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EFFECT OF DOSAGE COMPOSITIONS: SENSORY, CHEMICAL AND VOLATILE PROFILE ANALYSES OF DIFFERENT AGED CAVA

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ABSTRACT

Cava is the most prestigious sparkling wine from Spain. Nowadays, there is an upward in terms of consumption's increase while the research goes further on increasing its quality, being an example the recent distinction "Qualified Place Cava" for the most prestigious cava.

However, countries interested in healthy and balanced diet are also increasing. Their interest is not only for the variety of foodstuffs but also for their origin.

Organic farming and elaboration are gaining presence year to year worldwide. In recent years biodynamics also began to grow, and it has been able to differentiate from the organic farming.

To incorporate the cava to this production type, it obviously involves changes in the agriculture which give the principal raw material but also in the elaboration process.

In cava process a basic phase is the dosage, which needs some modification to be accepted in the regulation of these productions types.

The aim of this study is to compare the same cava with different modifications in their dosage compounds. It is essential that the products keep their basic oenological parameters and at the same time remain sensorial accepted.

Volatile profile allow to detect changes which, although not always detected by the senses, indicate the product evolution and also the effects that these additives have on the cava volatile profile. This profile allows to predict the evolution before it is detected by the senses.

During the study time, it was not observed significant differences between the samples. Thus, it suggests that in this period any dosage could be substituted by other. Four months later after the dosage was added the differences began to appear. It is necessary to extend the period of study to know for sure what happens over time.

RESUM

El cava és el vi escumós espanyol de més prestigi. Actualment a l'alça, tant pel seu consum com per la recerca continua per augmentar encara més la seva qualitat, sent un exemple la recent distinció "Cava de Paratge Qualificat" pels caves de més categoria.

D'altra banda són cada vegada més els països conscients de la importància d'una alimentació sana i equilibrada, no només per la varietat d'aliments sinó també per l'origen dels mateixos.

El cultiu i la producció ecològica fa anys que guanya presencia arreu del món. Els últims anys la biodinàmica també comença a agafar força, aconseguint-se diferenciar de l'agricultura ecològica.

Integrar el cava a aquest tipus d'elaboració implica evidentment canvis en l'agricultura, la qual donarà la matèria prima més important, però també en tot el procés productiu.

En el cas del cava un punt determinant és el licor d'expedició, el qual s'ha de modificar per poder estar acceptat dins del reglament d'aquest tipus de producció.

L'objectiu d'aquest estudi és la comparació d'un mateix cava al qual se li han modificat part dels additius del licor d'expedició. És imprescindible que aquest producte mantingui els seus paràmetres enològics bàsics i que a la vegada sigui acceptat sensorialment.

El perfil volàtil permet detectar canvis que, tot i que no sempre es detecten sensorialment, indiquen l'evolució del producte i l'efecte d'aquests additius al perfil volàtil del cava. D'aquesta manera permet preveure l'evolució abans de que es detecti sensorialment.

Durant el temps de l'estudi no s'han observat diferències significatives entre els licors, pel que fins als 4 mesos es podria substituir perfectament un licor per l'altre. Però als 4 mesos es comencen a insinuar les diferències, pel que caldria allargar el temps de l'estudi per saber amb certesa que passa al llarg del temps.

INTRODUCTION

Cava is the Spanish sparkling wine *par excellence*. Its production began in 1872 in Sant Sadurní d'Anoia, Catalonia, following the so-called traditional method. Nowadays Cava Designation Origin (D.O.) covers all the Spanish territory. However, the vast majority of cava production is concentrated in Catalonia, specifically in the area of Penedès (1).

According to the traditional method, cava is obtained as a result of two different fermentations (2). The first one is the same that takes place in a regular still wine. But this base wine have special characteristics for some chemical parameters: higher acidity, lower pH value and lower concentration of free SO_2 than a still one. Then, this wine undergoes a second fermentation in the bottle. This second one is possible thanks to the biological activity carried out by the wine yeasts added by the winemaker together with some sugar to the wine. The bottles are placed horizontally in a cave at a constant temperature of around 15 ºC for a minimum period of 9 months. The result of the second fermentation is the production of secondary aromas and carbon dioxide which are distinctive features of the final cava (3).

When the aging period has finished it is necessary to eliminate the lees and other sediments resent in the bottle through the disgorging process. In this moment a little quantity of cava is lost and it is necessary to re-establish the initial volume with the addition of the so-called dosage (3) which is mainly composed by wine and sucrose. But the International Organisation of Vine and wine (OIV) indicates than the dosage can also include other additives (4).

Cava is classified in seven categories depending on the amount of sugar that contains. While Brut Nature has not been added with any sugar and only contains 0-3 g/l of residual sugar, Sweet contains over 50 g of sugar per litre. Moreover, and depending on the aging in the bottle there is an additional cava classification: while Cava and Reserva present a minimum ageing of 9 and 15 months, respectively, Gran Reserva has been aged for a minimum period of 30 months (2).

More than 244 million of cava bottles were produced in 2015. However, the production only arrived to one million less than 100 years ago. The first data available about the national and the international cava market are dated in 1980. By that time, national market represented the vast majority of the total cava consumption and the international market only represented 12% of the total. Nevertheless, the trend has changed completely during the last three decades and in 2010 the international market

represented 61% of the total cava consumption. The most important European consumers in 2015 were Germany, Belgium and the United Kingdom whereas the United States and Japan were the most important consumers outside the European Union (5). This fact, together with some new food trends such as organic food, have forced cava producers to think about consumers from different countries, cultures and with different product demands.

Organic agriculture in Catalonia has increased considerably during the last 20 years. While in 1995 there were only 1,936 ha dedicated to this type of agriculture, nowadays, there are 142,024 ha. The biggest growth was registered in 2001 and since 2009 this growth has been constant. In the case of vineyards, in 2000 there were only 450 ha organically cultivated but today there are more than 11,700 ha. This is obviously proportional to the number of wineries that have embraced organic agriculture that has increased from 13 wineries in 2000 to 169 ones in 2015. The economics associated to organic grape, wine, sparkling wine and other organic alcoholic beverages produced in Catalonia was over 41,000,000 € in 2014 (6).

But it has to be noted that this trend does not only occurs in Catalonia. Different market studies (7, 8) show that organic farming is growing every year and that this growth is one of the fastest among the different agricultural sectors all over the world. The biggest demand of organic food takes place in North America and Europe and this demand was not affected by the 2008 economic crisis. Organic food consumers are mainly 30 to 40-years-old people of middle and high socioeconomic status who are willing to pay a higher price for these products because they are aware of their added value. These target consumers are similar to cava and other sparkling wines ones, therefore it is important to adapt these products to this market trend. However, the future hope for this type of agriculture should not be only based on a higher economic benefit but also on other factors such as a greater awareness of health issues and ethical concerns related to food (9).

In order to produce organic cava, not only organic grapes have to be used but it is also necessary to adapt the whole elaboration process to more restrictive regulations (10). The most important restrictions take place in the vineyard where many treatments are forbidden. There are also restrictions on the winemaking practices, such as partial dealcoholisation. Moreover, there has been another trend even more respectful with the environment that has become increasingly popular during the last years, the so-called biodynamic agriculture (11). But this trend cannot be really considered a new one, as it

was first proposed in 1924 by Rudolf Steiner (12). The rules and regulation of the biodynamic agriculture are even more restrictive than those for the organic agriculture. In this case, the agriculture and farming practices include the use of different natural preparations together with the follow-up of the philosophy of the cosmos. Winemaker and his team must respect the wine and should be reduced to the minimum the use of technology (13).

The Cava Regulatory Board specifies all the additives that winemakers can use to produce cava. Among them, neither sorbic acid nor copper sulphate are allowed by the organic specifications (10) and biodynamic practices only allow the addition of the minimum amount of sulphur dioxide (13), as it is shown in Table 1.

Table 1: Allowed additives according to the different elaboration regulations

Additive	**OIV[a]**	**CCPAE[b]**	**Demeter[c]**
Sulphur dioxide	X	X	Minium
Ascorbic acid	X	X	-
Sorbic acid	X	-	-
Metatartaric acid	X	X	-
Arabic gum	X	X[d]	-
Copper sulpate	X	-	-

[a]OIV: International Organisation of Vine and Wine

[b]CCPAE: Catalan Council of Organic Production

[c]Demeter:Biodynamic quality

[d]Only organic origen

X: Contains

-: Not contains

There are many ideal dosages recommended by different authors (14-19) but all of them contain wine (normally a secret of each winemaker) and different amounts of sugar depending on the type of cava to be produced (Brut Nature, Brut, Sweet…). Only a few authors explain why these ingredients are use or which their real function is. Some of them are added to equilibrate some parameters, such as acidity in the case of the addition of citric acid or to eliminate negative flavours, such as those related to reduction in the case of the addition of copper sulphate (20). In the case of SO_2, its antimicrobial and antioxidant activity is well-known and it has been deeply studied (21, 22). SO_2 is a common additive in food and wine, but is also considered an allergen. However, currently it does not exist any other additive that could replace SO_2.

The aim of this study is to compare the effect of four different dosage compositions in the evolution of cava along four months according to sensory, chemical and gas chromatography analyses. The knowledge of these changes is necessary to adapt the production of cava to the ecological and biodynamic methods.

MATERIAL AND METHODS

1. CAVA SAMPLES

All samples are from the same batch. They were produced following the traditional method with a blend of Xarel·lo (50%), Macabeu (40%) and Chardonnay (10%) from the 2010 harvest. All of them were aged in contact with the lees for 40 months at a constant temperature of around 15 ºC. The only difference between samples was the composition of the dosage added to each one of them once the second fermentation was finished.

This study compares four different samples and uses the usual liquor added to this product as the control sample (C). All the different dosages were made with the same wine and the same amount of sucrose as well as with the ingredients summarized in Table 2. When added, the concentration for the different ingredients was the same for each dosage (data not shown).

Table 2: Compounds added at each liquor

Liquor	Sulphur dioxide	Ascorbic acid	Sorbic acid
C (Control)	X	X	X
L1 (Liquor 1)	X	X	-
L2 (Liquor 2)	X	-	-
L3 (Liquor 3)	-	-	-

X: Was added

-: Wasn't added

All samples were analysed trough sensory, chemical and gas chromatography-mass spectrometry analyses three times along the study: just after the dosage was added and two and four months later. Every time three new bottles of each liquor were analysed for each type of analysis, so all the sensory, chemical and gas chromatographic analyses were made in triplicate.

2. REAGENTS AND CHEMICALS

Sulphuric acid (16% and 33%), NaOH (4%, 0.0204 M and 0.2041 M), starch indicator (1%), phenolphthalein (1%), cupric solution (0.168 M), tartrate-alkaline solution (0.886 M) and potassium iodide (1.6 M) were purchased from GAB (Barcelona, Spain) and ethanol absolute (HPLC grade), tartaric acid and sodium chloride (both reagent grade)

were purchased from Scharlab (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

The standards of the aroma compounds (isoamyl alcohol, 3-methyl-1-butanol, ethyl hexanoate, 2-phenylethanol, octanoic acid and decanoic acid) were purchased from Sigma-Aldrich (Madrid, Spain) and Fluka (Madrid, Spain), and their purity was over 99% in all cases.

3. ANALYSES

3.1. CHEMICAL ANALYSES

All samples were analysed in triplicate to determine the different oenological parameters indicated in Table 3, where also the methods applied are reported. These methods are the usual ones used in the winery to ensure that the basic parameters are not altered and that their values are consistent with the quality standards established by the winery.

After the analysis of the SO_2, all samples were filtered with a vacuum pump to avoid the CO_2 interference. No other sample pre-treatment was applied a part from the ones specifically required for each method of analysis.

Table 3: Analytical methods

Analyses	Method
Sulphur dioxide	Ripper method modified by GAB[a]
Reducing substances	Rebelein method modified by GAB[a]
Total acidity	Acid-Base volumetric[b]
Volatile acidity	García-Tena method[a]

[a] *Methods are indicated at reference 23*

[b] *Methods are indicated at reference 24*

The instruments used are a pHmeter GLP 21 (Crison, Barcelona-Spain) and a turbidimeter D-112 (DINKO Instruments, USA).

The colour evolution was analysed with a spectrophotometer Genesys 10S UV-Vis (Termo Scientific, Barcelona-Spain) following the OIV method (25) for the determination of the chromatic characteristics according to CIELab.

3.2. SENSORY ANALYSES

Sensory analyses were carried out by a panel of 10 trained assessors (2 women and 8 men), between 23 and 55 years old. All the panellists are used to taste cava products and take part in periodically training sessions.

The analysis consists in a triangular test. In each test the control sample (added with the control dosage) was contrasted with one of the other samples (each one of them added with the other liquors). All samples were identified with a random three-digit code and were analysed trough blind tasting.

3.3. VOLATILE PROFILE DETERMINATION

3.3.1. Headspace solid-phase microextraction (HS-SPME)

The soli-phase microextration (SPME) applied to the headspace (HS) of the sample was employed to extract and concentrate the volatile compounds of the samples in a single step and the StableFlex divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm from Supelco (Bellefonte, PA, USA) was the fibre used. Six different fibres were alternatively used to avoid any difference among the provided signals. All the fibres were conditioned before use according to the instructions given by the producer (30 minutes at 270 ºC) and thermally cleaned between analyses by inserting them into the GC injection port.

To avoid CO_2 interferences, all samples were decarbonized previously to their analysis. 100 ml of each sample were submitted for seven minutes to an ultrasonic bath at 0 ºC to avoid the loss of volatile compounds. Then, 10 ml of the decarbonized sample were placed in a 20-ml vial together with 2.9 g of NaCl (achieving saturation) to have a 1:1 sample/headspace ratio. Finally, the vial was tightly capped with a silicon septum under N_2 atmosphere.

The application of the HS-SPME was automatically carried out by a GC Injector 80 (Agilent, USA). The vial with the sample was pre-equilibrated for 15 min at 40 ºC. Afterwards, the SPME device was automatically pushed through the vial septum and the fibre was exposed to the headspace vial for 90 minutes at 40 ºC. All the procedure was made under mechanical stirring (500 rpm).

Finally, the SPME fibre was removed from the vial and automatically inserted into the injection port of the gas chromatograph for thermal desorption of the analytes at 270 ºC for 1 min in the splitless mode.

3.3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

To carry out the chromatographic separations, a Chrompack (Varian, Middelburg, The Netherlands) CP-WAX 57CB (50 m 0.25 mm i.d., 0.2 μm film thickness) fused silica capillary column was employed and the oven temperature was programmed as follows: 40 ºC (5 min), 3.5 ºC min^{-1} to 120 ºC, 5 ºC min^{-1} to 215 ºC (10 min). A Hewlett-Packard (HP, Palo Alto, CA, USA) 6890 gas chromatograph coupled to an HP-5973 mass selective detector (MSD) was used to perform the GC-MS analyses. The mass spectrometer operated in the electron impact ionization mode (70 eV), and the mass range was from 35 to 300 amu, while interface, source and quadrupole temperatures were 200 ºC, 230 ºC and 150 ºC, respectively. Helium was the carrier gas at a constant flow rate of 1.1 mL min^{-1}.

3.3.3. Stability of the chromatographic signal

To make sure that the chromatographic signal was consistent throughout the whole study a synthetic wine was prepared and analysed together with the samples applying the same chromatographic method. The standard aromas used to prepare the synthetic wine and their concentrations are shown in Table 4. Alcohol and pH were adjusted to the values presented by the cava samples.

Table 4: Standard aromas used to prepare the synthetic wine

Odorant	CAS no.	Concentration
Isoamyl alcohol	123-92-2	5 mg/l
3-Methyl-1-butanol	123-51-3	250 mg/l
Ethyl hexanoate	123-66-0	0.4 mg/l
2-phenylethanol	60-12-8	75 mg/l
Octanoic acid	124-07-2	5 mg/l
Decanoic acid	334-48-5	3 mg/l

3.3.4. Compounds identification

The identification of the volatile compounds was carried out by the comparison of the mass spectra obtained for each volatile compound when analysing the samples with the mass spectra given by the Willey software library (Hewlett-Packard, USA) for the

chromatographic signals, as well as by the comparison of the experimental retention indices (RI) obtained with the theoretical ones provided by the Flavornet data-base (26). The experimental RI were calculated from the retention times of a series of n-alkanes (from 6 to 26 carbon atoms) injected under the same chromatographic conditions as the samples.

4. STADISTICAL PROCEDURES

Binomial statistics were used to analyse assessors' tests.

The different oenological parameters for the dosages obtained at the three times studied were compared by simple regression analysis and one-way ANOVA analysis. Significant results were considered when $p<0.05$.

RESULTS AND DISCUSSION

Basic oenological parameters determination

The aim of the determination of the basic oenological parameters is to make sure that all the samples fulfil the quality standards established by the winery in order to eliminate those samples that could be out of these parameters.

Although the methods applied to carry out these analyses are not the official OIV ones they were selected as they are the usual methods applied in the winery where the samples were produced.

Turbidity analyses were done to make sure that the disgorgement process was correctly performed and that the bottles were clean. In all cases, the obtained values for the cava samples analysed in this study were lower than winery limits stablish for this parameter (data not shown).

ANOVA statistics were used in order to analyse all the chemical results. As expected, there were no significant differences between samples for the reducing substances, nor the acidity or the pH values at the three different times of analysis regardless the dosage considered. Regarding volatile acidity, and even though it has not changed in the studied time, it would probably change during the evolution of the cava as a result of the gas exchange through the cork.

Only SO_2 values were significate different for each dosage at the same time. As L3 dosage has not been added with SO_2, these samples only have the minimum amount of SO_2. This amount correspond to the SO_2 added in the previous process of production of the cava and to the SO_2 produced by different microorganisms during the elaboration process.

The expected decrease of free SO_2 observed for all samples through the study time it is not significantly different. However, it can be due to the fact that the amount of SO_2 is so minimal that the method applied is not sensitive enough to clearly show the drop trend along the time. Although this method is sensitive enough to carry out the usual control in the winery it could be interesting to find another method more sensitive. Moreover, ANOVA statistics are not reliable because these results present such a high dispersion among replicates for each sample that the differences founds will be probably due to the heterogeneity of the samples and the analysis method error.

Table 5: Basic oenological parameters determination

	C			L1		
	T0	T1	T2	T0	T1	T2
Reducing substances (g/l)	8,3 ± 0,3	7,8 ± 0,1	8,3 ± 0,2	6,8 ± 0,2	8 ± 0,1	6,8 ± 0,2
Free SO_2 (mg/l)	5,1 ± 0,4	3,3 ± 0,8	2,2 ± 0,5	5,3 ± 1,0	4,8 ± 0,8	3,1 ± 0,4
Total SO_2 (mg/l)	82,3 ± 2,5	79,4 ± 1,9	76,6 ± 0,9	77,3 ± 1,0	82,4 ± 0,9	78,3 ± 0,4
pH	3,00 ± 0,04	2,99 ± 0,01	2,98 ± 0,01	2,99 ± 0,05	2,98 ± 0,01	2,97 ± 0,01
Total acidity[a] (g/l)	3,9 ± 0,1	3,8 ± 0,0	4 ± 0,0	3,9 ± 0,0	3,8 ± 0,0	3,9 ± 0,1
Volatile acidity[b] (g/l)	0,3 ± 0,0	0,3 ± 0,0	0,3 ± 0,0	0,3 ± 0,0	0,3 ± 0,0	0,3 ± 0,0
	L2			**L3**		
	T0	T1	T2	T0	T1	T2
Reducing substances (g/l)	7,9 ± 0,5	8,6 ± 0,7	8,4 ± 0,1	7,5 ± 0,3	7,5 ± 0,3	8,3 ± 0,1
Free SO_2 (mg/l)	4,8 ± 0,5	1,8 ± 0,4	2,2 ± 0,6	1,7 ± 0,5	0,9 ± 0,6	0,4 ± 0,2
Total SO_2 (mg/l)	81,0 ± 0,8	82,4 ± 0,7	78,5 ± 1,5	57,8 ± 0,5	58,3 ± 0,8	57,2 ± 1,6
pH	2,99 ± 0,04	2,97 ± 0,02	3,02 ± 0,01	3,00 ± 0,04	3,00 ± 0,01	3,03 ± 0,01
Total acidity[a] (g/l)	3,9 ± 0,1	3,8 ± 0,0	3,9 ± 0,0	4 ± 0,1	3,7 ± 0,0	3,9 ± 0,0
Volatile acidity[b] (g/l)	0,2 ± 0,0	0,2 ± 0,0	0,2 ± 0,0	0,2 ± 0,0	0,2 ± 0,0	0,3 ± 0,0

[a]*Total acidity expressed as sulphuric acid (g/l)*

[b]*Volatile acidity expressed as acetic acid (g/l)*

CIELab method allows analysing the wine colour evolution through the spectrophotometer. Regarding the colour, it is known that it evolves along the aging of all wines, therefore the best comparison is between the different liquors at every time, but not between the same liquor at different time.

Four months after the dosage, the results of this analyses (data not shown) do not show differences between the samples at no time. However, this is coherent because the colour of cava is being stabilized before, during the aging process.

Moreover, these results are in accordance with assessors, who don't detected any different in the colour of the samples.

Sensory analyses

Just after the addition of the different dosages to the samples, the sensory assessors carried out a triangular test as well as a description tasting. Triangular sampling was used to establish if the samples were sensory equal or if the dosage could modify some organoleptic parameters. It was established that when triangular test shows differences between samples, the assessors have do a descriptive test too.

Binomial statistics were applied in order to analyse if the different samples analysed by the triangular test were significantly different.

Table 6: Standard aromas used to prepare the synthetic wine

Time	Samples	Error
T0	C-L1	18.3%[a]
	C-L2	74,6%[a]
	C-L3	18,3%[a]
T1	C-L1	27,5%[a]
	C-L2	55,0%[a]
	C-L3	10,4%[a]
T2	C-L1	3,8%[b]
	C-L2	3,8%[b]
	C-L3	0,8%[c]

[a]*Not significative(α5%)*

[b]*Significative (α5%)*

[c]*Significative (α1%)*

As expected, Table 6 shows that just after the dosage, assessors could not differentiate among the samples but two months later there were no significant differences either. It was not until four months after the addition of the dosage that this began to change. At this moment all liquors were significantly different to the control one ($p<0.05$), specially L3 ($p<0.01$). These results indicate that samples began to become sensory different four months after the dosage, so there will be necessary to include a descriptive tasting the next time of analysis (six months after the dosage) to really stablished which the differences are.

Sensory analyses are an important complement to basic oenological analyses, due to changes on organoleptic sensations could be consequence of minimum chemical changes and not were always reflected by the usual basic analyses.

For all the triangular tests the assessors preferred L1 to C and C to L3. However, when they were asked to compare C to L2, half of them preferred C whereas the other half prefer L2. The tasting also indicates that assessors preferred L1 to C, so winemaker could use the first dosage and fulfil the requirements of the organic production (sorbic acid is forbidden to produce organic wine since the 2012 harvest). Thanks to this study it has been seen that it does not have a real sensory effect, so it could be eliminated.

Differences between C and L2 began after four months from the addition of the dosage but still at that time some assessors preferred C and some others L2. In this case the samples differ in sorbic acid and also in ascorbic acid. Some authors (27) have indicated than sorbic acid presents an antioxidation activity only for a short period of time and that afterwards it can elicited an opposite effect and could promote wine browning although without oxidised aromas and flavours (28). In this case, it could be expected that in the next tasting evaluation more assessors prefer C than L2.

L3 was the most different liquor and the only one which was less appreciate than liquor C. This result suggest that it is necessary to add some SO_2 to keep the usual cava organoleptic perception or to found an alternative compound with the same sensory effect. However, in order to have consistent conclusions to apply these findings it would be necessary to increment the number of assessors.

Headspace solid-phase microextraction gas chromatography analysis

Headspace solid-phase microextraction (HS-SPME) was applied to obtain the volatile profile. The application of this extraction and concentration technique to different sparkling wines was compared to the simultaneous distillation extraction (SDE) and the

closed-loop stripping analysis (CLSA) by other authors (29). The HS-SPME allowed obtaining the most important volatile compounds for each chemical family in a reliable way and it demonstrated to be the fastest technique, as well as the most environmentally friendly.

The stability of the gas chromatography signal was confirmed by the periodical analysis of a synthetic wine. This wine was prepared with some usual wine components and according to their usual concentration. The variability of the chromatographic signal for all these compounds along the study was lower than 10%. Thus, this value was considered as the minimum percentage variation when analysing the chromatographic differences between samples and among the different times of analysis for one sample.

The gas chromatography mass spectrometry (GC-MS) analysis has allowed to detect more than seventy volatile compounds in each sample, some of them already reported as typical cava aroma compounds (29). As it can be seen in Figures 1 to 4, all volatile profiles seem to be nearly the same, whatever the sample and/or the time of analysis considered. However, there are some important differences.

The more evident one is related to the presence of ethyl sorbate and sorbic acid (picks number 13 and 28 in Table 7) in control sample (C) whereas they are not detected for the others three samples. Moreover, these two picks can be detected along the whole study for C sample, although it has to be said that they decrease their intensity gradually. This is consistent with the dosage composition: either L1 nor L2 or L3 have been added with sorbic acid and C dosage was the only one with this compound. For the other three liquors was not possible to identify any characteristic volatile compound which allowed the fast and unmistakable identification of each liquor.

But cavas are constituted by a complex mixture of a lot of different chemical compounds in a wide range of concentrations (from nanograms to grams per litre) so it would be possible that there were some characteristic volatile compounds for the other liquors not detectable by the current method of analysis applied.

The evolution for the volatile compounds that have shown a general chromatographic variation higher to 10% for each liquor is given in Table 7, where the compounds have been arranged following their retention indices in a polar column. For all of them it is shown their initial area at the dosage moment (time 0) and their variation two and four months later (time 1 and 2, respectively). Negatives numbers indicate a decrease in the chromatographic signal for the considered compound that will be related to a lower

concentration of the compound in the sample. Moreover, Figures 1-4 complete this information and show the volatile profiles for each liquor at the three times of the study.

It should be noted that three norisoprenoides have been identified in all samples: two vitispirane isomers (picks number 14 and 15) and 1,1,6-trimethyl-1,2-dihydronapthalene or TDN (pick number 21). These compounds have been previously related to cavas with a long aging in contact with lees (30).

In general, the concentration of the volatile compounds slightly increase two months after the dosage whatever the sample considered. However, it is detected that the vast majority of them decrease their concentration after four months from the dosage. This first evolution trend could be due to the stabilization process of the cava samples just after the addition of the dosage. The loss of the volatile compounds along time due could be explain due to the gas exchange through the cork.

However, there are also some compounds whose behaviour does not follow this pattern and their chromatographic signal gradually decrease from the beginning. This happens to benzaldehyde, ethyl 9-decenoate, TDN and ethyl dodecanoate (picks number 16, 20, 21 and 24, respectively) whose concentrations decrease and even disappear completely in some cases (ethyl dodecanoate) after four months from the dosage whatever the sample considered or in three of the four samples (as happens with benzaldehyde).

Headspace solid-phase microextraction gas chromatography analysis has demonstrated to be a really powerful technique to analyse cava samples and it allows identifying and quantifying volatile compounds. But it also has some important limitations. When analysing low concentration compounds, the signals obtained sometimes are not detectable enough either to carry out quantitative nor qualitative analyses. And as it has been said before, cava contains many different chemical compounds in a really wide range of concentration. Moreover, the sensitivity to detect some chemical families with low odour thresholds and usually present at ppt (such as pirazines) is also low as the mass spectrometer is not a specific detector for this type of compounds.

Furthermore, the chromatographic conditions to carry out the separation of the volatile compounds was not optimize because of a lack of time. By this reason, some of the compounds were not properly separated and the chromatographic signals obtained were overlap. This is the case for isobutyl alcohol and isoamyl acetate (picks number 5 and 6) and ethyl hexanoate and hexanol (picks number 9 and 10). So it would be a good idea to finish off these analyses applying other chromatographic parameters (temperature,

column, etc.) to completely separate these compounds and be able to follow up their evolution.

It should be pointed out that some of the volatile compounds that have not shown any chromatographic difference up to now for each sample can suffer a variation in their concentration in the future. So it would be necessary to consider all the volatile profile when doing the next analysis to control the whole evolution of the samples.

These results suggest that it is important to keep in reserve the cava for few months before it is commercially distributed to allow its stabilization and to guarantee its optimal quality to consumers. It is important to know for each dosage which is its quality evolution in order to determine the best moment for it to be consumed.

Table 7: Evolution of volatile *compounds* in the study time

			CONTROL			LIQUOR 1			LIQUOR 2			LIQUOR 3		
	COMPOST	CAS	C_T0[a]	T1-T0[b]	T2-T1[c]	L1_T0[a]	T1-T0[b]	T2-T1[c]	L2_T0[a]	T1-T0[b]	T2-T1[c]	L3_T0[a]	T1-T0[b]	T2-T1[c]
1	Ethyl butyrate	105-54-4	**7,54**	2,81	-41,14	**7,69**	-9,64	-20,66	**6,96**	15,43	-22,40	**6,53**	13,74	-91,33
2	Ethyl 2-methylbutanoate	7452-79-1	**1,32**	13,45	-46,53	**1,51**	-35,10	-9,21	**1,18**	16,50	-24,74	**1,00**	41,96	-75,65
3	Propanol	71-23-8	**0,88**	-22,62	-49,74	**0,81**	10,20	-38,14	**0,80**	14,55	-100,00	**0,67**	1,78	-83,96
4	Ethyl isovalerate	108-64-5	**2,75**	-2,29	-41,08	**2,84**	-16,94	-10,26	**2,40**	14,24	-21,87	**2,31**	9,95	-84,11
5	Isobutyl alcohol	78-83-1	**1,73**	115,69	-58,61	**1,63**	128,76	-46,53	**2,58**	30,19	-38,15	**1,26**	249,82	-51,64
6	Isoamyl acetate	123-92-2	**2,64**	-50,87	11,65	**2,25**	1,21	-26,25	**2,18**	5,18	-22,09	**1,59**	-17,38	-147,20
7	Butanol	071-36-3	**0,36**	51,79	-58,44	**0,42**	-72,00	22,22	**0,39**	22,14	-30,03	**0,33**	69,75	-27,15
8	Pentanol	71-41-0	**80,28**	16,75	-44,37	**87,54**	2,56	-66,48	**77,60**	24,96	-34,22	**72,66**	33,50	-66,84
9	Ethyl hexanoate	123-66-0	**151,32**	-0,11	-8,12	**186,53**	-43,98	14,65	**93,68**	51,11	-15,00	**120,93**	14,04	-98,84
10	Hexanol	111-27-3	**7,86**	9,33	-46,70	**7,61**	4,27	-72,24	**7,85**	-19,14	-62,54	**4,82**	26,27	-100,61
11	Ethyl octanoate	106-32-1	**437,41**	2,81	-34,38	**469,18**	-9,30	-28,25	**268,23**	23,69	0,23	**392,07**	9,28	-76,56
12	Furfural	98-01-1	**3,27**	15,22	-56,76	**3,44**	19,08	-16,68	**3,76**	-16,04	-9,22	**3,32**	12,87	-103,39
13	Ethyl sorbate	2396-24-1	**4,10**	26,56	-38,12	**1,35**	-18,38	9,51	**0,62**	96,14	-33,03	**1,16**	30,81	-54,80
14	Vitispirine a	-	**16,13**	-20,42	-34,07	**12,63**	-13,60	9,71	**10,46**	8,98	-2,82	**7,83**	53,22	-85,69
15	Vitispirine b	-	**10,45**	-1,09	-25,68	**7,85**	17,19	13,71	**9,72**	-22,51	23,03	**10,15**	1,15	-81,75
16	Benzaldehyde	100-52-7	**1,33**	-3,46	-2,65	**1,32**	-100,00	-	**1,29**	-100,00	-	**1,34**	-100,00	-
17	Ethyl 2-furancarboxylate	614-99-3	**1,85**	-12,68	-75,68	**1,89**	-25,46	-70,66	**1,53**	12,13	-32,09	**1,48**	-44,41	-146,16
18	Ethyl decanoate	110-38-3	**122,40**	19,72	-47,65	**134,31**	-7,51	-38,12	**71,94**	38,32	-7,03	**72,34**	68,25	-75,39

19	Diethyl succinate	123-25-1	**34,06**	-3,53	-22,12	**35,38**	-3,57	-9,77	**32,99**	-5,59	4,94	**22,13**	63,13	-92,66
20	Ethyl 9-decenoate	67233-91-4	**32,78**	-14,44	-28,75	**29,70**	-11,14	-29,07	**27,28**	-8,44	-9,80	**101,19**	-75,68	-94,04
21	TDN	30364-38-6	**8,30**	-10,80	-59,20	**8,84**	-23,47	-46,76	**6,55**	-12,51	-15,06	**7,45**	-28,01	-85,75
22	1-Decanol	112-30-1	**3,28**	10,88	-40,41	**2,76**	31,08	-37,19	**2,95**	-2,07	-13,91	**3,17**	-10,01	-88,78
23	B-damascenone	-	**3,17**	-12,49	-28,41	**3,32**	-23,37	-17,57	**2,73**	6,42	50,15	**2,15**	-8,35	-112,90
24	Ethyl dodecanoate	106-33-2	**3,11**	-100,00	-	**2,71**	-100,00	-	**2,14**	-100,00	-	**2,15**	-100,00	-
25	Hexanoic acid	142-62-1	**27,09**	18,04	-27,20	**24,27**	21,46	-23,15	**23,73**	42,30	-38,56	**21,36**	61,79	-60,36
26	2-Phenylethanol	60-12-8	**34,39**	-1,07	-14,98	**1,31**	-102,15	2,15	**34,15**	5,23	-17,67	**34,36**	4,28	-85,06
27	Octanoic acid	124-07-2	**105,17**	21,38	-35,13	**108,69**	13,69	-34,57	**115,14**	13,67	-30,61	**109,94**	20,57	-74,70
28	Sorbic acid	110-44-1	**13,42**	3,51	-60,45	**0,00**	-	-	**0,00**	-	-	**0,00**	-	-
29	Decanoic acid	334-48-5	**68,01**	9,96	-14,98	**71,39**	4,43	-38,26	**68,19**	10,10	-30,88	**66,96**	20,02	-81,40

[a]*The valour correspond to initial area of the chromatographic pick.*

[b]*The valour correspond to the difference of the area between T1 and T0. Are expressed in %.*

[c]*The valour correspond to the difference of the area between T2 and T1. Are expressed in %.*

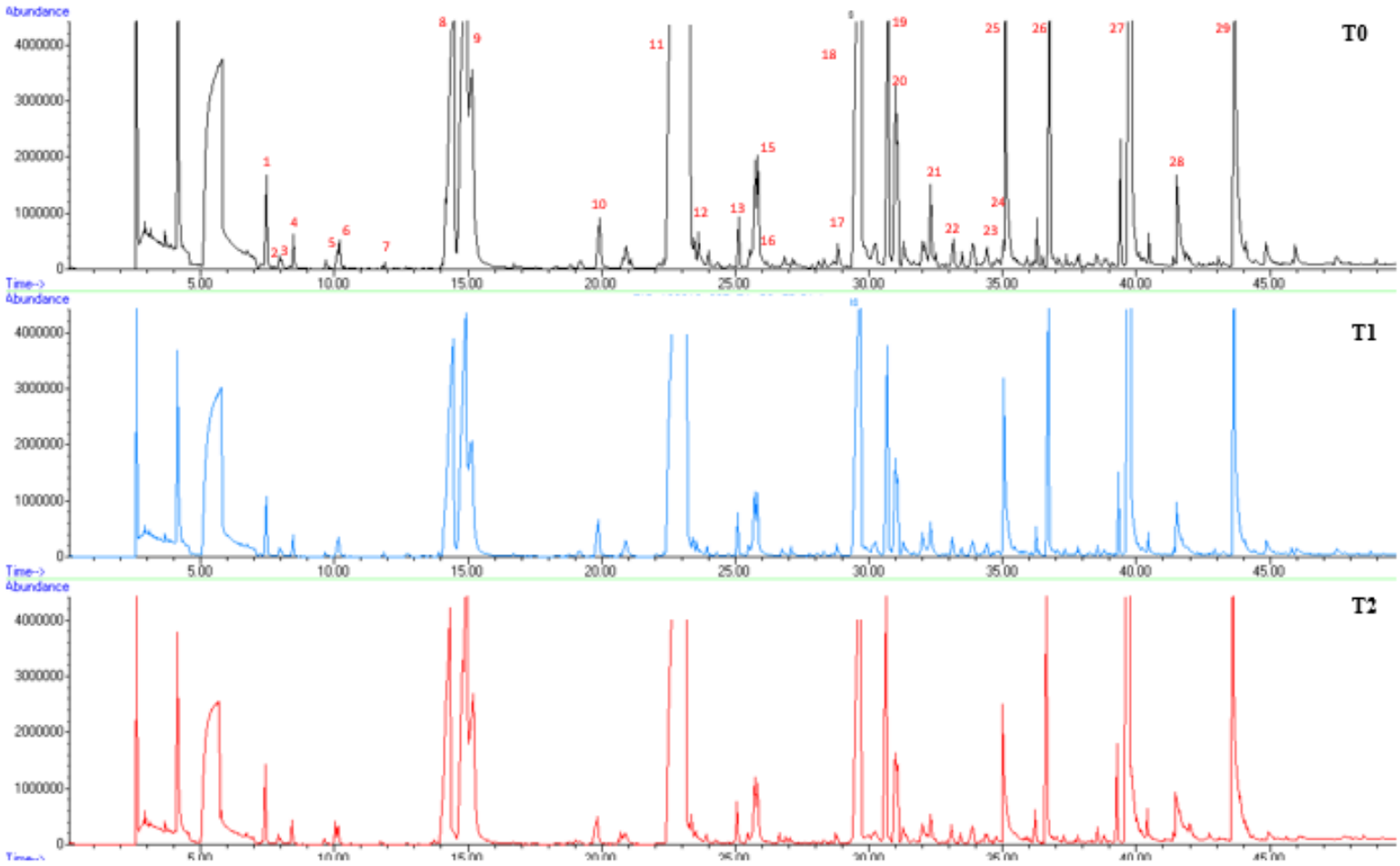

Figure 1: *Volatile profile evolution of Control*

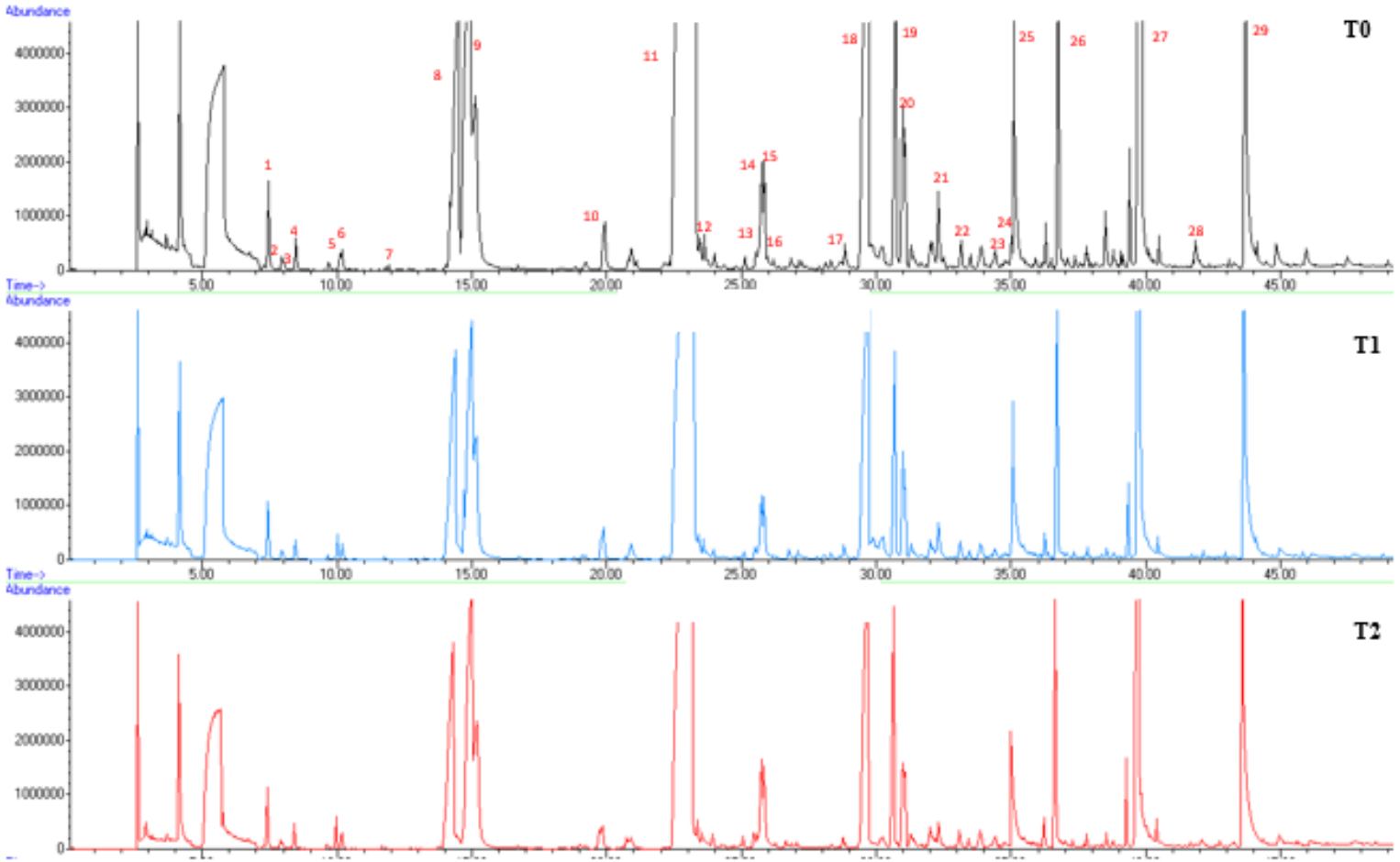

Figure 2: *Volatile profile evolution of Liquor 1*

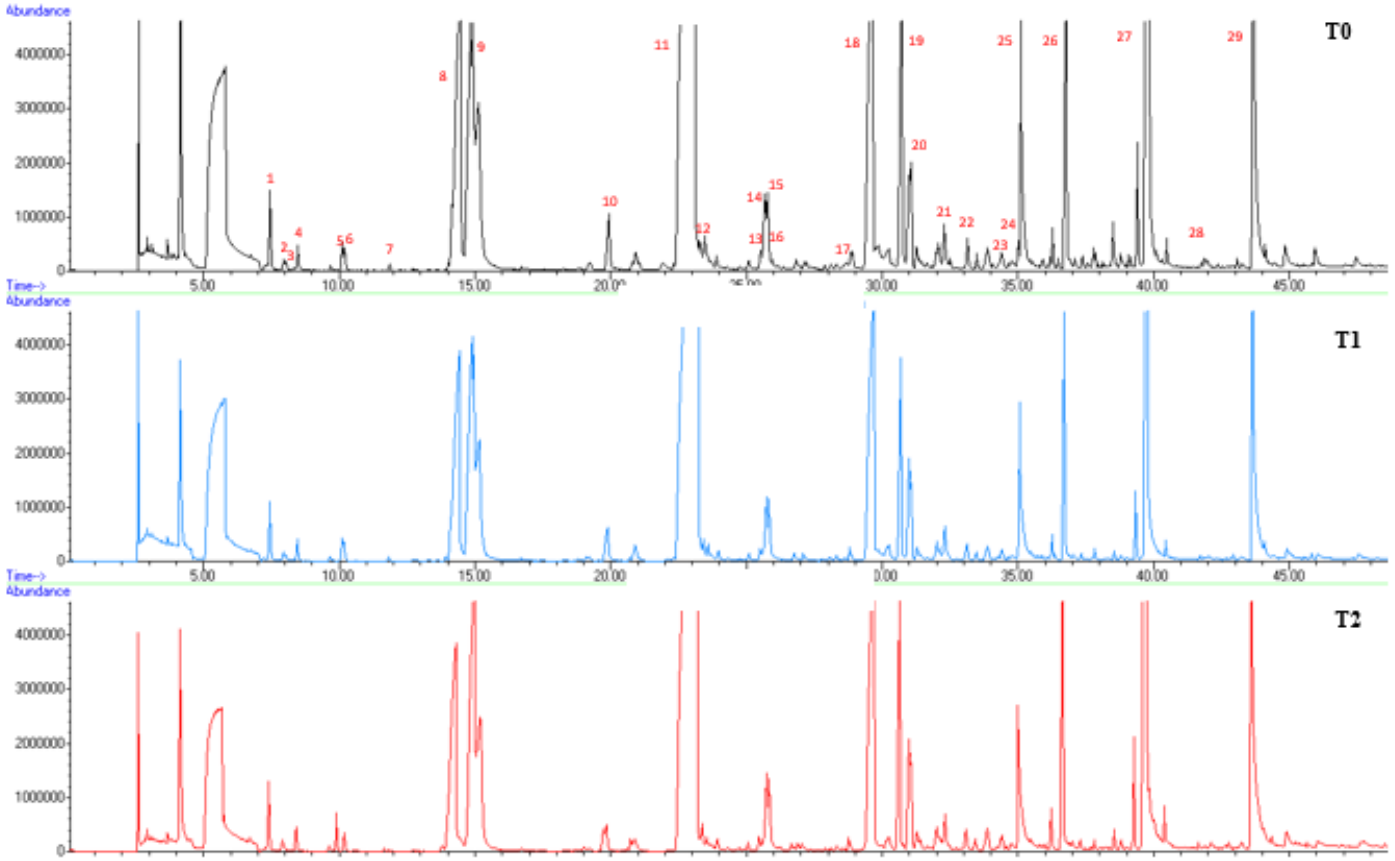

Figure3: *Volatile profile evolution of Liquor 2*

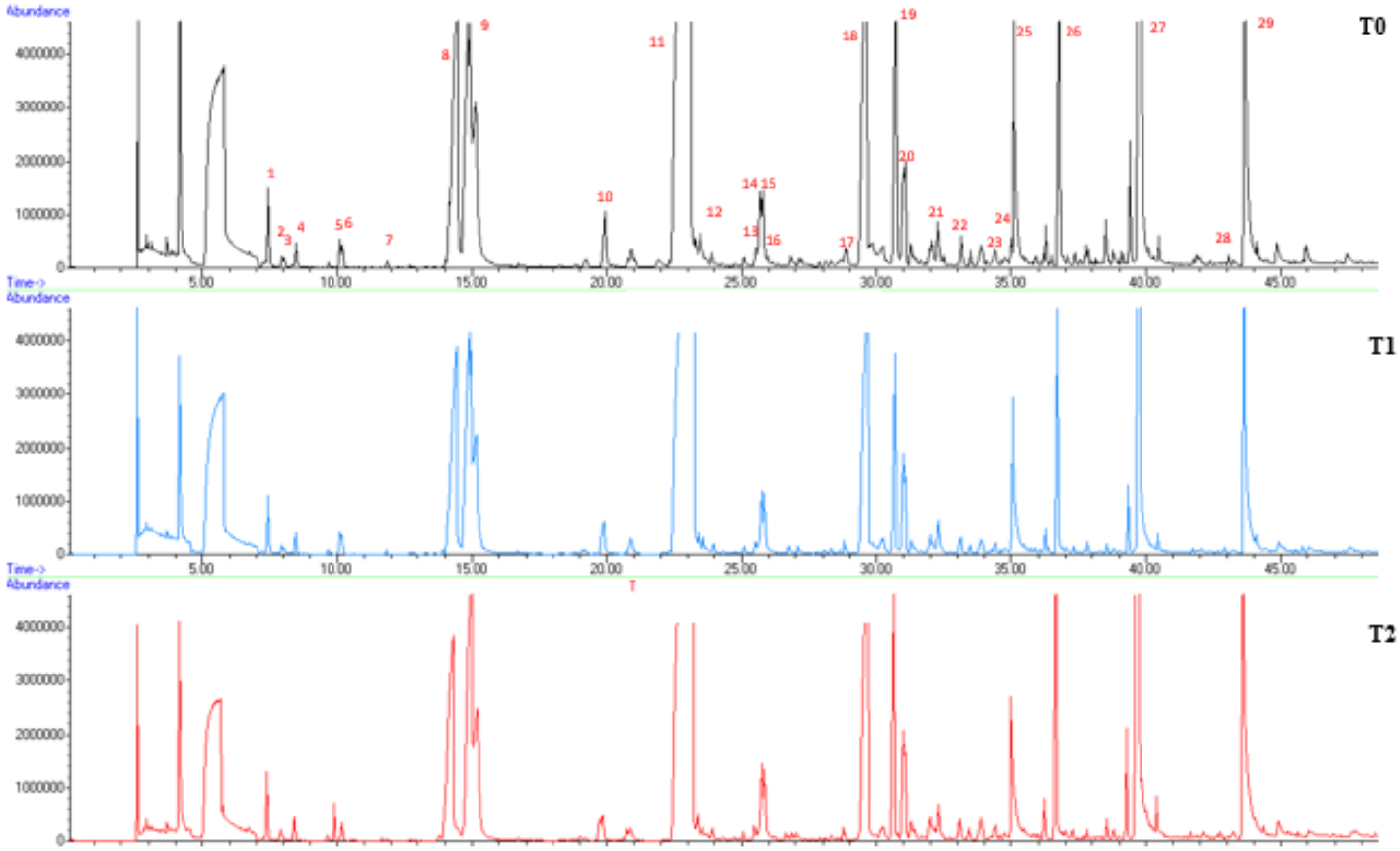

Figure 4: *Volatile profile evolution of Liquor 3*

CONCLUSIONS AND PERSPECTIVE

Up to the present date, four months after the dosage, all the samples fulfil quality parameters stablished by the winery, nevertheless panel assessors could differentiate the samples four months after the dosage. Assessors' results conclude that control liquor could be substituted by a most natural liquor (L1, L2, L3) without effect only if the cava is going to be consumed before 4 months.

Moreover, it is possible to eliminate sorbic acid because assessors could be differentiate C than L1 but the evaluation of L1 was over than the C. Four months it is not enough to know clearly if it is possible to eliminate ascorbic acid, therefore is needed more time to know how their evolution is. According to assessors preferences after four months from the dosage the activity of SO_2 or other alternative is necessary to achieve the same sensorial quality.

The volatile profile of all the samples were practically identical and, in the study time, their evolution are very similar in the three liquors. This is logical because the product are the same and only differs with 1% of their composition, corresponding to the dosage added.

In this study no microbiological analysis have been performed given that cava is a hostile medium, with high alcohol and low pH, and where two fermentations are made before. It is not expected any microbiological contamination, nevertheless if all the analysis indicate that these products can be saleable, it could be necessary one analysis to confirm that there is no microorganism.

It is important to continue with this study along more time because the vast majority of cava is not consumed four months after the dosage. Market logistics, and in especially international market, involves more time between the dosage, the last step in elaboration process, and their commercialization in a shop or their consume in a restaurant. On the other hand, is usual that consumers keep a bottle in their house for a long time, especially when is a prestigious cava. It is essential to know how long time the cava would be in the optimal conditions.

In order to follow the evolution of the different samples and to know from which moment each of them begins to lose their best qualities, the samples from the same batch were saved to allow the continuation of this study during few years.

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