Voluntary Composition of Wines Elaborated from Organic and Non-Organic Grapes

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Received date: May 26, 2015; Accepted date: Aug 18, 2015; Published date: Aug 21, 2015

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Abstract

The aim of this work was to study the evolution of volatile compounds during the alcoholic fermentation of organic and non-organic grapes. To do this, grapes were cultivated using organic and non-organic grapes; their tasting showed some differences between both types of Monastrell grapes. Throughout the alcoholic fermentation, the samples of organic grapes had higher concentration of total alcohols but lower concentration of esters and acids than the samples of conventional ones. Therefore, the volatile composition of wines from two different cultivated grapes using both agronomic practices was different. Moreover, regarding to the volatile compounds that contributed directly to wine aroma, generally organic wine had more chemicals and floral aromas, while the wines from conventional practices had more fruity aromas. Principal component analysis (PCA) showed that it is possible to differentiate between both types of wines in terms of concentration of volatile compounds formed during the alcoholic fermentation. Consequently, agronomic practices affected the grape taste, the wine volatile composition and its quality.

Keywords: Must; Grape; Red wines; Fermentative volatile compounds; Agronomic practices.

Introduction

The grape quality is the first factor that conditions the wine quality, so it is very important to make innovation in the current world of vine-growing in order to achieve a differential quality product. Although that be attempting innovate at the same time, agronomic practices have the organic viticulture philosophy that leads us to focus viticulture practiced by our ancestors in an encounter with nature and our vineyard patrimony (climate and soil). There is an increasingly raising concern regarding residues of fungicides in wine and effects on human and environmental health, so there is a new trend of making viticulture as before.

Moreover, consumers are becoming more and more concerned about choosing foods which are both healthy and respectful of the environment. For this reason, agriculture where neither fertilizers nor insecticides and other pest control synthetic substances are used has increased in popularity. The sustainable agronomic practices begin reuse cover crops and natural products such as manure or compost [1]. The presence of any chemical residue in wine is therefore avoided [2].

Aroma, which is due to a complex mixture of volatile compounds, is one of the most important characteristics for defining wine quality. Particularly, the fermentative volatile compounds, which are formed during the alcoholic fermentation, greatly influence the aroma quality of young wines, especially those which come from neutral varieties. The evolution of these compounds during the alcoholic fermentation from conventionally grown grapes has previously been studied [3,4], but we have not found any studies on the evolution of these compounds during the fermentation of ecologically grown grapes so far. Moreover, the contribution of each compound to wine aroma depends mainly on the quantity released to the headspace [5] and its odor threshold (OT). In order to determine the contribution of each compound to the aroma composition of wine, the parameter known as odor activity value (OAV) is usually used. This parameter is the ratio between the concentration of each compound in the wine and its respective OT. However, this ratio must also be studied as an approximation, since additive, synergic and antagonistic effects between compounds may take place. It is supposed that compounds which contribute to wine aroma are only those displaying OAVs greater than 1, although some authors have reported the relevance to the overall aroma of substances present at OAV>0.2 [6-8].

It is known that agronomic practices can modify the aroma composition of grapes [9-14]. Although there are studies on the differences between organic and non-organic grapes which focus on aspects such as antioxidant activity and phenolic composition [15], nitrogen composition [16], biogenic amines [17] and ochratoxin A [18], only one study has been found on volatile composition [2]. In this study, a comparison of odor-active compounds in Sherry wines from both types of cultivars was carried out. For this reason, the purpose of our study was to determine the volatile composition of wines elaborated with organic and non-organic grapes.

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Materials and methods

Samples and vinification

Both conventionally and ecologically grown Monastrell red grapes were used for this study. These grapes were harvested under optimum sanitary conditions. In the conventional agriculture system, the grapes were cultivated on trellises and were fitted with a drip irrigation system. They were fertilized with liquid fertilizer NPK 8-4-10 (% w/w) (Agribecco, Spain), applying a total of 250 g per vine. In the case of the grapes ecologically system, the vineyards were cultivated using the Goblet training system and were treated with “cultivit ecológico” fertilizer (Agribecco, Spain), consisting of dried granulated sheep manure with a composition of NPK 1.55-1.21-2.35 (% w/w), applying 200 g per vine. The ecologically system was not irrigated.

The must was inoculated with active dry Saccharomyces subsp. cerevisiae (U.C.L.M. S325, Springer Oenologie, France) in a proportion of 0.2 g/L according to commercial recommendations. To do this, 0.65 g of dry yeast was rehydrated in a sterile flask in 7.5 mL of distilled water with 0.07 g of sucrose (number of viable cells/ml ≥ 2 x10⁹); it was kept in this medium for 30 min at 35°C. The musts were inoculated while being mixed to obtain a homogeneous distribution. The musts were then sulphited with potassium metabisulphite to a final concentration of 70 mg/L in non-organic samples, and 20 mg/L in organic samples.

The fermentation-maceration was carried out under controlled temperature (28°C) in duplicate. The fermentation evolution was followed by the daily measurement of sugars through the refraction index at 20°C, using a refractometer CT (Soplem, France). The samples were taken at 50% of consumed sugars and at the end of fermentation (reducing sugars <2.5 g/L).

Grape sensory analysis

Grape samples were subjected to sensorial analysis by tasting separately berries, pulp, skins, and seeds. The method of sensory analysis was developed by Wine Cooperative Institute (ICV) from Montpellier [19]. In berries, crushing, threshing, and color were evaluated; in pulp, adherence, fruity aroma, sugar, acidity, and herbaceous aroma; ease of breakage, acidity, tannins or astringency, dryness, and herbaceous aroma in skin; and finally, color, ease of breakage, tannins or astringency, and aromas in seeds. The tasting panel consisted in five people (three men and two women).

Enological parameters

Total acidity, pH, volatile acidity, reducing sugars, and alcohol from different samples were measured following the methods established by the ECC [20]. Assimilable nitrogen was calculated as the sum of the ammonium and the amino nitrogen, without taking into account the proline concentration, analysed by HPLC [16]. The phenolic ripeness of the grapes was measured indirectly from the color intensity of the extract obtained by crushing 200 berries without breaking the seeds and then centrifugated at 3,500 rpm. The color intensity was determined by the sum of the absorbances at 420, 520, and 620 nm; this parameter is called the color index.

Analysis of volatile compounds by SBSE-GC-MS

The fermentative volatile compounds were analysed following the method describe by Lorenzo et al. [21]. These compounds were extracted by introducing the polydimethylosiloxane coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel, Mülheim and der Ruhr, Germany) into 10 ml of sample, to which 100 μl of internal standard 3-methyl-1-pentanol solution at 1 μl/ml in absolute ethanol (Merck, Damstard, Germany) was added. Samples were stirred at 500 rpm at room temperature for 60 minutes. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC–MS analysis. In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 290°C; final temperature of thermal desorption unit (TDU) as 330°C; desorption time, 4 min; cold trap temperature, -30°C; helium inlet flow 45 ml/min. The compounds were transferred into the Hewlett-Packard LC 3D mass detector (Palo Alto, USA) with a fused silica capillary column (BP21 stationary phase 30 m length, 0.25 mm i.d., and 0.25 μm film thickness; SGE, Ringwood, Australia). The chromatographic program was set at 40°C (held for 5 min), raised to 230°C at 10°C/min (held for 15 min). The total time analysis was 39 minutes. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from m/z 35 to 500 and the detector temperature was 150°C.

The analytical analysis of volatile compounds of each sample was twice injections to GC-MS systems for duplicated fermentation samples; therefore, the analytical results shown for each sample were average values of 4 analytical data. Identification was carried out using the NIST library and by comparison with the mass spectrum and retention index of chromatographic standards designed by us and data found in the bibliography. Quantification was based on five-point calibration curves of respective standards (R²>0.9) in a 12% ethanol (v/v) solution at pH 3.6.

Statistical analysis

Significant differences among wines for each of the parameters were assessed with a one-way analysis of variance (ANOVA) using the SPSS Version 19.0 statistical package for Windows (SPSS, Chicago, IL). Differences between means were compared using the least significant differences (LSD) test at 0.05 probability level. Principal Component Analysis (PCA) was carried out in order to identify underlying variables or factors that explain the pattern of correlations within a set of observed variables, besides to seek similarities and differences between data using InfoStat (www.infostat.com.ar).

Results and Discussion

The results from grape sensory analysis are shown in Figure 1. These results gave a value of 11 in the case of berries from Monastrell organic and 10 for non-organic at the same date. This resulted in more soft berries, with more black color and easier separation of the stalk in the case of Monastrell organic. In relation to the pulp, the values reached were the same in both types of grapes, 16. In this sense, both sugar increase and acidity decrease was evaluated, as well as adherence to the skin and aromatic evolution of it. When these results were related with the ratio °Baumé/total acidity in the harvest day, we found that there were few differences between Monastrell non-organic and organic (2.14 and 2.36, respectively). The maturity of berries and pulp is directly related with technological maturity, which starts at low levels and evolves in a constant way to its maximum in a few weeks. An increase of sweetness and loss of acidity is noticed, which provides more and more pleasant tasting [22].
samples from organic grown, volatile acidity was twice as high as in non-organic ones, probably due to the lower $SO_2$ concentration in musts from those. The pH in wines from non-organic grapes was higher than in those from organic ones. The must from non-organic grapes had lower reducing sugar content than the organic one, and the same occurred for wines processed from them. The wines elaborated with ecologically grown grapes showed higher alcohol than those from conventionally grown grapes, since the organic grapes had a higher content of reducing sugars. Organic wines also showed a higher color index, as the higher ethanol concentration, the greater concentration of phenolic compounds [23,24].

### Table 1: Enological parameters during the alcoholic fermentation of the different samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Monastrell non-organic</th>
<th>Monastrell organic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% of consumed sugars</td>
<td>Wines</td>
</tr>
<tr>
<td><strong>Volatile compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$-Propanol</td>
<td>11.57$^a$</td>
<td>28.58$^b$</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>35.31$^a$</td>
<td>50.34$^a$</td>
</tr>
<tr>
<td>1-Methyl-1-butanol</td>
<td>115.62$^a$</td>
<td>229.37$^b$</td>
</tr>
<tr>
<td>2-Methyl-1-butanol</td>
<td>71.82$^a$</td>
<td>87.97$^a$</td>
</tr>
<tr>
<td>3-Methyl-1-butanol</td>
<td>3.69$^a$</td>
<td>6.87$^b$</td>
</tr>
<tr>
<td>2-Phenylethanol</td>
<td>1.51$^a$</td>
<td>1.82$^a$</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>0.05$^a$</td>
<td>0.08$^a$</td>
</tr>
<tr>
<td>2-Hexen-1-ol</td>
<td>2.43$^a$</td>
<td>7.87$^a$</td>
</tr>
<tr>
<td>3-Methylthio-1-propanol</td>
<td>267.58$^a$</td>
<td>267.58$^a$</td>
</tr>
<tr>
<td>Total alcohols</td>
<td>241.99$^a$</td>
<td>412.91$^b$</td>
</tr>
</tbody>
</table>

### Table 2: Concentration of fermentative volatile compounds (mg/l) in the different samples (n=4). For each compound, different letters indicate significant differences between the samples (p<0.05).

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Monastrell non-organic</th>
<th>Monastrell organic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-Propanol</td>
<td>0.72$^b$</td>
<td>1.08$^a$</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>0.14$^{ab}$</td>
<td>0.12$^a$</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>0.47$^{ab}$</td>
<td>0.58$^b$</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>0.25$^a$</td>
<td>0.44$^b$</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>0.08$^{bc}$</td>
<td>0.28$^a$</td>
</tr>
<tr>
<td>Ethyl dodecanoate</td>
<td>0.02$^b$</td>
<td>0.05$^a$</td>
</tr>
<tr>
<td>Diethyl succinate</td>
<td>0.13$^a$</td>
<td>0.60$^a$</td>
</tr>
<tr>
<td>Total esters</td>
<td>1.83$^b$</td>
<td>3.12$^a$</td>
</tr>
</tbody>
</table>

**Table 1**: Results of sensorial analysis of Monastrell organic and non-organic grapes.

The main parameter that is taken into account when setting the harvest day is the ratio $\text{ºBaume}/\text{total acidity}$, which evaluates both, sugar increase and acidity decrease. For this reason, the coincidence between the maximum for the two parameters is logical ($\text{ºBaume}/\text{total acidity}$, and berries and pulp maturity measured by tasting). In reference to the skin tasting, Monastrell non-organic showed values slightly higher than Monastrell organic (Figure 1). By tasting skins, it was tested the ease of chewing and skin aromas, which were changing slightly higher than Monastrell organic (Figure 1). By tasting skins, it was tested the case of chewing and skin aromas, which were changing from herbaceous to fruity. Texture changes, being hard at first, and easily crushable at the end. Finally, the seeds tasting showed lower values for Monastrell non-organic than for Monastrell organic. The value of 7 for Monastrell non-organic indicated herbaceous aromatic characteristics and high astringency.

Table 1 shows the enological parameters in musts, 50% of consumed sugars, and final wines. The total acidity was higher in wines than in the musts, especially in wines from organic grapes. In
Table 2 shows the evolution of the concentration of fermentative volatile compounds in non-organic and organic samples during the alcoholic fermentation. The wines from ecologically grown grapes had a higher concentration of total alcohols than the wines from conventionally grown grapes, which may be related to the higher sugar content in the corresponding initial musts [25,26]. Moreover, organic musts had lower assimilable nitrogen content than non-organic musts (Table 1), which can explain the lesser synthesis of higher alcohols in the alcoholic fermentation of conventionally grown grapes. It is known that the higher the nitrogen concentration in the initial must, the lower the synthesis of higher alcohols during fermentation [27]. This was observed for n-propanol, isobutanol, 2-hexen-1-ol, and 2-phenylethanol (Table 2), compounds synthesized in greater quantities in the fermentation of the organic samples than in the fermentation of non-organic must. This last compound is the only alcohol described at a sensory level in pleasant terms [28]. In the case of isoamyl alcohols, 2-methyl-1-butanol and 3-methyl-1-butanol, their content did not show significant differences between both types of samples throughout the alcoholic fermentation (Table 2). These two compounds are the major higher alcohols in the wines [29]. However, the concentrations of 3-methyl-1-propanol and 1-hexanol were lower in wines from ecologically grown grapes than in wines from conventionally grown grapes (Table 2). On the other hand, the formation of isobutanol, 3-methyl-1-butanol, 2-hexen-1-ol and 1-hexanol in both alcoholic fermentations occurred mainly during the first half of the fermentation. Nevertheless, the concentration of n-propanol had a higher increase during the second half of fermentation, and the rise of total alcohols was similar throughout the alcoholic fermentation in both types of samples (Table 2). Normally, the formation of alcohols takes place during the first half of fermentation, coinciding with the greatest consumption of amino acids, which are their precursors [30].

Unlike the alcohols, the concentration of total esters was higher in samples from conventionally grown grapes than in samples from ecologically grown grapes, especially in the final wines (Table 2). All the esters studied showed higher concentration in non-organic wines, except ethyl hexanoate and 2-phenylethyl acetate. This is in accordance with the results obtained for Sherry wines by Moyano et al. [2], who explained the higher production of esters due to the higher concentrations of SO\textsubscript{2} in this type of wines, as did Valcarcel et al. [31]. The release of fatty acid ethyl esters by yeast has been linked to the availability of oxygen for the biosynthesis of unsaturated long-chain fatty acids. During alcoholic fermentation, unsaturated fatty acids can be assumed by yeast directly from must or can be synthesized by oxidation of free saturated fatty acids, with a process that involves the presence of free oxygen. The synthesis of unsaturated fatty acids is impossible if there is insufficient oxygen content and the whole process is stopped. This originates the accumulation of medium-chain acyl-CoA, an important product in the synthesis of esters and fatty acids. To recover free CoA, ester or acid formation is promoted by yeast, and the wine obtained under these conditions is richer in esters or acids containing the corresponding acyl group [32]. Hence, the higher the concentration of SO\textsubscript{2} (the non-organic must has 70 mg/l and the organic must has 20 mg/l), the lower the oxygen concentration, and therefore the formation of esters or acids is increased (Table 2). In non-organic and organic fermentations, the formation of ethyl hexanoate and 2-phenylethyl acetate was higher in the first half of the fermentation, the rise of diethyl succinate was higher in the second half of fermentation while ethyl octanoate formation was continuous throughout the alcoholic fermentation (Table 2). The evolution of the other esters was different for conventionally and ecologically grown samples. The formation of isoamyl acetate was higher in the second half of fermentation in organic samples, but in non-organic ones it was higher in the first half of the fermentation. The formation of ethyl decanoate and ethyl dodecanoate in conventional samples was higher during the second half of fermentation, but in organic samples it was almost the same throughout fermentation. Thus, the formation of these compounds during fermentation did not follow a clear trend.

In the case of acids, non-organic samples showed a higher concentration of total acids than organic ones (Table 2). This was mainly due to octanoic acid, as the concentration of decanoic acid was similar in both types of wines. The higher concentration of fatty acids in the wines from conventionally grown samples could be due to the higher concentration of SO\textsubscript{2} as explained above. These compounds are not associated with wine quality, although their presence plays an important role in the complexity of the aroma [33]. In ecologically grown samples, the formation of acids was continuous throughout alcoholic fermentation, but in non-organic samples the formation of acids was higher during the first half of fermentation (Table 2).
Table 3: Perception threshold and odor activity values for volatile compounds of wines obtained from conventionally and ecologically grown grapes.

Table 3 shows the perception thresholds and the odor activity values (OAVs) of fermentative volatile compounds from conventionally and ecologically grown grapes during alcoholic fermentation. The compounds which contributed most to the aroma of wines (i.e. those with OAV higher than 1) were almost the same in both types of samples. The alcohols that contributed directly to wine aroma were isoamyl alcohols (chemical aroma), 3-methylthio-1-propanol (raw potato aroma), 1-hexanol (grass flavour) and isobutanol (chemical aroma). Moreover, 2-phenylethanol, with floral aroma, only showed OAV>1 in the case of organic wines (Table 3). The OAV of 3-methylthio-1-propanol was especially high in non-organic wines, which can be due to the higher concentration of SO$_2$ in these types of wines. The esters that contributed actively to the aroma in all the cases were isoamyl acetate (banana aroma), ethyl hexanoate (green apple flavour) and ethyl octanoate (sweet aroma). In the case of wines from ecologically grown grapes, 2-phenylethyl acetate, with a floral aroma, also had OAV>1, and in the case of wines from conventionally grown grapes, ethyl decanoate, giving fatty aroma attributes (Table 3). In the case of the two acids analyzed, only octanoic acid (fruity and fatty aroma) showed OAV>1, especially in non-organic wines (Table 3). Therefore, wines from ecologically grown grapes had, in general, more chemical and floral aromas whereas wines from conventionally grown grapes showed fruitier, but also more unpleasant aromas.
Table 4 shows the correlation matrix for the fermentative volatile compounds in wines, where we can see the r values (Pearson coefficient). The strongest positive correlations (≥ 0.95) between fermentative volatile compounds were found in n-propanol with isobutanol and 2-hexen-1-ol; isobutanol with 2-hexen-1-ol; 2-methyl-1-butanol with ethyl octanoate, ethyl decanoate and diethyl succinate; 3-methyl-1-butanol with decanoic acid; 2-phenylethanol with 2-hexen-1-ol; 3-methylthio-1-propanol with ethyl hexanoate; isoamyl acetate with octanoic acid; ethyl hexanoate with octanoic acid. Likewise, the four esters, ethyl octanoate, ethyl decanoate, ethyl dodecanoate and diethyl succinate were correlated with each other.

Principal component analysis (PCA) was carried out, showing that the initial two principal components significantly contributed to explain the variance of the data, because both of them showed eigenvalues>1 (PC1: 9.98, PC2: 6.27) [34]. Moreover, they are sufficient to describe the data obtained, as they explain the 95.6% (PC1: 58.7%, PC2: 36.9%) of all the variation in the data (Figure 2).

Table 4: Correlation matrix for the fermentative volatile compounds. More significant correlations in bold with p ≤ 0.05. The name of the compounds is abbreviation, but the order is the same as in Table 3.

![Figure 2: Loading plot of the PC1 × PC2 corresponding to fermentative volatile compounds for the samples studied.](image)
4. Conclusions

Grapes from Monastrell organic were little softer, with more black color, showing skins with lower fruity aromas and seeds with lower herbaceous aromas and lower astrinjency than Monastrell non-organic. The concentration of total alcohols was higher in samples from ecologically grown grapes, but the concentration of esters and acids was higher in samples from conventionally grown grapes, and this was maintained throughout the alcoholic fermentation. Therefore, the volatile composition of wines was clearly different if grapes came from different grape types affected the volatile composition of wines but it did not affect the evolution of these compounds during the alcoholic fermentation. The OAVs showed that wines from ecologically grown grapes had, in general, more chemical and floral aromas whereas wines from conventionally grown grapes had more fruity aromas.

References


Table 5: Loadings for the two first principal components. The most significant values are in bold (loading ≥ 0.70). The name of the compounds is abbreviation, but the order is the same as in Table 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>0.36</td>
</tr>
</tbody>
</table>


