Universidade de Aveiro Departamento de Química
2013

João Pedro Barbosa David

Compostos de aroma em castas de uvas cultivadas na Polónia

Flavor compounds in grape varieties grown in Poland

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica, ramo da Bioquímica Alimentar, realizada sob a orientação científica de Doutor Henryk Jelén, Professor do Departamento de Ciência dos Alimentos e Nutrição da Poznan University of Life Sciences e de Doutora Sílvia Rocha, Professora Auxiliar do Departamento de Química da Universidade de Aveiro.

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~

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palavras–chave

Vitis vinifera L.*,* uvas brancas*,* compostos glicosidicamente ligados*,* compostos voláteis, extração em fase sólida, GC x GC-ToF-MS.

resumo

A Polónia é um país que não é habitualmente conhecido pelos os seus hábitos de vinicultura, no entanto, em determinadas regiões existe uma longa tradição de cultivo da vinha e produção de vinho. A vinha Golesz em Jasło, sudeste da Polónia, tem desenvolvido algumas variedades de uva interessantes com vista a resisterem à severidade do inverno.

Neste trabalho, as castas Mília, Merzling, Freiminer, Traminer, Jutrzenka, and Adalmiina foram analisadas com o objetivo de caracterizar o seu perfil volátil.

Na *Vitis vinifera* L. os compostos voláteis estão presentes na forma livre, contribuindo diretamente para o aroma da casta, ou na forma de conjugados de açúcar não voláteis, os quais podem sofrer hidrólise enzimática ou acídica libertando os voláteis que poderão potenciar o aroma.

Extração de fase sólida foi usada para separar os voláteis na forma livre dos ligados, de seguida foi feita uma análise por GC x GC-ToF-MS. A fração ligada foi submetida a hidrólise enzimática ou em condições acidicas. Todas as castas de uva mostraram ter terpenóides, álcoois aromaticos, C_{13} norisoprenoides, álcoois C_6 e aldeídos C_6. O seu padrão de distribuição entre as formas livres e ligadas, a sua quantidade e variedade de compostos é no entanto diferente de casta para casta. Os terpenóides representam a maior fração de voláteis nas castas Mília, Merzling, Freiminer, Traminer, e Jutrzenka, com especial atenção para o monoterpenol geraniol que é o mais abundante em todas as castas analisadas. A casta Adalmiina foi caracterizada principalmente por álcoois C_6 e aromáticos.

keywords

Vitis vinifera L., white grapes, glycosidically-linked volatiles, compostos voláteis, extração em fase sólida, GC x GC-ToF-MS.

abstract

Poland is not known as the country of wine producers; however, there is a long tradition of grapevine cultivation and wine making in certain regions. Golesz Wineyard in Jasło, southeastern part of Poland, has developed some interesting varieties in order to resist to the severity of the winter.

In this work, Mília, Merzling, Freiminer, Traminer, Jutrzenka, and Adalmiina grape varieties were analyzed with the purpose of characterizing their respective volatile profile.

In *Vitis vinifera* L. volatile compounds are present in the free form, which can contribute directly to varietal aroma, or as nonvolatile sugar conjugates, which can undergo acid or enzyme hydrolysis, releasing free volatiles and potentially enhancing aroma.

Solid phase extraction was used to fractionate the free from the bound form volatiles, followed up by GC x GC-ToF-MS analysis. The bound form was either hydrolyzed enzymatically or in acidic conditions. All grape varieties exhibited terpenoids, aromatic alcohols, C_{13} norisoprenoids, C_6 alcohols, and C_6 aldehydes. Each one of the varieties under study exhibited a specific free and glycosidically-bound pattern. Terpenoids represent the major fraction of volatiles in Mília, Merzling, Freiminer, Traminer, and Jutrzenka grape juice, with the monoterpenol geraniol standing out as the most abundant in every grape variety. Aldalmiina variety was characterized by aromatic alcohols, and C_6 alcohols.

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List of abbreviations

3MH	3-mercaptohexan-1-ol
3MHA	3-mercaptohexyl acetate
4MMP	4-mercapto-4-methyl-pentan-2-one
GC	Gas chromatography
HD	Dynamic Headspace
HPLC	High performance liquid chromatography
HS	Static headspace
IBMP	3-isobutyl-2-methoxypyrazine
IPMP	3-isopropyl-2-methoxypyrazine
LLE	Liquid-liquid extraction
MP	Methoxypyrazine
MS	Mass spectrometry
OT	Odor threshold
PDMS	Polydimethyl siloxane
SBMP	3-sec-butyl-2-methoxypyrazine
SBSE	Stir bar sorptive extraction
SDE	Steam distillation
SPE	Solid phase extraction
SPME	Solid phase microextraction
GCxGC-ToF-MS	Multi-dimensional Gas Chromatography-Time of Flight

1) Introduction

1.1) Grapes

The grape is unique: not only is it a major global horticulture crop but it also has ancient historical connections with the development of human culture. In the *Vitaceae* family, it is the *Vitis* genus that is of major agronomic importance. It consists of approximately 60 inter-fertile species that exist almost exclusively in the Northern Hemisphere. Among them, *Vitis vinifera* is the only species extensively used in the global wine industry. It is also the only species of the genus indigenous to Eurasia and is suggested to have first appeared 65 million years ago. Two forms still co-exist in Eurasia and in North Africa: the cultivated form, *V. vinifera* subsp. *vinifera* (or *sativa*) and the wild form *V. vinifera* subsp. *silvestris* (or *sylvestris*), sometimes referred to as a separate subspecies (1).

Grapes, the berries of *Vitis vinifera* L. ssp sativa, are used for various utilizations since ancient times. Also today, they are of worldwide interest for nutritional purposes including raw and dried consummation, wine production, but also extracts of their peels and seeds are used in pharmaceutical applications with advertised health beneficial properties due to polyphenolic and especially interesting resveratrol content. Production of grapes generally is situated in moderate-warm climate zones, e.g. Italy (9,256,814 mt/year), France (6,787,000 mt/year), USA (6,414,610 mt/year), Spain (5,880,800 mt/year) but also China (5,698,000 mt/year) in 2006 (2).

From an ecological point of view, the complete utilization of grapes including the grape pomace as byproduct from producing wine is an important aspect in waste reduction (2). This pomace consists of fruit skins, remnants from the fruit pulp, seeds, and, in certain cases, some stems, with the skins and seeds making up the major part. Grape seeds and grape skins are rich sources of ethanol, tartrates and malates, citric acid, grape seed oil, hydrocolloids, dietary fibers and phenolic compounds. Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols, and stilbenes are the principal phenolic constituents of grape pomace (3), known for providing beneficial substances for lowering incidence of atherosclerosis and coronary heart diseases (4). Grape pomace from wine processing is already used for the extraction of anthocyanins on an industrial scale. Anthocyanins have long been used as natural food colorants (2).

1.1.1) Grape physical structure

The internal structure of the grape is heterogeneous, three zones can be considered: central, pulp around the seeds and the fibrovascular bundles leading to the peciole, intermediate, and peripheral zone nearest to the skin (Figure 1) (5).

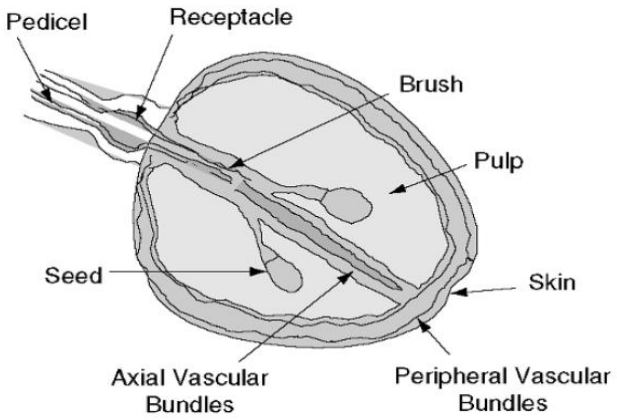

Figure 1 – Grape berry structure (6).

1.1.1.1) Seeds

Grape seeds contribute up to 6% of the total weight of the berry. A berry may have zero to four seeds. The more seeds produced, the larger the berry, which slows down the sugar accumulation and tends to prolong acid retention.

Seeds contain carbohydrates, nitrogen compounds, oils (oleic and linoleic), minerals, vitamin E, and phenolic compounds. The seeds contain approximately 20–50% of the total polyphenols in the berry, the greatest concentration of tannins. Seed tannins reach their highest concentration at véraison and diminish throughout the maturing process (7).

1.1.1.2) Skin

The berry skin contributes up to 20% of the total berry weight. The skin contains the essential anthocyanins required for red wine, along with flavonols and tannins. The major grape varieties are classified as red or white. The color differences are result of the lack of anthocyanins.

The skin is high in citric acid and contains benzoic and cinnamic acids. Aromatic substances, aroma precursors, and a small amount of sugar complete the profile (7).

1.1.1.3) Pulp

Berry pulp is the largest component, contributing up to 85% of the total berry weight. The pulp itself has very little solid parts but has a high density and soluble solids level because of the chemical substances within. Fructose, glucose, and other sugars are the heavyweights and contribute the greatest to the soluble solids content. Soluble proteins are also a contributing factor (8).

The primary acids contained in the pulp are tartaric, malic, and citric, with trace amounts of other acids. The concentration of potassium predominates with lesser alkaline metal concentrations of iron, calcium, magnesium, and sodium. Pulp pH is usually in the range 2.8–3.6. Nearly 25% of the total nitrogen is contained in the pulp as nitrogen compounds, including organic nitrogen. Pulp is high in the amino acids leucine, praline, arginine, threonine, and glutamic acid. There is a significant concentration of aromatic compounds such as aldehydes, esters, and terpenoids (7,8).

1.2) Polish grape varieties

Poland is not known as the country of wine producers; however, there is a long tradition of grapevine cultivation and wine making in certain regions, mainly western part of Poland. The other main region for wine production is southeastern part of Poland – Podkarpacie region, where small wineries are mainly localized near Jasło. The drawback for winemaking in Podkarpacie region is the severity of winters which implied the adaptation or development of grape varieties best suitable for this region and climate conditions and also works done for cross breeding resulting in some interesting varieties. The work was pioneered by Roman My¢sliwiec from Golesz Wineyard in Jasło, who developed among others Jutrzenka grape variety, which is used for production of white, mainly liquer wines for over 20 years. Similar climatic problems are encountered in Slovakia, so as a result of viticulture efforts vine hybrids, such as Devin, providing interesting aroma profiles were developed for white wine production (9).

1.2.1) Merzling

White wine hybrid grape variety with a parentage of Seyval blanc (or Seyve Villard 5-276) and a Riesling a Pinot Gris crossing, otherwise known as Fr 375-52. It was bred in Freiburg, Germany in 1960 by Dr. Johannes Zimmerman and registered as a protected varietal in 1933. Its high tolerance of cold weather, resistance to most disease, and tendency towards ultra-high yields means it is often used as a valuable blending component in cooler vineyards sites in various northern-european regions, including Germany and Poland (10).

1.2.2) Mília

Mília is an early ripening grape variety suitable for marginal regions since it is ripening early. It was crossed in 1973 in Senkvice with Müller-Thurgau *x Tramín červený.* Wines are aromatic, with interesting spiciness and pleasant, mild acidity (11).

1.2.3) Traminer

Traminer is one of the oldest European varieties and was even likely known by the Romans as *Vitis aminera*. Traminer was named after the South Tyrolean town of Tramin in north Italy. The variety is differentiated by its range of colors. Roter Traminer has red grapes; GewürzTraminer shows light red/pink grapes; and with yellow grapes is the Gelber Traminer. Name-wise, all of the Traminer types can be referred to as GewürzTraminer.

The grapes are round to oval with thick skins. Firm flesh with low acidity, high sugar content and an intense spicy taste. This is a special variety for highly ripe aromatic wines with rose, lemon, forest berry, raisin, dried fruit aromas (12).

1.2.4) Freiminer

Cultivars derived from interspecific crosses of French Seyve Villard 12-413 hybrids with Traminer variety of Freiburg, Germany. Grapes are able to accumulate more sugar. The wine is soft, accompanied by aromatic notes familiar to Traminer variety (13).

1.2.5) Jutrzenka

Jutrzenka is a hybrid of Seyve Villard 12-375 and Pinot Blanc. It can withstand low

temperatures (down to −25 ºC) and is resistant to fungal diseases. It gives fruits in second half of September and its berries are 16–18 mm, yellow-green. It has average sugar content in the must of 18–22 g/100 ml. The most prominent feature of this variety is the strong and fruity aroma (9).

1.2.6) Adalmiina

Developed by Elmer Swenson in mid-late 80 and never released with a name. Meeri Saario from Finland got it in the mid 90′s and got permission to name this vine. She named it Adalmiina. This variety is very disease resistant, though moderate winter hardiness to –35 ºC. Grows moderately fast with a good yield. Clusters are medium in size. Golden berries, the flesh is soft, sweet and without a clear aroma and taste (14).

1.3) Flavor

1.3.1) Definition

What is flavor? Flavor is usually the result of the presence, within complex matrices, of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties. Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (6,15,16).

Initially, flavors are released from food to the saliva phase. Nonvolatile flavors in saliva are sensed by taste buds on the tongue whereas volatile flavor compounds must be transported first from the saliva to the air phase in the mouth before travelling via the throat to the olfactory receptors located in the nose, where they are sensed (17). This interaction of sensory properties with the human senses is shown in Figure 2.

Whether flavor refers to the chemicals responsible for the stimulation or the biological receptor stimulation itself, is immaterial to the consumer of foods (6). Consumers consider

flavor one of the three main sensory properties decisive in their selection, acceptance, and ingestion of a particular food. The other two sensory properties are appearance and texture (6).

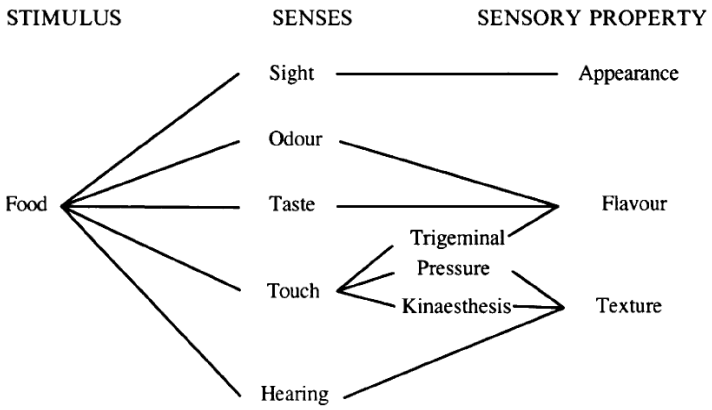

Figure 2 – Relation of the senses with sensory properties (15).

One important reason for the rapid growth of research interest on the topic of flavor perception in recent years stems from the light that gaining a better understanding of how the multisensory integration taking place in the context of food perception might shed on theories of multisensory integration in general (18). On the other hand, it is also widely believed that the study of the multisensory processes involved in flavor perception will have a number of important consequences for the food and beverage industries, such as, for example, a better understanding of the processes used by people to assess the acceptability and flavor of new products (18).

1.3.2) Flavor perception

The flavor of food is dependent on an array of volatile compounds – their number, character, and quantities. However, because flavor is related to perception of odorants by our olfactory system, unique features of volatile compounds have to be considered as well: their odor threshold being the most important and features that influence odor thresholds and aroma perception: chirality, concentration, synergistic effects and a type of matrix (food) from which the compounds are released (19).

Flavors can be classified by the general sensations that one feels when eating different foods (6). Flavor comes from three different sensations: taste, trigeminal and aroma (odor). It is generally agreed that taste sensations are divided into four major categories: saltiness, sweetness, sourness and bitterness. Trigeminal sensations give us the descriptors of astringency, pungency and cooling. Both taste and trigeminal sensations occur upon contact with food in the mouth, as most substances, which produce these flavors, are non-volatile, polar, and water-soluble (6). For aroma sensations to occur, an aromatic compound must be sufficiently volatile to allow detection at a distance. Aroma compounds can reach the pituitary via the nose during normal olfaction (via orthonasal) or via the pharynx when the food is taken in the mouth and swallowed (via retronasal). In the first case, we smell the odor of the product; in the second case, we perceive the flavor of the product (20).

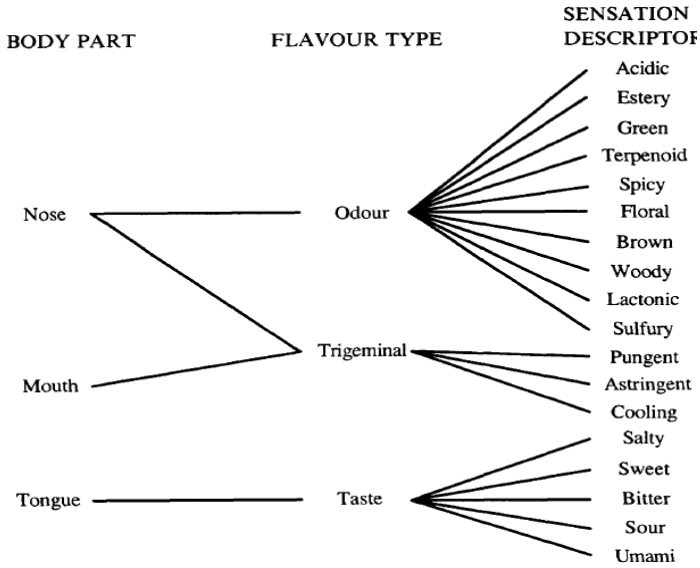

Figure 3 – Correlation of flavor types with sensation descriptors (15).

There is no doubt that the smell of the product is almost entirely due to the signals elicited by the aroma molecules in the olfactory receptors (pure odor), although there are also trigeminal nerve endings in the olfactory mucosa able to interact with some aroma chemicals to produce trigeminal stimulation. In fact, some aroma chemicals produce a sensory perception that is a mixture of odor and trigeminal stimulation (Figure 3.), such as acetic acid or menthol (20).

The flavor is a complex perception that integrates information from three different

sensory systems: odor, taste and the chemosensory receptors, responsible for “hot”, “cool”, “dry”, “irritant” or “pungent” attributes. In the case of aroma molecules, the amount and proportion of molecules reaching the olfactory receptors when the food is taken into the mouth and during swallowing is not exactly the same as that when the product is smelled, because the conditions for the release of the aroma molecules from the product are fairly different in both cases (e.g. temperature, dilution with saliva, aggregation). That complexity can make us think that flavor is totally different, while the truth is that most qualitative attributes of flavor are also produced by aroma molecules via the activation of the olfactory receptors (20).

The multiplicity of interactions between taste, smell, touch, and the trigeminal system (not to mention hearing and vision) has led numerous researchers to propose flavor as the term for the combinations of these systems, unified by the act of eating (18).

Flavor perception should be used as a term to describe the combinations of taste, smell, the trigeminal system, and touch, to which we add visual and auditory cues, which also influence our perception when tasting food. According to this view, the act of eating allows the different qualities of an object to be combined into a whole percept. According to this view, flavor is not defined as a separate sensory modality but as a perceptual modality that is unified by the act of eating (18).

1.3.3) Chemical compounds responsible for flavor

Food volatile flavor compounds are usually volatiles of MW not exceeding 300 Da and represent various chemical classes. Because of their character and molecule size, volatile flavor compounds have been analyzed using gas chromatography, although there are a limited number of publications where HPLC is used for the analysis of some odorants, mainly aldehydes (19). The many different possible flavors are due to interactions of chemical compounds with taste, trigeminal or aroma receptors (6). The characteristic task (including trigeminal stimulations) of a food is normally related to a single class of compounds. But, an odor is usually elicited by a combination of volatile compounds each of which imparts its own smells (6). Differences in characteristics of certain aromas can be equated to the varying proportions of these volatiles. However, some substances contain

trace amounts of a few volatile compounds that possess the characteristic essence of the odor. These are called character-impact compounds. One must also realize that the chemicals of a single compound class can elicit many diverse flavors, especially as their concentrations vary (6).

1.3.4) Flavor compounds in grapes

There has long been an interest in understanding the chemical origin of grape aromas. Such knowledge could provide useful information to predict wine quality (21,22), and benefit both grape growers and winemakers by permitting a more precise determination of a desirable harvest date (23).

Table 1 - Volatile compounds classes and their sensory character (3).

Compound Class	*Sensory Character*	*Examples*
Aldehydes	Fruity, green	Acetaldehyde, hexanal
	Oxidized, sweet	Decanal, vanillin
Alcohols	Bitter, medicinal	Linalool, menthol
	Piney, caramel	α-Terpineol, maltol
Esters	Fruity	Ethyl acetate, ethyl butyrate
	Citrus	Geraniol acetate
Ketones	Butter, caramel	Diacetyl, furanones
Maillard reaction products	Brown, burnt, caramel, earthy	Pyrazine(s), pyridine, furans
Phenolics	Medicinal, smokey	Phenol(s), guaiacols
Terpenoids	Citrus, piney	Limonene, pinene
	Citrus	Valencene

Grape aroma compounds are present as free volatiles (Table 1), which may contribute directly to odor, or as bound sugar conjugates, odorless precursors, which are nonvolatile. Conjugates (including glycosides) can undergo acid or enzyme hydrolysis, releasing free volatiles and potentially enhancing aroma (24). White wine aroma is especially interesting, as in its formation flavor compounds originating from grapes play a crucial role. Wine volatile profile is estimated to be around 1000 compounds (Table 2), and comprises compounds of different chemical classes and character, which occur in

concentrations ranging from ng/L to mg/L. Their contribution to the overall wine flavor is different, depending on their odor thresholds and concentrations (9).

Several families of compounds are responsible for primary aroma of grapes such as monoterpenols, abundant in Muscat varieties, methoxypyrazines, which characterize the Cabernet family, C_{13}-norisoprenoids, abundant in Chardonnay, volatile thiols in Sauvignon, volatile phenols in Traminer aromatico, and dimethyl sulfide in Syrah (24). These compounds, however, could also contribute significantly to the aroma of several other varieties (24). The sensory character of some of these families of compounds can be seen in Table 2.

Most of the volatile flavor components are produced after *véraison* until harvest (24). However, notable aroma compounds that are produced during the first period of growth, decline during fruit ripening. The concentration of varietal aroma compounds in grapes is influenced by several factors such as grape variety and degree of maturity, vintage, climate or vineyard management techniques (24–26). It is generally recognized that grape maturity will affect the flavor profile parallel to the sugar content. The knowledge of the grape varietal volatile composition offers a means of evaluating the aroma potential, and the period of time that the maximum potential is exhibited (27). Therefore it is important to determine the concentrations of varietal volatiles (terpenes, C_{13}-norisoprenoids and C_6-compounds) as a criterion to define the date of harvest (22). C_6-aldehydes and alcohols are formed from linoleic acid and linolenic acid when grapes enter into contact with the air, and are formed by the actions of lipoxygenase, peroxidase and alcohol dehydrogenase enzymes (28). C_{13}-norisoprenoid and terpene compounds are generated from carotenoids such as lutein and β-carotene (29).

1.3.4.1) Bound flavor compounds in grapes

Glycoconjugates of flavor compounds are present in several fruits such as grapes, apricot, peach, yellow plum, quince, sour cherry, passion fruit, kiwi, papaya, pineapple, mango, lulo, raspberry and strawberry (30). The occurrence of glycosidically-bound volatiles is typically two to eight times greater than that of their free counterparts (30,31). Many wine volatile compounds can be released from their flavorless glycoconjugate precursors by either acid or enzymatic hydrolysis.

Table 2 - Volatile compounds present in fruits (3).

Esters	Alcohols	Aldehydes	Ketones	Lactones	Terpenoids
Butyl acetate	Benzyl alcohol	Acetaldehyde	2,3-Butanedione	γ-Butyrolactone	β-Caryophyllene
Butyl butanoate	Butan-1-ol	Benzaldehyde	β-Damsenone	γ-Decalactone	1,8-Cineole
			eucalyptol		
Butyl hexanoate	(*E*)-cinnamyl alcohol	(*E*)-cinnamaldehyde	Eugenol	δ-Decalactone	Citral
Butyl-2-methyl butanoate	1-Hexanol	(*E*,*E*)-2,4-decadienal	2-Heptanone	γ-Dodecalactone	β-Damascenone
Butyl propanoate	(*E*)-2-hexenol	Hexanal	4-(*p*-Hydroxyphenyl)-2-butanone	δ-Dodecalactone	Dihydroedulan
Ethyl acetate	(*Z*)-3-hexenol	(*E*)-2-hexenal	3-Hydroxy-2-butanone	γ-Jasmolactone	Farnesyl acetate
Ethyl butanoate	1-Octanol	(*Z*)-3-hexenal	β-Ionone	γ-Octalactone	Geraniol
Ethyl 9-decenoate	(*Z*)-6-nonenol	(*Z*)-3-hexenal	Linalool	δ-Octalactone	Hotrienol
Ethyl hexanoate	Hexan-1-ol	Nonanal	6-Methyl-5-heptene-2-one		α-Ionone
Ethyl 2-methylbutanoate	(*Z*,*Z*)-3,6-nonadienol	(*Z*)-6-nonenal	Nerolidol		β-Ionone
Ethyl 3-methylbutanoate	1-Phenylethanol	(*E*,*Z*)-2,6-nonadienal	1-Octen-3-one		Limonene
Ethyl 2-methylpropanoate	2-Phenylethanol	(*E*)-2-nonenal	2-Pentanone		Linalool
Ethyl 2-methylbutanoate		Phenylacetaldehyde	(*Z*)-1,5-octadien-3-one		Myrtenol
Ethyl propanoate			Terpenes		Nerol
Ethyl 2-methylpropanoate					α-Phellandrene
Ethyl nonanoate					α-Pinene
(*E*)-2-hexenyl acetate					β-Pinene
(*E*)-3-hexenyl acetate					Terpinen-4-ol
Hexyl acetate					α-Terpineol
Hexyl butanoate					Terpinolene
Hexyl propanoate					α-Farensene
Hexyl-2-methyl butanoate					
Methyl acetate					
Methyl cinnamate					
Methyl butanoate					
Methyl hexanoate					
Methyl nonanoate					
Methyl octanoate					
Methyl-2-methylbutanoate					
Methyl-3-methylbutanoate					
2-Methylbutyl acetate					
3-Methylbutyl acetate					
2-Methylpropyl acetate					
(*Z*)-6-nonenyl acetate					
(*Z*,*Z*)-3,6-nonadienyl acetate					
Pentyl acetate					
Benzyl acetate					
Propyl acetate					
Propyl-2-methyl butanoate					

Glycosidically-bound volatiles identified in fruits and plants are highly complex and diverse, especially the aglycone moiety (30,32). Numerous aglycons have been identified from hydrolysis of wine and grape juice glycosides, including monoterpenes, norisoprenoids, aliphatics and phenolic compounds (33).

The sugar parts consist of *β*-D-glucopyranosides and different diglycosides: 6-O-*α*-L-arabinofuranosyl-*β*-D-glucopyranosides, 6-O-*α*-L-arabinopyranosyl-*β*-D-glucopyranosides (vicianosides), 6-O-*α*-L-rhamnopyranosyl-*β*-D-glucopyranoside (rutinosides), 6-O-*β*-D-apiofuranosyl-*β*-D-glucopyranosides, 6-O-*β*-D-glucopyranosyl-*β*-D-glucopyranosides and 6-O-*β*-D-xilopyranosyl-*β*-D-glucopyranosides (primeverosides). In rare cases, trisaccaharide glycoconjugates have been isolated (30).

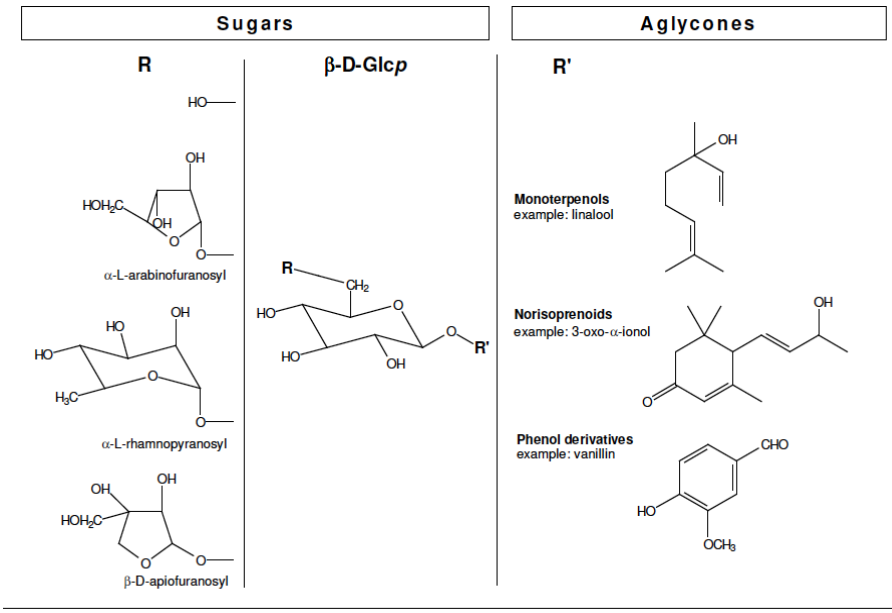

Figure 4 - Structure of some glycoconjugated aroma compounds (33).

The aglycon part is often formed with terpenols, but linalool oxides, terpene diols and triols can also been found (31),(30). However, other flavor precursors can occur such as linear or cyclic alcohols, e.g. hexanol, phenylethanol, benzyl alcohol, C_{13}-norisoprenoids, phenolic acids and probably volatile phenols such as vanillin (34).

Some structures of glycoconjugated aroma compounds are represented in Figure 4.

- **Terpenes**

Terpenes are an important group of aromatic compounds characterizing the odor of many flowers, fruits, seeds, leaves, woods, and roots (23). Chemically, terpenes are grouped together because of their distinctive carbon skeleton. It consists of a basic five-carbon isoprene unit (2-methyl-1,3-butadiene) which are generated from carotenoids such as lutein and b-carotene (23,35). Terpenes generally are composed of two, three, four, or six isoprene units. These are called monoterpenes, sesquiterpenes, diterpenes, and triterpenes, respectively (23).

In grapes, terpenes are important compounds as varietal aromas with floral and fruity notes and are present in green berries only in very small amounts, but their concentrations gradually rise during ripening until around maturity, after which concentration falls (29). In the berries, these compounds were found predominantly in skin tissue and typically stored as sugar or amino acid conjugates in the vacuoles of the exocarp cells. Studies show that geraniol and nerol, for example, were associated primarily with the skins of the berries, whereas linalool was more uniformly distributed between the juice and skin (15).

Terpenes contribute to some white wines aroma, especially these produced from Muscat grapes and others aromatic ones of high terpene contents (Gewürztramminer, Traminer, Huxel, Sylvaner). Terpenes are present in wine in free and bound (in a form of glycosides) forms (36). Glycosidically conjugated terpenes are not odorous and in most cases they are more abundant than unglycosylated free forms; they give a potential contribution to the aroma of the grape as they are varietal aroma precursors and, during the winemaking process, from these precursors some terpene compounds can be generated through slow enzymatic or chemical hydrolysis (37).

- **Monoterpenes**

In the aroma of white wines monoterpenes play an important role, being a group of flavor compounds characteristic for specific grapes used for wine production (7) . Monoterpenes were proposed to differentiate white wines into three classes (Muscat-type, Riesling-type and Silvaner type) (9) based on their free monoterpene concentration: (1) intensely flavored 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 monoterpenes for their flavor

(31,38).

At present, about 50 monoterpene compounds are known (Figure 5), the predominant monoterpenes in white wines are linalool, geraniol, nerol, α-terpineol, α-citronellol, hotrienol and limonene. Linalool (OT = 15 g L^{-1}) and geraniol (OT = 30 g L^{-1}) are the most abundant monoterpene alcohols in Muscat white wines (31).

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

Free aroma compounds, commonly dominated by linalool, geraniol, and nerol, together with the pyran and furan forms of the linalool oxides. Depending on how the juice has been treated and other factors like climate, many additional monoterpenes can be found in this group, i.e. citronellol, *α*–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 (31).

Second, there are the polyhydroxylated forms of the monoterpenes, or free odorless 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-dienesurveys 3,7-diol) can give ho-trienol and nerol oxide (31,38).

Third, there are the glycosidically conjugated forms of the monoterpenes, which also make no direct contribution to the aroma of the grape. Glycoterpene sides are, in most cases, more abundant than the unglycosilated forms of individual monoterpenes and polyols (31).

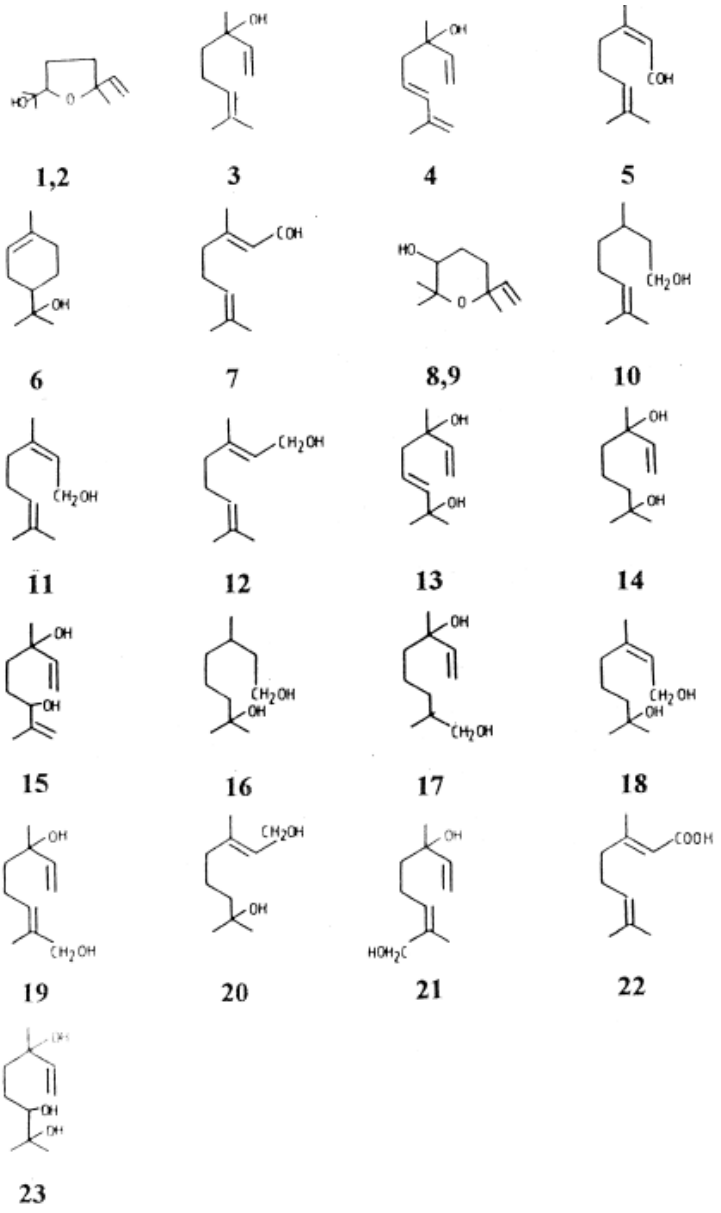

Figure 5 - Main monoterpenes in grape juice and wines. (1) *trans*-Furan linalool oxide, (2) *cis*-Furan linalool oxide, (3) Linalool, (4) Hotrienol, (5) Neral, (6) *α*-Terpineol, (7) Geranial, (8) *trans*-Pyran linalool oxide, (9) *cis*-Pyran linalool oxide, (10) Citronellol, (11) Nerol, (12) Geraniol, (13) Diol I, (14) Endiol, (15) Diol I, (16) Hydroxy-cityronellol, (17) 8-Hydroxydihydrolinalool, (18) Hydroxynerol, (19) *trans*-8-Hydroxylinalool, (20) Hydroxygeraniol, (21) *cis*-8-Hydroxylinalool, (22) Geranic acid, (23) Triol (31).

- **C_{13}-norisoprenoids**

Some of the most important key aroma components present in wine include C_{13}-norisoprenoids such as *β*-ionone or *β*-damascenone (Figure 6).

These molecules have an important sensorial impact on wine aroma as they have very low olfactory perception thresholds. Norisoprenoids contribute characteristic aromas to many varieties of *Vitis vinifera*. In Chardonnay, studies showed that "grassy", "tea", "lime", "honey", and "pineapple" aromas were derived from norisoprenoids and their precursors. Both red and white nonfloral varieties, including Chenin blanc, Semillon, Sauvignon blanc, Cabernet Sauvignon, and Syrah, are known to contain significant levels of norisoprenoids. Even in the floral varieties (e.g., White Riesling and Muscat), which derive most of their aroma impact from terpenes, norisoprenoid concentrations up to 40% higher than those of terpenes have been observed (39). C_{13}-Norisoprenoids are thought to arise from photochemical and enzymatic oxidation of carotenoids and occur in grapes as glycosidically-bound precursors; *β*-carotene and some xanthophylls (neoxanthin, flavoxanthin, and lutein) are abundant before *véraison* and subsequently decrease dramatically. These breakdown products of carotenoids are carbonyl compounds with 13, 11, 10 or 9 carbon atoms, and retaining the terminal group of their carotenoid parent, as it can be seen in Figure 7.

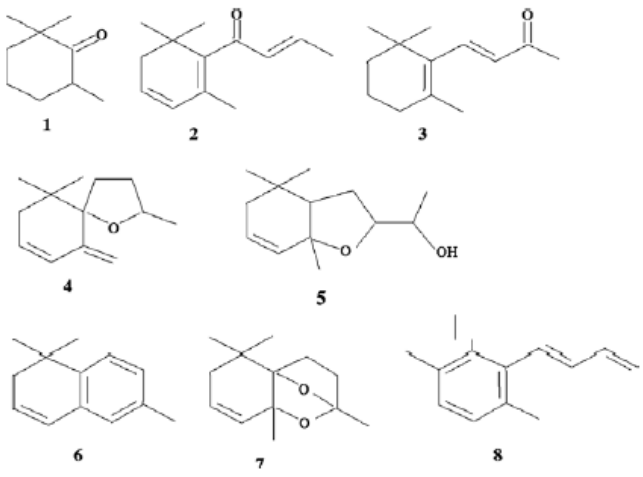

Figure 6 - Structures of norisoprenoids important to aroma. (1) TCH (2,2,6-trimethylcyclohexanone), (2) *β*-damascenone, (3) *β*-ionone, (4) vitispirane, (5) actinidiol, (6) TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), (7) Riesling acetal, (8) TPB (4-(2,3,6-trimethylphenyl)-buta-1,3-diene) (40).

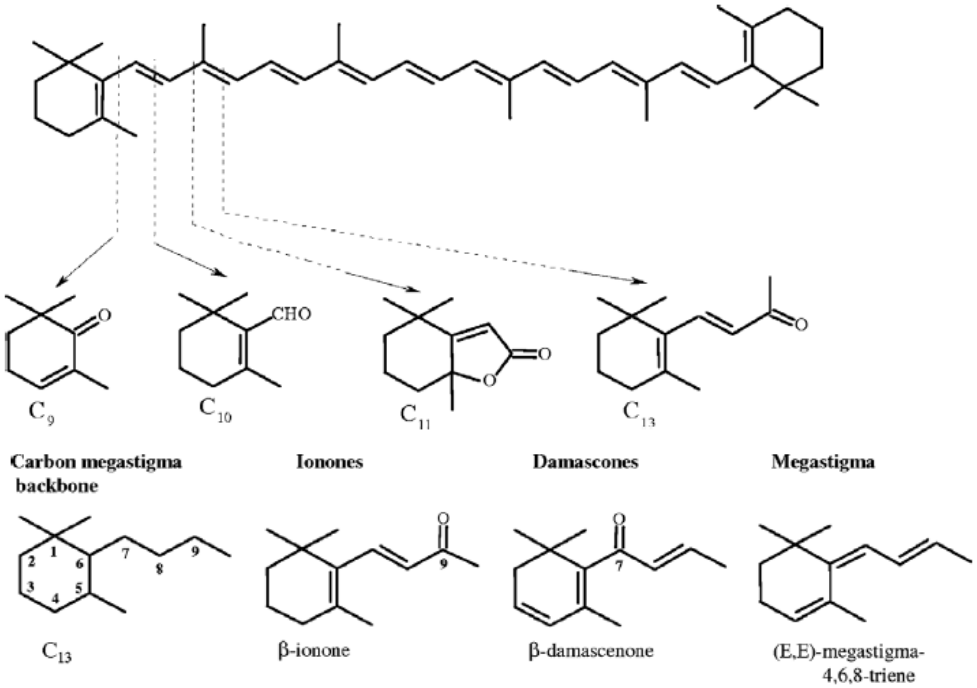

Figure 7 - Formation of C_9, C_{10}, C_{11}, and C_{13} norisoprenoid compounds from *β*-carotene and chemical structures of carotenoid-derived norisoprenoids with the megastigmane carbon backbone (35).

The C_{13} compounds are the most abundant norisoprenoids in nature. They can be divided into: (1) compounds with the megastigmane structure, including the family of ionones and damascones with oxygen at different positions e.g. with a keto group at C_9 as in *β*-ionone or at C_7 as in *β*-damascenone and (2) compounds with the megastigmane structure but without oxygen in the lateral chain, e.g. (E,E)-megastigma-4,6,8-triene (35,40,41). Compounds such as 2,2,6-trimethylcyclohexen-1-one, *β*-cyclocitral and DHA1 (dihydroactinidiolide) are examples of C_9, C_{10}, C_{11} norisoprenoids, respectively (35,40,41).

Some C_{13}-norisoprenoids that have been considered important to the aroma of wines, are represented in Figure 7, and are: TCH (2,2,6-trimethylcyclohexanone), *β*-damascenone [(2,2,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one], *β*-ionone [(2,2,6-trimethyl-1,3-cyclohexen-1-yl)-3-buten-2-one], vitispirane (2,10,10-trimethyl-6-methylene-1-oxaspiro[4.5]dec-7-ene), actinidiol, TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) and riesling acetal (2,2,6,8-tetramethyl-7,11-dioxatricyclo [6.2.1.01,6]undec-4-ene) (35).

The carotenoid breakdown reactions that occur during the maturation of grapes and the subsequent formation of norisoprenoids, as C_{13} varietal aromas, provide the contribution of

the free fraction of norisoprenoids to the wine aroma. This contribution can be more or less relevant to the final aroma of wine depending on the viticulture conditions, which affect the carotenoid profile of the grape (35).

- **Aldehydes, Alcohols, and esters**

Grapes produce few aldehydes important in the generation of varietal aromas. The C_6 aldehydes (hexanals and hexenals) appear to be the most significant. The patterns of production of *trans*-2-hexenal and hexanal from grapes throughout berry development are similar for both compounds: *trans*-2-hexenal, which is derived from linolenic acid (C18:3) and hexanal, which is derived from linoleic acid (C18:2), at the same level of the lipoxygenase pathway (23).

They may be involved in the grassy to herbaceous odor associated with certain grape varieties, such as “Grenache” and “Sauvignon blanc”, or with wines made from immature grapes. They appear to be formed during crushing by the enzymatic oxidation of grape lipids. The dienal, 2,4-hexadienal, may also be generated by the same process (23).

Alcohols with more than two carbon atoms are commonly called higher or fusel alcohols. They may be present in healthy grapes, but seldom occur in significant amounts. Hexanols are the major exception to this generalization. C_6 alcohols, hexanols and hexenols from plant tissues that give the herbaceous smells so characteristic of wines made from unripe grapes. Another of these compounds is octen-1-ol-3, with an odor reminiscent of mushrooms. Its presence in wine is due to the action of *Botrytis cinerea* on grapes (7).

Very few esters are present in grapes. Odoriferous molecules such as methyl anthranilate are responsible for the foxy odor in *Vitis labrusca* grapes and wines made from them (7).

C_6 alcohols, as well as aldehydes, were mainly found in free form (98% of total). In both tips and shoulders, they were mainly found in the berry skin. These compounds were approximately in the same amount in the skin of the shoulders and tips, but in the flesh were more abundant in the tips. Consequently, the berries from the tips had a higher content of C_6 alcohols than those from the shoulders.

Lactones may also come from grapes, as is the case in Riesling, where they contribute to the varietal aroma. Infection of grapes by *Botrytis cinerea* probably produces sotolon (4.5-dimethyl-3-hydroxy-2-furanone), involved in the toasty aroma characteristic of wines

made from grapes with noble rot. Concentrations present, on the order of 5 µg/l, are above the perception threshold (7).

- **Methoxypyrazines**

Methoxypyrazines are grape-derived compounds present in skin, pulp, and bunch stems of grape, and contribute with very characteristic vegetative, herbaceous, bell pepper, or earthy notes to the aroma of Sauvignon blanc, Semillon and Cabernet Sauvignon wines. Chemically, they are nitrogenated heterocycles produced by the metabolism of amino acids. In the late 1960s Buttery and co-workers identified 3-isobutyl-2-methoxypyrazine (IBMP) as the main impact compound responsible for the aroma of bell peppers, character associated with Cabernet Sauvignon and Sauvignon Blanc grape varieties. Even trace amounts of methoxypyrazines have been isolated from "Riesling" and "Chardonnay" (42).

Three methoxypyrazines have been identified, 3-isobutyl-2-methoxypyrazine (IBMP), 3-sec-butyl-2-methoxypyrazine (SBMP), and 3-isopropyl-2-methoxypyrazine (IPMP), their chemical structure can be seen in Figure 8. IBMP has been considered to be one of the main "varietal" volatiles reported in grape and wine, first identified in Cabernet Sauvignon wines and later found in wines of Sauvignon blanc and other *Vitis vinifera* grape varieties (43). IBMP is predominant, and they have structures consistent with a related biosynthetic origin. They occur at trace levels, with a combined concentration of, typically, 1-40 ng L^{-1}. There is a narrow concentration window that allows their flavor contribution to be evident yet not excessive.

IBMP, SBMP and IPMP have extraordinarily low sensory thresholds in wine, which are generally reported at low ng L^{-1} levels, but have been reported as low as the high pg L^{-1} range for IPMP in some wines and grape juices. In contrast, the concentration of grape-derived MPs in grape and wine can be well above their sensory thresholds. IBMP concentrations have been recorded at 307 and 56.3 ng L^{-1} in grapes and wines, respectively. IPMP has been up to 48.7 ng L^{-1} in grapes and up to 4.5 ng L^{-1} in wines. The highest reported concentration of SBMP in grapes and wines is 11.2 ng L^{-1}. The concentration of these MPs in grapes, and their impact in the resulting wines, is strongly and systematically influenced by viticultural conditions, such as the temperature during ripening, the berry maturity at harvest, and the fruit exposure to sunlight. Even though IBMP is generally more abundant than IPMP and SBMP in grapes and wine, high IPMP

concentrations are also considered undesirable in most wines and have been described as having a "pea-asparagus" type aroma (43).

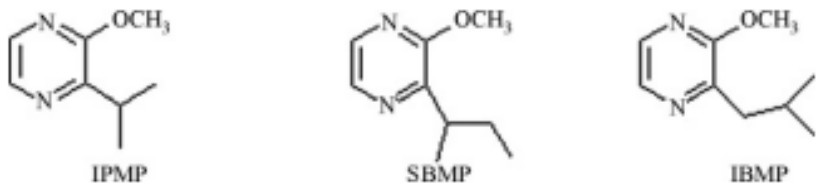

Figure 8 - Alkyl-2-methoxypyrazines chemical formulas. IPMP is the isopropyl, SBMP is the secondary butyl, and IBMP is the isobutyl group (43).

- **Volatile Thiols**

Sulfur compounds in the thiol family (or mercaptans) are generally held responsible for olfactory defects. However, their major contribution to the aromas of certain fruits and aromatic plants has been clearly established (7). The volatile thiols are thiol compounds with additional functional groups such as ketones, alcohols and esters (44). Since the early 1990s, a number of highly odoriferous thiols have been identified in Sauvignon Blanc wines. These wines have marked, characteristic aromas, featuring various herbaceous, fruity, and empyreumatic nuances. The first and second groups include green pepper, boxwood, broom, eucalyptus, blackcurrant buds, rhubarb, tomato leaves, nettles, grapefruit, passion fruit, white peaches, gooseberries, and asparagus broth, as well as acacia wood and blossoms (7). The first molecule found to be a characteristic component of the aroma of Sauvignon Blanc wines was 4-mercapto-4-methyl-pentan-2-one (4MMP). This extremely odoriferous mercaptopentanone has a marked smell of boxwood and broom. Its perception threshold in a model solution is 0.8 ng L^{-1}. It has an undeniable organoleptic impact, as concentrations may even exceed a hundred mg/l in Sauvignon Blanc wines with strong varietal character (44). Other volatile thiols responsible for the fruity or tropical organoleptic flavors of Sauvignon blanc wines are 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), responsible for the passion fruit, grapefruit and citrus aroma. 4-Mercapto-4-methylpentan-2-ol (4MMPOH) can also contribute to the characters of citrus, passion fruit and grapefruit, although its organoleptic role is more limited, due to its concentration in wines seldom exceeding its olfactory threshold of 55 ng

L^{-1} (44).

Specific thiols are involved in the characteristic aromas of fruits such as blackcurrant grapefruit, passion fruit and guava. Two mercaptans, ethyl-3-mercaptopropionate and ethyl-2-mercaptopropionate, have been identified as components in the aroma of *Vitis labrusca* grapes (7)

1.4) Determination of free vs bound compounds in grapes: methods overview

Food flavor compounds due to the complexity of food as a matrix, and usually their very low concentrations in a product, as well as their low odor thresholds, create a challenge in their extraction, separation and quantitation. Food flavor volatiles represent compounds of different polarity, volatility and chemical character, which determine method of extraction for their isolation from food (45).

Developments in sample preparation are aimed and find most applications in environmental analysis. The search for improved sample preparation has the following goals: (i) reduction of the number of steps in analytical procedure; (ii) reduction or elimination of solvents required for extraction; (iii) adaptability to field sampling; (iv) automation (45).

1.4.1) Solid phase extraction (SPE)

Accurate and precise methods are required to determine varietal aroma compounds and to establish their relative concentrations in wines and grapes. The analysis of the free and bound aroma compounds in wine and grapes requires fractionation of the sample and separation of the volatile (non-polar) fraction from the water-soluble, sugar-bound (polar) fraction. The vast majority of fractionation methods are based on solid-phase extraction (SPE), which is widely used as a sample preparation technique for the analysis of volatile compounds. SPE is a rapid, modern alternative to liquid-liquid extraction. With SPE, many

of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents (46). In SPE the analytes to be extracted are partitioned between a solid and a liquid (rather than between two immiscible liquids as in LLE) and these analytes must have a greater affinity for the solid phase than for the sample matrix (retention or adsorption step). Compounds retained on the solid phase can be removed at a later stage by eluting with a solvent with a greater affinity for the analytes (elution or desorption step) (47). By using SPE one can remove matrix interferences (these either pass through the cartridge or are subsequently washed off) and then isolate with selective enrichment one's target compounds. Solvent use is small (48). Among sorbents used in extraction of food aroma compounds several groups can be distinguished: silica gels (polar due to their hydroxyl groups), activated aluminas (polar), activated carbon (apolar), zeolites and polymers, such as polystyrene, polyacrylilc esters, PDMS and phenolic resins (47,49). Cartridges pre-packed with known quantities of adsorbent are on the market, and they are ready to use after simple conditioning (Figure 9) (48).

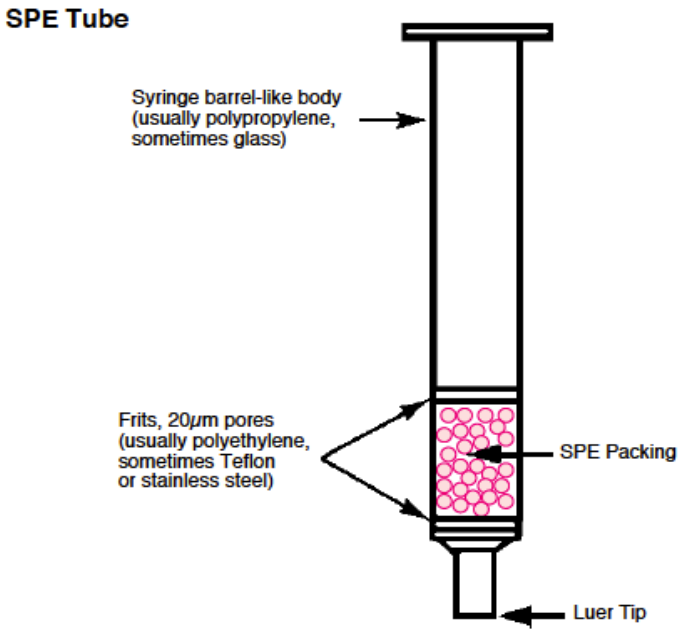

Figure 9 - General structure of a SPE tube (46).

Depending on the type of sorbent and on the characteristics of the analyte, a series of physical and chemical interactions are established that allow the analyte of interest to be separated from the rest of the components of the sample. The selectivity of the separation will be conditioned by the type of sorbent and eluent employed (50).

Reversed phase separations involve a polar (usually aqueous) or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Several SPE materials, such as the alkyl- or aryl-bonded silicas (LC-18, ENVI-18, LC-8, ENVI-8, LC-4, and LC-Ph) are in the reversed phase category. Here, the hydrophilic silanol groups at the surface of the raw silica packing (typically 60Å pore size, 40μm particle size) have been chemically modified with hydrophobic alkyl or aryl functional groups by reaction with the corresponding silanes. Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface. These nonpolar-nonpolar attractive forces are commonly called van der Waals forces, or dispersion forces. To elute an adsorbed compound from a reversed phase SPE tube or disk, use a nonpolar solvent to disrupt the forces that bind the compound to the packing (46).

Normal phase SPE procedures typically involve a polar analyte, a mid- to nonpolar matrix (e.g. acetone, chlorinated solvents, and hexane), and a polar stationary phase. Polar-functionalized bonded silicas (e.g. LC-CN, LC-NH2, and LC-Diol), and polar adsorption media (LC-Si, LC-Florisil, ENVI-Florisil, and LC-Alumina) typically are used under normal phase conditions. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, pi-pi interactions, dipole-dipole interactions, and dipole-induced dipole interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism usually a solvent that is more polar than the samples original matrix (46).

Ion exchange SPE can be used for compounds that are charged when in a solution (usually aqueous, but sometimes organic). Anionic (negatively charged) compounds can be isolated on LC-SAX or LC-NH2 bonded silica cartridges. Cationic (positively charged) compounds are isolated by using LC-SCX or LC-WCX bonded silica cartridges. The primary retention mechanism of the compound is based mainly on the electrostatic

attraction of the charged functional group on the compound to the charged group that is bonded to the silica surface. In order for a compound to retain by ion exchange from an aqueous solution, the pH of the sample matrix must be one at which both the compound of interest and the functional group on the bonded silica are charged. Electrostatic attraction of charged group on compound to a charged group on the sorbent surface. A solution having a pH that neutralizes either the compound functional group or the functional group on the sorbent surface is used to elute the compound of interest. Alternatively, a solution that has a high ionic strength, or that contains an ionic species that displaces the adsorbed compound, is used to elute the compound (46).

There are some disadvantages to solid-phase extraction, which are: (1) although solvent use is small, the solvent flow rate affects the recovery rate. (2) For samples which include suspended solid (SS), it is necessary to separate SS composition. (3) For samples, which are heavily contaminated, it is possible to get analyte break through. (4) In order to have high and stable recovery rates, it is important to choose the most appropriate solid phase for the target compounds (48).

Table 3 - Applications of SPE for volatile compounds analysis in different enological products (50).

Authors	Matrix	Column	Analytes	Extraction conditions
Wada and Shibamoto	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Solvent*: dichloromethane
López et al.	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume*: 50 mL; *Solvent*: dichloromethane
Culleré and others	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume*: 75 mL; *Solvents:* dichloromethane, pentane and pentane:dichloromethane (9:1)
Lukic et al.	Grape distillate	Octadecylsilica	Flavour	*Sample volume*: 3 mL diluted to 25 mL; *Solvent:* dichloromethane
Dieguez et al.	Spirits	Silica	Volatile organic acids	*Sample volume*: 50 mL; *Solvent:* dichloromethane
Genovese et al.	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Sample volume:* 50 mL; *Solvents:* pentane:dichloromethane (20:1) and dichloromethane
Karagiannis et al.	White wine	Silica	Terpenes	*Sample volume:* 25 mL; *Solvents:* dichloromethane and methanol
Piñeiro et al.	White wine	Styrene-divinylbenzene copolymer	Terpenes	*Solvent:* dichloromethane
Ferreira et al.	Wine	Styrene-divinylbenzene copolymer	Aliphatic lactones	*Sample volume:* 50 mL; *Solvent*: dichloromethane
Campo et al.	Wine, whisky and brandy	Styrene-divinylbenzene copolymer	Flavour	*Sample volume:* 100 mL; *Solvent:* dichloromethane
Insa et al.	Wine	Styrene-divinylbenzene copolymer	Anisoles	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Dominguez et al.	White wine	Styrene-divinylbenzene copolymer	Volatile phenols	*Sample volume:* 10 mL; *Solvent:* dichloromethane
Charles et al.	Vinegar	Styrene-divinylbenzene copolymer	Flavour	–
Morales et al.	Vinegar	Styrene-divinylbenzene copolymer	Flavour	*Solvent:* dichloromethane

SPE is a technique that has great applicability to enology, Wada and Shibamoto studied the extraction of odorant from red wines using Porapak Q columns (ethylvinylbenzene-divinylbenzene copolymer). Different solvents were tested, and dichloromethane was found to be the best, with recoveries near to 100% (50). A limited number of studies have

been performed with the view to systematically compare different SPE materials in terms of their extraction efficiency towards wine varietal aromatic compounds. In a study of the extraction of 100 aroma precursors in grape juice using C18, LiChrolut EN and Amberlite XAD-2 resins, maximum and minimum areas for terpenes and norisoprenoids were obtained with the C18 and Amberlite XAD-2 material, respectively (51).

Table 3 includes various different applications of SPE to wine and other enological products.

1.4.2) Static and dynamic headspace extraction

Headspace sampling techniques are frequently divided into two broad categories: static headspace, dynamic headspace (52). In each case, however, the fundamental principle is the same - volatile analytes from a solid or liquid material are sampled by investigation of the atmosphere adjacent to the sample, leaving the actual sample material behind. In static headspace techniques, a small sample (usually about 1 ml) of the atmosphere around the sample is injected directly onto the GC column (52). In dynamic techniques, the organic analytes from larger samples of the headspace are first concentrated, and then transferred to the GC. Dynamic headspace techniques in their simplest form, then, are just ways to transfer a headspace sample that is too large to inject directly. The term "dynamic headspace" is usually used when referring to the analysis of solid materials, and the term "purge and trap" generally refers to the analysis of liquid samples by bubbling the purge gas through them (53).

All headspace techniques share certain advantages and considerations.

The main advantage is that the analytes are removed from the sample matrix without the use of an organic solvent, so the resulting chromatogram has no solvent peak. This may be especially important when the compounds of interest are early eluters or are, in fact, solvents, and the presence of a solvent peak would both dilute and mask the analyte peaks (53). In addition, the effects of sample temperature, matrix solubility, and the volatility of the analyte are important considerations in optimizing a headspace assay, whether static or dynamic (53).

Recently a dynamic headspace method has been developed which permits the analysis of the volatile fraction of a wine by purging with an inert gas followed by thermal

desorption and gas chromatography. Coelho used HS-SPME to monitor volatile formation during maturation of Fernao-Pires grapes, sixteen monoterpenoids, two C_{13} norisoprenoids, two aromatic alcohols, two C_6 aldehydes, and three C_6 alcohols were identified as variety- and pre-fermentation-related volatile compounds of FP white grapes (27). Canuti also developed a headspace GC–MS method that was used to profile and quantify 27 free volatile compounds in the headspace of Cabernet Sauvignon fruit (54).

1.4.3) Solid phase micro-extraction (SPME)

Solid-phase micro-extraction (SPME) was introduced in 1990 by Pawliszyn's group as a (virtually) solvent-free preconcentration technique in which the analyte(s) is (are) adsorbed onto a fused-silica fiber coated with an appropriate sorbent layer by simple exposure of the fiber for a pre-selected time to the headspace of the sample or by direct immersion in a liquid sample (55). Despite been an equilibrium (i.e., non-exhaustive) technique and the initial limitations regarding the nature of the commercialized sorbent coating, SPME was rapidly accepted as a simple, reproducible, miniaturized and green technique, and its feasibility for fast and accurate analysis of compounds of different nature. Compared with conventional solvent extraction, solid-phase microextraction is a fast, easy to use, inexpensive and solvent-free procedure for aroma and flavor studies. The technique has been successfully applied to analyze volatile compounds of grapes and other fruit (55). Figure 10 shows the application of SPME in different food matrices.

To obtain profile of volatile compounds of particular food, SPME is since its invention the predominant method. The reason is that it offers a rapid way to obtain a "profile" of volatile compounds. However it has to be remembered that SPME profile does not reflect the actual composition of volatile compounds in a particular product, as it is a non-exhaustive method, but one based on the partition of analytes between phases (45).

Solid phase microextraction is based on the absorption–adsorption of analyte into a coating of an optic fiber (Figure 11), the variety of commercially available fiber coatings has increased significantly during the last year, something that has contributed to expand the range of analyte classes that can be successfully analyzed. Today, in addition to the originally introduced non-polar PDMS, semi-polar polydimethyl siloxane–divinylbenzene (PDMS–DVB), polar polyacrylate (PA), Carbowax–divinylbenzene (CW–DVB) liquid-

like phases, coated porous particle phases such a polydimethyl siloxane–Carboxen (PDMS–Carboxen), poly(3-methylthiophene) and Nafion are available (56).

However, when publications are screened for the type of SPME fibers used for extraction a domination of divinylbenzene/carboxene/polydimethylsiloxane fiber coatings can be observed. This type of fiber offers high efficiency in extracting food volatiles, and when several fibers are tested in the method development process, it often provides the highest peak areas of extracted compounds (45). SPME can be used for direct extraction of analytes from liquid phase, can be used for headspace extraction and in case of dirty matrices the fiber can be protected by membrane. Due to the specificity of food matrices – presence of sugars, lipids, proteins, colorants and other non-volatiles, SPME is used almost exclusively as a headspace extraction method (HS-SPME). Static headspace extraction (and also HS-SPME) is based on the partition of analytes and is a non-exhaustive extraction. However, static headspace can be an exhaustive method, when multiple extractions are performed from the same vial, allowing the sample to re-equilibrate after each extraction (45).

Two types of fiber SPME techniques can be used to extract analytes: HS – SPME and direct immersion (DI) - SPME. In HS - SPME, the fiber is exposed in the vapor phase above a gaseous, liquid, or solid sample. In DI - SPME, the fiber is directly immersed in liquid samples. Agitation of the sample is often carried out with a small stirring bar to increase the rate of equilibration. After a suitable extraction time, the fiber is withdrawn into the needle, the needle is removed from the septum and is then inserted directly into the injection port of the gas chromatograph or the desorption chamber of the SPME – high - performance liquid chromatography (HPLC) interface. HS - and DI - SPME techniques can be used in combination with any GC, GC– mass spectrometry (MS), HPLC, and HPLC - MS system. The desorption of analyte from the fiber coating is performed by heating the fiber in the injection port of a gas chromatograph or GC - MS, or by loading solvent into the desorption chamber of the SPME - HPLC interface, and then the analyte are transferred directly to the column for analysis (57).

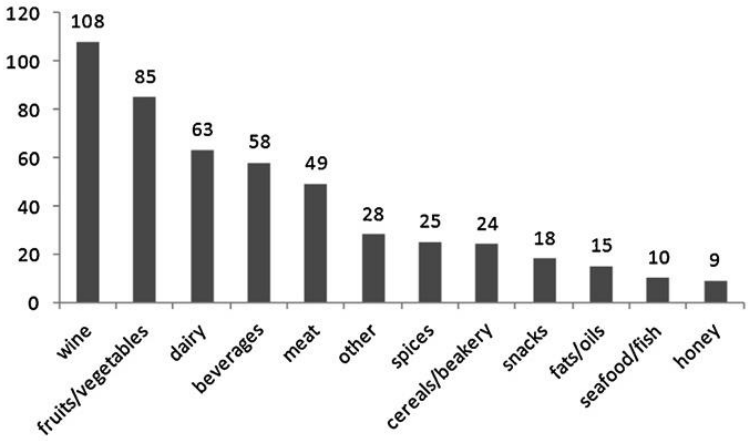

Figure 10 - Application of SPME to different food matrices. Number of papers based on Web of knowledge search for years 2006-2011 (45).

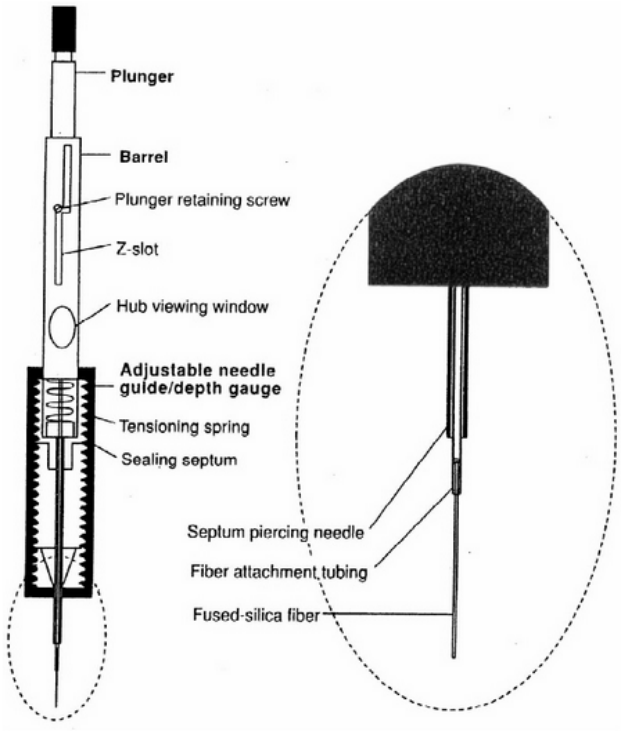

Figure 11 - Diagram of an SPME device (57).

1.4.4) Stir bar sorptive extraction (SBSE)

Stir bar sorptive extraction (SBSE) is a microextraction method introduced in 1999 by Baltussen, and is based, like SPME, on the use of an apolar sorbent polymer, polydimethylsiloxane (PDMS), as the medium of extraction of analytes in liquid and gaseous samples (45). In SBSE a coated stir bar can be added to the sample for stirring and extraction (direct SBSE) or exposed to the headspace (HS-SBSE). The amount of coating (PDMS) in SBSE is usually 50–250 times larger than in SPME, which increases the preconcentration efficiency, however increases equilibrium time due to the diffusion into the large volume of coating (45). PDMS presents a series of characteristics that have made it the sorbent material most commonly used for this type of technique. These include, in particular, its inert character, which reduces the risk that compounds may be generated on its surface; the relative ease with which it can be synthesized. Therefore the extraction times described in literature for SBSE are usually longer than that for SPME (50). Stir bar sorptive extraction found numerous applications in food flavors/volatiles analyses the main being wine analysis, beverages, detection of off-flavors, monitoring metabolism of flavor compounds (45). Due to the high volume of phase in SBSE stirrer, which results in low detection limits, it is a good tool for the analysis of off flavor (45).

SBSE presents a series of clear advantages. This technique is solvent-free, unlike SPE. This brings various additional advantages: the samples are not in contact with any solvent, and so are less likely to be altered by contamination or the formation of artifacts during the extraction process. For this same reason, the technique is much friendlier to the natural environment, since it does not generate residuals of any kind (50). Another advantage of this technique is that it can be almost completely automated thus making it very simple and fast to apply repeatedly. The technique requires almost no handling of the sample on the part of the analyst, nor does it require prior treatment of the sample. This means that the possibility of analytical error is considerably reduced (50). Compared with SPME, SBSE provides greater analytical sensitivity: it reaches much lower detection and quantification limits. The reason for this is that, in SBSE, the quantity of PDMS employed is rather greater, with the result that the extractive capacity is also greater (50).

Several studies have been published using this methodology to characterize the volatile fraction of wine and grape juice (58–61).

1.4.5) Analysis of free and glycosidically-linked flavors

Analysis of bound aroma compounds can be performed in two ways: (1) by analyzing whole glycosides or (2) by analyzing odoriferous aglycones after hydrolysis. Analysis of intact glycosides of aroma compounds performed by gas chromatography requires derivatization (due to their polarity and nonvolatility) and is performed rarely also because of lack of commercially available standards of glycosides and the need for their synthesis, usually using the methods of Koenig–Knorr and Schmidt (Figure 12). Compounds used most frequently for the derivatization are: N-methyl-bis-trifluoroacetamide; TMS 1% TMCS; N,O-bis(trimethylsilyl)-trifluoroacetamide 1% TMCS; and 1-trimethylsilylimidazole (19).

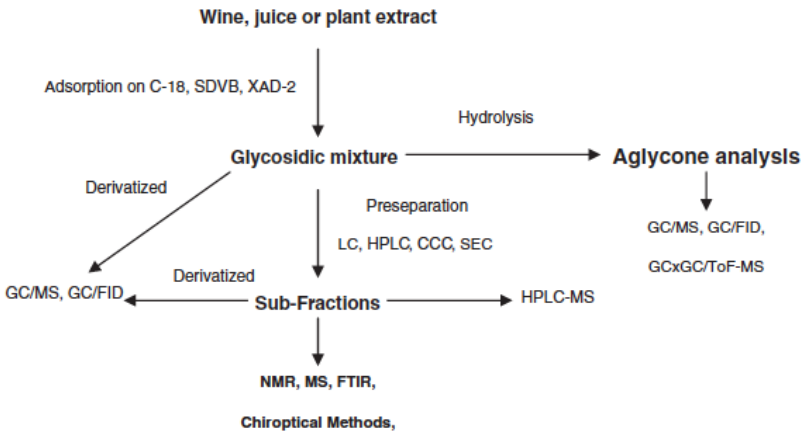

Figure 12 - Scheme of sample preparation and analysis of bound flavor compounds (19).

Different methodologies have been proposed to extract glycoside precursors from grape juices and wines. Williams et al. (1982) used glass column chromatography containing C-18 reversed-phase adsorbent to extract glycosides from juice or de-alcoholised 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-18 cartridges: hydrophilic compounds were eluted with water, free terpenes with dichloromethane and glycosides with methanol. This method has been improved in recent years, but it has a disadvantage, the separation is different depending on the commercial origin of the

cartridges. 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. This resin had been previously used to isolate naringin and limonin from grape juices.

1.4.5.1) Acidic hydrolysis of glycosides

Acidic hydrolysis of glycosides can induce a molecular rearrangement of the monoterpenols, which are transformed in other compounds (Figure 13). Nevertheless, these ways to liberate terpenes simulate the reactions taking place during ageing of wines, and the different terpenic alcohols were produced in similar quantitative rations. Experiments on both whole juice and monoterpene glycosides isolated from juice have demonstrated that significantly different patterns of volatile monoterpenes are produced when each is hydrolyzed at different pH values (30,31).

Most of the compounds given under hydrolytic conditions at pH 3.0 are the free terpenes of the juice. Grape glycosides are made up predominantly of geranyl, linalyl and neryl derivatives and only trace quantities of α-terpenyl glycosides, the hydrolysis products at pH 3.0 are dominated by linalool and α-terpineol, with geraniol relatively less abundant (30).

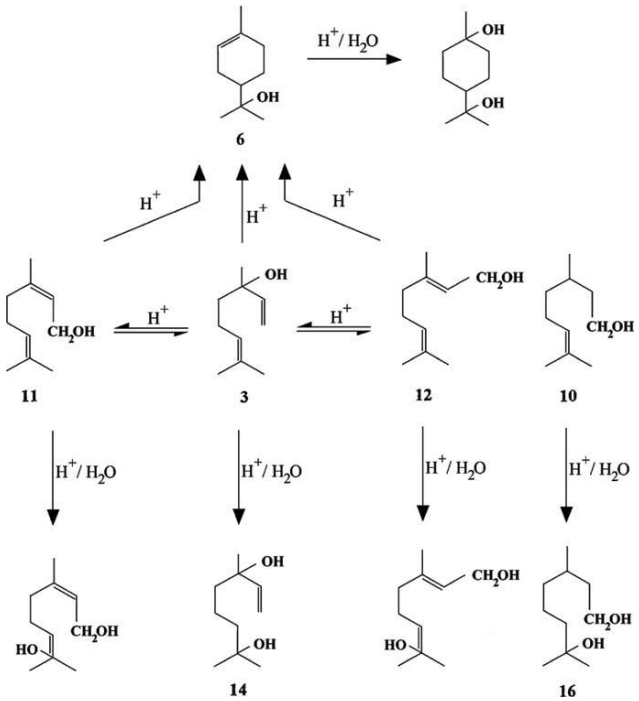

Figure 13 - Rearrangement of monoterpenes in acidic conditions (30).

1.4.5.2) Enzymatic hydrolysis

Glycosides can also be hydrolyzed by an enzymatic way, a more interesting way because it produces a more "natural" flavor. As an alternative, enzymatic methods hold the potential for increasing the concentration of free flavorants in grape juice with minimal change in the natural monoterpene composition. Studies conducted by Gunata et al. demonstrated that enzymatic hydrolysis of grape monoterpenyl diglycosides proceeds in two steps: firstly, the inter-sugar linkage is cleaved by either α-L-rhamnosidase, α-L-arabinosidase or β-D-apiosidase regardless of the structure of aglycon moiety and the corresponding monoterpenyl β-D-glucoside are released (Figure 14). The liberation of the aglycon moiety can only take place during the second step, which consists in the action of a β-D-glucosidase on the previous monoterpenyl β-D-glucosides (30)(33).

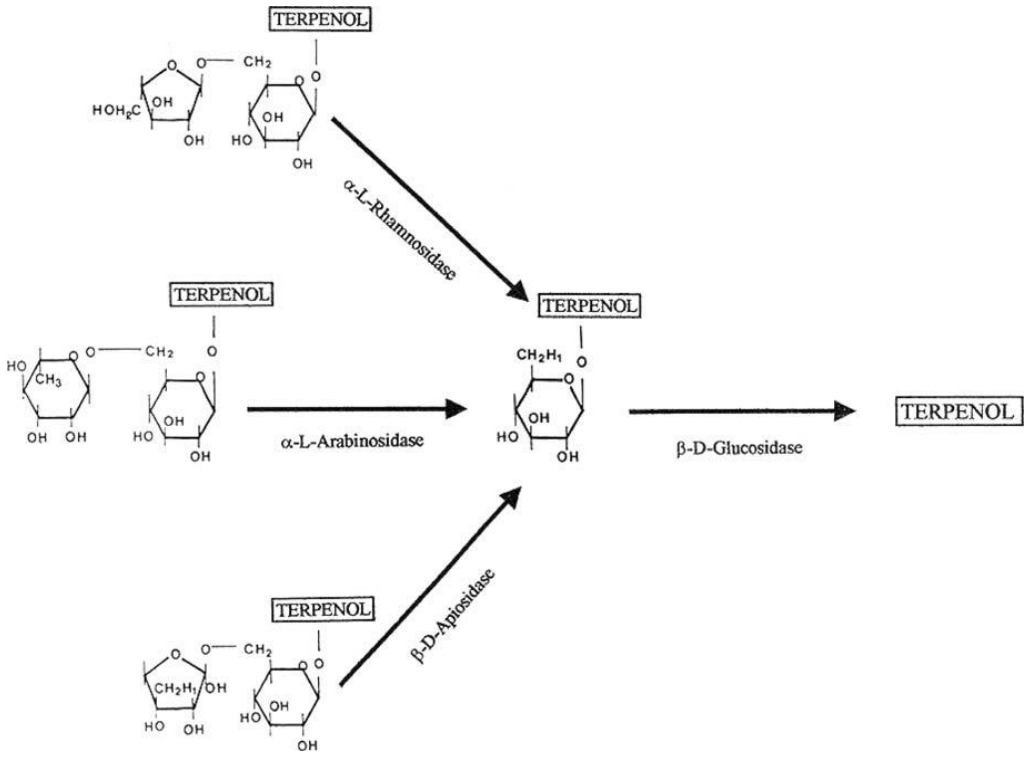

Figure 14 - Sequential enzymatic hydrolysis of disaccharidic flavor precursors (30).

Nevertheless, one-step hydrolysis of disaccharide glycosides has also been described by Gunata et al., enzymes catalyzing this reaction have been isolated from tea leaves and

grapes. This one step reaction occurs through the cleavage of the aglycone linkage, which yields a disaccharide, and aglycone, the identity of which have been confirmed by HPLC and GC/MS (30). Enzymatic hydrolysis of glycoside extracts from Muscat, Riesling, Semillon, Chardonnay, Sauvignon and Sirah varieties have provoked the liberation not only of terpenes, but also C_{13} norisoprenoids such as 3-oxo-α-ionol and 3- hydroxy-β-damascenone (30). These compounds are totally glycosylated in the grape and, as opposed to 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 flavor, because they have lower threshold values than terpenes, and they contribute characteristic aromatic features (31).

1.4.6) Analysis of free and bound compounds in grapes by Gas Chromatography

Separation methods are an important part of analysis, and chromatography has developed into the premier analytical separation technique. Chromatography rapid development can be attributed to its relative simplicity and the successful application of theory to practice. Furthermore, when equipped with sensitive detectors, chromatographs are capable of performing highly accurate quantitative analysis.

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) whole the other (the mobile phase) moves in a definite direction. Elution chromatography is a procedure in which the mobile phase is continually passed through or along the chromatographic bed and the sample is fed into the system in a definite slug. The separation process is achieved by distributing the components of a mixture between the two phases. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase ((62).

Developments in analytical methods have been closely linked to improved understanding of grape and wine flavor chemistry (42). Currently, there is much interest in rapid, high-throughput methods for quantifying volatile components and monitoring qualitative changes in volatile composition as a result of viticultural practices, winemaking

techniques, or storage processes. One-dimensional chromatographic processes are widely applied in the analysis of food products. Although such methods often provide rewarding analytical results, the complexity of many naturally occurring matrices exceeds the capacity of any single separation system. As a consequence, in the past years considerable research has been dedicated to the combination of independent techniques with the aim of strengthening resolving power (63,64). Due to the complexity of wine volatile fractions, identification and quantification of constituents (especially minor ones) using conventional one-dimensional (1-D) chromatography is hampered by frequent co-elutions, even when using high-efficiency capillary columns, selective stationary phases and programmed oven temperature conditions.

Two-dimensional techniques, especially the development of comprehensive GC x GC, have greatly improved the analysis of grape and wine volatiles (42).

Comprehensive two-dimensional gas chromatography (GC x GC) employs two orthogonal mechanisms to separate the constituents of the sample within a single analysis. The technique is based on the application of two GC columns coated with different stationary phases, such as one apolar and one polar, connected in series through a special interface (modulator) (64).

The interface cuts small (several seconds) portions of the first dimension eluate by cryofocusing, and re-injects it onto the second column. Each first dimension peak is modulated several times, which allows the preservation of the first dimension separation. The second column is very short and narrow and consequently each modulated portion is "flash" separated before the next modulation starts. Using this instrumental approach, compounds co-eluting from the first column undergo additional separation on the second one (64). Therefore, the separation potential is greatly enhanced when compared to the one-dimensional GC. Besides chromatographic separation, sensitivity and limits of detection are also improved due to the focusing of the peak in the modulator and the separation of analytes from chemical background. GC x GC also offers new opportunities to develop relationships between molecular structure and retentions in the two-dimensional separation space defined by the GC x GC retention in the combined dimensions (64).

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC x GC–ToF-MS) offers unprecedented separation power in multiresidue analysis. Combination of a long non-polar with a short and polar capillary column

connected in series through a thermal modulator provides enormous peak capacity, which is utilized in separating mixture of large number of compounds in single chromatographic run. The TOF mass analyzer further enhances the separation process on the basis of relative flight times of ions as decided by their mass/charge (m/z) ratio (65).

Ryan, Watkins, Smith, Allen and Marriott used GC x GC in combination with nitrogen phosphorus detection (NPD) and time-of-flight mass spectrometry (ToF-MS) for the identification of methoxypyrazines in Sauvignon Blanc wine (42). Other authors Rocha, Coelho, Zrostlíková, Delgadillo, and Coimbra applied GC x GC-ToF-MS for the analysis of grape volatiles in *Vitis vinifera* L. cv. "Fernão-Pires" white grape and identified 56 monoterpenoids, 20 of which were identified for the first time in grapes (27).

GC has become the premier technique for the separation and analysis of volatile compounds, and gas chromatographs have been the most widely used analytical instrument in the world, although HPLC is becoming more widely used. Clearly, GC is a major analytical method and it is complemented by the other major form of chromatography, HPLC, which is capable of handling the nonvolatiles not suited to GC. The currently accepted status between them is that GC can be used up to 350º C corresponding to an upper molecular weight limited of 600 D, and HPLC is used for higher molecular weight compounds. However, high temperature GC work has been done up to 450º C (19).

1.5) Aims of this work

During this work, six *V. vinifera* L. white grape varieties were collected with the aim of studying their individual volatile profile. After a careful sampling and obtainment of grape juice, solid phase extraction was used to fractionate the free volatile fraction from the glycosidically-bound fraction. Multidimensional gas chromatography coupled with time-of-flight mass spectrometry was used to analyse the volatile composition of each variety.
This study will allow us to characterize chemically each variety predicting their aroma, compare varieties with one another, evaluate the efficiency of two different methodologies of releasing glycosidically-bound volatiles, and draw some conclusions relatively to the

potential glycosidically-bound aroma that can be released during the process of winemaking.

2) Materials and Methods

2.1) Fruit supply

Six white wine grape varieties (Mília, Merzling, Traminer, Freiminer, Jutrenzka and Adalmiina) from 2011 harvest were collected in Jazło Appelation from Golesz vineyard, southeast Poland. Check section 1.3 for grape variety information. Samples were transported to the laboratory and were stored in a freezer at − 50 ºC until analysis.

2.2) Reagents and standards

Bond Elut C18 cartridges, 500 mg 6 mL were acquired from Agilent Technologies and used to perform Solid phase extraction. Standard of $^{2}[H]_{7}$-geraniol or a standards mixture of $^{2}[H]_{7}$ geraniol, $^{2}[H]_{8}$ naphthalene and $^{2}[H]_{2}$ β-ionone were used for quantification purposes. Pentane, dichloromethane and methanol (CHROMASOLV® HPLC grade) were obtained from Sigma-Aldrich.

Mc'Ilaine buffer was prepared using citric acid 0,1 M and Na_2HPO_4 0,2 M solutions in different proportions according to the desired pH, which was controlled using an Elmetron CP-411 pH meter. Rapidase AR 2000, composed of pectinases with glycosidases side activities, was obtained from DSM in order to hydrolyze grapes glycosides and enhance juice aroma.

2.3) Sample preparation

The grapes were destemmed then weighted, 1 to 1,5 kg of each grape variety were used for the analysis, 10 uL of internal standard and distilled water was also added. The grapes were then homogenized using a MPW-120 and a CAT Undrive X 1000 homogenizer in order to obtain the must. Two rounds of centrifugation were used in order to obtain a clear juice separated from the skins and seeds, the first round at 4000 rpm for 15 minutes, the second at 15000 rpm for 10 minutes. The second centrifugation was eventually replaced

with a vacuum centrifugation. The juice was then ready for analysis or short term storage to avoid fermentation.

2.4) Solid phase extraction of volatile compounds from grape juice

Solid phase extraction was used for extraction of volatile compounds from grape juice. The extraction was performed using Bond Elut C18, 500 mg, 6 mL cartridges and Supelco Visiprep SPE station. About 120 mL of grape juice was taken for analysis. The cartridges were preconditioned using methanol followed by deionized water (3 mL/min, pressure 0,67 atm). The juice was then applied to the column, after application the column was washed with deionized water. Non-polar faction was eluted using 20 mL of a mixture of pentane/dichloromethane 2:1 (v/v). Subsequently, polar faction was eluted using 20 mL methanol.

2.4.1) Analysis of non-polar fraction

The non-polar faction was salted out with disodium phosphate (Na_2HPO_4) in order to remove traces of water, the faction was then evaporated to 500 µL using a rotary evaporator at 40ºC, no reduced pressure was used. 1 µL of sample was introduced in a splitless mode into GC/MS and GCxGC-ToF-MS systems.

2.4.2) Analysis of polar fraction

2.4.2.1) Acid hydrolysis

Polar fraction was evaporated to dryness under a stream of nitrogen at 40ºC. The sample was then rehydrated using Mc'Ilaine buffer pH 2,5 and the hydrolysis was performed at 100ºC and lasted 1 hour. Posteriorly the vial was cooled down and 10 uL of internal standard was added. The hydrolyzate was loaded into a preconditioned (methanol and deionized water) SPE cartridge column and freed compounds were eluted with a pentane/dichloromethane mixture 2:1 (v/v). The sample was then concentrated to 500 uL in

a similar way as the non-polar fraction in 2.5, and 1 uL injected in a splitless mode into GC/MS and GCxGC-ToF-MS systems.

2.4.2.2) Enzymatic hydrolysis

Polar faction was evaporated to dryness under a stream of nitrogen at 40ºC. The sample was then rehydrated using a small portion of Mc'Ilaine buffer pH 5,5. A commercial enzyme preparation with glycosidase activity, Rapidase AR 2000 was used to perform the hydrolysis. One gram of the enzyme preparation was diluted in 50 mL Mc'Ilaine buffer pH 5,5 and then added to the sample. The hydrolysis reaction was carried out at the temperature of 40ºC during 21 h. Posteriorly the vial was vortexed to denature the enzyme and 10 µL of internal standard were added. The hydrolyzate was loaded into a preconditioned (methanol and deionized water) SPE cartridge column and freed compounds were eluted with a pentane/dichloromethane mixture 2:1 (v/v). The sample was then concentrated to 500 µL in a similar way as the non-polar fraction in 2.5, and 1 µL injected in a splitless mode into GC/MS and GCxGC systems.

2.5) GCxGC-ToF-MS analysis of samples prepared by SPE approach

Analyses of free and liberated after hydrolysis volatile compounds were performed using GC x GC–ToF-MS system (Pegasus IV, LECO, St. Joseph, IL) running in both one and two-dimensional modes. The GC was equipped with a DB-5 column (25 m x 0.200 mm x 0.33 µm), as a first dimension column and Supelcowax 10 (1.3 m x 0.1 mm x 0.1 um) as a second dimension column. For 1D analysis the secondary oven was kept at a temperature 30 ºC higher than the first oven for which a temperature program was used from 40 ºC (1 min) at 5 ºC/min to 220 ºC and kept for 5 min. Mass spectra were collected at a rate of 50 scans/s and the detector voltage was 1750 V. For two-dimensional (comprehensive) chromatography the temperature of the second oven was kept 5 ºC higher than first oven. Modulation time was optimized and set at 5 s, mass spectra were collected at a rate 100 scans/s.

2.6) Semi-quantification

The relative concentrations of grape volatiles in all 6 varieties were determined by GCxGC-ToF-MS (TIC) by comparison with concentrations of internal standards, assuming a response factor of 1. $^{2}[H]_{7}$-geraniol or a mixture of $^{2}[H]_{7}$ geraniol, $^{2}[H]_{8}$ naphthalene and $^{2}[H]_{2}$ β-ionone were used as the internal standards.

3) Results and discussion

In order to estimate the volatile composition of each grape variety, free and glycosidically-linked compounds from the musts were analyzed using solid phase extraction and GCxGC-ToF-MS. The bound fraction was obtained either by enzymatic treatment or acidic hydrolysis at high temperatures (100ºC). Concurrently, the volatile profile obtained from these two different treatments was subjected to a comparative study.

Concentrations of each compound were calculated based upon the corresponding obtained peak area, comparatively to internal standard peak area.

Total concentrations obtained in both free and bound fractions were calculated based on the sum of each individual concentration of the identified compound. This should be considered as a semi-quantitative analysis, which assigns approximate measurements to data, rather than an exact measurement.

All the grape varieties analyzed have shown to contain a large range of volatiles. Aliphatic and aromatic alcohols, ketones, terpenoid compounds, aliphatic acids, C_{13} norisoprenoids, were present in the grape juice in different concentrations and distribution.

After a careful analysis of the spectra the most relevant volatile compounds were selected to characterize each variety. Compounds not reported in the literature or present in trace quantities and not preponderant to varietal aroma were discarded.

Because of the considerable significance of volatile monoterpenes to flavor and varietal character of *V. vinifera* L. varieties (31), particular attention was devoted to these compounds. Alcohols were also the object of particular consideration because, quantitatively, the alcohol fraction was one of the main chemical groups present in the juices.

3.1 – Free and bound volatile compounds in Mília grape juice

The volatile composition of the Mília grape juice and its different distribution in the free form and bound form is shown in Table 4. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 15) and the most important chemical classes are highlighted.

The total free volatile compounds from Mília grape juice accounted for 2492,62 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 454,87 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 855,43 mg L^{-1} (Figure 16).

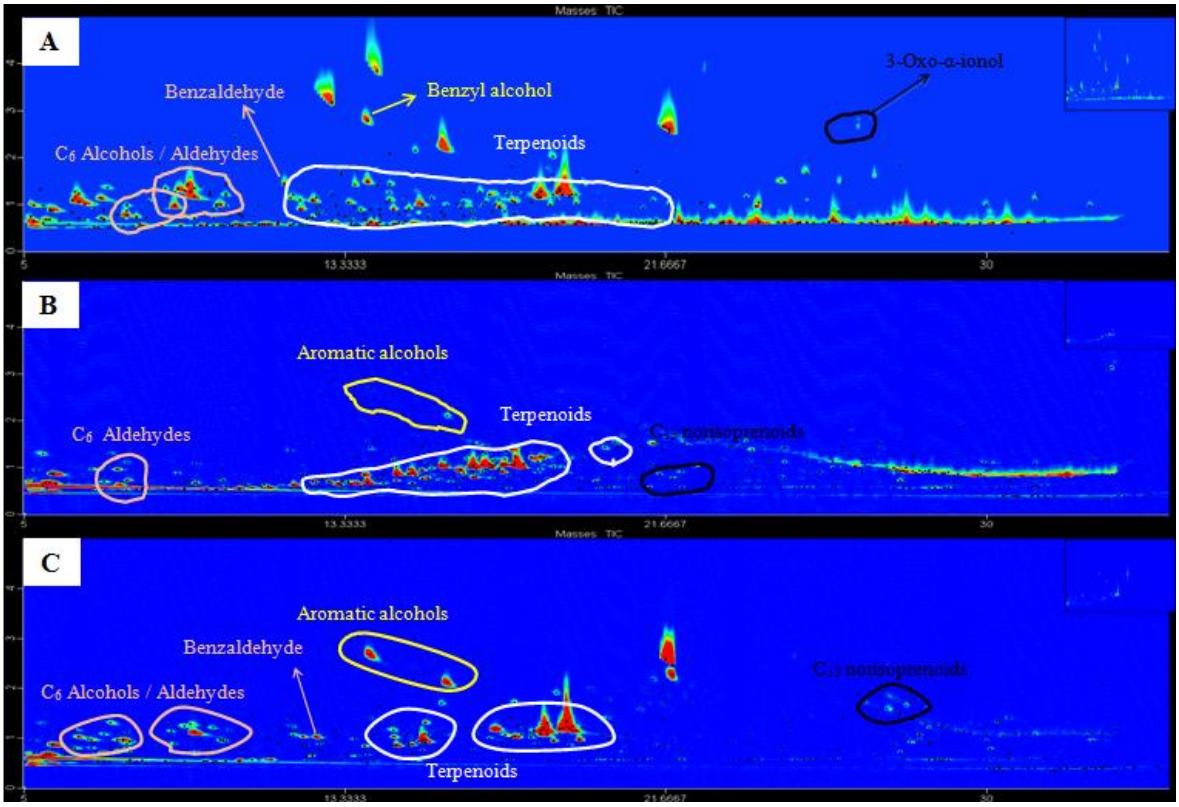

Figure 15 – GCxGC-ToF-MS chromatogram of the volatile compounds in Mília grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

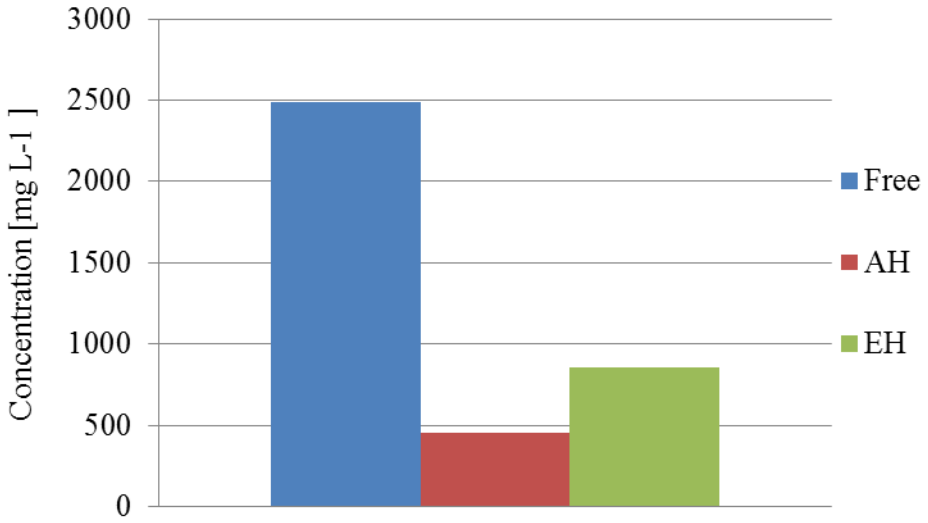

Figure 16 - Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Mília grape juice.

Table 4 - Free and bound volatile compounds identified in Mília grape juice, grouped by chemical classes.

No.	Compound [a]		Concentration		
			Free [μL^{-1}] (n=1)[b]	Bound [μL^{-1}]	
				Acidic (n=1)	Enzymatic (n=1)
	Terpenoids				
1	Camphene	11.3285 , 0.614	n.d.	671,58	n.d.
2	*β*-Myrcene[c]	12.4943 , 0.653	4798,49	n.d.	n.d.
3	*p*-Menthane	13.1605 , 0.653	n.d.	10854,96	n.d.
4	Carene	13.2437 , 0.647	n.d.	866,12	n.d.
5	Limonene	13.4103 , 0.693	7661,87	838,87	n.d.
6	*β*-Cymene	13.4103 , 0.726	n.d.	1381,2	n.d.
7	*β*-Phellandrene	13.4935 , 0.693	3544,47	n.d.	n.d.
8	Eucalyptol	13.4935 , 0.700	2827,67	21825,86	n.d.
9	*β*-Ocimene (isomer)	13.8266 , 0.713	2154,68	n.d.	941,06
10	*β*-Ocimene (isomer)	13.9099 , 0.680	n.d.	n.d.	51591,91
11	1,6-Dihydrocarveol	14.9924 , 0.865	n.d.	4195,5	n.d.
12	Linalool oxide (isomer)	14.9924 , 0.917	28245,52	61293,89	27033,54
13	Terpinolene	15.0757 , 0.680	n.d.	1771,81	n.d.
14	*p*-Cymene	15.1589 , 0.838	n.d.	1223,63	n.d.
15	Linalool	15.3255 , 1.003	119336,9	6015,16	107954
16	Rose oxide	15.492 , 0.759	4354,95	1113,02	252,71
17	Myrcenol	15.8251 , 1.043	n.d	36384,08	n.d.
18	*cis*-Thujane-4-ol	15.9916 , 0.997	10772,95	n.d.	n.d.
19	1-Terpinenol	16.2415 , 1.003	2264,24	n.d.	n.d.
20	*β*-Terpineol (isomer)	16.3247 , 0.950	n.d.	9636,49	n.d.
21	*p*-2-Menthen-1-ol	16.3247 , 0.950	n.d.	19584,79	n.d.

Table 4 (continued)

22	Lilac aldehyde (isomer)	16.3247 , 0.957	4972,78	n.d.	n.d.
23	Nerol oxide	16.3247 , 0.957	2663,35	10703,73	571,09
24	*cis*-2-*p*-Menthen-1-ol	16.408 , 1.076	18934,35	n.d.	n.d.
25	Lilac aldehyde (isomer)	16.9076 , 0.997	5170,28	n.d.	n.d.
26	Ocimenol	16.9076 , 1.069	n.d.	58695,43	n.d.
27	3-Pinanone	17.0741 , 0.620	14677,61	n.d.	n.d.
28	Epoxylinalol	17.0741 , 1.228	41283,79	1260,4	40941,06
29	Dihydro-*γ*-terpineol	17.2407 , 1.023	17617,07	n.d	n.d
30	Terpinen-4-ol	17.324 , 0.957	45684,99	7901,24	n.d
31	*α*-Cyclogeraniol	17.4905 , 1.076	n.d	19812,87	6114,31
32	*α*-Terpineol	17.657 , 1.089	40313,61	73088,14	18736,33
33	Hotrienol	17.7403 , 1.247	n.d	83734,41	6494,27
34	Myrtenol	17.8236 , 1.274	4208,053	n.d	545,62
35	Lilac alcohol (isomer)	17.9069 , 1.089	n.d	n.d	699,36
36	*cis*-*p*-Menth-1-en-3-ol	18.0734 , 1.148	4186,21	n.d	n.d
37	Lilac alcohol (isomer)	18.1567 , 1.109	n.d	n.d	584,43
38	Lilac alcohol (isomer)	18.2399 , 1.168	n.d	n.d	1501,96
39	Menthol acetate	18.3232 , 1.168	64098,85	n.d	n.d
40	Citronellol	18.4065 , 1.096	n.d	n.d	37873,68
41	6-Camphenol	18.4065 , 1.241	n.d	8454,6	n.d
42	*cis*-Geraniol	18.4065 , 1.274	154629,9	690,34	174001,9
43	*β*-Citral	18.6563 , 1.010	20479,84	n.d	7269,31
44	Carvone	18.8228 , 1.129	3988,19	n.d	n.d
45	Geraniol	18.9061 , 1.320	87583,47	n.d	n.d
46	*trans*-Geraniol	19.2392 , 1.214	95063,35	n.d	58956,75
47	*α*-Citral	19.3224 , 1.036	11525,23	n.d	13459,66
48	*trans*-Dihydrocarvone	19.9886 , 0.627	50834,1	n.d	n.d
49	Geranic acid	21.654 , 2.647	356696,4	n.d	114237
50	Nerolidol	28.4821 , 1.228	n.d	n.d	1102,51
Sub-total (µL^{-1})			**1244257,86**	**441998,11**	**670862,55**
Sub-total (%)			**49,92**	**97,17**	**78,42**
	C_{13} Norisoprenoids				
51	1,2-dihydro-1,1,6-trimethyl-naphthalene	21.4875 , 0.865	n.d	489,36	n.d
52	*β*-Damascenone	22.0704 , 0.917	n.d	724,23	n.d
53	3-Oxo-*α*-ionol	27.3996 , 1.868	n.d	n.d	1133,31
54	Dihydro-*β*-ionone	27.6494 , 1.775	n.d	n.d	393,83
Sub-total (µL^{-1})			**0**	**1213,59**	**1527,14**
Sub-total (%)			**0**	**0,27**	**0,18**
	Alcohols				
55	3-Methyl-1-butanol	6.41559 , 1.036	265786	n.d	14443,32
56	3-Hexanol	7.66464 , 0.904	n.d	n.d	2606,42
57	2-Hexanol	7.66464 , 0.990	10955,9	n.d	8444,74
58	3-Hexen-1-ol	8.91369 , 1.267	107591,1	n.d	5563,85
59	*(E)*-2-Hexen-1-ol	9.24677 , 1.426	125915,4	n.d	4609,81
60	1-Hexanol	9.41331 , 1.109	198429,6	450,27	45718,87
61	*(Z)*-2-Hexen-1-ol	9.41331 , 1.353	30779,9	n.d	n.d
62	2-Heptanol	10.0795 , 1.010	28337,79	n.d	n.d
63	2-Heptanol	10.1627 , 0.957	n.d	n.d	4874,8

Table 4 (continued)

64	3-Hepten-1-ol	10.246 , 1.208	21784,64	n.d	n.d
65	1-Heptanol	11.9114 , 1.142	23024,67	n.d	3840,5
66	2-Hepten-o1-ol	11.9947 , 1.241	n.d	n.d	1686,39
67	1-Octen-3-ol	12.2445 , 1.036	23024,67	316,22	6410,22
68	Benzyl Alcohol	13.9932 , 2.686	107406	81,64	40326,32
69	1-Octanol	14.5761 , 1.043	19250,04	n.d	5600,12
70	Phenylethyl alcohol	15.9916 , 2.112	n.d	1240,61	34648
Sub-Total (μL^{-1})			**951329,7**	**2088,74**	**178773,36**
Sub-Total (%)			**38,17**	**0,46**	**20,90**
	Aldehydes				
71	Hexanal	7.74791 , 0.739	115212,7	3017,18	1512,85
72	2-Hexenal	8.83042 , 0.884	125333,3	1005,9	n.d
73	2-Ethyl-hexanal	9.49658 , 0.686	n.d	2827,57	1410,64
74	Benzaldehyde	11.7449 , 1.393	49908,89	435,21	514,23
75	2,4-Heptadienal	12.5776 , 1.076	6577,98	n.d	n.d
76	Nonanal	15.3255 , 0.772	n.d	2280,6	825,06
Sub-Total (μL^{-1})			**297032,89**	**9566,46**	**4262,78**
Sub-Total (%)			**11,92**	**2,10**	**0,50**
Total (μL^{-1})			**2492620,45**	**454866,90**	**855425,84**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.1.1) Free and bound terpenoids in Mília grape juice

The terpenoid composition in Mília grape juice is the most relevant. Quantitatively, free terpenoids accounted for 1244257,86 µg/L representing nearly 50 % of the total considered amount of free volatiles. In contrast, both acidic and enzymatic bound fractions accounted for 97,17 % (441998,11µg L^{-1}) and 78,42 % (670862,55 µg L^{-1}), respectively, of their total analyzed concentration of volatiles. The concentration of free terpenoids is significantly higher in comparison to the concentration of released bound compounds (Figure 17).

Geranic acid, *trans*-geraniol, *cis*-geraniol, geraniol, *α*-citral, *β*-linalool are the main free terpenoids present in Mília grape juice. Other compounds such as menthyl acetate, epoxylinalool, terpinen-4-ol, linalool oxide, rose oxide, and *α*-terpineol were identified and are worth mention. The main bound terpenoids obtained with acidic hydrolysis are *α*-

terpineol, hotrienol, ocimenol, eucalyptol, and *cis*-linalool oxide, while *cis*-geraniol, *trans*-geraniol, geranic acid, citronellol, linalool oxide, and linalool.

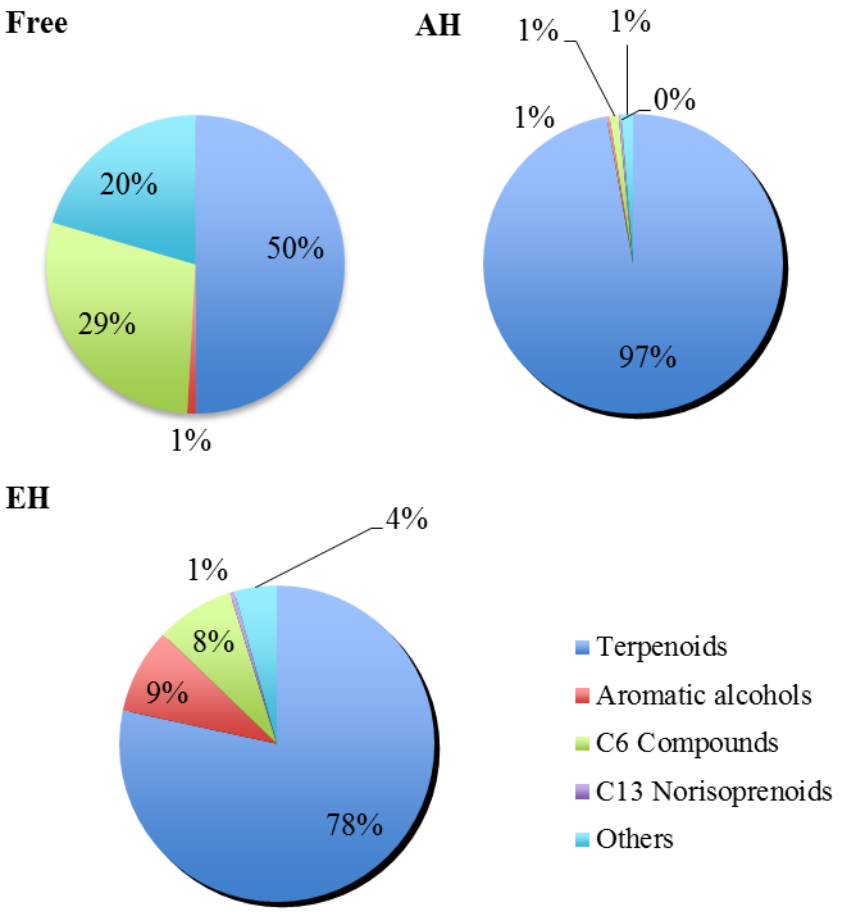

Figure 17 - Volatile composition of Mília grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.1.2) Free and bound alcohols in Mília grape juice

The alcohol composition of Mília grape juice was a major one, ranging from 38,17 % (951329,7 µg L^{-1}) in the free fraction to 20,90 % (178773,36 µg L^{-1}) in the enzymatic hydrolyzed fraction. The acidic hydrolysis fraction was particularly poor in alcohols

representing merely 0,46 %. This fraction is composed mainly by n-alcohols of C_6 chain length and aromatic compounds such and phenylethyl alcohol and benzyl alcohol.

1-Hexanol is the most abundant alcohol present in Mília grape juice, other C_6 alcohols such as 2-hexanol, *(E)*-2-hexen-1-ol, *(Z)*-2-hexen-1-ol, 3-hexen-1-ol, 3-hexanol. Phenylethyl alcohol is present in the bound form and is one of the main compounds of this class while benzyl alcohol is present abundantly in both free and bound forms.

3.1.3) Other compounds

C_{13} norisoprenoids are present in Mília grape juice only in its bound form, being completely absent in the free fraction. *β*-Damascenone and 1,2-dihydro-1,1,6-trimethyl-naphthalene were identified in the bound fraction hydrolyzed in acidic conditions while dihydro-*β*-ionone and 3-oxo-*α*-ionol were identified in the enzymatically hydrolyzed fraction.

Aldehydes, in Mília grape juice, are present mostly in the free form reaching nearly 12% of its total. Hexanal, 2-hexenal, and benzaldehyde are the most important aldehydes in Mília grape juice and are present abundantly in the free fraction and in smaller quantities in the bound fraction.

3.1.4) Concluding remarks

The results of this analysis show that Mília grape juice features a rich free volatile fraction, predominantly composed by terpenoids and alcohols. Comparatively, the bound fraction is considerable poorer in volatiles. Enzymatic hydrolysis yielded 40 compounds and shows to be more efficient, in releasing glycosidically bound volatiles, than acidic hydrolysis, which yielded 35 compounds. Terpenoids show to be the main class of compounds present in Mília grape juice and their contribution is particularly important in the bound fraction. The presence of terpenes, in their different forms, in grape juice

represents an enormous potential in a way to increase the varietal aroma, contributing with higher fruit-like characteristics (31).

The monoterpenol geraniol is the most abundant terpenoid in Mília grape juice and is above its perception limit of 30 μL^{-1} contributing with a floral, rose, and sweet odor (9).

α-Citral (OT=32 μL^{-1}), linalool (OT=15 μL^{-1}), citronellol (OT=40 μL^{-1}), rose oxide (OT=0,5 μL^{-1}), citronellol (OT=40 μL^{-1}), *α*-terpineol (OT=330-350 μL^{-1}), and hotrienol (OT=110 μL^{-1}) further contribute with floral fruity, rose, and citrus aromas in Mília grape juice.

C_6 compounds comprise alcohols and aldehydes, and are formed from linoleic acid and linolenic acid when grapes enter into contact with the air, and are formed by the actions of lipoxygenase, peroxidase and alcohol dehydrogenase enzymes (28). 1-Hexanol (OT=4,8 mg L^{-1}), the main identified C_6 alcohol, is above its sensory perception and its responsible for herbaceous and greasy odors. C_6 aldehydes, hexanal (OT=4,5-5 μL^{-1}) and 2-hexenal (OT=17 μL^{-1}) are also above their odor threshold and are responsible for green, grassy, and fruity aromas .

C_{13} norisoprenoids are glycosidically-bound in Mília grape juice and can contribute to varietal aroma when released. *β*-Damascenone is a powerful odorant with a low odor threshold of 0,002 µg L^{-1} in water and has been described as flowery.

Other important compounds such as benzyl alcohol with floral odors, benzaldehyde with almond like aroma and phenylethyl alcohol a common sweet, flowery odorant contribute to Mília grape juice varietal aroma.

3.2) Free and bound volatile compounds in Merzling grape juice

The volatile composition of the Merzling grape juice and its different distribution in the free form and bound form is shown in Table 5. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 18), the most important chemical classes are highlighted.

The total free volatile compounds from Merzling grape juice accounted for 127,03 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 18,15 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 400,36 mg L^{-1}. There was a noticeable decrease in released volatile compounds in the acidic treatment at high temperature. Conversely, enzymatic hydrolysis yielded a high amount of volatiles (Figure 19).

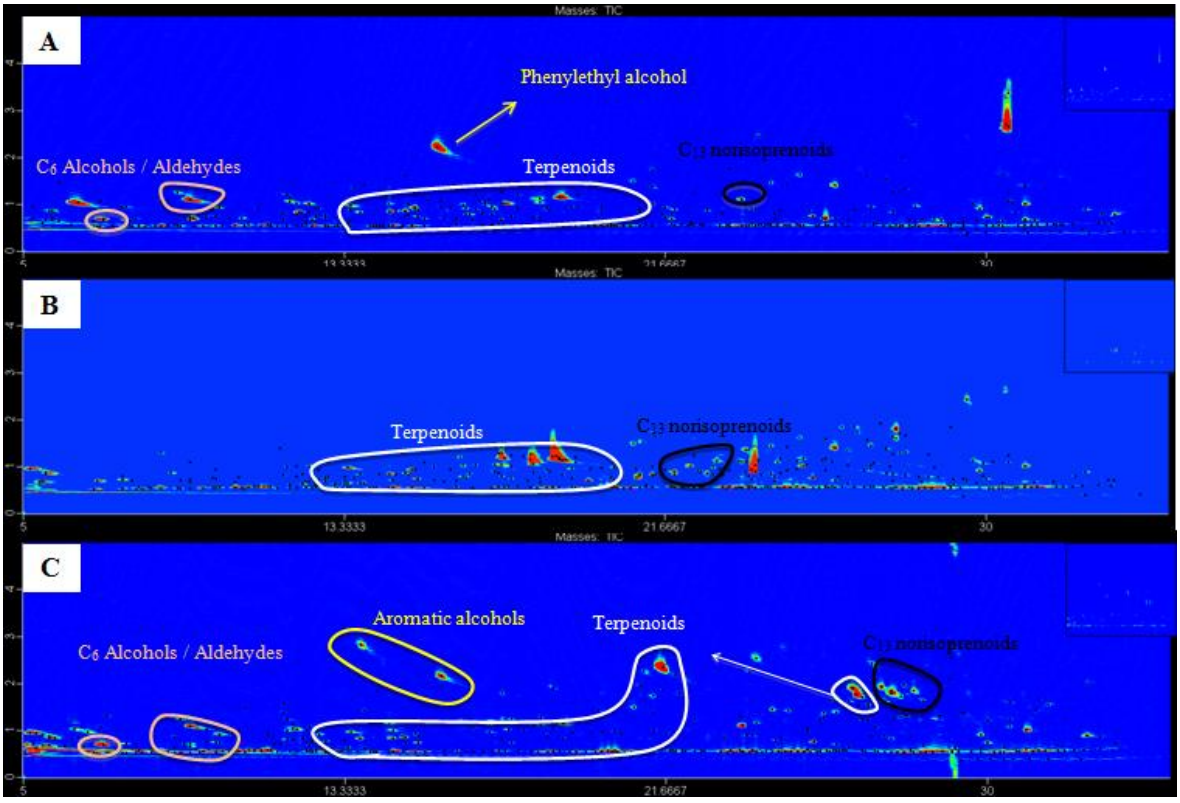

Figure 18 – GCxGC-ToF-MS chromatogram of the volatile compounds in Merzling grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

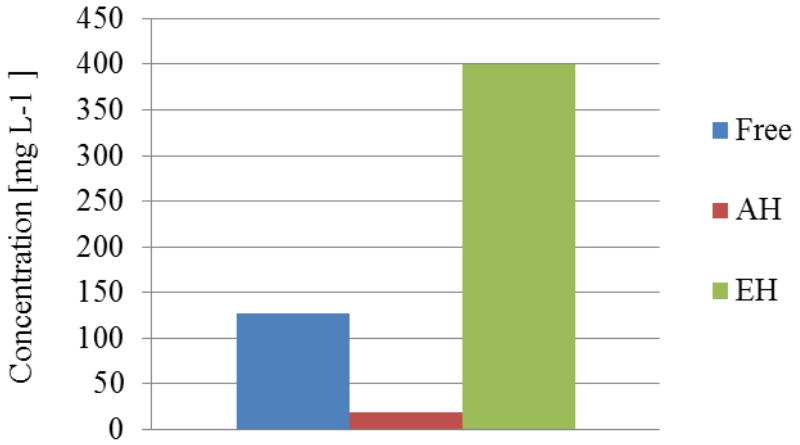

Figure 19 - Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Merzling grape juice.

Table 5 - Free and bound volatile compounds identified in Merzling grape juice, grouped by chemical classes.

No.	Compound [a]		Concentration		
			Free [µL^{-1}] (n=6)[b]	Bound [µL^{-1}]	
				Acidic (n=3)	Enzymatic (n=3)
	Terpenoids				
1	*cis*-Thujane-4-ol	13.0772 , 0.647	n.d	49,65 ± 31,57	n.d
2	Linalool oxide (isomer)	14.4928 , 0.858	1172,29 ± 582,22	726,34 ± 432,11	14740,09 ± 11650,34
3	Linalool	15.1589 , 0.944	1018,69 ± 482,02	37,45 ± 20,77	1112,09 ± 862,38
4	Rose oxide	15.4088 , 0.713	113,98 ± 58,33	29,53 ± 14,05	n.d
5	*α*-Terpineol	17.4905 , 1.049	1001,63 ± 618,94	n.d	2354,05 ± 1339,67
6	*β*-Terpineol	16.408 , 1.016	189,38 ± 40,77	n.d	n.d
7	Ocimenol	16.408 , 1.036	n.d	221,24 ± 170,01	n.d
8	Nerol oxide	16.4913 , 0.779	n.d	72,27 ± 49,00	n.d

Table 5 (continued)

9	Menthol	16.4913 , 0.931	142,67± 113,84	n.d	n.d
10	Epoxylinalool	17.0741 , 1.175	n.d	n.d	12294,66 ± 5954,31
11	Terpinen-4-ol	17.2407 , 0.911	124,32± 77,41	n.d	n.d
12	Myrcenol	17.4072 , 1.056	n.d	254,22 ± 318,20	n.d
13	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	17.4072 , 1.802	n.d	n.d	1754,36± 1202,35
14	Myrtenol	17.8236 , 1.221	40,45± 21,57	n.d	7008,49 ± 6205,17
15	Hotrienol	17.9901 , 1.003	n.d	n.d	868,15 ± 315,99
16	Citronellol	18.4065 , 1.049	454,63± 93,70	n.d	n.d
17	*cis*-Geraniol	18.4065 , 1.135	595,77± 172,46	n.d	3244,09± 639,10
18	*β*-citral	18.573 , 0.944	37,13± 18,07	71,37 ± 40,18	n.d
19	*trans*-Geraniol	18.9894 , 1.168	3641,13± 1899,44	10083,86± 9344,00	14346,63± 10463,44
20	*α*-Citral	19.2392 , 0.964	105,83± 23,88	141,00 ± 92,267	n.d
21	Eugenol	21.3209 , 1.742	n.d	n.d	2115,21 ± 1416,89
22	8-Hydroxylinalool	21.5707 , 2.356	n.d	n.d	120423,46 ± 82999,18
23	Nerolidol	25.4012 , 0.871	45,06± 22,73	n.d	n.d
24	*p*-Menthane	26.4837 , 1.907	n.d	n.d	31720,94 ± 24354,83
25	*γ*-Eudesmol	26.9833 , 0.944	n.d	79,70 ± 49,65	n.d
26	*α*-Bisabolol oxide	27.3164 , 0.884	n.d	n.d	911,78 ± 587,32
27	Farnesol	28.1491 , 0.970	243,36± 45,16	n.d	n.d

Table 5 (continued)

28	Neoisothujol	31.2301 , 1.452	n.d	n.d	3470,62 ± 2335,77
Sub-total (µL^{-1})			**8941,41**	**11766,66**	**216364,63**
Sub-total (%)			**7,04**	**64,85**	**54,04**
	C_{13}Norisoprenoids				
29	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	21.3209 , 0.851	n.d	429,91 ± 232,61	n.d
30	β-Damascenone	22.0704 , 0.917	159,53 ± 98,19	426,29 ± 304,55	365,40 ± 56
31	α-Ionone	22.7365 , 0.884	127,64 ± 14,11	n.d	n.d
32	3-Hydroxy-α-damascone	26.5669 , 1.775	n.d	n.d	42325 ± 33549,45
33	3-Oxo-α-ionol	27.2331 , 1.927	n.d	31,32 ± 7,11	18608,86 ± 13920,51
34	3-Hydroxy-5,6-epoxy-β-ionone	27.816 , 1.987	n.d	n.d	1099,41 ± 506,16
35	Blumenol C	28.0658 , 1.861	n.d	n.d	10163,42 ± 7571,54
Sub-total (µL^{-1})			**287,17**	**887,52**	**72562,09**
Sub-total (%)			**0,23**	**4,89**	**18,12**
	Alcohols				
36	3-Methyl-3-buten-1-ol	6.24905 , 1.201	n.d	n.d	1366,26 ± 970,43
37	1-Butanol, 3-methyl-	6.33232 , 1.056	11135,38 ± 1919,60	n.d	17669,19 ± 12231,82
38	3-Hexen-1-ol	8.99696 , 1.267	418,42 ± 184,65	n.d	4558,75 ± 3190,66
39	1-Hexanol	9.24677 , 1.122	4211,66 ± 1561,64	n.d	30543,18 ± 22000,63
40	1-Octen-3-ol	12.078 , 1.030	255,90 ± 149,65	30,69 ± 10	5885,89 ± 4579,74
41	2-Ethyl-1-hexanol	13.4103 , 0.990	1015,97 ± 79,39	830,01 ± 340,01	n.d

Table 5 (continued)

42	1-Octanol	14.5761 , 1.016	n.d	n.d	5394,17 ± 4067,70
43	2-Hexanol	15.2422 , 0.884	n.d	n.d	663,69 ± 438,22
44	Phenylethyl alcohol	15.7418 , 2.224	6818,26 ± 2798,11	n.d	33956,62 ± 23151,88
45	Nonen-1-ol	16.6578 , 1.082	n.d	n.d	808,46 ± 628,81
46	2-Butyl-1-octanol	28.3156 , 0.554	1647,29 ± 1300,73	3164,25 ± 2706,32	n.d
47	2,3-Butanediol	30.4806 , 0.482	94,07 ± 81,18	n.d	n.d
Sub-Total (μL^{-1})			**25596,95**	**4024,95**	**100846,21**
Sub-Total (%)			**20,15**	**22,18**	**25,19**
	Aldehydes				
48	Hexanal	7.58137 , 0.733	47,47 ± 15,87	371,96 ± 167,43	4126,25 ± 3116,40
49	2-Hexenal	8.83042 , 0.884	92035,56 ± 30724,21	266,73 ± 127,57	820,79 ± 593,61
50	Nonanal	15.2422 , 0.752	119,90 ± 64,40	401,82 ± 122,27 ±	5061,76 ± 3957,12
51	*cis*-4-Decenal	17.4072 , 0.812	n.d	138,42 ± 28,50	n.d
52	2,4-Decadienal	20.3217 , 0.997	n.d	287,06 ± 71,90	576,80 ± 520,26
Sub-Total (μL^{-1})			**92202,93**	**1465,99**	**10585,6**
Sub-Total (%)			**72,58**	**8,08**	**2,64**
Total (μL^{-1})			**127028,46**	**18145,12**	**400358,53**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.2.1) Free and bound terpenoids in Merzling grape juice

The terpenoid composition in Merzling grape juice is substantially different in between the free and the bound fractions. Quantitatively, free terpenoids accounted for 8941,41 µg L^{-1} representing 7% of the total considered amount of free volatiles. In contrast, both acidic and enzymatic bound fractions accounted for 65% (11766,66 µg L^{-1}) and 54% (216364,63 µg L^{-1}), respectively, of their total analyzed concentration of volatiles (Figure 20).

The terpenols *cis*-linalool oxide, *β*-linalool, *cis*-geraniol and *trans*-geraniol are the main terpenoids present in Merzling juice, and are present in higher abundance in the bound fraction obtained by enzymatic hydrolysis comparatively to the other analyzed fractions. Other important compounds such as hotrienol, eugenol, *α*-citral, *β*-citral, *α*-terpineol and rose oxide are present in the bound fraction, citronellol is present only in the free form fraction.

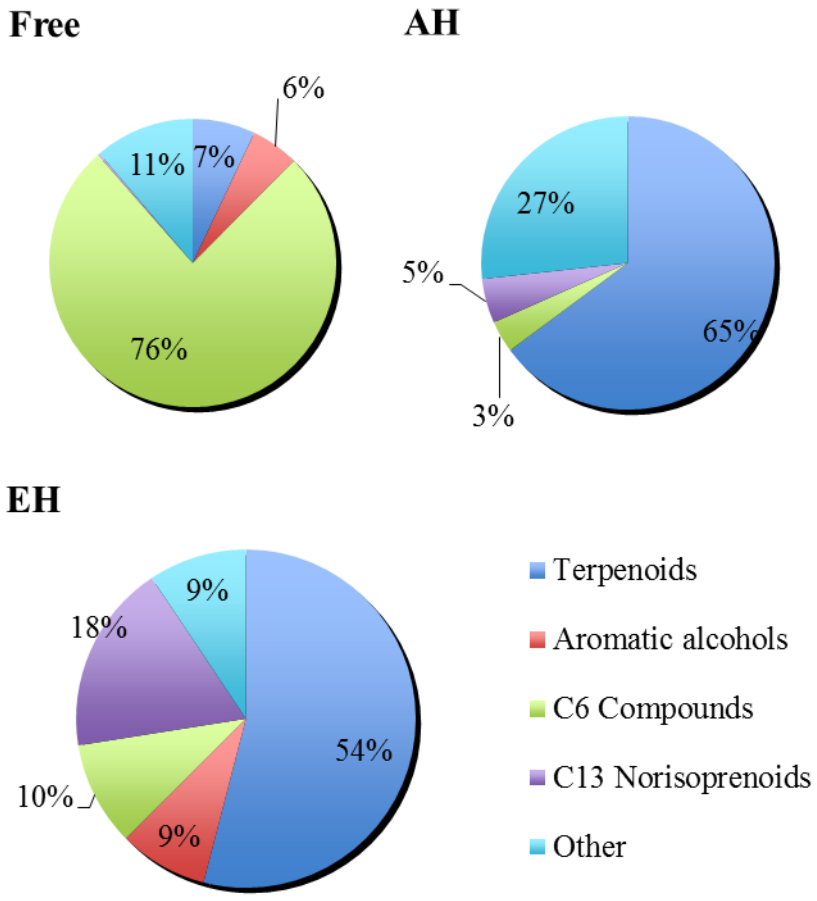

Figure 20 - Volatile composition of Merzling grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.2.2) Free and bound alcohols in Merzling grape juice

The alcohol composition of Merzling grape juice was a major one, ranging from 20,15% (25596,95 µg L^{-1}) in the free fraction to 22,18% (4024,95 µg L^{-1}) and 25,19% (100846,21 µg L^{-1}) in the acidic and enzymatic hydrolyzed fractions respectively. This fraction is composed mainly by n-alcohols of C_6 chain length and aromatic compounds such and phenylethyl alcohol. Enzymatic hydrolysis again proved to be more efficient in releasing glycosidically-bound compounds compared to the acidic hydrolysis.

Phenylethyl alcohol is the main alcohol present in the bound fraction obtained by enzymatic hydrolysis accounting for nearly 34% of the total alcohols obtained. C_6 alcohols, 3-hexen-1-ol, 1-hexanol, 2-hexanol and 2-ethyl-1-hexanol are present in Merzling grape juice predominantly bounded rather than in free form.

3.2.3) Other compounds

C_{13} norisoprenoids are also present in Merzling grape juice. They are almost absent in the free fraction accounting for 0,23% of the total volatile profile, only *β*-damascenone (159,53 µg L^{-1}) and *α*-ionone were identified (127,64 µg L^{-1}). They are mostly present in the bound fraction, acidic hydrolysis yielded a total of 887,52 µg L^{-1} and enzymatic hydrolysis a total of 72562,09 µg L^{-1}. The bound C_{13} norisoprenoids present in Merzling grape juice are *β*-damascenone, 3-oxo-*α*-ionol, 3-hydroxy-5,6-epoxy-*β*-ionone, blumenol C and 3-hydroxy-*α*-damascone.

Aldehydes are also present in Merzling grape juice, both in free and bound form, but mainly in the free fraction representing nearly 73% (92022,93 µg L^{-1}) of its total concentration of volatiles. Special attention should be given to C_6 aldehydes, hexanal and 2-hexenal, which are the main compounds of this chemical family present in Merzling grapes. Nonanal was identified and is the main component of the aldehyde bound fraction reaching 5061,76 µg L^{-1} after enzymatic hydrolysis.

3.2.4) Concluding remarks

The results of this analysis show that Merzling grape juice features a poor free volatile fraction, in comparison with the bound fraction. Enzymatic hydrolysis yielded 36 compounds and shows to be more efficient, in releasing glycosidically bound volatiles, than acidic hydrolysis, which yielded 35 compounds. Terpenoids show to be the main class of compounds present in Merzling grape juice and their contribution is particularly important in the bound fraction. Remarkably, bound terpenoids are 7 to 9 times more abundant than their free counterpart. This phenomenon is well documented and is not only characteristic to grapes (31).

The monoterpenols geraniol (OT=30 μL^{-1}) and linalool (OT=15 μL^{-1}) are the most abundant terpenoid in Merzling. They are fragrant compounds with floral and fruity aromas that play a significant role in the varietal flavor of wines (31). Both linalool and geraniol are present above their sensory perception limits. Linalool oxides such as *cis*-linalool oxide have flavor thresholds of 3000–5000 $\mu g\ L^{-1}$.

Rose oxide (OT=0,5 μL^{-1}), α-citral (OT= 32 μL^{-1}), citronellol (OT=40 μL^{-1}), α-terpineol (OT=330-350 μL^{-1}), and hotrienol (OT=110 μL^{-1}) further contribute with floral fruity, rose, and citrus aromas in Merzling grape juice. Eugenol (OT= 6-30 μL^{-1}) which can be regarded as a phenylpropanoid can give to the juice clove and aromatic notes (66).

Phenylethyl alcohol is the main bound alcohol in Merzling grape juice and also contributes with flowery, sweet aromas. C_6 compounds comprise alcohols and aldehydes, and are formed from linoleic acid and linolenic acid when grapes enter into contact with the air, and are formed by the actions of lipoxygenase, peroxidase and alcohol dehydrogenase enzymes (28). 3-hexen-1-ol, 1-hexanol, 2-hexanol and 2-ethyl-1-hexanol the main identified C_6 alcohols can be responsible for herbaceous and greasy odors. C_6 aldehydes, hexanal (OT=4,5-5 μL^{-1}) and 2-hexenal (OT=17 μL^{-1}) are also above their odor threshold and are responsible for green, grassy, and fruity aromas.

C_{13} norisoprenoids are abundantly present glycosidically-bounded in Merzling grape juice and can contribute to varietal aroma when released. β-Damascenone and α-ionone are powerful odorant with a low odor threshold of 0,002 $\mu g\ L^{-1}$ and 0,03 $\mu g\ L^{-1}$ and are responsible for flower and raspberry-like aromas.

3.3) Free and bound volatile compounds in Freiminer grape juice

The volatile composition of Freiminer grape juice and its different distribution in the free form and bound form is shown in Table 6. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 21), the most important chemical classes are highlighted.

The total free volatile compounds from Freiminer juice accounted for 23856,36 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 31,15 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 77,36 mg L^{-1}. There is a noticeable predominance of free volatile compounds in the analyzed juice, compared to the bound fraction (Figure 22).

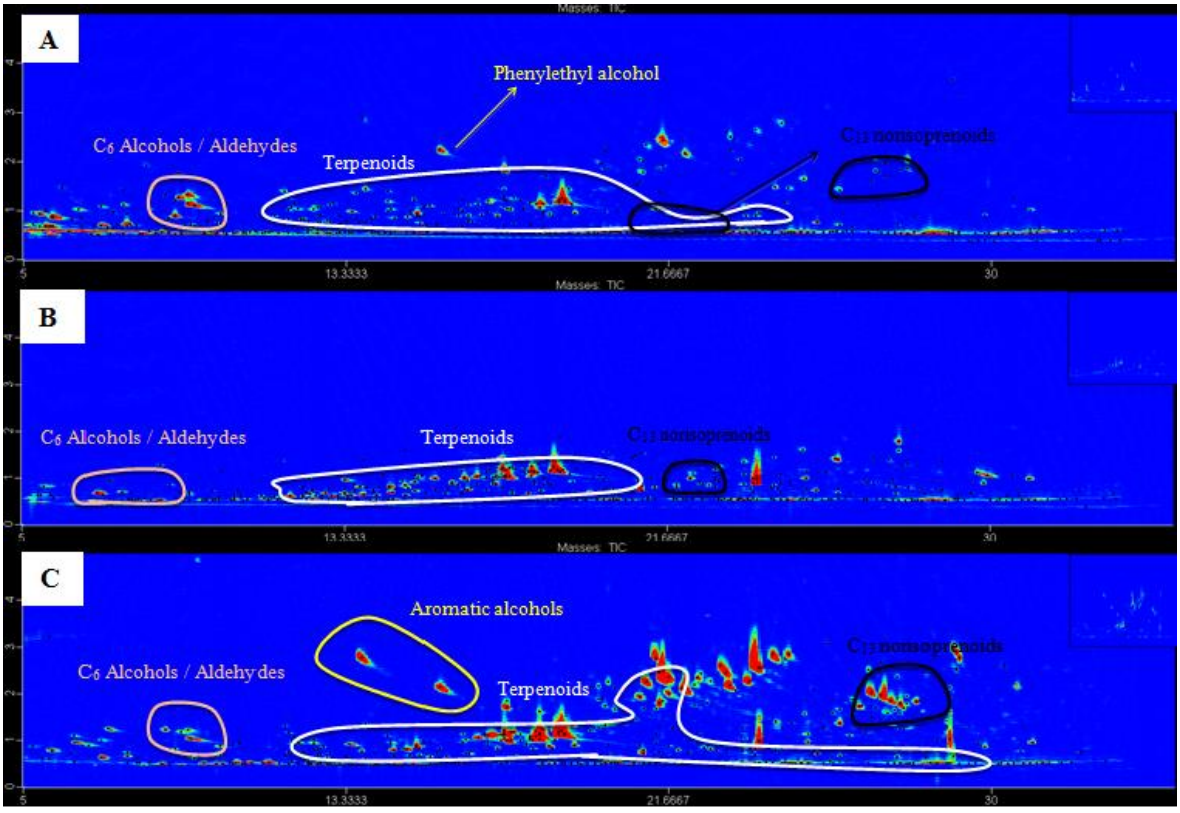

Figure 21 – GCxGC-ToF-MS chromatogram of the volatile compounds in Freiminer grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

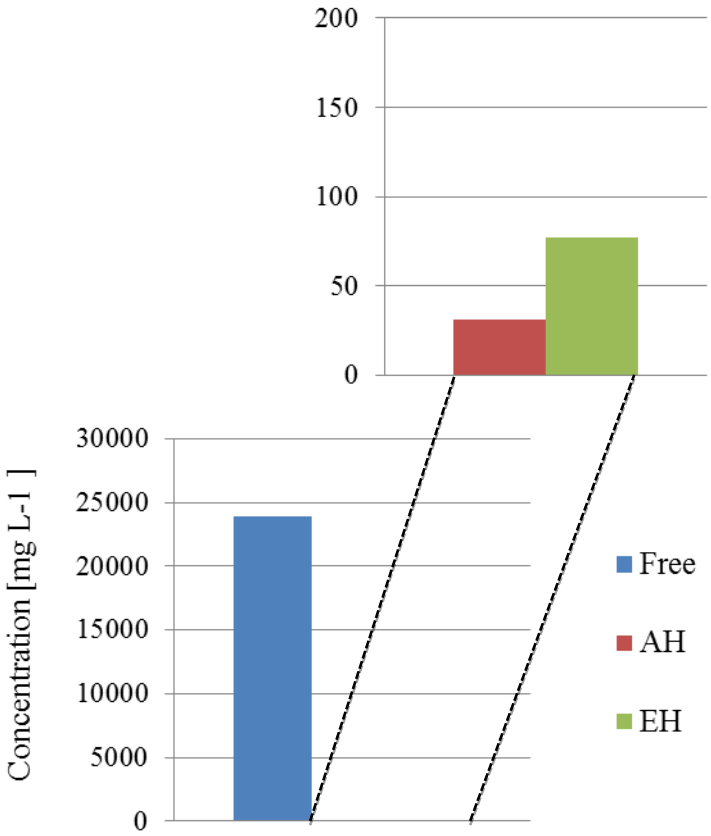

Figure 22 – Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Freiminer grape juice.

Table 6 - Free and bound volatile compounds identified in Freiminer grape juice, grouped by chemical classes.

No.	Compound [a] (n=6) [b]		Concentration		
			Free [μL^{-1}] (n=6)[b]	Bound [μL^{-1}]	
				Acidic (n=3)	Enzymatic (n=3)
	Terpenoids				
1	Camphene	11.4118 , 0.594	n.d	40,88 ± 16,07	n.d
2	Lilac alcohol (isomer)	11.4951 , 0.607	n.d	70,35 ± 28,97	267,23 ± 117,86
3	Lilac alcohol (isomer)	11.7449 , 0.614	n.d	22,14 ± 6,91	n.d

Table 6 (continued)

4	Geranic oxide	11.9114 , 0.574	n.d	n.d	42,14 ± 4,84
5	*β*-Cymene	13.327 , 0.700	n.d	319,45 ± 55,26	n.d
6	Limonene	13.4103 , 0.634	n.d	279,60 ± 31,62	n.d
7	Eucalyptol	13.4935 , 0.647	n.d	864,38 ± 222,14	n.d
8	*β-cis*-Ocimene	13.8266 , 0.614	19106,1± 5627,31	n.d	48,19 ± 13,48
9	*γ*-Terpinene	14.1597 , 0.647	n.d	320,73 ± 32,28	n.d
10	Artemiseole	14.5761 , 0.706	n.d	985,50 13,93	n.d
11	Linalool oxide (isomer)	14.9091 , 0.884	54913,0± 4413,56	1515,08 ± 239,61	2168,15 ± 391,91
12	*p*-Cymenene	14.9924 , 0.799	n.d	152,75 ± 25,72	n.d
13	Ocimenol	16.408 , 1.023	n.d	2905,23 ± 454,70	n.d
14	Linalool	15.1589 , 0.924	n.d	525,85 ± 47,46	1855,44 ± 350,81
15	Terpinolene	14.9091 , 0.653	n.d	263,18 ± 22,45	n.d
16	Hotrienol	15.2422 , 1.043	n.d	487,94 ± 190,88	68,18 ± 18,72
17	*trans*-Rose oxide	15.4088 , 0.719	2666,01 ± 587,02	n.d	n.d
18	Myrcenol	15.6586 , 1.003	n.d	865,05 ± 190,53	940,67 ± 18,72
19	3-pinanol	15.7418 , 0.851	n.d	n.d	39,90 ± 10,06
20	Rose oxide	15.9084 , 0.680	n.d	52,62 ± 15,19	190,88 ± 19,70
21	*trans-p*-Menth-8-en-2-one	16.2415 , 0.858	n.d	29,24 ± 8,85	n.d
22	Lilac aldehyde (isomer)	16.2415 , 0.904	14249,57± 1600,93	n.d	n.d
23	*β*-Terpineol	16.408 , 0.990	n.d	806,09 ± 348,27	n.d
24	Nerol oxide	16.4913 , 0.785	3711,42± 1032,09	592,77 ± 59,49	123,77 ± 16,09

Table 6 (continued)

25	Lilac aldehyde (isomer)	16.4913 , 0.924	33001,63± 15999,97	n.d	n.d
26	Verbenol	16.4913 , 1.129	15492,53± 4070,55	n.d	n.d
27	Lilac aldehyde (isomer)	16.8243 , 0.937	11575,91± 161,30	n.d	n.d
28	Epoxylinalol	17.0741 , 1.195	90096,4± 25514,19	23,80 ± 10,11	5452,53 ± 639,60
29	Terpinen-4-ol	17.2407 , 0.911	54122,49 ± 14738,61	1107,35 ± 103,91	209,27 ± 46,11
30	*α*-Cyclogeraniol	17.2407 , 1.063	n.d	497,55 ± 93,84	n.d
31	*p*-Cymen-8-ol	17.4072 , 1.452	19152,6± 8234,88	1001,27 ± 27,71	n.d
32	2,6-Dimethyl-3,7-octadiene-2,6-diol	17.4072 , 1.861	499654,73± 186670,92	n.d	1845,69 ± 249,61
33	*α*-Terpineol	17.5738 , 1.043	93840,2± 23398,48	6699,64 ± 129,36	3253,14 ± 726,67
34	Lilac alcohol (isomer)	17.8236 , 1.010	n.d	n.d	155,38 ± 92,50
35	Myrtenol	17.8236 , 1.148	7338,36± 1129,85	n.d	296,77 ± 155,01
36	Lilac alcohol (isomer)	18.0734 , 1.181	9044,20± 1378,42	n.d	n.d
37	*cis*-Carveol	18.1567 , 1.267	n.d	83,45 ± 16,45	153,79 ± 31,91
38	Citronellol	18.3232 , 1.076	218982,0± 134610,01	n.d	n.d
39	*cis*-Geraniol	18.4065 , 1.148	741732,7± 425227,94	n.d	8667,14 ± 3054,18
40	*β*-Citral	18.573 , 0.950	18755,84± 4865,91	154,88 ± 21,86	613,06 ± 88,03
41	*trans*-Carveol	18.573 , 1.221	n.d	n.d	206,56 ± 71,80
42	Geraniol	18.8228 , 1.195	n.d	3269,31 ± 890,44	7796,99 ± 1747,88
43	*trans*-Geraniol	18.8228 , 1.214	1167617± 21688409,46	4583,88 ± 424,13	9065,25 ± 1602,25
44	*β*-Myrcene	18.8228 , 1.234	236567,9± 294950,83	n.d	n.d

Table 6 (continued)

45	*α*-Ocimene	18.9061 , 1.261	961391,4± 848812,01	252,04 ± 84,26	n.d
46	*α*-Citral	19.2392 , 0.970	63335,79± 18675,34	84,09 ± 15,10	657,61 ± 118,85
47	*cis*-8-Hydroxylinalool	19.9053 , 1.973	56123,0± 25368,15	n.d	n.d
48	Verbenol	19.9886 , 1.340	n.d	n.d	219,22 ± 203,62
49	*p*-Cymen-7-ol	19.9886 , 1.855	10831,7± 256,28	n.d	120,76 ± 7,52
50	Terpin	20.2384 , 1.670	n.d	n.d	139,54 ± 9,65
51	Geranic acid	21.3209 , 2.752	n.d	n.d	2102,17 ± 223,02
52	Citronellol hydrate	21.4042 , 1.874	23273,24± 7445,90	n.d	1579,88 ± 170,22
53	8-Hydroxylinalool	21.4875 , 2.449	900270,1± 279874,47	n.d	4611,53 ± 2198,46
54	Menthol	22.0704 , 2.178	288865,98± 166976,91	n.d	n.d
55	Ledene oxide	22.3202 , 1.115	n.d	180,53 ± 27,07	n.d
56	Dihydrocitronellol	22.7365 , 0.574	8831,82± 11,83	n.d	n.d
57	Dihydro-*α*-terpineol	23.2361 , 2.244	2895,53± 122,28	n.d	n.d
58	8-Hydroxy carvotanacetone	23.4859 , 2.053	11523,34± 2605,35	n.d	n.d
59	2,6-Dimethyl-2,6-octadiene-1,8-diol	24.5685 , 2.785	28373,19± 1657,46	n.d	n.d
Sub-total (μL^{-1})			**18051098,38**	**29036,63**	**54119,95**
Sub-total (%)			**75,67**	**91,19**	**69,86**
	C_{13} Norisoprenoids				
60	*α*-Ionene	18.0734 , 0.693	n.d	471,76 ± 89,12	n.d
61	*β*-Damascenone	21.9038 , 0.871	n.d	148,80 ± 6,37	n.d
62	*β*-Ionone epoxide	23.9856 , 0.983	8611,13± 1159,75	n.d	n.d

Table 6 (continued)

63	3-Hydroxy-*β*-damascone	26.8167 , 2.521	n.d	n.d	578,11 ± 63,05
64	3-Hydroxy-7,8-dihydro-*β*-ionol	26.8167 , 2.521	n.d	n.d	190,87 ± 73,50
65	3-Oxo-*α*-ionol	27.2331 , 1.980	5568,24 ± 1157,41	n.d	2954,86 ± 703,47
66	Dihydro-*β*-ionone	27.3996 , 1.861	n.d	n.d	1353,03 ± 225,22
67	3-Hydroxy-5,6-epoxy-*β*-ionone	27.8993 , 1.940	137109,9± 42767,43	n.d	n.d
68	Blumenol C	28.0658 , 1.841	n.d	n.d	600,60 ± 121,36
Sub-total (μL^{-1})			**151289,334**	**620,53**	**8695,65**
Sub-total (%)			**0,63**	**1,95**	**11,22**
	Alcohols				
69	1-Butanol	5.16654 , 0.964	169860,91± 83758,04	n.d	n.d
70	3-Hexen-1-ol	9.08023 , 1.195	232164,46± 49061,47	n.d	397,42 ± 70,51
71	2-Hexen-1-ol	9.24677 , 1.234	503972,18± 131082,49	415,74 ± 95,63	415,74 ± 95,63
72	1-Hexanol	9.33004 , 1.049	1765575,5± 722219,79	n.d	1683,56 ± 317,66
73	2-Heptanol	10.246 , 0.911	n.d	n.d	267,11 ± 59,20
74	1-Octen-3-ol	12.2445 , 1.003	n.d	n.d	217,19 ± 56,20
75	1-Octanol	14.6593 , 0.997	n.d	n.d	160,04 ± 44,42
76	2-Ethyl-1-hexanol	13.5768 , 0.964	99585,05± 56088,72	475,09 ± 113,41	677,29 ± 66,73
77	Benzyl alcohol	13.9932 , 2.554	n.d	n.d	3465,73 ± 266,63
78	*α*-Methyl-benzenemethanol	14.3262 , 2.006	n.d	n.d	64,36 ± 22,62
79	Phenylethyl alcohol	15.7418 , 2.152	562062,54 ± 504300,60	n.d	3460,71 ± 480,29

Table 6 (continued)

80	2-Ethenyl-2,5-dimethyl-4-hexen-1-ol	16.3247 , 0.964	n.d	n.d	55,33 ± 17,00
81	1-Nonanol	16.9909 , 0.924	n.d	n.d	53,59 ± 7,39
82	2-Butyl-1-octanol	28.3989 , 0.521	716006,97± 521552,91	881,00 ± 88,71	2941,50 ± 291,43
Sub-Total (μL^{-1})			**4049227,7**	**1771,83**	**13859,57**
Sub-Total (%)			**16,97**	**5,56**	**17,89**
	Aldehydes				
83	Hexanal	7.58137 , 0.713	752856,22± 386429,75	176,21 ± 21,07	360,01 ± 122,14
84	2-Hexenal	8.91369 , 0.825	830856,00 ± 227244,50	130,86 ± 66,01	214,78 ± 82,71
85	2,2-Dimethyl-hexanal	9.33004 , 0.660	n.d	105,51 ± 39,43	89,12 ± 14,87
86	2-Heptenal	11.4951 , 0.898	21040,25 ± 5094,16	n.d	n.d
87	Nonanal	15.2422 , 0.700	n.d	n.d	135,30 ± 33,17
Sub-Total (μL^{-1})			**1604752,48**	**412,58**	**799,21**
Sub-Total (%)			**6,73**	**1,30**	**1,03**
Total (μL^{-1})			**23856367,87**	**31841,57**	**77474,40**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.3.1) Free and bound terpenoids in Freiminer grape juice

The terpenoid composition in Freiminer grape juice is present predominantly on the free form accounting for 18051098,38 µg L^{-1} representing nearly 76% of the total free fraction. The bound fraction is considerably less rich in its content of terpenoids, 29036,63 µg L^{-1} obtained with acidic hydrolysis and 54119,95 µg L^{-1} with enzymatic hydrolysis (Figure

23). Nevertheless, terpenoids show to be the major chemical component in the bound fraction ranging from 70% to 91%. The main terpenoids present in Freiminer grape juice are geraniol, *cis*-geraniol, *trans*-geraniol, *α*-terpineol, epoxylinalol, 8-hydroxylinalool, *cis*-8-Hydroxylinalool, 2,6-dimethyl-3,7-octadiene-2,6-diol, citronellol, *α*-ocimene, and menthol. Most of these terpenoids show to be present in both free and bound forms with the exception of menthol and citronellol. Geraniol stands as the most abundant terpenoids in the free and enzymatically treated bound fractions accounting for 11676170,08 µg L^{-1} and 9065,252129 µg L^{-1} respectively. *α*-terpineol is the most abundant terpenoid in the bound fraction obtained by acidic hydrolysis accounting for 6699,635124 µg L^{-1}.

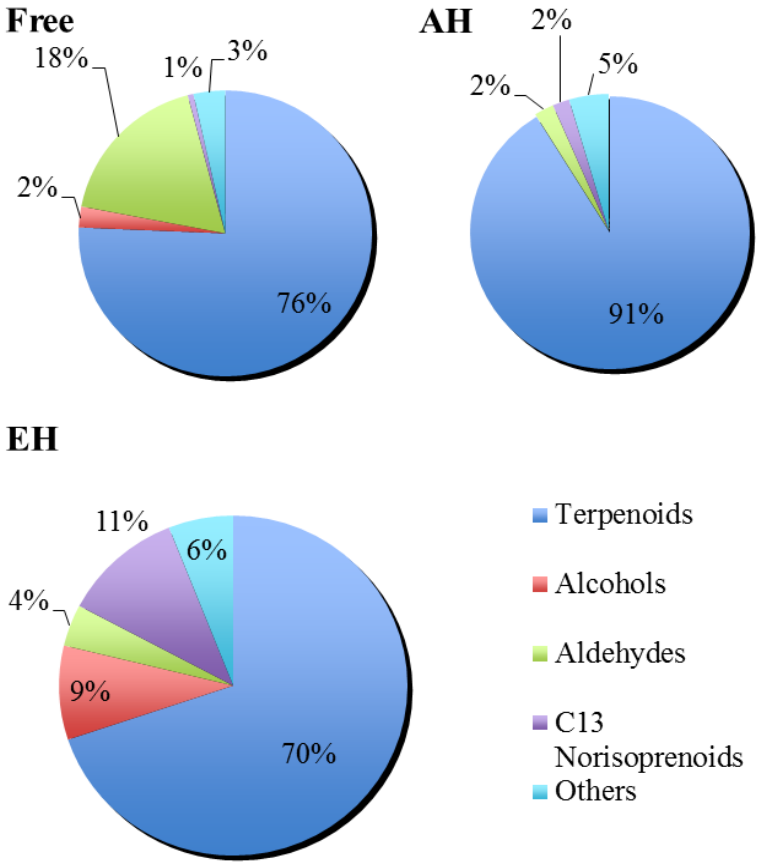

Figure 23 – Volatile composition of Freiminer grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.3.2) Free and bound alcohols in Freiminer grape juice

The alcohol composition of Freiminer grape juice is the second most abundant, ranging from 16,97 % (4049227,7 µg L^{-1}) in the free fraction to 5,56 % (1771,83 µg L^{-1}) and 17,89 % (13859,57 µg L^{-1}) in the acidic and enzymatic hydrolyzed fractions respectively. Aromatic alcohols, benzyl alcohol and phenylethyl alcohol, were detected and stand as the major alcohols in Freiminer grape juice. Phenylethyl alcohol is present in both free and bound forms accounting for 562062,54 µg L^{-1} and 3460,71 µg L^{-1} respectively. Benzyl alcohol is present merely in the bound form with 3465,73 µg L^{-1}. Comparatively, aliphatic alcohols are present in less abundance with emphasis on C_6 alcohols 2-hexen-1-ol, 1-hexanol, 2-ethyl-1-hexanol, and 3-hexen-1-ol.

3.3.3) Other compounds

In Freiminer grape juice, C_{13} norisoprenoids are present in the free form accounting for 151289,3362 µg L^{-1} , and bound form accounting for 620,53 µg L^{-1} and 8695,65 µg L^{-1} in the acidic and enzymatic fractions, respectively. Their contribution is more significant in the enzymatic hydrolyzed fraction representing 11,22 % of its total. *β*-Damascenone, *β*-ionone epoxide, *α*-ionene, dihydro-*β*-ionone, 3-oxo-*α*-ionol, 3-hydroxy-5,6-epoxy-*β*-ionone were the main identified compounds.

Aldehydes are also present in Freiminer grape juice, both in free and bound form, but mainly in the free fraction representing 6,73 % (1604752,48 µg L^{-1}) of its total concentration of volatiles. Special attention should be given to C_6 aldehydes, hexanal and 2-hexenal, which are the main compounds of this chemical family present in Freiminer grape juice.

3.3.4) Concluding remarks

The results of this analysis show that Freiminer grape juice features a rich free volatile fraction, in comparison with the bound fraction. Enzymatic hydrolysis yielded 31 compounds the same amount was released by than acidic hydrolysis, however, the volatile pattern obtained is qualitatively and quantitatively different. Terpenoids show to be the main class of compounds present in Freiminer grape juice in both free and bound forms.

The monoterpenols geraniol (OT=30 μL^{-1}) and *α*-terpineol (OT=330-350 μL^{-1}) are the most abundant terpenoids in Freiminer and contribute with floral, fruity, and lilac/ coniferous aromas, depending on the enantiomer of *α*-terpineol. The acidic conditions to which the terpenoids are subjected can result in the conversion of geraniol and limonene in *α*-terpineol, which explains the predominance of this monoterpenol (67,68).

Rose oxide (OT=0,5 μL^{-1}), *α*-citral (OT= 32 μL^{-1}) and citronellol (OT=40 μL^{-1}), further contribute with floral fruity, rose, and citrus aromas in Freiminer grape juice.

Menthol displays a minty, light, refreshing odor (69), but it was found in grapes in trace amounts, which was not the case (37).

Aromatic alcohols, phenylethyl alcohol and benzyl alcohol have been described as responsible for floral/sweet odors, and are the predominant alcohols in Freiminer grape juice. Benzyl alcohol was obtained only through enzymatic hydrolysis while phenylethyl alcohol is abundantly present in the free form (34).

C_{13} norisoprenoids are abundantly present, in the free and glycosidically-bounded forms, in Freiminer grape juice and can contribute to varietal aroma when released. *β*-Damascenone and *α*-ionone are powerful odorant with a low odor threshold of 0,002 $\mu g\ L^{-1}$ and 0,03 $\mu g\ L^{-1}$ and are responsible for flower and raspberry-like aromas and were obtained after acidic hydrolysis of the glycosidically-linked fraction. Dihydro-*β*-ionone, 3-oxo-*α*-ionol, 3-hydroxy-5,6-epoxy-*β*-ionone are precursors of *α*-ionone and *β*-ionone.

3.4) Free and bound volatile compounds in Traminer grape juice

The volatile composition of Traminer grape juice and its different distribution in the free form and bound form is shown in Table 7. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 24), the most important chemical classes are highlighted.

The total free volatile compounds from Traminer juice accounted for 10911,48 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 44,01 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 77,52 mg L^{-1}. There is a noticeable predominance of free volatile compounds in the analyzed juice, compared to the bound fraction (Figure 25).

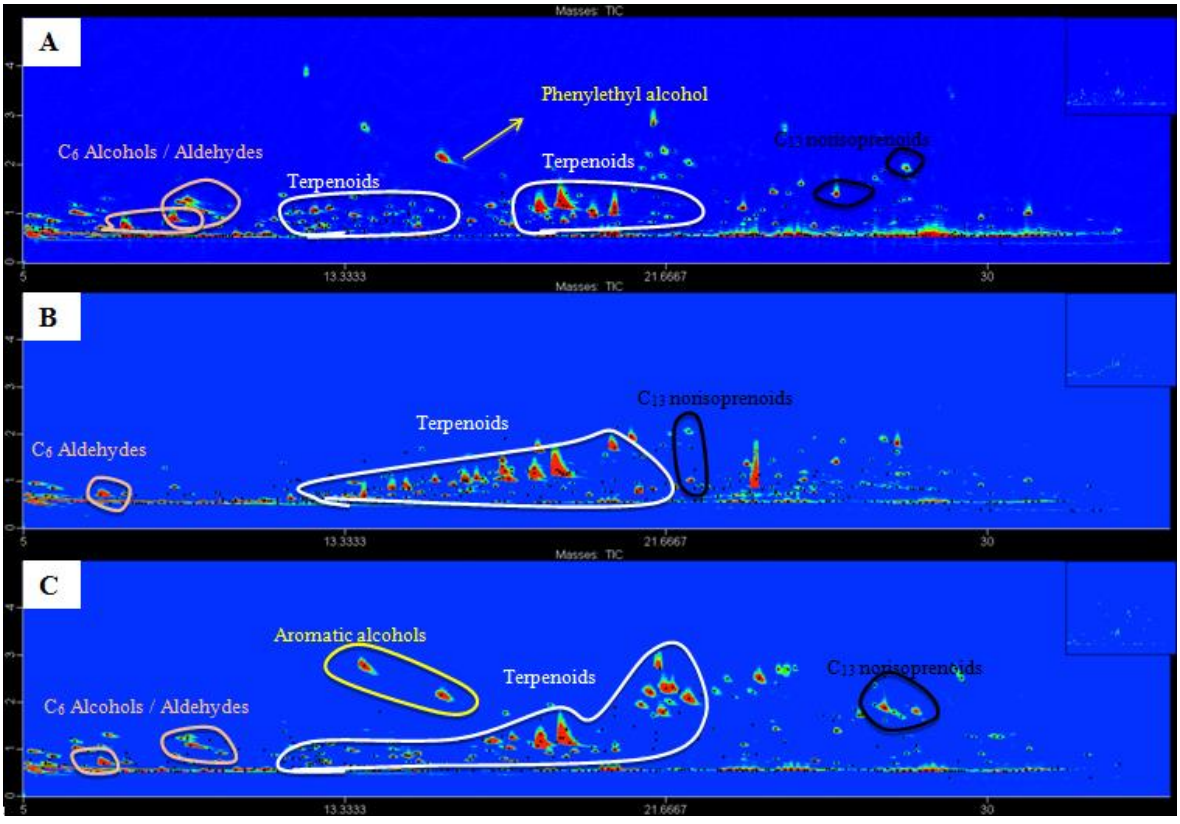

Figure 24 – GCxGC-ToF-MS chromatogram of the volatile compounds in Traminer grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

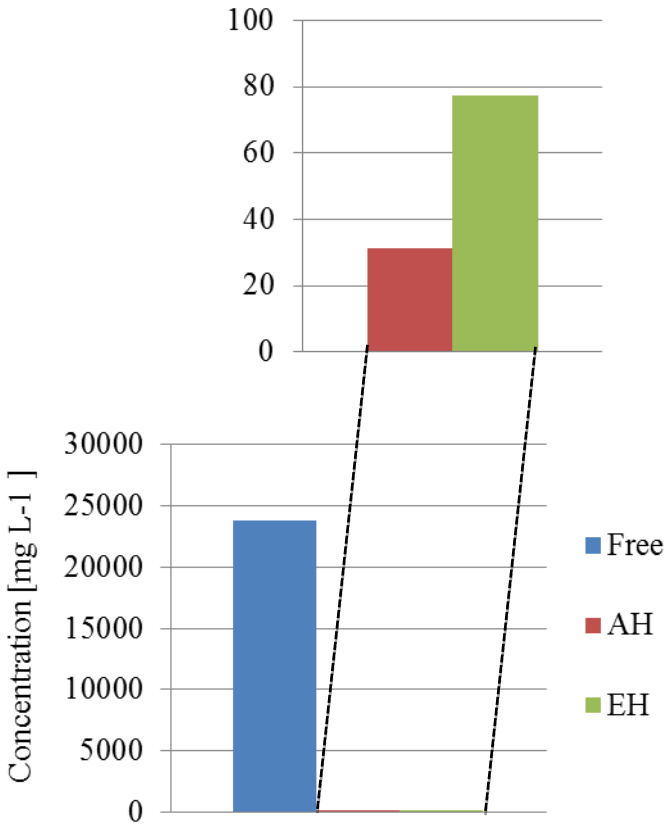

Figure 25 – Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Traminer grape juice.

Table 7 - Free and bound volatile compounds identified in Traminer grape juice, grouped by chemical classes.

No.	Compound [a]		Concentration		
			Free [μL^{-1}] (n=6)[b]	Bound [μL^{-1}]	
				Acidic (n=3)	Enzymatic (n=3)
	Terpenoids				
1	*β*-Myrcene	12.3278 , 0.647	29706,25± 5768,27	n.d	153,83 ± 23,73
2	*p*-Menthane, 1,4-epoxy	13.0772 , 0.640	n.d	709,55 ± 499,63	n.d
3	Limonene	13.4103 , 0.653	38267,09± 6040,65	586,32 ± 614,05	264,29 ± 79,57

Table 7 (continued)

4	Linalol oxide (furanoid)	14.5761 , 0.845	8310,30 ± 1026,65	n.d	n.d
5	Linalool oxide (isomer)	14.9091 , 0.871	23945,36± 8080,65	3642,46 ± 3357,39	1951,31 ± 404,01
6	Terpinolene	14.9924 , 0.660	356,64 ± 107,73	n.d	n.d
7	Linalool	15.1589 , 0.944	26949,90± 10190,00	154,63 ± 106,10	881,19 ± 172,13
8	*trans*-Rose oxide	15.4088 , 0.713	7895,09 ± 1382,16	n.d	n.d
9	*α*-Terpineol	17.5738 , 1.030	29631,51 ± 6845,09	3409,63 ± 2266,05	1793,69 ± 665,59
10	Myrcenol	15.6586 , 1.010	n.d	2052,79 ± 1319,28	398,57 ± 166,55
11	Rose oxide	15.9916 , 0.693	n.d	503,90 ± 362,90	266,64 ± 34,12
12	1-Terpinenol	16.2415 , 1.003	n.d	796,93 ± 561,76	n.d
13	*β*-Terpineol	16.408 , 1.016	n.d	1040,52 ± 1126,17	n.d
14	Nerol oxide	16.5745 , 0.772	n.d	490,06 ± 348,09	352,38 ± 38,93
15	Ocimenol	16.9076 , 1.069	n.d	7279,32 ± 5097,83	n.d
16	Linalool hydrate	18.573 , 1.643	n.d	2816,73 ± 1837,49	44,63 ± 17,58
17	Epoxylinalol	17.0741 , 1.175	178947,06± 48742,60	159,98 ± 159,11	3601,16 ± 1335,08
18	Terpinen-4-ol	17.2407 , 0.904	23174,13± 9338,59	n.d	n.d
19	*p*-Menth-1-en-9-al	17.324 , 1.043	n.d	569,07 ± 451,65	n.d
20	*p*-Cymen-8-ol	17.4072 , 1.432	7994,64 ± 1562,43	1094,52 ± 591,06	n.d
21	Citronellol	18.3232 , 1.076	323801,99± 95645,49	n.d	1875,28 ± 1213,87
22	*cis*-geraniol	18.3232 , 1.168	1126769,5± 358364,00	2743,69 ± 3486,05	14130,75 ± 5404,86
23	*p*-Menthan-2-ol	18.4065 , 1.327	n.d	n.d	383,63 ± 70,10

Table 7 (continued)

24	*β* -Citral	18.573 , 0.944	80313,14± 27636,82	n.d	805,87 ± 176,06
25	*trans*-Geraniol	18.8228 , 1.195	2055251,6± 655369,27	2330,24 ± 1688,32	15599,02 ± 4165,53
26	*α*-Ocimene	18.9061 , 1.261	736521,7± 868243,43	n.d	n.d
27	*α*-Citral	19.2392 , 0.964	128917,26± 35038,96	82,98 ± 78,49	808,60 ± 334,77
28	Terpin	20.5715 , 1.676	n.d	3878,34 ± 3275,28	n.d
29	Citronellol hydrate	21.5707 , 1.789	n.d	n.d	1877,62 ± 452,96
30	8-Hydroxylinalool	21.5707 , 2.317	94926,64± 56776,06	n.d	7387,18 ± 1629,70
31	Geranic acid	21.654 , 2.574	n.d	n.d	1219,82 ± 182,55
32	Menthol	22.2369 , 2.086	n.d	n.d	5131,08 ± 626,23
33	2-Acetyl-2-carene	22.4034 , 1.043	n.d	392,37 ± 371,48	n.d
34	*trans*-Geranylacetone	23.1529 , 0.832	7823,85 ± 4067,90	22,92 ± 6,17	n.d
35	*β*-Santalol	23.819 , 0.726	n.d	346,79 ± 128,78	n.d
36	Epoxy-linalool oxide	25.1513 , 0.851	n.d	224,90 ± 143,98	n.d
37	Epoxy-*α*-terpenyl acetate	27.7327 , 1.135	n.d	n.d	525,98 ± 350,72
38	*α*-bisabolol oxide	28.8985 , 1.162	n.d	n.d	408,06 ± 348,66
39	Ledene oxide	24.9015 , 0.752	n.d	52,13 ± 41,11	n.d
Sub-total (μL^{-1})			**4929503,71**	**38499,87**	**59860,58**
Sub-total (%)			**45,18**	**87,49**	**77,22**
	C_{13}Norisoprenoids				
40	1,2-Dihydro-1,1,6-trimethyl-naphthalene	21.4875 , 0.865	n.d	514,54 ± 355,74	n.d

Table 7 (continued)

41	*β*-Ionone epoxide	23.9856 , 0.977	15405,23± 7390,54	n.d	n.d
42	3-Hydroxy-*β*-damascone	26.6502 , 1.729	n.d	n.d	550,91 ± 13,78
43	3-Hydroxy-7,8-dihydro-*β*-ionol	26.9833 , 2.449	n.d	n.d	86,35 ± 8,15
44	3-Oxo-*α*-ionol	27.2331 , 1.914	11358,52± 2372,96	304,26 ± 238,90	2090,25 ± 357,31
Sub-total (μL^{-1})			**26763,75**	**818,8**	**2727,51**
Sub-total (%)			**0,25**	**1,86**	**3,52**
	Alcohols				
45	3-Hexen-1-ol	8.99696 , 1.267	186004,70± 27912,11	n.d	640,61 ± 79,40
46	2-Hexen-1-ol	9.24677 , 1.300	259705,48± 39133,80	n.d	398,22 ± 80,30
47	1-Hexanol	9.41331 , 1.129	1156564,5± 225551,65	n.d	2345,49 ± 878,11
48	2-Heptanol	10.0795 , 0.937	93811,17± 22458,16	n.d	689,72 ± 98,35
49	Heptanol	11.9947 , 1.063	44778,99± 25208,46	n.d	n.d
50	1-Octen-3-ol	12.2445 , 1.036	50257,89± 17726,26	n.d	740,75 ± 138,64
51	2-Ethyl-1-hexanol	13.4103 , 0.990	251436,93± 45066,81	667,61 ± 465,26	1151,11 ± 383,94
52	Benzyl alcohol	13.8266 , 2.779	n.d	n.d	4163,16 ± 1648,79
53	1-Octanol	14.4928 , 1.109	37889,34± 7230,70	n.d	460,71 ± 110,89
54	2-Octen-1-ol	14.5761 , 1.181	n.d	n.d	364,95 ± 88,93
55	2-Nonanol	15.2422 , 0.884	n.d	n.d	119,30 ± 36,45
56	Phenylethyl alcohol	15.8251 , 2.158	343936,87± 193982,95	n.d	n.d
57	1-Nonanol	17.0741 , 0.977	n.d	n.d	173,71 ± 44,60
58	2-Butyl-1-octanol	25.2346 , 0.587	633860,44± 242773,47	2231,61 ± 1201,84	n.d
Sub-Total (μL^{-1})			**3058246,27**	**2899,22**	**11247,73**
Sub-Total (%)			**28,03**	**6,59**	**14,51**

Table 7 (continued)

	Aldehydes				
59	Hexanal	7.66464 , 0.785	1335829,54 ± 407370,00	796,88 ± 545,24	742,33 ± 277,66
60	2-Hexenal	8.91369 , 0.878	n.d	607,48 ± 461,13	n.d
61	*(E)*-2-Hexenal	8.91369 , 0.891	1262117,29 ± 352311,55	n.d	n.d
62	Heptanal	10.0795 , 0.752	59618,47± 22153,91	n.d	373,14 ± 86,37
63	2-Heptenal	11.4951 , 0.891	21927,11± 1663,33	n.d	679,57 ± 319,70
64	2-octenal	14.0764 , 0.878	15909,88± 4118,80	n.d	n.d
65	Nonanal	15.2422 , 0.746	100675,32± 31418,79	384,15 ± 357,73	812,50 ± 77,46
66	2-Nonenal	16.5745 , 0.871	80726,93± 94804,12	n.d	204,26 ± 72,12
67	2-Decenal	19.0726 , 0.851	n.d	n.d	450,48 ± 31,48
68	2,4-Decadienal	20.3217 , 1.003	20166,46± 13027,76	n.d	419,31 ± 168,82
Sub-Total (μL^{-1})			**2896971**	**1788,51**	**3681,59**
Sub-Total (%)			**26,55**	**4,06**	**4,75**
Total (μL^{-1})			**10911484,73**	**44006,4**	**77517,41**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.4.1) Free and bound terpenoids in Traminer grape juice

The terpenoid composition in Traminer grape juice is present predominantly on the free form accounting for 4929503,71 µg L^{-1} representing 45% of the total free fraction. The bound fraction is considerably less rich in its content of terpenoids, 38499,87 µg L^{-1}

obtained with acidic hydrolysis and 59860,58 µg L^{-1} with enzymatic hydrolysis. Nevertheless, terpenoids show to be the major chemical component in the bound fraction ranging from 77% to 87%, slightly higher than in the free fraction (Figure 26).

The terpenoids *α*-ocimene, *cis*-geraniol, *trans*-geraniol, epoxylinalool, *α*-citral, and citronellol are the main compounds of this family, present in the free form. *cis*-linalool oxide, *α*-terpineol, terpin, terpin hydrate, ocimenol, *cis*-geraniol, and *trans*-geraniol are the main bound terpenoids obtained through acidic hydrolysis, while *cis*-geraniol, *trans*-geraniol, 8-hydroxylinalool, epoxylinalol, and menthol are the main bound terpenoids obtained through enzymatic hydrolysis. Other terpenoids worth mention such as limonene, *β*-citral, linalool, and rose oxide are present in Traminer grape juice in lower concentrations.

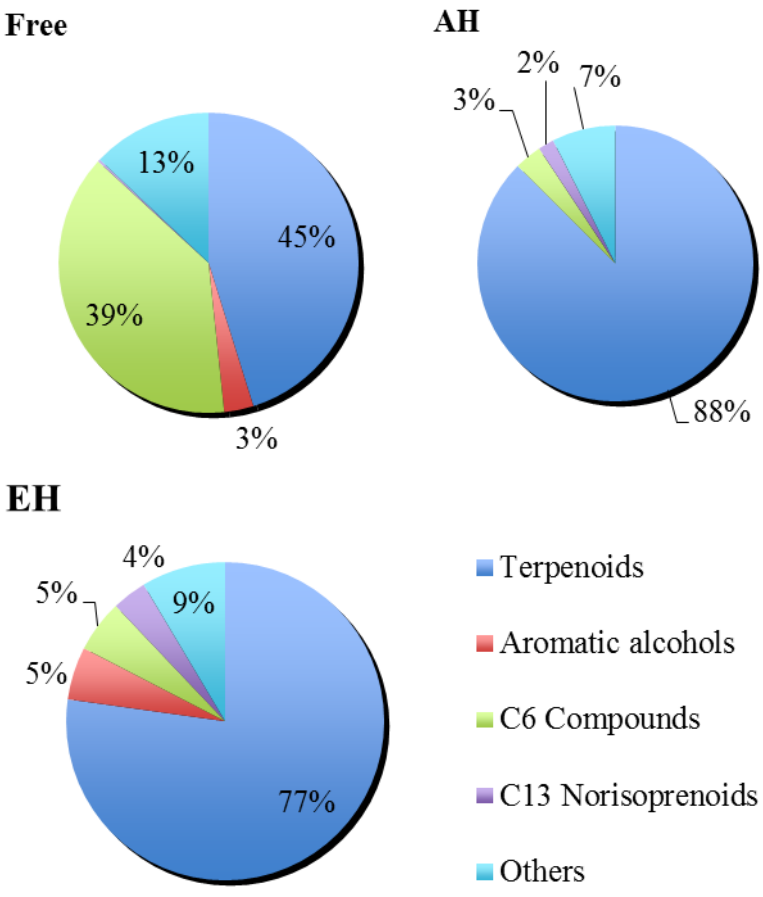

Figure 26 – Volatile composition of Traminer grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.4.2) Free and bound alcohols in Traminer grape juice

The alcohol composition of Traminer grape juice is the second most abundant, ranging from 28,03 % (3058246,27 µg L^{-1}) in the free fraction to 6,59 % (2899,22 µg L^{-1}) and 14,51 % (11247,73 µg L^{-1}) in the acidic and enzymatic hydrolyzed fractions respectively. Phenylethyl alcohol is present only in the free form accounting for 343936,87 µg L^{-1} while benzyl alcohol is the main alcohol present in the bound form with 4163,16 µg L^{-1}. Comparatively, aliphatic alcohols stand as the most abundant alcohols with particular focus on C_6 alcohols. 1-hexanol is the main alcohol in the free fraction and the second most abundant in the bound fraction obtained through enzymatic hydrolysis.

3.4.3) Other compounds

C_{13} norisoprenoids are also present in Traminer grape juice representing 0,25 % (26763,75 µg L^{-1}) in the free fraction and 1,86 % to 3,52 % in the acidic and enzymatic hydrolyzed fractions respectively. 3-oxo-*α*-ionol is present in both free and bound forms. *β*-ionone epoxide appears only in the free fraction. 3-hydroxy-7,8-dihydro-*β*-ionol, 3-hydroxy-*β*-damascone, and 1,2-dihydro-1,1,6-trimethyl-naphthalene are part of the bound fraction.

Aldehydes are also present in Traminer grape juice, both in free and bound form, but mainly in the free fraction representing nearly 27 % (2896971 µg L^{-1}) of its total concentration of volatiles. Special attention should be given to C_6 aldehydes, hexanal, and *(E)*-2-hexenal, which are the main compounds of this chemical family present in Traminer grape juice.

3.4.4) Concluding remarks

The results of this analysis show that Traminer grape juice features a rich free volatile fraction, in comparison with the bound fraction. Enzymatic hydrolysis yielded 36 compounds and shows to be more efficient than acidic hydrolysis, which yielded 29 compounds, in releasing bound volatiles. Terpenoids show to be the main class of compounds present in Traminer grape juice in both free and bound form, although their contribution is more significant in the bound fraction when compared with other chemical families.

The terpenoids *α*-ocimene, geraniol, epoxylinalool, *α*-citral, and citronellol contribute with pleasant, fruity, floral, citrus, and green aromas.

Menthol displays a minty, light, refreshing odor (69), but it was found in grapes in trace amounts, which was not the case (37).

1-hexanol (OT= 2500 µg L^{-1}) it"s by far the most abundant alcohol present in Traminer grape juice and contributes with leafy-grassy odor in Traminer grape juice. The corresponding C_6 aldehydes hexanal, and *(E)*-2-hexenal also have a pleasant grassy odor,

Aromatic alcohols, phenylethyl alcohol and benzyl alcohol have been described as responsible for floral/sweet odors. Benzyl alcohol was obtained only through enzymatic hydrolysis while phenylethyl alcohol is abundantly present in the free form (34).

C_{13} norisoprenoids are present mainly in the glycosidically-bounded form, in Freiminer grape juice and can greatly contribute to varietal aroma when released. 3-hydroxy-7,8-dihydro- *β*-ionol and 3-hydroxy- *β*-damascone are precursors of *β*-ionone and *β*-damascone.

1,2-dihydro-1,1,6-trimethyl-naphthalene is a typical undesired bottle-aged kerosene-like character of older Riesling wines, TDN was produced by acid hydrolysis (pH 1) and heating , but it was never found as an aglycon after enzymatic hydrolysis of grape and wine glycosides. This corroborates with the obtained results, TDN was identified after acidic hydrolysis and has an odor threshold of 20 µg L^{-1} and can contribute with petrol off-flavors (70).

3.5) Free and bound volatile compounds in Jutrzenka grape juice

The volatile composition of Jutrzenka grape juice and its different distribution in the free form and bound form is shown in Table 8. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 27), the most important chemical classes are highlighted. The total free volatile compounds from Jutrzenka juice accounted for 1723,92 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 1777,08 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 185,04 mg L^{-1} (Figure 28).

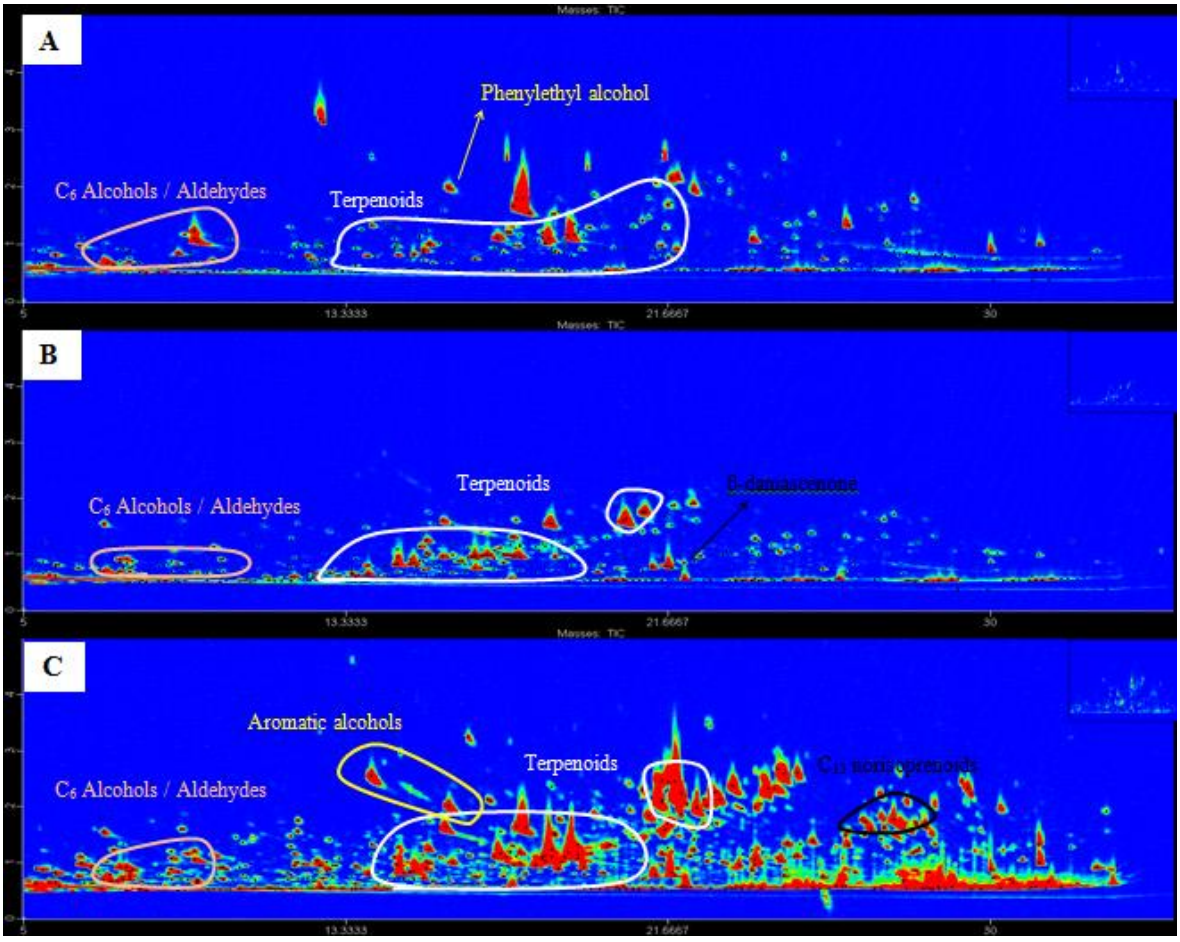

Figure 27 – GCxGC-ToF-MS chromatogram of the volatile compounds in Jutrzenka grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

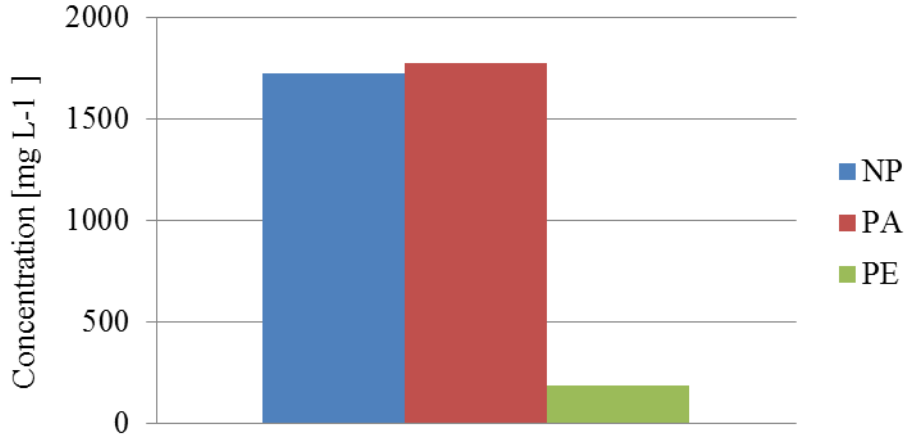

Figure 28 - Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Jutrzenka grape juice.

Table 8 - Free and bound volatile compounds identified in Jutrzenka grape juice, grouped by chemical classes.

No.	Compound [a]			Concentration		
				Free [μL^{-1}] (n=4)[b]	Bound [μL^{-1}]	
					Acidic (n=3)	Enzymatic (n=3)
	Terpenoids					
1	Limonene		13.5768 , 0.627	11136,75 ± 1227,43	47482,60 ± 43120,30	n.d
2	Eucalyptol		13.5768 , 0.640	3784,16 ± 105,01	92561,97 ± 58194,59	n.d
3	Terpinolene		14.9091 , 0.653	n.d	13637,44 ± 10992,30	n.d
4	linalool (isomer)	oxide	15.0757 , 0.845	89366,36± 28845,96	252564,66 ± 129788,72	8934,52 ± 142,33
5	linalool (isomer)	oxide	15.0757 , 0.858	n.d	105152,65 ± 18726,48	n.d
6	Myrcenol		15.8251 , 0.977	n.d	121717,22 ± 68956,01	n.d
7	Linalool		15.3255 , 0.904	124804,5 ± 36919,71	7827,73 ± 3803,32	9068,56 ± 1225,29
8	Rose oxide		16.0749 , 0.673	10033,34± 3327,16	15358,98 ± 10017,25	n.d
9	Nerol oxide		16.6578 , 0.759	23722,69 ± 9298,43	33321,43 ± 17642,15	2020,90 ± 827,14

Table 8 (continued)

10	Epoxylinalol	17.324 , 1.129	236825,68± 61348,12	24304,55 ± 10792,20	24934,24 ± 2446,75
11	Terpinen-4-ol	17.4072 , 0.865	9877,32± 3291,24	56425,69± 29040,82	n.d
12	*p*-cymen-8-ol	17.657 , 1.327	25986,63 ± 10036,31	44920,58 ± 24881,52	n.d
13	*α*-terpineol	17.8236, 0.970	27928,86 ± 11980,20	250723,74 ± 151735,22	4139,86 ± 1294,11
14	Hotrienol	18.1567 , 0.957	129992,56± 30989,03	n.d	13523,78 ± 1319,07
15	Citronellol	18.4897 , 1.036	46870,53± 15295,08	n.d	5069,21± 694,71
16	*cis*-geraniol	18.4897 , 1.148	298279,47± 88517,97	n.d	22298,46 ± 6503,52
17	*β* -Citral	18.7396 , 0.904	9069,91 ± 3436,41	n.d	5558,65 ± 1198,42
18	*trans*-geraniol	19.0726 , 1.162	591130,04± 131357,12	n.d	n.d
19	Geraniol	19.1559 , 1.142	n.d	n.d	28559,80 ± 6797,81
20	1,2-dihydro-8-hydroxylinalool	19.2392 , 1.162	n.d	n.d	2750,534 ± 605,647
21	*α* -citral	19.4057 , 0.924	10942,37± 4275,27	n.d	4724,85 ± 970
22	Terpin hydrate	20.5715 , 1.643	n.d	455240,10 ± 259091,60	n.d
23	Terpin	21.0711 , 1.775	n.d	216615,41 ± 111059,33	n.d
24	8-hydroxylinalool	21.4042 , 2.059	69118,66± 35547,50	n.d	39494,39 ± 763,63
25	Eugenol	21.5707 , 1.597	n.d	n.d	777,28 ± 231,59
26	Geranic acid	21.4875 , 2.587	5049,66 ± 6268,19	n.d	768,69 ± 240,18
27	Menthol	21.8205, 1.907	n.d	36707,56 ± 10075,57	12416,34 ± 9389,07
Sub-total (μL^{-1})			**1723919,57**	**1777077,72**	**185040,06**
Sub-total (%)			**64,15**	**86,15**	**75,86**

Table 8 (continued)

	C_{13} Norisoprenoids				
28	*β*-damascenone	22.0704 , 0.858	983,22 ± 722,04	20800,38 ± 12834,43	n.d
29	3-hydroxy-*α*-damascone	26.8167 , 1.630	n.d	n.d	3018,18 ± 433,90
30	3-oxo-*α*-ionol	27.4829 , 1.736	n.d	n.d	3873,44± 891,67
31	Dihydro-*β*-ionone	27.6494 , 1.670	n.d	n.d	3313,07 ± 939,84
Sub-total (μL^{-1})			**983,22**	**20800,38**	**6331,25**
Sub-total (%)			**0,0366**	**6,46**	**2,60**
	Alcohols				
32	3-methyl-1-butanol	6.49886 , 0.970	46802,05 ± 15906,80	n.d	6771,82 ± 2816,61
33	1-methyl-cyclopentanol	7.66464 , 0.950	n.d	n.d	7141,19 ± 2163,490
34	2-hexanol	7.74791 , 0.891	n.d	n.d	2780,21 ± 1257,69
35	3-hexen-1-ol	9.1635 , 1.188	23448,12 ± 6825,29	n.d	n.d
36	1-hexanol	9.41331 , 1.049	452136,15± 86896,46	n.d	5524,38 ± 1366,96
37	2-heptanol	10.1627 , 0.904	1317,07 ± 709,62	n.d	n.d
38	Benzyl alcohol	13.9932 , 2.521	n.d	n.d	10115,84 ± 5476,62
39	1-octanol	14.6593 , 0.977	13567,49 ± 1359,34	5898,25 ± 1079,02	n.d
40	*α*-methyl-*α*-[4-methyl-3-pentenyl]oxirane methanol	14.7426 , 0.832	n.d	n.d	13677,78 ± 4861,07
41	Phenylethyl mlcohol	15.9916 , 1.987	77672,39 ± 29496,82	n.d	10314,67 ± 3672,25
42	2-butyl-1-octanol	28.3156 , 0.541	97260,66 ± 23228,12	127283,43 ± 100355,15	n.d
43	11-hexadecen-1-ol	32.7289 , 0.898	5552,91 ± 1988,18	n.d	n.d
Sub-Total (μL^{-1})			**694308,72**	**133181,68**	**42648,11**
Sub-Total (%)			**25,84**	**6,46**	**17,48**

Table 8 (continued)

	Aldehydes				
44	3-methyl-2-Butenal,	7.4981 , 0.937	n.d	n.d	8103,31 ± 477,73
45	Hexanal	7.74791 , 0.706	29944,83 ± 10640,37	16194,34 ± 8733,92	1790,28 ± 393,53
46	2-hexenal	8.99696 , 0.845	92035,56± 30724,21	14301,09 ± 5368,06	n.d.
47	Nonanal	15.3255 , 0.746	17139,71± 10035,86	35078,61 ± 23649,63	n.d
Sub-Total (µL^{-1})			**139120,1**	**65574,04**	**9893,59**
Sub-Total (%)			**5,18**	**3,18**	**4,06**
Total (µL^{-1})			**2687218,05**	**2062690,21**	**243913,01**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.5.1) Free and bound terpenoids in Jutrzenka grape juice

The terpenoid composition in Jutrzenka grape juice is slightly more abundant on the bound form accounting for 1777077,72 µg L^{-1} with acidic hydrolysis. The enzymatic hydrolysis yielded less abundance and accounted for 185040,06 µg L^{-1} (Figure 29). Nevertheless, it represents 86 % and 72 % of their respective total fraction. The free fraction accounted for 1723919,57 µg L^{-1} representing 64 % of its total. The terpenoids *β*-linalool, *cis*-geraniol, *trans*-geraniol, epoxylinalool, hotrienol, and-limonene are the main compounds of this family, present in the free form. Linalool oxide (isomer), *α*-terpineol, terpin, terpin hydrate, myrtenol, and eucalyptol are the main bound terpenoids obtained through acidic hydrolysis, while *cis*-geraniol, geraniol, 8-hydroxylinalool, epoxylinalol, menthol, and hotrienol are the main bound terpenoids obtained through enzymatic hydrolysis. Other terpenoids worth mention such as *α*-citral, *β*-citral, citronellol, terpinen-4-ol and rose oxide are present in Jutrzenka grape juice in lower concentrations.

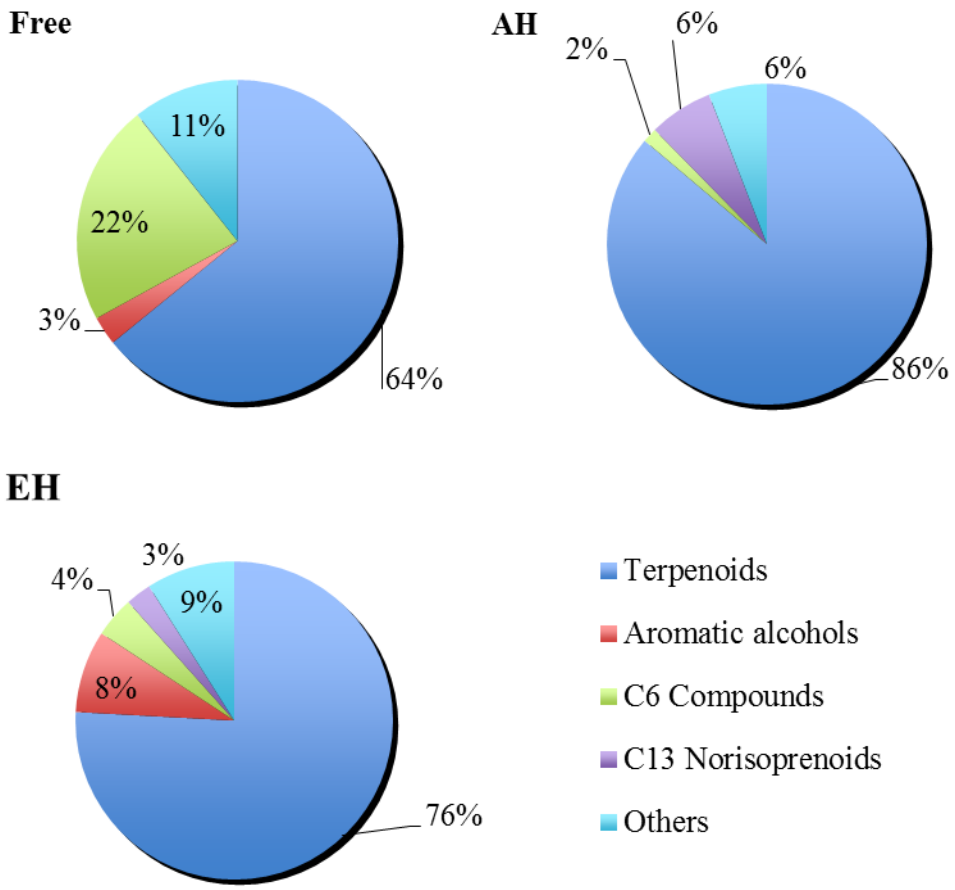

Figure 29 - Volatile composition of Jutrzenka grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.5.2) Free and bound alcohols in Jutrzenka grape juice

The alcohol composition of Jutrzenka grape juice is the second most abundant, ranging from 25,84 % (694308,72 µg L^{-1}) in the free fraction to 6,46 % (133181,68 µg L^{-1}) and 21,87 % (56325,89 µg L^{-1}) in the acidic and enzymatic hydrolyzed fractions respectively. Phenylethyl alcohol is one of the most abundant alcohols in Jutrzenka grape juice present in the free form with 77672,39 µg L^{-1} and bound form after enzymatic hydrolysis with 10314,67 µg L^{-1}. Benzyl alcohol is merely present in the bound form with 10115,84 µg L^{-1}. Comparatively, aliphatic alcohols stand as the most abundant alcohols with particular focus on C_6 alcohols such as 1-hexanol, 2-hexanol, and 3-hexen-1-ol.

3.5.3) Other compounds

C_{13} norisoprenoids are present in Jutrzenka grape juice in small quantities and mainly in the bound form. They are present in the free form in trace amounts representing 0,04 % (983,22 μg L^{-1}) of the free fraction. Acidic hydrolysis yielded 20800,38 μg L^{-1} representing 6,46 % of its total, while enzymatic hydrolysis yielded 6331,25 μg L^{-1} representing 2,6 %.

β-Damascenone is the main C_{13} norisoprenoid found in Jutrzenka grape juice peaking 20800,38 μg L^{-1}. Dihydro-*β*-ionone, 3-hydroxy-*α*-damascone were also identified.

Aldehydes are also present in Jutrzenka grape juice, both in free and bound form, but mainly in the free fraction representing 5 % (139120,1 μg L^{-1}) of its total concentration of volatiles. Special attention should be given to C_6 aldehydes, hexanal, and 2-hexenal, which are the main compounds of this chemical family present in Jutrzenka grape juice.

3.5.4) Concluding remarks

The results of this analysis show that Jutrzenka grape juice features a similar free and bound fraction. Enzymatic hydrolysis yielded 36 compounds and shows to be more eficient than acidic hydrolysis, which yielded 29 compounds, in releasing bound volatiles. Terpenoids show to be the main class of compounds present in Jutrzenka grape juice in both free and bound form, although their contribution is more significant in the bound fraction when compared with other chemical families. The main monoterpenols present in Jutrzenka grape juice, geraniol and linalool, are above their odor thresholds of 30 μg L^{-1} and 15 μg L^{-1}, respectively.

α-terpineol, terpin, terpin hydrate, and hotrienol contain is based on several reactions occurred during acidic hydrolysis (36). Rose oxide, hotrienol, limonene, epoxylinalool, *α*-citral, and citronellol contribute with pleasant, fruity, floral, citrus, and green aromas.

Menthol obtained through enzymatic hydrolysis, displays a minty, light, refreshing odor (69), but it was found in grapes in trace amounts, which was not the case (37).

Aromatic alcohols, phenylethyl alcohol and benzyl alcohol have been described as responsible for floral/sweet odors, and are prominent alcohols in Jutrzenka juice. Benzyl

alcohol was obtained only through enzymatic hydrolysis while phenylethyl alcohol is abundantly present in the free form and bound forms (34).

β-Damascenone is the main C_{13} norisoprenoids in Jutrzenka grape juice and can greatly contribute to varietal aroma when released. This norisoprenoid ketone is a ubiquitous compound and has a descriptor of "cooked apple/floral/quince" with an extremely low odor threshold of 0,002 μg L^{-1} in water (35).

3.6) Free and bound volatile compounds in Adalmiina grape juice

The volatile composition of Adalmiina grape juice and its different distribution in the free form and bound form is shown in Table 9. GC x GC chromatogram contour of total ion current is displayed bellow (Figure 30), the most important chemical classes are highlighted.

The total free volatile compounds from Adalmiina juice accounted for 1723,92 mg L^{-1}. Bound compounds obtained by acidic hydrolysis accounted for 1777,08 mg L^{-1} while the fraction obtained through enzymatic hydrolysis estimated a total of 185,04 mg L^{-1} (Figure 31).

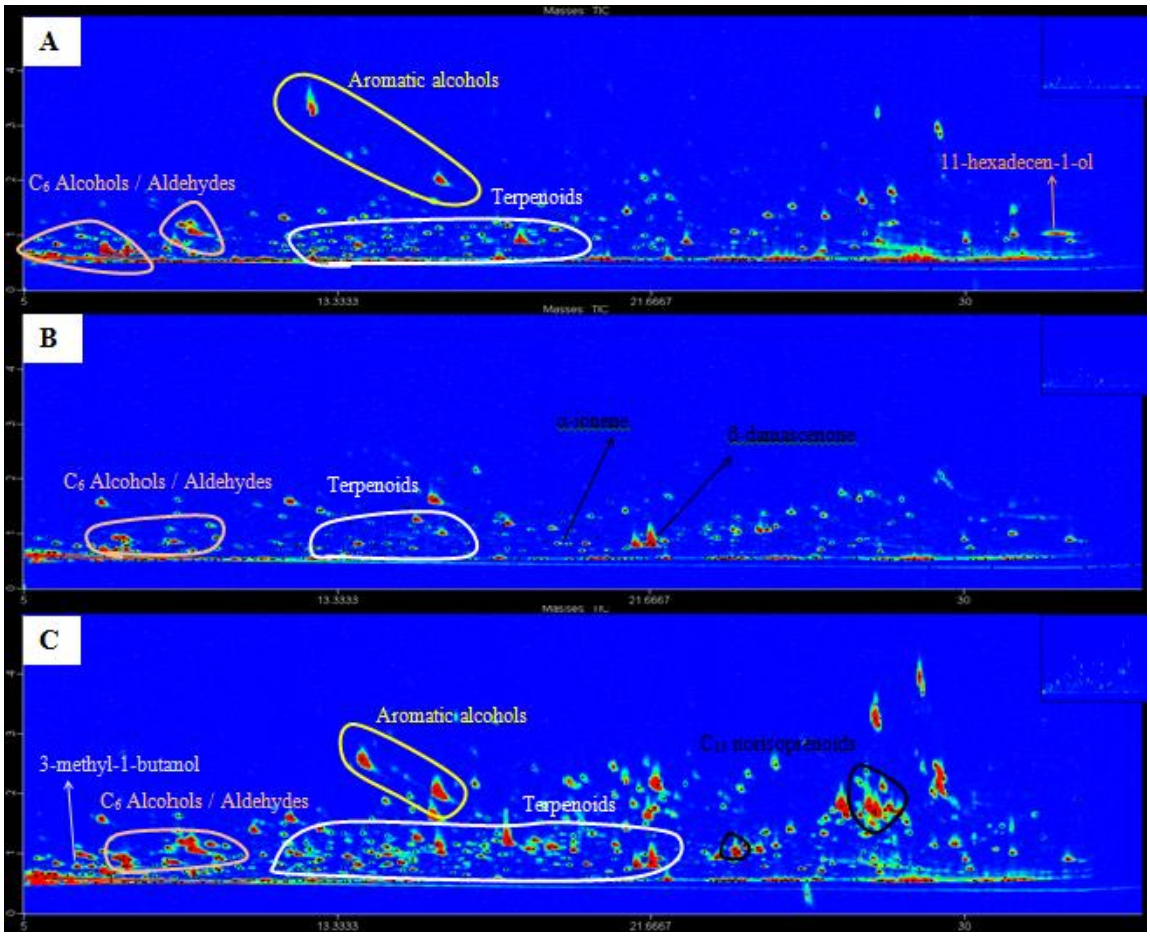

Figure 30 – GCxGC-ToF-MS chromatogram of the volatile compounds in Adalmiina grape juice, present in: (A) free form; (B) glycosidically-bound form obtained after acidic hydrolysis; and (C) glycosidically-bound form obtained after enzymatic hydrolysis.

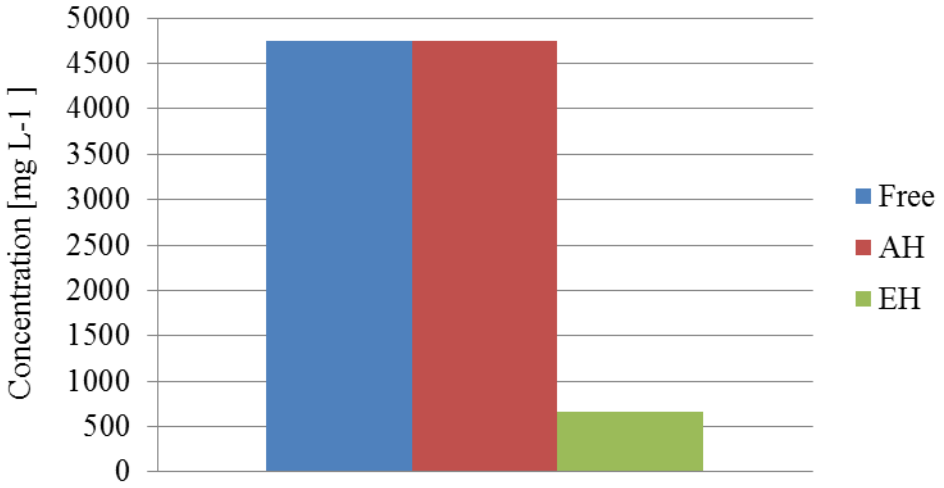

Figure 31 - Distribution of volatiles between the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH), in Adalmiina grape juice.

Table 9 - Free and bound volatile compounds identified in Adalmiina grape juice, grouped by chemical classes.

No.	Compound [a]		Concentration		
			Free [µL^{-1}] (n=4)[b]	Bound [µL^{-1}]	
				Acidic (n=3)	Enzymatic (n=3)
	Terpenoids				
1	Limonene	13.4103 , 0.634	n.d	24507,60 ± 9377,32	10338,30 ± 8989,05
2	Eucalyptol	13.4935 , 0.647	n.d	18124,46 ± 7042,67	2131,08 ± 509,87
3	*B*-Ocimene	13.8266 , 0.673	n.d	606,30 ± 11,84	n.d
4	Linalool oxide (isomer)	14.6593 , 0.838	29445,70 ± 904,25	5973,76 ± 169,84	5003,42 ± 2896,13
5	Linalool oxide (isomer)	15.0757 , 0.851	n.d	105152,64 ± 18726,48	6556,60 ± 2222,80
6	Guaiacol	15.2422 , 1.980	n.d	n.d	2713,67 ± 93,75
7	Linalool	15.3255 , 0.917	44545,60 ± 14633,60	n.d	2605,69 ± 1675,71

Table 9 (continued)

8	Epoxylinalol	17.0741 , 1.175	n.d	n.d	4998,08 ± 3933,70
9	*α*-Terpineol	17.5738 , 1.043	19523,83± 7006,90	n.d	2130,40 ± 905,01
10	*p*-Cymen-8-ol	17.657 , 1.327	n.d	6138,94 ± 1093,12	n.d
11	*cis*-Geraniol	18.573 , 1.089	n.d	n.d	5638,49 ± 1019,57
12	*p*-Menthan-2-ol	18.573 , 1.241	n.d	n.d	7431,36 ± 3392,46
13	*trans*-Geraniol	19.1559 , 1.115	195851,16± 60690,82	n.d	16152,56 ± 1261,27
14	Citronellol hydrate	21.4042 , 1.874	n.d	n.d	2994,13 ± 123,01
15	2,3-Pinanediol	21.4875 , 1.848	n.d	n.d	7135,08 ± 4103,99
16	8-Hydroxylinalool	21.7373 , 2.165	n.d	n.d	50314,11± 28701,58
17	*p*-Menth-1-en-9-al	23.1529 , 1.102	n.d	5006,21 ± 1660,08	n.d
18	*p*-Menthane	26.7335 , 1.756	n.d	8050,63 ± 2444,67	n.d
19	γ-eudesmol	27.2331 , 0.904	n.d	8254,15 ± 416,98	n.d
20	*β*-Eudesmol	27.5662 , 0.944	n.d	n.d	13427,55 ± 9517,31
21	Farnesol	32.8955 , 1.544	n.d	n.d	2645,22 ± 464,68
Sub-total (μL^{-1})			**396555,28**	**181814,69**	**151584,09**
Sub-total (%)			**8,35**	**61,01**	**22,76**
	C_{13}Norisoprenoids				
22	*α*-Ionene	18.2399 , 0.700	n.d	1914,95 ± 315,22	n.d
23	*β* –Damascenone	21.9038 , 0.871	n.d	12272,61 ± 2381,31	n.d
24	*β*-Ionone epoxide	23.9856 , 0.983	16690,10 ± 4992,57	n.d	3018,18 ± 433,90

Table 9 (continued)

25	3-Hydroxy-7,8-dihydro-*β*-ionol	27.2331 , 2.191	n.d	n.d	4468,51 ± 1007,30
26	3-Oxo-*α*-ionol	27.4829 , 1.789	n.d	n.d	52730,98 ± 8749,68
27	Dihydro-*β*-ionone	27.6494 , 1.696	n.d	n.d	31848,71 ± 15963,16
Sub-total (μL^{-1})			**16690,1**	**14187,56**	**52800**
Sub-total (%)			**0,035**	**4,76**	**7,93**
	Alcohols				
28	3-Methyl-1-butanol	6.49886 , 0.997	n.d	n.d	24651,29 ± 2061,63
29	3-Hexanol	7.66464 , 0.878	n.d	8656,83 ± 342,78	8812,64 ± 3188,83
30	2-Hexanol	7.83118 , 0.904	n.d	14471,95± 991,37	11676,19 ± 2837,87
31	3-Hexen-1-ol	9.1635 , 1.208	n.d	n.d	31259,42 ± 11186,75
32	1-Hexanol	9.41331 , 1.076	1288008,93 ± 159141,02	2169,67 ± 317,34	73303,00 ± 28082,44
33	*(E)*-2-Hexen-1-ol	9.41331 , 1.241	133572,61 ± 25840,54	n.d	9101,59 ± 3129,93
34	Cyclohexanol	9.91293 , 1.168	n.d	n.d	16343,23 ± 3059,80
35	*(Z)*-2-Hexen-1-ol	10.6624 , 0.792	n.d	3087,06 ± 469,53	n.d
36	1-Octen-3-ol	12.2445 , 1.003	110740,58 ± 48032,12	571,19 ± 135,72	7869,43 ± 4164,41
37	2-Ethyl-1-hexanol	13.5768 , 0.964	145847,70 ± 61096,62	n.d	5843,33 ± 864,05
38	Benzyl alcohol	13.9932 , 2.574	24904,61 ± 20961,57	n.d	82683,10 ± 2993,68
39	1-Octanol	14.6593 , 0.997	135523,50 ± 52581,79	2147,85 ± 295,53	14031,56 ± 388,39
40	*(E)*-2-Octen-1-ol	14.6593 , 1.122	75077,37 ± 16958,25	n.d	n.d
41	1-Nonen-4-ol	15.2422 , 1.181	38424,56 ± 12275,07	n.d	n.d
42	Phenylethyl alcohol	15.9916 , 2.033	659228,38 ± 174413,52	1573,50 ± 97,80	104246,04 ± 30266,79

Table 9 (continued)

43	1-Octanol, 2-butyl	24.1521 , 0.568	250632,72 ± 228272,48	n.d	n.d
44	11-Hexadecen-1-ol,	32.7289 , 0.904	162724,75 ± 22807,29	17967,74± 5287,95	n.d
Sub-Total (µL^{-1})			**3024685,71**	**50645,79**	**389820,82**
Sub-Total (%)			**63,66**	**17,00**	**58,54**
	Aldehydes				
45	Hexanal	7.74791 , 0.726	1277560,31± 93188,14	n.d	24910,88 ± 8565,64
46	*(Z)*-3-Hexenal	7.74791 , 0.799	n.d	n.d	29089,68 ± 11838,69
47	*(E)*-2-Hexenal	8.91369 , 0.891	n.d	36702,07± 5789,42	n.d
48	2-Hexenal	8.99696 , 0.865	n.d	n.d	17737,88 ± 6197,10
51	2-Ethyl-hexanal	9.49658 , 0.660	n.d	14672,74± 2690,60	n.d
52	2-Octenal	14.243 , 0.858	35731,70 ± 6199,24	n.d	n.d
Sub-Total (µL^{-1})			**1313292,01**	**51374,81**	**71738,43**
Sub-Total (%)			**27,64**	**17,24**	**10,77**
Total (µL^{-1})			**4751223,10**	**298022,85**	**665943,34**

[a] Identification based on NIST database library.
[b] Each value is the mean of the corresponding replicates.

3.6.1) Free and bound terpenoids in Adalmiina grape juice

The terpenoid composition in Adalmiina grape juice is substantially different in between the free and bound fractions. It represents merely 8,35 % of the total free fraction accounting for 396555,28 µg L^{-1}. In the bound fraction, terpenoids are present more

abundantly representing 61,01 % (181814,69 µg L^{-1}) in the acidic hydrolysis fraction and 22,76 % (151584,09 µg L^{-1})in the enzymatic hydrolysis fraction. Despite this, quantitatively the concentration of terpenoids is higher in the free fraction (Figure 32).

The terpenoids linalool, linalool oxide, and pinene are the main compounds of this family, present in the free form. Linalool oxide, limonene, and eucalyptol are the main bound terpenoids obtained through acidic hydrolysis, while *β*-eudesmol, 8-hydroxylinalool, *trans*-geraniol, *cis*-geraniol, and limonene are the main bound terpenoids obtained through enzymatic hydrolysis.

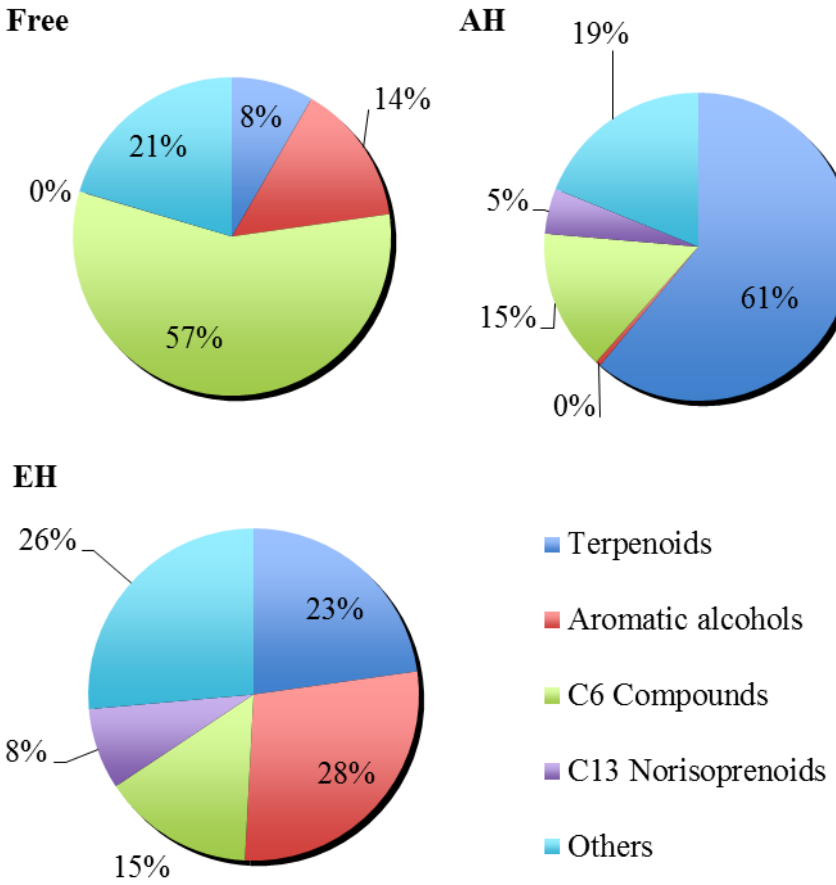

Figure 32 - Volatile composition of Adalmiina grape juice in the free and glycosidically linked forms after acidic (AH) and enzymatic hydrolysis (EH).

3.6.2) Free and bound alcohols in Adalmiina grape juice

The alcohol composition of Adalmiina grape juice is a major one. In the free fraction, alcohols reach 63,66 % (3024685,71 µg L^{-1}) of its total, while in the bound fraction enzymatically treated, alcohols reach 58,54 % (389820,82 µg L^{-1}). The alcohol composition is less significant in the acidic hydrolyzed bound fraction reaching only 17% (50645,79 µg L^{-1}) of its total. Phenylethyl alcohol stands as the most abundant alcohol in both free and bound forms, benzyl alcohol was also identified and is also present in the free and bound forms. C_6 alcohols represent the majority of the remaining alcohols in Adalmiina grape juice.

3.6.3) Other compounds

C_{13} norisoprenoids in Adalmiina grape juice are predominantly present in the bound form accounting for 52800 µg L^{-1} in the enzymatic hydrolyzed fraction and 14187,56 µg L^{-1} in the acidic hydrolyzed fraction. *β*-Ionone epoxide is the only compound present in the free form. *β*-Damascenone and *α*-ionene were identified after acidic hydrolysis while 3-hydroxy-*β*-damascone, 3-hydroxy-7,8-dihydro-*β*-ionol, and dihydro-*β*-ionone were identified after enzymatic hydrolysis.

Aldehydes, in Adalmiina grape juice, are specially significant in the free fraction representing 27,64 % (1313292,01 µg L^{-1}) of its total, where hexanal is the most abundant compound identified. Plenty of C_6 aldehydes such as 2-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenal, and 2-ethyl-hexanal are present in the bound form.

3.6.4) Concluding remarks

The results of this analysis show that Adalmiina grape juice features a rich free volatile fraction, in comparison with the bound fraction. Enzymatic hydrolysis yielded 36

compounds and shows to be more efficient than acidic hydrolysis, which yielded 29 compounds, in releasing bound volatiles. Terpenoids are present in lower abundance in Adalmiina grape juice, comparatively to other analyzed varieties. They were identified in both free and bound form, although their contribution is more significant in the bound fraction hydrolyzed in acidic conditions. The monoterpenol geraniol (OT=30 µ L^{-1}) and *β*-linalool (OT=15 µ L^{-1}) contribute with floral and fruity aromas.

Limonene (OT=10 µ L^{-1}) and *(-)*-*α*-pinene (OT=6 µ L^{-1}) are two other identified monoterpenes present abundantly in Adalmiina variety, these two compounds may provide a citrus-like aroma (71). Eucalyptol (OT=12 µ L^{-1}) can arise from limonene itself through sequence of chemical rearrangements, has a camphoraceous aroma. Eucalyptol is characterized by the typical eucalyptus odor (fresh, camphoraceous, cool).

Aromatic alcohols, phenylethyl alcohol and benzyl alcohol have been described as responsible for floral/sweet odors, and are the predominant alcohols in Freiminer grape juice. Benzyl alcohol was obtained only through enzymatic hydrolysis while phenylethyl alcohol is abundantly present in the free and bound forms (34).

C_{13} norisoprenoids are mainly present in the glycosidically-bounded form, in Adalmiina grape juice and can contribute to varietal aroma when released. *β*-Damascenone has a descriptor of “cooked apple/floral/quince” with an extremely low odor threshold of 0,002 µg L^{-1} in water (35). Dihydro-*β*-ionone, 3-oxo-*α*-ionol, 3-hydroxy-5,6-epoxy-*β*-ionone are precursors of *α*-ionone and *β*-ionone.

4) Conclusion

Throughout this study, the volatile profile of *Vitis vinifera* L. Mília, Merzling, Freiminer, Traminer, Jutrzenka, and Adalmiina varieties was characterized. There are some significant differences in between each grape variety, but also some resemblances. In grapes, the varietal volatile content is distributed by free and glycosidically-linked forms.

For Mília, Freiminer, Traminer, and Adalmiina grape juice, volatiles appear predominantly distributed in its free form. As for Merzling and Jutrzenka grape juice, volatiles are mostly present as glycosidically-bound. Terpenoids, C_{13} norisoprenoids, alcohols and aldehydes are the main chemical families and were identified in all the analysed grape varieties. Their pattern of distribution in between the free and bound forms, quantities, and variability of compounds is however different from variety to variety.

A comparative study was carried out in order to test the effectiveness of two types of hydrolysis with the purpose of releasing the glycosidically-bound volatile compounds, which can greatly contribute to the varietal aroma of the studied grape juices. Enzymatic hydrolysis shows to be more efficient in releasing bound compounds, giving rise to a wider range of compounds.

Terpenoids represent the major fraction of volatiles in Mília, Merzling, Freiminer, Traminer, and Jutrzenka grape juice. Adalmiina is quantitatively richer in alcohols aliphatic and aromatic alcohols.

Bound fractions are mainly characterized by terpenoids, monoterpene alcohols more specifically. Geraniol stands out as the most important monoterpenol in every grape variety. Linalool, linalool oxides, citronellol, rose oxide, α-citral were other key volatiles identified in the analysed grape varieties. C_{13} norisoprenoids are intrinsically related with the bound fraction as shown in previous grape studies as this was indeed verified. Through enzymatic hydrolysis, β-damascenone, α-ionone, and β-ionone precursors were mostly identified. The powerful odorant β-damascenone was primarily identified after acidic hydrolysis in Mília, Merzling, Freiminer, Jutrzenka, and Adalmiina grape juice.

Aromatic alcohols, C_6 alcohols are particularly important in in characterizing Adalmiina grape juice, although they are also present in all the other varieties and above their sensory perception limit. Last but not least, C_6 aldehydes are present in every grape variety but with

special emphasis on Merzling variety, where these compounds represent the majority of the free fraction volatiles.

In conclusion and taking in consideration the volatiles obtained from the odorless glycosidically-bound precursors, Mília, Freiminer, Traminer, and Jutrzenka can be characterized by sweet and flowery notes and a pleasant fruity aroma. Merzling due to its richness in aldehydes may show some green, grassy, and fruity aromas along with some contribution from terpenoids present in the bound fraction. Adalmiina may show prominent floral/sweet odors derived from aromatic alcohols.

As a future work, this analysis could be corroborated with a GC-Olfactometry analysis in order to identify the potential key odorants of the varietal aroma of each grape variety. It would be also interesting to use alternative methodologies in the analysis of the glycosidically-linked fraction, liquid chromatography–mass spectrometry could be employed, discarding the hydrolysis step in sample preparation. This could avoid monoterpene rearrangement during harsh acidic hydrolysis, and show to which extent was enzymatic hydrolysis efficient in releasing glycosidically-bound volatiles. It would also make possible the study of bound sugars and the whole glycoside structure.

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