing recommendation that consumers want to see the product before buying it, although scientists point out that light can damage food quality and nutritional value. This work revealed that light can dramatically damage the aroma profile and sensorial identity of varietal white wine in less than a week of shelf-life in flint glass bottles. We proposed a mechanism of the lightstrike off-odor development, which includes the decrease of the fruity and flowery aroma able to mask off-odors, the rapid loss of aroma enhancers, and the catalyzation of photodegradations. It is our considered opinion that flint glass bottles should be avoided.

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are no studies on the primary volatiles (e.g., terpenes, norisoprenoids) that characterize each cultivar identity.

Because the volatile metabolomic profile (also known as the volatilome) includes compounds that belong to various chemical classes with different functional groups and several of them are of paramount importance to explain wine aroma and sensorial character, it is of great interest to study their global behavior caused by light exposure when flint glass bottles are used. Lately, an untargeted analysis proved to be a powerful tool to explore global metabolomic changes, without focusing on a small group of analytes (16–19).

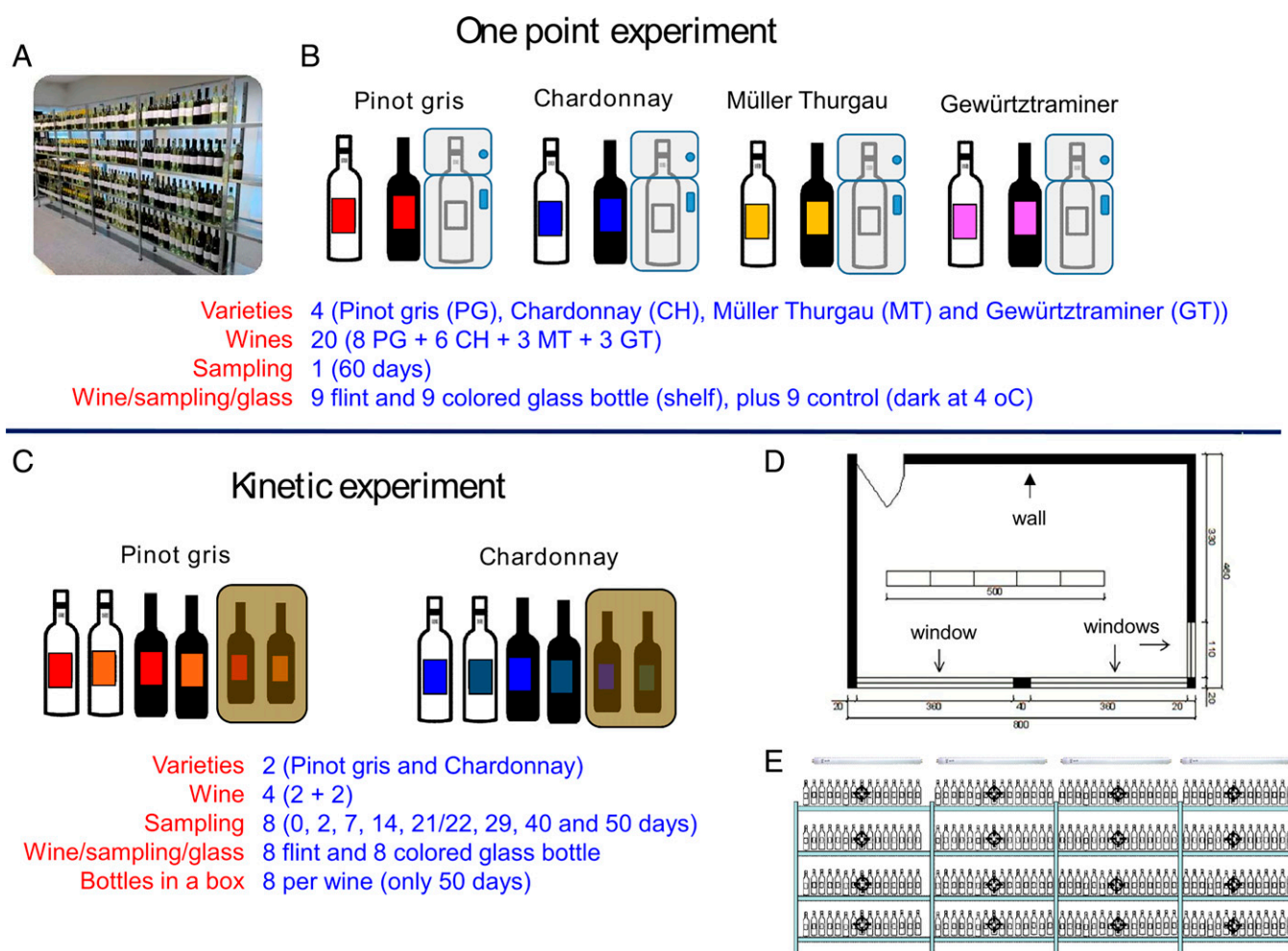
The aim of this work was to study the influence of light exposure on the white wine volatilome under the typical supermarket shelf conditions and to monitor the primary aroma compounds that characterize the sensorial identity and flavor of each cultivar in particular. To achieve robust and wide results, an experimental design based on the use of a room that mimics the typical supermarket conditions; the use of a big number of bottles from several different wines, providing high biological diversity; and a fingerprinting method able to maximize the number of volatiles detected, via comprehensive gas chromatography, was considered essential.

## Results

This study was part of a wider project, whose main aim was to understand white wine behavior under the typical/realistic

supermarket shelf-life conditions in various glass bottle packaging. The present study, schematized in Fig. 1, was based on 1,052 samples (all commercial wines in 750-mL bottles), coming from 2 separate experiments run in 2 different years, and included 24 different white wines from 4 different cultivars. The previous results (10) of this project focused on the lightstrike sensorial analysis and the light and temperature that each sample received, whereas this study referred to the volatile compound investigation by using a comprehensive gas chromatography combined with time-of-flight mass spectrometry (GC×GC-ToF-MS) instrument. For the realization of the project, a room that mimics the supermarket shelf conditions, with controlled temperature, artificial lights turned on 12 h per day, and small amounts of natural light entering through the curtains, was prepared. To enhance the realistic parameters of the project further, all the wines selected for this project were produced and bottled under the typical oenological industrial conditions by commercial wineries.

The first experiment (single point experiment), which also served as a pilot for the second one, was based on 20 monocultivar white wines. As shown in Fig. 1, all the wines were bottled in both flint and colored glass bottles, and the control samples were stored at 4 °C in the dark during the period of the experiment (90 d), with 9 replicates from each wine/condition being used (540 samples). The second experiment (kinetic experiment in Fig. 1), which was the core one, was based on four monocultivar white wines, bottled in flint and colored glass bottles, and included eight sampling points in 2 mo. For each wine/color/



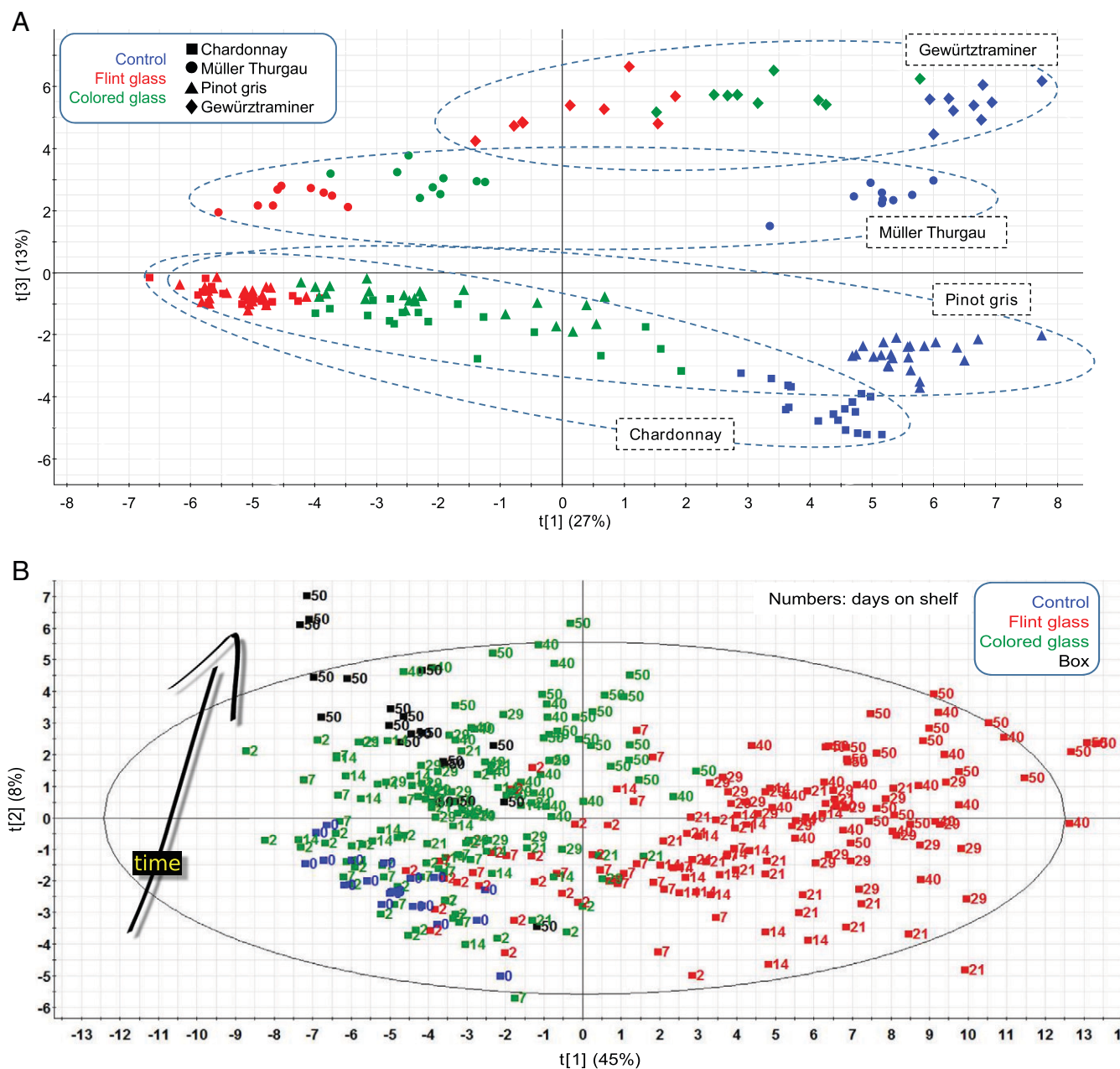
**Fig. 1.** Experimental design. The room that mimics the supermarket shelf conditions (A and D), how the sensors were distributed in along the shelf (E), the single point experiment (B), and the kinetic experiment (C) that this study is based on.

time condition, 8 samples were used, and by considering the 2 controls (4 °C in dark vs. room temperature in dark), the sample set included 512 samples. The same experiment also investigated the influence of storing wine samples with shelf-life exposure at 4 °C in dark, for a variable period.

Using GC×GC-ToF-MS, more than 1,700 features were extracted. The following filtration steps were performed in order to find out just the most important markers: 1) column and fiber breakdown features were eliminated, 2) only statistically significant features ( $P \leq 0.05$ ) were kept, 3) peak and spectra were controlled manually, and 4) only features present as putative markers in at least two wines were considered. The annotation process of the putative markers was made with authentic standards, when available, injected under identical conditions. When the authentic compound was not available, commercial libraries were used. Between the 84 putative markers, 23 monoterpenes, 9 sesquiterpenes, 14 norisoprenoids, 10 esters,

4 benzenoids, 5 alcohols, 3 ketones, 1 furan, 2 sulfur compounds, 2 hydrocarbons, 1 aldehyde, and 1 lactone, and 9 unknowns (Dataset S1) were annotated.

The principal component analysis (PCA) plots of the filtered data sets, where each point refers to a different wine bottle, demonstrated a clear separation of the various experimental conditions for both experiments (Fig. 2). Fig. 2A, which corresponds to the single time point experiment, shows a clear separation of the three experimental conditions; thus, the control samples are located on the right part of the x-axis, colored glass bottles are in the middle, and flint glass bottles on the left. This separation was achieved by considering all 20 wines coming from 4 different cultivars, and the figure shows that all wines had the same general behavior for the selected markers. The results obtained with such a deductive study design should be considered, in our opinion, of general validity, considering the great biological variability represented in this experiment.



**Fig. 2.** PCA plots based on the selected markers. (A) Single time point experiment based on 20 wines coming from four cultivars. (B) Kinetic experiment based on four wines coming from Chardonnay and Pinot gris.

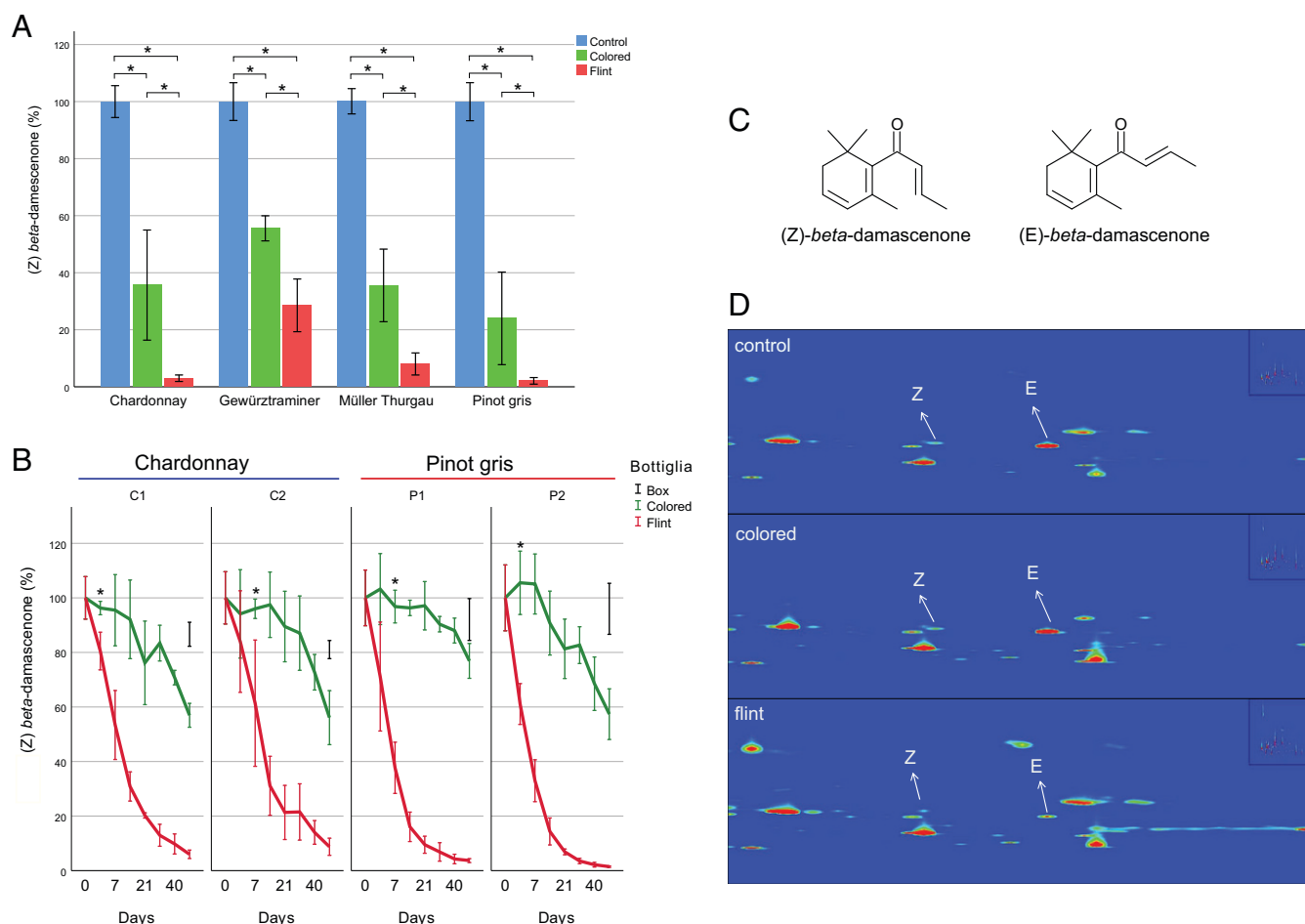
On the other hand, the y-axis makes it possible to highlight the separation caused by the four cultivars and gives us information about their individual behavior. The wines are well separated in the control samples and therefore in the optimal conservation conditions, whereas wine stored on the shelf in the green bottle already shows a partial loss of chemical identity between Chardonnay and Pinot gris, and, finally, a complete loss of identity in wines stored in flint bottles, except for the two more aromatic varieties. We have to underline that this PCA plot included wines coming from various production lines and bottled in glass bottles of various types and colors and that different closures were used (Dataset S2).

Fig. 2B shows the distribution of the kinetic experiment in a PCA plot. PC1 allows the separation of the flint glass bottled wines from all the other conditions, by considering all four wines from the two cultivars and all sampling points. All wines bottled in flint glass were distinguishable from all the others after 14 d of shelf life. This is in accordance with our sensorial analysis experiment where the people from the panel test were able to distinguish them after 21 d of shelf life (10). On the other hand, PC2 provides information about how many days each bottle remained on the supermarket shelf, since samples with zero or minimum storage are shown at the bottom of the plot and the samples with maximum storage life (50 d) are shown at the top. In this case, too, due to the wide biological

variability used, the data set demonstrated its potential to provide results not specific to a particular cultivar of wine, with extensive validity.

The wine science literature is rich in research results reporting the influence of shelf-life temperature conditions and of packaging oxygen level on varietal aromas (20–23). Here, we shed light on the influence of light and glass color on the most important and well-known wine aromatic volatile compounds. In the following lines and graphs, we will try to demonstrate the decidedly negative effect of wine storage in flint glass bottles on the most important wine aromatic compounds, under typical supermarket shelf conditions.

Norisoprenoids are a group of important aromatic compounds, especially for the varietal wines, which originate from the chemical and enzymatic degradation of the nonvolatile carotenoids in the grapes and are further released from their non-volatile precursors during wine maturation. This group includes several known metabolites, such as  $\beta$ -damascenone,  $\beta$ -ionone, TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), vitispirane, and safranal. In Fig. 3, as a typical example of the results obtained, we reported the behavior of the *cis* isomer of  $\beta$ -damascenone in the various wines of the two experiments. The two isomers of  $\beta$ -damascenone were described as having different odor descriptors, such as baked apple, quince, and floral, depending on their concentration and on the matrix, and with a very low



**Fig. 3.** Behavior of (Z)- $\beta$ -damascenone during the shelf life experiments. (A) The single point experiment; the data represent the mean values of at least 9 independent experiments (9 for Gewürztraminer and Müller Thurgau, 18 for Chardonnay, and 24 for Pinot gris), and error bars indicate SD. (B) Shows the results of the kinetic experiment, each point represents the mean value of five independent experiments, and error bars show the 95% confidence intervals. (C) Presents the chemical structures of the two isomers of  $\beta$ -damascenone. (D) Shows the separation achieved by GCxGC and how the bottle glass influenced the peak intensity in the single point experiment for *cis*-(Z) and *trans*-(E)  $\beta$ -damascenone. \*Indicates statistical significance ( $P \leq 0.05$ ) in A, and the first time point that a statistical significance occurred in B.

sensory threshold (*SI Appendix*, Table S1).  $\beta$ -Damascenone occurs in wines from nearly all varieties and could have a synergistic effect or act as an aroma enhancer (24). In the single time point experiment, after 90 d of storage, we registered a loss of 66% ( $\pm 18$ ) of *cis*- $\beta$ -damascenone from the wines bottled in colored glass, and 93% ( $\pm 10$ ) for the flint (Fig. 3*A*). The situation was even more dramatic in the kinetic experiment, since for the Pinot gris wines bottled in flint glass, the average loss of *cis*- $\beta$ -damascenone was 34% after 2 d and 65% after 7 d (Fig. 3*B*). For the same storage period (2 to 7 d) in colored glass bottles, the loss was negligible and was  $\sim 40\%$  only after 50 d. Therefore, the damage caused by light passing through flint glass after 2 d was comparable to the one of colored glass after 50 d. The dramatic effect of the light on  $\beta$ -damascenone stability in the “naked” wines in flint bottles was confirmed also by the observation that the samples kept in the dark, inside a cardboard box (same room condition apart from the light), maintained concentrations like those of the control samples. This was expected, because colored glass does not offer full protection and allows the transmission of some light. Both isomeric forms of  $\beta$ -damascenone had the same behavior, and that made us hypothesize that the reaction caused by light exposure was not a *cis-trans* isomerization (*Datasets S3–S7*).

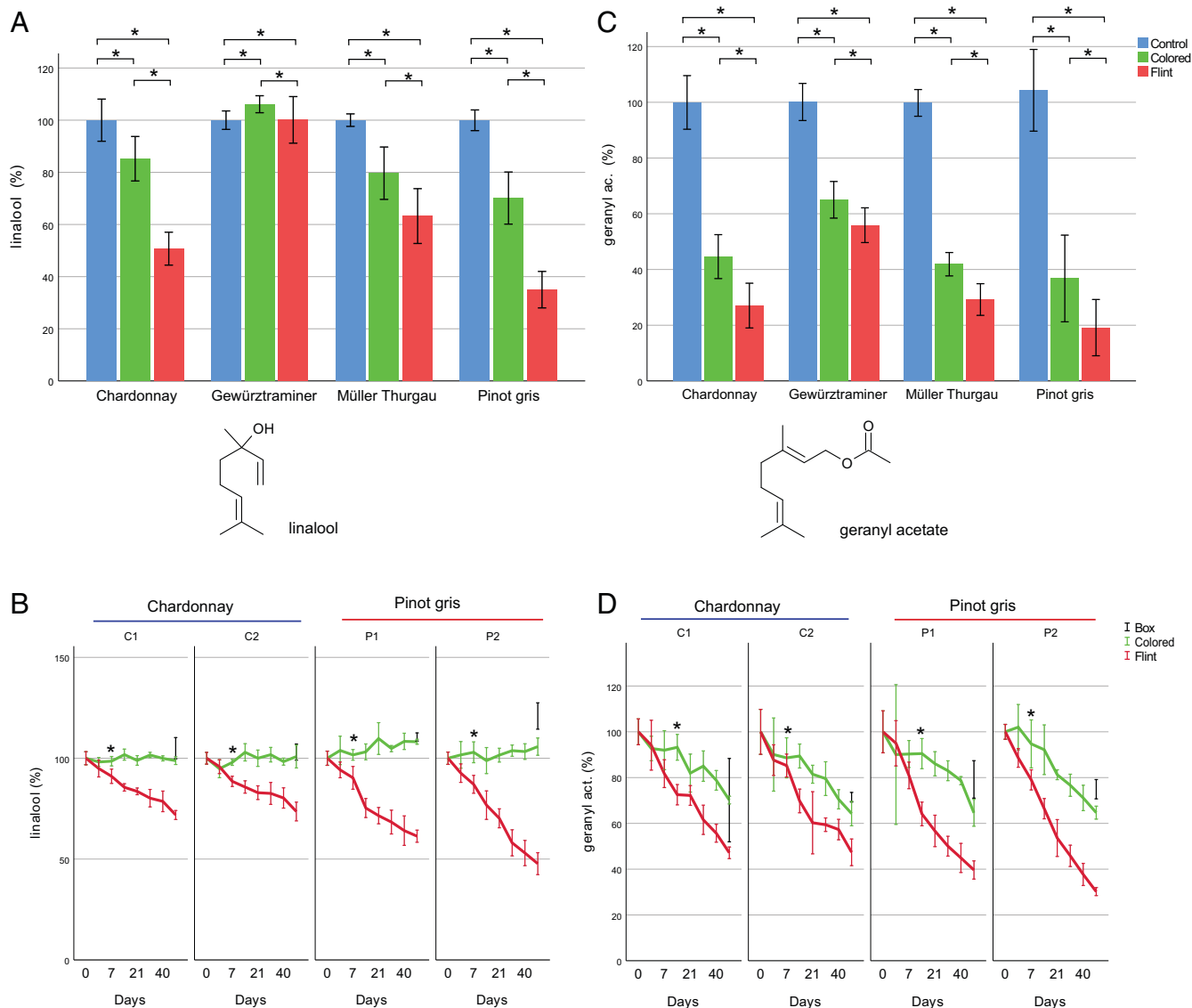
The use of two-dimensional separation (GC $\times$ GC) allowed us to increase the chromatographic resolution, separate the two isomers of  $\beta$ -damascenone, and demonstrate that the peak intensity of both compounds decreased with light exposure (Fig. 3*D*). From the *Datasets S3–S7*, it was clear that the same trend was apparent for most of the other norisoprenoids. The *trans*- $\beta$ -damascenone concentration decreased about 70% in all the 20 wines bottled in green glass and in 95% of those bottled in flint glass, on the single time point experiment, with Gewürztraminer being the more resistant. Similarly, in the kinetic experiment, about 66% of its concentration for Pinot gris and 48% for Chardonnay was lost after 7 d of storage in flint glass, and similar values were registered for the colored glass bottled wines after 50 d of shelf life (*SI Appendix*, Fig. S1). Safranal, responsible for the saffron aroma notes, also suffered from light exposure in both experiments. The wines housed in flint glass bottles in the single point experiment had a safranal loss of about 60% after 90 d of shelf life, again with the Gewürztraminer wines being the most resistant. Colored glass, on the other hand, protected this compound in Gewürztraminer wines and diminished its concentration loss for the others (average loss limited to about 13%) (*SI Appendix*, Fig. S2). Vitispirane and TDN are well-known heavy aromatic compounds that increase with wine aging and storage at higher temperatures and give kerosene, petrol, or camphor notes (25). In fact, in both experiments we noticed a stability or increase in their concentration for the wines bottled in colored glass (*SI Appendix*, Figs. S3 and S4 and *Datasets S3–S7*). According to a previous publication on the same experiment, the temperature of the colored bottles was higher by about 2 °C than that of flint bottles (10). Generally, wines bottled in flint glass had a decrease of about 20 to 40% in the concentration of these compounds during the first 1 to 3 wk of shelf life, and then they stabilized. This could be explained by their susceptibility to light, like the other norisoprenoids, initially, but their loss was later compromised by their released from their glycosylated precursors (26).

The second group of volatile compounds influenced by the light exposure were the terpenes, also derived from the grapes, which are considered the primary source of aroma in wines. Indeed, grapes are divided into aromatic and neutral varieties based on their terpene concentration (27); linalool and geraniol

belong to this group and enrich wines with floral, fruity, citrus, and sweet character (28). The aromatic cultivars (e.g., Muscat, Gewürztraminer) are rich sources in such molecules and their presence could possibly mask or delay the appearance of light-strike wine faults. On the other hand, for the wines produced from neutral and lightly aromatic grape cultivars, like Pinot gris and Chardonnay, terpenes play a paramount importance in their sensorial quality, and the winemakers' main aim is to maintain their concentration. Terpenes are sensitive compounds, and their concentration is temperature, pH, oxygen, and time dependent (23, 28, 29). According to our results, we should add that light also plays an important role in their stability in wine. In the first experiment, wines bottled in flint glass lost on average, and considering all 20 wines, 46% ( $\pm 23$ ) of their linalool and 63% ( $\pm 17$ ) of geraniol concentration (Fig. 4*A* and *Dataset S3* and *SI Appendix*, Fig. S5). The decrease for the wines housed in colored glass bottles was 18% ( $\pm 15$ ) of their linalool and 40% ( $\pm 13$ ) of geraniol. Gewürztraminer wines one more time demonstrated to be more resistant to the light, probably due to their high concentration of free and glycosylated terpenes (Fig. 4*A*). On the other hand, Pinot gris and Chardonnay had the bigger decrease. In the kinetic experiment, we noticed that in colored glass bottles linalool was protected, and geraniol decreased about 25% for both Pinot gris and Chardonnay wines only after 50 d on the shelf. The wine stored inside the cardboard box maintained their entire initial content. The situation of flint glass bottles was again dramatic because linalool decreased by about 20 to 30% after 3 wk and by 30 to 50% after 50 d and geraniol by about 35 to 45% and by 50 to 70% correspondingly (Fig. 4*B* and *Datasets S4–S7* and *SI Appendix*, Fig. S5). Studies focused on wine storage temperature effect often registered an initial increase on free terpenes due to acidic hydrolysis of their glycosylated forms, which probably is accelerated by the temperature, and later a decrease. According to the results of our experiment, wines bottled in flint glass were continuously losing their terpene potential, and therefore, light might also have damaged their glycosylated analogs.

During alcoholic fermentation, terpenes react with the produced acids and deliver acetate esters, which are also molecules with a positive aromatic character already at trace levels and belong to the wine secondary aromatic profile; being very light-sensitive compounds, they were severely affected by the shelf life. Geranyl acetate, a typical example of this chemical group, decreased in all samples but more severely in wine bottled in flint glass (Fig. 4). In the first experiment, wines in flint glass bottles had about 71% ( $\pm 15$ ) less geranyl acetate than the control and wines in colored glass about 56% ( $\pm 14$ ) less. Pinot gris and Chardonnay were again the more sensitive wines, and Gewürztraminer was the most resistant wine. The kinetic experiment showed similar results because after 50 d on the supermarket shelf, only 30 to 50% of geranyl acetate remained in the flint glass bottled wines and 70 to 80% in the colored glass bottled wines. The behavior of the ethyl ethers of the corresponding monoterpenes (linalyl, geranyl, neryl, and  $\alpha$ -terpenyl ethyl ethers), which showed an increase in wines bottled in the colored glass, was especially interesting. These molecules are organoleptically very active as extremely volatile and are produced by the substitution of an ethanol molecule for that of sugar during the hydrolysis of heterosides (30). The same compounds in flint glass bottled wines showed a mixed behavior, with linalyl and geranyl ethyl ether tending to decrease and neryl to increase (*SI Appendix*, Fig. S6).

4-Vinylguaicol, which has a spicy note of cloves or wheat beer and characterizes the aromatic profile of Gewürztraminer

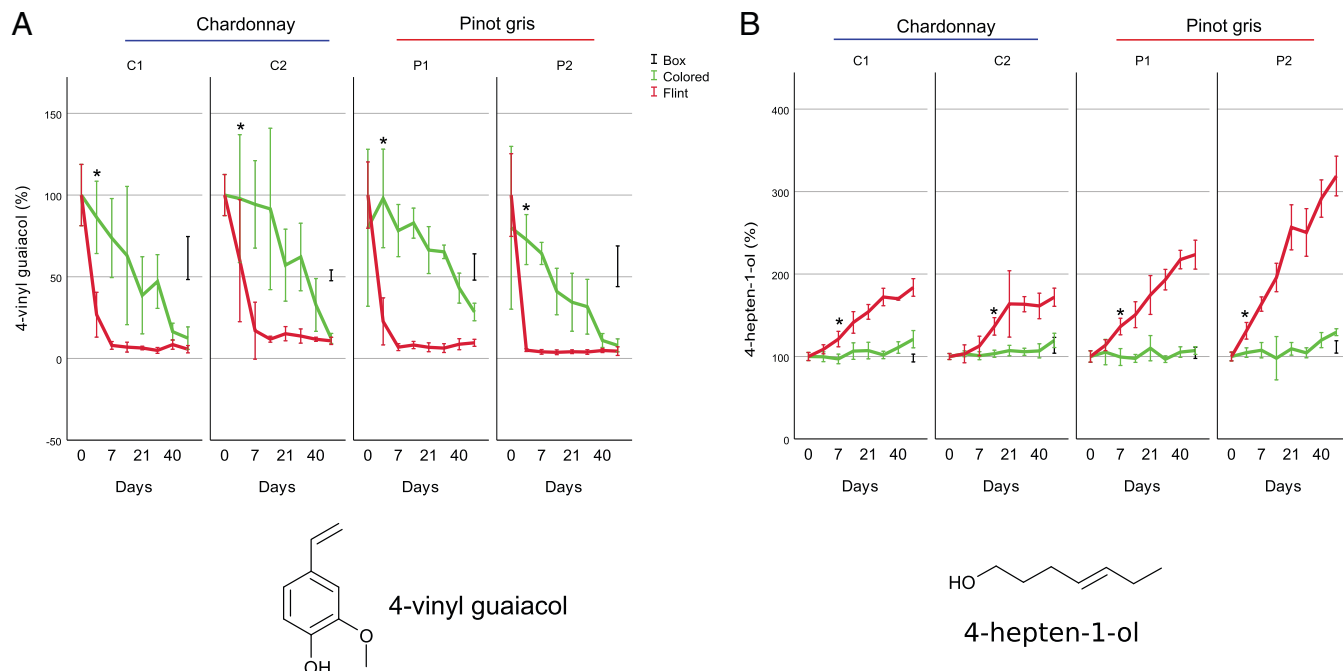


**Fig. 4.** Behavior of linalool and geranyl acetate in the two experiments. (A and C) One point experiment based on 20 wines coming from 4 cultivars. The data represent the mean values of at least 9 independent experiments (9 for Gewürztraminer and Müller Thurgau, 18 for Chardonnay, and 24 for Pinot gris), and error bars indicate SD. (B and D) The kinetic experiment based on four wines coming from Chardonnay and Pinot gris. Each point represents the mean value of five independent experiments, and error bars shows the 95% confidence intervals. \*Indicates statistical significance ( $P \leq 0.05$ ) in A and the first time point that a statistical significance occurred in B.

wines, was also a strong marker for the light exposure. Interestingly, although in most of the wines 4-vinylguaiacol is not important and sometimes is even unwanted, it plays a key role in explaining the typicality of Gewürztraminer wines (31). Especially in the kinetic experiment, we observed a fast degradation (50 to 90%) of 4-vinylguaiacol already after 2 d in flint glass bottles, whereas in colored glass, it was relatively more protected (Fig. 5A). Considering the high amounts of 4-vinylguaiacol in Gewürztraminer and the abovementioned resistance of terpenes and norisoprenoids in the wines of this cultivar, we hypothesize that the fast degradation of 4-vinyl guaiacol could play a protective role of the other volatile compounds.

Although several volatile compounds decreased due to the light exposure, the number of the compounds with the opposite behavior was very limited. The strongest identified marker positively correlated to light exposure was 4-hepten-1-ol, whose content increased significantly in wine bottled in flint bottles, doubling in content at the end of the experiment for Chardonnay wines and tripling in Pinot gris 2 (Fig. 5B). Already after

1 wk of storage in flint glass bottles, the concentration of 4-hepten-1-ol was significantly higher in respect to the same wines bottled in colored glass. On the other hand, the wines stored at the same temperature but in the dark did not develop this aromatic compound, which has a fish/rancid oil odor (32). More weakly, positively correlated markers of light exposure were (E)-2-hexen-1-ol-acetate, epoxy-calamecene, and some S-volatile compounds. Generally, the S-volatile compounds may have both positive and negative influences on the aromatic profile of wines and foodstuffs (33). It is widely accepted that the light-induced off-flavor could be attributed to a volatile sulfur compound (7) and in particular to the production of hydrogen sulfide, methanethiol, and dimethyl disulfide. These stinky compounds appear to be responsible for the cooked cabbage off-flavor and sometimes form in wine exposed to a source of light. Among the markers that increased in all wines bottled in flint and colored bottles, we also found furfural, a derivative of the carbohydrate dehydration in Maillard reactions (34), which has been positively correlated with the cooked cabbage odor peculiar



**Fig. 5.** Behavior of 4-vinyl guaiacol (A) and 4-hepten-1-ol (B) in the kinetic experiment. The variability considered for each point was based on five different bottles. The data represent the mean value, and error bars show the 95% confidence intervals. \*Indicates the first time point that a statistical significance ( $P \leq 0.05$ ) occurred.

to “sunlight” off-flavor (35) (*SI Appendix, Figs. S7 and S8*). The values of 4-hepten-1-ol and epoxy-calamemene showed a good correlation with the light exposure data registered by the sensors (*SI Appendix, Figs. S9–S19*).

Interestingly, as previously demonstrated (10), none of the basic oenological parameters (e.g., pH, titratable acidity, volatile acidity, total  $\text{SO}_2$ , and free  $\text{SO}_2$ ) and oxygen consumption indicated any statistically significant difference due to bottle color choice.

## Discussion

The two shelf-life experiments described above, conducted in two consecutive years on a very large sample set and organized under realistic conditions, shed light on a previously unknown phenomenon in wine chemistry. The concentration of dozens of volatile compounds with paramount importance in wine quality and identity, because they are responsible for the floral and fruity notes, was dramatically reduced by light exposure in flint glass bottled wine (*SI Appendix, Table S1*). As expected, the emergence of aroma compounds normally produced during wine aging, which have less welcome notes, was similar for both flint and colored glass bottled wines.

Flint glass allowed UV-Vis light to pass through and catalyzed several unwanted reactions. A common structural characteristic of all the markers was the presence of C-C double bonds, often conjugated, which can take part in several photochemical reactions such as photo-oxidation, isomerization, hydrogen abstraction and addition, cycloaddition, and polymerization (36). If the markers underwent isomerization and hydrogen abstraction modifications, their products should be detectable by our analytical method. Because that was not the case, we believe that the light-catalyzed reactions most probably delivered products with poor volatility, or nonvolatiles, such as polymerized compounds with high mass or oxidized polar molecules or degraded to very small volatiles. The involvement of terpenoids in photodegradation was not unexpected because exposure to photochemical

oxidation was found to be the major driver in long-term photochemical transformations, i.e., reaction times of several hours up to days, of terpene mixtures in secondary organic aerosols (37). In a small experiment, described in *SI Appendix*, where a wine was spiked with linalool,  $\beta$ -damascenone, and 4-vinyl-guaiacol at levels 3 to 50 times higher than the common concentrations in wines, it was hard to detect any of the degradation products described in the literature (23, 38–43).

The great majority of the annotated markers negatively correlated with light exposure belong to the group of varietal/primary wine aromatic bouquet, which include compounds synthesized in grape from isoprene or carotenoids and thus have conjugated double bonds. The major wine aromatic/aliphatic esters, secondary aroma compounds which are formed during the alcoholic fermentation and participate in the fruity aromatic character of the wine, were not among the light exposure markers. However, among the markers of the present experiment were some aliphatic esters with longer chains. Some authors have shown that these fermentative esters may decrease due to light exposure in the presence of riboflavin, in both model wine solutions and red or white wines (12, 15). Other authors registered that the behavior of the aliphatic esters in real wine under light exposure is cultivar/wine dependent because they registered decreases, increases, and mixed behaviors (44). Therefore, the fact that the major fermentative aliphatic esters were not markers in our experiments could be due to the limitations of the analytical method (45) or the absence of double bonds between the carbons of their structure. Without a doubt, aliphatic esters and higher alcohols represent the main wine aroma compounds in terms of concentration, which is often 2 to 3 orders of magnitude higher than for terpenes and norisoprenoids. However, they also have higher odor thresholds and do not characterize the varietal identity of wine. On the other hand, terpenes and norisoprenoids—strong light exposure markers according to this study—might have generally lower concentrations in wines, but they are also characterized by a lower odor threshold (*SI Appendix, Table S1*) and are strongly related to wine varietal identity (34).

In wine science and winemaking, very aromatic wines are well known to be able to mask wine faults, by using various mechanisms. For example, Gewürztraminer wines, which have a rich aromatic profile, are rarely studied in wine fault experiments because they are not considered susceptible. On the other hand, wines with a poor, limited, or neutral aromatic profile are often susceptible to atypical aging, oxidation, and lightstrike. The results of a sensorial panel on the same wines demonstrated that Gewürztraminer masked or did not develop the lightstrike off-flavor, whereas Chardonnay and Pinot gris resulted to be very sensitive wines. Thus, an abundant back up of varietal aromatic molecules and their precursors can at least partially mask off-flavor molecules. Nevertheless, a second synergic mechanism could also be plausible. According to our results, compounds with conjugate double bonds (e.g.,  $\beta$ -damascenone and 4-vinylguaiacol) decreased faster, probably offering a kind of transient protection to the compounds with isolated double bonds (e.g., terpenes). However, the high photoreactivity of the volatiles rich in double bonds, and especially conjugated double bonds, could also have a negative side. It has been demonstrated that riboflavin, which is the only known nonvolatile marker of lightstruck wines (3, 4, 9), also has several double bonds and may promote the production of off-flavors. Whether this chemical group of markers has analogous properties should be the subject of future studies. Finally, a third important mechanism of synergy between the wine volatile constituents should be noted because  $\beta$ -damascenone has the ability to enhance the fruity and flowery character of the other molecules. The loss of such aromatic enhancers in a very complex matrix, like wine, could also have a negative effect by unmasking off-flavors.

Temperature, which is also a factor of wine aging acceleration and wine shelf-life shortening, revealed a less aggressive impact on the wine aromatic profile. Several studies on wine accelerated aging at high temperature reported a loss of fruity and floral notes in young white wine, associated with the hydrolysis of the fermentative esters, and the increase of other compounds linked to the aging, such as TDN and vitispirane and other terpenoids, due to the hydrolysis of their precursors (31, 46, 47). Generally, at high temperatures (50 °C), their concentration initially increases due to glycoside hydrolysis and then decreases because of different rearrangements that lead to molecules such as  $\alpha$ -terpineol, linalool oxides, and cyclic terpenes (23, 48, 49). Therefore, to have comparable results to our shelf-life experiment for the free monoterpenes, we need to store the wine at 50 °C. As far as norisoprenoids are concerned, it has been noted (48, 49) that the amount of  $\beta$ -damascenone increases initially and then decreases. In short, we can assume that the negative impact of bottling in flint glass on some varietal aromatic compounds is much worse than that of storage at 50 °C. However, although wine has a relative resistance to heat, the plasticity of its aromatic profile turns very fragile when exposed to light. Indeed, the sensorial damage caused by light is irreversible and additive (10).

The outputs of the experiments indicated also that the boxed samples changed compared to the control samples, of course on a much smaller scale. Taking into consideration that the optimal white wine storage temperature is below  $\sim 14$  °C, the suboptimal supermarket conditions ( $\sim 20$  °C) should have favored acid hydrolysis and/or rearrangement reactions. This enforces our theory that light is by far more dangerous than heat for the white wine aroma profile, under the typical shelf-life conditions.

In the first publication of this project (10), we pointed out that even though the bottles facing the wall on the shelf received about 2 to 3 time less light (UV-Vis) than bottles

facing the window, we could not detect any difference between the two sides of the shelf in relation to lightstrike odor. Moreover, we recommended a limit of 20 to 30 UVI of UV light to avoid the fault, considering the relative UV light in relation to sensor measurements and glass type. The same dataset showed us that the wines bottled in flint glass received about 1 to 2 UVI (UV index) of UV light after 2 d, 4.5 to 12 UVI after 7 d, and 33 to 80 UVI after 50 d (*SI Appendix, Figs. S23–S25*). Therefore, if the aim is simply to avoid the appearance of lightstrike, the recommended limit could be 20 to 30 UVI, but if the aim is to avoid a considerable loss of varietal aroma compounds, the limit should be below 10 UVI.

In conclusion, we registered a dramatically unexpected loss of the major wine aroma compounds after only 7 d of shelf life in flint glass. Such a substantial decrease has tremendous consequences for neutral or low-aromatic white wine quality, typicality, and identity in an extremely short period, given the wine's commercial life. The decrease, first, yields a product with a lesser aromatic bouquet and, second, leaves the wine naked of metabolites that could mask possible off-flavors. Thus, flint glass bottles damage the wine quality in two ways, as follows: directly, by diminishing the positive organoleptic characteristics, and indirectly, by enhancing the negative ones. Years of extensive and hard work from the vine to the bottle, to deliver sensorially identifiable quality products, in terms of grape cultivar used, can be lost in a few days. Flint glass bottles bring no benefit to the wines, while the multiples changes in the aroma composition can jeopardize the quality, depriving the wine of the identity of the variety and terroir. In other words, the wine is naked.

We clearly demonstrate the negative effect of transparent packaging on foodstuff in a detailed, realistic, and vigorous manner. Therefore, it is of paramount importance for the scientific community to study the depth and length of this problem in all foodstuff and provide valuable knowledge to the food industry, in order to redesign packaging and shelf-life recommendations and rules. Special attention should be paid to products where the presence of terpenes contributes to their nutritional or health-promoting value.

## Materials and Methods

**Wines.** All samples were commercial wines (750-mL bottles), and all their metadata and basic oenological parameters are included in [Dataset S2](#). As shown in Fig. 1, the project was divided into two experiments, as follows: 1) a pilot single point experiment and 2) a kinetic experiment. For the first experiment, wines from the 2014 vintage and produced in 2015 were used, and the second experiment used wines from the 2015 vintage produced in 2016. Fig. 1A shows the room and the shelf used to mimic the typical supermarket conditions, which were used in both experiments. The room layout included windows with curtains (closed), shelves with four levels and five columns (5 m  $\times$  2.5 m  $\times$  35 cm), air conditioner fixed at 20 °C, and four tube lamps (TL-D 58W/33-640 1SL) turned on from 8 AM to 8 PM (Fig. 1A, D, and E). The single point experiment was based on 540 monocultivar white wine bottles, coming from 4 cultivars (Pinot gris, Chardonnay, Müller Thurgau, and Gewürztraminer). For each of the 20 wines, 9 bottles were made of colored glass and stayed on the shelf for 90 d, 9 were flint glass and stayed on the shelf for 90 d, and 9 colored glass bottles stayed at 4 °C in the dark for the entire period. After the end of the experiment, all wines were stored at 4 °C until analysis.

The kinetic experiment was previously described (10). Briefly, it was based on four monocultivar white wines from the 2015 harvest, namely, two Pinot gris and two Chardonnay. A map with the position of each bottle on the shelf was prepared in advance, following a randomized order (<https://www.random.org/>). For each wine/bottle color, eight bottles were sampled at seven time points (2, 7, 14, 22, 29, 40, and 50 d). The samples from the first four time points



were divided as follows: five samples per wine/bottles were placed on the shelf at day 0 and sampled after 2, 7, 14, and 22 d, while three samples per wine/bottle were placed on the shelf 21, 14, 7, and 2 d before the end of the experiment and sampled at day 50. Time 0 was the eighth time point, so these bottles were stored directly at 4 °C and in the dark. For each wine, eight bottles were left in the room, simulating supermarket conditions, but in a cardboard box, to have samples stored under the same environmental conditions but in complete darkness.

**Basic Oenological Analysis.** All the basic oenological analyses were performed within 1 wk from the end of each experiment, at the winery quality-control laboratory. A WineScan FT120 was used to measure alcohol, sugars, pH, total acidity, and volatile acidity. Free and total SO<sub>2</sub> were measured using a Mettler T70 instrument.

**Aroma Compound Analysis.** The volatolome was extracted by solid-phase microextraction (SPME) and GC×GC-ToF-MS analysis were done according to a previously published protocol (18, 19). Briefly, the SPME fibers used were 2 cm long (50/30 DVB/CAR/PDMS), from Supelco, conditioned according to the manual. Two internal standards (2-octanol and ethyl hexanoate d11) have been added to cover the two most important classes of the wine volatolome (alcohol and ester), which also have a different affinity for the fiber. The monitoring of the standard areas allowed us to evaluate the technical variability and the instrumental stability, during and after the measurements. A Gerstel multipurpose sampler (Gerstel GmbH & Co. KG Mülheim an der Ruhr Germany) with an agitator and SPME fiber was used to extract the volatiles from the sample vial headspace. The GC×GC system was the Agilent 7890 A (Agilent Technologies). Equipped columns were VF-Wax column (100% polyethylene glycol; 30 m × 0.25 mm × 0.25 μm, Agilent J&W Scientific Inc.) as the first dimension and Rxi-17Sil MS 1.50 m × 0.15 mm × 0.15 μm, Restek) as the second dimension. A nonmoving quad jet dual-stage thermal modulator was used to couple the two columns. The MS signal was obtained with Pegasus IV time-of-flight mass spectrometer (Leco Corporation, St. Joseph, MI). For additional details, see the [SI Appendix](#).

Quality control (QC) samples, namely, one for each experiment, consisting of an equal proportion of each sample, were placed at the beginning of the run ( $n = 5$ ) and thereafter every 10th sample. For the single point experiment, four bottles per condition were analyzed according to a randomized order. The samples were analyzed in three independent sequences, namely, one for the Chardonnay samples ( $6 \times 3 \times 3 = 54$  bottles), one for the Pinot gris (8 wines × 3 conditions × 3 bottles = 72 bottles), and a last one combined the Müller Thurgau and Gewürztraminer ( $3 \times 2 \times 3 \times 3 = 54$  bottles). For the kinetic

experiment, five bottles were selected for every wine/time point/condition, as we tried to cover the shelf surface. For the time points of 2, 7, 14, and 21/22 d, three bottles were from the samples that were initially placed on the shelf, and two bottles were from the samples that were placed later and removed at the end of the experiment. The samples were analyzed in four independent sequences, one for each wine, that included 80 bottles each ((7 time points × 2 bottle glass × 5 bottles) + 5 box + 5 time 0). The samples of each sequence were randomized before the sample preparation and analyzed according to this randomized order. All wines were analyzed within 1 mo of the end of the experiment, with an untargeted analysis protocol that included randomization before the sample preparation and the use of a pooled sample as a QC, according to our previous experience in the field (17–20). The percent coefficient of variation (%CV) of the internal standard areas was 7.13% for the first and 4.47% for the second experiment.

**Data Processing, Statistical Analysis, and Visualization.** ChromaTOF software version 4.32 was used to perform baseline correction, chromatogram deconvolution, and peak alignment (18, 19). The NIST (National Institute of Standards and Technology) 2.0, Wiley 8 and FFNSC 2 (Flavour and Fragrance Natural and Synthetic Compounds 2) libraries were used for the metabolite annotation. Based on the identification level, following assignments were given authentic standards, injected under identical GC×GC-ToF-MS conditions, (first level annotation); mass spectra and linear retention index, (second level of annotation); annotated based on their chemical group, (third level of annotation); considered unknowns, (fourth level of annotation) (50). For further statistical analysis and data visualization, the following tools were used: EZinfo version 2.0 (Umetrics' SIMCA), SPSS V28 (IBM Statistics), Metaboanalyst (51), and Statistica V13 (TIBCO Software Inc.). For additional details see [SI Appendix](#).

**Data Availability.** The management of all data and metadata was made according to the FAIR (Findable, Accessible, Interoperable and Reusable) guidelines for grapevine and wine studies. Metabolomics data have been deposited to the database (<https://www.ebi.ac.uk/metabolights>) with the identifier [MTBLS3201](#) (52). The annotation results for all metabolomics datasets are provided as supporting information in [SI Appendix](#).

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