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Chemo-sensory approach for the identification of chemical compounds driving green character in red wines

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Abstract

The present work seeks to define the “green character” of red wines and characterise the groups of molecules potentially involved in that perception. Fifty-four wines were screened by wine experts for different levels of green character. Six different phenolic fractions were obtained by liquid chromatography (LC) and further submitted to sensory and chemical characterisation. The volatile fraction was screened by semipreparative LC, Gas Chromatography-Olfactometry (GC-O) and quantitative analysis. The green character was linked to vegetal aroma, astringency, green and dry tannins according to experts of the Somontano region. Non-volatile fractions containing tannins with mean degree of polymerisation of ten and smaller anthocyanin-derivative pigments (<tetramers) imparted astringency-related sensations such as stickiness and dryness, respectively. No specific aroma compounds were identified in the GC-O study of green wines, however the wines contained significantly higher levels of fusel alcohols. The interaction between isoamyl alcohol and the anthocyanin-derivative fraction and/or tannins is suggested to be involved in the formation of green character in red wines.

Key words: green character; sensory analysis; tannins; anthocyanins; aroma; isoamyl alcohol
1. Introduction

Flavour in food and beverages is the result of sensory interactions between sensory active volatile and non-volatile molecules (Prescott, 2012). Understanding the formation of food flavour is of paramount importance for the food industry in general and especially for the industry of complex beverages such as beer or wine. Increasing knowledge about flavour formation allows to have objective tools to manage the production process and optimise the quality of the final product. Traditionally, flavour chemists use two main strategies. The first, involves the complete chemical quantification of known sensory-active molecules and in parallel the sensory description of the product. Both data sets are submitted to statistical analysis with the aim of building models able to predict perceived sensory properties from chemical composition (Regueiro, Negreira, & Simal-Gándara, 2017). Main drawbacks are that a wide range of compounds have to be quantified by a diverse range of chemical methods and the most important is that key unknown sensory active molecules are most probably not being considered. To overcome these drawbacks, flavour chemists use sensory-directed methodologies targeting only compounds eliciting sensory properties in the product object of study and further focusing in the identification and quantification of molecules in sensory-active fractions. For volatile compounds, Gas Chromatography coupled to an olfactory detector (GC-O), which involves the use of GC for separation and the nose of trained judges as detector, has been demonstrated to be a powerful tool to identify sensory-active volatile compounds and is widely used in flavour chemistry (Chin & Marriott, 2015; d’Acampora Zellner, Dugo, Dugo, & Mondello, 2008). Concerning non-volatile compounds, the separation of molecules or group of molecules is usually carried out by preparative liquid chromatography (LC), collected fractions are further sensory described in terms of taste and mouth-feel properties (Sáenz-Navajas et al., 2017; Scharbert, Holzmann, & Hofmann, 2004). The study of the sensory activity of non-volatile molecules is less explored than volatiles, mainly due to two reasons: it is difficult to describe mouth-feel properties because there is a lack of references and the second reason is that it is more time and resource consuming than GC-O in terms of both fractionation and sensory fatiguing. Once sensory active molecules involved in the formation of flavour are identified by either GC-O or LC followed by sensory evaluation of
fractions, reconstitution experiments are carried out to evaluate the real sensory impact of compounds in the matrix object of study. This helps to confirm the complex relationships existing between chemical composition of foods and flavour perception (Regueiro et al., 2017).

This strategy comprising the steps of 1) isolation and identification of sensory-active molecules or groups of molecules and 2) reconstitution experiments to confirm their sensory role has been applied in the present work to identify fractions of both volatile and non-volatile compounds responsible for a recurring undesirable sensory property found in red wines and named by wine experts: “green character”.

Due to climate change, there is a difference in time between technological (related to sugar and acidity content) and phenolic maturity of grapes. Based on declarations of winemakers, the fact that technological maturation is achieved, while phenolics and aroma precursors are still unripe, lead to green wines. During winemaking, the oenologist has to make decisions related to the elaboration of grapes that enter the winery with an acceptable content in sugars or acids but with immature tannins. Such grapes will generate wines with green character, which will induce a decrease of consumers’ acceptance of the product. Given the lack of objective criteria to this concern, such decisions are mainly based on empirical experience. An increase in the chemical and sensory knowledge of such character will allow managing grapes and/or wines with maximum efficiency during winemaking processes.

In this context, the main aims of the present work are to: 1) understand the meaning of green character in red wines and establish relationships with simpler sensory descriptors, 2) select wines with different levels of green character and 3) identify volatile and non-volatile compounds involved in the green character in red wines.
2. Material and methods

Supplementary material 1 shows a fluxogram which graphically explains the steps followed in the material and methods section.

2.1. Screening for wines with different levels of green character

2.1.1. Samples, participants and procedure

A total of 54 red wines were selected in consultation with wine experts. The selection criterion was to have a wide range of samples with different levels of green character according to winemakers from Somontano region. Wines were elaborated mainly in Spain (46), but there were also wines from Italy (3), France (3), Portugal (1) and Chile (1). The alcoholic content ranged from 11.9 to 15.2% (v/v), vintage from 2009 to 2015 and their time in oak barrels from 0 to 24 months. The detailed list of wines is shown in supplementary material 2 and 3.

The panel of experts was composed of fifteen established winemakers of Somontano region (8 women, from 33 to 56 years, median = 45).

Two different tasks were carried out for the screening of wines with green character. The first task (1st screening task) was mainly exploratory. It aimed at globally understanding the term “green character” and linking it to sensory (aroma and/or in-mouth) terms usually employed by wine experts. The second task (2nd screening task) was focused on the screening of aroma compounds driving “green character”.

In the first task, held in year 2015, thirty-eight red wines (supplementary material 2) were presented simultaneously. Wine experts were asked to taste each sample from left to right and to score the multidimensional terms: green character and preference. Then, they were presented with the same wines but with different codes and order of presentation. They had to score the intensity of 12 attributes: six aroma (aroma intensity, oxidation, vegetal, fresh fruit, ripe fruit and wood) and six in-mouth (sweet, sour, astringent, oily, green tannins and dry tannins) terms. The intensity was rated on a five-point scale, from 1 (absent) to 5 (very intense). They were free to compare wines before scoring if they wanted. The selection of the intensity scale and attributes was carried out in an independent session in consultation with three experts of the region. These participants were different from those that carried out the description. In this session, they were firstly asked...
to freely cite terms related to green character. Then, the participants were presented with a global list for all the terms pooled together and a final list was selected by consensus. The scale and terms were those usually employed by the experts in the region and thus more familiar to them. The second task was held in the year 2016 which involved expanding the space of aroma terms related to green character. It was focused on finding wine exemplars high in green character based exclusively on aroma properties. Therefore, wine experts were presented with 16 red wines (supplementary material 3) simultaneously and were asked to smell each sample from left to right and to score the green character. Then, they were presented with a list of 14 aroma terms (white/yellow fruit, citrus, tropical fruit, red fruit, black fruit, dried fruit, fresh vegetal, cooked vegetables, herbal, reduction, wood, spicy, roasted, animal) compiled from other lists employed in the description of red wines (Noble et al., 1987; Saenz-Navajas, Fernandez-Zurbano, Martin-Lopez, & Ferreira, 2011). Wine experts were asked to rate the intensity of terms that applied exclusively to the particular wine sample on a seven-point scale (1 = not intense; 7 = very intense) according to Rate-all-that-Apply (RATA) methodology (Ares et al., 2014; Reinbach, Giacalone, Ribeiro, Bredie, & Frost, 2014). Terms that did not apply to the sample were allocated a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomized order for each assessor.

In both tasks (1st and 2nd screening tasks), wines were presented at room temperature, in clear ISO glasses identified only by random three-digit codes. The poured volume per sample was 30 mL. Samples were presented in random order and different for each participant. Mineral water and unsalted crackers were available for palate rinsing. For the first task, participants were asked not to swallow the samples but to expectorate into wine spittoons.

2.1.2. Data analysis

Two-way ANOVAs with assessors as random factor and wine as fixed factor were calculated on the scores of preference, green character, aroma and in-mouth attributes. For significant effects, Fischer post-hoc pairwise comparison (95%) test was performed.
Two principal component analysis (PCA) were calculated with data derived from 1\textsuperscript{st} (38 wines) and 2\textsuperscript{nd} (16 wines) screening tasks and the mean scores (of the 15 winemakers) of the significant in-mouth and/or aroma attributes (active variables) and green character and preference (supplementary variables). All analyses were carried out with XLSTAT (2015 version).

2.2. Screening for non-volatile fractions driving green character

2.2.1. Preparation of wine fractions

Based on the results of the 1\textsuperscript{st} screening task, two wines with high (High Green-HG: wines 37 and 38) and two with low (Low Green-LG: wines 1 and 19) green character were selected. These four wines were fractionated. A total of six fractions per wine were obtained by a two-step methodology as described elsewhere (Sáenz-Navajas et al., 2017). Briefly, in the first step, four fractions were collected by a preparative LC method adapted from Remy, Fulcrand, Labarbe, Cheynier, and Moutounet (2000) and Gonzalo-Diago, Dizy, and Fernandez-Zurbano (2013). Therefore, 200 mL of wine were dealcoholized in a rotary evaporator (15 min at 28 °C). Then, the sample was freeze-dried in 500 mL-rounded flasks. The extract was redissolved in 20 mL of hydroalcoholic solution (12% ethanol, v/v) and the whole volume was injected into a preparative Millipore LC column (gel: Toyopearl HW-50F; dimensions: 120 mm x 22 mm id; flow rate: 4 mL min\textsuperscript{-1}). A first fraction (F1) was eluted with 720 mL of ethanol/water/formic acid (55:45:1, v/v/v). The second fraction (F21) was recovered by elution with 80 mL of acetone (100%). The third (F22) and fourth (F23) fractions were eluted with 160 and 80 mL of acetone/water at rates of 80:20 and 60:40, respectively. Solvents present in the four fractions were evaporated under vacuum and samples were further freeze-dried.

In the second step, F1 was redissolved in 200 mL of hydroalcoholic solution (12%, v/v) and further submitted to solid-phase extraction (SPE) using an extraction unit (VAC ELUT 20 Station from Varian). SPE cartridges filled with 500 mg of Bond Elut LRC-C18 resins were firstly conditioned by passing 5 mL of methanol followed by 10 mL of an aqueous solution at pH 2.5 (5 g L\textsuperscript{-1} of tartaric acid, pH adjusted to 2.5 with 0.1 M NaOH). This fraction has been described to be especially sour due to the presence of organic acids, which masks other in-mouth attributes
such as bitterness or astringency (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014). Thus, sugars and organic acids were removed by loading 5 mL of F1, which was further washed with 10 mL of aqueous solution at pH 2.5. Then, F11 was eluted with 5 mL of diethyl ether, F12 with 5 mL of ethyl acetate and F13 with 10 mL of methanol. Each cartridge was used a maximum of 5 times. Finally, after F13 was eluted, 10 mL of acetonitrile were used as pre-conditioning solvent before conditioning to remove any impurities on the SPE cartridge. The SPE-procedure was repeated until the 200 mL of F1 were extracted. The extracts were evaporated prior to freeze-drying. The total absence of solvents was assessed by headspace solid phase micro extraction using a 75 um Carboxen/PDMS fiber (75 µm at 30 ºC x 10 min) and GC with a MS detector (overall system detection limit 1 ng/sample).

The six freeze-dried fractions (F11, F12, F13, F21, F22, F23) were stored at 4 ºC prior to sensory and/or chemical analysis. Fractions (coming from 200 mL of original wine) were dissolved in 100 mL of hydroalcoholic solution (7% ethanol, v/v; 50 mg L\(^{-1}\) of SO\(_2\); 80 mg L\(^{-1}\) of ascorbic acid) to have fractions twice concentrated in order to facilitate sensory description. The level of ethanol (7%) was selected in preliminary tests, in which the range from 5% to 11% was evaluated. This level fulfilled two criteria: 1) it did not induce a burning effect able to mask other sensations (they are simple fractions with no aroma, which results in an enhanced burning sensation elicited by ethanol in comparison with real wines) and 2) it was as similar as possible to ethanol content in real wines.

2.2.2. Sensory characterisation of non-volatile fractions

2.2.2.1. Samples, participants and procedure

Four original wines (HG1-wine 37, HG2-wine 38, LG1-wine 1 and LG2-wine 19) and 24 fractions were sensorily evaluated: six fractions (F11, F12, F13, F21, F22, F23) x 4 wines (HG1, HG2, LG1, LG2) by sixteen wine experts (11 women and 5 men, ranging from 23 to 62 years of age, average = 37). Participants attended two sessions spread over two different days. Each session was split into two parts (ca. 30 min each), which were separated by a break of 15 min. First session included 16 fractions and second session eight fractions and four wines, respectively.
The absence or limited availability of reference materials with defined mouthfeel properties makes Rate-all-that-Apply (RATA) methodology an interesting procedure to describe in-mouth properties by wine experts with no specific training phase as described in Sáenz-Navajas et al. (2017). Therefore, participants were presented with a list of 23 terms (four for taste, 18 for mouthfeel and persistence) developed in previous work (Sáenz-Navajas et al., 2017). Participants were asked to taste and rate the intensity of exclusively those terms that applied to the sample on a seven-point scale according to RATA methodology. Participants were instructed to sip the samples via a dark straw (to control the volume they had -2 mL each sip- and to limit carry-over effects) and gently spread out the liquid over the whole mouth cavity. After one minute, they were told to expectorate the sample. The use of a sip (rinsing solutions: water and 1 g L\(^{-1}\) pectin solution) and spit protocol between each sample was imposed as described elsewhere (Colonna, Adams, & Noble, 2004). Participants tasted samples in a sequential monadic manner. Ten-mL samples were served in dark ISO-approved wine glasses labelled with 3-digit random codes and covered with plastic Petri dishes according to a random arrangement, different for each participant. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC). Participants were informed that samples were not commercial wines but fractions obtained in the laboratory, in case they accepted to continue in the experiment they signed a consent form. Participants were not paid for their participation.

2.2.2.2. Data analysis

To determine discriminant attributes for the four original wines (HG1, HG2, LG1 and LG2) a two-way ANOVA (panellists as random and wines as fixed factors) was calculated for each of the 23 terms on the list. Pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. Next, principal component analysis (PCA) was carried out on the mean intensity scores of the significant terms and the four wines.

For fractions, two-way ANOVAs (panellists as random and fractions as fixed factors) were performed on the intensity ratings of the 24 fractions. Pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. Then, PCA was carried out on the mean intensity scores.
of the significant terms and the 24 fractions. A hierarchical cluster analysis (HCA) with the Ward criteria was finally applied to all PCs. Clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centres. The terms that best characterised each cluster were identified by using the test-value parameter (Lebart, Morineau, & Piron, 1995). The test-value corresponds to a statistical criterion akin to a standardized variable (zero mean and unit variance). Significance is obtained when the absolute test-value is \( \geq 1.96 \), which corresponds to an error threshold of 5%.

All statistical analyses were performed using XLSTAT (2015) and SPAD (version 5.5).

2.2.3. Chemical characterisation of non-volatile fractions and wines

Chemical analyses were performed in four original wines (HG1-wine 37, HG2-wine 38, LG1-wine 1 and LG2-wine 19) and their corresponding fractions (F11, F12, F13, F21, F22, F23). All samples were analysed in duplicate.

2.2.3.1. Conventional oenological parameters

Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and colour intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). For TPI determination, the abs at 280 nm of samples diluted 1:100 in deionized water was measured in 1-cm-quartz cuvettes. For CI, absorbance of undiluted samples was measured in 2-mm-crystal cuvettes.

2.2.3.2. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) in wines and fractions was carried out as described elsewhere (Harbertson, Picciotto, & Adams, 2003). MPs were the group of compounds bleachable with bisulphite, while SPP and LPP were resistant to bisulphite bleaching. SPP did not precipitate with ovalbumin, different to LPP. Levels of MP, SPP, and LPP were expressed as absorbance at 520 nm.

2.2.3.3. Thiolysis assay

Acid-catalysed degradation in the presence of toluene-\( \alpha \)-thiol was performed according to the method described by Gonzalo-Diago et al. (2013). The mean degree of polymerization (mDP) as
well as the percentage of procyanidins (%PC), prodelphinidins (%PD), and galloylation (%G) were calculated as the molar ratio of the total units to terminal units.

2.2.3.4. Characterisation of tannins

Concentration and activity of tannins were estimated by a HPLC-UV-Vis method following the method proposed by Revelette, Barak, and Kennedy (2014).

2.3. Screening for volatile fractions driving green character

2.3.1. Preparation of volatile fractions and sensory characterisation

2.3.1.1. Samples, participants and procedure

Based on the results of the 1st screening task, one wine with high (HG3-wine 18) and one (LG1-wine 1) with low vegetal aroma and green character were selected. The aroma extract of both wines was obtained and fractionated by semipreparative reversed-phase liquid chromatography using a water–ethanol gradient system as mobile phase as described by Ferreira, Hernández-Orte, Escudero, López, and Cacho (1999). A total of 17 fractions per wine were obtained. Fifteen of them (the first and last fractions were not considered as they were odourless) together with two duplicates were sensory evaluated by sorting task. Therefore, 16 trained subjects (9 women, ranging from 23 to 70 years of age, average = 38), belonging to the laboratory staff and with large experience in aroma analysis, were asked to orthonasally smell the 32 fractions and sort them on the basis of their similarity attending to their aroma properties. They could make as many groups as they wished. Upon completion, participants recorded the codes of the samples of each group on a paper sheet and described each group with a maximum of three attributes.

One mL of each fraction was presented in 6-mL flasks covered with aluminium foil and labelled with 3-digit random codes according to a random arrangement, different for each participant. Fractions were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC).

2.3.1.2. Data Analysis

For each participant, results were encoded in an individual similarity matrix (fraction x fraction), in which 1 stands for two wines set in the same group and 0 for two wines put in different groups.
These individual matrices were summed across subjects; the resulting co-occurrence matrix represents the global similarity matrix where larger numbers indicate higher similarity between samples. The underlying assumption for this method is that samples plotted together are more similar than samples plotted far away. The resulting co-occurrence matrix was submitted to an MDS analysis in order to derive a spatial representation of fractions. All MDS dimensions were submitted to Hierarchical Cluster Analysis (HCA). All analyses were performed with XLSTAT (2015 version).

2.3.2. Head Space-Gas Chromatography-Olfactometry (HS-GC-O)

2.3.2.1. Samples, participants and procedure

Fractions (F9, F12 and F13) of wines 1-LG1 and 18-HG3, which were scored low and high for green character and vegetal aroma, respectively, were submitted to HS-GC-O. The complete aroma extracts of wines 1 and 19 (low scores for green character) and wines 18, 39-42 (high scores for green character) were also screened by HS-GC-O.

The dichloromethane/methanol extract to be used for GC-O was obtained in a purge and trap system and then screened by GC-O system equipped with a flame ionization detector (FID) and a sniffing port ODO-I from SGE (Ringwood, Australia), connected by a flow splitter to the column exit as described elsewhere (Escudero, San-Juan, Franco-Luesma, Cacho, & Ferreira, 2014).

Sniffing was carried out by a panel composed of six trained subjects, four women and two men belonging to the laboratory staff. Each participant evaluated the sample extract once in two time segments of 30 min to avoid fatigue (one session per day). They were asked to evaluate the time, description and the odour intensity of each aromatic sensation. An intensity scale of 0–3 was used (0 = no odour, 1 = weak odour, low intensity, 2 = clear perception of odour, strong intensity, 3 = extremely strong intensity of odour; intermediate values of 0.5, 1.5 and 2.5 were allowed).

2.3.2.2. Data Analysis

The data processed from GC-O were a mixture of intensity and frequency of detection (labelled as “% modified frequency”, %MF), which was calculated with the formula proposed by Dravnieks (1985):
where %F is the detection frequency of an aromatic stimulus expressed as a percentage and %I is the average intensity expressed as percentage of the maximum intensity. The identification of the odorants was carried out by comparison of their odours, chromatographic retention index in both DB-Wax and DB-5 columns, and MS spectra with those of pure reference compounds.

### 2.3.3. Quantitative analysis of fusel alcohols

Fusel alcohols were quantified in wines 1-LG1 and 19-LG2 (low green character) and wines 18-HG3 and 39-42 (high green character based exclusively on aroma perception). Therefore, major volatile compounds were isolated by liquid-liquid extraction and analysed in a gas chromatograph with flame ionization detector following the method described by (Ortega, Lopez, Cacho, & Ferreira, 2001). Analytes were referred to a selected internal standard, and response factor was the selected method for calibration.

### 2.3.4. Sensory analysis

Based on the results derived from quantitative studies, the sensory importance of methionol (7.3 mg L\(^{-1}\)) and isoamyl alcohol (454 mg L\(^{-1}\)) was checked in two different wines with different sensory profile by addition experiments. The wines were one young red wine (YW-Tempranillo 2016; 12.5% ethanol v/v) representative of a neutral red wine in terms of aroma and in-mouth properties (taste and mouthfeel) and an oaked red wine (OW-Tempranillo 2011; 13.5% ethanol v/v) representative of an aged wine with oak, oxidation and ripe fruit aromas and no outstanding taste or mouthfeel properties. Both, isoamyl alcohol and methionol, were firstly purified as described by De-La-Fuente-Blanco, Sáenz-Navajas, and Ferreira (2016) as commercial standards always contain traces of their corresponding aldehydes, which have a very high odorant power. A first set of triangle tests was carried out to evaluate the individual sensory impact of methionol and isoamyl alcohol and in a second set, their sensory interaction was evaluated. Thereafter, a trained sensory panel composed of 20 subjects (11 women, ranging from 24 to 70 years of age) performed tests in duplicate. Samples presented in the triangle tests were the red wine (YW or

\[
%\text{MF} = \sqrt{\%F \times \%I}
\]
OW) and the same sample spiked with methionol, isoamyl alcohol or methionol + isoamyl alcohol. Three glasses were presented to each participant and they were asked to select the different sample based exclusively on orthonasal aroma properties. The number of correct answers was compared with tabulated values to evaluate the presence of significant sensory differences between original and spiked samples. In all tests, samples (10 mL, 20 °C) were presented in random order in coded black tulip shaped wine glasses covered with a Petri dish.

2.4. Evaluation of the sensory impact of fractions and compounds on green character

2.4.1. Samples, participants and procedure

Based on screening steps, the sensory impact of two non-volatile fractions and isoamyl alcohol on the green character was evaluated in two very different wines in terms of sensory properties. These wines were the same young (YW) and oaked red (OW) wines used in the triangle tests (section 2.3.4). Table 1 shows the 16 wines evaluated attending to a full factorial design with three variables (isoamyl alcohol-IA-, F13_HG1 and F22_HG2+F23_HG2) at two levels (not spiked and spiked) in the two different wines. Considering that non-volatile fractions F22_HG2 and F23_HG2 contain similar compounds, basically proanthocyanidins (Sáenz-Navajas et al., 2017), and with the aim of reducing the number of variables, both fractions were pooled and added together in wines (F22+F23_HG2 or F2_HG2).

Fourteen established winemakers of Somontano region (7 women, from 33 to 56 years, median = 45) carried out the experiment.

Participants completed two sessions separated by a break of 30 min. They were presented with the 16 samples (Table 1) simultaneously and asked to taste each sample from left to right. In the first session they had to score the green character of wines on a seven-point scale (1=not intense and 7 = very intense). In the second session participants were asked to describe each sample for aroma and in-mouth properties by using a list of 9 aroma, 3 taste and 19 mouthfeel terms. These last terms were developed in a previous work (Sáenz-Navajas et al., 2017). They were asked to rate the intensity of exclusively those terms that applied to the sample on a seven-point scale according to RATA methodology.
Wines were presented at room temperature, in dark ISO glasses identified only by random three-digit codes. The poured volume per sample was 10 mL. Samples were presented in random order and different for each participant. Mineral water and unsalted crackers were available for palate rinsing. Participants were asked not to swallow the samples but to expectorate into wine spittoons.

2.4.2. Data analysis

Two-way ANOVA with assessors as random factor and type of wine (young or oaked red wine) as fixed factor was calculated on the scores of green character to evaluate the effect of wine matrix on green character.

Then, one two-way ANOVA for each wine matrix (young or oaked red wine) was calculated with the scores of green character and aroma and in-mouth attributes (assessors as random factor and wine as fix factor). For significant effects, Fischer post-hoc pairwise comparison (95%) test was performed.

Finally, two principal component analysis (PCA) were calculated (one for each type of wine) with the mean scores (of the 14 winemakers) of the significant in-mouth and/or aroma attributes (active variables) and green character (supplementary variable). All analyses were carried out with XLSTAT (2015 version).

3. Results and discussion

3.1. Screening for wines with different levels of green character

Wine experts were first asked (1st screening task) to score the green character, the preference and 12 additional sensory terms, usually used by experts, referring to aroma and in-mouth properties of the 38 wines included in the first part of the study. All terms were significant and in fact, the factor wine was found to be significant for all of them at P<0.001 in all cases except for sourness, for which it was significant at P<0.05. The projection of the terms on the two first dimensions of the PCA is shown in Figure 1. The illustrative variables, green character and preference, are negatively correlated (r =-0.70; P<0.001) between themselves, which confirms that the multidimensional descriptor green character has a negative valence for wine experts in the region.

The green character term seems to be highly multidimensional and it was correlated to both aroma
and in-mouth attributes. Green character was positively correlated to the aroma term vegetal and
to the in-mouth attributes astringency, green and dry tannins and, and negatively correlated to the
aroma term woody and to the in-mouth sensations oily and sweet.

Cluster analysis yielded four groups of wines (supplementary material 4) with different aroma
and/or in-mouth (taste and mouthfeel) properties. As seen in Table 2, the first cluster is mainly
classified by the aroma terms oxidation, woody and ripe fruit and by the in-mouth terms sweet
and oily, and it is negatively correlated to green character (P<0.01). In contrast, cluster 4 which
is mainly described with vegetal aroma, astringency and green tannins, is positively correlated to
the green character (P<0.001) and negatively to preference (P<0.01). Clusters 2 and 3 do not show
significant correlations with green character. Cluster 2 is mainly defined by terms such as dry
tannins and astringency as well as ripe fruit, woody and high aroma intensity. Cluster 3 has mainly
fresh fruity aroma and sourness, and is weakly (P<0.1) and positively correlated to preference.

Based on these results, one wine from each cluster was selected (wines 1, 19, 37 and 38) to be
screened for non-volatile compounds involved in green character. The selected samples represent
the global sensory space of the studied wines and present significant different scores for the green
character. As shown in Figure 2, samples 37 and 38 have, together with sample 18, the highest
scores for this attribute, while 1 and 19 the lowest. Sample 37 is representative for the term dry
tannins and wine 38 for green tannins.

Regarding aroma terms, wines 1 and 18 were selected for the screening of volatile compounds
(semipreparative LC, GC-O and quantitative analysis) involved in green character as they
presented the lowest and highest scores for vegetal aroma, respectively, as can be seen in Figure
2. Besides, in order to expand the space of aroma terms related to the green character, 15
additional wine samples plus sample 18 from the previous task (supplementary material 3) were
evaluated for their aroma properties (orthonasal perception only) in an independent second
session (2nd screening task). Results confirmed again that the green character is positively
correlated (P<0.001) to fresh vegetal aroma (r = 0.88). Wines 39-42 and 18, with the highest
scores for green character (Figure 3), were further submitted to GC-O and quantitative analysis.
of volatiles to screen for aroma compounds potentially involved in green character and compared to the composition of non-green wines.

3.2. Screening for non-volatile compounds driving green character

3.2.1. Sensory characterisation of fractions

The four wines selected for their different levels of green character (hereinafter coded as 1_LG1, 19_LG2, 37_HG1 and 38_HG2) were sensory described by RATA methodology, using a predefined list of terms related to in-mouth attributes (4 tastes, 18 mouth-feel-related terms and persistence) which was developed in a recent work (Sáenz-Navajas et al., 2017). Significant differences (P<0.05) were found in 4 out of the 23 in-mouth attributes: sticky, prickle, dry and dry-on-the tongue side. As shown in Figure 4, samples with high green character scores (37_HG1 and 38_HG2) had the highest scores for the terms dry and sticky, with 38_HG2 the highest score for dry on the tongue side.

These four wines were further fractionated by a liquid chromatography procedure to obtain 6 fractions per wine (coded F11, F12, F13, F21, F22, F23). The 24 fractions were sensorily described using the previous methodology. In this case, 14 out of the 23 attributes differed significantly (P<0.05) among fractions. The higher discriminant power of the descriptors must be attributed to the higher precision attainable in the sensory assessment of simplified non-volatile fractions. Results are summarised in Figure 5 which shows the PCA calculated with the significant attributes and the 24 fractions classified into six clusters derived from cluster analysis. As can be seen in Figure 5a, the first PC retains nearly 50% of the original variance, and confronts the terms dry, dry on the palate, dry on the tongue side, persistent, coarse, to silky, watery, sweet and greasy. The second PC, explaining almost 15% of the original variance, is mainly negatively correlated to the term sticky, and positively to burning, sour and bitter tastes. Figure 5b shows that clusters 1 and 2, which are formed by fractions F22 and F23 of wine 38_HG2 and F13 of wines 1_LG1, 19_LG2 and 38_HG2, respectively, are especially dry, dry on the palate, dry on the tongue side, persistent and coarse. Conversely, cluster 6 is mainly watery, silky, sweet and greasy. Fractions F11 (cluster 4), mainly sour, F12 (cluster 3), especially bitter, and the fractions within cluster 5,
sticky and coarse, are in the centre of the plot. Interestingly, equivalent fractions from the four wines are plotted very close together, indicating that they present similar sensory properties. The exceptions are F22_HG2 and F23_HG2 (Supplementary material 5a-b), which are significantly coarser (only for F22_HG2), drier, drier on the palate and more persistent than the equivalent fractions from the other three wines, as well as F13 HG1, which is significantly stickier than the equivalent fractions from the other wines (Supplementary material 5c).

These results strongly suggest that compounds in fractions F22_HG2 and F23_HG2 should be responsible for the high green character of wine 38_HG2, related to astringency and green tannins (Figure 2) as well as to dry and dry on the tongue-side (Figure 4). Similarly, compounds eluting in F13 HG1 are suggested to be the drivers of the green character in wine 37_HG1, which is linked to astringency and dry tannins (Figure 2) and dryness (Figure 4).

3.2.2. Chemical characterisation of fractions driving green character

Fractions F13 retain a large proportion of the original colour intensity (at least 66% of CI is retained in F13 in the four wines) and also a large proportion of the phenolic compounds measured by absorbance at 280 nm (at least 50% of TPI is retained in F13 in the four wines) (supplementary material 6), in agreement with previous work (Sáenz-Navajas et al., 2017). Table 3 shows that fraction F13 contains mainly anthocyanin-derivative pigments, including a major part of the monomers bleachable with sulphite (at least 87% of MP are retained in F13 in the four wines), and pigments resistant to sulphite and not precipitable with ovalbumin (at least 77% of SPP are retained in F13 in the four wines), as well as pigments resistant to sulphite and precipitable with ovalbumin (between 43% for 38_HG2 and 77% for 19_LG2 of LPP are retained in F13). The mean degree of polymerisation (mDP) of these fractions is below 4 in all cases, which is in accordance with previous works performed with this fractionation method (Gonzalo-Diago et al., 2014; Sáenz-Navajas et al., 2017). This means that F13 is free of tannins and contains small flavanols (dimers and trimers of flavanols) mainly composed of catechin or epicatechin units (%PC up to 68%) (Table 3). Interestingly, F13 HG1, with the highest stickiness, contains the highest levels of anthocyanin-derived pigments resistant to sulphite and able to precipitate proteins (LPP), as shown in Table 3. This is in accordance with previous results suggesting that
certain trimers of anthocyanins able to react with proteins could be responsible for certain astringent-related attributes perceived in red wines (Sáenz-Navajas et al., 2017).

Concerning fractions F22+F23, they contain tannins with degrees of polymerisation up to 15 units as well as up to 35% of total LPP in F22+F23_HG2 (Table 3). The tannin concentration in these fractions is given in Table 3, and as can be seen, the highest level of dry and dry on the tongue-side attributes of the fraction F22+F23_HG2 could then be related to its highest concentration of tannins with a mean degree of polymerisation of 10. However, these astringent-related attributes could not be related either to the activity of tannins as determined as “stickiness” after Revelette et al (2014) or to the level of galloylated units (Table 3) as suggested by other authors (Ferrer-Gallego, García-Marino, Miguel Hernández-Hierro, Rivas-Gonzalo, & Teresa Escribano-Bailón, 2010; Revelette et al., 2014; Soares, Mateus, & De Freitas, 2007).

3.3. Screening for volatile compounds driving green character

Cluster analysis (supplementary material 7) showed that equivalent aroma fractions from wines 1 (low green score) and 18 (high green score and the highest for vegetal aroma) are plotted very close together, indicating that they present similar aroma properties. The exception is F9, which for wine 1 is mainly sweet and floral, while alcoholic and solvent-like for wine 18. However, the GC-O analysis of these fractions did not show any difference in the aroma zones of both fractions and no relevant compound with vegetal aroma in the green wine (wine 18).

Most surprisingly, the GC-O screening of the samples (wines 18, 39-42) showing vegetal notes did not reveal the presence of any outstanding differences in the aroma profiles of non-green wines (wines 1 and 19) (data not shown). A remarkable difference was, however, that green wines seemed to have smaller GC-O scores in fruity esters and slightly higher scores in fusel alcohols. Significantly higher levels of fusel alcohols, was corroborated by quantitative analysis, as summarized in Table 4.

As seen in the table, 1-hexanol in green wines is significantly above the levels found in non-green wines (1 and 19) and in a high-quality reference sample set, but it is anyway well below its odour threshold. Levels of z-3-hexenol, with a clear green-leafy character, in green wines were however,
within the range of occurrence observed for the non-green wines 1 and 19 and the reference sample set and also below the sensory threshold. Most differently, isoamyl alcohol, methionol and β-phenylethanol were at significantly higher concentrations (P<0.001) in green wines. On the bases of previous research, it can be concluded that those levels of β-phenylethanol are not sensorily significant (De-La-Fuente-Blanco et al., 2016). Thus, the potential sensory role played by methionol and isoamyl alcohol was further tested by simple addition experiments with highly purified standards. In the case of methionol, the standard contained some other non-identified odour impurities which were removed by semipreparative HPLC. As previously found for β-phenylethanol, the reported odour threshold for methionol resulted to be strongly overestimated and the odour threshold for the completely pure standard was found to be around 9.5 mg L$^{-1}$, nearly 20 times above the previously reported threshold. Addition experiments at the 7.3 mg L$^{-1}$ maxima levels found in green wines in two different matrices resembling one young and one oaked red wine, did not have any sensory effect, which makes us conclude that methionol levels, per se, are not directly involved in the green character of wines.

Quite differently, the addition of purified isoamyl alcohol to both wine models, bringing their levels to the maxima concentrations found in green wines (454 mg L$^{-1}$, Table 4) was significantly detected by the test panel (P<0.01). Confirming previous results (De-La-Fuente-Blanco, Sáenz-Navajas, & Ferreira, 2017) some individuals resulted to be extremely sensitive to the addition of this chemical and reported it to add a pungent and metallic note to the wine. Furthermore, some of them described it as geranium-like via orthonasal and as harsh and astringent in the mouth, which made us wonder whether this compound could be involved in the formation of green character in red wines.

3.4. Sensory impact of isoamyl alcohol and astringent fractions on green character

Attending to previous results, the compounds present in fractions F22_HG2 (fraction F22 of Wine 38), F23_HG2 (fraction F23 of Wine 38) and F13_HG1 (fraction F13 of Wine 37) are suggested to be involved in green character; isoamyl alcohol could also play some role, not only in the aroma perception, but in the general green character perception. Besides, compounds responsible for
woody, ripe fruit and oxidation aroma could also counteract the green perception (Figure 1). This hypothesis is well in line with the fact that for winemakers in general, and also in some scientific papers, it is generally accepted that problems related to green character are solved or masked by ageing wines with oak (Llaudy, Canals, González-Manzano, Canals, Santo-Buelga, & Zamora, 2006) even if there is no sound scientific evidence for this fact. The present experiment could provide a clue about these assumptions. In order to check these hypotheses, two wine models with different aroma profiles were selected: one young red wine (YW) with fruity aromas, and one oaked aged red wine (OW) described with oaked, ripe fruit and oxidation aromas. These wines were spiked with the fractions F22_HG2/F23_HG2, F13_HG1 and/or with isoamyl alcohol following a factorial design and were further sensory evaluated for their green character by the panel of experts of Somontano region.

Results showed that young wines had significantly (F=11.8; P<0.001) higher scores (average = 4.9±1.6) for green character than oaked wines (average = 4.0±1.6). This result confirms that the green character is wine dependent and suggests that the oaked, ripe fruit and oxidation aromas of the oaked wine could mask green character.

Interestingly, Figures 6a and 6b show that the single addition of isoamyl alcohol, proanthocyanidins (F22+F23) or anthocyanin-derivative compounds (F13) to a young or oaked aged red wines (base samples) does not generate a significant increase (P>0.1) of the green character. Results indicate that in the young wine model, only the joint addition of both phenolic fractions (F22+F23 and F13) or the joint addition of F22+F23 and isoamyl alcohol generates a significant increase (P<0.05) on the green character of the wine. The maximum green character is observed in the sample containing the three elements (F13, F22+F23 and isoamyl alcohol). In the oaky wine model, all effects are less evident, since the green character is comparatively much reduced. Results clearly demonstrate that there is a significant increase in green character only in the samples containing isoamyl alcohol and one of the phenolic fractions (IA-F13 or IA-F22+F23). Maximum green character is observed in the sample containing the three elements simultaneously.
The panel of experts carried out additionally a quantitative description of the wine models. Results (data not shown) confirmed that the green character of the reconstituted wine models was significantly correlated to mouthfeel sensations such as sticky ($r=0.66; P<0.05$) in the young wine model and to green tannins ($r=0.71; P<0.01$) in the oaked red wine model. No significant correlation to any aroma term was found, which suggests that aromatic characteristics associated to the green character are due to additional aroma molecules not included in our models.

4. Conclusions

The green character is a multivariate character associated to both aroma and mouthfeel descriptors such as vegetal, astringency, green and dry tannins.

Although a direct link between chemicals and sensory perception has not been yet established, wine fractions containing small anthocyanin derivative pigments (<tetramers) and tannins with higher degree of polymerisation (average of decamers) seem to be the most important non-volatile drivers imparting astringent-related sensations. Similarly, fractions containing anthocyanin-derived pigments resistant to SO$_2$ and precipitating with ovalbumin seem to be related to stickiness (called dry tannins by experts), while tannin fractions seem to be responsible for dry sensations (called green tannins by experts).

The interaction between isoamyl alcohol and the anthocyanin-derivative fraction and/or tannins is suggested to contribute to the green character and to enhance it in red wines. These three elements apparently explain the sticky and dry character, but cannot explain vegetal odour nuances related to the green character. Besides, the intensity of green character is demonstrated to be wine-dependent and it is suggested to be masked by woody, oxidation and/or ripe fruit aromas present in oaked aged red wines.

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References


Figure Captions

**Figure 1.** Projection of active (in red) and supplementary –or illustrative- (in blue) variables on the two first principal components of the PCA calculated with significant attributes scored for the 38 wines evaluated in the 1st screening session (attribute_n: evaluated in nose-orthonasally; attribute_m: evaluated in mouth)

**Figure 2.** Mean scores of attributes significantly (P<0.05) discriminating among 5 wines with high (HG1-HG3) and low (LG1 and LG2) green character scores. Error bars are calculated as s/(n)\(^{1/2}\) (s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).

**Figure 3.** Green character scores for the 16 wines based on orthonasal aroma in the 2nd screening session. Error bars are calculated as s/(n)\(^{1/2}\) (s, standard deviation; n, number of panellists).

**Figure 4.** Mean scores of in-mouth attributes evaluated by RATA and significantly (P<0.05) differing among the 4 selected wines (LG1, LG2, HG1 and HG2). Error bars are calculated as s/(n)\(^{1/2}\) (s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).

**Figure 5.** Projection on the first two principal components of the PCA of a) significant attributes and b) 24 fractions (6 fractions x 4 wines) and the six clusters and significant attributes yielded from the HCA calculated with all dimensions of the PCA.

**Figure 6.** Green character scores (average of individual scores given by the panel of experts) for a) young red and b) oaked red wines spiked with two phenolic fractions (F13 and/or F22+F23) and/or isoamyl alcohol. Error bars are calculated as s/(n)\(^{1/2}\) (s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).
Figure 1.
Figure 2.

Figure showing bar charts for different attributes such as vegetal, fresh fruit, ripe fruit, wood, oily, sweetness, astringency, green tannins, dry tannins, and green character. The bars are labeled with different categories and subcategories, and some are marked with letters (a, b, c) to indicate statistical significance. The categories include 1 (LG1), 18 (HG3), 19 (LG2), 37 (HG1), and 38 (HG2).
Figure 3.
Figure 4.
Figure 5.

a) Factor 2 - 14.14%

Factor 1 - 48.18%

- burning
- bitter
- sour
- coarse
- persistent
dry tongue
- granular
dry palate
- dry
- sticky
- sweet
- greasy
- watery
- silky
Cluster 1
dry-palate, dry tongue-side
persistent, dry, granular, coarse

Cluster 2
sour, dry-palate, dry, dry tongue-side
bitter, coarse

Cluster 3
bitter

Cluster 4
sour

Cluster 5
sticky, coarse

Cluster 6
watery, silky, sweet, greasy
Figure 6

(a) addition experiments in young red wine

(b) addition experiments in oaked aged red wine
Table 1. Samples evaluated in the confirmation task aimed at evaluated the impact of isoamyl alcohol (IA), fractions F22 +F23 of wine HG2 (F2) and/or fraction F13 of wine HG1 (F13) in the green character of two different wines (YW: young and OW: oaked red wines). Y_base and O_base were not spiked (“0” means not spiked). The rest of samples were spiked (“1” means spiked with the corresponding compound or fraction) with IA, F2 and/or F13.

<table>
<thead>
<tr>
<th>Spiked wine</th>
<th>sample</th>
<th>code</th>
<th>IA (isoamyl alcohol)</th>
<th>F2 (F22_HG2 + F23_HG2 )</th>
<th>F13 (F13_HG1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young red wine (YW)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Y_base</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Y_IA</td>
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<td>0</td>
</tr>
<tr>
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<td>Y_IA_F2</td>
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<td>1</td>
<td>0</td>
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<tr>
<td></td>
<td>4</td>
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<td>1</td>
<td>1</td>
</tr>
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<td>5</td>
<td>Y_F2</td>
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<td>1</td>
<td>0</td>
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<tr>
<td></td>
<td>6</td>
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<td></td>
<td>8</td>
<td>Y_F13</td>
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<td>0</td>
<td>1</td>
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<tr>
<td><strong>Oaked red wine (OW)</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>O_base</td>
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</tr>
<tr>
<td></td>
<td>10</td>
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<td>15</td>
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<td>0</td>
<td>1</td>
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<td></td>
<td>16</td>
<td>O_F13</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tbody>
</table>
Table 2. Clusters in which wines were classified by HCA. Descriptors significantly (P<0.05) defining each cluster and correlation to the illustrative variables green character and preference. Wines marked with an asterisk are those closest to the center of gravity of the cluster and were further selected for the study of the non-volatile fraction.

<table>
<thead>
<tr>
<th>cluster</th>
<th>attributes positively correlated</th>
<th>Positive correlation with green character and/or preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1*, 27, 32, 9, 22, 10, 36, 29, 17, 5, 14, 2, 21 oxidation_n, sweetness_m, oily_m, wood_n, ripe fruit_n</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37*, 23, 30, 6, 16, 35, 26, 34, 8 dry tannins_m, astringency_m, ripe fruit_n, wood_n, aroma intensity_n</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19*, 24, 3, 7, 20, 15, 13, 11, 33, 25, 12, 31 fresh fruit_n, sourness_m</td>
<td>Preference (t-value=+1.38; P&lt;0.1)</td>
</tr>
<tr>
<td>4</td>
<td>38*, 4, 18, 28 green tannins_m, vegetal_n, astringency_m</td>
<td>Green character (t-value=+3.93; P&lt;0.001)</td>
</tr>
</tbody>
</table>

*attributes followed by _n and _m means evaluated in nose and in-mouth, respectively
Table 3. Mean degree of polymerization (mDP), percentage of flavanols constituted by procyanidins (%PC), prodelphinidins (%PD), gallates (%G) and linked to malvidin (%Mv) calculated by thyolysis. Monomeric anthocyanins (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) analysed as proposed by Harbertson et al. (2003) and expressed in absorbance units. Tannin activity and tannin concentration determined as described by Revelette et al (2014). Different letters for a parameter and the same fraction (F13 or F22+F23) means significant differences (P<0.05) among wine fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>mDP</th>
<th>%PC</th>
<th>%PD</th>
<th>%PG</th>
<th>%Mv</th>
<th>MP</th>
<th>SPP</th>
<th>LPP</th>
<th>tannin activity (J mol⁻¹)</th>
<th>tannin concentration (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F13_LG1</td>
<td>3.1±0.0ᵃ</td>
<td>75±1ᵇ</td>
<td>14±1</td>
<td>2±0</td>
<td>9±0ᵇ</td>
<td>0.47±0.03ᶜ</td>
<td>0.47±0.02ᵇ</td>
<td>0.10±0.03ᵇ</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>F13_LG2</td>
<td>1.7±0.0ᵇ</td>
<td>69±0ᵇ</td>
<td>14±0</td>
<td>2±0</td>
<td>15±0ᵃ</td>
<td>1.22±0.05ᵃ</td>
<td>0.57±0.01ᵃ</td>
<td>0.08±0.01ᶜ</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>F13_HG1</td>
<td>2.8±0.0ᵃ</td>
<td>93±0ᵃ</td>
<td>12±1</td>
<td>4±0</td>
<td>8±0ᵇ</td>
<td>0.68±0.04ᵇ</td>
<td>0.60±0.04ᵃ</td>
<td>0.25±0.04ᵃ</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
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<tr>
<td>F13_HG2</td>
<td>2.6±0.0ᵃ</td>
<td>76±0ᵇ</td>
<td>13±0</td>
<td>4±0</td>
<td>7±0ᵇ</td>
<td>0.61±0.02ᵇ</td>
<td>0.60±0.01ᵃ</td>
<td>0.12±0.02ᵇ</td>
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<td>&lt;LD</td>
</tr>
<tr>
<td>F22+F23_LG1</td>
<td>15±1ᵃ</td>
<td>71±1ᵇ</td>
<td>23±1ᵃ</td>
<td>5±0ᵃ</td>
<td>1±0ᵃ</td>
<td>&lt;LDᵇ</td>
<td>&lt;LDᵇ</td>
<td>&lt;LDᵇ</td>
<td>24526±825ᵇ</td>
<td>384±7ᶜ</td>
</tr>
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<td>F22+F23_LG2</td>
<td>&lt;LDᶜ</td>
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<td>ndᵇ</td>
<td>ndᵇ</td>
<td>&lt;LDᵇ</td>
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<td>&lt;LDᵇ</td>
<td>29499±754ᵃ</td>
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<td>13498±460ᵈ</td>
<td>584±26ᵇ</td>
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<tr>
<td>F22+F23_HG2</td>
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<td>5±0ᵃ</td>
<td>1±0ᵃ</td>
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<td>0.07±0.01ᵃ</td>
<td>0.09±0.00ᵃ</td>
<td>15732±582ᶜ</td>
<td>919±13ᵃ</td>
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</tbody>
</table>
Table 4. Concentration, concentration ranges and odour thresholds of fusel alcohols in non-green wines (wine 1 and 19), green wines (wine 18 and 39-42; n=5) and the reference sample set (n=25). For a given compound, different letters mean significant differences (P<0.001) among wines.

<table>
<thead>
<tr>
<th>compound</th>
<th>non-green wine (wine 1)</th>
<th>non-green wine (wine 19)</th>
<th>green wines (n=5)</th>
<th>reference sample set (n=25)</th>
<th>odour threshold**</th>
</tr>
</thead>
<tbody>
<tr>
<td>isobutanol</td>
<td>42120</td>
<td>45720</td>
<td>33800-43060</td>
<td>21400-61400</td>
<td>40000 1</td>
</tr>
<tr>
<td>1-butanol</td>
<td>1230</td>
<td>960</td>
<td>892-1294</td>
<td>530-960</td>
<td>150000 2</td>
</tr>
<tr>
<td>isoamyl alcohol</td>
<td>285270</td>
<td>280550</td>
<td>358220-454190</td>
<td>111000-305000</td>
<td>30000 3</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>1940</td>
<td>1380</td>
<td>2550-2960</td>
<td>520-1560</td>
<td>8000 4</td>
</tr>
<tr>
<td>z-3-hexenol</td>
<td>190</td>
<td>90</td>
<td>40-170</td>
<td>&lt;4.47-290</td>
<td>400 5</td>
</tr>
<tr>
<td>methionol</td>
<td>2560</td>
<td>2900</td>
<td>3870-7310</td>
<td>88.6-1148</td>
<td>500 6</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>1760</td>
<td>4620</td>
<td>390-1360</td>
<td>70-5400</td>
<td>200000 7</td>
</tr>
<tr>
<td>β-phenylethanol</td>
<td>44850</td>
<td>34850</td>
<td>51610-98150</td>
<td>18700-80500</td>
<td>14000 8</td>
</tr>
</tbody>
</table>

*Reference sample set of Spanish Premium red wines (n=25) referred to San-Juan et al. (2012). **Odour thresholds calculated in red wine if available; otherwise threshold in synthetic wine is given. Concentrations are expressed in micrograms per litre. Reference in which the odour threshold value has been calculated is given as superscript. 1Guth (1997); 2Etievant (2000); 3Ferreira et al (2000); 4Escudero et al (2007)

Graphical abstract

Highlights

- Green character is related to vegetable aroma, astringency, green and dry tannins
- Small anthocyanin derivatives and large tannins retain stickiness and dryness
- Fractions with anthocyanins precipitating with ovalbumin retain dry tannins
• Interaction of isoamyl alcohol and 2 phenolic fractions enhances green character
• Role of isoamyl alcohol and phenolic fractions in green character is matrix-dependent