Factors affecting the presence of 3-alkyl-2-methoxypyrazines in grapes and wines. A review.

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Abstract

This review discusses the presence of 3-alkyl-2-methoxypyrazines in grapes, musts and wines, their effect on wine flavor and the influence of environmental conditions, viticultural practices and winemaking techniques on their contents. These compounds have been identified in the following varieties of *Vitis vinifera*: Sauvignon blanc, Cabernet sauvignon, Cabernet franc, Merlot noir, Pinot noir, Muscat, Semillon, Chardonnay and Riesling, Zweigeltrebe and Xynomavro. 3-isopropyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine are the most important, the last one being the more abundant. Reported contents of this compound in grapes and wines are up to 307 and 56.3 ng/L, respectively. Levels of 3-isopropyl-2-methoxypyrazine can be up to 48.7 ng/L in grapes and up to 4.5 ng/L in wines. Finally, the concentration of 3-*sec*-butyl-2-methoxypyrazine in grapes and wines has been found to be lower than 11.2 ng/L. Sunlight exposure and grape maturity at harvest are the main factors affecting the contents of 3-alkyl-2-methoxypyrazines in wines.

Keywords

3-alkyl-2-methoxypyrazines, aroma, grape, *Vitis vinifera*, wine

Introduction

3-alkyl-2-methoxypyrazines (MPs) (Fig. 1) have been found to play an important role in the aroma of several varieties of *Vitis vinifera*. The study of the aroma compounds of some of them, mainly Sauvignon blanc, Cabernet sauvignon, Cabernet franc and Merlot noir, is of great interest in the field of enology due to their economical importance. The most important pyrazines in wines are 3-isobutyl-2-methoxypyrazine (IBMP), 3-*sec*-butyl-2-methoxypyrazine (SBMP), and 3-isopropyl-2-methoxypyrazine (IPMP).

Some excellent reviews have been published which have concentrated on the broad field of pyrazines in foods *(1-6)* and on the aroma of Sauvignon blanc *(7)*. The knowledge of what factors can influence MP contents in grapes, how they evolve throughout the berry development and how they do pass to wines may provide a means for controlling the flavor and quality of the final wines. The present review summarizes and discusses the sensory properties of MPs, their occurrence in grapes, musts and wines; their influence on wine flavor and the viticultural and winemaking factors affecting their concentrations.

Sensory properties

The reported odor descriptions and olfactory thresholds of the main MPs in several media: air, water, and (synthetic, white and red) wine are summarized in Table 1. The difficulties for determining these properties are well known to the analysts dedicated to sensory evaluation. Differences in the results obtained can be due to biases linked to sensory analysis, differences in the methodologies used, in the sensitivity of the panel members, the influence of the medium, the concentration levels and the environmental conditions.

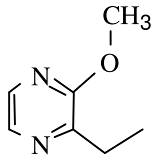
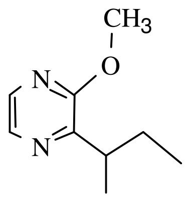
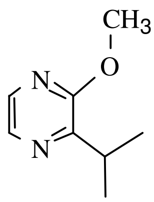
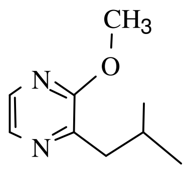
Nevertheless, it is generally agreed that the threshold of IBMP in water is 2 ng/L. The threshold of this compound has been found to be as low as 1-6 ng/L in white and synthetic wines and of 10-16 ng/L in red wines (Table 1). Wines containing a higher amount of this compound tend to have a strong 'vegetative' character *(8)*. A study of MP addition to red wine has indicated a higher odor threshold for IBMP (16 ng/L) than for IPMP (2 ng/L) *(3)* (Table 1). The threshold of this last pyrazine is of 1-2 ng/L in water and wine (synthetic, white and red). Finally, data on sensory threshold of SBMP is scarce (Table 1), though in water 1-2 ng/L have been reported, so that values are likely to be higher in wines.

MPs have strongly green, somewhat earthy odor characteristics with roasted and nutty notes *(9)*. IBMP, SBMP, and IPMP are the most important pyrazines regarding their potential influence in wine flavor. Their smell is generally described as 'bell pepper', 'leafy', 'ivy leaves', 'vegetable', 'peas', 'asparagus', 'galbanum', 'ginseng', 'roasted', 'musty' and 'raw potatoes'. 'Green pepper' aromas are considered more typical of IBMP, and 'pea-asparagus' aromas typical of IPMP *(10)*. The olfactory notes of pyrazines are determined by their alkyl chain: the compounds with the smaller chains are described as 'roasted, nutty and sweet', whereas MPs with the longest side chain tend to have green-like odors *(11-12)* (Table 1).

Influence in wine aroma

MP contents in grapes, musts and wines reported in the literature are summarized on Tables 2 and 3. Data about their region of origin, vintage, climatic conditions and degree of grape maturation is also provided. IBMP is generally more abundant than IPMP and SBMP. This is also the compound more likely to influence wine flavor because its contents in the final wines are more often above the sensory threshold. For all these reasons, IBMP is the most relevant MP in grapes, musts and wines.

Figure 1: Main 3-alkyl-2-methoxypyrazines found in grapes, musts and wines.

3-ethyl-2-methoxypyrazine (ETMP)	3-sec-butyl-2-methoxypyrazine (SBMP)
	
3-isopropyl-2-methoxypyrazine (IPMP)	**3-isobutyl-2-methoxypyrazine (IBMP)**
	

The fact that SBMP occurs in wines at very low levels suggests that it will seldom contribute significantly to the sensory character of wines *(13)*. However, in some Cabernet sauvignon wines it reaches values of 2-6 ng/L, and the highest values registered are in Merlot noir wines, with 4-16 ng/L (Table 3). Considering its sensory threshold in water (1 ng/L), the possibility of the compound having an influence in wine aroma can not be discarded, though further work is needed to know more about its sensory threshold in wines (Table 1).

The presence of 3-ethyl-2-methoxypyrazine (ETMP) was reported in Sauvignon blanc *(14)* grapes. However, this compound was not found in an extensive research of 22 wines of this variety from Australia, New Zealand and France *(15)*. Consequently, it is unlikely that ETMP, even if present in wines, would occur at a concentration sufficient to contribute to wine aroma, because its odor threshold in water has been reported to be 400-425 ng/L (Table 1). Therefore, authors analyzing MPs in grapes and wines tend not to look for this compound.

Analysis of a particularly MP rich wine (containing an average IBMP level of 35 ng/L) established an upper limit for 3-methyl-2-methoxypyrazine of 0.1 ng/L *(16)*. As its odor threshold has been reported to be 4000-7000 ng/L (Table 1), any contribution of this compound to Sauvignon blanc wine aroma is unlikely to happen.

A too strong 'vegetative', 'green bell pepper' aromas found in wines containing IBMP are generally considered as detrimental to the quality of red wine *(8, 17-21)*. It can be associated to unripe grapes and poor quality wines *(21)*.

Table 1. Reported odor descriptions and olfactory thresholds (ng/L) of the 3-alkyl-2-methoxypyrazines.

MP	Medium	Odor description	Threshold	Ref.
2-	water	roasted, nutty, sweet	400,000-700,000	*(47-48)*
3-methyl-2-	water	nutty, roasted, sweet, roasted peanuts, earthy	3,000-7,000	*(9, 11, 47-49)*
3-ethyl-2-	red/synthetic wine		>64	*(3)*
	water	sweet, brown, nutty, roasted	10,000	*(47)*
	water	raw potato, earthy, green bell pepper	400-425	*(48-50)*
3-propyl-2-	water	green, earthy, ginseng-like, vegetable	120	*(11, 47)*
	water	bell pepper	6	*(48-49)*
3-isopropyl-2-	white wine	earthy, cooked or canned asparagus, green beans	2	*(37)*
	air	earthy, roasted	0.0005-0.005	*(9, 51)*
	water	galbanum, earthy, musty, potato, green pepper, roasted, pea, mouldy, cellar	1-2	*(3, 9, 32, 48-49)*
	olfactometry	grassy, earthy		*(40)*
	red wine	leafy	2	*(3)*
	synthetic wine	musty, earthy, leafy	2	*(3)*
3-butyl-2-	red wine	green pepper, leafy	8	*(3)*
	synthetic wine	musty, green pepper	2	*(3)*
	water	green, earthy, ginseng-like, burdock-like, bell pepper	50	*(3, 11, 47, 49)*
3-*sec*-butyl-2-	water	green (peas, bell pepper, galbanum), ivy leaves, bell pepper	1-2	*(9, 11, 49, 52-53)*
3-isobutyl-2-	air	earthy	0.002-0.005	*(9, 51, 54)*
	water	green, bell pepper, musty, earthy	10	*(9)*
	water	green, bell pepper	2	*(3, 48-49, 54)*
	white wine	fresh vegetables, bell pepper, green gooseberries, herbaceous, vegetative	1	*(37)*
	olfactometry	herbaceous, earthy		*(40)*
	water	strongly green, bell pepper	16	*(55)*
	water		0.5	*(19)*
	red wine	leafy	10, 15, 16	*(3, 8, 19)*
	synthetic wine	musty, green pepper	2, 6	*(3, 8)*
3-pentyl-2-	water	green, earthy, ginseng, burdock	20	*(11, 47)*
3-hexyl-2-	water	green, earthy, burdock, vegetable	70	*(11, 47)*
	water	bell pepper	1	*(1, 48-49)*
3-heptyl-2-	water	green, earthy, burdock, vegetable	26	*(47)*
3-octyl-2-	water	green, earthy, burdock	60	*(47)*
3-decyl-2-	water	green, earthy, burdock, waxy	40,000	*(47)*

However, even when exceeding its sensory threshold, the presence of IBMP might be compatible with high quality Cabernet and Merlot wines *(22)* due to the flavor complexity of this variety *(23)*.

IBMP is, together with certain sulfur-containing odorants like 4-mercapto-4-methylpentan-2-one, 3-mercaptohexyl acetate or 3-mercaptohexan-1-ol *(24-27)*, one of the most important varietal aromas of Sauvignon blanc *(7, 10, 28)*. A positive correlation between wine scores and MP levels was found for some Sauvignon blanc wines. However, to achieve a good wine quality, it is important that such varietal aroma is in balance with other sensory features. In fact, the presence of IBMP may provide flavor complexity and varietal character to the resulting Sauvignon blanc wines, being a positive quality factor, as long as it is not too dominant, but in balance and complemented by other herbaceous and fruity aromas *(7, 10, 29)*.

Grape ripening

The 'herbaceous-vegetative' aroma of Cabernet, Merlot noir and Sauvignon blanc grapes is known to decrease as grape maturity increases *(14, 21)*. In agreement with this sensory perception, it has been found that MP levels declined progressively and rapidly with grape maturity in these varieties *(15, 23, 30)*. Finally, once maturity is reached, IBMP levels remain consistent *(22)*. As a consequence of these changes in concentrations throughout the ripening process, a very wide range of MP levels can be found in grapes. One of the major factors influencing IBMP contents in wines is grape maturity, which might vary with climatic conditions year by year: samples made from well-ripened grapes tend to have lower IBMP contents *(31)*. A harvest delay of 15 days can diminish significantly the concentration of IBMP *(32)*.

Although contents of both IPMP and IBMP increase in the early developmental stage, thereafter IPMP decreases before veraison, while IBMP decreases rapidly after veraison *(33)* (Table 2). Then, the major decrease in IBMP contents happens mainly at the initial stages of grape maturation. Finally, as grapes ripen, a very good correlation between the breakdown of malic acid and IBMP has been observed *(8, 15)*.

Light exposure seems to have two opposite effects on the concentration of MPs in grapes: (a) promoting the biological formation of MPs in immature grapes, and (b) photo-decomposing the MPs in ripening grapes. The actual contents of MPs in grapes would be the result of a balance between these two opposite effects *(33-34)*.

Climate and soil

Variations in MP contents due to differences in climatic conditions, region of origin and vintage are difficult to isolate, because all these factors are connected and actually all of them have to do with macro, meso and microclimatic conditions. However, it is generally acknowledged that wines from colder regions or vintages tend to exhibit a more pronounced 'vegetative-herbaceous' aroma and contain higher amounts of MPs *(7-8, 10, 13, 15, 20, 22, 30, 35-36)*. This might be due to the increased vine vigor and canopy shading generated by better water availability and greater soil fertility *(8, 13, 15, 37)*.

The influence of factors like vine vigor, pruning and leaf removal, all of them determining fruit exposure to sunlight, are of especial interest because they can be manipulated to control the concentrations of these aromas. Climate and viticultural practices can have an influence on the decrease of IBMP contents throughout grape ripening and the final concentration reached by this compound at harvest *(8, 21)*. MP concentrations decrease more under increased sunlight and temperature conditions *(7)*.

Ripening temperature has a strong influence in IBMP levels in wines *(10, 30)*. Cool ripening conditions can lead to higher MP levels and therefore to enhanced 'vegetative' aromas *(7, 13, 15, 19, 31, 38)*. Ripening temperature seems to have a greater effect on IBMP levels than on sugar accumulation, leading to a lower IBMP concentration in the warmer year than in the cooler year at comparable stages of sugar accumulation. This means that, in relatively warm areas, MP levels might fall below the aroma threshold before the end of grape maturity *(13, 15)*. A high humidity level, particularly in the pre-veraison month, can cause higher IBMP contents *(8, 22, 33)*. Increased canopy shading can increase the 'vegetative' aromas in Cabernet sauvignon *(10, 38-39)*.

Differences in water holding capacity, draining and nutritional richness of the soil can determine vine vigor, which in turn affects the sunlight available to the grape and IBMP contents. Wines with higher 'vegetative' aromas have been associated to the deep, clay-rich soils that are nutrient rich and have a high water holding capacity. On the other hand, fruitier wines, richer in berry aromas are linked to shallow, sandy soils that are nutrient poor and have a lower water holding capacity *(20)*. Finally, Cabernet sauvignon grapes from vineyards grown on sandy-silt soils can contain higher IBMP amounts than grapes from gravel soils *(8)*.

Viticultural Practices

It has been proven by sensory evaluation that leaf removal (prior to veraison) of Sauvignon blanc vines decreased the 'herbaceous' character of the resulting wine *(10)*. MP concentrations can decrease with an increase in sunlight exposure obtained by a reduction in the number of leaf layers *(38)*. Severe leaf removal seems to be most effective at reducing the vegetal character of Sauvignon blanc wine, and earlier treatments would be more effective than later treatments *(17)*.

Different pruning techniques may cause significant differences in IBMP levels *(30, 38)*. This might be explained by the influence the pruning system has upon fruit exposure to sunlight. Minimal pruned vines can produce grapes with eight-fold lower concentrations than fruits from spur-pruned vines. This can be explained by the fact that this pruning system provides a relatively loose and open canopy, with many well-exposed small clusters of small-berried fruit *(30)*.

Winemaking

Skin, pulp and bunchstems contain more MPs than the corresponding grape juices *(31, 40-41)*. Consequently, pressing technique can have a very important influence on MP contents in wines. Press wines are known to present a stronger 'herbaceous' character and higher IBMP amounts *(19, 32, 41)*. The addition of stems to the vinification process can increase the levels of IPMP, SBMP and IBMP in the final wines, particularly in the case of this last compound.

Table 2. Reported contents of IBMP, IPMP and SBMP (averages, ng/L) in grapes.

Variety	Origin	Stage	HY	MTV	IBMP	IPMP	SBMP	Ref.
Sauvignon blanc	Australia				0.5-79			*(30)*
	Australia			19.8	8 - 16			*(30)*
	Australia			23.9	< 3			*(30)*
	Japan	30a	1997		101.2	48.7		*(33)*
	Australia	e	1987	22.2	35.4	1.1		*(15)*
	Australia	e	1988	27.0	30.7	1.6		*(15)*
	Australia	e	1987	19.4	78.5	6.8	0.6	*(15)*
	Australia	e	1987	20.2	8.6	0.6		*(15)*
	Australia	e	1987	19.6	12.1	0.6	0.1	*(15)*
	Australia	l	1987	22.2	1.3	1.0		*(15)*
	Australia	l	1988	27.0	0.6	0.3		*(15)*
	Australia	l	1987	19.4	13.4	1.3		*(15)*
	Australia	l	1987	20.2	11.5	0.5		*(15)*
	Australia	l	1987	19.6	9.5	0.5	0.5	*(15)*
	Japan	h	1997		0.5	0.2		*(33)*
Cabernet sauvignon	*Australia*			23.9	0.5-189			*(30)*
	Japan	10a	1997		18.6	6.9		*(33)*
	Japan	30a	1997		96.2	16.2		*(33)*
	Japan	50a	1997		143.0	2.7		*(33)*
	Japan	70a	1997		23.0	0.6		*(33)*
	Spain	v	1998	23.5	18.4 - 42.5	< 5.4	4.0 – 9.6	*(42)*
	France	e	1997		100			*(8)*
	France	e	1996		30-45			*(8)*
	Spain	e	1998	23.5	10.9 - 20.0	< 8.0	2.4 – 13.0	*(42)*
	Spain	m	1998	23.5	5.6 – 18.0	< 15.0	3.0 – 18.2	*(8)*
	France	l	1996		10 - 20			*(40)*
	Spain	l	1998	23.5	3.7 – 7.0	< 3.0	2.3 – 4.6	*(33)*
	Japan	h	1995		17.5			*(42)*
	Japan	h	1997		1.5	< 0.2		*(22)*
	Spain	h	1998	23.5	< 3.9	< 4.7	< 4.1	*(42)*
	France	must	1996		4-20			*(33)*
	Spain	must	1998	23.5	4.3-10.0	< 2.8	< 3.4	*(8)*
Merlot noir	*Japan*	30a	1997		*227.3*	15.4		*(42)*
	France	e	1997		60			*(42)*
	Spain	e	1998	23.5	*21.7*	15.8	11.2	*(8)*
	Spain	l	1998	23.5	*10.4*	10.7	6.3	*(42)*
	France	l	1997		20			*(33)*
	Spain	h	1998	23.5	*5.6*	2.7	2.1	*(22)*
	Japan	h	1997		*0.9*	< 0.2		*(42)*
	France	must	1996		*5-12.9*			*(33)*
	Spain	must	1998	23.5	*19.4*	18.1	8.2	*(33)*
Pinot noir	*Japan*	30a	1997		*1.4*	0.4 ± 0.2		*(33)*
	Japan	h	1997		*< 0.2*	< 0.2		*(33)*
Muscat Baley A	*Japan*	30a	1997		0.3	<0.2		*(33)*
	Japan	h	1997		< 0.2	< 0.2		*(33)*
Semillon	*Japan*	30a	1997		307.0	40.3		*(33)*
	Japan	h	1997		2.6	0.3		*(33)*
Chardonnay	*Japan*	30a	1997		33.3	2.3		*(33)*
	Japan	h	1995		5.3			*(33)*
	Japan	h	1997		0.2	<0.2		*(33)*
Riesling	*Japan*	30a	1997		54.7	6.4		*(30)*
	Japan	h	1997		0.3	<0.2		*(30)*

HY: Harvest Year; MTV: Mean temperature at the month of veraison (°C)

10a, 30a, 50a and 70a: 10, 30, 50 and 70 days after anthesis, respectively; v: veraison; e: grape ripening, early; m: grape ripening, middle; l: grape ripening, late; h: harvest

Table 3. Reported contents of IBMP, IPMP and SBMP (averages, ng/L) in wines.

Variety	Origin	HY	MTV	IBMP	IPMP	SBMP	Ref.
Sauvignon blanc				18	2	< 1	[02]
	Australia		19.4-27.0	6.8	1.3	< 1	[18]
	Australia & New Zealand	1984/85		4.7			[01]
	France			6-38	2-4	0-2	[18]
	New Zealand			25.9	4.4		[18]
	New Zealand	1985		35	< 6	< 0.5	[09]
Cabernet sauvignon	Australia		18.0	3.6 – 56.3			[02, 03, 05]
	France			5-30			[19]
	France	1995		11 – 15.4			[16, 19]
	France	1996		8-19			[16]
	Japan	1975-88		5-28			[11]
	Japan	1989	23.5	17			[11]
	Japan	1990	23.3	4			[11]
	Japan	1991	23.2	10			[11]
	Japan	1992	22.6	9			[11]
	Japan	1993	21.7	35			[11]
	Japan	1994	23.6	3			[11]
	Japan	1995		25.3			[25]
	Japan	1996		6.5	0.2		[12]
	Spain	1998	23.5	3.4 – 15.3	< 1	< 5.8	[20-23]
> 80% Cabernet sauvignon	Australia		22.7	3.6			[03, 05]
	Australia		23.6	6.2 –7.6			[03, 05]
	Australia		21.2	9.1			[03, 05]
	Australia		19.8	11.2			[03, 05]
	Australia		20.0	12.3			[03, 05]
	Australia		22.7	17.1			[03, 05]
	Australia		18.0	26.1			[03, 05]
	New Zealand		17.9-18.8	27.6 - 28.6			[03, 05]
Merlot noir	France			4-23			[19]
	France			5.1-10.8			[13, 14, 17]
	France	1993		9-16			[13, 14, 17]
	France	1994		6.5-13			[13, 14, 17]
	France	1995		3.7 – 7.5			[16]
	France	1996		*3-16*			[16]
	France	1996		6-12			[15, 16]
	Japan	1989-1994		11			[11]
	Japan	1996		0.8	0.2-0.3		[12]
	Spain	1998	23.5	*25.2-27.3*	3.9 - 4.5	9.8 - 10.3	[20-21]
Cabernet franc	France			6-34			[19]
	France			8.5-12.1			[13, 14, 17]
	France	1995		4-5			[16]
	France	1996		*2-11*			[16]
	Japan	1987-1992		14			[11]
Pinot noir	France	1983		<0.7		0.12 - 0.26	[03]
Muscat Baley A	Japan	1989-1994		1			[11]
Chardonnay	Japan	1995		11.6			[25]
Zweigeltrebe	Japan	1983-1991		3			[11]
Xynomavro	Greece	1995		15-33			[13, 14, 17]

HY: Harvest Year; MTV: Mean temperature at the month of veraison (°C)

Grape cluster stems can be present during vinification due to incomplete stemming. Consequently, care must be taken since these vegetative aromas, particularly IBMP can be enhanced by the vinification technique *(31, 40)*.

Changes in MP levels take place through the fermentation process. MP concentrations in freshly fermented red wines of MP containing varieties are higher (in some cases, twofold and threefold) than the corresponding grape juice prior to fermentation *(13, 23, 31, 42-43)*. MPs in Cabernet sauvignon red wines do not only derive from the pulp but also from other parts of the grape berries *(31)*. If the same vinification process is followed, IBMP levels in red wines depend only on the levels of IBMP in their corresponding grapes. They would pass to wine due to maceration and, if the contents in grapes were known, the final levels of IBMP in wines might be predictable *(22)*. Maceration duration has an important influence in the MP contents in red wines *(32)*. IBMP levels in wines obtained by fermentation under pressure have been found to be lower than the ones obtained with the traditional technique *(44)*.

Other factors

MPs are sensitive to photo-degradation *(34)*. Light conditions, as well as the color of bottles may determine MPs levels in wines after a period of storage or aging. MP concentrations decrease more rapidly in the presence of light over 12 months' storage in clear glass bottles (up to 60%) than in green and amber colored bottles (up to 40%). MP contents are stable in bottles stored for 12 months in the darkness, regardless of glass color *(3)*.

IPMP can be produced by some microorganisms *(45)*, sometimes in relatively high concentrations *(46)*. Within a batch of wine bottles, one particular bottle of wine was found to contain a very high amount of IPMP (9.7 ng/L). It has been proposed that bottle-specific microbial contamination (perhaps linked to the cork) might be responsible for such bottle variation in IPMP contents *(23)*.

Wines from grapes infected with *Botrytis cinerea* have been found to contain high IBMP levels and it has been suggested that the fungus would shrink the grapes, their skin would be more fragile and IBMP extraction from the skins during fermentation would be increased *(22)*.

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Literature cited

(01) Gallois, A. Pyrazines in Food: A review. *Sci. Aliments.* **1984**, *4*, 145-146.

(02) Maga, J. A. Pyrazines in foods: an update. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 1-48.

(03) Maga, J. A. Sensory and stability properties of added methoxypyrazines to model and authentic wines. In *Flavours and Off-Flavours*, Proceedings of the 6th International Flavor Conference; Charalambous, G. Elsevier, Amsterdam, **1990**, 61-70.

(04) Maga, J. A. Pyrazine update. *Food Rev. Int.* **1992**, *8*, 479-558.

(05) Shibamoto, T. Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.* **1980**, *28*, 237-243.

(06) Rizzi, G. P. The biogenesis of food-related pyrazines. *Food Rev. Int.* **1988**, *4*, 375-400.

(07) Marais, J. Sauvignon blanc cultivar aroma - a review. *S. Afr. J. Enol. Vitic.* **1994,** *15*, 41-45.

(08) Roujou-de-Boubée, D.; Leeuwen, C. Van; Dubordieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on Red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830-4834.

(09) Boelens, M.H.; Gemert, L.J. Structure-activity relationships of natural volatile nitrogen compounds. *Perf. Flav.* **1995**, *20*, 63-76.

(10) Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Sauvignon Blanc varietal aroma. *Austr. Grapegr. Winem.* **1988**, *292*, 51-56.

(11) Mihara, S; Masuda, H. Structure-Odor Relationships for Disubstituted Pyrazines. *J. Agric. Food Chem.* **1988**, *36*, 1242-1247.

(12) Mihara, S.; Masuda, H.; Tateba, H.; Tuda, T. Olfactive properties of 3-substituted 5-alkyl-2-methylpyrazines. *J. Agric. Food Chem.* **1988**, *39*, 1262-1264.

(13) Allen, M. S.; Lacey, M. J.; Boyd, S. Determination of Metoxypyrazines in Red Wines by Stable Isotope Dilution Gas Chromatography-Mass Spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734-1738.

(14) Augustyn, O. P. H.; Rapp, A.; Wyk, J. Some Volatile Aroma Components of *Vitis vinifera* L. cv. Sauvignon blanc *S. Afr. J. Enol. Vitic.* **1982**, *3*, 53-60.

(15) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V. Methoxypyrazines in Sauvignon blanc Grapes and Wines. *Am. J. Enol. Vitic.* **1991**, *42*, 109-112.

(16) Harris, R. L. N.; Lacey, M. J.; Brown, W. V.; Allen, M. S. Determination of 2-methoxi-3-alkylpyrazines in wine by gas chromatography / mass spectrometry. *Vitis.* **1987**, *26*, 201-207.

(17) Arnold, R.A.; Bledsoe, A.M. The effect of various leaf removal treatments on the aroma and flavor of Sauvignon blanc wine. *Am. J. Enol. Vitic.* **1990**, *41*, 74-76.

(18) Colagrande, O.; Fumi, M. D. The use of cork in closing bottles: a scientific survey. *Ind. Bevande.* **1993**, *22*, 393-409.

(19) Kotseridis, Y.; Anocibar, A.; Bertrand, A.; Doazan, J. P. An Analytical Method for Studying the Volatile Compounds of Merlot noir Clone Wines. *Am. J. Enol. Vitic.* **1998**, *49*, 44-48.

(20) Noble, A.C.; ElliottFisk, D.L.; Allen, M.S. Vegetative flavor and methoypyrazines in Cabernet Sauvignon. *Fruit Flavors.* **1995**, *596*, 226-234.

(21) Roujou-de-Boubee, D. The 'plant' aroma characteristic of wines. *Vigne Vin.* **1999**, *33*, 145-146.

(22) Kotseridis, Y.; Baumes, R. L.; Bertrand, A.; Skouroumounis, G. K. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chromatogr. A.* **1999**, *841*, 229-237.

(23) Allen, M. S.; Lacey, M. J.; Boyd, S. Methoxypyrazines in red wines: occurrence of 2-methoxy-3-(1-methylethyl) pyrazine. *J. Agric. Food Chem.* **1995**, *43*, 769-772.

(24) Tominaga, T.; Peyrot des Grachons, C.; Dubordieu, D. A new type of flavor precursors in Vitis vinifera L. Cv. Sauvignon blanc: s-Cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215-5219.

(25) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubordieu, D. A powerful aromatic volatile thiol, 2-furanethiol, exhibiting roast coffee aroma in wines made from several Vitis vinifera Grape varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799-1802.

(26) Tominaga, T.; Guimbertau, G.; Dubordieu, D. Contribution of benzenemethanethiol to smoky aroma of certain Vitis vinifera L. wines. *J. Agric. Food Chem.* **2003**, *51*, 1373-1376.

(27) Schneider, R.; Kotseridis, Y.; Ray, J-L; Augier, C.; Baumes, R. Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.* **2003**, *51*, 3243-3248

(28) Hashizume, K.; Tozawa, K.; Endo, M.; Aramaki, I. S-Adenosyl-L-methionine-dependent O-Mehylation of 2-Hydroxy-3-alkylpyrazine in Wine Grapes: a putative final step of methoxypyrazine biosynthesis. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 795-801.

(29) Allen, M. S. What level of methoxypyrazines is desired in red wines? The flavour perspective of the classic red wines of Bordeaux. *Austr. Grapegr. Winem.* **1995**, *381*, 7-9.

(30) Allen, M. S.; Lacey, M. J. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Wein-Wissenschaft.* **1993**, *48*, 211-213.

(31) Hashizume, K.; Umeda, N. Methoxypyrazine content of Japanese red wines. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 802-805.

(32)* Kotseridis, Y.; Anocibar-Beloqui, A.; Bayonove, Cl.; Baumes, R. L.; Bertrand, A. Effects of selected viticultural and enological factors on levels of 2-methoxy-3-isobutylpyrazine in wines. *J. Int. Sci. Vigne Vin. 1999, ***33*, 19-23.**

(33) Hashizume, K.; Samuta, T. Grape maturity and light exposure affect berry methoxypyrazine concentration. *Am. J. Enol. Vitic.* **1999**, *50*, 194-198.

(34) Heymann, H.; Noble, A. C.; Boulton, R. B. Analysis of Methoxypyrazines in Wines. 1. Development of a Quantitative Procedure. *J. Agric. Food Chem.* **1986**, *34*, 268-271.

(35) Slingsby, R. W.; Kepner, R. E.; Muller, C. J.; Webb, A. D. Some volatile components of *Vitis vinifera* variety Cabernet sauvignon wine. *Am. J. Enol. Vitic.* **1980**, *31*, 360-363.

(36) Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet sauvignon wines from California. Am. J. Enol. Vitic. **1987**, *3*, 41-44.

(37) Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Contribution of Methoxypyrazines to Sauvignon blanc Wine Aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109-112.

(38) Allen, M. S. Viticultural effects on methoxypyrazine grape flavour: current research directions. *Austr. Grapegr. Winem.* **1993**, *354*, 10-12.

(39) Morrison, J. C.; Noble, A.C. The effects of leaf and cluster shading on the composition of Cabernet sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* **1990**, *41*, 193-200.

(40) Hashizume, K.; Samuta, T. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. *J. Agric. Food Chem.* **1997**, *45*, 1333-1337.

(41) Roujou-de-Boubee, D.; Cumsille, A.M.; Pons, D. Dubordieu, D. Location of 2-methoxy-3-isobutylpirazine in Cabernet sauvignon bunches and its extractability during vinification. Am. J. Enol. Vitic. **2002**, *53*, 1-5.

(42) Sala, C.; Mestres, M.; Martí, M.P.; Busto, O.; Guasch, J. Headspace solid-phase microextraction method for determining 3-alkyl-2-methoxypyrazines in musts by means of polydimethylsiloxane-divinylbenzene fibres. *J. Chromatogr. A.* **2000**, *880*, 93-99.

(43) Sala, C.; Mestres, M.; Martí, M.P.; Busto, O.; Guasch, J. Headspace solid-phase microextraction analysis of 3-alkyl-2-methoxypyrazines in wines. *J. Chromatogr. A.* **2002,** *953*, 1-6.

(44) Kotseridis, Y.; Anocibar, A.; Bertrand, A. 3-Isobutyl-2-methoxypyrazine, responsible of vegetative aroma in wines, viticultural and winemaking factors. In: In vino Analytica Scientia, First Symposium, Bordeaux, **1997**, 12-14 June.

(45) Leete, E.; Bjorklund, J. A.; Reineccius, G. A.; Cheng, T. B. *Biosynthesis of 3-Isopropyl-2-methoxypyrazine and other alkylpyrazines: widely distributed flavour compounds*. Bioformation of flavours, Patterson, R.L.S., Charlwood, B. V., MacLeod, G. and Williams, A. A, Eds.; Royal Society of Chemistry, Cambridge, U. K, **1992,** 75-95.

(46) Gallois, A.; Kergomard, A.; Adda, J. Study of the Biosynthesis of 3-Isopropyl-2-Methoxypyrazine Produced by Pseudomonas taetrolens. *Food Chem.* **1988**, *28*, 299-309.

(47) Masuda, H; Mihara, S. Olfactive Properties of Alkylpyrazines and 3-Substitued 2-Alkylpyrazines. *J. Agric. Food Chem.* **1988**, *36*, 584-587.

(48) Seifert, R. M.; Buttery, R.G.; Guadagni, D.G.; Black, D.R.; Harris, J.G. Synthesis of Some 2-Methoxy-3-Alkylpyrazines with Strong Bell Pepper-Like Odors. *J. Agric. Food Chem.* **1970**, *18*, 246-249.

(49) Parliament, T.H.; Epstein, M.F. Organoleptic properties of some alkyl-substituted alkoxy- and alkylthiopyrazines. *J. Agric. Food Chem.* **1973**, *21*, 714-716.

(50) Buttery, R. G.; Seifert, R.M.; Ling, L. C. Characterization of some volatile potato components. *J. Agr. Food Chem.* **1970**, *18*, 538-539.

(51) Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of Arabica Coffee. *Z. Lebensm Unters Forsch.* **1992**, *195*, 239-245.

(52) Murray, K. E.; Shipton, J.; Whitfield, F. B. 2-Methoxypyrazines and the flavour of green peas (*Pisum sativum*). *Chem. Ind.* **1970**, *7*, 897-898.

(53) Murray, K. E.; Whitfield, F. B. The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973-986.

(54) Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of Some Volatile Constituents of Bell Peppers. *J. Agr. Food Chem.* **1969**, *17*, 1322-1327.

(55) Takken, H. J.; van der Linde, L. M.; Boelens, M.; van Dort, J. M. Olfactive Properties of a Number of Polysubstituted Pyrazines. *J. Agric. Food Chem.* **1975**, *23*, 638-642.

(56) Hashizume, K.; Kida, S.; Samuta, T. Effect of steam treatment of grape cluster stems on the methoxypyrazine, phenolic, acid, and mineral content in red wines fermented with stems. *J. Agric. Food Chem.* **1998**, *46*, 4382-4386.

(57) Kotseridis, Y.; Baumes, R.; Skouroumounis, G. K. Synthesis of labelled [2H4] β-damascenone, [2H2] 2-methoxy-3-isobutylpyrazine, [2H3] α-ionone, and [2H3] β-ionone, for quantification in grapes, juices and wines. *J. Chromatogr. A.* **1998**, *824*, 71-78.