

Influence of Vine Training and Sunlight Exposure on the 3-Alkyl-2-methoxypyrazines Content in Musts and Wines from the *Vitis vinifera* Variety Cabernet Sauvignon

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The influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines contents in musts and wines was studied by means of two previously reported methods based on headspace solid-phase micro-extraction. Experimental samples were monitored throughout grape ripening and wine making. 3-Isobutyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine were identified. The 3-isobutyl-2-methoxypyrazine content decreased throughout grape ripening in all of the sample types studied. After 1 day of maceration with the skins, there was an increase, but after racking, no further increase was observed. No significant differences between samples were found during grape ripening. Wines from goblet-trained vines, however, contained significantly less 3-isobutyl-2-methoxypyrazine. Clusters protected from sunlight since the beginning of the veraison resulted in wines with a significantly lower content of this compound than the control samples.

KEYWORDS: 3-Alkyl-2-methoxypyrazines; Cabernet sauvignon; gas chromatography; grape; ripening; solid-phase micro-extraction; sunlight; training; *Vitis vinifera*; wine

INTRODUCTION

The different types and amounts of volatile aroma compounds in berries are the major source of flavor distinction in varietal wines. Thus, primary aromas can influence wine quality and have an important economic impact. This is the case of 3-alkyl-2-methoxypyrazines (MP), which are associated with the green, herbaceous or vegetative aromas typical of certain cultivars. 3-Isobutyl-2-methoxypyrazine (IBMP), together with 3-*sec*-butyl-2-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), have been identified in Cabernet sauvignon, Merlot noir, and Sauvignon blanc (2–6).

Sensory thresholds of some MPs are very low. IBMP, SBMP, and IPMP can be perceived by the human nose at 1–2 ng/L in distilled water (7–12). IBMP is detected at 10 ng/L in red wines (5) and IPMP at 2 ng/L in red wine (10). Because MP can be present in grapes and wines at higher levels, they can have an important sensorial impact on wine quality.

Among the most important aroma compounds of the Sauvignon blanc variety, some sulfur-containing compounds have been found (13–14). Nevertheless, MP, and in particular IBMP, are considered to be mainly characteristic of this variety. Their contribution to the quality of Sauvignon blanc wines can be positive as long as it is not too dominant and it is balanced and

complemented by other aromas (15–16). However, the “vegetative” character it provides is generally considered unacceptable in red wines (5, 17), though its presence in moderate amounts (2–15 ng/L) is not incompatible with the high quality of Bordeaux wines (16, 18).

It has been found that a balance between biological formation and photodegradation may determine the MP content in grapes throughout the ripening process. MP might form largely in the earlier stages of grape development, and photodegradation might be more important in the ripened fruits (19). This hypothesis would explain how some viticulture factors, like the effect of the weather conditions on ripening, the exposure of the fruit to sunlight, and the degree of grape ripening can affect the MP content in grapes and wines.

It is generally accepted that wines from warm areas tend to have lower “vegetative-herbaceous” aromas and a lower IBMP content than wines from cool areas (5–6, 15, 20–22). Cool ripening conditions can lead to higher MP levels and thus enhance these aromas in Sauvignon blanc grapes (6, 20). Likewise, a greater humidity in the pre-veraison month can result in higher IBMP contents (17). This is in accordance with the theory that unripe grapes determine the IBMP content of final wines (19).

Producers know that the exposure of grapes to light has a strong influence on the character of wines (22). They are interested in factors such as vine vigor, leaf removal, and

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pruning, all of which determine the exposure of the fruit to sunlight, because they can be manipulated to manage wine flavor and quality from the beginning of grape growth. Indeed, increased vine vigor and the resulting canopy shade can significantly change the aromas in Cabernet sauvignon and Sauvignon blanc wines (20, 22–23) by increasing their vegetative character. Severe leaf removal seems to be effective at reducing these notes in Sauvignon blanc wine, and earlier treatments are more effective than later treatments (22, 24). Light exposure has been found to have an effect on IBMP levels, which were three times higher in heavily shaded clusters than in the well exposed fruits of Cabernet sauvignon (20). Finally, minimally pruned vines gave fruits with IBMP concentrations that were eight times lower than those in fruits from spur-pruned vines. The authors explained that minimal pruning provided greater exposure to sunlight (20).

Grape growers are familiar with the fact that Cabernet and Merlot noir grapes have a vegetative taste when they are not ripe (25). Also, it has been reported that, in Cabernet sauvignon and Sauvignon blanc, the riper the grapes are, the lower the IBMP levels (4, 26) and the herbaceous-vegetative character (3, 6, 20). Thus, a very wide range of MP levels can be found in grapes (27), and consequently in the final wines obtained.

Assessing how viticultural factors affect the contents of MP requires accurate analytical methods because some differences may be too small to be noticed by sensory analysis. Because the contents of these compounds in grape juices and wines are so low (ng/L), their determination is methodologically difficult. We have developed and reported two methods based on HS-SPME with GC-NPD for quantifying MP in musts and wines. They have high recoveries and detection limits at 0.1–0.3 ng/L for IBMP, SBMP, and IPMP (26, 28). The results presented here were obtained by means of these procedures.

The purpose of this work was to study the influence that training and sunlight exposure can have on the MP content of Cabernet sauvignon musts and wines. MP concentrations were monitored throughout grape ripening and winemaking.

MATERIALS AND METHODS

All samples were produced and collected at the experimental vineyard and wine cellar of the Faculty of Enology of the Rovira i Virgili University in Constantí (Tarragona) in 1998. The weather in the region (average June, July, and August, respectively) was as follows: temperature, 20.6, 23.6, and 23.5 °C; maximum temperature, 26.1, 29.2, and 29.9 °C; minimum temperature, 15.2, 17.8, and 17.6 °C; daily solar irradiation, 21.7, 22.6, and 17.2 MJ/m²; rainfall, 1.0, 3.5, and 35.0 L/m².

Two plots of Cabernet sauvignon vines were used in this study. Both plots were planted 7 years before in the same parcel, on the same type of ground, with the same orientation and at the same elevation. The first plot is goblet-trained and has a total of 365 plants, distributed in 5 rows. The second plot is trellised, bilateral cordon trained, and has 502 plants distributed in 12 rows. Both plots have a density of plantation of 2500 plants per hectare. None of them was irrigated.

To study the effect of vine training on MP concentrations, samples from (sunlight-exposed) clusters of trellised-trained vines were compared with samples from (sunlight-exposed) clusters of goblet-trained vines.

Sunshine Protection. Samples for studying the influence of sunshine exposure on MP content were collected from the plot of trellised-trained vines. One cluster on every two vines was protected from the sunlight from the beginning of the veraison by means of pieces of sackcloth. These clusters were randomly selected, and special care was taken to make sure that the more and less sunlight-exposed clusters were correctly represented. The pieces of sackcloth were wrapped round the cluster, but the bottom was left open to facilitate gas movement and to

prevent the temperature from increasing due to grape respiration. The temperature of the fruits was monitored, and no important differences between wrapped and nonwrapped fruits were found during the ripening process. Samples of sunlight-protected clusters (from trellised-trained vines) were compared with sunlight-exposed clusters from the same vines. Both series of samples were common at veraison. For the other sampling stages during fruit ripening, both types of samples were collected from clusters belonging to the same plant.

Sampling. To obtain random samples and not use the same vine twice at the different sampling times, a mark was put every five vines of each vineyard. The first sample was collected only from marked vines. The second sample was collected from the vine immediately next to the marked vine. The third to fifth samples were collected from the plants on the third to fifth places after the marked vine. The clusters sampled were also randomly selected within the vine, to ensure a homogeneous distribution between clusters that had been exposed more or less to sunlight. Three berries from each cluster were collected, up to a total of 100. They were collected at random: one from the top, one from the bottom, and one from the middle of the cluster. Special care was taken to obtain a good distribution between berries from inside and outside the cluster. Three replicates of each kind of sample were collected in all cases.

During the grape ripening period, grapes were collected at the following times: veraison, every 5–12 days (ripening stages 1, 2, and 3), and on the harvest day. Samples were manually pressed in the laboratory, NaF (1 g/L) was added as preservative, and they were stored in dark bottles at –20 °C. At the harvest day, grapes from goblet-trained vines presented a potential alcoholic content of 11.8% and a pH of 3.73, while trellised trained grapes presented a slightly lower potential alcoholic content 11.2–11.3% and a similar pH of 3.70–3.71. A total of nine different micro-vinifications were monitored: three replicates of each type of sample. Classical red winemaking was used for all the samples. The final volumes of each micro-vinification were about 2.5 L. The sampling times during winemaking were the following: after 1 day of maceration, at the end of alcoholic fermentation, and after the malolactic fermentation. Fermented samples were preserved with SO₂ (25 mg/L) and stored in dark bottles at 4 °C.

Sample Preparation. MP contents were determined in accordance with the previously published HS-SPME procedures (26, 28). Every determination was made in duplicate. In the case of musts, 3 g of NaCl and 1 mL of a 100 ng/L solution of 3-isopropyl-2-ethoxypyrazine (internal standard) was placed in a 20 mL vial for SPME together with an aliquot of 10 mL of must and a little magnetic stirrer. It was thickly capped, put in an isothermal bath at 30 °C and continuously stirred. The SPME fiber was then introduced into the headspace. After 4 h, it was analyzed by GC. In the case of wines, 10 mL of the sample was spiked with the internal standard, acidified with HCl, and distilled at low pressure and room temperature. After the volume had been reduced to 50%, the resulting solution was neutralized with NaOH and transferred to the 20 mL SPME vial containing the NaCl and the magnetic stirrer. Finally, the SPME extraction was performed as for the musts.

Chromatographic Conditions. Chromatographic analysis was performed with a Hewlett-Packard 5890 II gas chromatograph equipped with a nitrogen-phosphorus detector. Injection (splitless, 1 min) was performed with an inlet of 0.75-mm ID and at 250 °C. The analytical column was a CP-WAX 57 CB (50-m × 0.25-mm ID, 0.2- μ m FT). The carrier gas was high purity helium flowing at 0.8 mL/min. The oven temperature was 30 °C (1 min), 25 °C/min to 100 °C (20 min). 3-Isopropyl-2-ethoxy-pyrazine (Pyrazine Specialties, Atlanta, Georgia), more than 97% pure, was used as internal standard (IS). The SPME device and the poly(dimethylsiloxane)/divinylbenzene, 65 μ m, fibers used in this study were purchased from Supelco (Bellefonte, PA). Each fiber was conditioned before use, as well as cleaned afterward by insertion into a GC injector at 260 °C for a minimum of 5 min. They were used immediately to prevent contamination.

Statistics. All the data are expressed as the arithmetic average \pm standard deviation from three replicates. Two-factor ANOVA and Fisher test were carried out using Statview (software for Macintosh).

Table 1. Evolution of 3-Isobutyl-2-methoxypyrazine (IBMP) Contents (ng/L) throughout Grape Ripening

	IBMP: Grape Ripening ^a		
	sunlight/trellised	sunlight/goblet	protected/trellised
veraison	26.0 (7.5) ^b	18.4 (2.7) ^b	26.0 (7.5) ^b
ripening 1	18.8 (5.4) ^b	13.7 (4.4) ^{bc}	13.4 (3.9) ^{bc}
ripening 2	16.3 (4.7) ^{bc}	13.0 (2.9) ^c	18.0 (4.8) ^c
ripening 3	4.1 (1.0) ^c	5.4 (1.1) ^d	3.6 (1.4) ^d
harvest	2.8 (0.6) ^c	3.2 (0.8) ^d	bl ^e

Two-Factor Anova:
Ripening $p < 0.0001$; Treatment $p = 0.4867$

	sunlight/trellised	sunlight/goblet
sunlight/goblet	0.0665	
protected/trellised	0.4646	0.2822

^a Average values and standard deviations (in brackets). ^{b-d} Statistical analysis: Two-factor ANOVA and Fisher test (both, $p = 0.05$). Same letter in the same column indicates the absence of statistically significant differences ($p > 0.05$). ^e bl, below quantification limits.

Table 2. Evolution of 3-Isobutyl-2-methoxypyrazine (IBMP) Contents (ng/L) throughout Winemaking

	IBMP: Winemaking ^a		
	sunlight/trellised	sunlight/goblet	protected/trellised
harvest	2.8 (0.6) ^b	3.2 (0.8) ^b	bl ^e
1 day macer.	10.0 (2.2) ^c	6.4 (0.8) ^c	7.3 (2.0) ^b
end alc. ferm.	12.3 (2.8) ^c	9.3 (1.1) ^d	3.4 (0.9) ^c
end mal. ferm	12.0 (2.1) ^c	7.8 (1.1) ^{cd}	4.6 (1.4) ^{bc}

Two-Factor Anova:
Winemaking $p < 0.0001$; Treatment $p < 0.0001$

	sunlight/trellised	sunlight/goblet
sunlight/goblet	0.0133	
protected/trellised	< 0.0001	0.0002

^a Average values and standard deviations (in brackets). ^{b-d} Statistical analysis: Two-factor ANOVA and Fisher test (both, $p = 0.05$). Equal letter in the same column indicate the absence of statistically significant differences ($p > 0.05$). ^e bl, below quantification limits.

RESULTS AND DISCUSSION

Tables 1–4 show the MP content of experimental musts and wines, and **Figure 1** shows several typical chromatograms. IBMP, SBMP, and IPMP were identified in the three series of samples. The content of SBMP and IPMP was very low throughout winemaking in all cases and often below quantification limits. Similarly, 3-ethyl-2-methoxypyrazine was identified in some grape samples, but the content was generally too low to be quantified.

IBMP was the most abundant methoxypyrazine in almost all the samples studied, which agrees with the literature (4, 6, 7, 20, 29). SBMP levels were generally higher than IPMP levels. It should be pointed out that data on SBMP concentrations in musts and wines is scarce in the literature. According to the sensory thresholds stated in the Introduction, only IBMP might have an influence on the flavor of the final wines obtained from the sunlight exposed grapes of the trellised-trained vines. The content of SBMP in final wines was higher than its detection threshold in water, but we do not have data about the threshold of SBMP in red wines.

Grape Ripening. IBMP levels are reported to vary considerably in Cabernet sauvignon and Sauvignon blanc musts (0.5–

Table 3. Evolution of 3-sec-Butyl-2-methoxypyrazine (SBMP) Contents (ng/L) throughout Grape Ripening

	SBMP: Grape Ripening ^a		
	sunlight/trellised	sunlight/goblet	protected/trellised
veraison	4.3 (1.8) ^b	9.6 (4.1) ^b	4.3 (1.8) ^b
ripening 1	11.4 (4.7) ^c	13.0 (6.6) ^b	9.2 (3.6) ^c
ripening 2	16.2 (3.8) ^c	13.9 (2.9) ^b	18.2 (4.3) ^d
ripening 3	2.8 (0.8) ^b	4.0 (0.8) ^c	2.6 (0.4) ^a
harvest	bl ^e	4.1 (2.0) ^c	bl ^e

Two-Factor Anova:
Ripening $p < 0.0001$; Treatment $p = 0.5437$

	sunlight/trellised	sunlight/goblet
sunlight/goblet	0.8848	
protected/trellised	0.3397	0.2736

^a Average values and standard deviations (in brackets). ^{b-d} Statistical analysis: Two-factor ANOVA and Fisher test (both, $p = 0.05$). Same letter in the same column indicate the absence of statistically significant differences ($p > 0.05$). ^e bl, below quantification limits.

Table 4. Evolution of 3-Isopropyl-2-methoxypyrazine (IPMP) Contents (ng/L) throughout Grape Ripening

	IPMP: Grape Ripening ^a		
	sunlight/trellised	sunlight/goblet	protected/trellised
veraison	bl ^d	5.4 (2.7) ^b	bl ^d
ripening 1	4.5 (0.4) ^b	8.0 (5.4) ^{bc}	3.1 (1.0) ^b
ripening 2	10.7 (3.7) ^c	12.3 (1.7) ^c	13.7 (6.8) ^c
ripening 3	bl ^d	3.0 (0.5) ^b	bl ^d
harvest	bl ^d	4.7 (3.6) ^b	bl ^d

^a Average values and standard deviations (in brackets). ^{b-d} Statistical analysis: Fisher test ($p = 0.05$). Equal letter in the same column indicate the absence of statistically significant differences ($p > 0.05$). Results could not be analyzed by means of two-factor ANOVA test due to the lack of data above quantification limits. ^d bl, below quantification limits.

189 ng/L) (20). The results presented here support this (**Table 1**), although an IBMP content no higher than 26 ng/L was found in the monitored samples. The content of this compound changed significantly during grape ripening, and its evolution was similar in all cases: it decreased dramatically after the veraison and then gradually throughout the process so that the levels were lowest on harvest day. Our results match those in the literature, which reported that the IBMP content in Cabernet sauvignon, Merlot noir, and Sauvignon blanc varieties decreased with increasing grape maturity, and mainly during the first stage of the ripening process (6, 17, 20, 26).

Interestingly, the SBMP and IPMP content evolve differently during grape ripening (**Tables 3** and **4**). They tend to increase during the first part of the process and then decrease. Consequently, although at veraison and grape ripening stage 1, the IBMP content is higher than both SBMP and IPMP, at ripening stage 2, the concentration of all these MP is similar, (between 11 and 16 ng/L). After reaching this maximum level, the concentrations of both SBMP and IPMP drop, so the average levels of the three MPs are below 5 ng/L at harvest.

Winemaking. The IBMP concentration increases significantly in all musts on the first day of maceration (**Table 2**). However, no further significant increase was observed after racking. These results suggest that IBMP seems to be released from the solid parts of the grapes (30). Finally, malolactic fermentation proved not to change the IBMP content.

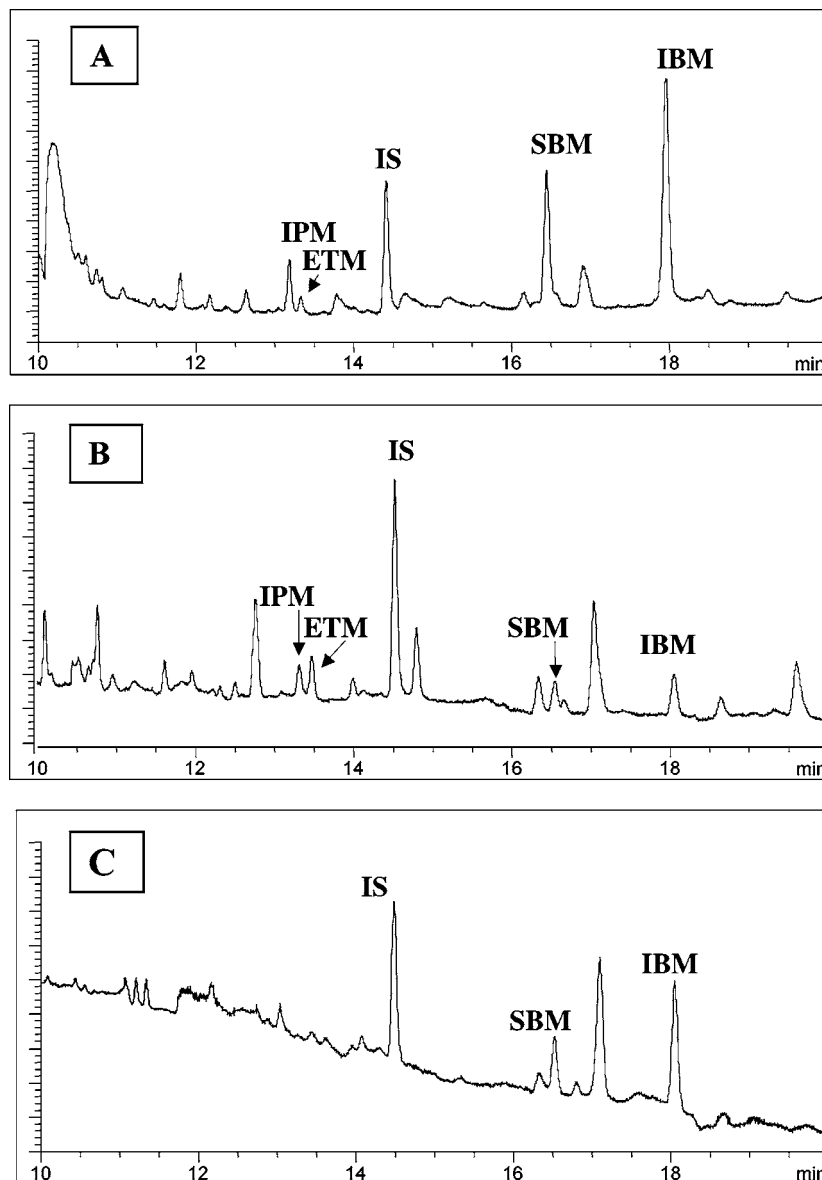


Figure 1. Chromatograms of samples from sunlight-exposed grapes of trellised-trained vines. Grape juices at an earlier (A) and at a later (B) grape ripening stage, and the final wine obtained from them (C). Internal standard (IS): 10 ng/L.

The increase in IBMP during the winemaking process has also been reported in the literature: Cabernet sauvignon wines showed a higher MP content than did the juice before fermentation (21, 27). It has been reported that the skins, seeds, and stems of grapes contain MP and that they can partly pass into the juice during fermentation/maceration (4, 19, 26, 30). The extraction by ethanol during fermentation and/or the release from precursors by specific yeast strains (15, 21) have been considered responsible for this compound passing from the solid parts to the juice. However, the results presented here show that the IBMP levels mainly increased during the first 24 h of maceration, before the alcoholic fermentation actually started. These results indicate that ethanol or specific yeasts are unlikely to have a major influence. The destruction of the berry structure due to destemming and crushing means that there is a considerable contact between skin/seeds/stems and juice, which increases the levels of these compounds in musts, because MP occur mainly in the solid parts of the fruit. So, if extraction by ethanol has an influence, it would be lower and slower.

Surprisingly, neither the SBMP nor the IPMP content increases significantly during the winemaking process (data not

shown), even on the first day of maceration. This suggests that the contents of these MP in the skins of the fruits might be lower than the IBMP content. This, together with the fact that they evolve differently during grape ripening, indicates that the dynamics of both SBMP and IPMP are totally different from that of IBMP.

The MP content in final wines agrees with the literature. The IBMP contents of 4.6–12.0 ng/L do not differ from the reported 3.6–56.3 ng/L (20, 27). Finally, the SBMP and IPMP content, which is close to or below quantification limits, agree with the levels mentioned in the literature: 2.0–4.9 ng/L and 0.92–10.1 ng/L, respectively (2).

Vine Training. The results do not show statistically significant differences in IBMP contents between samples from goblet and trellised trained vines during grape ripening (Table 1). The evolution of all MP is similar throughout this process in both types of vines.

Nevertheless, the training factor produces significant differences in the IBMP content of final wines (Table 2). The relative increases in samples from both goblet and trellised-trained vines during the winemaking process was similar, in fact almost

parallel. However, in the winemaking stages, the IBMP content of samples from the trellised-trained vines was significantly higher. Eventually, the average content in final wines was higher in these samples. This difference can be critical because the amount of IBMP in final wines from trellised trained vines is actually higher than its reported odor threshold in red wines (5), whereas in samples from goblet-trained vines it is not. These differences may be related to the fact that grapes from goblet-trained vines presented a slightly higher maturation level at harvest.

Finally, musts from goblet trained vines contain enough SBMP to be determined, but in musts from trellised-trained vines, the amount of SMBP is below determination limits (Table 3).

Sunshine Exposure. During ripening, IBMP levels in grapes exposed to sunshine were not significantly different from the ones that had been covered with pieces of sackcloth (Table 1). Interestingly, however, the increase in IBMP content during maceration was significantly lower in the samples from the sunlight-protected clusters (Table 2). And the amount of IBMP in the final wines was clearly lower. Such differences cannot be due to different maturity levels, inasmuch as both samples, sunlight exposed, and sunlight protected grapes, presented similar pH and potential alcoholic content at harvest. Therefore, the results reveal that less sunshine exposure resulted in wines with a lower IBMP content.

These results are surprising because sunlight protection was expected to lead to higher levels of MPs. Indeed, according to the literature, fruit that is exposed less to the sun has higher MP levels (20) and a different aroma, with a stronger vegetative accent (23, 31). It has also been proven that light can photodegrade of IBMP in grape tissues (19). Our findings point to the hypothesis that the lower sunshine exposure obtained by means of the artificial protection of the berries would result in lower levels of IBMP in the skins. These results suggest that the balance between MP formation and degradation (19) is complex and can be influenced by several factors. Thus, the biological formation of MPs in grapes would require sunlight not only in the earlier stages of grape development (19) but also during grape ripening. In terms of wine flavor, the reported differences in IBMP content might be critical. In wines from sunlight-protected clusters, these levels are below the sensory threshold for this compound in red wines (5), so the final product is likely not to have the vegetative character, whereas wines from the sunlight-exposed clusters might have this character. Finally, no significant differences in the SBMP levels (Table 3) were found between the samples from the sunlight exposed and sunlight protected clusters during grape ripening.

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