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## Review

# Monoterpenes in grape juice and wines

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### Abstract

The importance of monoterpenes on varietal flavour of wines has been reviewed. These compounds were mainly found linked to sugar moieties in the grape juice and wines, showing no olfactive characteristics. In this way, mechanisms to liberate terpenes were studied, making a comparative study between acidic and enzymic hydrolysis of terpene glycosides. Finally, analytical techniques developed to study these compounds, in both free or glycosidically forms, and also to fractionate glycosidic precursors, have been discussed. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Reviews; Wine; Fruit juices; Food analysis; Monoterpenes; Terpenes; Glycosides

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## 1. Introduction

It has emerged from numerous studies that the terpenoid compounds form the axis for the sensory expression of the wine bouquet which is typical of its variety and that they can therefore be used ana-

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lytically for varietal characterization. Apart from the hitherto known compounds (terpene ethers, monoterpene alcohols) [1–5] numerous monoterpene compounds, in particular monoterpene diols, in grape must and wine, has been identified. At present, about 50 monoterpene compounds are known of which the most important are shown in Fig. 1. The dominating monoterpene alcohols, particularly from Muscat varieties, are linalool, geraniol, nerol, citronellol,  $\alpha$ -terpineol. In the case of koshu, an indigenous Japanese variety, terpinene-4-ol was identified as the dominating monoterpene compound [6]. The flavour threshold of nerol and  $\alpha$ -terpineol is three to four times higher than that of linalool (100  $\mu\text{g}/\text{l}$ ). The linalool oxides have flavour thresholds of 3000–5000  $\mu\text{g}/\text{l}$  [7].

Terpene compounds belong to the secondary plant constituents, of which the biosynthesis begins with acetyl-coenzyme A (CoA) [8]. Microorganisms are also able to synthesize terpene compounds [9] but the formation of terpenes by *Saccharomyces cerevisiae* has not yet been observed. Terpenes are not changed by the yeasts metabolism during fermentation [7]. Several authors have shown that terpenes play a significant role in the varietal flavour of wines and, in the berries, they are located in skin and linked to sugars [10–12].

### 1.1. Classification of grape varieties

A number of surveys have been made of monoterpene concentration in different grape varieties [11,13–16]. However, since the reported quantitative data were obtained by different techniques and from samples of fruit from diverse areas, direct comparison on the analytical figures from different surveys is not feasible. Nevertheless, a general classification of those varieties which have been screened is possible allowing division into (1) intensely flavoured muscats, in which total free monoterpene concentrations can be as high as 6 mg/l; (2) non-muscat but aromatic varieties with total monoterpene concentration of 1–4 mg/l; and (3) more neutral varieties not dependent upon terpenes for their flavour (Table 1).

Data for many of these assignments were made from a limited number of samples (often only one) and, in most cases, the fruit was taken from a single

viticultural region. The classification, however, indicates those cultivars for which analysis for monoterpenes are likely to give data which could be useful in an investigation of fruit varietal character, i.e., those listed in groups (1) and (2). In the cultivars listed under (3), monoterpenes are at such low concentration that these compounds could only play a minimal role in determining varietal flavour. It is from among the more numerous cultivars of group (3) that a large volume of the world's wine is produced.

### 1.2. Classification of monoterpenes

Three types of categories of monoterpenes exist in grapes with some interrelationships between the categories.

On the top of the complex are the free aroma compounds, commonly dominated by linalool, geraniol, and nerol, together with the pyran and furan forms of the linalool oxides. However, depending on how the juice has been treated and on factors, which may include climate, many additional monoterpenes can be found in this group, i.e. citronellol,  $\alpha$ -terpineol, ho-trienol, nerol oxide, myrcenol, the ocimenols plus several other oxides, aldehydes and hydrocarbons. In wines, several monoterpene ethyl ethers and acetate esters have also been found among the free aroma compounds.

Second, there are the polyhydroxylated forms of the monoterpenes, or free odourless polyols. A most significant feature of the polyols is that, although these compounds make no direct contribution to the aroma, some of them are reactive and can break down with great ease to give pleasant and potent volatiles, i.e. diendiol (3,7-dimethylocta-1,5-diene-3,7-diol) can give ho-trienol and nerol oxide [5].

Third, there are the glycosidically conjugated forms of the monoterpenes which also make no direct contribution to the aroma of the grape. Glycosides are, in most cases, more abundant than the unglycosilated forms of individual monoterpenes and polyols.

### 1.3. Quantitation of monoterpenes

The occurrence of terpene glycoside precursors in grapes was better demonstrated. Glycoside precur-

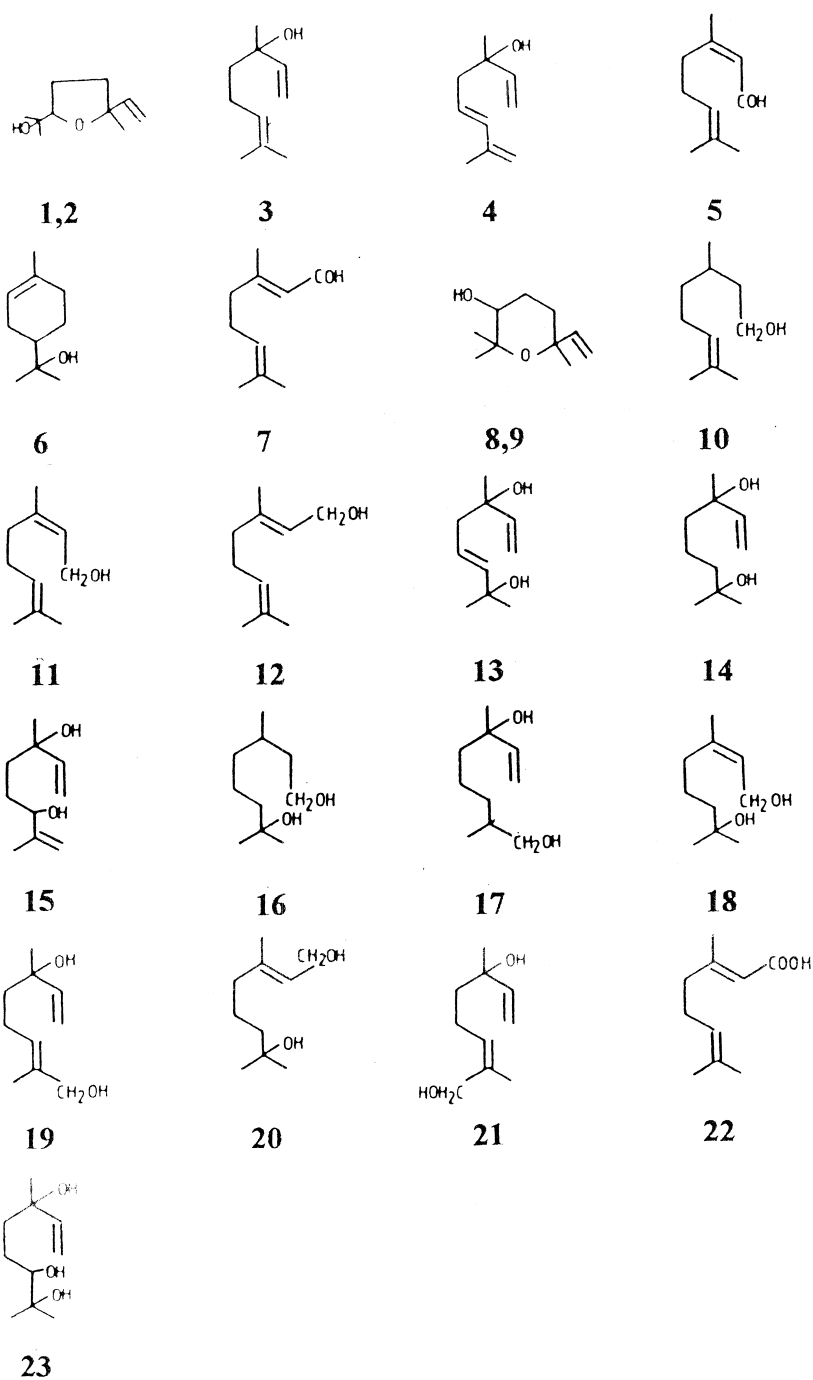


Fig. 1. Main monoterpene compounds in grape juice and wines. Numbers correspond to Table 2.

Table 1  
Classification of some grape varieties based on monoterpene content

(1) Muscat varieties	(2) Non-muscat aromatic varieties	(3) Neutral varieties
Canada Muscat	Traminer	Aryan
Gewurztraminer	Huxel	Bacchus
Muscat of Alexandria	Kerner	Bobal
Muscat de Frontignan	Morio-Muskat	Cabernet-Sauvignon
Muscato Bianco del Piemonte	Müller-Thurgau	Carignan
Muscat Hamburg	Riesling	Cencibel
Muscat Ottonel	Schurebe	Chardonnay
Moscato italiano	Schonburger	Chasselas
	Siegerebe	Chenin Blanc
	Sylvaner	Cinsault
	Wurzer	Clairette
		Dattier de Bevrouth
		Doradillo
		Forta
		Merlot
		Nobling
		Rkaziteli
		Ruländer
		Sauvignon Blanc
		Semillon
		Shiraz
		Sultana
		Terret
		Trebbiano
		Verdelho
		Viognier

sors are numerous and fairly abundant as, in flavourant grapes, they are evaluated between 6.5 and 28 mg/l juice. Most grape varieties contain free and bound glycoside terpenes but concentrations are higher in flavourant cultivars. In general, bound glycosides forms are more abundant than the free ones [11,16].

The sugar moieties were identified to rutinose (6-*O*- $\alpha$ -L-rhamnopyranosyl- $\beta$ -D-glucopyranoside), 6-*O*- $\alpha$ -L-arabinofuranosyl- $\beta$ -D-glucopyranosides, 6-*O*- $\beta$ -D-apiofuranosyl- $\beta$ -D-glucopyranosides or  $\beta$ -D-glucopyranosides [17–19]. The aglycon part is often formed with terpenols, but linalool oxides, terpene diols and triols can also be found. However, other flavour precursors can occur such as linear or cyclic alcohols, e.g. hexanol, phenylethanol, benzyl alcohol, C<sub>13</sub> norisoprenoids, phenolic acids and probably volatile phenols such as vanillin [20–22].

If we consider only glycosides with the most

flavourant aglycons (i.e., with the same state of oxidation than linalool) the most abundant are apiosylglycosides (up to 50% according to grape variety) followed by rutinoids (6–13%) and finally glucosides (4–9%). A more accurate analysis indicates that all glycosides are not present in all cultivars and that their proportions also differ according to grapes [23].

The glycoside flavour potential from grapes remain unfortunately quite stable during winemaking and in young wines as well.

## 2. Glycoside hydrolysis

Terpene glycosides can be hydrolysed by acids [24,25] or enzymes [10,11,17]; under the former conditions, rearrangements of the monoterpene may occur, whereas under the latter conditions the

changes in the natural monoterpenol distribution are minimal.

### 2.1. Acidic hydrolysis

Experiments on both whole juice and monoterpene glycosides isolated from juice [26,27] have demonstrated that significantly different patterns of volatile monoterpenes are produced when each is hydrolysed at different pH values. Furthermore, there appears to be a pH-dependent interrelationship between several of the grape monoterpenes. Thus e.g. the isomeric ocimenols appear to be formed hydrolytically in juice at pH 1 at the expense of linalool, nerol and geraniol, the last three compounds being pH 3 products [24].

For acidic hydrolysis, samples, with no free volatile compound, were dissolved in a buffer at acidic pH and solutions were heated and resulting free volatile compounds extracted and analysed by GC [24,25,27–29].

Acidic hydrolysis of terpene glycosides can provoke a molecular rearrangement of the monoterpenols, which were transformed in other compounds. Nevertheless, this way to liberate terpenes simulate the reactions which takes place during ageing of wines and the different terpenic alcohols were produced in similar quantitative rations.

### 2.2. Enzymic hydrolysis

To enrich wine flavour by release of free aromatic compounds from natural glycoside precursors, particularly pathways are required. Enzymic hydrolysis was most interesting because it produces a more “natural” flavour in the wine [10,11,29]. Enzymic hydrolysis of glycosides is carried out with various enzymes which act sequentially according to two steps: firstly,  $\alpha$ -L-rhamnosidase,  $\alpha$ -L-arabinosidase or  $\beta$ -D-apiosidase make the cleavage of the terminal sugar and rhamnose, arabinose or apiose and the corresponding  $\beta$ -D-glucosides are released; subsequently liberation of monoterpenol takes place after action of a  $\beta$ -D-glucosidase [30].

Enzymic hydrolysis of glycoside extracts from Muscat, Riesling, Semillon, Chardonnay, Sauvignon and Sirah varieties have provoked the liberation not only of terpenes, but also C<sub>13</sub> norisoprenoids, such

as 3-oxo- $\alpha$ -ionol and 3-hydroxy- $\beta$ -damascenona [31]. These compounds are totally glycosilated in the grape and, opposite with terpenes, they are found in the same quantities in all the grape varieties, aromatics or neutral, and they are capable of awarding certain typicity to the wine flavour because they have lower threshold values than terpenes and they contribute characteristic aromatic features [32].

#### 2.2.1. Grape glycosidases

The potential use of enzymic systems of grapes to liberate terpenes from grape juices or wines has been always the subject of different works regarding the enzymic hydrolysis of terpene glycosides.

Grapes have an enzyme with  $\beta$ -glucosidase activity (Table 2) [31,33–37] but only low  $\alpha$ -rhamnosidase,  $\alpha$ -arabinosidase or  $\beta$ -apiosidase activities have been detected [31]. On the other hand, grape  $\beta$ -glucosidase was not quite stable and showed low activity at grape juice or wines pH values [38]. Similarly, certain  $\beta$ -glucosidasae activity has been observed in grape leaves [35–37].

In general,  $\beta$ -glucosidases with a vegetal origin show a low activity on monoglucosides of terpenes with a tertiary alcohol group (linalool,  $\alpha$ -terpineol), and they are only capable to hydrolyse monoglucosides of terpenes with a primary alcohol group (geraniol, nerol, citronellol) [34,36,39].

Recently, some evidence has been obtained on the presence of an endoglycosidase in grape skins able to split the heterosidic linkage of disaccharide glycosides releasing disaccharide and aglycon; the enzyme was quite tolerant to glucose inhibition [40].

#### 2.2.2. Yeast glycosidases

Regarding yeast  $\beta$ -glucosidases, yeasts of the *Hansenula* species isolated from fermenting must were reported to have an inducible  $\beta$ -glucosidase activity, but this enzyme was inhibited by glucose [41]. Among other yeast strains,  $\beta$ -glucosidases from *Candida molischiana* [42] and *C. wickerhamii* [43] also possess activities towards various  $\beta$ -glucosides and they were little influenced by the nature of aglycon [44].

Finally, the situation regarding *Saccharomyces cerevisiae* is more complex because this yeast is capable of modifying the terpenic profile of the wine; so, it can produce citronellol from geraniol and nerol,

Table 2

Results obtained by hydrolysis of terpene glycosides treated with different enzymatic preparations. Data are normalized to 100 in untreated wines

No.	Terpenes	Grape skin	<i>S. cerevisiae</i> glucosidase	Exogenous glycosidases				
				Klerzyme 200	Pectinol C	Rohapect C	Hemicellulase	
							Sweet wine	Dry wine
1	<i>trans</i> -Furan linalool oxide	108.2	122.9	247.3	174.3	943.1	109.7	129.7
2	<i>cis</i> -Furan linalool oxide	124.3	296.8	149.2	133.8	456.1	87.0	117.6
3	Linalool	101.2	204.6	113.6	111.0	187.8	107.5	108.8
4	Ho trienol			93.3		113.2	130.8	87.1
5	Neral							
6	$\alpha$ -Terpineol	230.0	150.8		190.8	113.8	141.7	
7	Geraniol							
8	<i>trans</i> -Pyran linalool oxide	124.9		109.3	109.1	113.1	104.2	104.4
9	<i>cis</i> -Pyran linalool oxide	104.5		88.9		136.3	111.4	98.0
10	Citronellol	177.6	176.6	551.9	239.8	131.8	146.5	335.4
11	Nerol	593.0	847.6	424.5	720.0	1173.2	190.6	642.0
12	Geraniol	381.9	833.4	491.8	633.2	806.2	107.0	359.1
13	Diol I	104.2	91.4	151.9	112.7	152.3	113.8	98.0
14	Endiol		133.0					
15	Diol II	101.4	12.5	174.7	114.0	124.3	100.1	97.6
16	Hydroxy-cityronellol	152.6	159.0		178.4	552.9		
17	8-Hydroxydihydrolinalool	275.9	230.8	152.9	397.6		105.9	93.2
18	Hydroxynerol		141.4					
19	<i>trans</i> -8-Hydroxylinalool	133.4	466.0	1343.0	431.5			
20	Hydroxygeraniol		140.2			475.0		
21	<i>cis</i> -8-Hydroxylinalool	271.8	688.9	4083.0	990.0	916.9		
22	Geranic acid	362.6	522.8		410.2			
23	Triol							

the intensity of this transformation depends on the yeast strain used [45]. Other authors propose a more complex scheme: geraniol was transformed by these yeasts into geranyl acetate, citronellyl acetate and citronellol, while nerol was transformed into neryl acetate; in addition, geraniol was transformed into linalool and nerol was cyclized to  $\alpha$ -terpineol at must pH [46].

Few data are available regarding glycosidase activities of enological yeast strains and the technological properties of the enzymes. Last published results [47] have detected  $\beta$ -glucosidase activity in different *Saccharomyces* strains on the basis of its hydrolytic activity on *para*-nitrophenyl- $\beta$ -D-glucoside (pNPG) and terpene glycosides of Muscat juice (Table 2); it is quite glucose independent but is inhibited by ethanol. These results could open new pathways regarding other glycosidase activities in *S. cerevisiae*;  $\alpha$ -rhamnosidase,  $\alpha$ -arabinosidase or  $\beta$ -apiosidase activities could be induced in wine yeast

by changing the composition of the medium including inductive compounds, as well as in filamentous fungi [48,49].

### 2.2.3. Exogenous (fungal) glycosidases

Taking into account that enzymic systems of grapes are not suitable to hydrolyse terpene glycosides in grape juice or wine and that more studies are needed regarding the ability of *S. cerevisiae* to produce all the enzymes which take part in this process, several exogenous enzymes, mainly with a fungal origin, have been developed to liberate terpenes in wines.

The most suitable enzymic preparations to be used during winemaking process are those which possess all  $\beta$ -D-glucopyranosidase,  $\alpha$ -L-arabinofuranosidase,  $\alpha$ -L-rhamnopyranosidase and  $\beta$ -D-apiofuranosidase activities (Table 2) [39]. These enzymes are present only in low quantities in the majority of commercial fungal enzymic preparations, mainly regarding  $\beta$ -D-

apiosidase activity [50]. Determination of free volatile compound terpenols, norisoprenoids and volatile phenols indicate that concentration in enzyme treated wines highly increase, not only in aromatic varieties, but also in neutral ones, from 265 to 2000% (Table 2) [31].

The ability of purified enzymes, synthetic substrates and suitable analytical methods are needed for the rigorous progress in the field of enzymic hydrolysis of terpenyl glycosides [11,19,30,51–54]. Enzymes have been isolated from fungal enzymic preparations, vegetal extracts or synthetic culture media inoculated with fungal cultures (mainly *Aspergillus niger*), and have been purified by different chromatographic techniques (gel filtration, ion-exchange chromatography, affinity chromatography, chromatofocusing) [31,49,55–57].

The method used to actually enrich wine in volatile compounds is only effective when dry wines are used, because  $\beta$ -glucosidase in fungal enzymic preparations is hardly inhibited by glucose; so, the hydrolysis of terpene glycosides is not completed in sweet wines obtained from Muscat de Frontignan grapes (Table 2) [31].

When enzymic preparations have been used to improve the aromatic characteristics of the wines, undesirable odours have been sometimes detected, even if liberation of terpenes has been produced, showing high concentrations in vinyl-phenols (4-vinyl-phenol, 4-vinyl-guayacol) [50].

On the other hand, fungal enzyme preparations generate oxidation artefacts during the hydrolysis of glycosides [58]; this fact implies that, perhaps, the review of all the information regarding the use of these enzymic preparations will be necessary.

### 3. Methodology

Glycosides of monoterpenes have been recognised as plant constituents and a variety of techniques have been used to isolate these hydrophylic compounds. Croteau and Martinkus [59], after lyophilizing aqueous leaf extracts, used TLC, ion-exchange chromatography and gel permeation chromatography to purify neomethyl- $\beta$ -D-glucoside. Francis and Allcock [60] isolated  $\beta$ -D-glucosides of geraniol, nerol and citronellol from aqueous extracts of rose petals by

solvent extraction followed by silica gel chromatography and preparative thin layer chromatography. Subsequently, Banthorpe and Mann [61] applied the same technique to petals of *Tanacetum vulgare*. Similarly other authors [62–64] extract plant material directly with organic solvents, after clean-up of the extracts on silica gel, conventional chromatographic procedures were used to isolate  $\beta$ -D-glucosides and derivatives of several terpenoids. Unfortunately, these techniques were either inappropriate or found to be unsuitable for the isolation of monoterpene glycosides from grape juice and wines. In the case of grape juice, the presence in aqueous solution of large amounts of glucose and fructose, together with other free sugars, makes the problem of small amounts of glycosides extremely difficult. On the other hand, the isolation from wines in which most of the sugars have been removed by fermentation is also complicated by the presence of glycerol and the isomeric 2,3-butanediols. Several techniques were tried unsuccessfully, including solvent extraction and subsequent TLC, gel filtration of both juice and wines and ion-exchange chromatography of juice on a column of cation exchanger in the calcium form [65] and on an anion exchanger in the bisulphite form [66,67].

#### 3.1. Isolation of glycosides

The isolation of glycosides from wines and juices by selective retention of the compounds on a solid-phase adsorbent is a commonly used technique. By washing the adsorbent with water following the adsorption step, free sugars and other polar constituents can be removed while the less polar glycosides are retained. Elution with an organic solvent then give the glycosidic fraction.

Different methodologies have been proposed to extract glycoside precursors from grape juices and wines.

Williams et al. [51] used glass column chromatography containing  $C_{18}$  reversed-phase adsorbent to extract glycosides from juice or dealcoholised wine. After washing with water and eluting free compounds with 20% aqueous acetic acid, precursors were eluted in two fractions with 30% aqueous acetic acid and methanol. A modification of the methodology has been proposed by using 1 g solid-phase

extraction C<sub>18</sub> cartridges; hydrophylic compounds were eluted with water, free terpenes with dichloromethane and glycosides with methanol [26]. This method has been improved in latter years, but it has a disadvantage because separation is different depending on the commercial origin of the cartridges [68].

A second approach to the problem has been proposed by Montpellier researchers by using Amberlite XAD-2 resin because it possesses an excellent capacity for adsorption of free terpenols from grape juice [11]. This resin had been previously used to isolate naringin and limonin from grape juices [69]. Once wine has been eluted through resin, free compounds were eluted with pentane and glycosides with ethyl acetate. Amberlite XAD-2 resin displays extraction capacities similar to those of coated octadecyl silica. Furthermore, it has the advantage of being sold in large particle sizes; it is thus possible to use it in a wide preparative column at atmospheric pressure. A modification of this method has been suggested and free compounds were eluted with pentane:dichloromethane to improve extraction [70]. Nevertheless, even with extensive washing of the adsorbent beds both Amberlite XAD-2 and Amberlite XAD-16 (the Amberlite XAD-16 resin has a surface area and capacity greater than that of Amberlite XAD-2) retained free glucose. Both of these adsorbents had the disadvantage of retaining free glucose in addition to the adsorbed glycosides [71].

Reversed-phase silica gel has been found to be a particularly suitable adsorbent for the isolation of glycosidic terpenes [72]. The commercial availability of this adsorbent in uniform, prepacked cartridges was an additional advantage in development of the isolation step.

More recently, a method has been proposed to extract glycosylated flavour precursors from grape juices by microwaves [73]. This method was compared to the method using a column of Amberlite XAD-2 resin. Although a further purification of the extracts was required, this method provided the advantages of rapidity, ease of extraction and possibility of extract berries without deseeding or crushing.

### 3.2. Analytical determination of monoterpenes

Investigations of the precursor compounds isolated

from must or wine, be they acidic [16,24], with the well-known drawback of terpene rearrangements, or enzymic [74,75] are all based on the analysis of monoterpenols released [76]. Since the monoterpenols must be first isolated from the hydrolysed sample, such methods require at least two steps preceding the final analysis by GC (Fig. 2). Furthermore, they elicit no information about the glycosidic conjugation of each monoterpene, allowing only gross characterization of the bound aroma fraction.

In order to achieve a method suitable for the direct and individual analysis of monoterpenyl glycosides, both GC and high-performance liquid chromatography (HPLC) have been investigated.

HPLC is a priori a well suited technique as the compounds of interest are not volatile. Liquid chromatography of monoterpenyl glycosides has already been considered for extraction and sample clean-up purposes, using adsorption on silica gel [60,64] Also, reversed-phase chromatography on C<sub>18</sub> bonded silica [51,76] and apolar resin copolymer [11] as well as combined gel permeation and hydrophobic interaction chromatography [77] have been investigated for the same purpose. HPLC has also been used for the chromatography of heavier glycosides, e.g. with diterpenyl [78,79] or triterpenyl [80] aglycones.

Among all the liquid chromatography methods studied, the best involved a reversed-phase HPLC system although good results were also obtained by using another apolar packing, a polystyrene–divinylbenzene copolymer [81].

GC is recognised as a highly efficient and resolute separation technique but it can only be applied to volatile derivatives of monoterpenyl glycosides. Comparing both techniques, similar results are obtained by HPLC techniques and by GC of trimethylsilyl derivatives of the monoterpenyl glycosides [52]. So, when severe chromatographic conditions are not considered as restrictive, GC turns out to be a good alternative to HPLC; however, as glycosides are not volatile, an additional derivatisation step is required.

The use of GC–MS for determination of synthetic glycosides can be a way to establish satisfactory conditions for the separation and identification of monoterpenyl glycosides, to facilitate detection of new glycosides through identification of characteristic fragmentation patterns and to determine individual glycosides and their aglycones [53]. Trifluoroacetylation derivatization proved to be more suitable



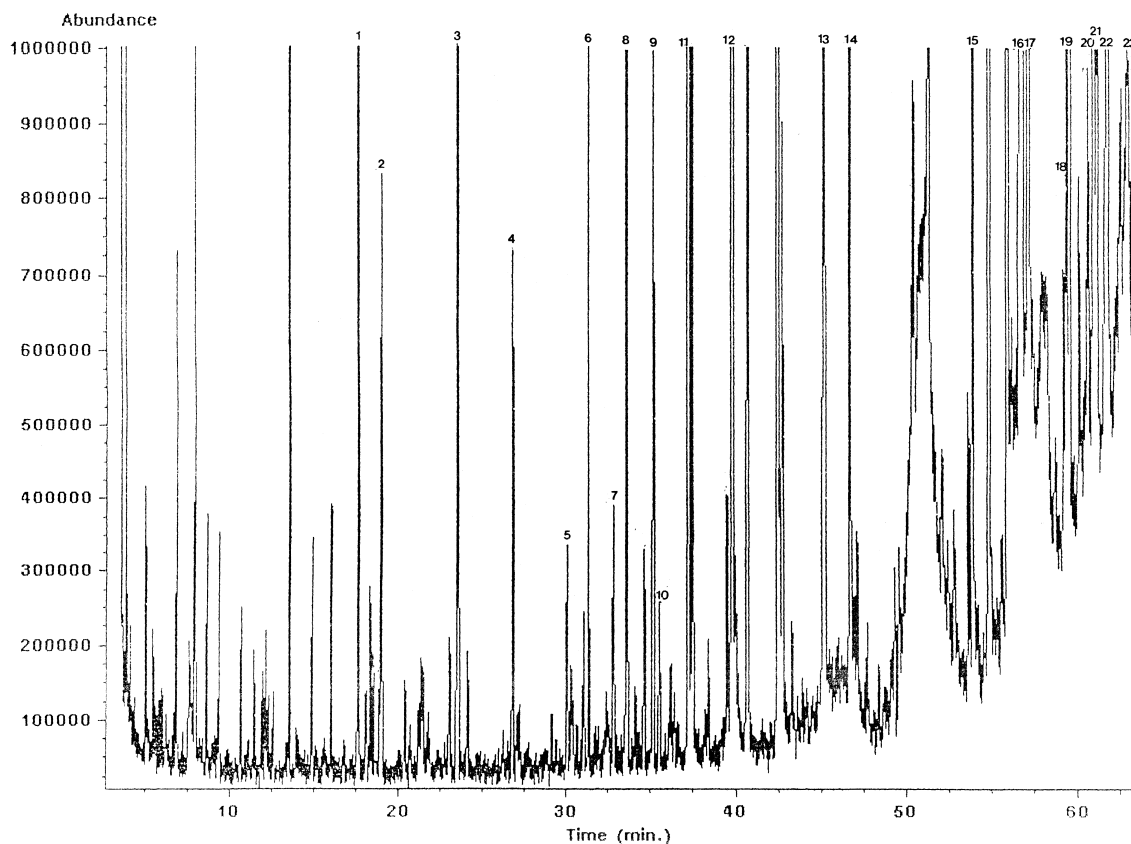


Fig. 2. GC-MS chromatogram of terpenes obtained by enzymic hydrolysis of glycosides. Peak numbers correspond to Table 2.

for the qualitative and quantitative analysis of mono-terpenyl diglycosides but trimethylsilylation, as well as HPLC methods, provided complementary results [52,81].

### 3.3. Glycoside fractionation

Williams et al. [51], laid stress on the existence, in the aromatic varieties of grapes, of two classes of precursors of  $C_{10}$  and  $C_{13}$  components with different polarity, separable by chromatography on  $C_{18}$  cartridges. The method described from these authors about the separation of two glycosides classes about the absorption of dealcoholized wine on  $C_{18}$  cartridges of adequate size to the wine volume used, the elimination of hydrophilic components with water, the elution of more polar precursors with 30% acetic acid and the elution of monohydroxylated terpenic alcohols with methanol. The components in both fractions were recuperated by evaporation of

solvent and subjected to another purification or hydrolysis.

The critical points of this method are: (a) the incomplete elimination of volatile components during the evaporation of wine alcohol under vacuum; (b) the partial recuperation of the precursors with an acidic solvent, with the opportunity of hydrolytic adulterations and chemical transformations during the solvent evaporation.

Besides, Strauss et al. [82] fractionated by doplet counter-current chromatography (DCCC) the  $C_{10}$ ,  $C_{13}$  and aromatic ring precursors in must previous isolation on  $C_{18}$  RP column, and Bittner et al. [52] described an analytical HPLC method with  $C_{18}$  column to fractionate aroma precursors, using acetonitrile and water as solvents.

Following developments of chromatographic techniques in totally liquid phase [83] led to the fractionation of the precursors in leaves of Renan Riesling by using multilayer coil counter-current

chromatography (MLCCC). In this case, the precursors were previously isolated on Amberlite XAD-2 resin column. This technique has been used to isolate two novel terpenoid glucose esters from Riesling wines [84].

A separation system for volatile precursors using commercial C<sub>18</sub> RP columns and inert solvents has been recently performed. The glycosides of mono, di and trihydroxylated terpene and norisoprenoid alcohols as well the related shikimate pathway ones have been isolated on C<sub>18</sub> RP cartridges and fractionated in classes having different polarity with eluents at increasing percentages of methanol. The benzyl alcohol glycosides appear the most polar, while those of terpene monohydroxylated alcohols and of geranic acid the least polar. The terpene diols, linalool furanoid and pyranoid oxides as well norisoprenoid precursors show intermediate polarity and place themselves in well fixed fractions according their polarity [28].

#### 4. Conclusion

The presence of terpenes, in their different forms, in grape juices and wines represents an enormous potential in a way to increase the varietal characteristics of the wines, contributing the final product with higher fruit-like characteristics. Actually, researchers have sufficient information and tools to study the presence of terpenes and their evolution in grape juices and wines, but it is not yet possible to translate all of the acquired knowledge to the wineries because an efficient methodology to improve the terpene content of all the wines present in the market has not been found. So, much work remains to be done to obtain a methodology, probably of enzymic nature, which allows that wine consumer sense of smell can, at last, appreciate the whole organoleptic richness of the product that he has on his hand or in his mouth.

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