Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences III, 2013: 45-60 ISBN: 978-81-7895-605-3
Editors: Diego Muñoz-Torrero, Amparo Cortés and Eduardo L. Mariño

3. *Cava* (Spanish sparkling wine) aroma: Composition and determination methods

Montserrat Riu-Aumatell, Jordi Torrens[1], Susana Buxaderas and Elvira López-Tamames

Departament de Nutrició i Bromatologia, Xarxa de Referència en Tecnologia dels Aliments (XaRTA), Facultat de Farmàcia, Universitat de Barcelona; Campus de l'Alimentació de Torribera, Av. Prat de la Riba, 171, 08921-Santa Coloma de Gramenet, Spain and Av Joan XXIII s/n, 08028-Barcelona, Spain
[1]Freixenet S.A., C/ Joan Sala 2, 08770-Sant Sadurní d'Anoia, Barcelona, Spain

Abstract. *Cava* (Spanish sparkling wine) is one of the most important quality sparkling wines in Europe. It is produced by the traditional method in which a base wine is re-fermented and aged in the same bottle that reaches the consumer. The special ageing in contact with lees gives the *cava* a particular bouquet with toasty, sweet or lactic notes. These nuances could be related with the chemical composition of aroma. The methods required to analyze the flavor of *cava* are revised. Three approaches are necessary to obtain a wider profile: chemical, olfactometric and sensory.

Introduction

A sparkling wine is defined as one in which the carbon dioxide, produced exclusively by fermentation, is released when the bottle is opened. To obtain a European Union sparkling wine designation, the product must have excess pressure, due to carbon dioxide in solution, of no less than 3 bars when kept

Correspondence/Reprint request: Dr. Montserrat Riu-Aumatell, Departament de Nutrició i Bromatologia Facultat de Farmàcia, Universitat de Barcelona, 08028-Barcelona, and Campus de l'Alimentació de Torribera 08921-Santa Coloma de Gramenet, Spain. E-mail: montseriu@ub.edu

at a temperature of 20ºC in a closed container. In addition, the total alcoholic strength of the base wine (*cuvée*) used in sparkling wine preparation should not be less than 8.5% vol. For quality sparkling wines, the European regulation requires a minimum excess pressure of 3.5 bars and a minimum alcoholic degree of the base wine of 9% vol. [1]. The best quality sparkling wines produced in Europe are *Champagne* (from Reims, France), *Cava* (mainly from Penedès, Catalonia, Spain) and *Talento* (from Italy).

Cava is a Spanish sparkling wine produced by the traditional method (*Méthode Champenoise,* classical or traditional) in which a base wine is re-fermented in a sealed bottle [2]. The second fermentation is followed by an aging process in which the wine acquires its complex and particular bouquet. Aging is carried out in anaerobic conditions and always in contact with lees. Much of the chemical and sensory composition of *Cava* is acquired during aging. This evolution over time is attributed to yeast autolysis, the deterioration of yeast cells after their death, and the exchange between yeast cells and wine [3].

One of the first quality factors of a wine is its aroma. The analysis of aroma is a multidisciplinary science as it should involve three approaches: chemical composition, sensory analysis and an olfactometric study. All of these techniques are necessary to obtain an overall view of the aroma of wine. The characterization of aroma and its evolution during aging could be an interesting tool for winemakers. The present chapter describes the most useful methods for analyzing the aroma of sparkling wine and recent knowledge about the aroma of *cava* gained by our research group.

1. Méthode traditionelle or champenoise

C*ava* or Spanish sparkling wine is produced in two stages, following the traditional method. The first stage consists of white wine vinification and the second stage consists of a second fermentation in the same bottle that reaches the consumer, followed by a period of aging in contact with lees.

The first step begins with the harvest of the grape. The autochthonous V*itis vinifera* from Macabeu, Xarel·lo and Parellada varieties are the main grapes used in *cava* elaboration, which follows the typical vinification process of white wines. For high quality sparkling wines, must is obtained by soft pressure and is then clarified. The acidity of the must is corrected with tartaric acid or citric acid, and sulfur dioxide is also added.

Usually, alcoholic fermentation takes place in stainless steel tanks at a controlled temperature, which is always below 20ºC to prevent the loss of aroma molecules. The fermentation process takes a few days and results in a wine with no more than 1.5 g/L of residual sugar. Then, physical sedimentation of colloids clarifies the wine; this can also be achieved by adding fining agents, such as bentonite.

If necessary, malolactic fermentation can be induced to decrease the acidity of the wine. Tartaric stabilization usually takes place by reducing the temperature of the tank below 0ºC in order to precipitate the tartrate crystals. The precipitate is usually eliminated by filtration. At this point in the process, the oenologist chooses the optimum blend or *coupage* of the monovarietal wines, in order to obtain the definitive base wine. The base wine composition is determined by the intended final product, the physicochemical composition (which must comply with the legislation) and the tasting notes, in particular.

The characteristic of the traditional method that distinguishes it from alternative methods (such as Charmat or Granvas) is the secondary fermentation in a closed bottle and the aging time in contact with yeast. A *liqueur de tirage* is added to the base wine, which is composed of yeast cells (yeast starter), sucrose, bentonite, and other compounds to stimulate yeast growth. The second fermentation of base wine is also called *prise de mousse*, because the carbon dioxide and effervescence are acquired in this phase. The second fermentation takes 2 or 3 months and can be considered finished when the sugar concentration is less than 1.5 g/L. Afterwards, the *cava* remains in contact with the lees for at least 9 months.

During ageing, autolysis of lees takes place. This involves the enzymatic self-degradation of cell components that is associated with cell death. The process leads to the release of some compounds to the wine and their interaction with wine constituents [4-6]. The wine is enriched by low molecular weight compounds and colloids [7], including nitrogen compounds, which are the most widely studied. In the first stages of autolysis, proteins and high molecular weight peptides are released to the wine and are subsequently hydrolyzed to smaller peptides and free amino acids [8]. The role of amino acids as aroma precursors is well known [9]. Degradation continues with the hydrolysis of glucans and mannoproteins [8]. Factors such as the time and temperature of *sur lie* ageing modulate the release of polysaccharides to the sparkling wine [7, 10]. In particular, the process of yeast autolysis is involved in the qualitative and quantitative composition of aromatic compounds [11].

The final stage of the traditional method begins with the riddling process, to eliminate the yeast lees from the bottle. It consists in bringing the yeast lees to the bottleneck through the addition of fining agents such as bentonite. The riddling process is usually performed with automatic riddlers, in which the bottles are placed in a cage and shaken to simulate the classical manual riddling process. Finally, less removal, which is called disgorging, is performed by submerging the neck of the bottle in a freezing bath of polyethylene glycol (45%). The bottle is opened and frozen, and the lees come out of the top. Then a dose of expedition liqueur is added to restore the lost volume. The composition of the expedition liqueur defines the different commercial products on the market. Depending on the sugar added with the expedition liqueur, different indications must be cited on the label (Table 1). The last stage is closure with a cork, the capsule and the wire and the labeling of the bottle.

Table 1. List of indications for quality sparkling wine according to the sugar content.

Term	Conditions of use
Brut nature	Its sugar content is < 3 g/L; no sugar has been added after the secondary fermentation
Extra brut	Sugar content between 0 and 6 g/L
Brut	Sugar content < 12 g/L
Extra Dry	Sugar content between 12 and 17 g/L
Sec	Sugar content between 17 and 32 g/L
Semi-sec	Sugar content between 32 and 50 g/L
Doux	Sugar content > 50 g/L

During fermentation, and principally during ageing in contact with lees, the wine develops its characteristic foam and its particular bouquet. The factors that can influence the quality of sparkling wine and its chemical composition include the following: the grape varieties, the vineyard yield, the quality and composition of the base wine and the yeast strain. The second fermentation and the ageing in contact with yeast are key factors to explain the quality of sparkling wines like *cava* [8].

2. Aroma characteristics

Although the main attraction of sparkling wines is the CO_2, aroma is the sensorial aspect that is most appreciated by the consumer and is one of the main quality factors of a sparkling wine. The aroma substances that comprise flavors are found in nature as complex mixtures of volatile compounds. More than 6,000 compounds have been identified in the volatile fraction of foods, including over 800 in different types of wine. The concentration of these volatile compounds varies from high concentrations (hundreds of mg/L) to lower levels (ng/L). The impact of each individual compound in the odor profile can be different. Most volatile compounds do not have a significant impact on flavor, although some that only occur in traces can have a significant influence. Here, the aroma properties of Spanish sparkling wine are reviewed.

According to some authors, such as Belitz *et al.* [12] or Ferreira *et al.* [13], volatiles from a wine can be classified according to the role they play, as follows:

- ✓ Genuine impact compound (individual compound associated with a specific aroma nuance)
- ✓ Major contributor (individual or family of aroma that provides a generic descriptor for the wine as citric or red wine. When they are removed, there is a qualitative change in the flavor of the wine)
- ✓ Net contributors (individual or family of aroma that provides a generic descriptor for a wine, but when they are removed only a decrease in the intensity of the descriptor is noticed)
- ✓ Secondary or subtle contributor (a compound found in wine at low concentrations, to provide an individually generic descriptor)
- ✓ Aroma enhancer (individual or family of aroma that does not provide the specific descriptor, but enhances the specific aroma of other molecules in the wine)
- ✓ Aroma depressor (molecule that decreases the intensity of an odor note)

In addition, wine volatile compounds can be classified in different ways: by chemical family (e.g. aldehydes, esters, ketones, terpenes, alcohols, norisoprenoids) or by sensory note (e.g. fruity, lactic, chemical, floral). However the most usual way to classify the aroma of wine is the stage in which the compounds are formed. The volatile compounds that can be found in sparkling wines come from grape (pre-fermentative origin), from yeast during the first or second fermentation or from ageing in contact with lees. This special ageing of *cava* provides a more complex profile, which is produced by chemical and enzymatic reactions.

The pre-fermentation volatile compounds depend largely on the grape variety. In addition, some compounds can be found that are formed by chemical or enzymatic reactions in must during grape processing (crushing and pressing). Other factors that have an influence are the degree of ripeness and the environment. The most usual varietal compounds are terpenes, which are typical of Muscat varieties, and methoxypyrazines, which are typical of *Cabernet sauvignon*, *Sauvignon blanc* and *Semillon* wines [14]. Methoxypyrazines provide these varieties of wines with a characteristic note of green pepper. In the family of terpenes, the main compounds of interest in the olfactory field are alcohols and aldehydes. The monoterpenes can be found as free and glycosidically bound forms in grape berries [9]. The ratio between the free and bound form of monoterpenes varies during ripening, with a higher proportion of bound forms in mature grapes.

Carotenoids also play an important role in varietal aroma as precursors of C13-norisoprenoids such as TDN and vitispirane [15]. The oxidation of carotenoids releases some important strong odor fragments. According to some authors, these can be found in grape berries linked to a sugar molecule [16] or may be produced by direct carotenoid degradation [14].

Quantitatively, the most important portion of the aroma is that which comes from fermentation by yeast. Yeast influences the wine aroma as a consequence of microbiological transformations during alcoholic fermentation. One of the most important factors is the yeast strain of *Saccharomyces cerevisiae* that is used [17]. In addition, factors such as pH, temperature and must composition determine the biosynthesis of volatile compounds. Besides glycerol, ethanol, acetaldehyde and diacetyl, the main compounds formed are ethyl esters of fatty acids. Other fermentation compounds are higher alcohols that come from amino acid metabolism and associated ethyl esters, and volatile acids such as isovaleraldehyde, amyl alcohol, isobutanol, and 2-phenyl acetate. The Ehrlich reaction is responsible for the formation of these compounds.

Volatile esters are one of the most important classes of volatiles and are responsible for fruity notes in wine. The formation of these compounds differs according to the yeast strain [17], and factors such as fermentation temperature, nutrient availability and oxygen level must be taken into account [9]. In addition, the grape variety and the harvest year have a great influence on the volatile composition of wines. When six yeast strains were tested to evaluate the ability to produce optimum aromatic characteristics, those with high ethyl ester content showed a fresh sensory profile, with citric and green fruit notes, while the yeast strains that produce more acetate esters showed a profile with notes such as tropical and ripe fruit (data not shown).

Higher alcohols are related with yeast metabolism and, for this reason, wines fermented by different yeast strains can be differentiated [18]. Some authors, including Mateo *et al.* [19], have shown that the alcohol profile is dependent not only on the yeast strain but also directly on the inoculum concentration. According to these authors, the connection between the two chemical families, esters and higher alcohols, is related with the olfactory quality of wine:

$$[long\ chain\ esters] \times \frac{400}{[higher\ alcohols]}$$

This equation takes into account the relationship between esters and alcohols, according to Mateo *et al.* [19]. When the concentration of alcohol is above 400 mg/L is considered a negative quality factor.

The study by Torrens *et al.* [17] revealed that varietal compounds such as terpenes (linalool, citronellol), norisoprenoids (β-damascenone), and C_6 alcohols (hexanol and *cis*-3-hexenol) are linked with the yeast strain used in the vinification process. The liberation of terpenes and C_6 alcohols from their glycosylated conjugates is likely to be modulated by the enzymatic activity of the yeast strain.

However, it seems that other minority compounds, such as methionol or acetaldehyde, are independent of the yeast strain. Methionol seems to be produced by methionine catabolism and acetaldehyde is dependent on alcohol dehydrogenase activity and interaction with SO_2 concentration.

Finally, the third fraction of aroma is created during ageing in contact with lees. Usually, the evolution of aroma during ageing consists of loss of characteristic varietal notes and fermentation nuances, and the occurrence of the characteristic ageing notes, such as toasty and lactic [9, 18]. The process of autolysis seems to be a key factor in the development of the flavor profile of aged sparkling wines. Torrens *et al.* [18] compared the base wine and its corresponding young *cava* and reserve (> 15 months) to find the volatiles responsible for the characteristic and complex bouquet of *cava*. Three analytical methods were applied: physicochemical (SPME-GC-MS), olfactometric (GC-O) and sensory analysis, to obtain complementary information about the sensory profile of wines. When the sensory analysis was performed, the fruity profile of base wines evolved into a more complex bouquet with toasty, lactic and yeasty notes. These nuances were more pronounced in reserve than in young *cavas*.

When the behavior of volatiles in relation to ageing time was studied, some compounds were found to be useful as ageing markers. Acetate esters decrease during ageing [15, 20], while ethyl esters of high molecular weight, such as diethyl succinate and ethyl lactate, seem to be typical of aged *cavas* and could be used as age markers. Diethyl succinate, defined by the tasters as fruity or floral [18], could be a good ageing marker during the entire ageing period, while ethyl lactate was defined as a cheese note.

During ageing in contact with lees, the content of acetate esters decreases [15], which results in a loss of freshness of bouquet. According to some authors [21], ageing in contact with lees for 18 months has an impact on the sensory profile, leading to enrichment with butter or vanilla aroma in white varieties. Torrens *et al.* [18] found that the lactic note increases significantly in reserve *cava*, which can be related with acetoin and diacetyl content.

In contrast, the content of some compounds of varietal origin, such as furans, thiol and norisoprenoids, increases during ageing [15, 18, 22]. Furans are derived from sugar degradation and they are described by tasters as toasted, dried fruit or caramel notes. Although they are not detected by GC, and only furfural has been identified and quantified, they play an important role in the bouquet of *cava*. Other extraction methods, such as SDE or CLSA, seem to be more adequate for the chemical determination of furan compounds [22]. The content of other varietal compounds such as lactones increases, according to Torrens *et al.* [18]. These compounds are difficult to identify and quantify in wines due to their low concentration, but they could be detected by olfactometric analysis due to their characteristic and desirable nuances (fruity, floral, and caramel).

In conclusion, ageing in contact with lees is a key factor in the development of the bouquet of *cava*. According to studies carried out to date, the bouquet of *cava* is described as toasty, lactic and sweet with enrichment in furan, lactone and norisoprenoid compounds in the volatile profile.

3. Aroma analysis

Aroma is one of the most important quality factors of a wine. Wine aroma analysis is a multidisciplinary science, which can be carried out following three principles: chemical analysis to obtain the qualitative and quantitative volatile profile, usually by means of gas chromatography-mass spectrometry; sensory analysis that uses people as the measuring instrument; and sniffing techniques that use gas chromatography coupled to an olfactometric detector.

3.1. Chemical analysis

The characterization of aromatic compounds is one of the toughest challenges in the analysis of *cava*. The main difficulty is due to the fact that the volatile fraction is composed of multiple substances of varying physical and physicochemical nature. However, the fact that the molecules are more or less volatile means that the instrumental technique of choice is gas chromatography (GC), preceded by an extraction phase and concentration of the volatile compounds. Since the 1950s, the most usual techniques for the determination of volatile composition are chromatographic analysis coupled with various detectors, such as the flame ionization detector (FID). Since the emergence of mass spectrometry detection (MSD), which can be used to identify a large number of compounds, GC-MS has become the reference in aroma assessment. Before analysis, an extraction method is needed that is usually based on volatility (headspace techniques) or solubility (solvent extraction) of aromatic compounds, or a combination of the two.

Distillation methods including steam distillation (SD), simultaneous distillation-extraction (SDE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) have been previously applied to the analysis of wine aroma. In particular, SDE (the Likens-Nickerson technique) combines the advantage of liquid-liquid extraction with steam distillation techniques, and has been successfully used in wine analysis [22]. The use of solvents in the sample during the extraction increases the risk of contamination and the formation of artifacts. In addition, thermolabile compounds can be decomposed or transformed into other compounds. Prior to the chromatographic analysis, a concentration stage is needed to remove the solvent excess; this action can cause the loss of some volatile compounds.

Headspace techniques isolate volatile compounds according to their volatility. They mainly identify the most volatile compounds, so odor-active compounds are obtained. The method can be classified into two main kinds of extraction: static or dynamic headspace. Static headspace (SH) is based on static equilibrium under given conditions, and only a small fraction is extracted from the sample. The headspace composition depends on the partition of volatiles between air and the matrix in which they can be found [23]. SH usually gives a low yield of volatile compounds (the most volatile ones), so its combination with other techniques such as vacuum distillation is a good alternative. The use of dynamic headspace (DH) minimizes the low yield of some compounds [24]. One of the most commonly used DH techniques is the Purge-and-Trap analysis. The trapping stage of the analysis enhances the detection power of this technique. The stage consists of controlled flow of an inert gas, which sweeps the volatile compounds from the matrix. Then, they are trapped in an adsorbent where they will be retained until the following solvent extraction or thermal desorption [23].

In recent years, solid-phase microextraction (SPME) has become the most widely used method for food analysis, particularly for wine or sparkling wine [15, 22, 18, 25]. Initially, SPME was developed by Pawliszyn and coworkers in 1990 [26] for pesticide analysis in water. It is a solvent-free, simple, rapid, inexpensive and selective method for analyzing volatile and semi-volatile compounds. It can be applied in solid, liquid and gas matrices and is based on the equilibrium between the analyte concentration in the sample matrix and a stationary phase (a fused silica fiber coated with a suitable stationary phase) (Fig 1). Subsequently, target analytes are desorbed

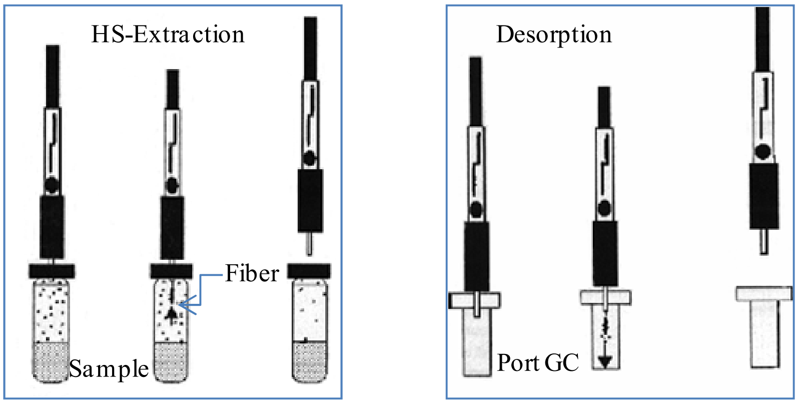

Figure 1. Scheme of the extraction and desorption process using the SPME technique.

immediately before chromatographic analysis by introducing the fiber into the liner of a GC-MS injector, normally in splitless mode (Fig. 1). The SPME can be applied by immersing the fiber into the sample (DI-SPME) or by exposing it to the sample headspace (HS-SPME).

The amount of analyte extracted by the fiber is determined by the partition coefficient between the sample matrix and the fiber coating. The fiber is coated by an absorbent polymer, such as Polydimethylsiloxane (PDMS) or Polyacrylate (PA), or an adsorbent polymer such as Carbowax (CAR) or Divinylbenzene (DVB). The fiber that currently provides the best results is the triple phase (CAR-DVB-PDMS), which combines three coatings with different polarities that can extract a wide range of compounds. Torrens *et al.* [25] found that this fiber had a better response; 76% more of the area than the other fibers tested (PDMS, PDMS-CAR and PDMS-DVB) in white and red wines aroma analysis.

3.2. Sensory analysis

Flavor perception is the result of multiple interactions between a wide range of chemical and sensory receptors located in the olfactory epithelium. The volatiles that reach the pituitary via the nose comprise the odor of a product, but when the chemicals reach the pituitary via retronasal stimulation, i.e. through the mouth, we talk about the flavor of the product [13]. Sensory analysis can be used to evoke, measure, analyze and interpret the reactions to stimuli perceived through the senses [27]. Sensory analysis still remains an efficient tool for assessing the properties of sparkling wine [21, 28, 29].

The current sensory analysis techniques can be divided into three categories: discrimination tests, consumer tests, and descriptive analysis. Discrimination tests are used to determine whether two products are perceived differently by human senses. The most common are the triangle test or the duo-trio, which is used when the differences between products are very small. When the differences between products are greater and should be defined, the pair test is used to find out which sample is stronger in a specific attribute [30]. The triangle test is used in the oenology industry to evaluate the impact of the yeast strain used in the first fermentation on the sensory perception of *cava*. The triangle test is performed for the base wine, young *cava* and reserve *cava*. Fig. 2 shows that the influence of yeast strain on the differentiation of sparkling wine decreases with ageing time.

Other discrimination tests are the threshold test and the intensity ranking test. The threshold test is generally used to determine the importance of a component in a flavor, for example the taint of a product. The intensity ranking test is similar to a pair test, but more than two products are compared for a single attribute.

The second method that could be applied in the wine industry is the consumer test, also named the affective test, and is based on studying which product the consumer population likes. The affective test can be qualitative and quantitative. Usually, these tests are performed using a pair preference test between two samples. The scale used is hedonic or unstructured [27].

Finally, the most sophisticated tool in sensory analysis is descriptive analysis (DA). Descriptive analysis objectively characterizes and differentiates a product, using sensory descriptors. It has been used to analyze numerous alcoholic beverages, such as *champagne* [29], *cava* [18], gin [31], beer [32] and whisky [33, 34]. Descriptive analysis quantitatively characterizes appearance, aroma, taste and mouthfeel. The first requirement is to develop a lexicon to describe a target food. Vocabulary development is usually performed by a panel of experts from the industry, who have extensive experience in sensory analysis. The closed list used in the descriptive analysis is obtained by consensus in preliminary sessions and through discussion between the panelists and the panel leader. Synonyms, hedonistic or irrelevant terms must be eliminated from the preliminary list.

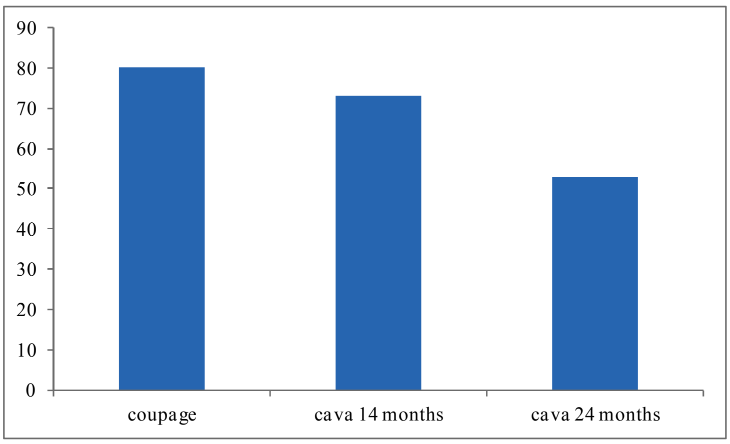

Figure 2. Percentage % of significant triangular tests among the six yeast strains [17].

According to some authors, the number of panelists required to perform a descriptive analysis is between 8 and 12. Panelists are trained with fortified wines at different concentrations [18] or through the qualitatively discrimination of artificial flavor standards [29]. The samples must be tasted at 20-22ºC to increase the perception of the aroma and flavor descriptors. In addition, quantitative descriptive analysis uses statistical tools, including multivariate methods such as principal components analysis (PCA) and ANOVA, to determine the appropriate terms, procedures and panelists for a specific product.

A few studies on sparkling wine (*cava* or *champagne*) can be found in the literature [18, 29]. The special ageing of sparkling wines through the traditional method in contact with lees leads to an evolution of the sensory profile different from that of table wines. The sensory profile of *cavas* was described empirically by the winemakers as toasted, yeasty and sweet.

Table 2 shows the olfactory descriptors evaluated by the panelists in the study by Vannier *et al.* [29] on *Champagne*, and by Torrens *et al.* [18] on base wine and *cava*. In the study by Vannier *et al.* [29], 16 descriptors were evaluated. In the study by Torrens *et al.* [18] on *cava*, fewer descriptors were used. In *cava* sparkling wines [18], the profile of the *cava* was more complex than that of the base wine, with toasty, lactic, yeasty and sweet nuances. These attributes are found to be significantly stronger in reserve *cava* than in young *cavas* (as confirmed by De la Presa-Owens, *et al.* [21]). In young *champagnes* the predominant attribute is herbaceous and in old *champagnes* the profile is similar to the profile of *cava*, with chemical and yeasty notes. In the same way, according to Vannier *et al.* [29], the exotic fruit notes decrease with ageing, while the butter and toasty notes increase in old *Champagnes*.

Table 2. Descriptors used in the studies by Vannier *et al.* [29] and Torrens *et al.* [18].

Champagne	Base wine	*Cava*	Reference aroma
Butter	Lactic	Lactic	Milk, cheese, butter
Caramel	Sweet	Sweet	Honey, caramel
Toast		Toast	Almond, nuts
Moss			
Floral	Floral	Floral	Rose, geranium,
Fruity lemon	Citrus fruit		Lemon, grapefruit
Fruit		Fruit	
Exotic fruits	Tropical fruit		Banana, pineapple
Ripe fruits	Ripe fruit		Jam, stewed fruit
Apple	Tree fruit		Apple, pear
Dust			
Herbaceous			
Animal			
Mould	Yeast	Yeast	Bread, baker's yeast
Spice			
Rubber	Chemical	Chemical	Petroleum, plastic

3.3. Olfactometric analysis (GC-O)

Olfactory GC techniques (GC-O) detect the influence of individual compounds on the sensory properties of food. Some compounds at very low concentrations (ng/L) can have a great impact on the aromatic profile, whereas other compounds at high concentrations may have very little influence.

GC-O is based on the sensory evaluation of eluate from the chromatographic column in order to detect the odor-active compounds. The judges who carry out olfactometric analyses are experts in wine aroma and are trained in the detection and approximate quantification of aromatic nuances. Usually, the same people perform the olfactometric and the sensory analyses. Olfactometric analyses can be used to detect whether a compound is odor active in the sample concentration, in which case the note is perceived by the judge, and the intensity of the odor [35]. The device consists of an olfactometric port connected in parallel with a conventional detector (Fig. 3). The eluate is split and reaches both detectors simultaneously in conventional detectors or with a slight time difference in mass spectrometer detectors. The available commercial ports are similar. An auxiliary gas (moist) is needed to prevent drying of the judges' nasal mucous membranes and the eluate passes the transfer line and is smelled in a conical port of PTFE fitted to the nose shape.

The appearance of olfactograms depends largely on the isolation method, as this affects the amount and composition of the final eluate. Isolation methods that reflect the release of volatile compounds from a food matrix can be considered more useful. In this context, static and dynamic headspace are useful, but the dynamic methods are used to a greater extent [36, 37, 38]. In the case of wine, the flavor profile is composed of a complex mixture of flavor compounds. To prevent the reduction of sensitivity due to fatigue, the analysis should be divided into several parts.

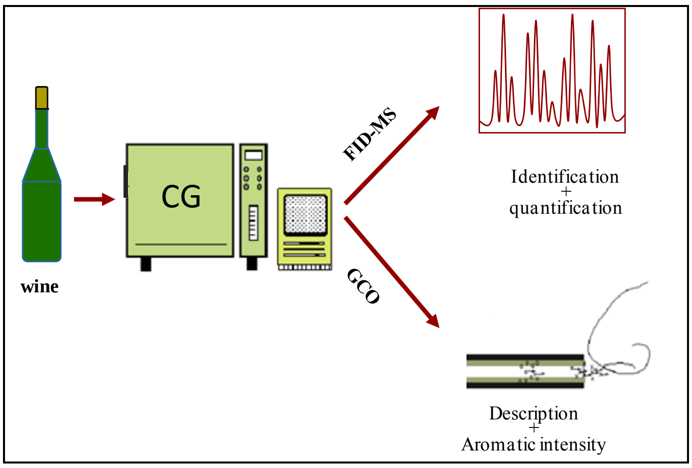

Figure 3. Schematic representation of GC-O analysis.

Various approaches are used to quantify volatiles in the sample:

1) Detection frequency methods (based on NIF or SNIF)
2) Dilution to threshold methods (CHARM and AEDA)
3) Direct intensity methods (including time-intensity, OSME and the finger span method)

In detection frequency methods (1), the percentage of judges who detect a determined odor at a determined retention time is calculated. The results are quantified using the nasal impact frequency (NIF) or surface of nasal impact frequency (SNIF), taking into account the stimulation time.

Dilution threshold methods (2) are the most commonly used techniques in analyses of alcoholic beverages. They include combined hedonic aroma response measurement (CHARM) and aroma extract dilution analysis (AEDA). These methods provide a quantitative description of the odor potential of a target compound, based on the ratio between its concentration in the sample and the sensory threshold in the air. The odor potential is usually counted as the odor activity value (OAV):

$$OAV = \frac{Concentration}{Sensory\ detection\ Threshold}$$

The AEDA method measures the highest sample dilution at which the odor is still detectable, while the CHARM method determines the duration of the odor sensation. Finally, direct intensity methods (3) measure the intensity and the duration of stimuli. The most usual is the odor magnitude specific estimation (OSME) method, in which the measure is dynamic: the appearance of an odor, the maximum intensity and the declination are all measured.

The methods that are usually employed in wine analysis [39] are the dilution to threshold methods CHARM and AEDA, and the direct intensity method OSME. However, only a few studies can be found about the olfactometric profile of sparkling wines such as *cava* or *champagne* [18, 36, 40].

4. Conclusion

Cava (Spanish sparkling wine) is a special wine produced mainly in the Penedès region (Catalonia, Spain) by the traditional method. Special ageing in contact with lees gives this beverage a particular bouquet with toasty, lactic and sweet notes. These nuances could be related with some chemical components, such as furan compounds with toast notes, lactones and norisoprenoids. The content of acetate esters decreases with ageing, which reduces the more complex fruity notes in the base wines (tropical, tree and ripe fruits) so that the flavor evolves to a single note in the *cava*.

Due to the complexity of aroma, three approaches are required to determine the bouquet of *cava*: chemical, sensory and olfactometric. Further studies are needed to deepen knowledge of the compounds responsible for the flavor and notes of a product as complex as *cava*.

Acknowledgements

The authors are grateful to Freixenet S.A. wineries for their support in providing samples. They are also grateful for funding from the *Comisión Interministerial de Ciencia y Tecnología* (CICYT) (Spain), project AGL2011-23872, and the Generalitat de Catalunya, Project 2009SGR-606.

References

1. EU 2008. Regulation (EC) No. 479/2008 of the European Parliament and of the Council on the common organization of the market in wine. *Off. J. Eur. Union* 148, 1.
2. EU 2009. Regulation (EC) No. 607/2009 of the European Parliament and of the Council laying down certain detailed rules for the implementation of Council Regulation (EC) No 479/2008 as regards protected designations of origin and geographical indications, traditional terms, labelling and presentation of certain wine sector products. *Off. J. Eur. Union* 193, 60.
3. Charpentier, C. 2010, Ageing on lees (*sur lie*) and the use of speciality inactive yeast during wine fermentation. In Managing wine quality, Volume 2: Oenology and wine quality, pp 164-187. Edited by Reynolds, A.G.. Woodhead Publishing. Cambridge.
4. Gallardo-Chacón, J.J., Vichi, S., López-Tamames, E., Buxaderas, S. 2009, *J. Agric. Food Chem*., 57, 3279.
5. Gallardo-Chacón, J.J., Vichi, S., López-Tamames, E., Buxaderas, S. 2010, *J. Agric. Food Chem*., 58, 12426.
6. Tudela, R., Gallardo-Chacón, J., Rius, N., López-Tamames, E., Buxaderas, S. 2012, *FEMS Yeast Res*., 12, 466.
7. Guilloux-Benatier, M., Chassagne, D. 2003, *J. Agric. Food Chem*., 51, 746.
8. Pozo-Bayón, M.A., Martínez-Rodríguez, A., Pueyo, E., Moreno-Arribas, M.V. 2009, *Trends Food Sci. Technol*., 20, 289.
9. Styger, G., Prior, B., Bauer, F.F. 2011, *J. Ind. Microbiol. Biotechnol*., 38, 1145.
10. Caridi, A. 2006, *I. J. Gen. Mol. Micro*., 89, 417.
11. Pérez-Serradilla, J.A., Luque de Castro, M.D. 2008, *Food Chem*., 111, 447.
12. Belitz, H.D., Grosch, W., Schieberle, P. 2009, Aroma substances. In Food Chemistry, pp. 342-408. Edited by Belitz, H.D., Grosch, W., Schieberle, P. Springer-Verlag. Berlin.
13. Ferreira, V. 2010, Volatile aroma compounds and wine sensory attributes. In Managing wine quality, Volume 1: Viticulture and wine quality, pp. 3-28. Edited by Reynolds, A.G.. Woodhead Publishing. Cambridge.

14. Rapp, A. 1998, *Nahrung*, 42, 351.
15. Riu-Aumatell, M., Bosch-Fusté, J., López-Tamames, E., Buxaderas, S. 2006, *Food Chem.*, 95, 237.
16. Silva-Ferreira, A.C., Guedes de Pinho, P. 2004, *Anal. Chim. Acta*, 513, 169.
17. Torrens, J., Urpí, P., Riu-Aumatell, M., Vichi, S., López-Tamames, E., Buxaderas, S. 2008, *Int. J. Food Micro.*, 124, 48.
18. Torrens, J., Riu-Aumatell, M., Vichi, S., López-Tamames, E., Buxaderas, S. 2010, *J. Agric. Food Chem.*, 58, 2455.
19. Mateo, J.J., Jiménez, M., Pastor, A., Huerta, T. 2001, *Food Res. Int.*, 34, 307.
20. Francioli, S., Torrens, J., Riu-Aumatell, M., López-Tamames, E., Buxaderas, S. 2003, *Am. J. Enol. Vitic.*, 54, 158.
21. De la Presa-Owens, C., Schlich, P., Davies, H.D., Noble, A.C. 1998, *Am. J. Enol. Vitic*. 49, 289.
22. Bosch-Fusté, J., Riu-Aumatell, M., Guadayol, J.M., Caixach, J., López-Tamames, E., Buxaderas, S. 2007, *Food Chem.*, 105, 428.
23. Biniecka, M., Caroli, S. 2011, *Trends Anal. Chem.*, 11, 1756.
24. Stephan, A., Bücking, M., Steinhart, H. 2000, *Food Res. Int.*, 33, 199.
25. Torrens, J., Riu-Aumatell, M., López-Tamames, E., Buxaderas, S. 2004, *J. Chromatgr. Sci.*, 42, 310.
26. Arthur, C.L., Pawliszyn, J. 1990, *Anal. Chem.*, 62, 2145.
27. Lesschaeve, L., Noble, A.C. 2010, Sensory analysis of wine. In Managing wine quality, Volume 1: Viticulture and wine quality, pp. 189-217. Edited by Reynolds, A.G.. Woodhead Publishing Limited. Cambridge.
28. De la Presa-Owens, C., Noble, A.C. 1995, *Am. J. Enol. Vitic.*, 46, 5.
29. Vannier, A., Brun, O.X., Feinberg, M.H. 1999, *Food Qual. Pref.*, 10, 101.
30. Noble, A.C. 2006, Sensory analysis of food flavor. In Flavour in food, pp. 62-80. Edited by Voilley, A., Etiévant, P.. Woodhead Publishing. Cambridge.
31. Riu-Aumatell, M., Vichi, S., Mora-Pons, M., López-Tamames, E., Buxaderas, S. 2008, *J. Food Sci.*, 73, 86.
32. Da Silva, G.A., Maretto, D.A., Bolini, H.M.A., Teófilo, R.F., Augusto, F., Poppi, R.J. 2012, *Food Chem.*, 134, 1637.
33. Guy, C., Piggott, J.R., Marie, S. 1989, *Food Qual. Pref.*, 1, 69.
34. Jack, F.R. 2012, Whiskies: composition, sensory properties and sensory analysis. In Alcoholic beverages. Sensory evaluation and consumer research, pp. 379-392. Edited by Piggott, J.R.. Woodhead Publishing. Cambridge.
35. Plutowska, B., Wardencki, W. 2008, *Food Chem.*, 107, 449.
36. Campo, E., Cacho, J., Ferreira, V. 2008, *J. Agric. Food Chem.*, 56, 2477.
37. Ferreira, V., San Juan, F., Escudero, A., Culleré, L., Fernández-Zurbano, P., Sáenz-Navajas, M.P., Cacho, J. 2009, *J. Agric. Food Chem.*, 57, 7490.
38. Sáenz-Navajas, M.P., Campo, E., Culleré, L., Fernández-Zurbano, P., Valentin, D., Ferreira, V. 2010, *J. Agric. Food Chem.*, 58, 5574.
39. Plutowska, B., Wardencki, W. 2012, Gas-chromatography-olfactometry of alcoholic beverages. In Alcoholic beverages. Sensory evaluation and consumer research, pp. 101-130. Edited by Piggott, J.R.. Woodhead Publishing. Cambridge.
40. Escudero, A., Charpentier, C., Etievant, P.X. 2000, *Sci. Aliments*, 20, 331.