

Natural *versus* conventional production of Spanish white wines: an exploratory study

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Abstract

BACKGROUND: Natural wine (NW) lacks an official or agreed definition, but it can be generally described as a wine produced with organic or biodynamic grapes with minimal intervention in the cellar, and with minimal or no use of oenological additives. The present study aimed to test the hypotheses that self-defined NWs differ from conventional wines (CW) in their chemical composition and main sensory characteristics. The levels of conventional oenological parameters, turbidity, biogenic amines, ochratoxin A, ethyl carbamate, sulphites, chlorides, some metals, major, trace and Strecker aldehyde volatile compounds were determined in 28 wines, including natural and conventional Spanish commercial white wines. Wines were also sensory described following a labelled free sorting task.

RESULTS: NWs presented higher pH, volatile acidity (VA) and turbidity values, and a more intense yellow colour, whereas they have a lower malic acid content compared to their conventional counterparts. NWs presented lower levels of total sulphur dioxide but significantly higher levels of biogenic amine putrescine, although both compounds are within the legal limits in all cases. None of the dimensions of the similarity space discriminated NWs from CWs. However, 70% of the NWs were grouped on the basis of various aromatic defects related to their higher content in 4-ethylphenols and VA. The remaining 30% were not differentiated from their conventional counterparts.

CONCLUSION: It could be confirmed that NW can be globally differentiated from CW with respect to their chemical and their sensory profiles, whereas the content in toxicants was not significantly different, with the exception of total sulphur dioxide and putrescine levels.

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Supporting information may be found in the online version of this article.

Keywords: natural wine; sensory description; Spain; wine; sorting task; toxics

INTRODUCTION

In recent years, consumers have been paying more attention to the effects of conventional agriculture on the environment, human health, and food and beverage sustainability.¹ One of the main reasons for this is a change in consumer behaviour. Customers are increasingly more knowledgeable about what they buy and consume regarding not only the intrinsic characteristics of the product, but also its environmental and social influence.² Greater consumer awareness of the negative effects of conventional/industrial agriculture on human health and the environment has led to a growing demand for 'natural' or healthier foods and beverages, which are perceived as safer and with a lower environmental impact.³

This trend has affected all sectors, particularly that of wine. As a result, a new category called 'natural wine' (NW) has emerged in

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recent years, which extends beyond organic and biodynamic wines.⁴ The use of different production methods greatly influences the style and composition of wine.⁵ In addition to the wine itself, such methods can influence the perception and conceptualisation of food and beverage products, modifying consumer expectations about hedonic benefits associated with organoleptic properties, health and environmental benefits.⁶ In the case of NW, the controversies are growing⁷ and the present study aimed to address them. NWs do not comply with any internationally endorsed regulations, being a declaration of the individual producer or an association of winemakers, who follow their own specific interpretation of naturalness in both the vineyard and winery.⁸ Moreover, winemakers declare that they use no commercial yeasts, chemical additives or SO₂ (or minimal doses of it) during the process. A French winemakers' union managed to officially regulate the *Vin Méthode Nature* logo in 2020, although several NW associations with their own respective internal regulations had operated for decades in Europe.^{9,10}

There is heated debate about the organoleptic quality of NWs. For NW supporters, it represents more closely the 'terroir' where it comes from, being more 'expressive', 'pure' or 'authentic'.¹¹ Another controversy regarding NW production is that they may be perceived as healthier products. There is growing public attention to health issues and so the consumer is likely to associate the concepts of NW with a healthier product because it has fewer additives than a conventional wine (CW). However, it should also be noted that the restricted use of sulphur dioxide as a preservative, as well as the fact that no selected yeasts or lactic acid bacteria are added during production, could lead to greater undesired microbial alterations in wine. This lack of microbiological control could generate higher levels of toxic products, such as biogenic amines from the nitrogen nutrition of lactic bacteria,¹² as well as ethyl carbamate produced by reaction of ethanol with compounds having a carbamyl group¹³ or ochratoxins. Moreover, because natural winemakers are more likely to continue fermentation on grape skins even in white wines, higher levels of the toxic methanol can be expected in natural over conventional wines.¹⁴

Despite growing interest in NWs, specific scientific information on them is scarce. Indeed, knowledge of their physicochemical composition (including conventional oenological parameters and toxic-related compounds) and sensory profile compared to their conventional counterparts is practically non-existent, thus requiring in-depth analysis. The general objective of the present study was to explore the sensory, oenological and toxicological profiles of a set of natural Spanish white wines compared to their conventionally produced counterparts. Implementation of both instrumental and sensory analyses was aimed at increasing the validity of results, in line with recent calls to reduce the subjective rhetoric that prevails in wine assessment by experts.¹⁵

The first starting hypothesis was that self-defined NWs differ from CWs in their chemical composition regarding both oenological and toxicological parameters [e.g. level of total sulphur dioxide, biogenic amines, ochratoxin A (OTA), ethyl carbamate, certain heavy metals, methanol, chlorides and sulphates]. The second hypothesis was that NWs differ from CWs in their sensory profile, given their different self-declared production methods.

MATERIALS AND METHODS

Wines

A balanced sample set of NW and CW was selected from the market. In total, 28 commercial white wines, half NW and half CW,

were included in the study (Table 1). For each NW, a CW sharing vintage, origin and variety was selected to obtain a balanced sample. It is important to consider that NW is not a regulated Appellation or Denomination in wine production; thus, the samples included in the experiment were from wines for which producers declared they intervene minimally during grape and wine production, including the principles proposed by the French *vin méthode nature* regulations. These include: (i) production of organic or biodynamic grapes; (ii) handpicking; (iii) use of indigenous yeasts; (iv) no added external oenological products; (v) no intentional modification of grape composition; (vi) no use of aggressive physical practices (reverse osmosis, filtration, tangential flow filtration, flash pasteurisation, thermovinification, centrifuging); and (vii) no added sulphite either before or after fermentation (maximal 30 mg L⁻¹ of total SO₂ in final wines). The selected wines were all commercial wines from 2018–2019 vintages.

The sample set included different varieties (Macabeo-Mac, Godello-God, Verdejo-Ver, Xarello-Xar, Garnacha Blanca-Gar, Malvasía-Mal, Parellada-Par, Zalema-Zal or Airén-Air) and origin (Catalonia-Cat, Castilla La Mancha-CM, Andalucía-And, Castilla y León-CL, Bierzo-Bie, Galicia-Gal and North-Central Spanish region-NC). All wines were fermented in stainless steel tanks and had no or limited contact with oak barrels (Table 1).

Physicochemical characterisation of wines

Conventional oenological analysis

Alcohol content was determined by a near infrared technique (Spectraalyzer 2.0; Zeutec, Rendsburg, Germany), volatile acidity and reducing sugars by a QuAatro 39 segmented flow autoanalyser (Bran+Luebbe, Norderstedt, Germany), pH and total acidity by potentiometry (ATP 3000; Tecnología Difusión Ibérica, S.L., Barcelona, Spain), and malic acid following the method proposed by the International Organisation of Vine and Wine (OIV) (OIV-MA-AS313-10). These parameters were analysed by accredited procedures at Estación Enológica de Haro (La Rioja, Spain) following the UNE-EN ISO/IEC 17025 standards.

Colour of wines was determined by calculating CIELAB coordinates. For this, the spectra of centrifuged and filtered samples (0.45 µm, recorded every 1 nm between 380 and 780 nm) were acquired in a UV-1800 spectrophotometer (Shimadzu, Milan, Italy) using 0.2-cm path-length quartz cuvettes. From the spectra, colour coordinates were calculated using the CIE method with the CIE 1964 10° standard observer and the illuminant D65, according to the OIV. Turbidity of wines was measured by a portable turbidimeter (HI 93703-11; Hanna Instruments, Woonsocket, RI, USA).

Analysis of OTA and other toxics

Ethyl carbamate was quantified as described by Alberts et al.¹⁶ using an HPLC instrument (model 1200 Infinity; Agilent, Santa Clara, CA, USA) coupled to a triple Quadrupole mass spectrometer detector (model 6490; Agilent) (limit of quantification, LOQ = 10 µg L⁻¹). Ochratoxin A (LOQ = 0.1 µg L⁻¹) was quantified by HPLC-tandem mass spectrometry (MS) (model 1200 Infinity HPLC coupled to a 6490 triple quadrupole mass detector, using *n*-propylcarbamate as internal standard), and biogenic amines (LOQ = 1.0 mg L⁻¹) by HPLC with *o*-phthalaldehyde precolumn derivatisation and fluorescence detection (Nexera; Shimadzu) as described previously,^{16,17} respectively.

Methanol content was measured by gas chromatography-flame ionisation detection (GC-FID) (Clarus 500; Perkin Elmer, Waltham, MA, USA), total and free SO₂ levels by the titration/aspiration method, and chlorides and sulphates by potentiometry

Table 1. Twenty-eight commercial white wines [14 natural (NW) and 14 conventional (CW)] sampled in the present study

| Code | Sorting | Variety | Origin | Type | Vintage | Oak |
|------------|---------|--------------------|----------------------|------|---------|------------------------------|
| Mac1_Cat_N | S1 | Macabeo | Catalonia | NW | 2018 | No |
| Mac1_Cat_C | S1 | Macabeo | Catalonia | CW | 2018 | No |
| Xar2_Cat_N | S1 | Xarel-lo | Catalonia | NW | 2019 | No |
| Xar2_Cat_C | S1 | Xarel-lo | Catalonia | CW | 2019 | No |
| Par_Cat_N | S1 | Parellada | Catalonia | NW | 2019 | No |
| Par_Cat_C | S1 | Parellada | Catalonia | CW | 2019 | No |
| Xar1_Cat_N | S1 | Xarel-lo | Catalonia | NW | 2018 | 9-month acacia barrel |
| Xar1_Cat_C | S1 | Xarel-lo | Catalonia | CW | 2018 | 6-month oak barrel |
| Gar_Cat_N | S1/S2 | Garnacha Blanca | Catalonia | NW | 2019 | Short period oak barrel |
| Gar_Cat_C | S1/S2 | Garnacha Blanca | Catalonia | CW | 2019 | |
| God_Bie_N | S2 | Godello | Castilla y León | NW | 2019 | No |
| God_Bie_C | S2 | Godello | Castilla y León | CW | 2019 | No |
| God_Rib_N | S2 | Godello | Galicia | NW | 2018 | No |
| God_Rib_C | S2 | Godello | Galicia | CW | 2018 | No |
| Ver1_CL_N | S2 | Verdejo | Castilla y León | NW | 2019 | No |
| Ver1_CL_C | S2 | Verdejo | Castilla y León | CW | 2019 | No |
| Gar_NC_N | S2 | Garnacha Blanca | North-Central region | NW | 2019 | No |
| Gar_NC_C | S2 | Garnacha Blanca | North-Central region | CW | 2019 | No |
| Ver2_CL_N | S2/S3 | Verdejo | Castilla y León | NW | 2019 | No |
| Ver2_CL_C | S2/S3 | Verdejo | Castilla y León | CW | 2019 | No |
| Mal_CL_N | S3 | Malvasía, Palomino | Castilla y León | NW | 2019 | No |
| Mal_CL_C | S3 | Malvasía | Castilla y León | CW | 2019 | No |
| Zal_An_N | S3 | Zalema | Andalucía | NW | 2019 | No |
| Zal_An_C | S3 | Zalema | Andalucía | CW | 2019 | 5-month oak barrel |
| Air_CM_N | S3 | Airén | Castilla La Mancha | NW | 2019 | Oak barrel |
| Air_CM_C | S3 | Airén | Castilla La Mancha | CW | 2019 | No |
| Mac2_Cat_N | S3 | Macabeo | Catalonia | NW | 2019 | No |
| Mac2_Cat_C | S3 | Macabeo | Catalonia | CW | 2019 | 12% fermented in oak barrels |

(Ag/AgCl) and gravimetry, respectively. The following heavy metals were also quantified by atomic absorption spectroscopy: lead, copper, zinc, arsenic and iron.

All analyses were carried out by accredited procedures following the UNE-EN ISO/IEC 17025 standards at the laboratories of *Estación Enológica de Haro* (La Rioja, Spain).

Quantification of volatile compounds with sensory activity

Major volatile compounds present in the concentration range of 10 to 200 mg L⁻¹ including higher alcohols, acetates and ethyl esters, and volatile fatty acids were quantified by solid phase extraction-GC-FID as described previously.¹⁸

Minor compounds present between 0.1 and 1000 µg L⁻¹ consisted of acetate and ethyl esters, vanillin derivatives, volatile phenols, terpenes, norisoprenoids and lactones. These were quantified by GC-MS as described previously¹⁹ with some modifications.²⁰

Concentrations of major and minor compounds were obtained from the relative response factor of each compound related to its corresponding internal standard.

The total concentration of most relevant Strecker aldehydes (isobutyraldehyde, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde) were quantified by GC-MS as described by Castejón-Musulén and colleagues.²¹ Their amounts in free forms were estimated using the apparent equilibrium constants (K_a) for the sulphur dioxide-carbonyl adducts, as reported elsewhere.²²

The reagents used for volatile analysis are specified in the Supporting information Appendix S1.

Data analysis

To evaluate the effect of the type of production (CW or NW) on the sample set studied (28 wines), one-way analysis of variance (ANOVA) for oenological parameters, mycotoxins and other toxics, turbidimetric measurements, and volatile compounds was conducted, considering the type of production (CW versus NW) as a fixed factor.

To identify volatile compounds inducing sensory differences between NW and CW and among the clusters of wines generated in the sorting task, the volatile compounds were grouped in vectors as described in the Supporting information (Appendices S1 and S2). Furthermore, one-way ANOVA was calculated with aroma vectors considering the type of wine production (NW or CW) or the cluster for each sorting task as fixed factors. A Fisher's post-hoc means comparison test was carried out whenever the ANOVA was significant.

$P < 0.05$ was considered statistically significant. Analyses were carried out using XLSTAT, version 19.03 (<https://www.xlstat.com/en>).

Sensory characterisation of wines

Participants

In total, 16 established winemakers from Rioja area took part in the study (11 women, and five men, ranging in age from 26 to

45 years, average of 34 years). On average, they had 17 years of experience (range 10–30 years) in wine production and tasting.

Procedures

The 28 wines studied were selected to constitute three sets of samples to be evaluated in three free sorting tasks followed by free description of the groups. Each sample set included wines with *a priori* different sensory variability, from minimal (Sorting 1) to maximal variability (Sorting 3), as indicated in Table 1. Sorting 1 included 10 samples from the same region and four grape varieties, Sorting 2 was constituted of 12 wines from five different origins and three different grape varieties, and Sorting 3 was formed by 10 samples from four different origins and five different varieties.

Each participant carried out the three sorting tasks on the same day, each lasting about 20 min separated by at least 10 min. The three tasks were performed in different order for each expert in order to avoid priming effects. Water and unsalted crackers were available during each session for rinsing purposes. Participants were provided with the samples of each sorting task simultaneously (20 mL) in dark wine glasses coded with different three-digit numbers and arranged in random order. Participants were asked to taste and group the wines on the basis of similarity, attending to olfactory and gustatory stimuli. Participants could make as many groups as they wished. Upon completion, they recorded the three-digit codes of the samples of each group on a paper sheet, and they were further asked to describe each of the groups formed with a maximum of three attributes. All wines were served at room temperature and evaluated in individual booths. The sessions took place in a ventilated and air-conditioned tasting room (at around 20 °C). No information was given about the wines or the purpose of the study.

Ethical approval for the involvement of human subjects in the present study was granted by the Research Ethics Committee of the Consejo Superior de Investigaciones Científicas (CSIC), Ref. 211/2020, in February 2021.

Data analysis for sensory characterisation

Raw data were encoded in 16 individual matrices (one by subject), each consisting of a wines \times wines matrix. These individual matrices were summed across subjects and the resulting co-occurrence matrix was submitted to a multidimensional scaling (MDS) analysis with a non-parametric scaling algorithm (absolute method). For significant dimensions (stress value > 0.2) derived from each MDS, one way-ANOVA was performed on the scores of wines considering the type of wine production (NW or CW) as fixed factor, aiming to identify sensory dimensions linked to the type of production. The stress value (varies from 0 = perfect fit to 1 = worst possible fit) is based on the sum of the squares of distances between objects observed in the raw data and objects observed in the p -dimensional MDS space and measures the quality of the fit of the MDS configuration.

Finally, all dimensions derived from the MDS configuration were analysed using hierarchical cluster analysis with the Ward criterion. All analyses were carried out with XLSTAT, version 19.03.

The descriptions of groups formed in the sorting tasks were analysed following as described in the Supporting information (Appendix S1).

RESULTS AND DISCUSSION

Physicochemical characterisation of wines

Conventional oenological parameters

Table 2 shows that white NWs presented significantly higher pH levels and volatile acidity than CWs, whereas the opposite was observed for malic acid. The higher pH value in NWs may be a result of their lower levels of malic acid or the correction of pH levels in CW by adding tartaric acid. The latter is a usual and permitted practice in conventional production in warm climate areas,²³ such as Spain. The higher levels of volatile acidity in NWs may be a result of the fact that alcoholic fermentation takes place with indigenous yeasts and lactic acid bacteria, which are linked to higher levels of acetic acid compared to CWs made with selected microorganisms.²⁴ In addition, by not using sulphur

Table 2. Mean \pm SD and range of occurrence of conventional oenological parameters and colour coordinates of the 14 conventional (CW) and 14 natural (NW) wines studied

| Parameter | CW | | NW | | P |
|--|-------------------|-----------------|--------------------|--------------|----|
| | Mean \pm SD | Range | Mean \pm SD | Range | |
| pH | 3.29 \pm 0.11 | 3.10–3.50 | 3.42 \pm 0.18 | 3.20–3.91 | * |
| Volatile acidity (g L ⁻¹) ^a | 0.41 \pm 0.13 | 0.22–0.78 | 0.72 \pm 0.44 | 0.25–2.00 | * |
| Total acidity (g L ⁻¹) ^b | 5.66 \pm 0.56 | 4.80–6.50 | 5.99 \pm 1.28 | 4.6–10.00 | NS |
| Reducing sugars (g L ⁻¹) | 1.96 \pm 1.42 | 1.00–5.20 | 1.87 \pm 1.43 | 1.00–4.80 | NS |
| Malic acid (g L ⁻¹) | 1.09 \pm 0.76 | 0.10–2.40 | 0.33 \pm 0.51 | 0.10–1.60 | ** |
| Alcohol content (% v/v) | 12.87 \pm 0.57 | 12.04–13.56 | 12.79 \pm 1.29 | 9.28–14.74 | NS |
| Free SO ₂ (mg L ⁻¹) | 10.29 \pm 6.27 | < 5.00–23.00 | 6.93 \pm 4.01 | < 5.00–17.00 | NS |
| a* | −1.46 \pm 1.26 | −4.01 – (−0.31) | −1.83 \pm 2.56 | −4.88 – 5.20 | NS |
| b* | 3.51 \pm 4.13 | 0.55–13.62 | 11.87 \pm 11.15 | 0.65–41.66 | * |
| L* | 99.86 \pm 0.29 | 99.00–100.00 | 97.99 \pm 3.75 | 86.40–100.00 | NS |
| C* | 3.81 \pm 4.31 | 0.63–14.11 | 12.31 \pm 11.09 | 0.74–41.98 | * |
| H | 117.31 \pm 4.62 | 105.20–122.20 | 108.55 \pm 12.35 | 82.88–121.60 | * |

Significance (P) was calculated according to one-way-ANOVA with the type of wine (CW, NW) as main fixed factor (* P < 0.05; ** P < 0.01; NS, no significant difference).

^a Expressed as g L⁻¹ of acetic acid.

^b Expressed as g L⁻¹ of tartaric acid.

dioxide after alcoholic fermentation or in only very small quantities, *Acetobacter* bacteria can develop, transforming ethanol into acetic acid. Finally, NWs showed lower malic acid content, which may be a result of the presence of lactic acid bacteria in NW production, transforming malic acid into lactic acid.²⁵ Indeed, NW makers often seek malolactic fermentation (MLF) for greater wine stabilisation, whereas generally Spanish white wines do not follow MLF given their relatively low acid contents, which is the case for the wine samples investigated in the present study.

Regarding the CIELAB coordinates (Table 2), NWs showed higher values of b^* and C^* (Chroma) coordinates, which indicates that they present a yellower, stronger or more saturated colour than the CWs. The NWs showed lower values of h^* ($= \arctan a_{10}^*/b_{10}^*$), which is related to their higher levels of yellow colour (or b_{10}^* coordinate). This could result from the non-use of SO_2 in NWs, leading to oxidation of musts and wine, particularly of phenolic compounds through formation of yellow species (i.e. o-quinones) with varying degrees of polymerisation.^{26,27} Maceration promoted in NW could explain the yellow-brownish colour of NW, as well as their significantly (10×) higher ($F = 10.7$; $P < 0.05$) levels of turbidity, also explained by lack of filtration and clarification.

These results confirm our initial hypothesis that NWs can be differentiated from CWs according to their conventional oenological parameters, including pH, malic acid, volatile acidity, turbidity and colour.

Toxicological parameters

Regarding the mycotoxin analysis, in the determination of biogenic amines, OTA and ethyl carbamate (Table 3), NWs showed only significant higher levels of putrescine than CWs. This could be explained by the presence of endogenous lactic acid bacteria in NWs, promoted by the lower levels of SO_2 . Moreover, higher amounts of amino acids extracted during maceration of NWs, which are precursors of biogenic amines, could explain the higher levels of putrescine in NWs.²⁸ This biogenic amine has been reported to lower blood pressure, enhancing the negative effects on human health.²⁹ Besides its effect on health, the presence of 10–15 mg L⁻¹ of this amine in white wines produces an unpleasant taste and, at a presence > 30 mg L⁻¹, this becomes foul-smelling and putrid.³⁰ Only one of these NWs (with 14.1 mg L⁻¹) exceeds the sensory threshold and could therefore be affected by its negative aroma. By contrast, none of the CWs exceed

7.8 mg L⁻¹. Significant correlations have been found between low levels of total SO_2 and higher content of biogenic amines,³¹ which could explain the higher levels of putrescine in NW. The OTA and ethyl carbamate contents were both below the LOQ (0.1 and 10 µg L⁻¹, respectively) for both wine types.

The effect of the production method related to the use of pesticides, fungicides or fertilisers in the vineyard, as well as to the winemaking practices and the use of additives in the cellar on the presence of other toxic compounds, such as methanol, sulphates, chlorides, total sulphur dioxide and heavy metals, was also evaluated. Interpreting the ANOVA, significant differences between the two wine categories were only observed for total sulphur dioxide content (Table 3). It is noteworthy that 12 out of the 14 NWs studied present levels of total sulphur dioxide lower than the LOQ (< 10 mg L⁻¹). However, there are two samples that contain levels as high as 82 mg L⁻¹ (Xar2_Cat_N) and 120 mg L⁻¹ (Gar_NC_N), which contradicts declarations by these two NW producers. One would expect certain sulphur dioxide produced naturally by the yeasts, but such high levels are undoubtedly a result of the addition of this compound. The question of possible fraud arises because of the absence of monitoring institutions and certifications. This raises doubts as to whether the wines are as natural as they are claimed to be, calling for the need of certification.¹⁰

Finally, the determination of metals, sulphates and chlorides was in all cases within the established legal limits and did not show any significant difference between the two wine types (Table 4).

These results partially confirm our initial hypothesis regarding the possible differences between NWs and CWs in terms of toxicological parameters. However, the lower levels of toxicological products in NWs could not be confirmed because, although they present lower levels of total SO_2 content, higher contents of some biogenic amines were detected.

Sensory characterisation

The flavour of the 28 wine samples was characterised using a labelled sorting task. To evaluate the generalisability of our results, three sorting tasks were performed on three sets of wines, with different expected sensory variability. The results obtained for the three sets were similar, with three dimensional solutions showing stress values of 0.150, 0.169 and 0.150 for set 1, set 2 and set 3 respectively (data not shown). Figure 1 shows the

Table 3. Mean \pm SD and range of occurrence of biogenic amines, ochratoxin A, ethyl carbamate and total SO_2 in the 14 conventional (CW) and 14 natural (NW) wines studied

| | LOD | CW | | NW | | P |
|--|-----|-------------------|------------------|-------------------|------------------|----|
| | | Mean \pm SD | Range | Mean \pm SD | Range | |
| Histamine (mg L ⁻¹) | 1.0 | $< 1.00 \pm 0.00$ | < 1.00 | 1.44 ± 1.04 | < 1.00 –4.50 | NS |
| Tyramine (mg L ⁻¹) | 1.0 | $< 1.00 \pm 0.00$ | < 1.00 | 2.22 ± 3.13 | < 1.00 –12.20 | NS |
| Phenylethylamine (mg L ⁻¹) | 1.0 | $< 1.00 \pm 0.00$ | < 1.00 | $< 1.00 \pm 0.00$ | < 1.00 | NS |
| Putrescine (mg L ⁻¹) | 1.0 | 2.80 ± 1.89 | < 1.00 –7.80 | 5.36 ± 4.20 | < 1.00 –14.10 | * |
| Cadaverine (mg L ⁻¹) | 1.0 | $< 1.00 \pm 0.00$ | 1.00 | 1.00 ± 0.00 | < 1.00 | NS |
| Ochratoxin-A (µg L ⁻¹) | 0.1 | $< 0.1 \pm 0.00$ | < 0.1 | $< 0.1 \pm 0.00$ | < 0.1 | NS |
| Ethyl carbamate (µg L ⁻¹) | 0.1 | $< 0.1 \pm 0.00$ | < 0.1 | $< 0.1 \pm 0.00$ | < 0.1 | NS |
| Total SO_2 (mg L ⁻¹) | 20 | 86.71 ± 36.31 | < 10.00 –133.0 | 37.07 ± 32.56 | < 10.00 –120.0 | ** |

Significance (P) was calculated according to one-way-ANOVA with the type of wine (CW, NW) as main fixed factor (* $P < 0.05$; ** $P < 0.01$; NS, no significant difference).

Table 4. Mean \pm SD and range of occurrence of methanol, metals, chlorides and sulphates in the 14 conventional (CW) and the 14 natural (NW) wines of the study

| | CW | | NW | | P |
|---|---------------------|---------------|--------------------|--------------|----|
| | Mean \pm SD | Range | Mean \pm SD | Range | |
| Methanol (mg L⁻¹) | 49.79 \pm 15.18 | 26.00–91.00 | 62.43 \pm 63.19 | 29.00–271.00 | NS |
| Lead (μg L⁻¹) | 9.79 \pm 6.47 | < 5.00–19.60 | 7.85 \pm 6.36 | < 5.00–28.00 | NS |
| Copper (mg L⁻¹) | 0.08 \pm 0.03 | 0.05–0.13 | 0.09 \pm 0.06 | 0.03–0.20 | NS |
| Zinc (mg L⁻¹) | 0.47 \pm 0.29 | < 0.10–1.13 | 0.36 \pm 0.24 | < 0.10–0.90 | NS |
| Arsenic (μg L⁻¹) | < 10.00 \pm 0.00 | < 10.00 | < 10.00 \pm 0.00 | < 10.00 | NS |
| Iron (mg L⁻¹) | 0.70 \pm 0.37 | 0.27–1.40 | 1.90 \pm 2.66 | 0.16–9.48 | NS |
| Chlorides^a | 128.43 \pm 345.23 | 13.00–1320.00 | 22.21 \pm 22.97 | 3.00–77.00 | NS |
| Sulphates^b | 0.31 \pm 0.11 | 0.20–0.50 | 0.32 \pm 0.14 | < 0.10–0.50 | NS |

Significance (P) was calculated according to one-way-ANOVA with the type of wine (CW, NW) as main fixed factor (*P < 0.05; **P < 0.01; NS, no significant difference).

^a Expressed as mg L⁻¹ of sodium chloride.

^b Expressed as g L⁻¹ of potassium sulphate.

dendrograms derived from the cluster analysis calculated with all the MDS dimensions obtained from the three sorting tasks.

Figure 1(a) shows the results for Sorting Task 1 (S1), which consisted of grouping a total of 10 wines (half NWs and half CWs), with all sharing their origin (Catalonia). The hierarchical cluster analysis shows the presence of three main groups of wines differing in their sensory profile. The first group, or cluster 1, consisted of three natural wines (Mac1_Cat_N, Par_Cat_N and Xar1_Cat_N), elaborated with three different varieties (Macabeo, Parellada and Xarel.lo). The attributes that defined this cluster were default-related including: 'faulty', 'vinegar' and 'animal'. This sensory profile is consistent with their significant higher levels of ethyl acetate [odour activity values (OAV) = 10] and ethylphenol (OAV = 25.6) aroma vectors shown in Table 5. Cluster 2 consisted of two CWs and one NW (Xar1_Cat_C, Mac1_Cat_C and Xar2_Cat_N), two of the Xarel.lo variety and one Macabeo. The attributes that defined this group were: 'fruity', 'floral', 'woody/toasty' and 'balanced'. This cluster presents the highest levels in the fatty acid (OAV = 51.9) and vinylphenol (OAV = 2.2) vectors, which could explain the fruitiness and floral character of this cluster, respectively. Although, in isolation, fatty acids may present some unpleasant rancid cheese-like aroma, they have been reported to be involved in the formation of positive fruity aroma of wines.³² Furthermore, this cluster presents the highest levels of the whisky lactone vector. However, the levels are quite discrete with OAV of 0.4, which could explain the subtle woody/toasted profile of these wines. Finally, Cluster 3 comprised four wines from three different varieties (Garnacha, Parellada and Xarel.lo). Here, three NWs and only one CW are characterised by the terms 'fruity', 'vegetable' and 'sour'. Although the fruity character could be related to their high levels of damascenone (OAV = 177.7) and fatty acid (as high as in cluster 2: OAV = 41.8) vectors, we cannot rule out other aroma compounds not quantified in the present study, responsible for the 'vegetable' aroma notes in this cluster. Despite the fact that 60% of NWs were grouped together in the first cluster sharing faulty aromas, our initial hypothesis was not confirmed by the ANOVA results because no significant effect of wine production type (NW or CW) was observed for any of the dimensions derived from this sorting task.

Sorting Task 2 (Fig. 1b) consisted of a total of 12 wines, comprising six NWs and six CWs from different regions and three grape

varieties (Garnacha, Verdejo and Godello). Cluster 1 consisted of five NWs from five different regions and three grape varieties. The attributes that defined this group were: 'dried/candied fruit', 'oxidation/evolved' and 'vegetable'. Low levels of fruitiness and the appearance of these typical notes of oxidation are usually related with higher levels of Strecker aldehydes.³³ However, no significant differences were observed in comparison with the other two clusters, neither in total amount, nor in that of free forms (data not shown). This lack of significant differences could be explained by the formation of aldehydes over time at different production stages, depending on the levels of SO₂. On the one hand, it has been observed that fermentative formation of Strecker aldehydes is positively influenced by total SO₂³⁴ because this molecule prevents a fraction of the Strecker aldehydes produced within the Ehrlich pathway from being enzymatically reduced or oxidised by the corresponding dehydrogenases. This would suggest that CWs would have higher levels of Strecker aldehydes because they contain higher levels of SO₂ during alcoholic fermentation. On the other hand, Strecker aldehydes formation during ageing at low SO₂ levels³⁵ would be consistent with development of the Fenton reaction once SO₂ cannot prevent H₂O₂ accumulation³⁶ and thus with higher Strecker aldehyde levels in NWs. It is possible that, in the present study, a lower amount of aldehydes was formed during fermentation in NW as a result of its low SO₂ content; however, this same low amount of total SO₂ during bottle ageing would produce more aldehydes at this stage, finally leaving a balanced and similar content of Strecker aldehydes to that in CW. From the sensory point of view, the oxidation notes of this Cluster 1 may be explained by perceptual interactions because it has a significantly lower level of the fruity isoamyl acetate vector and of the floral-like cinnamate vector compared to the other two clusters, which could lead to a clearer perception of the oxidation-related aldehyde vector than in the other two clusters. Second, Cluster 2 consisted of two CWs from Castilla y León and Catalonia and two different varieties (Garnacha and Verdejo). The attributes that defined this group were 'tropical fruit', 'woody/toasted' and 'sour'. This group of wines presented high levels of isoamyl acetate that could be responsible for a generic fruity aroma to these wines. Nonetheless, this tropical fruit character detected for the group, particularly Verdejo, could be induced by the presence of

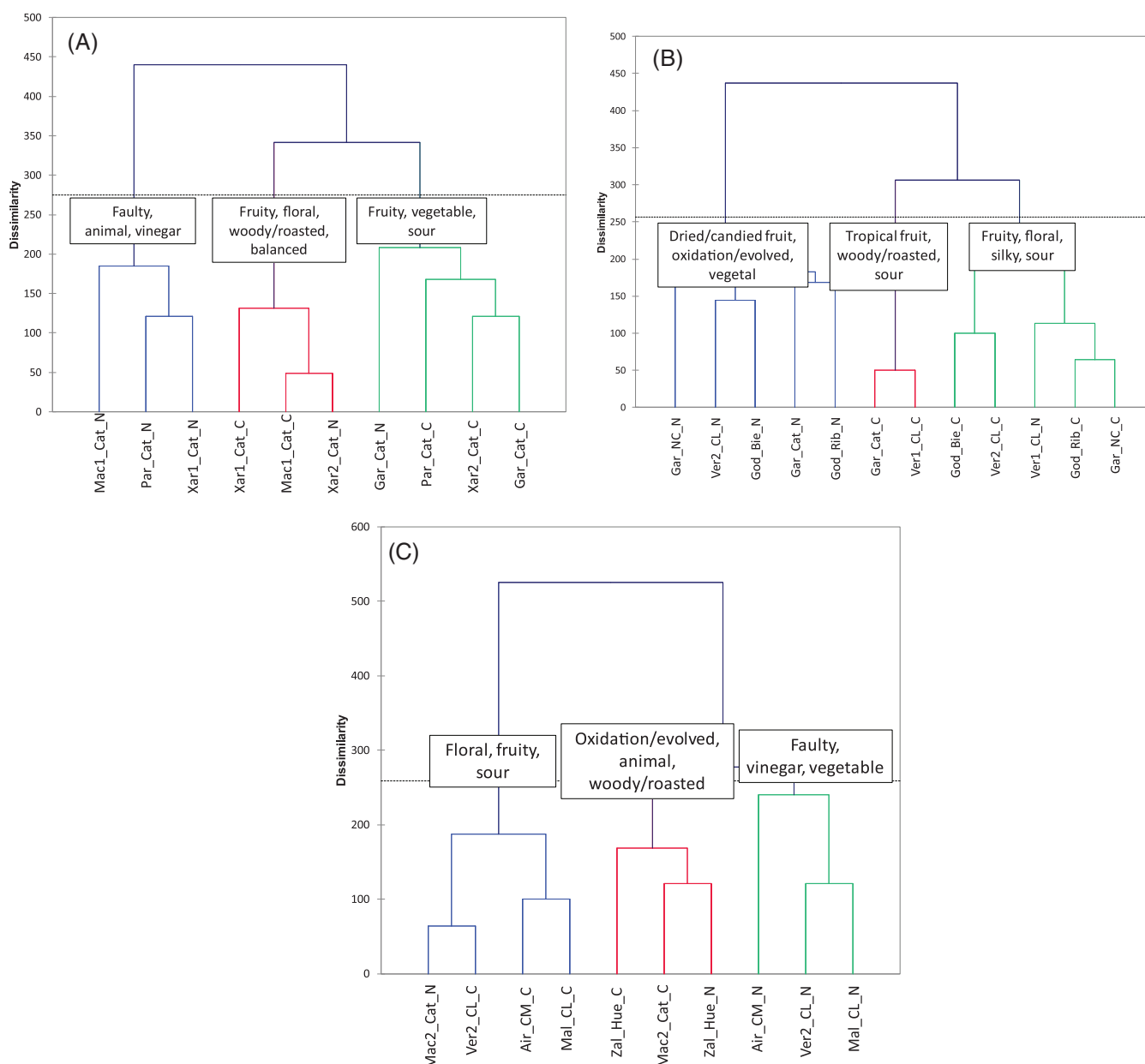


Figure 1. Dendrogram showing the groups of wines derived from the hierarchical cluster analysis calculated on all dimensions of the MDS derived from: (a) Sorting Task 1 with 10 wines (five conventional and five natural) from the same region; (b) Sorting Task 2 with 12 wines (six conventional and six natural) sharing variety; and (c) Sorting Task 3 with 10 wines (five conventional and five natural) mixing variety and region. The attributes describing each group are those with significantly higher scores within each.

polyfunctional mercaptans, such as 3-mercaptohexyl acetate,³⁷ not quantified in the present study. Finally, the third cluster consisted of five wines: four conventional and one natural. It was mainly described as 'fruity' (high levels in isoamyl acetate vector) (Table 5), 'floral', 'silky' and 'sour'. Once again, based on the ANOVA (fixed factor wine production) of the scores, there was no significant effect of production type (NW or CW) on the dimensions derived from the MDS. However, it cannot be neglected that 83% of NWs are grouped together in the cluster described with oxidation-related aroma notes.

Finally, Fig. 1(c) shows the tree diagram derived from Sorting Task 3. The test consisted in grouping 10 wines (five natural and five conventional) from different regions and varieties, and thus

maximal sensory variability according to their sensory similarity. Cluster 1 comprised four wines, three conventional and one natural, each from a different variety (Macabeo, Verdejo, Airén and Malvasía) and from the regions: Castilla y León, Castilla La Mancha and Catalonia. The perceived attributes of the group were 'fruity', 'floral' and 'sour'. Cluster 2 consisted of three wines (two conventional and one natural). Again, a NW is positioned within a group of CWs indicating sensory similarity between them. This cluster included negative descriptors such as: 'oxidation/evolved' and 'animal', associated with their higher levels in isaldehydes (OAV = 4.5) and ethylphenol (OAV = 20.5) vectors (Table 5), and 'woody/roasted'. Moreover, two of them belong to the same region (Huelva) and variety (Zalema) but one is conventional

Table 5. Odour activity values (OAVs) of aroma vectors significantly differing among clusters derived from Sorting Tasks 1, 2 and 3, according to one-way ANOVA with cluster as fixed factor (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

| | | Clusters derived from sorting tasks | | |
|---|--------------|---|--|-----------------------------|
| | | Sorting Task 1 (S1) | | |
| Aroma vectors | Significance | Cluster 1 (3 NW) | Cluster 2 (1 NW/2 CW) | Cluster 3 (1 NW/3 CW) |
| | | Faulty, animal, vinegar | Fruity, floral, woody/roasted, balanced | Fruity, vegetable, sour |
| Ethyl acetate | * | 10.0 a | 4.3 b | 4.4 b |
| Fatty acids | * | 24.5 b | 51.9 a | 41.8 a |
| β -damascenone | * | 79.2 b | 106.7 b | 177.7 a |
| Whisky lactones | * | 0.0 b | 0.4 a | 0.1 b |
| Ethylphenols | *** | 25.6 a | 0.1 b | 0.0 b |
| Vinylphenols | ** | 0.6 b | 2.2 a | 1.8 ab |
| | | Sorting Task 2 (S2) | | |
| Aroma vectors | Significance | Cluster 1 (5 NW) | Cluster 2 (2 CW) | Cluster 3 (1 NW/4 CW) |
| | | Dried/candied fruit, oxidation/evolved, vegetable | Tropical fruit, woody/roasted, sour | Fruity, floral, silky, sour |
| Isoamyl acetate | * | 11.3 b | 23.8 a | 31.6 a |
| Acetic acid | *** | 1.9 a | 0.6 b | 0.8 b |
| Cinnamates | ** | 1.0 b | 5.1 a | 2.5 ab |
| | | Sorting Task 3 (S3) | | |
| Aroma vectors | Significance | Cluster 1 (1 NW/3 CW) | Cluster 2 (1 NW/2 CW) | Cluster 3 (1 NW) |
| | | Floral, fruity, sour | Oxidation/evolution, animal, woody/roasted | Faulty, vinegar, vegetable |
| Isoaldehydes | * | 2.6 ab | 4.5 a | 1.3 b |
| Cinnamates | * | 3.1 a | 2.1 ab | 0.7 b |
| Spicy phenols | ** | 0.7 b | 2.6 a | 1.9 ab |
| Ethylphenols | * | 0.03 b | 20.3 a | 5.8 b |
| Ethyl acetate | * | 4.2 b | 6.8 b | 21.5 a |
| OAVs calculated as averages for wines belonging to the same cluster. Different lowercase letters after a given aroma vector indicate significant differences (Fisher's test). The highest OAV value for given aroma vector (and sorting task) is indicated in bold. The number of NW and CW in each cluster is given in brackets. | | | | |

and the other natural. In this case, the production process does not appear to be responsible for their sensory profile. Finally, cluster 3 was formed by three NWs, each from a different variety (Airén, Verdejo and Malvasía), two from Castilla y León and the third from Castilla La Mancha. The attributes that defined this group of NWs were: 'vinegar', consistent with the highest levels of ethyl acetate vector (OAV = 21.5) (Table 5), 'faulty' and 'vegetable'. Once again, none of the three MDS dimensions discriminated significantly NWs from CWs.

In sum, no significant effect of the type of wine production (NW or CW) was found for any of the dimensions derived from any of the three sorting tasks. Notwithstanding these results, it cannot be neglected that 70% of the NW were characterised by aroma 'defects' or non-positive attributes such as 'vinegar', 'oxidation', 'evolved' and 'animal'. Instead, 30% of NWs could not be differentiated from CWs and were grouped on the basis of their sensory similarity with positive attributes such as 'fruity' and/or 'floral'. Regarding their profile of sensory-active compounds (see Supporting information, Appendix S2), NWs tend to present systematically higher levels of animal-like ethyl phenols and volatile acidity compared to their conventional counterparts, supporting the evidence from their differential sensory characterisation. No further significant effects of other fault-related volatile compounds were detected.

CONCLUSIONS

The present study advances knowledge on the rather unexplored issue of NWs, an expanding concept and approach to winemaking among the also growing market of wines with sustainability attributes. Supporting the commonly held assumption that NWs present distinct oenological, toxicological and sensory profiles, the present study highlights various parameters where differences are clear with regard to CWs.

First, oenological composition of NWs differed from their conventional counterparts, NW presenting higher pH and volatile acidity values and lower malic acid content. Regarding toxic-related composition, the results only partially confirm our first hypothesis because, although NW presented lower overall total sulphur dioxide levels, they had higher biogenic amine content. Therefore, we could not confirm the commonly-held belief that they are healthier wines, at least regarding the compounds quantified in the present study.

The present study also confirms our second hypothesis that NWs differ from CWs in their sensory profile, showing significantly higher levels of 4-ethylphenols and volatile acidity in NWs, noted as animal and vinegar-like aromas. Nonetheless, although there was a high percentage of NWs with olfactory defects, 30% of them

reached similar quality standards to CWs, showing that low-sulphite wines can potentially achieve top quality results.

This prospective work, limited to 28 commercial white wines, shows very interesting preliminary results, offering data for policy-makers to regulate the NW market. It suggests that NWs do not present toxicological profiles calling for a specific monitoring strategy. Furthermore, these results open the door to further studies to identify the key points in producing wines with minimal intervention (e.g. not adding sulphur dioxide). These can show high organoleptic quality, as in some of those rated in the present study. Further research should also explore other chemical contents such as pesticides and other heavy metals, and incorporate a wider variety of international wines and experts beyond the Spanish context.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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