

# Clues about the Role of Methional As Character Impact Odorant of Some Oxidized Wines

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A young white wine that had undergone spontaneous oxidation and showed a strong off-flavor reminiscent of cooked vegetables was demixed by salting out to obtain an ethanolic extract that retained the off-flavor of the wine, as was demonstrated by sensory analysis. This extract, together with a second one obtained from a non-oxidized wine sample, were chromatographed into a reversed-phase HPLC column using a water/ethanol gradient. The column effluents were collected in 14 different fractions that were evaluated for smell. Four fractions were found to differ between oxidized and non-oxidized wine, but only one showed a clear cooked vegetables off-flavor and, when added to a non-oxidized wine, made it significantly more similar to the oxidized wine. The fraction was analyzed by GC-MS-olfactometry, which made it possible to identify methional as the single important odorant of the fraction. The odor threshold of methional in a synthetic wine was found to be  $0.5 \mu\text{g L}^{-1}$ . The analytical determination of methional through GC-FPD in several oxidized and non-oxidized wines showed that, in the former, methional can reach more than 200 Odor Units, whereas in the non-oxidized samples, it was not possible to detect methional. The methional concentration was found to increase in wines spiked with both methionol or methionine, which suggests that it can be formed from direct peroxidation of methionol or via Strecker degradation of methionine mediated, probably, by o-quinones formed during wine oxidation.

**Keywords:** *Methional; wine; oxidation aroma; off-flavor; GC-FPD*

## INTRODUCTION

It has recently been described that the young white wines undergoing oxidation develop several aroma nuances reminiscent of wood, cider, liquor, pungent, and cooked vegetables (Asensio, 1998; Escudero et al., submitted). The presence of some of these aroma nuances can have a nasty effect on the quality of the wine, particularly in the case of the "pungent" and "cooked vegetables" notes, whose appearance mark the end of the shelf life of the wine. Despite their importance, the chemical compounds responsible for these flavor notes are not known. Previous efforts made to find any relationship between these aroma nuances and several aroma compounds developed during wine oxidation (Ferreira et al., 1997a, 1997b, 1998a) were unsuccessful. The use of olfactometric techniques in a subsequent study has revealed that, during the oxidation of wine, some very powerful odorants, not previously reported nor quantified, can be produced (Escudero et al., in press). One of the most important odorants found in that study was methyl-thio-propanaldehyde (methional). Because methional has an unpleasant aroma, it was thought that it might be an important contributor to some of the off-flavors formed during wine oxidation. However, due to the nature of olfactometric data (Acree, 1997; Ferreira et al., 1998b), it still is not possible to draw precise conclusions about its importance in the aroma of the wine, nor about its relationship with the oxidation process. These two questions are tackled in this paper, the main aims of which are to verify whether

**Table 1. Wines Used in This Study, Characteristics, and Concentration of Methional**

wines	characteristics <sup>a</sup>	origin <sup>b</sup>	grapes	methional ( $\mu\text{g/L}$ )
1	2 year old spontaneously oxidized wine	cariñena	macabeo	140
2	normal, non-oxidized	cariñena	macabeo	n.d.
2 ox	wine 2, oxidized	cariñena	macabeo	9.5
3	normal, non-oxidized	cariñena	macabeo	n.d. <sup>c</sup>
3 ox	wine 3 oxidized	cariñena	macabeo	11.8
4	normal, non-oxidized	borja	macabeo	n.d.
4 ox	wine 4 oxidized	borja	macabeo	10.7
5	2 year old rosé spontaneously oxidized wine	navarra	grenache	41
6	wine 4 + 0.1 mmol methionol, oxidized	borja	macabeo	18
7	wine 4 + 0.1 mmol methionine, oxidized	borja	macabeo	31

<sup>a</sup> Wines were spontaneously oxidized, oxidized in the lab, or normal samples. <sup>b</sup> Spanish winemaking regions. <sup>c</sup> n.d. = Non detected.

methional can be formed during wine oxidation and, if so, whether it can reach a level high enough to be clearly perceived and whether it has something to do with some of the off-flavors formed during wine oxidation.

## MATERIAL AND METHODS

**Wine Samples.** The wines used in this study are shown in Table 1. Wines 1 and 5 reached a high degree of oxidative deterioration spontaneously in the bottle. Wines 2, 3, and 4 were normal wines and showed the characteristic fruity notes

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of non-oxidized young white wines. Volumes of these three wines were oxidized under the conditions described below.

**Wine Oxidation.** 750 mL of wine were transferred from the bottle to a 1 L sterilized amber bottle, and saturated with pure oxygen (99.999%). The remaining headspace of the bottle was also saturated with oxygen to ensure enough oxygen availability along the oxidation process. The bottles were sealed and stored at 20 °C in the dark for 1 month. To determine the nature of the precursors of methional, wine 4 was made 0.1 mM in methionol (wine 6) or in methionine (wine 7) and oxidized under the conditions described above.

**Wine HPLC Fractionation.** The HPLC fractionation of the wine was accomplished with an HPLC Waters (Milford, MA), integrated by two 510 pumps, an automated gradient controller, a manual injector U6K, and a Lambda-Max Model 481 LC Spectrophotometer. The column used was a Kromasil, 5  $\mu$ m, 25 cm long, and 4.6 mm i.d., from Análisis Vínicos (Tomelloso, Spain). The chromatographic conditions that were used include a flow rate of 1 mL/min, detection at 254 nm, and an injection volume 1500  $\mu$ L. The program gradient involved Phase A, water; Phase B, ethanol; Min 0, 100% A, which was linearly programmed until there was 20% B in min 8, 50% B in min 28, and 100% B in min 40.

Alcoholic extracts were prepared by salting out 250 mL portions of the wines adjusted at 13%(v/v) alcohol with 33 g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  and 135 g of  $(\text{NH}_4)_2\text{SO}_4$ . The demixed ethanolic phase (about 13 mL) was collected and further reconstituted until a final volume of about 1–2 mL by washing with a brine composed of 13.2 g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 54 g of  $\text{NH}_4\text{SO}_4$ , and 87 mL of water. These extracts were sensorily tested to evaluate their representativity and injected into the HPLC system under the conditions given above. The HPLC eluate was recovered in 14 separated fractions that were rediluted with synthetic wine, added to a non-oxidized wine (wine sample 2), and evaluated for their smell following the procedures described below. Those fractions showing remarkable odors were re-extracted and analyzed.

**Flavor Fractions Re-extraction.** The alcoholic content of the fractions eluted from the HPLC was adjusted to 13% (v/v). Then, to the solution was added 0.3 g of  $(\text{NH}_4)_2\text{SO}_4$  per mL, which was finally extracted with  $\text{CH}_2\text{Cl}_2$  (organic/aqueous phase ratio 1:10, v/v). The dichloromethane extract was concentrated under Nitrogen until 100  $\mu$ L. This extract was analyzed by GC-MS with simultaneous sniffing detection.

**GC-MS-Olfactometry.** A Star 3400CX (Varian) gas chromatograph fitted with a Saturn 4 electronic impact MS detector and equipped with a sniffing port (Open Split Interface, makeup flow 4 mL/min He). The columns that were used include a Carbowax 20 M (J&W, Folsom, USA), 30 m in length  $\times$  0.32 mm i.d. with a 0.5  $\mu$ m film thickness, and a BP-5 column (J&W), 50 m in length  $\times$  0.32 mm i.d. with a 1.0  $\mu$ m film thickness. The chromatographic conditions involve carrier He at 1.2 mL/min. One  $\mu$ L of the sample was injected into a 1093 Septum-equipped programmable injector held 6 s at 20 °C and then raised to 190 °C at 200 °C/min. The initial column temperature, 40 °C, held for 5 min and then raised to 190 °C at 2 °C/min. The olfactometry was performed by three different trained tasters.

**Quantitative Analysis of Methional.** The wine sample to be analyzed (100 mL) was diluted 1 to 1 with MilliQ water (Waters) and then extracted in a 20  $\times$  1 cm Amberlite XAD-4 (Supelco) column, previously conditioned with methanol (2 volumes) and water (2 volumes). The methional was eluted with 80 mL of diethyl ether/pentane (1:1). This extract was then spiked with 5  $\mu$ L of an Internal Standard solution (60 mg/L of allyl isothiocyanate, supplied by Aldrich-España, Madrid, in absolute ethanol), dried with  $\text{Na}_2\text{SO}_4$  and concentrated at 40 °C in a Kuderna-Danish fitted with a 3-ball Snyder column until a final volume of 100  $\mu$ L was reached. This extract was analyzed by GC-FPD. The yield of the extraction method was tested by the extraction of synthetic wines with known amounts of methional and wines spiked with known amounts of methional.

**GC-FPD.** A 3300 gas chromatograph (Varian) was fitted with a flame photometric detector (FPD) maintained at 250

°C. The detection conditions are as follows:  $\text{H}_2$ , 150 mL/min; air 1, 83 mL/min; air 2, 250 mL/min; makeup  $\text{N}_2$ , 14 mL/min. The column was a MFE-73 (Análisis vlnicos, Tomelloso, Spain), 50 m  $\times$  0.53 mm with a 3  $\mu$ m film thickness. The chromatographic conditions involve carrier  $\text{H}_2$  at 10 mL/min, injection from a splitless injector at 220 °C, a splitless time of 2 min, and an injected volume of 5  $\mu$ L. The initial column temperature, 40 °C, was held for 2 min and then raised to 200 °C at 10 °C/min. The relative area of methional vs the internal standard was interpolated in a calibration graph built by the analysis of synthetic wine samples containing known amounts of methional (standard from Aldrich-España, Madrid) following the entire procedure.

**Sensory Analysis.** The sensory panel consisted of 15 experienced individuals (8 women and 7 men whose average age was 30). All the tests were carried out in standardized booths with tulip glasses containing 30 mL of wine, synthetic wine, or wine reconstituted from the HPLC fractions or ethanolic extracts (in these cases, the alcoholic degree was adjusted to 12% v/v and the pH fixed at 3.4).

**Representativity of the Extracts.** Triangular tests (AFNOR, 1983) were performed to compare a non-oxidized wine (Wine 2), a wine of the same brand, and a type spontaneously oxidized (Wine 1), and the ethanolic extracts of each of the wines (ext 1 and ext 2). After demonstrating that the panel could perceive clear differences between the two wines and the two extracts, two tests of similarity were carried out as follows. First, the oxidized wine was presented as the reference sample, and the extracts were presented in a random order. The panel members were instructed to sniff and to memorize the aroma of the reference sample, and then to sniff the first coded tulip glass (containing one of the two extracts) and determine the similarity of their odors. A 100 mm unstructured scale was used for this, anchored with "identical to the reference sample" on the left and "different from the reference sample" on the right. The panelists were then asked to repeat the evaluation using the same reference sample and the second coded tulip glass. On the other hand, a new similarity test was made with the non-oxidized wine.

**Sensory Analysis of the HPLC Fractions.** The fractions were rediluted with water (buffered at pH 3.4 with 5 g/L tartaric acid and NaOH) to form synthetic wines. In a first experiment, triangular tests were made to detect differences between similar fractions obtained from oxidized (wine 1) or non-oxidized (wine 2) wine samples. After this, and only with those fractions that were found to significantly differ between wines, similarity tests were carried out as explained before. In this case, the reference taken was the oxidized wine, and the samples tested were the non-oxidized wine spiked with volumes of the HPLC fractions (volumes taken to make the non-oxidized wine contain amounts of odorants equivalent to that of the oxidized one) and the non-oxidized wine.

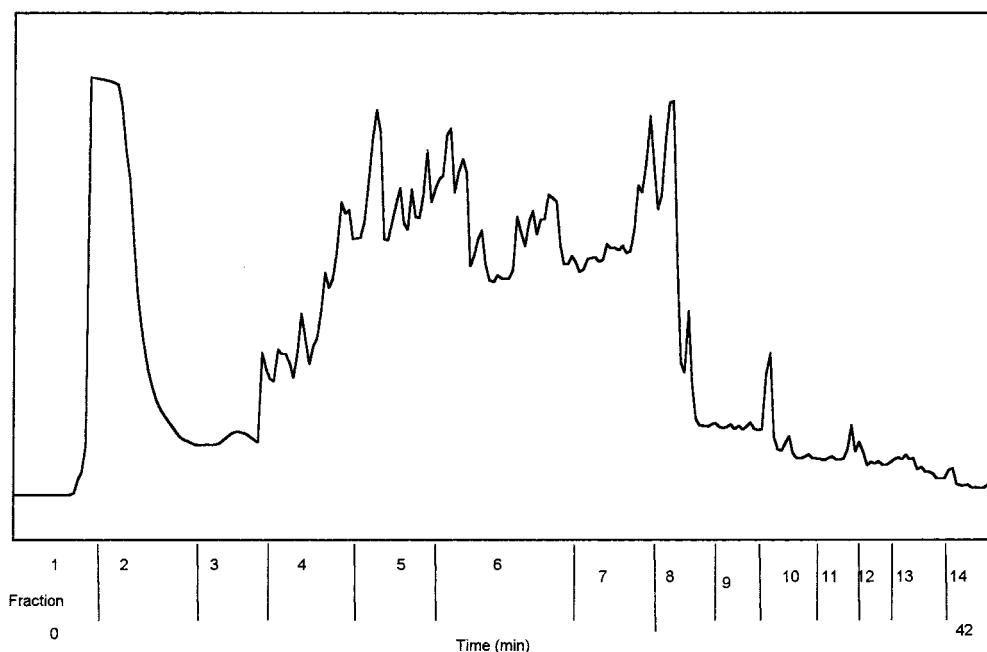
The olfactory threshold of methional was determined through triangular tests comparing synthetic wine vs synthetic (11% (v/v) ethanol, 5 g/L tartaric acid, 7 g/L glycerin, pH 3.4) wine spiked with different amounts of methional. Besides, the odor threshold was determined by a nonexperienced jury (16 women and 10 men whose average age was 35). The samples were presented following a decreasing concentration order.

**Data Analyses. Triangle Tests.** In these tests, the number of total and correct answers were used to test if there were differences of odors between the samples with a binomial law table settled for 1/3 probability.

**Similarity Tests.** In these tests, the marks on the unstructured scales were transformed into distances in millimeters from the left anchor. An univariate analysis of the variance was performed on these distances for the sample effect to test the significance of the difference of the mean answers of the panel.

## RESULTS AND DISCUSSION

Previous experience had shown us that the aroma compounds responsible for the off-flavors found in some



**Figure 1.** HPLC chromatogram of the extract from the oxidized wine (wine 1) showing the fractions collected for further sensorial analysis and GC-MS-O study.

**Table 2. Triangle Tests Carried out to Determine the Existence of Differences between Wine 1 (spontaneously oxidized) and Wine 2 (same brand as 1 but normal, non-oxidized) and Also between Their Corresponding Extracts**

pair tested	correct answers vs total answers	p value
ext 1 vs ext 2	11/15	0.005
W 1 vs W 2	13/15	<0.001

**Table 3. Similarity Tests to Measure the Difference between the Odors of the Wines and of the Extracts<sup>a</sup>**

sample evaluated	ext 2	ext 1	p value
wine 2	40.4 (27.8)	66.5 (23.5)	<0.05
wine 1	56.6 (29.9)	36.4 (23.2)	<0.05

<sup>a</sup> The data are the distance measured in mm ( $\sigma_{n-1}$ ).

oxidized wines were very difficult to extract, probably due to a highly polar nature. This led us to carefully control all of the isolation steps and choose those isolation techniques that are known by their ability to extract polar compounds. Demixture fulfills this requisite (Cacho et al., 1992) and is known to produce highly representative extracts (Priser et al., 1997). Consequently, it was chosen as the method of isolation. Nevertheless, comparison tests were carried out to ensure the representativity of the wine extracts. First, it was demonstrated through triangle tests that two extracts coming from different wines (both made with the same grapes in the same cellar, but the one was oxidized and the other was non-oxidized) are perceived by the sensory panel as different. These results are shown in Table 2. Once this result was obtained, two similarity tests were performed to measure how much the aroma of each extract resembled the wine from which it was obtained. These results are shown in Table 3. They demonstrate, in both cases, that each extract is more similar to the wine from which it comes, whereas it is clearly different from the other wine. This indicates that the extracts retain those properties of the wines that make the members of the panel recognize both

samples as different, i.e., retain the sensory properties of the oxidized wine.

The following isolation step was a HPLC chromatography in a C18 reversed phase column. This was selected because previous experiences with normal phase fractionations were unsuccessful due, likely, to the irreversible adsorption of some important odorants in the columns. An additional advantage of the reversed-phase system is that it allowed us to use only water and ethanol as mobile phases, thus simplifying the sensory analysis of the fractions (Ferreira et al., 1999). The eluates of both extracts, oxidized and non-oxidized, were divided into 14 different fractions taken at different times, as is indicated in the chromatogram shown in Figure 1. The reconstituted fractions (see Material and Methods) were sensorily tested, via triangular tests, to detect significant differences between equivalent fractions coming from the oxidized and the non-oxidized wine, respectively. The results of this experiment, together with the aromatic descriptors of those fractions obtained from the oxidized wine, are presented in Table 4. As is shown in the table, only the third, fourth, tenth, and fourteenth fractions were found to differ between wines. The most intense difference was found between the third fractions, namely, that the one coming from the oxidized wine clearly smelled of cooked vegetables, thus reminiscent of the off-flavor of the oxidized wine. A further sensory experience was carried out with these four fractions. In this case, aliquots of the fractions were added to a non-oxidized wine, and the degree of sensory similarity between each of the spiked samples and the oxidized wine was measured. The results of the experiment are shown in Table 5, and clearly indicate that the only fraction powerful enough to produce a change in the non-oxidized wine that made it more similar to the oxidized wine was fraction 3.

This fraction was then re-extracted with dichloromethane and analyzed by GC-MS with simultaneous sniffing detection. The olfactometry of the fraction showed a single odor (R. I. 1487 in a carbowax column, and 911 in a DB-5) whose mass spectra confirmed that

**Table 4. Results of the Triangular Tests Comparing Equivalent HPLC Fractions from a Non-oxidized Wine (wine 2) and an Oxidized One (wine 1)<sup>a</sup>**

fraction	correct answers vs total answers	p value	aromatic description
1	7/15	>0.05	heavy, flowery, sweet
2	6/15	>0.05	nice, very weak
3	12/15	0.001	cooked french beans, very intense
4	11/15	0.005	pungent, acid, chickpea water, green, intense
5	7/15	>0.05	unpleasant, weak
6	8/15	>0.05	oily
7	4/15	>0.05	fusel, weak
8	6/15	>0.05	fusel
9	8/15	>0.05	honey
10	9/15	0.05	honey, very sweet, flowers of almond trees, intense
11	5/15	>0.05	sweet
12	8/15	>0.05	olive oil, weak
13	7/15	>0.05	sweet, weak
14	9/15	0.05	additive of butane gas, weak

<sup>a</sup> The aromatic descriptors of the fractions refer to those coming from the oxidized sample.

**Table 5. Similarity Tests Comparing the Odor of Oxidized Wine (wine 1) with Those of the Non-oxidized Wine (wine 2) Spiked with Equivalent Amounts of Each of the Four Selected HPLC Fractions<sup>a</sup>**

fraction	non-oxidized wine + fraction	non-oxidized wine	p value
3	29.75 (25.2)	75.17 (30.9)	<0.05
4	44.9 (33.6)	62.7 (28.4)	>0.05
10	50.1 (29.0)	50.7 (31.3)	>0.05
14	58.4 (28.5)	63.0 (24.8)	>0.05

<sup>a</sup> Data are the distance measured in mm ( $\sigma_{n-1}$ ).

it was methional. This result clearly demonstrates that methional was the key odorant contained in the oxidized wine that causes an off-flavor reminiscent of cooked vegetables.

To check if these results can be generalized, the following step of this research looked for the determination of the Odor Unit values of methional in several wines. As the scientific literature gives different figures for the odor threshold of methional in beer and water, which range from 40  $\mu\text{g/l}$  (Anderson and Howard, 1974) to 1.6  $\mu\text{g/L}$  (Jansen et al., 1971) or even 0.2  $\mu\text{g/L}$  (Buttery et al., 1971), we decided to estimate it in a synthetic medium closest to wine. The odor threshold determined by us was 0.25  $\mu\text{g/L}$  for a well-trained panel (15 individuals, 10 out of 15 correct answers) and 0.75  $\mu\text{g/L}$  for a panel composed of nontrained individuals (26 individuals; 14 out of 26 correct answers). Consequently, we decided to use 0.5  $\mu\text{g/L}$  as odor threshold. Other important observations of this study were found, namely, that this compound can produce smells not usually associated with sulfur compounds and a nauseating and creamy taste; and that some individuals seem to be absolutely insensitive to this odor, and were unable to recognize it at concentrations as high as 50  $\mu\text{g/L}$ .

Methional was isolated by solid phase extraction with a XAD-4 Amberlite resin, and further determined by GC with flame photometric detection. In this case, demixture was not used in the isolation process because it does not make it possible to obtain clean and concentrated enough extracts. Amberlite XAD-4 resins are the best sorbents for the extraction of polar compounds and have been previously used by some authors to extract polar

aroma compounds from wine (Guichard et al., 1993), and in fact, recovery experiments showed that 100% of methional is extracted from wine in the conditions used. Several wines were analyzed, and the quantitative results are given in Table 1. In no case could methional be detected in the non-oxidized samples, which means that its concentration in these samples must be below the method detection limit, which was estimated as 1.5  $\mu\text{g/L}$  in wine through the injection of both standards and spiked extracts. On the other hand, the levels of methional in the oxidized wines were considerably higher than its odor threshold, and data in the table show that it can reach around 200 Odor Units.

Methional has been previously reported as the cause of an off-flavor in beer (Anderson and Howard, 1974; Markl et al., 1987), dry spinach (Masanetz et al., 1998), cheddar cheese (Milo and Reineccius, 1997), and milk and dairy products (Bosset et al., 1993). It is possible to find some reports in which methional is referenced as a possible constituent of the wine volatile fraction (Lavigne et al. 1992), but this is the first time that methional has been found to be a key odorant responsible for an off-flavor of wine.

To determine its chemical origin, several wines were added with methionine or with methionol. Results, shown in Table 1, clearly indicate that both compounds can act as precursors of methional. We can propose a Strecker degradation of methionine as the most likely pathway for forming methional from methionine. Wine contains two possible sources of dicarbonyl compounds able to induce the Strecker reaction. The first one is the oxidation of wine ortho-diphenols, which have been demonstrated to form ortho-quinones (Singleton, 1987), which are, in turn, able to induce the Strecker reaction of amino acids, as Saijó and Takeo (1970) found working with tea leaves extracts. The second source of dicarbonyls would be the naturally occurring wine dicarbonyls, such as diacetyl, glyoxal, or 2,3-pentanedione, as Pripis-Nicolau et al. (2000) have demonstrated. However, the fact that in the case considered in this paper, methional formation is directly linked to oxidation and, the present understanding of the wine oxidation process (Singleton, 1987, Cheynier and Fulcrand, 1998), make us think that the most likely pathway is that described by Saijó and Takeo. Finally, methional would be formed from the peroxidation of methionol, just as acetaldehyde is formed from ethanol (Wildenradt and Singleton, 1974).

#### ABBREVIATIONS USED

GC-FPD, gas chromatography coupled with a flame photometric detector; GC-MS, gas chromatography coupled with mass spectrometry; HPLC, high performance liquid chromatography; R. I., retention index.

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#### LITERATURE CITED

- Acree TE, GC/Olfactometry. *Anal. Chem.* **1997**, March 1: 170A-175A.  
 AFNOR. Analyse sensorielle. Méthodologie. Directives générales. Norme 09-001, **1983**.

- Anderson, R. J.; Howard, G. A. The origin and occurrence of volatile sulphur compounds in British ales and lagers. *J. Inst. Brew.* **1974**, *80*, 357–370.
- Asensio, E. Caracterización analítica y sensorial del proceso de degeneración oxidativa del aroma del vino blanco. Graduate thesis, University of Zaragoza, Spain, 1998.
- Bosset, J. O.; Gallmann, P. U.; Sieber, R. Influence of light transmittance of packing materials on the shelf life of milk and dairy products. *Lait* **1993**, *73* (1), 3–49.
- Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of additional volatile components of tomato. *J. Agric. Food Chem.* **1971**, *19*, 3, 524–529.
- Cacho, J.; Ferreira, V.; Fernández, P. Microextraction by demixing for the determination of volatile compounds in aqueous solutions. *Anal. Chim. Acta* **1992**, *262*, 311–317.
- Cheynier, V.; Fulcrand, H. Oxidation des polyphénols dans les moûts et les vins. In *Oenologie, Fondements Scientifiques et Technologiques*; Flanzy, C., Ed.; Tec&Doc: Paris, 1998. pp 581–596.
- Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Descriptive and quantitative sensory analysis of the aroma of young wines undergoing oxidation. An assessment of the role played by acetaldehyde. *Food Sci. Technol. Int.*, submitted for publication.
- Escudero, A.; Cacho, J.; Ferreira, V. Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography-olfactometric study. *Z. Lebensm. Unters. Forsch. A* **2000**, in press.
- Ferreira, V.; Escudero, A.; Fernández, P.; Cacho, J. Changes in the profile of volatile compounds in wines stored under oxygen and their relationship with the browning process. *Z. Lebensm. Unters. Forsch. A* **1997a**, *205*, 392–396.
- Ferreira, V.; Asensio, E.; Escudero, A.; Lopez, R.; Cacho, J. Analytical characterization of the flavor of oxygen-spoiled wines through the CG-Ion Trap MS of ultratrace odorants. *8th Symposium on Handling of Environmental and Biological Samples in Chromatography and 26th Scientific Meeting of the Group of Chromatography and Related Techniques of the Spanish Royal Society of Chemistry*. Almerimar-Eljido, Almeria. **1997b**, pp 119.
- Ferreira, V.; Escudero, A.; Lopez, R.; Cacho, J. Analytical characterization of the flavor of oxygen-spoiled wines through the gas chromatography-ion-trap mass spectrometry of ultratrace odorants: Optimization of conditions. *J. Chromatogr. Sci.* **1998a**, *36*, 284–292.
- Ferreira, V.; Ardanuy, M.; López, R.; Cacho, J. Relationship between flavor dilution values and odor unit values in hydroalcoholic solutions: The role of volatility and a practical rule for its estimation. *J. Agric. Food Chem.* **1998b**, *46*, 434–440.
- Ferreira, V.; Hernández-Orte, P.; Escudero, A.; López, R.; Cacho, J. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. *J. Chromatogr. A* **1999**, *864*, 77–88.
- Guichard, E.; Pham, T.; Etiévant, P. Quantitative determination of sotolon in wines by HPLC. *Chromatographia* **1993**, *37*, 539–542.
- Jansen, H. E.; Strating, J.; Westra, W. M. *J. Instit. Brew.* **1971**, *77*, 154.
- Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Formation des composés soufrés lourds au cours de la vinification des vins blancs secs. *J. Int. Sci. Vigne Vin* **1992**, *26*, 2, 75–85.
- Markl, K. S.; Iverson, W.; Nauert, S. M. Characterization of an off-flavor in beer: a case study. *Technical-Quarterly, Master Brewers, Association of the Americas* **1987**, *24* (1), 9–13.
- Masanetz, C.; Guth, H.; Grosch, W. Fishy and hay-like off-flavors of dry spinach. *Z. Lebensm. -Unters.-Forsch.* **1998**, *206* (2), 108–113.
- Milo, C.; Reineccius, G. A. Identification and quantification of potent odorants in regular-fat and low-fat mild cheddar cheese. *J. Agric. Food Chemistry* **1997**, *45* (9), 3590–3594.
- Pripis-Nicolau, L.; De Revel, G.; Bertrand, A. Les composés dicarbonylés et carbonylés du vin: précurseurs possibles d'arômes. In *Oenologie 99, 6e symposium international d'oenologie*; Lonvaud-Funel, A., Ed.; Tec&Doc: Paris, 2000; pp 425–427.
- Priser, C.; Etievant, P. X.; Nicklaus, S.; Brun, O. Representative champagne wine extracts for gas chromatography olfactometry analyses. *J. Agric. Food Chem.* **1997**, *45*, 3511–3514.
- Saijó, R.; Takeo, T. The formation of aldehydes from amino acids by tea leaves extracts. *Agric. Biol. Chem.* **1970**, *34*, 277–233.
- Singleton, V. L. Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69–77.
- Wildenradt, H. L.; Singleton, V. L. The production of aldehydes as a result of oxidation of polyphenolic compounds in relation to wine aging. *Am. J. Enol. Vitic.* **1974**, *25*, 119–126.

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