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ARTICLE

# Optimizing the Process of Making Sweet Wines To Minimize the Content of Ochratoxin A

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During the drying process of raisins, the grapes are subjected to climatic variations that can result in heavy infections of some fungal species that produce ochratoxin A (OTA), a powerful toxic metabolite, whose maximum permitted content is set by the European Union at 2.0 $\mu$g/L for grapes, wine and other drinks derived from the grape. The aim of this paper is to optimize the process of making sweet wines in order to minimize the content of ochratoxin A. The results reflect a reduction of the OTA content in grapes dried under controlled conditions in a climatic chamber up to 24% compared to those sunned in the traditional way. A decrease of the concentrations of OTA is also observed during the processes of vinification. Those wines with prefermentative maceration reached a higher OTA content than the wines without maceration, but unexpectedly were not those preferred from a sensorial point of view. In addition, the process of aging in oak casks has been shown to serve as a natural method for reducing the OTA content of these wines.

**KEYWORDS: Ochratoxin A; food safety; sweet wine; Muscat; climatic chamber; raisining; pellicular maceration; oak aging; sensory evaluation**

## INTRODUCTION

The sweet wines of Andalusia are made from grapes of white varieties, particularly *Muscat* and *Pedro Ximen*, dried in the open air exposed sun, in the process known as *soleo* or sunning.

In this sunning process, after harvesting, the bunches of grapes are spread out on mats of esparto grass, known as *redores*, or on large areas of plastic sheeting that can cover as much as fifteen hectares of surface area. When subjected to hours of intense sunshine, the grapes gradually lose water, resulting in a significant increase in the concentration of sugars and a variation in the aromatic profile (*1*). Depending on the external conditions, the process can last from seven to twenty days (*2*, *3*). However, this traditional system is susceptible to climatological variations that can alter the final product; in particular, rains during this period can cause the grapes to rot.

In the disease known as gray mold, the grape is infected by *Botrytis cinerea*, one of the pathogenic agents with the most serious adverse consequences for the quality of wines, which affects the composition, stability and sensorial properties; it can cause the loss of the typical fruity character of these wines and instability of the aromas of fermentation, and it gives the wines a bitter flavor (*4*).

Similarly rain during the sunning process makes the grapes more disposed to infection by bacteria, which cause acetic disease, and facilitates the development of other molds such as *Penicillium* and *Aspergillus* species, which give rise to the formation of ochratoxin A (OTA) (*5*), a highly toxic metabolite.

During the drying of the grape, there is an increase in the incidence of *Aspergillus nigri* section (*A. carbonarius* and *A. niger*) compared with other types of fungi. *A. carbonarius* presents a greater ochratoxigenic potential, whereas *A. niger* is the predominant strain (*6*).

The growth of these fungi in the grape is subject to the climatic conditions of the region (*7*−*9*). *A. carbonarius*, which is highly resistant to solar radiation and high temperatures, up to a maximum in excess of 40 °C, is the major source of OTA in grapes, wine and other enological products (*10*−*13*). Furthermore the existence of high levels of ochratoxin A has been demonstrated in wines originating from grapes dried in the sun (*14*).

It would be therefore useful to devise alternative systems of raisining that allow a greater control of the process (*15*), but which would not have any negative influence on the sensorial properties of the final product. The use of a climatic chamber for the raisining of grapes (*16*, *17*) allows the temperature and humidity of the drying process to be adjusted, reduces the length of time required for adequate raisining, and makes the process independent of the external meteorological conditions. The results obtained in the studies cited demonstrated that the employment of the climatic chamber allows the process of drying to be carried out in a controlled way, and that the content of polyphenols and volatile compounds in the resulting musts is similar for both types of grape drying.

About OTA pathologies and toxic effects (*18*−*20*), OTA causes chronic nephropathies in diverse species of mammals and birds.

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**Table 1.** Conditions of the Different Vinification Experiences and Aging Assays and Their Codes

| | | | | | | aging | |
|---|---|---|---|---|---|---|---|
| fermentation experiences (vessel no.) | yeast | fermentation temp[a] | nutrients (diammonium phosphate) | pellicular maceration with pectolytic enzymes (4 °C, 24 h) | control (without aging) | with oak casks | with oak shavings(chips) |
| 1 | *S. cerevisiae* | rt | no | no | 1_C | 1_OC1 1_OC2 | 1_Ch |
| 2 | *S. cerevisiae* | rt | yes | no | 2_C | 2_OC | |
| 3 | *S. cerevisiae* | rt | no | yes | 3_C | 3_OC | |
| 4 | *S. bayanus* | lt | no | no | 4_C | 4_OC1 4_OC2 | |
| 5 | *S. bayanus* | rt | no | yes | 5_C | 5_OC | |

[a] rt, room temperature (<30 °C); lt, low temperature (<10 °C).

In the human, it has been associated with a nephropathy endemic to the Balkans region, although this is still an unconfirmed hypothesis. OTA also possesses teratogenic, hepatotoxic, neurotoxic and immunotoxic effects, and is classified by the *International Agency for Research on Cancer* as a possible human carcinogen (class 2B). In the European Union the OTA content is regulated in foods susceptible to contamination. In the case of wine, other drinks based on wine and/or musts, the maximum limit is set at 2.0 $\mu$g/L (*21*, *22*).

In order to optimize the process of making sweet wines to minimize the content of ochratoxin A, a study of the evolution of OTA content throughout the entire process of production of sweet wine, from the raisining of the grapes to vinification and the aging of the resulting wine in oak barrels, is presented. An alternative to the traditional sun-drying is proposed.

## MATERIALS AND METHODS

**Samples.** The musts analyzed were obtained from grapes of the *Muscat* variety, subjected to two different drying procedures: natural sunning on a roof terrace and artificial sunning in a climatic chamber. In the first, bunches were placed on esparto mats exposed to the air, without any type of protection against environmental conditions. In the artificial sunning, bunches were placed in a climatic chamber with controlled temperature of 40 °C and humidity of 10%.

In both systems, the loss of weight of the grapes was monitored periodically and the assays were completed when this loss reached about 30%. Samples were taken at the start and the end of both the traditional sun-drying and the raisining in the climatic chamber; these samples were pressed and the must obtained was preserved at −20 °C for subsequent analysis.

Five different conditions (two of them by duplicate) were tested for the vinification of the must obtained from grapes dried in the climatic chamber (experiences 1 to 5, **Table 1**). The sweet wines obtained in the various experiments were aged in 30 L oak casks (_OC). In addition, one of them was aged by contact with oak shavings (chips, _Ch).

Samplings were performed for each of the assays at the finish of the fermentation (V01) and after desludging and clarification (V02 or _C); at the beginning of the aging stage (A01, after 30 days) and after 60 (A02) and 90 (A03) days of aging.

**Chemicals.** Hydrochloric acid 37%, ammonia 30%, sodium bicarbonate and glucose were obtained from Panreac (Panreac Quimica S.A., Barcelona, Spain). Polyethylene glycol 8000 and methanol were supplied by Scharlau (Scharlab S.L., Barcelona, Spain). Acetonitrile (HPLC gradient grade), tartaric acid (analytical quality) and acetic acid (analytical quality) were obtained from Merck (Darmstadt, Germany). The OTA standard and malic acid were supplied by Sigma (Sigma-Aldrich, St. Louis, MO, USA). The ultrapure water employed was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

**Analysis of Ochratoxin A.** The methods chosen for the determination of ochratoxin A in musts and wines was devised by the "Analytical Chemistry of Wine and Agrofood Products" Research Group (PAI AGR122) (*23*, *24*). They are based on an initial extraction followed by separation using liquid chromatography (HPLC) and subsequent detection of the toxin by fluorescence. The extraction in musts was performed by the addition of sodium bicarbonate and polyethylene glycol. Since wine is a more complex matrix, solid phase extraction (SPE) was used for the wines. The chromatographic conditions set were the same in both cases. All the analyses were performed in duplicate.

*Sample Preparation*. For the preparation of must samples aliquots each of 5 g of the musts were taken. To each was added 75 mg of polyethylene glycol 8000 and 250 mg of sodium bicarbonate. Each preparation was then mixed using a magnetic agitator for 30 min and centrifuged for 5 min at 4000 rpm. The supernatant obtained was passed through syringe filters of 0.45 $\mu$m (Millipore). The extraction of the wine samples was performed using solid phase extraction (SPE) following the method proposed by Hernández et al. (*24*). Bond Elut C-18 cartridges (500 mg, 3 mL) were employed; the solid phase extraction was performed in several stages in a "Rapid Trace, SPE Workstation" system (Zymark). First the cartridge was conditioned with 5 mL of methanol and 5 mL of Milli-Q water, both at a flow rate of 2 mL/min. A volume of 10 mL of a 1:2 dilution of the sample in Milli-Q water was passed through the cartridge at 0.5 mL/min. The cartridge was then washed with 2 mL of Milli-Q water and 2 mL of methanol/water (60/40 v/v), and dried under a stream of $N_2$ for 10 min. Lastly the extract was collected in 2 mL of methanol at a flow rate of 0.5 mL/min.

*Chromatographic Separation*. The HPLC system employed for the analysis was a "Waters Alliance 2695", with a 110A C18 Gemini reverse phase column, of 250 mm length × 4.6 mm i.d. and particle size of 5 $\mu$m (Phenomenex). The alkaline mobile phase was composed of acetonitrile/ammonium chloride−ammonia buffer (205 mM, pH 9.4), in a proportion 20:80 (v/v), by isocratic flow at 30 °C and at a flow rate of 0.75 mL/min. The volume of sample injected was 25 $\mu$L. A Waters 474 scanning fluorescence detector was used for the measurement of fluorescence. The wavelengths of excitation and emission were 333 and 460 nm, respectively. The data acquisition time for each of the samples was set at 14 min; the software employed for the recording and processing of the data was Empower Pro 2002 (Waters). **Figure 1** shows typical chromatograms of the samples studied, a must and a wine, with a retention time of 10.2 min for OTA.

*Calibration*. For the quantification in musts, a calibration curve was obtained from eight different concentrations of a standard solution of OTA (0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 $\mu$g/kg). All the dilutions were prepared in duplicate, in a matrix of synthetic must (1 g/L of malic acid, 6 g/L of tartaric acid and 200 g/L of glucose), and were treated in the same way as the real samples. For the samples of wines, the calibration curve was obtained in HPLC grade methanol, from known volumes of a working solution of 50 $\mu$g/L (0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 $\mu$g/L). Lastly, the OTA peak areas obtained in the chromatograms were represented graphically against their corresponding concentrations. Two replicates of all the dilutions were prepared, and these were analyzed in duplicate.

*Validation of the Analytical Methods*. Samples of musts and wines are of great chemical complexity and are difficult to reproduce accurately. In addition, the fluorescence detection is a very sensitive method that could cause interference from the matrices. As a result, two different studies, one for each type of sample under study, were carried out to ensure the matrix effects did not affect the quantitation. The method of standard additions to the real samples was used. The slope values of the regression curves obtained with standard solutions were then compared with the slope values obtained by the method of standard additions using the Student *t* test. In both studies, for musts and wines, the slopes obtained were similar for a 95% confidence limit, so it was concluded that matrix effects were absent.

Regarding the validation of other analytical parameters, it was performed in a different way for musts and wines. On one hand, two studies of

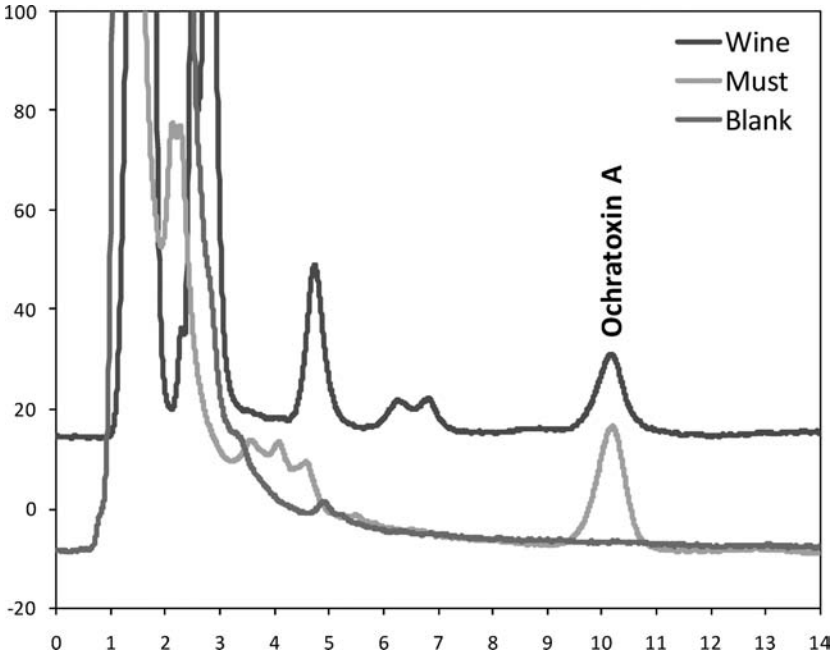


**Figure 1.** Typical chromatograms of must and wine samples under study, with the one of a blank superimposed, showing the OTA peak at 10.2 min.

the repeatability of wines were carried out, concluding that relative standard deviations (RSD) both intraday repeatability and interday were low (1.11% and 1.14% respectively). In addition, a series of standard additions (at four levels, in duplicate) from 0.75 to 6 $\mu$g OTA/L was prepared in order to obtain the recoveries. These were from 93 to 99%. Finally, the limit of detection and limit of quantification were established at 0.22 and 0.77 $\mu$g/L, respectively. On the other hand, samples of grapes and musts spiked with 2 $\mu$g/kg of OTA were used for the studies of repeatability performed. The relative standard deviations (RSD %) of the intraday and the interday studies were both 0.14%. The accuracy of the analysis method was established by using the method of standard additions. Five levels of OTA addition, from 0.5 to 5 $\mu$g/kg, and two samples per level were prepared by evaporation of known volumes of the OTA working solution under nitrogen stream, followed by dissolution in real samples of grape. The method presented recovery values close to 100% for most of the concentrations analyzed and low values of standard deviations (from 94.7 $\pm$ 0.8 to 107.8 $\pm$ 0.9). About the limits of detection and quantification, they were established at 0.13 and 0.43 $\mu$g/kg of OTA respectively.

**Sensory Analysis.** The sessions were carried out in a standard tasting room (*25*). All evaluations, exclusively orthonasal, were carried out at 22 °C. Twenty milliliters of sample were presented in blue glasses, generally used for olive oil sensory analysis, and covered with a glass top in order to minimize the possible losses of aroma. The panel judges, all of them laboratory personnel, were submitted to a training period about general and specific sensorial aspects. During this stage, the judgment repeatability and the panel homogeneity were evaluated. Duplicated discrimination testing and descriptive profiling were employed to validate the judges' reproducibility, whereas for each descriptor, a two factor ANOVA (judges $\times$ samples) allowed study of the homogeneity of the panel.

Triangular test and descriptive profiling were performed. For the first case, the triangular test (*26*), the judges were asked about the quantitative value of the detected differences. A scale with five points was used: not present, poor, regular, strong, and very strong. For the second study, the descriptive profiling (*27*), as a preliminary stage of generating appropriate descriptors to define the samples, the judges were presented with a group of musts and wines, representative of the complete set, and were asked to describe them qualitatively. The final selection included those with a frequency of appearance greater than 5. A structured nine-point scale was employed to evaluate each descriptor (*28*). The descriptors evaluated were as follows: *aromatic intensity*, *fruity*, *citric*, *ripe fruit*, *sweet*, *raisin*, *floral*, *honey*, *herbaceous*, *oak*, *wine character*, *dairy*, *wetness*, *chemical character*, and *general impression*. A ranking test (*29*) was carried out to assess the judges' preferences among the different wines produced. This type of discrimination test is indicated when there are several samples to compare, since it minimizes the consumption of samples and the sensorial fatigue of the judges. After presenting the series of wines to the judges, they were asked to assess them from left to right, and then to rank them by preference from least to most. The test of Friedman was applied for the interpretation of the results obtained.

**Statistical Analysis.** One and two factor variance analysis (ANOVA) and multivariate analysis of data included factor analysis (FA) using the statistical computer package Statistica 7.0 (Tulsa, OK, USA) were performed.

## RESULTS AND DISCUSSION

**Musts.** The OTA contents in the musts from the two different systems of raisining tested suggested that a previously existing fungal contamination in the grape could occur, and this contamination, associated with the conditions of the drying process itself, led to the growth of the fungus and, consequently, of ochratoxin A in both systems. This finding is related to the data provided by Valero et al. (*14*) which demonstrated that it is after the harvesting, and particularly in the process of raisining, when the *Aspergillus* species becomes predominant over other fungi present on the grape, due principally to greater tolerance of high temperatures and low activity of the water ($a_w$).

However, the OTA concentration of the must from grapes dried in the climatic chamber (6.9 $\pm$ $\mu$g/kg) was lower than that obtained in the natural process (28.8 $\pm$ $\mu$g/kg), the difference being statistically significant ($p < 0.05$), so it seems the increased control over the conditions of raisining resulted in better hygienic conditions for the final must. The must obtained in the traditional system presented a very high concentration of OTA which is not suitable for subsequent fermentation since it will be difficult to achieve a legally acceptable OTA content in the finished wine.

With respect to the sensory analysis, the musts obtained from the grapes dried by the traditional method of sunning were compared by triangular test with those from grapes dried under controlled conditions in the climatic chamber. A significant difference between them was detected (according to 10 of the 14 judges, $p < 0.05$), with the intensity of difference reported ranging between weak and moderate with a mean $\pm$ standard deviation value equal to 1.9 $\pm$ 0.8. Then, with the aim of identifying the descriptors that best explained the differences detected between

**Table 2.** Analysis of Variance Applied to the Intensity Values of the Attributes Used To Describe the Sensory Profiles of the Musts under Study[a]

| descriptors | traditional sun drying | climatic chamber | *F* | *p* |
|---|---|---|---|---|
| **aromatic intensity** | 4.5 ± 1.4 | 4.2 ± 1.3 | 7.54 | **0.052** |
| **fruity** | 3.9 ± 1.2 | 4.7 ± 0.8 | 8.95 | **0.045** |
| citric | 1.1 ± 1.4 | 0.9 ± 1.3 | 2.45 | 0.215 |
| ripe fruit | 3.4 ± 2.2 | 2.6 ± 2.0 | 2.10 | 0.276 |
| sweet | 2.9 ± 1.6 | 3.0 ± 1.6 | 2.51 | 0.138 |
| **raisin** | 3.3 ± 1.2 | 4.5 ± 1.5 | 7.80 | **0.049** |
| floral | 2.0 ± 1.9 | 1.7 ± 1.6 | 0.12 | 0.557 |
| honey | 1.2 ± 1.6 | 2.3 ± 2.5 | 1.06 | 0.345 |
| herbaceous | 1.9 ± 1.4 | 1.2 ± 1.9 | 2.41 | 0.219 |
| wine character | 0.9 ± 1.7 | 1.2 ± 1.9 | 1.18 | 0.337 |
| dairy | 0.4 ± 0.9 | 0.5 ± 1.1 | 2.45 | 0.216 |
| **wetness** | 1.9 ± 2.2 | 1.3 ± 1.8 | 4.57 | **0.099** |
| chemical character | 0.1 ± 0.5 | 0.2 ± 0.5 | 3.00 | 0.125 |
| **general impression** | 3.8 ± 1.9 | 3.6 ± 1.7 | 3.12 | **0.115** |

[a] Mean values ± standard deviation ($n$ = 14) are shown. The differences are significant at $p < 0.05$. The lowest $p$ (in bold) indicate the most significant descriptors in order to discriminate the musts obtained both by traditional sun-dried and climatic chamber.

the systems of traditional and controlled raisining, an analysis of the variance was performed for the various descriptors (**Table 2**). Only *raisin* and *fruity* descriptors differentiated the musts to any extent, and a greater but not significant *aromatic intensity* and *wetness* in the traditionally sunned grapes was confirmed. According to the *general impression*, the must obtained from grapes naturally sunned on the roof terrace was slightly preferred to that obtained from grapes dried in the climatic chamber, with a stastistically nonsignificant difference.

**Vinification.** During the vinification of the musts made from grapes dried in the climatic chamber a considerable decrease of the OTA content was observed (averages and standard deviations shown in **Figure 2** and **Table 3**). A large part of the solid matter that was in suspension, and with it part of the toxin present in the grape marc, was eliminated in the desludging and clarification (*30*), the difference being statistically significant between the two wines before (V01) and after (V02) these enological practices ($p < 0.05$). Similar results have been obtained during the vinification of musts of red grapes (*31*). In addition recent investigations suggest a possible link between ochratoxin A and the cellular surface of yeasts (*32*). Similarly, in those wines with prefermentative maceration (experiences 3 and 5), a smaller decrease in OTA content than in the wines without maceration was exhibited. In these two experiences the must was in contact with the solid matter (skins, etc.) for 24 h, and this facilitated the transfer of the toxin to the must.

The wines made under different conditions of vinification were compared by sensory analysis. The analysis of variance of the descriptive data (**Table 4**) suggested that the descriptors *floral* and *citric* were the best to discriminate between some of the wines. Regarding the preference, the 5 wines were ordered from the lowest to the highest preference as shown: 2_V02, 5_V02, 3_V02, 1_V02, 4_V02. The wine fermented at low temperature with *S. bayanus* yeast (4_V02) and characterized by more intense *citric* and *floral* notes was assessed as the best, whereas the wine made with an additional source of nitrogen (2_V02) was the least liked and presented the lowest intensities in both *fruity* and *citric* notes and a certain *chemical character*. Unexpectedly, the wines with pellicular maceration (3_V02 and 5_V02), characterized by high both floral and fruity notes, were not those preferred.

**Aging.** During the aging in oak barrels a decrease was observed in the OTA content (**Figure 2** and **Table 3**); in all the cases, lower concentrations than in the corresponding controls were found, the difference being statistically significant ($p < 0.05$). The OTA

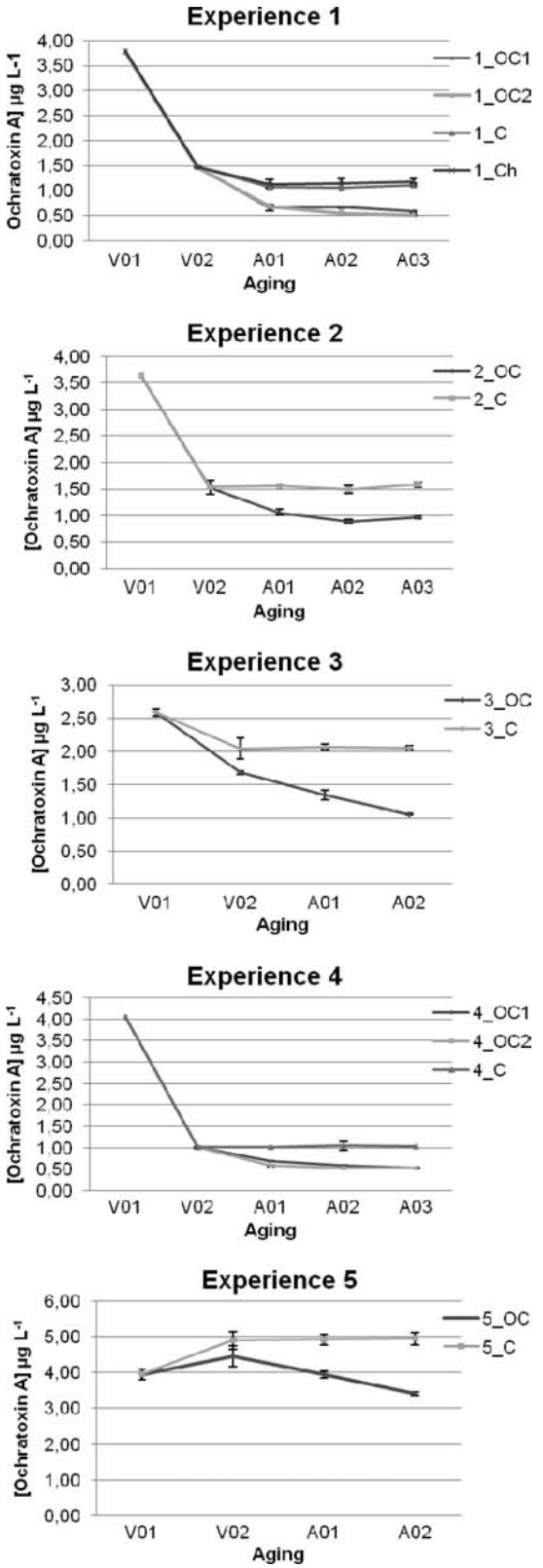


**Figure 2.** Time evolution of the OTA contents in the five sweet wines elaborated according to the fermentation conditions specified in **Table 1**. V01, wine after fermentation; V02, wine after desludging and clarification; A01, wine aged for 1 month in oak cask; A02, wine aged for 2 months in oak cask; A03, wine aged for 3 months in oak cask; C, wine without aging; Ch, wine aged with oak chips; OC, wine aged in oak cask.

content in these controls hardly varied at all during the assays, while those of the wines aged in oak barrels showed a large decrease at the beginning, with a slight progressive decrease

throughout the aging time in the case of the wines with the higher contents (experiences 3 and 5). The decrease of OTA during the 90 days of aging in the wines of the different assays ranged from 8.7% in experience 2 to 37.2% in experience 3. The aging of red wines in bottle was evaluated by Grazioli et al. (*29*), and no short-term effect was found, but there was a decrease of 17% in OTA after 12 months. With regard to our samples, in four of the seven barrels studied this decrease was even greater than 17%. In addition, in experiences 1 and 4, it was observed that wines of the same assay aged in different oak barrels (1_OC1−1_OC2 and 4-OC1−4_OC2) showed a very similar trend over time. In the experiment conducted with wood shavings (1_Ch), the OTA content of the wine remained constant, showing behavior more comparable to that of the control than the wine aged in barrel. All the aged wines, with the exception of that obtained in experiment 5, showed an OTA content of less than 2.0 $\mu$g/L, the legal maximum set by the European Union; therefore these wines are suitable for consumption. However, given that the aging in oak barrel reduces the OTA content, it is quite possible that the wine of experiment 5 would also fall within the legally acceptable limit in the next samplings, although this concern needs to be confirmed.

**Table 3.** Mean and Standard Deviation Values of the OTA Contents in the Studied Sweet Wines through the Different Stages of the Elaboration Process[a]

| | V01 | V02 | A01 | A02 | A03 |
|---|---|---|---|---|---|
| | | | Experience 1 | | |
| OC1 | 3.75 ± 0.03 | 1.44 ± 0.02 | 0.64 ± 0.05 | 0.65 ± 0.05 | 0.62 ± 0.02 |
| OC2 | | | 0.69 ± 0.03 | 0.58 ± 0.02 | 0.55 ± 0.02 |
| C | | | 1.12 ± 0.08 | 1.12 ± 0.10 | 1.14 ± 0.06 |
| Ch | | | 1.10 ± 0.02 | 1.08 ± 0.01 | 1.13 ± 0.01 |
| | | | Experience 2 | | |
| OCC | 3.64 ± 0.01 | 1.51 ± 0.11 | 1.11 ± 0.03 | 0.86 ± 0.02 | 0.97 ± 0.01 |
| C | | | 1.52 ± 0.01 | 1.48 ± 0.03 | 1.60 ± 0.02 |
| | | | Experience 3 | | |
| OC | 2.55 ± 0.02 | 1.71 ± 0.01 | 1.29 ± 0.03 | 1.07 ± 0.01 | |
| C | | 2.01 ± 0.06 | 2.03 ± 0.02 | 2.03 ± 0.02 | |
| | | | Experience 4 | | |
| OC1 | 4.09 ± 0.02 | 0.97 ± 0.01 | 0.73 ± 0.01 | 0.58 ± 0.02 | 0.50 ± 0.02 |
| OC2 | | | 0.62 ± 0.02 | 0.56 ± 0.02 | 0.52 ± 0.01 |
| C | | | 1.00 ± 0.01 | 1.01 ± 0.07 | 1.00 ± 0.02 |
| | | | Experience 5 | | |
| OC | 3.99 ± 0.03 | 4.44 ± 0.4 | 3.95 ± 0.01 | 3.40 ± 0.01 | |
| C | | 4.92 ± 0.2 | 4.92 ± 0.02 | 4.98 ± 0.02 | |

[a] V01, wine after fermentation; V02, wine after desludging and clarification; A01, wine aged for 1 month in oak cask; A02, wine aged for 2 months in oak cask; A03, wine aged for 3 months in oak cask; C, wine without aging; Ch, wine aged with oak chips; OC, wine aged in oak cask.

The sensorial data of all the wines were analyzed from a multivariate perspective. A factor analysis was performed including the base wines and those aged in barrel and with wood shavings; from this, three major factors were extracted that explained 68% of the total variability. According to what can be deduced from the graph of loadings of the factors presented as **Figure 3** (loadings values shown in **Table 5**), factor 1 (which accounts for 36.8% of the variance) was correlated positively with the *general impression*, to which the sweet notes (especially *raisin*, *sweet*, *ripe fruit*, and *honey*), together with the *oak* note due to the aging, seemed to contribute in particular. On the other hand, factor 2 (16.1% of the explained variance) presented high loadings for *fruit*, *citric* and *floral* notes; therefore, this could be a measure of the primary and variety aromas, which are fully expressed after the fermentation. Factor 3 (14.5% of explained variance), for its part, was a measure of the defects, since it included the descriptors

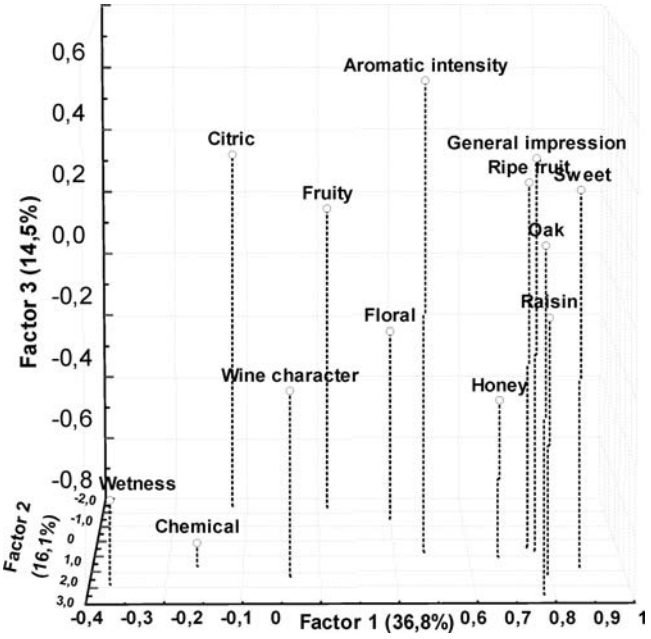


**Figure 3.** Factor analysis of sensory data of sweet wines made from raisins dried in a climatic chamber. Location of the sensorial attributes onto the new space defined by the three major factors: *x* axis, factor 1 (36.8%); *y* axis, factor 2 (16.1%); and *z* axis, factor 3 (14.5%).

**Table 4.** Analysis of Variance Applied to the Intensity Values of the Attributes Used To Describe the Sensory Profiles of the Sweet Wines Produced[a]

| descriptors | 1_V02 | 2_V02 | 3_V02 | 4_V02 | 5_V02 | *F* | *p* |
|---|---|---|---|---|---|---|---|
| aromatic intensity | 4.1 ± 0.5 | 4.2 ± 0.6 | 4.4 ± 0.6 | 4.1 ± 0.5 | 4.2 ± 0.5 | 0.114 | 0.97 |
| fruity | 4.2 ± 0.3 | 2.7 ± 0.4 | 3.8 ± 0.3 | 4.1 ± 0.4 | 3.8 ± 0.5 | 2.164 | 0.11 |
| **citric** | 2.2 ± 0.7 | 1.4 ± 0.5 | 2.8 ± 0.3 | 3.1 ± 0.4 | 2.7 ± 0.3 | 3.184 | **0.04** |
| ripe fruit | 1.9 ± 0.4 | 1.5 ± 0.6 | 2.1 ± 0.4 | 1.7 ± 0.5 | 1.4 ± 0.4 | 0.307 | 0.87 |
| sweet | 2.3 ± 0.6 | 2.0 ± 0.6 | 3.5 ± 0.6 | 2.3 ± 0.7 | 1.4 ± 0.5 | 1.312 | 0.37 |
| raisin | 1.9 ± 0.5 | 1.6 ± 0.5 | 2.2 ± 0.4 | 2.3 ± 0.4 | 1.1 ± 0.3 | 0.349 | 0.84 |
| **floral** | 3.6 ± 0.8 | 3.0 ± 0.7 | 3.9 ± 0.5 | 4.1 ± 0.6 | 4.0 ± 0.8 | 2.647 | **0.07** |
| honey | 0.9 ± 0.2 | 0.8 ± 0.4 | 1.5 ± 0.5 | 1.2 ± 0.3 | 1.1 ± 0.5 | 0.806 | 0.54 |
| herbaceous | 1.1 ± 0.4 | 2.1 ± 0.6 | 1.3 ± 0.6 | 1.4 ± 0.5 | 1.9 ± 0.7 | 0.351 | 0.83 |
| wine character | 2.5 ± 0.5 | 2.3 ± 0.7 | 2.5 ± 0.5 | 2.9 ± 0.6 | 2.8 ± 0.6 | 0.298 | 0.87 |
| dairy | 0.2 ± 0.3 | 0.5 ± 0.3 | 0.5 ± 0.3 | 0.5 ± 0.3 | 0.5 ± 0.4 | 0.033 | 0.99 |
| wetness | 0.6 ± 0.3 | 1.1 ± 0.4 | 1.1 ± 0.6 | 0.9 ± 0.4 | 1.5 ± 0.5 | 0.322 | 0.86 |
| chemical character | 0.8 ± 0.3 | 2.1 ± 0.3 | 0.6 ± 0.3 | 1.0 ± 0.3 | 0.8 ± 0.2 | 0.908 | 0.43 |
| **general impression** | 4.4 ± 0.4 | 2.5 ± 0.6 | 3.1 ± 0.5 | 4.5 ± 0.5 | 3.8 ± 0.5 | 2.994 | **0.05** |

[a] Values are given as mean ± standard deviation ($n = 17$). The differences are significant at $p < 0.05$. The lowest $p$ (in bold) indicate the most significant descriptors in order to discriminate the 5 sweet wines produced.

**Table 5.** Loadings of the Sensory Descriptors from Raisins Dried in a Climatic Chamber in the New Space Defined by the Three Major Factors 1, 2 and 3[a]

| descriptor | factor 1 (36.8% expl. var.) | factor 2 (16.1% expl. var.) | factor 3 (14.5% expl. var.) |
|---|---|---|---|
| aromatic intensity | 0.480 | 0.243 | 0.447 |
| fruity | 0.226 | **0.867** | −0.035 |
| citric | −0.039 | **0.889** | 0.135 |
| ripe fruit | **0.758** | 0.303 | 0.128 |
| sweet | **0.885** | 0.046 | 0.144 |
| raisin | **0.790** | −0.044 | −0.223 |
| floral | 0.398 | **0.703** | −0.399 |
| honey | **0.677** | 0.179 | −0.516 |
| oak | **0.774** | −0.296 | 0.033 |
| wine character | 0.122 | −0.067 | −0.437 |
| chemical | −0.122 | 0.068 | **-0.925** |
| wetness | −0.344 | −0.159 | **-0.742** |
| general impression | **0.776** | 0.258 | 0.209 |

[a] Numbers in bold indicate the descriptors with the highest correlations to each factor.

**Table 6.** Loadings of the Wines Made from Raisins Dried in a Climatic Chamber in the New Space Defined by the Three Major Factors 1, 2 and 3

| wine | factor 1 (36.8% expl. var.) | factor 2 (16.1% expl. var.) | factor 2 (14.5% expl. var.) |
|---|---|---|---|
| 1_V02 | −0.041 | 0.740 | −0.882 |
| 2_V02 | −1.651 | 0.403 | −2.824 |
| 3_V02 | −0.748 | 1.824 | −0.945 |
| 4_V02 | −2.748 | 0.053 | −0.250 |
| 5_V02 | −0.088 | 2.726 | −0.748 |
| 1_Ch_A01 | −1.112 | 0.820 | 1.038 |
| 1_OC2_A01 | −0.107 | −1.104 | 1.033 |
| 1_OC1_A01 | −0.992 | 0.030 | 0.495 |
| 2_OC1_A01 | −0.153 | −0.916 | −0.085 |
| 3_OC1_A01 | 0.488 | −0.577 | 1.047 |
| 4_OC1_A01 | 0.170 | −1.612 | 1.110 |
| 4_OC2_A01 | −0.557 | −0.943 | 0.870 |
| 5_OC1_A01 | −0.621 | −0.422 | 1.099 |
| 1_OC2_A02 | 0.963 | −0.283 | 0.204 |
| 1_OC1_A02 | 1.185 | 0.711 | 0.761 |
| 1_Ch_A02 | −0.312 | −0.214 | 0.141 |
| 2_OC1_A02 | 1.154 | 0.184 | 0.062 |
| 3_OC1_A02 | 1.026 | 0.582 | 0.613 |
| 4_OC1_A02 | 0.363 | 0.820 | 0.796 |
| 4_OC2_A02 | 0.511 | −1.651 | 0.490 |
| 5_OC1_A02 | 0.238 | −0.518 | −0.163 |
| 1_OC1_A03 | 1.553 | −0.899 | −0.313 |
| 1_OC2_A03 | 1.244 | 0.683 | −0.741 |
| 1_Ch_A03 | −0.581 | 0.006 | −1.462 |
| 2_OC1_A03 | 1.653 | 0.029 | −0.758 |
| 3_OC1_A03 | 1.459 | −0.800 | −0.525 |
| 4_OC1_A03 | 0.892 | −0.472 | −0.696 |
| 4_OC2_A03 | 1.407 | −0.747 | −0.149 |
| 5_OC1_A03 | 1.346 | −0.303 | −0.811 |

*chemical* and *wetness* with high and negative loadings. When the samples were placed in the new space (**Table 6** and **Figure 4**), the interpretation of the dimensions of the factors was confirmed, since the wines increase their time of aging in oak barrel to the right of factor 1 (from A01 to A03), while the control wine produced with an additional nitrogen supply (2_V02) reaches the lowest values for factor 3, confirming the results obtained in the preference test. Additionally, it seems that the wine aged with wood shavings (1_Ch) acquired an aromatic defect along the time, as a decreasing value for factor 3 indicates. Regarding factor 2 associated with primary aromas, as expected, the initial wines produced with pellicular maceration (3_V02 and 5_V02) obtained high scores.

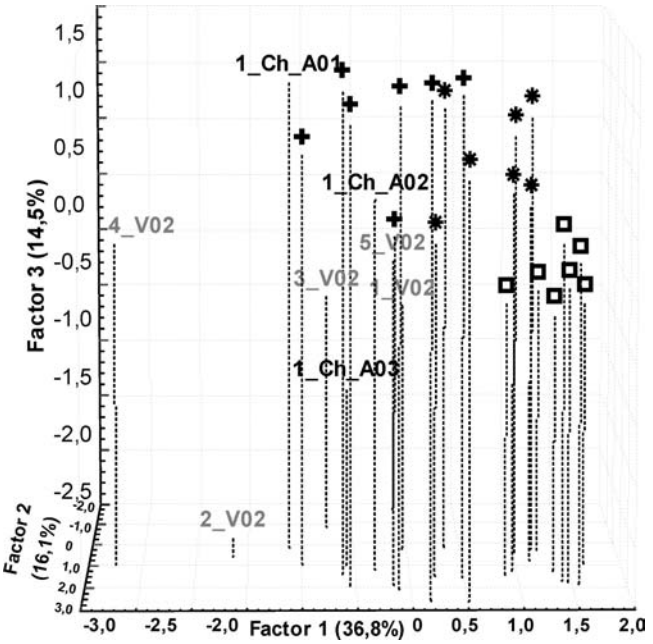


**Figure 4.** Factor analysis of sensory data of sweet wines made from raisins dried in a climatic chamber. Location of all the sweet wines (aged or not) onto the new space defined by factors 1, 2, and 3. Symbols: cross, A01, sweet wines aged for 3 months; starburst, A02, sweet wines aged for 6 months; box, A03, sweet wines aged for 9 months.

In conclusion, throughout the aging period, the *oak* note increased, and those of the primary aromas (*citric*, *floral*, and *fruity* notes), together with the initial defects, generally diminished. The wines became more acceptable with aging in barrel (the *general impression* of all the wines tested improved), and this stage had the effect of diminishing the initial differences between the different methods of vinification.

We can conclude that the final concentration of ochratoxin A in the must obtained after controlled drying was much less than that found in the musts originating from the traditional system. Although it was not possible to eliminate completely the growth of the fungus, the ability to control more precisely the temperature and humidity of the grapes dried in the climatic chamber reduced the production of OTA. Therefore, the use of the climatic chamber for drying grapes for the production of sweet wines, combined with the traditional techniques of vinification (without pellicular maceration), and a final stage of aging in oak barrels has yielded wines with a low content in OTA and well-accepted from a sensorial point of view.

## ABBREVIATIONS USED

OTA, ochratoxin A; TSD, raisin obtained by traditional sun drying; CC, raisin dried in the climatic chamber; V01, wine after fermentation; V02, wine after desludging and clarification; A01, wine aged for 1 month in oak cask; A02, wine aged for 2 months in oak cask; A03, wine aged for 3 months in oak cask; C, wine without aging; Ch, wine aged with oak chips; OC, wine aged in oak cask; HPLC, high performance liquid chromatography; ANOVA, analysis of the variance; FA, factor analysis.

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