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ORIGINAL RESEARCH ARTICLE

Yeast metabolic activity is sufficient to create a wine like aromatic feature in a synthetic grape must —a sensory-driven approach

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ABSTRACT

Wine is characterised by an underlying aromatic volatile profile, which allows human subjects to easily recognise the product as "wine" by smell alone. Yeast metabolism significantly contributes to wine organoleptic properties, and some yeast metabolism-derived compounds contribute to the vinous character of wine. However, the relative contribution of yeast and grape-derived metabolic compounds to the sensory perception of a product as "wine-like" remains unexamined. This study explores the possibility of creating a wine-like aroma by yeast metabolic activity alone. For this purpose, we fermented a simple synthetic media without any grape-derived aromatic compounds or precursors thereof. Fermentation products were evaluated for the degree of wine-like sensory perception. The synthetic grape juice nitrogen, sterol and fatty acid composition were altered to improve the recognition of this character. Initial fermentations resulted in products that were not recognised as wine-like, but over several reiterations, more wine-like associations were observed, with some products judged as similar to real wine. The data suggest that the wine-like character responsible for the recognition of a product as "wine" is largely the result of the de novo synthesis of aromatic compounds by yeast and does not require the contribution of grape-derived volatile compounds.

KEYWORDS: sensory-driven approach, *Saccharomyces cerevisiae*, chemically defined synthetic grape juice, wine-like aroma

INTRODUCTION

Wine aroma is the function of the interaction of several hundred volatile and non-volatile compounds (Sáenz-Navajas *et al.*, 2010), which have three general origins: Grape (varietal or grape processing), microbial i.e. formed during alcoholic and malolactic fermentation, and lastly ageing or storage associated aromas (Drawert, 1974; Rapp and Versini, 1995). This is indeed an overly simplistic view, as a dynamic interplay exists between compounds across "origins" as they undergo chemical changes and modifications throughout the entire winemaking process (Ferreira and Lopez, 2019; Robinson *et al.*, 2014). A significant portion of the volatile compounds produced during alcoholic fermentation are products of yeast metabolism. The production of these compounds is greatly influenced by the chemical composition of the grape juice, in particular, the nitrogen (Barrajón-Simancas *et al.*, 2011; Burin *et al.*, 2015; Dickinson *et al.*, 1997; Gutiérrez *et al.*, 2012; Torrea *et al.*, 2011; Vilanova *et al.*, 2007), fatty acid and sterol composition (Duan *et al.*, 2015; Rollero *et al.*, 2014; Rollero *et al.*, 2016; Varela *et al.*, 2012), as well as by the natural microflora and the inoculated yeast and bacterial strains (Mauricio *et al.,* 1997; Miller *et al.,* 2007; Rossouw *et al.,* 2008). The volatile compounds may interact with each other (Francis and Newton, 2005; Robinson *et al.et al.,* 2014) and with the non-volatile matrix (Sáenz-Navajas *et al.*, 2012; Villamor and Ross, 2013) by masking (De-La-Fuente-Blanco *et al.*, 2017), enhancing (Atanasova *et al.*, 2005a) or altering each other's sensory perception (De-La-Fuente-Blanco *et al.*, 2017). Consequently, it remains largely impossible to predict a specific sensory outcome based on chemical data alone (Francis and Newton, 2005; Robinson *et al.,* 2014).

In part because of this diversity and complexity, wine is one of the most widely studied and described food items, yet the very feature that is required for a product to be identified as wine has not been fully characterised. This wine-like aroma is present in every wine, regardless of wine quality, cultivar or winemaking practices. Importantly, it underlies and interacts with all the other aromatic features discussed above (Atanasova *et al.*, 2005b; De-La-Fuente-Blanco *et al.,* 2017; Sáenz-Navajas *et al.*, 2010).

This study aimed to explore the extent to which the yeast volatilome contributes to this wine-like perception. Synthetic grape juice is a chemically defined medium widely used in wine research to mimic natural grape juice. It contains only non-volatile nutrients essential for yeast growth, including sugars (glucose and fructose), acids (malic, citric and tartaric acid), minerals, vitamins, anaerobic factors (sterols and unsaturated fatty acids) as well as numerous nitrogen sources (amino acids and ammonium) the concentrations of which are easily modified. In research, synthetic juice is commonly used to reduce the overall complexity of real grape juice and to allow for reproducibility and easy comparison between data sets. Most importantly, it does not contain any volatile aromas or direct precursors of varietal aroma compounds. Therefore, it permits the evaluation of the contribution of the yeast volatilome on the wine-like feature in isolation, without the contribution of grape-derived precursors, grape varietal odorants, ageing or the impact of other microflora, including malolactic bacteria. Using a reiterative process, fermentation products were evaluated for the degree to which they resembled wine and these sensory outcomes drove the decision-making rather than the usual chemical data-driven approach.

MATERIALS AND METHODS

1. Fermentation media, conditions, and treatments

1.1. Temporal evolution of the wine-like feature during alcoholic fermentation

To establish the temporal evolution of the wine-like feature throughout fermentation, fermentations were conducted in a chemically defined juice (CDJ) adapted from Ciani and Ferraro (1996), containing ammonium chloride as the nitrogen source (200 mg/L). Fermentations (1 L) were conducted in modified 2 L Schott bottles fitted with a port for sampling and CO_2 egress. These fermentations were mediated by *Saccharomyces cerevisiae* QA23 (Lallemand, Montreal, Canada), Zymaflore VL1 (Laffort-Oenologie, Bordeaux, France), a "cachaça" strain (L328) and a yeast isolate from the Douro region, Portugal (ZA). Fermentation vessels were inoculated with a pre-culture in the logarithmic growth phase (OD 640nm ≈ 1) to an OD 640 nm of 0.1 (final cell density of approximately 10^6 CFU/mL). To evaluate the temporal expression of the wine-like feature, samples were collected daily for sensory analysis and to monitor yeast growth (OD 640 nm).

1.2. Modulating the expression of the wine-like feature at the end of alcoholic fermentation

VIN13 (Anchor Yeast, Cape Town, South Africa), an *S. cerevisiae* yeast strain, was rehydrated (20 g/hL) for 20 min at 37 °C in warm water and subsequently cooled to within 10 °C of the medium's temperature before inoculation, according to the supplier's instructions. Fermentations were conducted using a synthetic grape juice (Henschke and Jiranek, 1993) specifically formulated for wine research (100 g/L glucose and 100 g/L fructose), containing 10 mg/L ergosterol and 0.5 mL/L Tween 80 as the anaerobic factor (SGJ), as previously described (Henschke and Jiranek, 1993) unless indicated otherwise. This synthetic grape juice was formulated to mimic a natural grape must and was used in subsequent fermentations to construct a wine-like aroma.

The nitrogen composition consisted of various amino acid classes, but the concentration of fermentable nitrogen was maintained at 200 mg N/L and each amino acid provided equal amounts of fermentable nitrogen. The nitrogen treatments were classified based on their ability to support yeast growth, namely all amino acids ("all AA"), preferred ("preferred"), branched-chain and aromatic (BCAA), not utilised ("not utilised"), utilised but not preferred ("utilised") and ammonium chloride ("ammonium") (Table 1).

Static fermentations took place at 20 °C, in duplicate, and were monitored daily (CO_2 evolution).

Based on the data obtained in the previous fermentations, a final set of nitrogen and anaerobic factor treatments were selected, as summarised in Table 2, to be evaluated in combination with each other (1.5 L). Nitrogen treatments include 200 mg N/L of fermentable nitrogen in the form of ammonium chloride, MS200 amino acids (Bely *et al*., 1990) and the BCAA's (Table 1). The SGJ, ergosterol with oleic acid, phytosterol, and phytosterol with ergosterol treatments were used in combination with the nitrogen treatments as described in Table 2. Static fermentations took place at 20 °C in triplicate and were monitored daily (CO_2 evolution).

TABLE 1. Fermentations were supplemented with different nitrogen treatments, classified on how well the nitrogen sources support yeast growth or volatile aroma production.

All amino acids			Preferred amino acids			Branched-chain & aromatic amino acids			Not utilised amino acids			Utilised but not preferred amino acids			Ammonium chloride		
	mg N/L	mg/L		mg N/L	mg/L		mg N/L	mg/L		mg N/L	mg/L		mg N/L	mg/L		mg N/L	mg/L
NH_4Cl	50.0	191.7	NH_4Cl	50.0	191.7	NH_4Cl	50.0	191.7	NH_4Cl	50.0	191.7	NH_4Cl	50.0	76.7	NH_4Cl	200	766.7
ALA	7.5	47.8	ARG	30.0	93.2	ILE	30.0	200.0	HIS	50.0	150.0	ALA	30.0	100.0			
ARG	7.5	23.3	ASN	30.0	141.5	LEU	30.0	300.0	LYS	50.0	250.0	GLY	30.0	50.0			
ASN	7.5	35.4	ASP	30.0	285.7	PHE	30.0	150.0	PRO	50.0	500.0	SER	30.0	400.0			
ASP	7.5	71.4	GLN	30.0	156.3	TYR	30.0	20.0				THR	30.0	350.0			
CYS	7.5	64.9	GLU	30.0	315.8	VAL	30.0	200.0				TRP	30.0	100.0			
GLN	7.5	39.1															
GLU	7.5	78.9															
GLY	7.5	40.3															
HIS	7.5	27.7															
ILE	7.5	70.1															
LEU	7.5	70.1															
LYS	7.5	39.1															
MET	7.5	79.8															
PHE	7.5	88.2															
PRO	7.5	61.5															
SER	7.5	56.4															
THR	7.5	63.6															
TRP	7.5	54.7															
TYR	7.5	97.4															
VAL	7.5	62.5															

Table 2. Combinations of various nitrogen and anaerobic factor treatments were used.

Nitrogen (200 mg/L)	Anaerobic factor
Ammonium (NH_4)	Ergosterol, 10 mg/L & Tween80, 0.5 mL/L (SGJ)
Ammonium (NH_4)	Phytosterol, 10 mg/L (PHY)
Ammonium (NH_4)	Ergosterol, 5 mg/L & phytosterol, 5 mg/L (ERG+PHY)
Ammonium (NH_4)	Ergosterol, 10 mg/L & oleic acid, 0.5 mL/L (ERG+OLE)
Branched chain & aromatic amino acids (BCAA)	Ergosterol, 10 mg/L & Tween80, 0.5 mL/L (SGJ)
Branched chain & aromatic amino acids (BCAA)	Phytosterol, 10 mg/L (PHY)
Branched chain & aromatic amino acids (BCAA)	Ergosterol, 5 mg/L & phytosterol, 5 mg/L (ERG+PHY)
Branched chain & aromatic amino acids (BCAA)	Ergosterol, 10 mg/L & oleic acid, 0.5 mL/L (ERG+OLE)
Standard amino acids (MS200)	Ergosterol, 10 mg/L & Tween80, 0.5 mL/L (SGJ)
Standard amino acids (MS200)	Phytosterol, 10 mg/L (PHY)
Standard amino acids (MS200)	Ergosterol, 5 mg/L & phytosterol, 5 mg/L (ERG+PHY)
Standard amino acids (MS200)	Ergosterol, 10 mg/L & oleic acid, 0.5 mL/L (ERG+OLE)

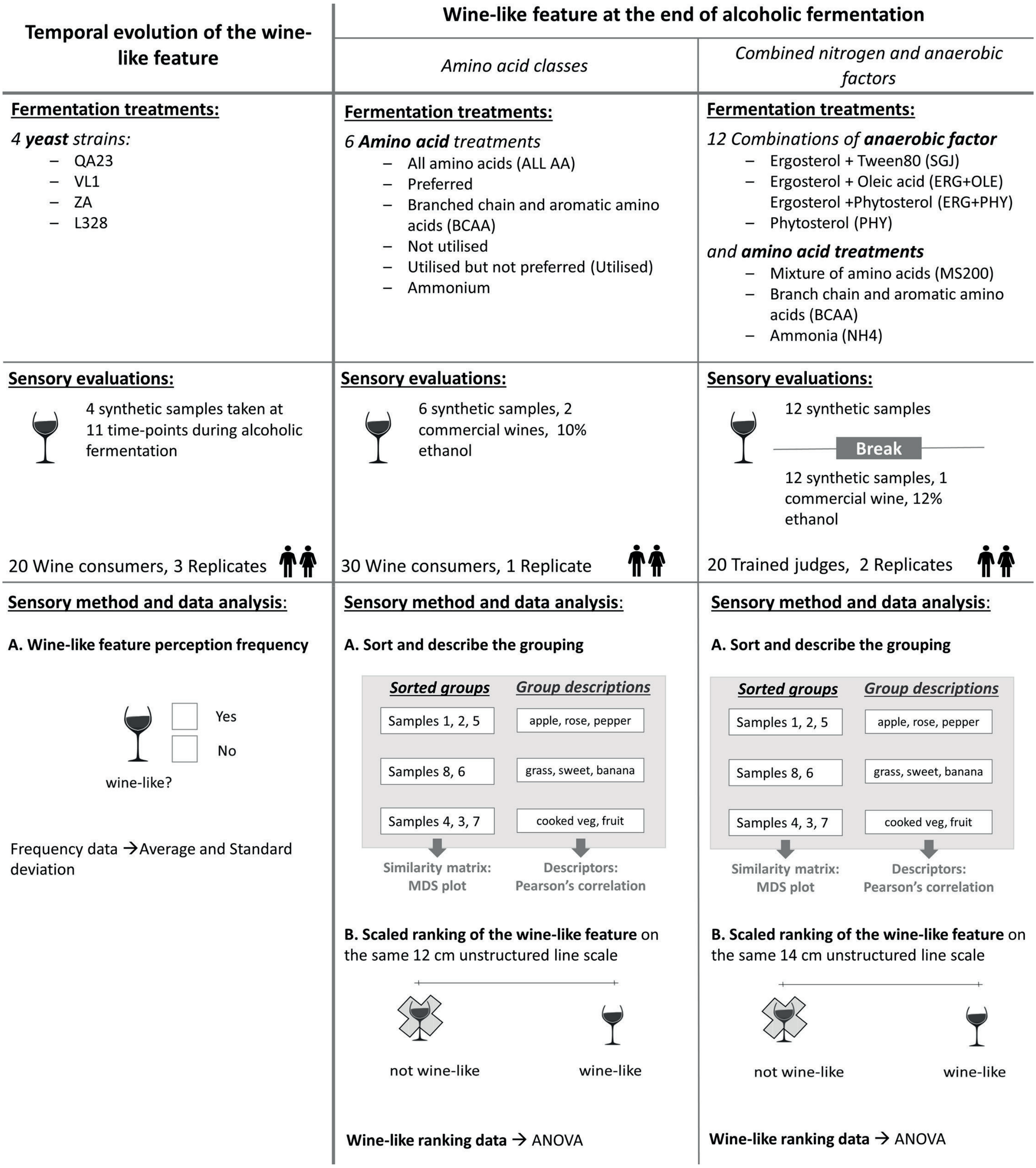

FIGURE 1. An overview of the sensory evaluations, including the fermentation parameters and the sensory methods used.

2. Sensory analyses

An overview of the sensory evaluations performed is summarised in Figure 1

2.1. Temporal evolution of the wine-like feature throughout fermentation

The testing sessions were conducted in individual booths under conditions in accordance with ISO 8589 (facilities) and ISO11037 (lighting). Eleven informal sensorial tests were performed (one for each fermentation time point). In each session, 20 untrained panellists, recruited within the department at Escola Superior de Biotecnologia, between the ages of 20 and 46 years old, evaluated the "wine-like aroma" of the four strains. The panellists evaluated the same four samples of 25 mL each in black international tasting glasses (ISO NORM 3591, 1977), coded with random three-digit codes and covered with a watch glass. Samples were randomised across panellists using a balanced complete block design. Panellists were asked whether they perceived a wine-like aroma or not. All data were collected on paper and processed using Microsoft Excel (Microsoft, Redmond, USA).

2.2. Wine-like feature at the end of alcoholic fermentation

The sensory evaluations were conducted (within 3 weeks of the completion of alcoholic fermentation) in compliance with ASTM standards (8589) in an odourless, well-ventilated room, secluded from excess noise, with controlled lighting and temperature (20 °C ±2). All samples and wines were served at room temperature and all assessments were conducted in off-white individual tasting booths. For each experiment, samples (25 mL) were presented simultaneously in black ISO glasses (ISO NORM 3591, 1977) in a randomised order using a balanced complete block design. Each glass was labelled using a 3-digit code and covered with a Petri dish. The commercial wines used were unwooded fruity Chenin blanc wines, without any perceptible faults, in addition to 10 % or 12 % ethanol samples.

Samples were only evaluated via orthonasal olfaction using a free sorting task (Cartier *et al*., 2006; Chollet *et al*., 2011; Valentin *et al*., 2012). Free sorting involves the classification of samples into distinct groups based on their perceived similarities (Valentin *et al*., 2012), which inherently requires a degree of product comparison; panellists were also asked to describe the reason for the grouping. Finally, each sample was rated with respect to the degree to which they resembled wine on the same unstructured line scale (12 cm or 14 cm) anchored at "wine-like" and "not wine-like". In the case of the combined evaluation of anaerobic factors and amino acids, panellists first evaluated the synthetic products, and after a short break, they evaluated the second set of replicates of the same synthetic samples in addition to a commercial wine and 12 % ethanol (Figure 1).

For the sensory evaluation of amino acid classes, 30 naive wine consumers were used between the ages of 18 and 65 to affirm that the wine-like character is a precept that does not require defining or training to identify it and the suitability of the wine-like scales used. These naïve wine consumers were staff and postgraduate students recruited from Stellenbosch University who did not have any formal winemaking training. In subsequent evaluations, the panel used was not trained on this matrix but had received regular and extensive training for other white wine descriptive analyses. These 20 participants (24 to 62 years of age) were recruited annually and trained to participate in sensory evaluations. Consequently, we relied on their previous experience with line-scale rating to inform the intuitive rating of the wine-like feature. All data were collected on paper and processed using Microsoft Excel.

3. Chemical analyses

3.1. Gas chromatography-flame ionisation detector analyses at the end of alcoholic fermentation

The quantification of several fermentation-derived volatile compounds was conducted using gas chromatography equipped with a flame ionisation detector (GC- FID), as described previously by Louw *et al*. (2009). Briefly, five millilitres of the sample were used with 4-methyl-2-pentanol (internal standard, 100 µL of 0.5 mg/L solution). The volatile compounds were extracted with diethyl ether (1 ml) and this mixture was then placed in an ultrasonic bath for 5 min. Following this, samples were centrifuged at 4000 g for 3 min. A Hewlett Packard 6890 Plus GC-FID instrument (Agilent, Little Falls, Wilmington, USA) with a split/splitless injector was used for major volatiles quantification. The split flow rate was set at 49.4 mL/min and the split ratio was set to 15:1 at a temperature of 200 °C. The separation of compounds was done using a J and W DB-FFAP capillary GC column (Agilent, Little Falls, Wilmington, USA) with the dimensions of 60 m × 0.32 mm and a 0.5 µL coating film thickness with the flow rate of the hydrogen carrier gas set at 3.3 mL/min. An initial oven temperature of 33 °C was held for 17 min; the temperature was then increased by 12 °C/ min to 240 °C and held for 5 min. Once the FID oven temperature reached the temperature of 240 °C, three microliters of the extracted sample was injected into the gas chromatograph. A post-run step at the end of each sample was carried out at 240 °C for 5 min. The column was cleaned with an injection of hexane after every 10 samples. Authentic reference standards (Ethyl acetate (Sigma-Aldrich, Germany); methanol (Merck, Germany); ethyl 2-methyl propanoate (Sigma-Aldrich); ethyl propionate (Sigma-Aldrich); 2-methyl propyl acetate (Sigma-Aldrich); ethyl butyrate (Fluka, Switzerland), n-propanol (Sigma-Aldrich); ethyl 2-methylbutyrate (Sigma-Aldrich); ethyl isovalerate (Sigma-Aldrich); isobutanol (Fluka); isoamyl acetate (Riedel de Haën, Germany); n-butanol (Fluka); isoamyl alcohol (Sigma-Aldrich); ethyl hexanoate (Sigma-Aldrich); pentanol (Sigma-Aldrich); hexyl acetate (Fluka); acetoin (Sigma-Aldrich); 4-methyl-1-pentanol (Fluka); 3-methyl-1-pentanol (Fluka); ethyl lactate (Fluka); hexanol (Merck); 3-ethoxy-1-propanol (Sigma-Aldrich); ethyl octanoate (Sigma-Aldrich); 1-octen-3-ol (Sigma-Aldrich); acetic acid (Sigma-Aldrich); ethyl-3-hydroxybutanoate (Merck); propionic acid (Sigma-Aldrich); isobutyric acid (Fluka); butyric acid (Fluka); ethyl decanoate (Sigma-Aldrich); isovaleric acid (Fluka); diethyl succinate (Fluka); n-valeric acid (Sigma-Aldrich); ethyl phenylacetate (Sigma-Aldrich); 2-phenylethyl acetate (Sigma-Aldrich); hexanoic acid (Sigma-Aldrich); 2-phenylethanol (Merck); octanoic acid (Sigma-Aldrich); decanoic acid (Sigma-Aldrich)) were used to calibrate for each of the compounds using the internal standard compound 4-methyl-2-pentanol (Fluka, Switzerland) 10 mg/L). A six-point calibration was performed by spiking model wine with the volatile stock solution and performing the liquid–liquid extraction as described above. Manual data collection and peak integration were done using the HP ChemStation software (Rev. B01.03 [204]).

4. Data analyses

4.1. Statistical analysis of the sensory data relating to the temporal evolution of wine-like aroma

For the sensory data, mean and standard deviations were calculated in Microsoft Excel.

4.2. Statistical analysis of the sensory data at the end of alcoholic fermentation

Participants sorted (grouped) samples based on their similarities and then described the groups made using their own words (Chollet *et al*., 2011). This sorting data was recorded for each participant in a similarity matrix which was summed for all participants. The descriptors participants provided to describe each group was used to construct a contingency table which summarised how frequently a descriptor was used to describe each of the products. Where appropriate, similar descriptors were combined using lemmatisation and categorisation, and descriptors used by fewer than 15 % of the panel were discarded (Lawrence *et al.*, 2013; Valentin *et al*., 2012). The summed similarity data matrix was evaluated using multidimensional scaling (MDS) in the case of the sorting data sets using XLSTAT 2017 (XLSTAT, Paris, France). In multidimensional scaling (MDS), samples that are close to each other are similar, whereas those that are further apart are dissimilar (Valentin *et al*., 2012). Additionally, the descriptors used to describe the products in the sorting task were projected onto the MDS plot using Pearson's correlation coefficients.

The wine-like rating data were evaluated using analysis of variance (ANOVA) (type III) paired with the Fisher LSD post hoc test ($P < 0.05$) to determine which samples are significantly different with respect to the wine-like rating (Statistica, version 13, Statsoft Inc., Tulsa, USA). A mixed-model ANOVA was used with the judges treated as a random effect and treatments as fixed effects.

4.3. Statistical analysis of the chemical data (GC-FID) at the end of alcoholic fermentation

Principal component analyses (PCA) were performed on the chemical data obtained using XLSTAT 2017, following autoscaling.

RESULTS AND DISCUSSION

1. Temporal evolution of the wine-like feature

The evolution of the wine-like feature during alcoholic fermentation was evaluated for four different *S. cerevisiae* strains (Figure 1). The data show an increase in the perception of a wine-like character over time, which reaches a peak and declines towards the end of fermentation (Figure 2). The wine-like feature is most clearly recognisable after six days. After reaching this peak, and while the product begins to appear less wine-like, the reproducibility between biological repeats also diminishes drastically. This change may be linked to a loss due to evaporation or the transition from active growth to stationary phase fermentative metabolism, which has been reported to result in shifts in volatile aroma production (Rossouw *et al*., 2008; Rossouw *et al*., 2010). During the latter stages of fermentation (238 to 334 hours), wine-likeness continues to decrease, but the agreement between panellists regarding the degree of this wine-likeness is again highly reproducible (Figure 2). The sensory data collection strategy (sample shared among participants) prevents any substantive data analysis or interpretation, but these data importantly serve as proof of the existence

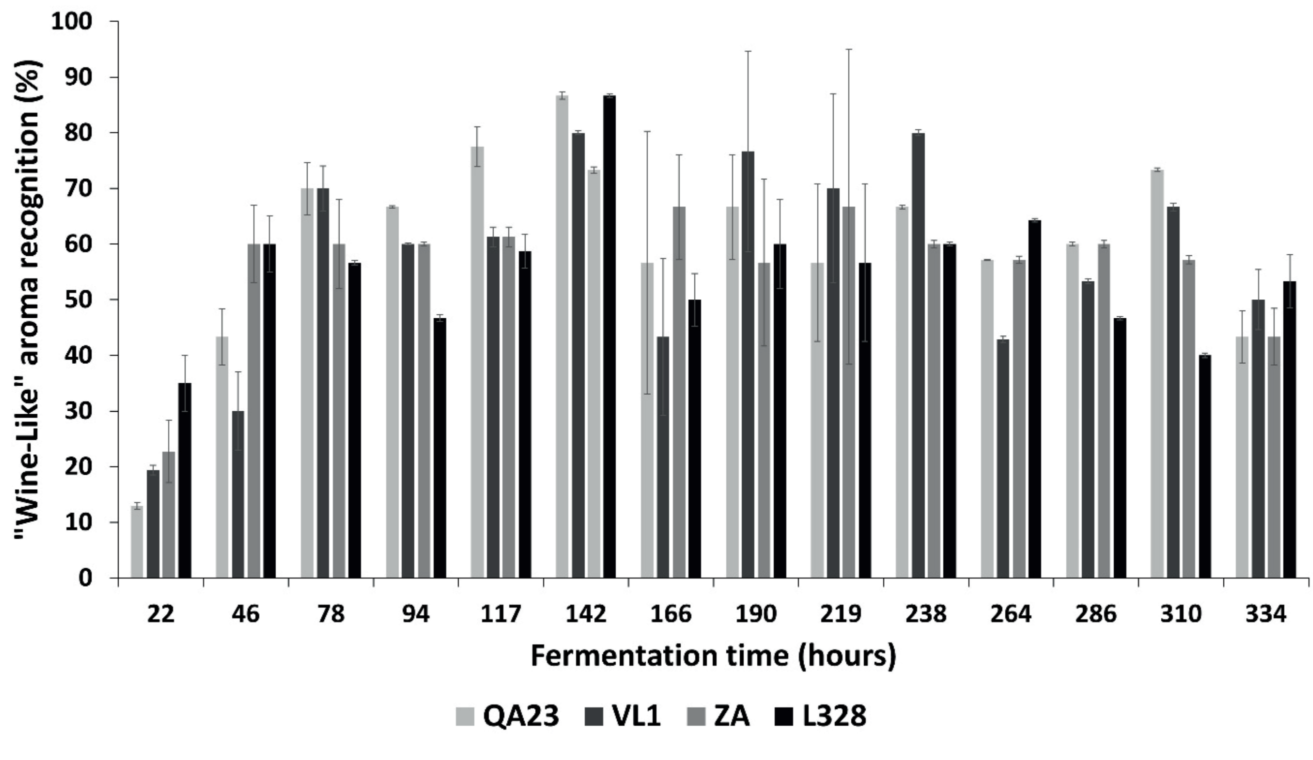

FIGURE 2. The evolution of the wine-like feature is shown by the frequency (%) with which panellists perceived a "wine-like" aroma. Values are the average of three biological repeats, with the error bars denoting the standard deviation.

of this wine-like concept. As well as suggesting that yeast metabolism of synthetic grape juice resulted in sensory features that were associated with "wine" by the panel, but that the final products of these fermentations were not considered to be "wine-like". Furthermore, the wine-like character was observed regardless of the yeast strain used (Figure 2), and consequently, only *S. cerevisiae* yeast strain VIN13 was used in subsequent fermentations as this yeast and its aromatic impact have been better characterised in other ongoing projects. Furthermore, since the aim was to obtain a final product with a wine-like character, all sensory evaluations were only carried out on the final product of each fermentation treatment, as panellists were once again in agreement with the degree of wine-likeness at this stage of fermentation.

2. Wine-like feature at the end of alcoholic fermentation

2.1. Amino acid classes

Yeast nitrogen metabolism has been shown to directly impact the successful completion of alcoholic fermentation as well as the production of volatile compounds influencing the wine volatilome (Bell and Henschke, 2005). Amino acids assimilated from the medium can be directly incorporated into proteins or catabolised to free the amine group used for the de novo synthesis of other amino acids. The "preferred" amino acid treatment (Table 1) contains amino acids (ARG, ASN, ASP, GLN and GLU) that support yeast growth well when they are the sole nitrogen source (Ljungdahl and Daignan-Fornier, 2012). The amino acids in the "not utilised" treatment (HIS and LYS) do not support growth as well as the others when they are the only nitrogen source (Ljungdahl and Daignan-Fornier, 2012) or require oxygen (PRO) to be catabolised (Duteurtre *et al.*, 1971). The "utilised" but not preferred treatment contains amino acids (ALA, GLY, SER, THR and TRP) that are not fully depleted from grape must during fermentation (Beltran *et al.*, 2004; Beltran *et al.*, 2005; Smit, 2013). Branched-chain and aromatic amino acids (BCAAs) range from average (VAL and PHE) to poor (ILE, LEU and TYR) supporters of growth (Ljungdahl and Daignan-Fornier, 2012). These BCAA amino acids may be catabolised via the Ehrlich pathway (Fairbairn *et al.*, 2017; Hazelwood *et al.*, 2008) into corresponding higher alcohols, esters and volatile fatty acids. At low concentrations, volatile fatty acids contribute positively to wine aroma, but at high concentrations, they may generate a rancid-sweaty character (Francis and Newton, 2005), whereas esters are generally associated with fruity or floral aromas (Bell and Henschke, 2005).

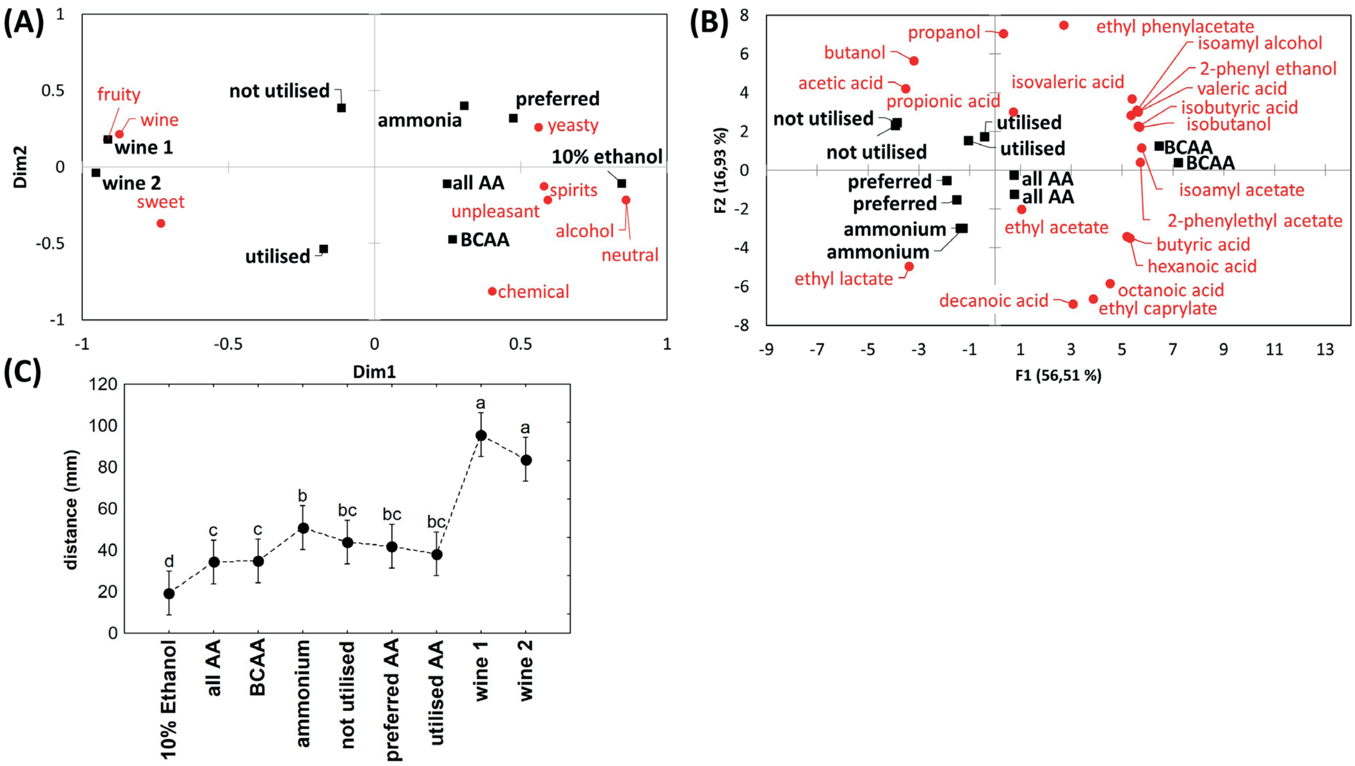

FIGURE 3. The sensory and chemical impact of amino acid class supplementation. The multidimensional scaling (Kruskal's stress of 0.128) (A) with attributes projected by calculating Pearson's correlation coefficients describing the sorting of treatments: All (all AA), preferred (preferred), branched-chain and aromatic (BCAA), not utilised (not utilised), and the utilised but not preferred amino acids (utilised), in addition to ammonium chloride (ammonium), two commercial wines and 10 % ethanol. Principal component analyses of the GC-FID data (B). The wine-like rating summarises how similar or dissimilar the products are to being wine-like (evaluated on the same 12 cm unstructured line scale); the greater the distance, the more wine-like they are (C).

In contrast, higher alcohols are greatly affected by the sensory description of the matrix, having little influence when in a neutral background but reducing the intensity of fruity and woody descriptors whilst increasing the intensity of spirit and solvent-like traits (De-La-Fuente-Blanco *et al.*, 2016; Ferreira *et al.*, 2016). Moreover, at concentrations above 284 mg/L higher alcohols are considered to have a negative impact on product preference (De-La-Fuente-Blanco *et al.*, 2017).

We used a similar sensory approach (free sorting and typicality rating) as was used to evaluate the typicality of Chardonnay and Muscadet wines (Ballester *et al*., 2008) to evaluate a wine-like character. To generate a wide variety of fermentation products with significantly different sensory profiles, entire classes of amino acids were either added in excess to or omitted entirely from the synthetic grape must, as shown in Table 1 (Figure 1). The outcome of the sensory analysis of these fermentation products, together with some real wine samples and a 10 % ethanol solution, is shown in Figure 3A. Panellists were asked to sort the synthetic samples based on their similarities and differences and to describe the reasons for their groupings using their own words (Figure 3A). This sorting data is visualised using a multidimensional scaling (MDS) plot; samples that are close to each other are similar (i.e. frequently grouped), whereas those that are further apart are dissimilar (Valentin *et al*., 2012). The commercial wines were considered similar to each other and different from the 10 % ethanol sample, with the synthetic products falling between these control samples (commercial wines and 10 % ethanol) in the first dimension (Figure 3A).

The wine samples were associated with positive descriptors such as fruity and sweet, as well as the descriptor "wine" (Figure 3A). The synthetic products were mostly described as unpleasant, yeasty and chemical. Interestingly, the "not utilised" and "utilised" treatments were more frequently described as fruity and less frequently as chemical than the other synthetic products (Figure 3A).

These differences in sensory perception between synthetic juice samples are not surprising, as the nitrogen treatments were selected to generate distinct chemical profiles, which in this case translated to sensory differences (Figure 3A, Supplementary Table S1). The fermentations treated with BCAA's contained very high levels of several volatile compounds (2-phenyl ethanol, isoamyl alcohol, isobutanol, isoamyl acetate, isobutyric acid and isovaleric acid), which are directly associated with the catabolism of these amino acids (Supplementary Table S1). Additionally, the yeast is also able to de novo synthesise these volatile compounds; the same volatile compounds are therefore also present in all treatments, albeit at comparatively lower concentrations.

While the synthetic grape juice fermentations clearly and as intended resulted in sensorially and chemically distinct products, they did not differ significantly on a wine-like rating scale. Indeed, all samples, while appearing more wine-like than the 10 % ethanol solution, remained closer to this control than to the real wines, which were readily identified as such (Figure 3D). Nevertheless, the ammonium treatment resulted in the most wine-like synthetic product. This data set suggests that the synthetic grape juice fermentations clearly resulted in distinct products based on the descriptors generated, yet no clear wine-likeness was apparent.

In a similar manner, the decreasing amounts of BCAA were evaluated (Supplementary Figure S1), as was the impact of various combinations of anaerobic factors on the expression of a wine-like aroma, yet the wine-like ratings closely resembled those shown in Figure 3C (Supplementary Figure S1A).

The inclusion of the commercial wines was useful as a means of segmenting the panellists based on whether they could recognise the wine-like concept, but it also served as a reference for comparison. Due to their more chemical nature, the synthetic products may be more negatively judged, as hedonic judgements may take precedence or confound the wine-like ratings (Charters and Pettigrew, 2007; Parr *et al*., 2010). Therefore, in subsequent sensory evaluations, the synthetic samples were first rated against a memorised abstraction of what a wine-like aroma is, and, following a short break, these same samples were again rated along with a commercial wine (Figure 1).

2.2. Combinations of nitrogen and anaerobic factor treatments

The variation in nitrogen composition led to a series of chemically different fermentation products (Figure 3) but did not significantly impact the wine-likeness of these products. Thus, the unsaturated fatty acid and sterol composition was changed together with the nitrogen composition. Unsaturated fatty acid and sterol composition of grape must have previously been shown to significantly impact fermentative volatile aroma production (Duan *et al*., 2015; Fairbairn *et al*., 2019; Mauricio *et al*., 1997; Rollero *et al*., 2014; Varela *et al*., 2012). Panellists were tasked with rating and sorting (also describing) the synthetic products (Figure 4A) and after a short break, these tasks were repeated using a second fermentation replicate with commercial wine and ethanol samples (Figure 4B). In contrast with the previous data set, the wine-like rating data generated using combinations of nitrogen and lipid treatments (Figure 4C and D) showed a progression from less to more wine-like. This evaluation was replicated, and similar trends were observed (Supplementary Figure S2). All treatments in Figure 3 contained the SGJ anaerobic factors and their low wine-like ranking mirrors the trends seen in Figure 4. For each nitrogen treatment, the SGJ sample was rated as comparatively less wine-like (Supplementary Figure S2), particularly the BCAA treatment.

Interestingly, the inclusion of a commercial wine had little impact on the relative rating of the synthetic products (Figure 4B). The commercial wine was rated as being more wine-like (Figure 4B) than the BCAA_SGJ and 12 % ethanol; however, it was not rated as more wine-like than several of the synthetic products.

When the synthetic products were evaluated on their own (Figure 4A), the MDS plot shows that each nitrogen treatment largely falls into a separate quadrant.

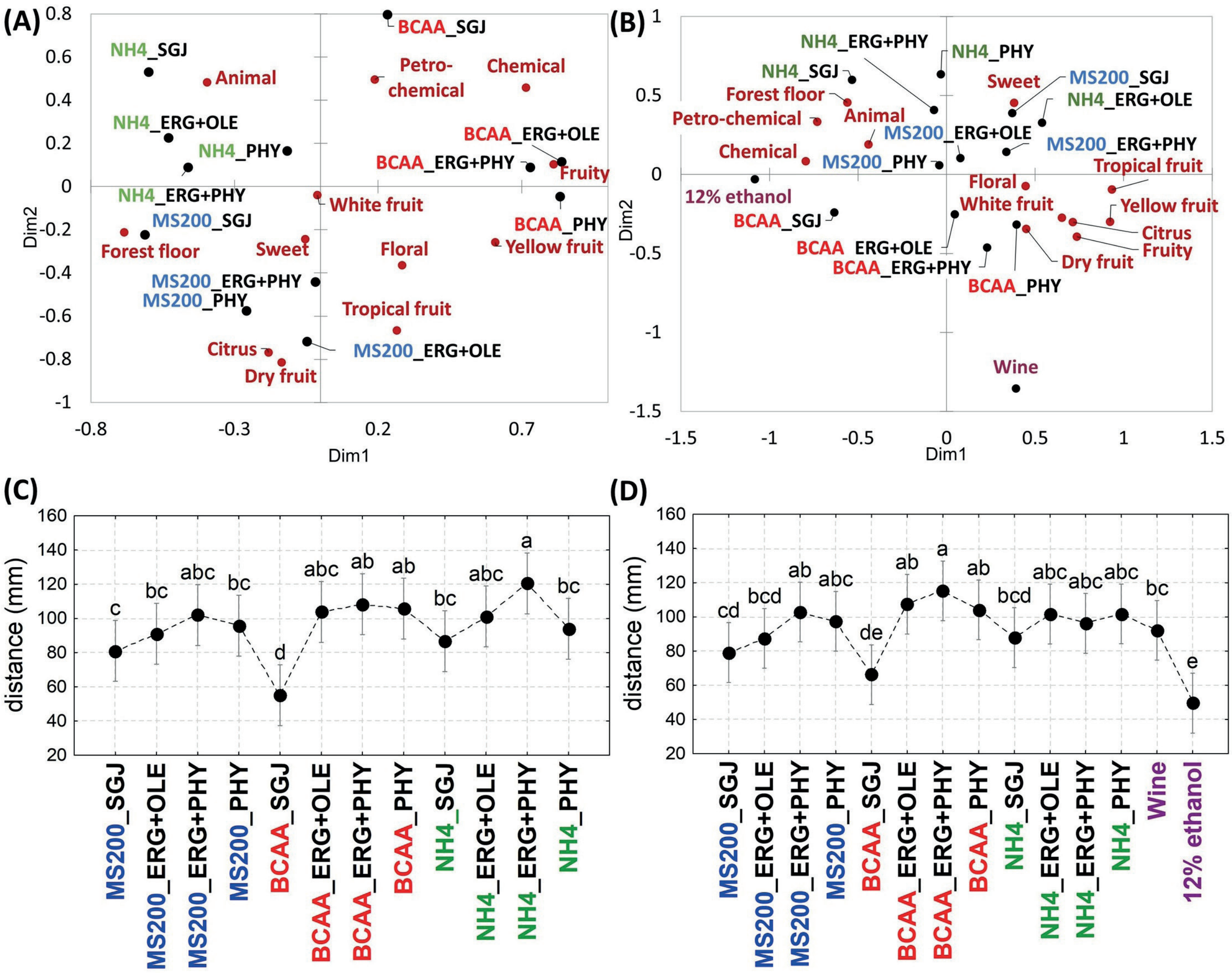

FIGURE 4. MDS analyses were performed on the sorting data with aroma attributes projected onto the space using Pearson's correlation coefficients for the synthetic products (Kruskal's stress of 0.132) (A), as well as their description relative to 12 % ethanol and commercial wine (Kruskal's stress of 0.190) (B). The wine-like rating was obtained on the same 14 cm unstructured line scale for the synthetic samples treated with various combinations of anaerobic factors (C) (phytosterol (PHY), ergosterol and oleic acid (EGR+OLE), ergosterol and phytosterol (ERG+PHY), and SGJ anaerobic factor mixture) and nitrogen compositions (BCAA, Ammonium, and MS200 amino acids), as well as rating them relative to ethanol and commercial wine (D).

The first dimension illustrates the differences between the BCAA and the ammonia and MS200 treatment grouping, and the second dimension describes the separation of the MS200 treatment from the others. Cluster analysis shows that the BCAA treatment forms a distinct grouping with the ammonia and MS200 treatments forming a second cluster (data not shown). This second cluster generally forms a continuum with the ammonium treatment at one end and the MS200 treatment at the other. The BCAA samples generally correlate with fruity and yellow fruit attributes. The MS200 products were also described as fruity (tropical fruit, citrus, dry fruit), in addition to sweet and floral. The ammonium treatment was associated with an animal aroma. In all instances, the SGJ samples were somewhat removed from the other anaerobic factor treatments. The SGJ modality was also associated with negative sensory attributes (BCAA—chemical/petro-chemical, MS200—forest floor and NH4—animal). It is possible that the SGJ treatment results in a chemical matrix whose sensory impact is enhanced to a greater degree by the high concentration of higher alcohols in the BCAA treatment than in the other nitrogen treatments (De-La-Fuente-Blanco *et al.*, 2016).

With the inclusion of the controls (ethanol and commercial wine), the first dimension describes the separation of the ethanol solution from the synthetic products, and the second dimension shows how the synthetic products and ethanol differ from wine (Figure 4B). As seen in Figure 4A, the BCAA treatments generally clustered together, with the ammonium and MS200 samples forming a second cluster (Figure 4B).

The descriptor data shows that the BCAA treatments were more frequently associated with fruity aromas (fruity, white fruit, citrus and dry fruit), except when the BCAA nitrogen was combined with the SGJ lipids. Interestingly, the other SGJ treatments were also associated with petrochemical and forest floor descriptors (Figure 4C, 4D and Supplementary Figure S2), although to a lesser extent. The inclusion of the controls had little impact on the relative distribution of the synthetic samples. Nonetheless, the improved recognition of the wine-like feature is certainly in part because of the changes to the sensory approach used, suggesting that by evaluating the synthetic samples independently first (Figure 1), the panellists were better able to evaluate the less complex synthetic samples for the presence or absence of the wine-like feature. This is confirmed by the poor wine-like rating of various anaerobic factors when evaluated with wine (Supplementary Figure S1).

Overall, the data shown in Figure 4 suggests that although most of the samples were rated as being wine-like, each also had its own specific sensory profile.

CONCLUSION

This study sought to answer a simple question, "What makes a wine a wine?" by exploring the wine-like concept using a novel reiterative and sensory-driven approach. The volatile fermentation products generated in synthetic must fermentations ultimately resembled wine. The simplified nature of synthetic juice, which only contains non-volatile compounds, and the lack of direct precursors or conjugated forms of aromatic products means that only de novo-produced yeast metabolites impact the sensory perception of the volatile wine-like feature. The data also provide for an optimised formulation of the synthetic juice for future experiments, which can expand on the current work to analyse and evaluate the sensory impact of combinations of aroma compounds.

This study shows the value of using sensory data as a driver of experimental approaches and also raises some methodological questions regarding the inclusion of standards in an experimental layout designed to assess a mental concept. In our case, it is likely that the inclusion of commercial wine in the initial experiments magnified a bias against the synthetic wines resulting in a compression of the data. In addition, it is likely that individuals have significantly different precepts of what wine-like means, and any such multidimensional quality is not easily translated into a simple linear scale. It is remarkable in this context that some of the synthetic products were clearly perceived as wine-like. These wine-like products are derived from several synthetic juices with significant differences in composition between them, suggesting that several chemical signatures will lead to a wine-like perception. Indeed, a more comprehensive characterisation of these chemical signatures is now the object of follow-up investigations, as is the sensory comparison of synthetic products to other fermented alcoholic beverages.

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REFERENCES

Atanasova, B., Thomas-Danguin, T., Langlois, D., Nicklaus, S., Chabanet, C., & Etiévant, P. (2005a). Perception of wine fruity and woody notes: Influence of peri-threshold odorants. *Food Quality and Preference*, *16*(6), 504–510. https://doi.org/10.1016/j.foodqual.2004.10.004

Atanasova, B., Thomas-Danguin, T., Chabanet, C., Langlois, D., Nicklaus, S., & Etiévant, P. (2005b). Perceptual interactions in odour mixtures: Odour quality in binary mixtures of woody and fruity wine odorants. *Chemical Senses*, *30*(3), 209–217. https://doi.org/10.1093/chemse/bji016

Ballester, J., Patris, B., Symoneaux, R., & Valentin, D. (2008). Conceptual vs. perceptual wine spaces: Does expertise matter? *Food Quality and Preference*, *19*(3), 267–276. https://doi.org/10.1016/j.foodqual.2007.08.001

Barrajón-Simancas, N., Giese, E., Arévalo-Villena, M., Úbeda, J., & Briones, A. (2011). Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. *Food Chemistry*, *127*(2), 441–446. https://doi.org/10.1016/j.foodchem.2010.12.151

Bell, S. J., & Henschke, P. a. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research*, *11*(3), 242–295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Beltran, G., Esteve-Zarzoso, B., Rozès, N., Mas, A., & Guillamón, J. M. (2005). Influence of the timing of nitrogen additions during synthetic grape must fermentations on fermentation kinetics and nitrogen consumption. *Journal of Agricultural and Food Chemistry*, *53*(4), 996–1002. https://doi.org/10.1021/jf0487001

Beltran, G., Novo, M., Rozès, N., Mas, A., & Guillamón, J. M. (2004). Nitrogen catabolite repression in *Saccharomyces cerevisiae* during wine fermentations. *FEMS Yeast Research*, *4*(6), 625–632. https://doi.org/10.1016/j.femsyr.2003.12.004

Bely, M., Sablayrolles, J. M., & Barre, P. (1990). Description of alcoholic fermentation kinetics: its variability and significance. *American Journal of Enology and Viticulture*, *41*(4), 319–324. http://www.ajevonline.org/content/41/4/319.short

Burin, V. M., Gomes, T. M., Caliari, V., Rosier, J. P., & Bordignon Luiz, M. T. (2015). Establishment of influence the nitrogen content in musts and volatile profile of white wines associated to chemometric tools. *Microchemical Journal*, *122*, 20–28. https://doi.org/10.1016/j.microc.2015.03.011

Cartier, R., Rytz, A., Lecomte, A., Poblete, F., Krystlik, J., Belin, E., & Martin, N. (2006). Sorting procedure as an alternative to quantitative descriptive analysis to obtain a product sensory map. *Food Quality and Preference*, *17*(7–8), 562–571. https://doi.org/10.1016/j.foodqual.2006.03.020

Charters, S., & Pettigrew, S. (2007). The dimensions of wine quality. *Food Quality and Preference*, *18*(7), 997–1007. https://doi.org/10.1016/j.foodqual.2007.04.003

Chollet, S., Lelièvre, M., Abdi, H., & Valentin, D. (2011). Sort and beer: Everything you wanted to know about the sorting task but did not dare to ask. *Food Quality and Preference*, *22*(6), 507–520. https://doi.org/10.1016/j.foodqual.2011.02.004

Ciani, M., & Ferraro, L. (1996). Enhanced glycerol content in wines made with immobilized Candida stellata cells. *Applied and Environmental Microbiology*, *62*(1), 128–132.

De-La-Fuente-Blanco, A., Sáenz-Navajas, M. P., & Ferreira, V. (2017). Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions. *Australian Journal of Grape and Wine Research*, *23*(2), 162–169. https://doi.org/10.1111/ajgw.12273

De-La-Fuente-Blanco, A., Sáenz-Navajas, M. P., & Ferreira, V. (2016). On the effects of higher alcohols on red wine aroma. *Food Chemistry*, *210*, 107–114. https://doi.org/10.1016/j.foodchem.2016.04.021

Dickinson, J. R., Lanterman, M. M., Danner, D. J., Pearson, B. M., Sanz, P., Harrison, S. J., & Hewlins, M. J. E. (1997). A 13C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, *272*(43), 26871–26878. https://doi.org/10.1074/jbc.272.43.26871

Drawert, F. (1974). Winemaking as a Biotechnological Sequence. *Chemistry of Winemaking, American Chemical Society*, 1–10.

Duan, L., Shi, Y., Jiang, R., & Yang, Q. (2015). Effects of adding unsaturated fatty acids on fatty acid composition of *Saccharomyces cerevisiae* and major volatile compounds in wine. *South African Journal of Enology and Viticulture*, *36*(2), 285–295. https://doi.org/10.21548/36-2-962

Duteurtre, B., Bourgeois, C., & Chollot, B. (1971). Study of the assimilation of proline by brewing yeasts. *Journal of the Institute of Brewing*, *77*(1), 28–35. https://doi.org/10.1002/j.2050-0416.1971.tb03350.x

Fairbairn, S., Mckinnon, A., Musarurwa, H. T., Ferreira, A. C., & Bauer, F. F. (2017). The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae strains*. *Frontiers in Microbiology*, *8*(DEC), 1–12. https://doi.org/10.3389/fmicb.2017.02554

Fairbairn, S., Silva Ferreira, A. C., & Bauer, F. (2019). Modulation of yeast-derived volatile aromas by oleic acid and sterols. *South African Journal of Enology and Viticulture*, *40*(2). https://doi.org/10.21548/42-2-3264

Ferreira, V., & Lopez, R. (2019). The actual and potential aroma of winemaking grapes. *Biomolecules*, *9*(12). https://doi.org/10.3390/biom9120818

Ferreira, V., Sáenz-Navajas, M. P., Campo, E., Herrero, P., de la Fuente, A., & Fernández-Zurbano, P. (2016). Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. *Food Chemistry*, *199*, 447–456. https://doi.org/10.1016/j.foodchem.2015.12.048

Francis, I. L., & Newton, J. L. (2005). Determining wine aroma from compositional data. *Australian Journal Of Grape And Wine Research*, *11*(2), 114–126. https://doi.org/10.1111/j.1755-0238.2005.tb00283.x

Gutiérrez, A., Chiva, R., Sancho, M., Beltran, G., Arroyo-López, F. N., & Guillamon, J. M. (2012). Nitrogen requirements of commercial wine yeast strains during fermentation of a synthetic grape must. *Food Microbiology*, *31*(1), 25–32. https://doi.org/10.1016/j.fm.2012.02.012

Hazelwood, L. A., Daran, J.-M. M., van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology*, *74*(8), 2259–2266. https://doi.org/10.1128/AEM.00934-08

Henschke, P. A., & Jiranek, V. (1993). Yeast: Metabolism of nitrogen compounds. In G. H. Fleet (Ed.), *Wine Microbiology and Biotechnology* (Issue August, pp. 77–164). Harwood Academic.

Lawrence, G., Symoneaux, R., Maitre, I., Brossaud, F., Maestrojuan, M., & Mehinagic, E. (2013). Using the free comments method for sensory characterisation of Cabernet Franc wines : Comparison with classical profiling in a professional context. *Food Quality and Preference*, *30*(2), 145–155. https://doi.org/10.1016/j.foodqual.2013.04.005

Ljungdahl, P. O., & Daignan-Fornier, B. (2012). Regulation of amino acid, nucleotide, and phosphate metabolism in *Saccharomyces cerevisiae*. *Genetics*, *190*(3), 885–929. https://doi.org/10.1534/genetics.111.133306

Louw, L., Roux, K., Tredoux, A., Tomic, O., Naes, T., Nieuwoudt, H. H., & Van Rensburg, P. (2009). Characterization of selected South African young cultivar wines using FTMIR Spectroscopy, Gas chromatography, and multivariate data analysis. *Journal of Agricultural and Food Chemistry*, *57*(7), 2623–2632. https://doi.org/10.1021/jf8037456

Mauricio, J. C., Moreno, J., Zea, L., Ortega, J. M., & Medina, M. (1997). The effects of grape must fermentation conditions on volatile alcohols and esters formed by *Saccharomyces cerevisiae*. *Journal of the Science of Food and Agriculture*, *75*(2), 155–160. https://doi.org/10.1002/(SICI)1097-0010(199710)75:2<155::AID-JSFA853>3.0.CO;2-S

Miller, A. C., Wolff, S. R., Bisson, L. F., & Ebeler, S. E. (2007). Yeast strain and nitrogen supplementation: Dynamics of volatile ester production in chardonnay juice fermentations. *American Journal of Enology and Viticulture*, *58*(4), 470–483.

Parr, W. V., Valentin, D., Green, J. A., & Dacremont, C. (2010). Evaluation of French and New Zealand Sauvignon wines by experienced French wine assessors. *Food Quality and Preference*, *21*(1), 56–64. https://doi.org/10.1016/j.foodqual.2009.08.002

Rapp, A., & Versini, G. (1995). Influence of nitrogen compounds in grapes on aroma compounds in wine. *Developments in Food Science*, *37*, 1659–1694.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture*, *65*(1), 1–24. https://doi.org/10.5344/ajev.2013.12070

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M. M., Dequin, S., & Mouret, J.-R. R. (2014). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Applied Microbiology and Biotechnology*, *99*(5), 2291–2304. https://doi.org/10.1007/s00253-014-6210-9

Rollero, S., Mouret, J.-R., Sanchez, I., Camarasa, C., Ortiz-Julien, A., Sablayrolles, J.-M., & Dequin, S. (2016). Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. *Microbial Cell Factories*, *15*(1), 32. https://doi.org/10.1186/s12934-016-0434-6

Rossouw, D., Næs, T., Bauer, F. F., Naes, T., Bauer, F. F., Næs, T., & Bauer, F. F. (2008). Linking gene regulation and the exo-metabolome: A comparative transcriptomics approach to identify genes that impact on the production of volatile aroma compounds in yeast. *BMC Genomics*, *9*(1), 530. https://doi.org/10.1186/1471-2164-9-530

Rossouw, D., Van Den Dool, A. H., Jacobson, D., & Bauer, F. F. (2010). Comparative transcriptomic and proteomic profiling of industrial wine yeast strains. *Applied and Environmental Microbiology*, *76*(12), 3911–3923. https://doi.org/10.1128/AEM.00586-10

Sáenz-Navajas, M.-P., Fernández-Zurbano, P., & Ferreira, V. (2012). Contribution of Nonvolatile Composition to Wine Flavor. *Food Reviews International*, *28*(4), 389–411. https://doi.org/10.1080/87559129.2012.660717

Sáenz-Navajas, M. P., Campo, E., Culleré, L., Fernández-Zurbano, P., Valentin, D., & Ferreira, V. (2010). Effects of the nonvolatile matrix on the aroma perception of wine. *Journal of Agricultural and Food Chemistry*, *58*(9), 5574–5585. https://doi.org/10.1021/jf904377p

Smit, A. Y. (2013). *The impact of nutrients on aroma and flavour production during wine fermentation*. (Doctoral dissertation, Stellenbosch University) https://scholar.sun.ac.za/handle/10019.1/80076%5Cnpapers2://publication/uuid/B5107A7C-2B29-4AB9-9BF2-B9D2245DF9E4

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice - Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry*, *127*(3), 1072–1083. https://doi.org/10.1016/j.foodchem.2011.01.092

Valentin, D., Chollet, S., Lelièvre, M., & Abdi, H. (2012). Quick and dirty but still pretty good: A review of new descriptive methods in food science. *International Journal of Food Science and Technology*, *47*(8), 1563–1578. https://doi.org/10.1111/j.1365-2621.2012.03022.x

Varela, C., Torrea, D., Schmidt, S. A., Ancin-Azpilicueta, C., & Henschke, P. a. (2012). Effect of oxygen and lipid supplementation on the volatile composition of chemically defined medium and Chardonnay wine fermented with *Saccharomyces cerevisiae*. *Food Chemistry*, *135*(4), 2863–2871. https://doi.org/10.1016/j.foodchem.2012.06.127

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. a. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Applied Microbiology and Biotechnology*, *77*(1), 145–157. https://doi.org/10.1007/s00253-007-1145-z

Villamor, R. R., & Ross, C. F. (2013). Wine matrix compounds affect perception of wine aromas. *Annual Review of Food Science and Technology*, *4*, 1–20. https://doi.org/10.1146/annurev-food-030212-182707