Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration.

Arancha de-la-Fuente-Blanco, María-Pilar Sáenz-Navajas, Dominique Valentin, Vicente Ferreira

PII: S0308-8146(19)31677-2
DOI: https://doi.org/10.1016/j.foodchem.2019.125553
Reference: FOCH 125553

To appear in: Food Chemistry

Received Date: 12 April 2019
Revised Date: 12 September 2019
Accepted Date: 16 September 2019

Please cite this article as: de-la-Fuente-Blanco, A., Sáenz-Navajas, M-P., Valentin, D., Ferreira, V., Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration, Food Chemistry (2019), doi: https://doi.org/10.1016/j.foodchem.2019.125553

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.
Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration.

Arancha de-la-Fuente-Blanco¹, María-Pilar Sáenz-Navajas¹, Dominique Valentin², Vicente Ferreira¹*

¹Laboratorio de Análisis del Aroma y Enología (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Associate unit to Instituto de las Ciencias de la Vid y el Vino (ICVV) (UR-CSIC-GR), c/ Pedro Cerbuna 12, 50009 Zaragoza, Spain

²Centre des Sciences du Goût et de l'Alimentation, INRA, Universidad Bourgogne Franche-Comté, Dijon, Francia

*corresponding author: Vicente Ferreira (vferre@unizar.es)
Abstract

Aroma contribution of individual esters has been studied in complex mixtures mimicking red wine models. A mixture containing 14 ethyl esters at concentrations found in wine (V1) was prepared and kept as reference. Isointense and qualitatively similar aroma vectors with a reduced number of esters (V2-V7) were prepared. Those vectors were introduced in two reconstituted wines to assess whether simpler vectors could replace V1 without compromising wine quality. In the simpler young wine model, V1 could be replaced by a vector containing just 3 odorants (ethyl 2-methylbutyrate, ethyl butyrate and hexanoate). In the oaky model, a vector containing just ethyl 2-methylbutyrate (V7) could replace V1 without any discernible sensory change. Results also reveal that sub- or perithreshold odorants play outstanding roles on the overall odour intensity of the mixture and that aroma simplification concomitantly implies an increase in the amount of odorant required to keep the intensity of the aroma vector.

Key words: fruity; isointensity; aroma integration; odour quality; OAV; subthreshold; aroma enhancement; aroma suppression
1. Introduction

Ethyl esters have been considered to be relevant odorants in the aroma of wine for several decades due to their fresh and fruity odours (Etievant, 1991). Most of them are the result of the esterification of fatty acids produced by yeasts during alcoholic fermentation and constitute one of the largest and most important groups of compounds involved in the flavour of fermented beverages (Lambrechts & Pretorius, 2000). Research has shown that, leaving aside ethyl esters of major acids, such as ethyl lactate or diethyl succinate, wines contain around 14 different ethyl esters bearing slightly different aroma descriptors. These ethyl esters include ethyl esters of linear and branched fatty acids and also of some hydroxy acids (Escudero, Campo, Farina, Cacho, & Ferreira, 2007). Interestingly, only some of them (ethyl isobutyrate, ethyl 2- and 3-methylbutyrates, ethyl butyrate, ethyl hexanoate and ethyl 2-hydroxy-4-methylpentanoate) have been found to be at concentrations above or close to their sensory thresholds (San Juan, Cacho, Ferreira, & Escudero, 2012).

The quality of red wines has been found to be linked to their red and black berry character and particularly to their blackberry and blackcurrant odour notes (Pineau, Barbe, van Leeuwen, & Dubourdieu, 2010; Saenz-Navajas, Avizcuri, Ballester, Fernandez-Zurbano, Ferreira, Peyron, et al., 2015). Different researches have demonstrated that these notes are related to the interaction between fruity esters and other aroma compounds, notably dimethyl sulphide (Escudero, Campo, Farina, Cacho, & Ferreira, 2007; Segurel, Razungles, Riou, Salles, & Baumes, 2004), polyfunctional mercaptans (Rigou, Triay, & Razungles, 2014) and other basic wine aroma vectors (Ferreira, Sáenz-Navajas, Campo, Herrero, de la Fuente, & Fernández-Zurbano, 2016). Other researches have explored whether those berry notes are rather linked to the existence of specific profiles of ethyl esters including odorants at subthreshold
concentrations. It should be considered, however, that some of the conclusions reported may be difficult to fully generalise, because they are based on observations made at threshold level, where hyperaddition and synergism are more frequent (Atanasova, Thomas-Danguin, Langlois, Nicklaus, & Etievant, 2004; Ferreira, 2012a). Other conclusions derive from studies with simple aroma solutions containing just the esters, where the effects of major wine odorants are not present.

Pineau, Barbe, Van Leeuwen, and Dubourdieu (2009) suggested that the type of berry fruit perceived is related to the specific profile of ethyl esters. Wines containing more than average levels of lineal esters would display red-berry character, while wines containing more branched esters would have blackberry character. These authors report that increases as little as 3 or 24 μg/L (0.05% or 1.3% of the respective reported thresholds) of ethyl 2-methylbutyrate and ethyl 2-methylpropanoate, respectively, would lead to a modification of the wine odour profile. In subsequent works, Falcao, Lytra, Darriet, and Barbe (2012) showed that 2-hydroxy-4-methylpentanoate at slightly subthreshold levels may play an outstanding role in fresh blackberry aromas. Lytra, Tempere, de Revel, and Barbe (2012, 2015) found that specifically the R:S 95:5 mixture of enantiomers of this compound is responsible for the aroma enhancement of fresh fruit notes and for the partial suppression of jammy fruit notes. In another research paper, Lytra, Tempere, Le Floch, de Revel, and Barbe (2013) reported that subthreshold levels of ethyl propanoate, butyl acetate, ethyl 3-hydroxybutyrate, and isobutyl acetate also act as fruity aroma enhancers. Ethyl 3-hydroxybutyrate and isobutyl acetate would act via hyperaddition, and ethyl propanoate and isobutyl acetate would significantly impact the perception of fresh fruit and blackberry notes. In contrast, Lytra and collaborators (Lytra, Tempere, de Revel, & Barbe, 2015; Lytra, Tempere, Le Floch, de Revel, & Barbe, 2013) have shown that it is the S/R 85:15 ratio of the enantiomers of ethyl 3-
hydroxybutyrate which is responsible for an enhancement of red berry descriptors. In addition, Lytra, Tempere, de Revel, and Barbe (2014) showed that the S enantiomer of ethyl 2-methylbutyrate specifically enhances blackberry descriptors. Finally, Cameleyre, Lytra, Tempere, and Barbe (2017) showed that the S enantiomer of 2-methylbutyl acetate acts as aroma enhancer specifically of black, fresh and jammy-fruit notes.

It should be noted that if all those perceptual effects hold completely in real wines, predicting wine aroma properties from its chemical composition would be hardly possible, because of the many odorants and many non-linear and hardly predictable effects involved. In contrast, previous research (Aznar, Lopez, Cacho, & Ferreira, 2003; San-Juan, Ferreira, Cacho, & Escudero, 2011) has suggested that individual ethyl esters may act in a concerted and integrated way forming what can be identified as an aroma vector. The level of integration would be high enough so that the specific aroma nuances of individual components would be difficult to perceive. The concept of aroma vector, even if implicitly used in previous works, has been just recently defined (Ferreira, de-la-Fuente-Blanco, & Sáenz-Navajas, 2019) as “a perceptual unit constituted by one or several molecules with similar aroma descriptors, which altogether and in an integrated form, are responsible for a specific set of sensory features of a type of products” and is meant to be a basic concept for modelling aroma perception.

In this context, the main goal of the present work is to demonstrate that the specific sensory nuances of minor esters have, if any, small relevance on the overall wine aroma perception and that for modelling purposes, ethyl esters constitute a single aroma vector conveying a generic fruity-estery note well represented by a simple mixture of odorants or even by a single odorant. Regarding aroma intensity, the goal is to assess how the
progressive simplification of the aroma vector affects to the intensity of the mixture, and to verify whether minor esters have a relevant impact on the overall aroma intensity.

2. Materials and methods

2.1. Chemicals and standards

Solvents. Ethanol of LiChrosolv quality was purchased from Merck (Darmstadt, Germany) and 1-butanol of the maximum purity available (99.4%) from Thermo Fisher Scientific (Kandel, Germany). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

Standards. The chemical standards were supplied by Sigma - Aldrich (Madrid, Spain) and Firmenich (Geneva, Switzerland). They were of the maximum purity available.

Reagents. Tartaric acid, NaOH, mono- and di-potasium phosphate and sodium sulphate anhydrous were supplied by Panreac (Barcelona, Spain).

2.2. Preparation of wine models

2.2.1. Purification of esters

Step 1: Liquid-liquid extraction: 500 microlitres of each ester solution prepared with commercial standards were mixed with 2 mL of a 5 % bicarbonate solution. This mixture was shaken for 10 minutes and the aqueous phase was removed. This step was carried out in triplicate. The last mixture was centrifuged for 5 minutes with a speed of 4000 rpm and a temperature of 10 ºC to assure the complete separation. Purified compounds were transferred to a vial and it was dried with sodium sulphate anhydrous until the confirmation of the absence of water in the purified compound. They were preserved and analysed by gas chromatography with a flame ionization detector to confirm the absence of impurities.
Step 2: GC-FID method. Confirmation of the purity of standards: One microlitre of a solution containing the purified ester and methyl octanoate (internal standard), was injected in a gas chromatograph and purity was calculated according to the method described by Tissot, Rochat, Debonneville, and Chaintreau (2012) for unstable substances. Only when the purity achieved was higher than 96% and the content of the corresponding fatty acid was below its sensory threshold the standard was considered pure.

2.2.2. Preparation of isointense vectors with different number of esters

A first V1 vector containing the 14 ethyl esters naturally occurring in wine at maximum concentrations found in a set of Spanish red wines (Table 1) (San Juan, Cacho, Ferreira, & Escudero, 2012) was prepared. Then, progressively simpler solutions containing a smaller number of esters (named as V2, V2’, V3, V4, V5, V6 and V7, as seen in Table 2) with odour intensities similar to that of V1 were prepared. Each simplified vector was built, first selecting the esters to be removed, then adjusting the concentration of the mixture to ensure that its odour intensity equals that of V1, and finally by adjusting the proportions of those to be retained in order to have aroma quality as similar as possible to V1.

A total of eight different pools of esters (V1-V7, V2’), from now on called vectors, were prepared with the purified compounds. These vectors contained different number of esters, from the most complex (V1 with 14 esters) to the simplest (V7 with 1 ester). The more complex ester vector V1, contained 5 lineal and 9 branched ethyl esters (ethyl propanoate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl 2-methylpentanoate, ethyl 3-methylpentanoate, ethyl 4-methylpentanoate, ethyl hydroxybutyrate, ethyl cyclohexanoate and ethyl 2-hydroxy-4-methylpentanoate).
Vectors V2 and V2’, corresponding to the second level of complexity, were prepared with 6 esters (ethyl butyrate, ethyl hexanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate), V3 with 4 (ethyl butyrate, ethyl hexanoate, ethyl 2-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate), V4 with 3 (ethyl butyrate, ethyl hexanoate and ethyl 2-methylbutyrate), V5 and V6 with 2 (ethyl butyrate and ethyl 2-methylbutyrate for V5 vector, and ethyl hexanoate and ethyl 2-methylbutyrate for V6 vector) and V7 represents the simplest vector, including only one ester (ethyl 2-methylbutyrate).

Vectors were prepared with the aim of being as similar as possible to the most complex vector (V1) in terms of both odour intensity and quality. Isointensity was evaluated by means of the 1-butanol reference scale described in section 2.3.3.1. Qualitative similarity has been achieved by deleting esters with similar aroma quality and considering the relative concentration of components (this idea is developed in the results and discussion section). The sensory similarity between simplified vectors and V1 was evaluated via triangular tests.

To avoid the spontaneous hydrolysis of the purified esters, which should yield little amounts of acids with important sensory activity, pure ester solutions were assessed in 1% hydroalcoholic solutions kept at pH 6.0.

2.2.3. Wine model (WM) preparation

Two WMs were generated mimicking: a) a young red wine and b) an oaked red wine. They were prepared by mixing a pool of common non-volatile, and volatile red wine components. Non-volatiles were obtained by lyophilisation of a dearomatised red wine (Total Polyphenol Index measured as absorbance at 280 nm = 59.5 a.u., reducing sugars = 5.8 g L⁻¹, total acidity = 3.6 g L⁻¹ expressed in sulphuric acid; lactic acid = 1.9 g L⁻¹ and malic acid = 0.3 g L⁻¹). The concentration of volatile compounds (Table 3)
corresponds to the average concentration of aroma compounds found in a previous work (San Juan, Cacho, Ferreira, & Escudero, 2012). Two base wines, one imitating a young red and the other an oaky wine, were further spiked with one of the ester vectors (V1, V2’, V3, V4, V5, V6 or V7). In total ten wine models were prepared, six mimicking young red wine models (W-V1, W-V2’, W-V3, W-V4, W-V5, W-V6) and four mimicking oaky red wine (oW-V1, oW-V5, oW-V6 and oW-V7). The final ethanol content of WMs was adjusted to 12 % (v/v) and pH = 3.5.

2.3. Sensory evaluations

2.3.1 Participants

Twelve participants (four men and eight women, ranging from 23 to 60 years of age, average = 33 years old), belonging to the laboratory staff and with a long experience in wine aroma description, carried out the sensory tasks.

2.3.2 Assessment of literature sensory thresholds

Three 10% ethanolic solutions buffered at pH 6.0 containing one purified ester at concentrations equal, 10 x and 0.1 x those reported as threshold were prepared. These solutions were confronted versus a blank via triangular tests. In the cases in which results suggested that the literature threshold could be far from that of our panel, the threshold was accurately determined following the procedure described in the reference (Lawless & Heymann, 2010). 2.3.3 Evaluation of odour intensity and quality of vectors

Isointensity of ester vectors was confirmed by using a 1-butanol reference scale adapted from Atanasova, Langlois, Nicklaus, Chabanet, and Etievant (2004) as described in section 2.3.3.1. Overall similarity of vectors was evaluated by triangle tests (section 2.3.3.2).
2.3.3.1. Evaluation of odour intensity of vectors

**Selection of the 1-butanol reference odorant scale**

**Procedure:** This first step aimed at choosing the range of concentration of the 1-butanol scale. Serial 1:2 dilutions of 1-butanol (geometrical dilution) from 6.25 to 12800 mg L\(^{-1}\) were prepared according to the method described in Atanasova, Langlois, Nicklaus, Chabanet, and Etievant (2004). Accordingly, 10 mL of each one of the twelve solutions prepared were presented in dark ISO approved wineglasses labelled with 3-digit random codes and covered with plastic Petri dishes. These solutions were compared with the control sample (V1 vector in a phosphate buffered solution, pH 6 and 1% ethanol). The dilution series of 1-butanol were presented in ascending order of concentration to minimise the effects of olfactory adaptation and with a 1-min break after each pair. Participants were instructed to indicate the similarity in intensity between each 1-butanol standard and control on a 13-cm long linear scale labelled on the right side with “totally similar” and on the left side with “totally dissimilar”.

Results showed that the highest similarity scores were for solution containing around 2500 mg L\(^{-1}\) of 1-butanol. Thus, the scale was selected to cover the range between 0 and 6400 mg L\(^{-1}\).

**Training session**

**Procedure:** It comprised two parts. The first part consisted of two 15-min sessions (held in different days). It aimed at (1) familiarising participants with the 1-butanol solutions and (2) qualifying participants. In the first session, participants were familiarised with the five different intensities of 1-butanol scale (12.5, 550, 1802, 3773 and 6400 mg L\(^{-1}\)) and were instructed to memorise them. To match the perceived intensity with the 1-butanol scale intensities, the participants were asked to use a 13-cm line scale labelled at
each end (zero intensity to very strong intensity) on which the 5 positions corresponding
to the 5 levels of 1-butanol intensities were indicated. Level 0 corresponds with water.
In the second session, participants’ ability to correctly match 1-butanol concentrations
with their intensity in the scale was checked. Participants were presented with the five
1-butanol samples together with water and were asked to classify them in order of
intensity. This activity was carried out in triplicate. The order of presentation was
randomised for each series and repetition.

The second part of the training session consisted in the evaluation of the use of the
scale. Participants were presented with 24 samples. The samples corresponded to four
identical series of the six solutions of 1-butanol and water presented in dark ISO
approved wineglasses labelled with 3-digit random codes and covered with plastic Petri
dishes. Panellists were asked to locate the perceived intensity of each solution on the
13-cm line scale. All the responses were collected in paper ballots. Participants were
qualified when they were able to correctly identify the intensity of at least 90% of
solutions. If they were disqualified, they repeated the training session from the
beginning.

*Evaluation of the odour intensity of vectors*

**Procedure:** Before each session, participants smelled the six different solutions of the 1-
butanol scale generated in the previous step. When they considered they had memorised
them, wineglasses were removed. In a session, participants had to evaluate the odour
intensity of a given vector (V2, V3, V4, V5, V6, V7 and V2’) together with the control
(V1) sample (5 repetitions for each vector were presented) on the 13-cm line scale
employed in training sessions.
Data analysis: the scores obtained for odour intensity were submitted to two-way analysis of variance (with panellists as random and vector as fix factor). Seven ANOVAs comparing odour intensity of V1 with the given vector (V2-V7, V2’) were calculated to evaluate the presence of significant intensity differences (P < 0.05) among vectors. All statistical analyses were performed using XLSTAT (Addinsoft, version 2018).

2.3.3.2 Evaluation of sensory differences of vectors in isolation by triangle tests

The evaluation of the overall sensory differences between V1 and the simplified vectors (V2’-V7) was carried out by means of triangular tests.

Samples: The same pools of esters (V1 and V2’-V7) were prepared with the purified compounds in 1% hydroalcoholic solutions kept at pH 6.0. The V2 vector was not evaluated because its intensity was significantly different from V1, as detailed in the results section.

Procedure: A total of six comparisons (tests A.1-A.6 in Table 4) were performed by confronting simpler vectors to V1 in triangular tests (V1 vs V2’; V1 vs V3; V1 vs V4; V1 vs V5; V1 vs V6; V1 vs V7). Two sets of three wineglasses were presented to each participant (V1-Vi-Vi and Vi-Vi-V1), resulting in 24 responses for each test. They were asked to orthonasally smell each sample and they had to select the one that was different.

In all tests 10 mL of samples (20 ± 1 °C) were presented in dark ISO approved wineglasses labelled with 3-digit random codes and covered with plastic Petri dishes according to a random order and different for each participant.
Data analysis: The number of correct responses was compared with tabulated values (binomial distribution) to evaluate the presence of significant sensory differences (P < 0.05) between confronted samples.

2.3.4. Evaluation of sensory differences induced by vectors in red wine models by triangle tests

Samples: Ten wine models were prepared with the base wines (young and oaked, as detailed in Table 3) spiked with one of the isointense ester vector (V1, V2’, V3, V4, V5, V6 and V7): six mimicking young red wine models (W-V1, W-V2’, W-V3, W-V4, W-V5 and W-V6) and four mimicking oaky red wines (oW-V1, oW-V5, oW-V6 and oW-V7).

Procedure: In a first set of five triangular tests, the young red wine model containing the most complex vector (W-V1) was confronted to young red wine models containing simpler vectors from W-V2’ to W-V6 (tests B.1-B.5 in Table 4). Comparison W-V1 vs W-V7 was not carried out because wine models containing more complex vectors (W-V5 and W-V6) presented significant sensory differences hence, it was assumed that simpler vectors (W-V7) would generate more important sensory differences.

In a second set of three triangular tests, oaky red wine model containing the most complex vector (oW-V1) was confronted to the simpler oaky red wine models from W-V5 to W-V7 (tests C.1-C.3 in Table 4). The work was made under the assumption that sensory differences would be more evident in simpler contexts (young red wine model) than in more complex models (oaky red wine model). Thus, comparisons of oW-V1 to oW-V2’, oW-V3 and oW-V4 were not carried out because no significant sensory differences were observed between W-V1 and W-V2’, W-V3 and W-V4 in the simpler young red wine context.
Two sets of three wineglasses were presented to each participant (W-V1/W-Vi/W-Vi and W-Vi/W-Vi/W-V1), resulting in 24 responses for each test. In all tests, 10 mL of samples (20 ± 1 ºC) were presented in dark ISO approved wineglasses labelled with 3-digit random codes and covered with plastic Petri dishes according to a random order and different for each participant.

**Data analysis**: The number of correct responses was compared with tabulated values (binomial distribution) to evaluate the presence of significant sensory differences (P < 0.05) between confronted samples.

### 3. Results

#### 3.1. Validation of the purity of the esters

The use of aroma models requires the use of pure standards free from any olfactory impurity that could bias the conclusions. In the present work, commercial standards were prepurified by washing them with a 5% bicarbonate solution in order to remove traces of fatty acids, some of which are very powerful aroma compounds. This procedure allowed to have pure ester standards. Additionally, and for avoiding the spontaneous hydrolysis of the purified esters, which should yield little amounts of acids, pure ester solutions were assessed in 1% hydroalcoholic solutions kept at pH 6.0. With the purified esters, thresholds reported in the literature were partially checked just to identify potential large discrepancies. In the cases of ethyl 2- and 3-methylbutyrate, thresholds were recalculated (Table 1).

#### 3.2. Development of the ethyl ester vectors and study of effects on red wine models

The main goal of the present work was to assess whether the 14 fruity ethyl esters found in normal wines could be replaced by simpler ester vectors containing a reduced number of components without compromising wine sensory characteristics. For that a vector
containing the 14 ethyl esters at the maxima levels found in commercial Spanish wines, V1, was built and further used to reconstitute hypothetical red wines containing maxima levels of fruity esters. Therefore, the first step consisted in building vectors with a lower number of esters than V1 but presenting similar intensity and quality (evaluated by triangle tests A.1-A6, Table 4). Then, the sensory differences induced by these simplified vectors in reconstituted wine models were evaluated by triangle tests (B.1-B.6 for young red models and C.1-C.3 for oaky red wines, Table 4).

3.2.1. Simplification of V1 to V2 and V2’

Preparation of an isointense simplified aroma vector: In the first step of simplification (from V1 to V2) the eight esters in V1 with concentrations below their odour thresholds (given in Table 1) were removed keeping for V2 just six esters: ethyl butyrate, ethyl hexanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate. Four of the eliminated esters (ethyl propanoate, ethyl octanoate, ethyl decanoate and ethyl 4-methylpentanoate) were at concentrations close to threshold (often referred as perithreshold concentrations), while the other four were at concentrations more than one order of magnitude below the threshold. In OAVs terms, which are not a psychophysical measurement since they do not measure sensory response, but a way to express concentration of odorants, the amount eliminated is 2.3, which is barely a 1.2% of the OAV in V1. These OAV calculations are given in Table 5. Initially, the concentrations of some the remaining odorants in V2 were slightly increased to compensate for such “aroma loss” so that total OAV in V2 was not smaller than that of V1. In spite of that, the simplified vector V2 (Table 5) had a significantly (F = 5.86, P < 0.01) much lower odour intensity (6.14) than V1 (7.68). Because of that, a second vector V2’ with concentrations of odorants a 50% higher than those of V2 was prepared to match the odour intensity of V1. Interestingly,
V2’ presented an OAV of 305, more than 50% higher than that of V1 (Table 5). This V2’ vector was isointense to V1 (6.76 for V1 and 6.77 for V2’, F = 0.522, P = 0.792).

**Quality evaluation:** Sensory differences between V1 and the isointense simplified vector V2’ were compared via triangular tests. Results revealed that both vectors were slightly but significantly (P < 0.05) different (Table 4, test A.1).

**Effects on red wine models:** It was further checked whether V2’ was able to replace V1 in a wine context. In order to do that, two semi-synthetic wine models (WMs) were built and compared. Those WMs contained non-volatile material directly extracted from a red wine and aroma compounds added at the levels indicated in the first part in Table 3 plus either the ethyl ester vector V1 or V2’. The two red wine models (W-V1 and W-V2’) differing just in the ester composition were confronted in a triangular test, and as shown in Table 4 (test B. 1), no significant differences (P > 0.05) were found, indicating that the simplification from V1 to V2’ does not lead to noticeable sensory differences in the wine context studied. Because of that, more simplified ester vectors were sought.

### 3.2.2. Simplification of V1 to V3

For further reducing the number of components achieving the minimal sensory change, it was necessary to guarantee that the simplified mixture retained all the main odour features of the original V1 mixture and of its first simplification, V2’: fruity, apple and strawberry. The simplification was based on main descriptors found in the bibliography (Table 1). For this, it was observed that the six components in V2’ can be roughly classified into two pairs based on their odour characteristics: ethyl butyrate and ethyl 2-methylpropanoate that share strawberry, sweet, or bubble gum descriptors and ethyl 2-methylbutyrate and ethyl 3-methylbutyrate that present common apple descriptors. Taking this into account, V3, a mixture containing just four components was prepared.
The esters removed were ethyl 2-methylpropanoate and ethyl 3-methylbutyrate. The concentrations of the compounds remaining in the solution were re-adjusted trying to reproduce qualitatively and quantitatively V1.

**Isointensity evaluation:** It can be seen that concentrations of ethyl hexanoate and ethyl 2-hydroxy-4-methylpentanoate have been decreased in comparison to those in V2´ and that concentrations of ethyl butyrate and ethyl 2-methylbutyrate have been much increased. The overall OAV of the V3 mixture is very similar to that of V2´, as can be seen in Table 5. The aroma intensity of V3 (6.82) was not significantly different from the intensity of either V1 (6.76) or V2´ (6.77), F = 0.522, P = 0.792.

**Quality evaluation:** The evaluation of the sensory differences between V1 and the simplified vector V3 was measured; V1 was found not significantly (P > 0.05) different from V3 based on results of triangle tests (Table 4, test A.2).

**Effects on red wine models:** Triangle tests confronting W-V3 and W-V1 (test B.2) showed no significant sensory difference (P > 0.05) between both wine models (Table 4). Thus, the next step in simplification of ester vectors was sought.

### 3.2.3. Simplification of V1 to V4

**Isointensity evaluation:** V4, the mixture containing just three components, was further prepared by removing ethyl 2-hydroxy-4-methylpentanoate, as explained in the next paragraph. In this case there was no need even for changing the relative contents, so the three aroma compounds in V4 have the same proportion as they had in V3 at slightly higher concentrations (between 6 or 7 % increase). The OAV of V4 was quite similar to those of V2´ and V3 (Table 5), its odour intensity (7.43) was not significantly different from either V1 (6.77), V2´(6.77) or V3 (6.82), F = 0.522, P = 0.792.)
Quality evaluation: As previously mentioned, for further reducing the number of components it was necessary to guarantee that the simplified mixture retained all the main odour features of the original V1 mixture and of its next simplifications: V2' and V3 with fruity, apple and strawberry descriptors. Thus, it was observed that the four components in V3 maintain their odour characteristics: ethyl butyrate with strawberry, sweet, or bubble gum descriptors, ethyl 2-methylbutyrate with apple and blackberry descriptors and hexanoate strawberry and fruity descriptors (Table 1). For this reason, leaving out ethyl 2-hydroxy-4-methylpentanoate (with fruity, strawberry and blackberry descriptors) was expected not to generate noticeable differences. Triangle tests showed no significant difference (P > 0.05) between V1 and V4 (Table 4, test A.3).

Effects on red wine models: Triangle tests confronting W-V4 and W-V1 (test B.3) showed no significant sensory difference (P > 0.05) between both wine models (Table 4). Thus, the next step in simplification of ester vectors was sought.

3.2.4. Simplification of V1 to V5 and V6

Isointensity evaluation: two different binary vectors were generated: V5 and V6, in which either ethyl butyrate or hexanoate were omitted, while ethyl 2-methylbutyrate was maintained. Their concentrations were increased in order to keep isointensity. No significant difference (F = 0.522, P = 0.792) in intensity was observed between V1 (6.76) and V5 (6.93), or V6 (7.07). It can be observed in Table 5 that OAVs of V5 and V6 exceed by more than a factor 2 the one of V1 and by factors around 1.5 and 1.6, respectively, those of V2', V3 and V4.

Quality evaluation: Reducing to two components seemed a priori far more difficult, since the elimination of any of the three components in V4 seemed to inevitably involve a remarkable change in quality (remove one of the main features of the mixture: fruity,
apple and strawberry). Anyway, two different binary vectors were generated: V5 and V6. In both cases, the best solutions (i.e., the ones giving aroma qualities the least dissimilar from those of V1) consisted of increasing levels of ethyl butyrate or ethyl hexanoate by a 15 and a 28%, for V5 or V6 respectively, and those of ethyl 2-methylbutyrate by 39 and 50%. Once isointensity with V1 was ensured, these vectors were confronted via triangular tests with V1, and surprisingly, they could not be significantly discriminated (P > 0.05) (Table 4, tests A.4 and A.5).

Effects on red wine models: Although V5 and V6 were not significantly different from V1 when they were isolated (Table 4, tests A.4 and A.5), significant sensory differences between W-V1 and W-V5 (P < 0.01) as well as between W-V1 and W-V6 (P < 0.01) were observed via triangle tests (Table 4, tests B.4 and B.5). In order to check whether these qualitative differences could pass unnoticed in a more sophisticated aroma context, a second reconstituted wine model was prepared. This model was similar to the young red wine, but contained wood extractables at the levels indicated in Table 3, which are within the normal range of occurrence of these compounds in wine. In this case, the model containing V1 (oW-V1) was not significantly different from V5 nor V6 (oW-V5 and oW-V6) based on triangle tests, as shown in Table 4 (tests C.1 and C.2).

3.2.5. Simplification of V1 to V7

Isointensity evaluation: Finally, V7, the last vector with just one component, contained only ethyl 2-methylbutyrate. In order to achieve similar intensity to V1, the concentration of its single odorant had to be increased to 1.7 mg L\(^{-1}\) (see Table 2), around twice the amount of this odorant in vectors V5 and V6, which lead to a OAV of 867 (Table 5). In spite of these differences in OAV values, the evaluation of the isointensity between V1 (6.76) and V7 (6.80) was confirmed (F = 0.522, P = 0.792).
Quality evaluation: As for V5 and V6 vectors, the elimination of any of their components, seemed to inevitably involve a remarkable change in quality. The only compound that maintains the three descriptors of the mixture: fruity, apple and strawberry seems to be ethyl 2-methylbutyrate (Table 1). This vector, V7, was significantly (P < 0.05) different from V1 (Table 4, test A.6).

Effects on red wine models: The comparison W-V1 vs W-V7 was not carried out, assuming that a simpler vector (V7), which was already significantly different in isolation from V1 (Table 4, test A.6), would induce also significant sensory differences in the same context of the young red wine (W-V7). For this reason, the effect of V1 in a more complex context such as an oaky red wine model (oW-V1) was evaluated by confronting this sample to the simpler oaky red wine models (oW-V7) in a triangular test. In spite of the differences found in the comparison of the isolated vectors V1 vs V7, results showed that oW-V7 was not significantly different from oW-V1 in the red wine model with oaky notes (Table 4, test C.3).

4. Discussion

4.1. Effect of diversity of ethyl esters on quality of overall wine aroma

A first remarkable and unexpected result is the large odour similarities between V1 and the simplified vectors (except for V2’and V7) when smelled in isolation in a hydroalcoholic solution kept at pH = 6 (Table 4, tests A.2 - A.5). However, if all the comparisons are taken together (considering the sum of tests A, with 65/144, correct/total responses), it can be said that globally, the simplified vectors are significantly different (P < 0.01) from V1. Moreover, a second interesting observation is that the assessors found significantly more difficult to pick the difference in comparisons Vi-Vi-V1 (the most complex solution, V1, is the odd one) than in the V1-
V1-Vi (the simpler vector, Vi, is the odd one) condition (with 28/72 vs 37/72 correct/total responses in the first set of trials, respectively, being the significance (P < 0.05) level at 32/72). These two observations highlight the existence of subtle differences, not easily identified, linked to the elimination of secondary aroma compounds.

Results obtained when the ester vectors were integrated in the young red wine model (Table 4, tests B.1 - B.5) showed that only V2’, V3 and V4 were able to replace V1 without introducing noticeable sensory changes (P > 0.05). However, the replacement could be extended until V5, V6 and V7 when they were integrated in an oaked red wine model also without sensory changes (Table 4, tests C.1 - C.3). This implies that the mixture of 14 esters could be replaced by just three esters (ethyl butyrate, ethyl hexanoate and ethyl 2- or 3-methylbutyrate) in a young red wine model and just by one ester (ethyl 2- or 3-methylbutyrate) in an oaky red wine model.

The results in the hydroalcoholic solution suggest that, in qualitative terms, odour interactions between the different esters in the mixture can be interpreted in terms of the theory of dominance. According to this theory, the odour of a mixture is very close to the odour of its most intense odorant. As it is shown in Table 5, ethyl 2- or 3-methylbutyrate (whose odours are very similar, and which in fact can be easily interchanged) is the most abundant in the mixture. The rest of components, qualitatively, would add secondary odour nuances which are not essential. This fact can be also explained in terms of aroma quality. Berglund, Berglund, and Lindvall (1976) reported that when the percept induced by a mixture is heterogeneous, at least some of the component odours can be perceived within the mixture. This is due to an analytical processing of olfactory information (Berglund & Olsson, 1993) also qualified as elemental (Kay, Crk, & Thorngate, 2005). In that case, some perceptual interactions
may be observed, such as perceptual dominance or partial overshadowing (Atanasova, Thomas-Danguin, Langlois, Nicklaus, & Etievant, 2004; Ferreira, 2012b; Kurtz, Lawless, & Acree, 2009). According to this theory, as suggested by Romagny, Coureaud, and Thomas-Danguin (2018), it is possible to perceive some individual elements present in the ester mixture in a hydroalcoholic solution, in spite of the fact that the similarity between the whole mixture (V1) and the elements is strong. However, with the addition of other compounds to form the wine context, the elements are no more perceived though their own identity and create a key association, the “fruity” concept. In red wine models, the elimination of secondary compounds in the ester mixture can produce a partial loss in the identity of the “fruity” concept which leads to significant differences (V5 and V6 were significant different, P < 0.01, from V1 in a young red wine context, Table 4, test B.4 – B.5). The “fruity” concept follows the hypothesis that the characteristics of ethyl esters are only completely expressed when they coexist with some other aroma compounds, which naturally concur with them in natural fruits. These concurrent odorants could be volatile fatty acids, such as hexanoic acid and isovaleric acids or furaneol (Romano, Perello, Lonvaud-Funel, Sicard, & de Revel, 2009; San-Juan, Ferreira, Cacho, & Escudero, 2011).

On the other hand, the differences between V1 and V5 and V6 disappeared in an oaky red wine model (Table 4, test C.1 – C.2), because in this new context, the dominant concept is not the "fruity" but the “woody” aroma. Atanasova, Thomas-Danguin, Langlois, Nicklaus, Chabanet, and Etievant (2005) had already reported that woody notes tend to dominate over fruity notes, but their work was done on binary solutions and so this issue would deserve additional specific research to be generalised to more complex contexts.
In any case, results indicate that the perception of some secondary odour features of the original mixture is strongly dependent on the aroma context. Too simple aroma contexts, such as hydroalcoholic solution, decrease the perception of subtle differences, and in too complex aroma contexts, those subtleties may be completely lost. A practical corollary is that the role of odorants or ingredients should be always checked in the final product, since some of the differences or particularities observed or missed in simpler aromatic contexts may or may not finally translate into the overall perception. This is likely the case of some of the observations about the role of specific esters in the sensory characteristics of wines (Lytra, Cameleyre, Tempere, & Barbe, 2015; Lytra, Tempere, de Revel, & Barbe, 2012, 2014, 2015).

But the most relevant consequence of all the previous observations is the existence of aroma vectors as perceptual units. Results have shown that the signals corresponding to the 14 ethyl esters integrate in a single whole with at least three different qualitative dimensions in simple aromatic contexts, and with just one in most complex contexts. Aroma vectors should constitute a basic concept for the interpretation and modelling of complex aroma mixtures and suggest that the modelling of complex mixtures should be a difficult, but not impossible task. However, for making full use of the concept a deeper understanding of the qualitative similarity leading to a unitary perception should be first acquired. In the present experiment, just ethyl esters with a relatively high degree of similarity between their attributes have been intentionally grouped into the vector, leaving out of it some other esters bearing specifically different aroma notes, such as ethyl acetate (solvent, vinegar), isoamyl acetate (banana) or ethyl cinnamate (flowery), which were present as components in the wine models (see Table 3). This was done in this way because previous experience with wine models had shown that these components could transmit to the wine aroma buffer (the aroma mixture formed
by ethanol and by the major volatile metabolites of alcoholic fermentation) their specific odour nuances, if present at adequate levels. It is obvious that a more systematic approach should be developed in order to generalize the use of aroma vectors in flavour chemistry.

4.2. Effect of diversity of ethyl esters on intensity of overall wine aroma

A third remarkable result is that the role of subthreshold or perithreshold odorants on the intensity of the odour mixture is undeniable, which is supported by the important decrease in intensity when simplifying V1 to V2 by removing compounds below their sensory threshold. This result is in agreement with some observations from Lytra, Tempere, Le Floch, de Revel, and Barbe (2013) and with most recent results presented by Niu, Wang, Xiao, Zhu, Sun, and Wang (2019). These authors have found that the removal of subthreshold levels of ethyl decanoate from an ester solution imitating the composition of cherry wines brought about a strong decrease in the odour intensity of the mixture ($I_{\text{final}} = 0.72 I_{\text{original}}$). This odour enhancement or, in psychophysical terms, hyperaddition effect, can be related to previous observations, such as the strong effects caused by small amounts of 4-methyl-4-mercaptopentanone in the aroma of white wine (Escudero, Gogorza, Melus, Ortin, Cacho, & Ferreira, 2004), or by subthreshold amounts of ethyl salicylate (Niu, Wang, Xiao, Zhu, Sun, & Wang, 2019). However, the intensity enhancement observed in these two last cases can be attributed to the specific features, such as fresh, cool or green, introduced by those odorants. Such specific features may complete or improve the odour concept represented by the mixture, enhancing its intensity. It is however intriguing which special aroma feature would develop by adding ethyl decanoate in the experiment of Niu, Wang, Xiao, Zhu, Sun, and Wang (2019), or any of the 7 other odorants removed here. This question would deserve additional specific research.
A fourth remarkable result is that the process of simplification inevitably implies an increase in the concentration of the remaining odorants in a proportion well above that corresponding to a strict replacement. This is revealed by the continuous and strong increase in OAVs seen in Table 5. For instance, in the most simplified model V7, the OAV was 4.4 times higher than that of V1. The aroma simplification concomitantly involves an increase of the mass of odorant. It should be acknowledged that most of the thresholds in which this observation is based are from literature and that their accuracies with the panel used here were only partially assessed, which inevitably introduces some uncertainty. However, it can be seen that for the cases of ethyl hexanoate and ethyl butyrate, even if the thresholds of the panel used here were 10 times below those given in the table, the conclusions would not change.

Such result is the simple consequence of the nature of the psychophysical functions relating odour intensity and the concentration of the odorant. The function relating psychophysical odour intensity to the logarithm of the stimulus concentration should have a sigmoid shape, and can be described by the function proposed by Chastrette, Thomas-Danguin, and Rallet (1998):

\[
I = \frac{I_m \cdot C^n}{C_{ip}^n + C^n}
\]

where \(I\) is the calculated intensity of the response, \(I_m\) is the maximum value of perceived intensity, \(C\) is the stimulus concentration, \(C_{ip}\) is the concentration at the inflection point and half intensity and \(n\) is the Steven’s exponent for the odorant (which varies between 0 and 1). This sigmoid function is represented in Figure 1A.

As the most relevant sigmoid function is the logistic function, whose first derivative is the Gaussian function which reaches maximum value at the inflection point (or half
concentration; z=0), it can be naively thought that in all sigmoid functions, the first derivative will be maximum at the inflection point. This is, however, not the case for the psychophysical function described by equation 1 and represented in log scale in Figure 1A and in linear scale in Figure 1B. The first derivative of this function, representing each slope in logarithmic or linear scales in Figures 1C and 1D, respectively, takes maxima values at smallest values of concentration and continuously decreases with the concentration (C). This implies, that as the concentration of the odorant increases, equal increments in intensity imply much higher increments of concentration, and hence of OAVs. For example, in a hypothetic situation ($I_m = 10$, $C_{ip} = 450 \text{ mg L}^{-1}$, odour threshold = 6.77 mg L$^{-1}$ and $n = 0.54$), the removal of an odorant present at threshold levels (6.77 mg L$^{-1}$, ●) would mean a decrease in intensity of 0.068 units (Figure 1C and 1D). However, the concentration at the inflection point and half intensity ($I=5$) (450 mg L$^{-1}$, ●) represents an intensity of just 0.003 units. It should be remarked that this result will take place regardless of the nature of the type of interaction between the odorants in the mixture. Except in the rare cases in which the intensity of the mixture is below the intensity of the most intense odorant (in isolation), which is often defined as compromise, the outcome will be the same in hypoaddition, perfect addition and hyperaddition or synergy. Of course, as the degree of additivity increases the divergence between the OAVs of the simpler and those of the most complex mixtures of odorants will increase.

One of the practical corollary of this fact is that OAVs are quite limited measures to predict not only the actual importance of an odorant in a mixture but, as seen here, also as criteria for combining odorants into vectors. The limitations about OAVs were first formulated by Frijters (1978) 40 years ago but the practical consequences are usually ignored by aroma researchers (including ourselves), who keep on using OAVs –a
physical measurement- to infer psychophysical properties. Rather, odour intensity is the parameter that has to be used, which makes it advisable to determine the psychophysical curves of main odorants and to study the additivity of the signals.

A similar caution should be equally raised about the observations made at threshold levels, at which more interaction between odorants are expected (Lytra, Tempere, Le Floch, de Revel, & Barbe, 2013).

5. Conclusions

Results show that differences between the aroma vector containing 14 ethyl esters at maximum red wine levels and other isointense aroma vectors containing a reduced number of esters can be perceived just in some aromatic contexts. When the vectors are smelled in isolation, they are barely perceived. However, when the vectors are smelled in a young red wine context, vectors with just 1 or 2 components were easily identified; but in an oaky red wine context, differences were not noticeably perceived. In practical terms this implies that the mixture of 14 ethyl esters can be satisfactorily replaced by just 3 ethyl esters in a young red wine, or by just 1 –the most intense in the original vector- if woody notes are present. This result questions the alleged importance of some minor esters or specific enantiomeric forms on the specific odour descriptors of wine and confirms the need to work with complex mixtures similar to the target product in suprathreshold areas.

Notwithstanding this, results confirm that subthreshold or perithreshold odorants play an outstanding role on the overall odour intensity of the mixture. Besides, it has been found that the process of aroma simplification concomitantly implies an increase in the amount of odorant (in OAV terms) required to keep the intensity of the aroma vector. It is suggested that such effects are the simple consequence of the power nature of
psychophysical functions, evidencing the limited value of OAV for predicting importance or combining aroma vectors.

Finally, the fact that olfactory signals from 14 compounds are *de facto* integrated into one vector with three or just one dimensions, suggests that complex odour mixtures could be interpreted and modelled making use of the concept of aroma vectors as basic perceptual units carrying specific qualitative information. This result constitutes a proof of concept for the existence of aroma vectors which should be of general interest for the food and flavour industries.

**Acknowledgements**

Funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (projects AGL2014-59840 and AGL2017-87373-C3-1-R). M.P.S.N. acknowledges the MINECO for her postdoctoral fellowship (JCI-2015-23444). LAAE acknowledges the continuous support of Diputación General de Aragón (T53) and European Social Fund. Authors also want to thank panellists for their interest and diligence during their participation in the sensory sessions.

**References**


San-Juan, F., Ferreira, V., Cacho, J., & Escudero, A. (2011). Quality and Aromatic Sensory Descriptors (Mainly Fresh and Dry Fruit Character) of Spanish Red Wines can be Predicted from their Aroma-Active Chemical Composition. *Journal of Agricultural and Food Chemistry, 59*(14), 7916-7924.


Figure captions

Figure 1. Theoretical psychophysical function and its first derivative, representing an odorant with threshold 6.77 mg L\(^{-1}\), a concentration at half intensity of 450 mg L\(^{-1}\) and a Stevens coefficient of 0.54. The function has been represented using the equation proposed by Chastrette, Thomas-Danguin, and Rallet (1998). A) Standard representation with concentration in logarithmic scale; B) Representation in linear scale; C) First derivative of the function in logarithmic scale; D) First derivative of the function in linear scale.

Table 1. Concentration (μg L\(^{-1}\)) of different esters found in different red wines.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Descriptor(^a)</th>
<th>Concentration ranges (average) (b)</th>
<th>Odor threshold (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESTERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear fatty acid derivates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl propanoate</td>
<td>Fruity, solvent, acetone</td>
<td>85-1966 (260) (^1)</td>
<td>5500 (^1)</td>
</tr>
</tbody>
</table>
Ethyl butyrate  Fruity, strawberry, sweet, bubble gum  87-252 (152)  125  1
Ethyl hexanoate  Fruity, strawberry, pineapple, anise  78-337 (169)  62  1
Ethyl octanoate  Soapy, fatty, fruity  26-225 (103)  580  3
Ethyl decanoate  Fruity, grape  29-163 (72)  200  2

Branched fatty acid derivates

Ethyl 2-methylpropanoate  Fruity, strawberry, sweet, bubble gum  <4.0-419 (114)  15  2
Ethyl 2-methylbutyrate  Fruity, strawberry, apple, blackberry  6.5-69 (14)  2  6
Ethyl 3-methylbutyrate  Fruity, strawberry, apple  11-89 (26)  0.7  6
Ethyl 3-hydroxybutyrate  Nutty, coconut, grape  100-370 (221)  21000 (S), 63000 (R)  4
Ethyl 2-methylpentanoate  Fruity, strawberry  <0.0007 (<0.0007)  10  1
Ethyl 3-methylpentanoate  Fruity, strawberry  <0.0006 (<0.0006)  0.50  1
Ethyl 4-methylpentanoate  Fruity, strawberry  <0.0005-0.53 (0.110)  1  0.75  1
Ethyl cyclohexanoate  Anise, strawberry  <0.0008 (<0.0008)  0.030  1

Other esters

ethyl 2-hydroxy-4-methylpentanoate  Strawberry, blackberry, fruity  135 (135)  126 (R), 55 (S), 51 (mx)  5

Table 2. Concentration (μg L⁻¹) of different esters found in the different vectors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>V1</th>
<th>V2</th>
<th>V2’</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl propanoate</td>
<td>1966</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>252</td>
<td>296</td>
<td>444</td>
<td>690</td>
<td>740</td>
<td>850</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Compound</td>
<td>337</td>
<td>411</td>
<td>617</td>
<td>434</td>
<td>460</td>
<td>-</td>
<td>589</td>
<td>-</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>225</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>163</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylpropanoate</td>
<td>419</td>
<td>419</td>
<td>629</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylbutyrate</td>
<td>59</td>
<td>72</td>
<td>108</td>
<td>593</td>
<td>630</td>
<td>877</td>
<td>943</td>
<td>1733</td>
</tr>
<tr>
<td>Ethyl 3-methylbutyrate</td>
<td>89</td>
<td>89</td>
<td>134</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylpentanoate</td>
<td>0.0007</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 3-methylpentanoate</td>
<td>0.0006</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 4-methylpentanoate</td>
<td>0.53</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 3-hydroxybutyrate</td>
<td>370</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl cyclohexanoate</td>
<td>0.0008</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-hydroxy-4-methylpentanoate</td>
<td>135</td>
<td>137</td>
<td>206</td>
<td>137</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 3. Wine aroma base models composition (µg L⁻¹).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoamyl alcohol</td>
<td>180000</td>
</tr>
<tr>
<td>β-phenylethanol</td>
<td>30000</td>
</tr>
<tr>
<td>acetic acid</td>
<td>150000</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>50000</td>
</tr>
<tr>
<td>hexanoic acid</td>
<td>2000</td>
</tr>
<tr>
<td>3-methylbutanoic acid</td>
<td>300</td>
</tr>
<tr>
<td>2,3-butanedione</td>
<td>400</td>
</tr>
<tr>
<td>isoamyl acetate</td>
<td>1000</td>
</tr>
<tr>
<td>ethyl vanillate</td>
<td>250</td>
</tr>
<tr>
<td>vanilla</td>
<td>70</td>
</tr>
<tr>
<td>γ-nonalactone</td>
<td>20</td>
</tr>
<tr>
<td>guaiacol</td>
<td>10</td>
</tr>
<tr>
<td>β-damascone</td>
<td>4.0</td>
</tr>
<tr>
<td>β-ionone</td>
<td>0.30</td>
</tr>
<tr>
<td>4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)</td>
<td>30</td>
</tr>
<tr>
<td>ethyl cinnamate</td>
<td>0.43</td>
</tr>
<tr>
<td>linalool</td>
<td>7.0</td>
</tr>
<tr>
<td>geraniol</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Pool of compounds conforming the common aroma base**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>whisky lactone</td>
<td>300</td>
</tr>
<tr>
<td>vanilla</td>
<td>100</td>
</tr>
<tr>
<td>eugenol</td>
<td>15</td>
</tr>
<tr>
<td>guaiacol</td>
<td>15</td>
</tr>
<tr>
<td>4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)</td>
<td>100</td>
</tr>
</tbody>
</table>

**Woody vector**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)</td>
<td>100</td>
</tr>
</tbody>
</table>

*The woody vector was only added to aged oaked red wines.*
Table 4. Triangle tests performed to check the effect of the simplification of esters composition on simple and complex aroma contexts. In A tests, the vectors were directly confronted. In B tests, vectors were part of a neutral red wine model. In C tests, they were part of an oaky red wine model.

<table>
<thead>
<tr>
<th>Test A</th>
<th>Media</th>
<th>Confronted vectors</th>
<th>P a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 1</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V2'</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>A. 2</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V3</td>
<td>n.s.</td>
</tr>
<tr>
<td>A. 3</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V4</td>
<td>n.s.</td>
</tr>
<tr>
<td>A. 4</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V5</td>
<td>n.s.</td>
</tr>
<tr>
<td>A. 5</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V6</td>
<td>n.s.</td>
</tr>
<tr>
<td>A. 6</td>
<td>Water 1% ethanol pH 6</td>
<td>V1 vs V7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>V1 vs Vi</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test B</th>
<th>Wine models</th>
<th>Confronted wines</th>
<th>P a</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. 1</td>
<td>Young red wine</td>
<td>W-V1 vs W-V2'</td>
<td>n.s.</td>
</tr>
<tr>
<td>B. 2</td>
<td>Young red wine</td>
<td>W-V1 vs W-V3</td>
<td>n.s.</td>
</tr>
<tr>
<td>B. 3</td>
<td>Young red wine</td>
<td>W-V1 vs W-V4</td>
<td>n.s.</td>
</tr>
<tr>
<td>B. 4</td>
<td>Young red wine</td>
<td>W-V1 vs W-V5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>B. 5</td>
<td>Young red wine</td>
<td>W-V1 vs W-V6</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test C</th>
<th>Wine models</th>
<th>Confronted wines</th>
<th>P a</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. 1</td>
<td>Oaked red wine</td>
<td>oW-V1 vs oW-V5</td>
<td>n.s.</td>
</tr>
<tr>
<td>C. 2</td>
<td>Oaked red wine</td>
<td>oW-V1 vs oW-V6</td>
<td>n.s.</td>
</tr>
<tr>
<td>C. 3</td>
<td>Oaked red wine</td>
<td>oW-V1 vs oW-V7</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Significance of the effect; ns: not significant. Significant effects marked in bold (P < 0.05).
### Table 5. Odor Aroma Value (OAV) of different esters found in the different vectors.

<table>
<thead>
<tr>
<th>OAV</th>
<th>V1</th>
<th>V2</th>
<th>V2'</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl propanoate</td>
<td>0.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>2.0</td>
<td>2.4</td>
<td>3.6</td>
<td>5.5</td>
<td>5.9</td>
<td>6.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>5.4</td>
<td>6.7</td>
<td>10</td>
<td>7.0</td>
<td>7.4</td>
<td>-</td>
<td>9.5</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>0.39</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>0.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylpropanoate</td>
<td>28</td>
<td>28</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylbutyrate</td>
<td>30</td>
<td>36</td>
<td>54</td>
<td>297</td>
<td>315</td>
<td>439</td>
<td>472</td>
<td>867</td>
</tr>
<tr>
<td>Ethyl 3-methylbutyrate</td>
<td>127</td>
<td>127</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 2-methylpentanoate</td>
<td>0.000070</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 3-methylpentanoate</td>
<td>0.0012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 4-methylpentanoate</td>
<td>0.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl 3-hydroxybutyrate</td>
<td>0.018</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl cyclohexanoate</td>
<td>0.027</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ethyl 2-hydroxy-4-methylpentanoate</td>
<td>2.6</td>
<td>4.0</td>
<td>4.0</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**TOTAL OAV** | 197 | 203 | 305 | 312 | 328 | 445 | 481 | 867 |

**Highlights**

- 14 ethyl esters of red wine can be replaced by just 3 esters in young red wine
- Or just by 1 (ethyl 2 or 3-methylbutyrate) in an oaky red wine
- Aroma integration allows defining Aroma Vectors as basic perceptual units
- Sub/peri thresholds esters play an outstanding role on aroma intensity
- Aroma simplification requires an increase of Odor Activity Value to keep intensity
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: