

Article

Profiling the Aroma of Grape Spirits for Port Wine Using a Multi-Analytical GC Approach and Sensory Analysis

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Abstract

Port wine production involves the addition of grape spirit to halt fermentation and retain natural sweetness. This spirit, produced by distilling wine and its by-products, must comply with legal standards, including a mandatory sensory assessment. Because grape spirit influences Port wine's volatile composition, this study investigated the odour-active compounds present in several grape spirits intended for fortification. Volatile compounds were extracted by liquid–liquid extraction, concentrated, and analysed using gas chromatography–olfactometry (GC-O) and gas chromatography–mass spectrometry (GC-MS). In GC-O, based on frequency detection, a panel of assessors sniffed the extracts to determine the presence of aroma compounds. The results revealed a wide range of odour-active compounds in grape spirits, belonging to several chemical families such as esters, alcohols, terpenic compounds and acids. These compounds exhibited both pleasant aromas, such as *fruity*, *floral* and *caramel* notes as well as undesirable ones like *cheese* and *foot odour*. Most of these compounds originate from the fermentation process and are also found in other unaged distilled beverages, including young Cognac, Calvados and fruit spirits. This research highlights the aromatic complexity of grape spirits and, for the first time, determined the aroma thresholds for 25 of 36 the compounds studied at an ethanol content of 20%.

Keywords: grape spirits; volatiles; odorant compounds; sensory thresholds; sensory profile



Academic Editors: Valentina Terio,
Patrizio Lorusso and
Pandiscia Annamaria

Received: 16 December 2025

Revised: 12 January 2026

Accepted: 13 January 2026

Published: 16 January 2026

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1. Introduction

Port wine, commonly referred to simply as “Port,” is a fortified wine originating from the demarcated Douro region in Portugal. Like other fortified wines, Port wine production, includes the addition of a grape spirit to the fermenting grape juice at a certain point, a process known as fortification [1–3]. This process halts fermentation, preserving a

significant portion of the natural grape sugars and resulting in the characteristic sweetness of the wine. Additionally, fortification raises the alcohol content of the wine, typically to around 19–22% *v/v*.

The composition of Port wine is influenced by several factors, including the grape varieties used, the vinification process, the ageing process and also the fortification with distilled grape spirit, as summarised in several works [1–3]. While it has long been understood that the addition of grape spirit primarily serves to increase the ethanol content, it was traditionally believed to have minimal impact on the final sensory profile of the wine. However, research by Rogerson and de Freitas [4] challenges this view, demonstrating that grape spirits influence the volatile composition of Port wine. Their study showed that fortification increases the levels of several key odorant compounds, thereby enhancing the aromatic complexity of the wine. This effect could also result, as mentioned by Tredoux and Ferreira [1] to the fact that in contrast with other fortified wines, in Port wine the added fortified spirit is not highly rectified (77% *v/v*). Despite these findings, Ribeiro et al. [2] note that only four studies have investigated the volatile composition of grape spirits used in Port wine production. These studies [4–7] have identified a total of 23 volatile compounds, including two alcohols, nine aldehydes, eight esters, one phenol, and three terpenic compounds. Furthermore, studies on unaged wine spirits obtained from *Vitis vinifera* grape varieties have revealed a wide range of volatile compounds, encompassing several chemical families, including higher alcohols, esters, aldehydes and ketones [8–10], with similar findings reported for distillates from other grape varieties [11,12].

Given the diversity of chemical compounds involved, which are influenced by factors such as grape variety, fermentation conditions and distillation methods, the study of grape spirit aroma requires a multifaceted approach. A comprehensive, multi-analytical methodology is essential to unravel the complexities of its aroma profile. One of the most effective tools for this analysis is gas chromatography (GC), which, when combined with a range of complementary techniques, provides a detailed molecular fingerprint of the volatile compounds present in grape spirits.

In recent years, the combination of Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Olfactometry (GC-O) has gained prominence as a powerful approach for identifying and quantifying volatiles and odorant compounds in wine and spirits [4,8–12] as well as in other foods [13]. These techniques enable not only the chemical identification of aroma-active compounds but also provide insights into their contribution to the sensory properties of the spirit. When combined with sensory analysis, this approach bridges the gap between instrumental data and human perception, enhancing the understanding about grape spirits composition.

The aim of this study is to characterise the aroma profile of grape spirits intended to Port wine production through a comprehensive GC-based analytical approach, complemented by sensory evaluation. The identification of key volatile compounds and the assessment of their contribution to the overall aroma profile will provide a deeper understanding of the grape spirits compounds with the greatest sensory relevance and impact on quality. Ultimately, the findings may support the development of more precise techniques for controlling and analysing aroma compounds during the production of grape spirits for Port wine.

2. Materials and Methods

2.1. Reagents

Anhydrous sodium sulfate was obtained from AppliChem GmbH (Darmstadt, Germany). Dichloromethane (99.9% purity) was supplied by Honeywell Riedel-de Haën (Steinheim, Germany) and silanised glass wool was sourced from Supelco (Steinheim, Germany).

GC-FID, GC-O and GC-MS used standards: acetic acid from Riedel-de-Haën; isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, benzyl alcohol, 2-phenylethanol, pentanoic acid, butanoic acid, isovaleric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, isobutyl acetate, ethyl 2-methylbutyrate, ethyl hexanoate, ethyl lactate, 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol; *trans*-2-hexen-1-ol, 1-heptanol, linalool, ethyl decanoate, diethyl succinate, α -terpineol, nerol, phenethyl acetate, ethyl dodecanoate, ethyl hydrocinnamate and ethyl tetradecanoate from Fluka, all acquired from Honeywell (Seelze, Germany); ethyl butanoate, 1-propanol, 2-ethyl-1-hexanol, isobutyric acid and ethyl octanoate from Merck, ethyl isobutanoate, ethyl isovalerate, isoamyl acetate, 2-methylbutyl acetate, ethyl pentanoate, 1-butanol, methyl hexanoate, 3-methyl-3-buten-1-ol, 5-methyl-2-hexanol (internal standard), 1-octen-3-ol, furfural, methyl decanoate, 3,4-dimethylphenol (internal standard) from Aldrich and 2,3 butanedione and ethyl hexadecanoate from Sigma-Aldrich were acquired from Merck KGaA (Darmstadt, Germany); 3-methyl-1-pentanol was acquired from TCI (Oxford, UK).

2.2. Samples

Two sets of grape spirit samples (23 in total), comprising twelve and eleven distinct samples, were supplied by Symington Family Estates Vinhos S.A. (Vila Nova de Gaia, Portugal). All samples had an ethanol content of 77% *v/v* and, according to company's experience, exhibited distinct characteristics. Additional information from their general analyses is provided in the Supplementary Materials (Table S1). Each sample set was evaluated by the INIAV tasting panel and analysed for its volatile composition.

2.3. Sensory Analysis

2.3.1. Aroma Descriptive Analysis of Grape Spirits

The grape spirit samples were diluted to an ethanol content of 20% *v/v* using a 5% hydroalcoholic solution one day prior to the tasting session. This procedure was intended to mimic the fortification process in Port wine production, in which a partially fermented wine ($\approx 5\%$ *v/v*) is blended with a grape spirit (77% *v/v* ethanol) to achieve a final ethanol content of 20%. The diluted samples were stored in the dark at 20 °C until analysis. Sensory evaluation was performed by a panel of eleven trained tasters during 2021 and 2022.

Owing to the pandemic situation, health and safety measures were implemented. Tasters entered the tasting room wearing masks, which were only removed once they were seated and ready to begin the sensory evaluation. To comply with public health regulations, the tasting room was operated at 50% capacity to ensure appropriate physical distancing between participants.

The tasters evaluated orthonasal aroma attributes, including *alcohol*, *fruity*, *floral*, *dried fruits*, *smoke*, *toasted*, *oily*, *sweet*, *green*, *tails*, *glue/varnish* and *caoutchouc*. Each attribute was rated using a structured scale ranging from 0 (no perception) to 5 (strong perception). Overall aroma quality, corresponding to the tasters' perception of the sample in terms of the absence or presence of defects and aroma balance, was also rated on a scale from 0 to 20. The tasting sessions were conducted in sensory room at Polo de Inovação de Dois Portos, Instituto Nacional de Investigação Agrária e Veterinária (INIAV), using individual white booths. Each taster evaluated 30 mL of each sample, served in standard ISO 3591:1977 wine-tasting glasses [14]. Sessions were held in the morning between 10:00 a.m. and 12:00 a.m. Samples were coded with three-digit random numbers and presented in a balanced order to minimise first-order carryover effects.

2.3.2. Orthonasal Odour Thresholds of Identified Odorants and OAV Calculation

Orthonasal detection thresholds (OTs) of key aroma compounds were determined in a 20% *v/v* ethanol-water solution using triangle sensory tests, as described in the

literature [15,16]. The resulting threshold values were subsequently used to calculate the corresponding odour activity values (OAVs), which were obtained by dividing the concentration of each compound by its respective OT, as proposed by Guth [17].

For the OT determination procedure, each sample (15 mL) was presented in a screw-capped 60 mL amber glass vial (height: 90.5 mm; internal diameter: 35 mm) and maintained at a controlled temperature of 20 °C. Five concentrations of each compound were tested in descending order, with each concentration being 2.5 times lower than the previous one. At each concentration level, one spiked sample was presented together with two blanks, forming a triangle. The position of the spiked sample within the triangle (AAB, ABA, or BAA) was randomised and counterbalanced across tasters and all samples were coded with three-digit random numbers.

A total of fourteen trained tasters participated in the sensory evaluation. They were instructed to sniff all three samples in each triangle and to identify the odd (spiked) sample based on orthonasal aroma perception. Each 1 h sensory session, conducted in individual booths of the INIAV sensory room, involved the evaluation of three compounds per taster.

Detection thresholds for individual tasters were determined using the Best Estimate Threshold (BET) method, following the procedure described by Piornos et al. [16]. For each taster, the BET was calculated as the geometric mean between the highest concentration at which the sample was incorrectly identified and the next lower concentration correctly identified. When tasters failed to identify the spiked sample at the highest concentration, their BET was estimated as the geometric mean between the highest tested concentration and a hypothetical higher concentration, obtained by multiplying the highest analysed concentration by the factor of 2.5. Conversely, for tasters who correctly identified the spiked sample at all concentration levels, the BET was estimated as the geometric mean between the lowest tested concentration and a hypothetical lower concentration, calculated using the same factor. The final group BET for each compound was calculated as the geometric mean of all individual BETs.

All tasters provided informed consent, confirming their voluntary participation in the sensory sessions conducted as part of this study. The sensory evaluations were performed in accordance with the ethical guidelines approved by the INIAV Executive Board.

2.4. Volatiles Analysis

2.4.1. Extraction of Volatiles

Volatile compounds were extracted according to a previously validated methodology [18]. For each grape spirit sample (100 mL), previously diluted to 20% (*v/v*) ethanol content, 1.6 mL of 5-methyl-2-hexanol (internal standard, 81.0 mg/L in a 50% (*v/v*) ethanol solution) and 0.5 mL of 3,4-dimethylphenol (internal standard, 100 mg/L in ethanol) were added. The samples were then extracted with dichloromethane in three successive volumes (30 mL, 10 mL, and 10 mL).

The combined organic phases were concentrated using a rotary evaporator (Büchi Rotavapor R-114; Büchi Labortechnik AG, Flawil, Switzerland) at 42 ± 0.5 °C, without vacuum, to a final volume of approximately 0.25 mL. Each sample was extracted in duplicate. The resulting extracts were stored at -20 °C until analysis by GC-O, GC-FID, and GC-MS.

2.4.2. Gas Chromatography-Olfactometry (GC-O) Analysis

GC-Olfactometry (GC-O) analysis was performed using an Agilent Technologies 6890 Series gas chromatograph (Wilmington, DE, USA), equipped with a fused silica capillary column coated with polyethylene glycol (DB-WAX, JW Scientific, Folsom, CA, USA; 30 m length, 0.32 mm i.d., 0.50 μ m film thickness). Hydrogen was used as the carrier gas and each extract was injected in splitless mode. At the end of the capillary column, the

effluent was split between the flame ionization detector (FID) and the olfactory detection port (ODP; Gerstel, Mülheim, Germany) maintained at 220 °C to prevent condensation of volatile compounds. Humidified air (17 cm³/min) was introduced at the sniffing cone to minimise nasal fatigue and dryness in the sniffers. The operating conditions were as follows: injector temperature, 250 °C; FID, 260 °C and ODP, 220 °C. Hydrogen (H₂ ≥ 99.9992%) was used at a constant flow of 2.4 mL/min. The oven temperature programme was: initial hold at 35 °C for 6 min, increased by 3.5 °C/min to 55 °C, followed by 10 °C/min to 85 °C, 7.5 °C/min to 100 °C, 10 °C/min to 130 °C (held for 1 min), and finally 5 °C/min to 210 °C, held for 30 min.

A trained panel of seven sniffers, aged between 26 and 57, was employed to identify odour-active compounds. Each sniffer was seated at the sniffing port and instructed to smell the column effluent. Upon perception of an aroma, the sniffer pressed an “olfactometer button” (Gerstel, Mülheim, Germany) to register the detection, thereby generating an aroma-gram simultaneously with the FID chromatogram (Supplementary Materials, Figure S3). Odour-active compounds were characterised by the number of sniffers detecting the odour simultaneously (detection frequency method [19]), the odour descriptions by the whole panel and the retention indices calculated from the retention times of a homologous series of n-alkanes (C8–C26, C28, and C30) analysed under similar conditions [20]. A peak was considered odour-active if it was detected by at least three judges in the same sample, in accordance with previously established criteria [21].

Due to pandemic-related safety protocols, the GC-O control computer was relocated to an adjacent room, separated from the GC-O equipment by a glass partition, allowing physical distancing between the operator and sniffers. Between each session, the olfactometer button, seating area, and table were thoroughly cleaned and disinfected.

2.4.3. GC-FID Analysis

Gas chromatography with flame ionisation detection (GC-FID) was performed using an Agilent Technologies 6890 N GC System (Santa Clara, CA, USA) equipped with an FID, operating under similar conditions to those used in GC-O analysis and employing a HP-INNOWAX (30 m × 0.320 mm i.d. × 0.25 µm; Agilent J&W Technologies, Santa Clara, CA, USA). The quantification of volatile compounds was carried out based on the analysis of standards and the corresponding calibration curves.

2.4.4. GC-MS and GC-TOF/MS Analysis

Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a Finnigan MAT Magnum instrument (San Jose, CA, USA). A 0.4 µL aliquot of each extract was injected, and volatile compounds were separated using a fused silica capillary column coated with polyethylene glycol (HP-INNOWAX, 30 m × 0.25 mm i.d. × 0.25 µm film thickness; Agilent J&W Technologies, Santa Clara, CA, USA). The GC conditions were similar to those of GC-O but helium (He ≥ 99.9992%) was employed as the carrier gas. The mass spectrometer was equipped with an ion trap detector and operated in electron impact (EI) mode at 70 eV. Data were acquired in full-scan mode over a mass range of *m/z* 30–340. Linear retention index (LRIs), were calculated through analysis of a commercial hydrocarbon mixture (C8–C26; C28; C30; Supelco, Bellefonte, PA, USA), using the same chromatographic conditions [20].

Compound identification was performed by matching the mass spectra with those from the NIST and Wiley libraries. Identification was further supported by comparing calculated linear retention indices (LRIs) with those reported in the NIST database and in relevant scientific literature. For the majority of the compounds, identification was confirmed by injection of the standard compounds. Additionally, matching the odour

attributes reported by the sniffers with those of the sensory panel, during the determination of sensory thresholds, provided further confirmation.

Owing to the difficulty in identifying all the odorants compounds, the extracts were further analysed on a GC-TOFMS system consisting of an Agilent 8890GC System (Agilent Technologies, Santa Clara, CA, USA) with a BenchTOF-Select detector (Markes International, Bridgend, UK). An automatic sampler injector (CTC Analysis autosampler PAL-System; SepSolve Analytical, Peterborough, UK) was used and the data were acquired and analysed with TOF-DS 4.1 of Markes International (Bridgend, UK). Chromatographic separation was achieved on a ZB-WAX capillary column (60 m × 0.25 mm i.d. and 0.25 µm df; Phenomenex, Torrance, CA, USA). The oven temperature program began at 40 °C hold for 5 min, raised at 4 °C min⁻¹ up to 240 °C. Helium was used as carrier gas and hold for 10 min and a split ratio of 50:1. The MS transfer line and source temperatures were set at 250 °C. Electron ionization (EI) at 70 eV was used to record full scan mass spectra of the analytes over the range m/z 30–400 Da. The linear retention index values were calculated through analysis of the commercial hydrocarbon mixture (C7–C30), using the same chromatographic conditions. The volatile compounds were identified by matching mass spectra with spectra of reference compounds in NIST mass spectral library (NIST MS Search Program Version 2020), also taking into consideration structure and molecular weight, and additionally, by comparing linear retention indices calculated with the LRI described in the literature.

2.5. Statistical Analysis

The sensory and chromatographic data were subjected to multivariate analysis, including clustering and principal component analysis (PCA). All calculations were performed using the NTSYS-pc package, version 2.1 [22] and the Statistica software, version 10 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Grape Spirits Aroma

To evaluate whether the aroma attributes could help to distinguish between the different wine spirits samples, multivariate analysis, specifically PCA, was applied to the sensory data of the 23 samples. The first three components explain 66% of the total variability of the data. The projection of the samples and variables on the planes formed by components 1 and 2, and by components 1 and 3, is shown in Figure 1.

The first component appears to separate the samples according to the overall quality, with samples exhibiting higher overall quality on the positive side of this component and those with lower overall quality on the negative side. The descriptors *fruity*, *floral*, *sweet* and *dried fruits* seem to contribute to the positive side and are more strongly correlated with higher overall quality. In contrast, descriptors associated with lower overall quality include *tails*, *oily*, *toasted*, *smoke* and *herbaceous*. These findings are supported by the cluster analysis results (Figure 2). The samples marked with an asterisk correspond to those analysed by GC-O and are distributed across all quadrants of the planes formed by components 1 and 2, and by components 1 and 3, confirming that these samples have distinct sensory profiles and corresponding differences in overall quality, as also shown by the cluster analysis (Figure 2). The 12 selected samples adequately represent the variability under study, as illustrated by the PCA performed using only these samples (Supplementary Materials, Figure S1). In this analysis, the variability associated with each component increases, with component 1 explaining 40% and component 2 explaining 25% of the total variance.

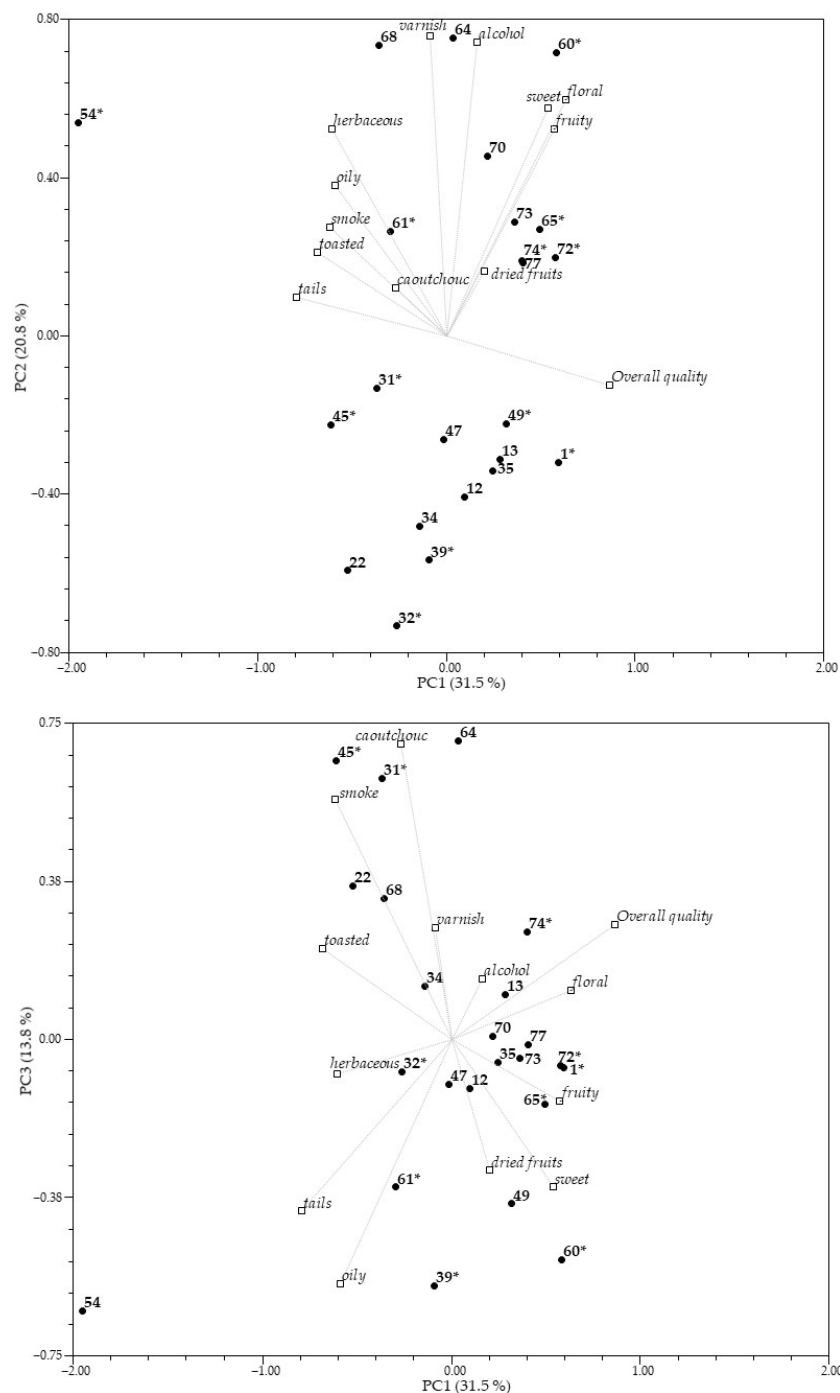


Figure 1. Biplot projection of the samples (grape spirits) and variables (aroma attributes and overall quality) onto the planes defined by the first and second components (PC1 × PC2), and the first and third components (PC1 × PC3). Sample number assignments are described in Table S1. Sample names marked with an asterisk correspond to those selected for GC-O analysis.

Taking these results into account and considering the applicability and practicality of the GC-O technique, a subset of 12 samples was selected for GC-O analysis. These samples exhibited distinct organoleptic characteristics and a corresponding range of overall quality values. The 12 samples marked with an asterisk in Figures 1 and 2 were thus subjected to GC-O analysis. Among these, some were of lower quality (overall quality < 12), namely samples 54, 39 and 45. Notably, sample 54, which exhibited the lowest overall quality, showed high intensities of *tails* and *oily* descriptors. Samples 39 and 45 exhibited low intensities of *fruity*, *floral* and *sweet* notes, but displayed some herbaceous character-

istics (see Supplementary Materials, Figure S2). In contrast, the highest-rated samples (overall quality > 13.0), namely samples 49, 74, 72, 65 and 1 displayed higher intensities of *fruity*, *floral* and *sweet* descriptors (Supplementary Materials, Figure S2). Meanwhile, the intermediate-quality spirits (overall quality > 12 and <13), such as samples 31, 61, 60 and 32 presented an intermediate sensory profile (Supplementary Materials, Figure S2).

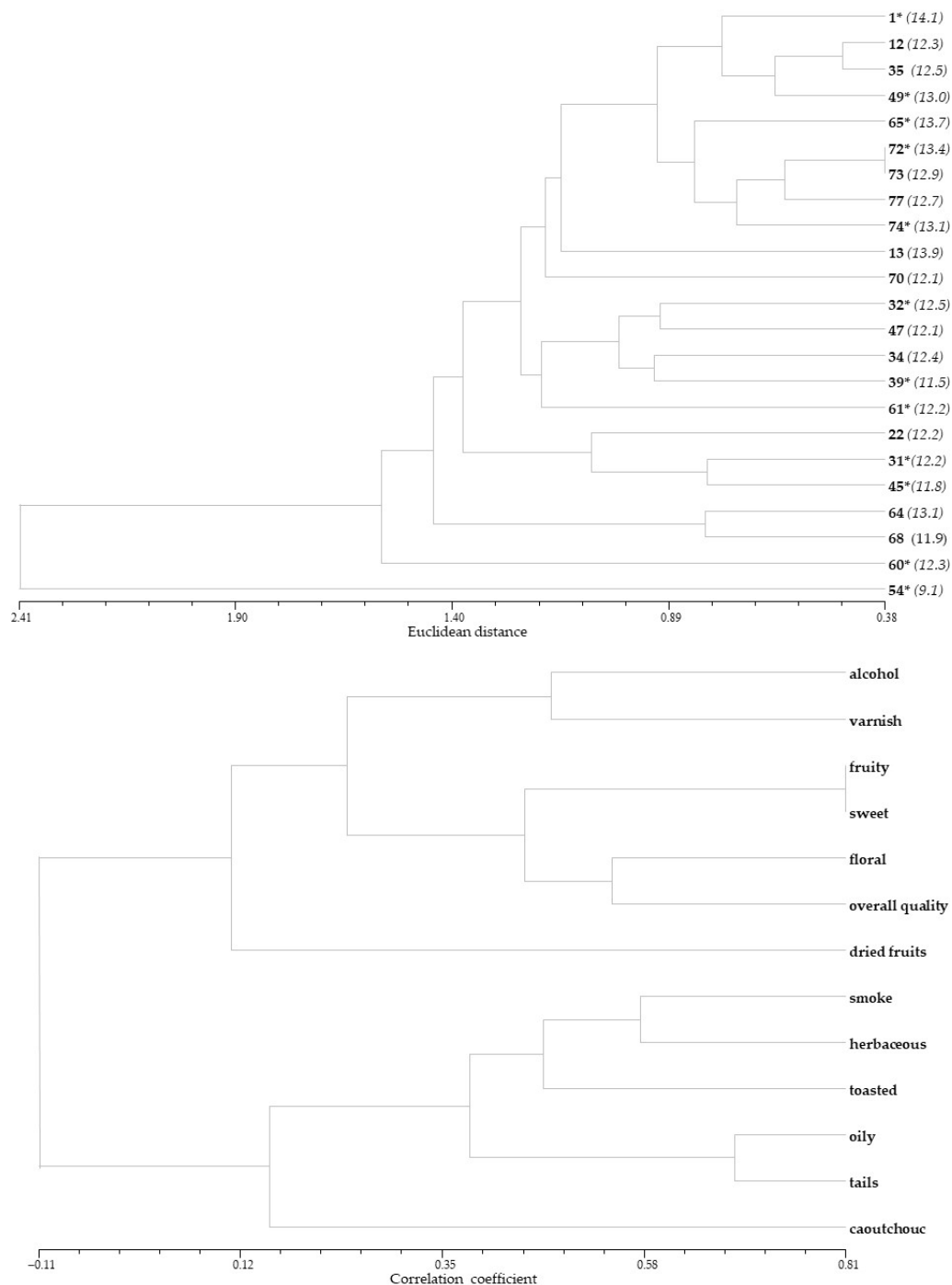


Figure 2. Dendrogram of the grape spirits based on sensory data, constructed from the distance matrix calculated using the initial results matrix (23 spirits \times 13 variables), and dendrogram of the variables, obtained from the correlation matrix derived from the same initial results matrix. The average overall quality attributed by the INIAV tasting panel is shown in parentheses, and sample names marked with an asterisk correspond to those selected for GC-O analysis. Sample number assignments are described in Table S1.

3.2. GC-O Analysis of Grape Spirits

The spirits analysed by GC-O exhibited a rich and complex volatile profile, as illustrated by the chromatogram and aromagram provided in the Supplementary Materials (Figure S3), in accordance with previous results [4]. In total, several dozen volatile compounds were identified, some of which were odourless, such as ethyl lactate or ethyl decanoate, while 38 compounds from various chemical families were identified as odour-active compounds (Table 1), as they were detected by at least by three sniffers in at least one grape spirit sample analysed. However, because sniffing revealed odour-active zones in areas with little or no corresponding chromatographic peaks (see Supplementary Materials, Figure S3), some compounds could only be identified using GC-TOF/MS.

The results of GC-O analysis of the twelve samples of grape spirits for the 38 identified odorant active compounds are summarised in Table 1. Most of the compounds identified as odour-active correspond to those previously reported in studies on wine distillates [8–10] and in distillates used in the production of Port wines [4]. However, ethyl hydrocinnamate was only identified in spirits intended for Port wine by Rogerson and de Freitas [4]. The exceptions are compounds j, l, ai, r, aa, ab, and al, which, although previously identified in other distillates and wine distillates [23,24] have only now been identified as odour-active compounds in this study. These differences may result from variations in the methodologies used to obtain extracts for GC-O analysis, but they may also be attributable to the broader range of grape spirit samples analysed in this study, which exhibited different organoleptic profiles, as discussed previously.

Table 1. Frequency of detection (%) from GC-O analysis, including odour attributes reported by sniffers, calculated linear retention indices (LRIs), compound codes and details of the identification criteria.

Odour Attributes (from Sniffers at GC-O Runs)	Identification *	LRI	Identified Compound	Codes	Detection Frequency (%) Data in Grape Spirit Samples											
					61	65	54	60	72	74	1	39	45	49	31	32
Esters																
Fruity	MS, LRI, S	955	ethyl isobutanoate	a	0	14	71	0	0	0	14	14	29	0	0	0
Fruity, floral, geranium	MS, LRI, S	1032	isobutyl acetate	c	43	29	0	71	71	57	43	57	57	71	100	71
Sweetish, fruity	MS, LRI, S	1045	ethyl butanoate	d	43	57	57	43	71	43	86	71	57	29	43	14
Fruity, red fruits, floral	MS, LRI, S	1060	ethyl 2- methylbutanoate	e	100	86	100	100	71	86	57	43	100	86	71	0
Floral, cooked, not defined	MS, LRI, S	1077	ethyl isovalerate	f	86	86	100	86	86	86	100	100	86	100	100	29
Fresh floral/ fruity, sweetish	MS, LRI, S	1134	2-methyl butanol acetate	h	0	0	0	14	14	0	0	29	14	0	14	43
Fruity, banana	MS, LRI, S	1139	isoamyl acetate	i	43	14	14	43	57	57	43	14	14	71	29	86
Fruity, floral	MS, LRI, S	1143	ethyl pentanoate	j	29	14	57	14	29	57	43	29	29	29	29	71
Fruity	MS, LRI, S	1192	methyl hexanoate	l	0	0	29	0	0	14	43	0	29	14	0	14
Fruity, strawberry, watermelon	MS, LRI, S	1254	ethyl hexanoate	q	71	71	100	71	71	86	100	71	86	86	0	57
Fruity, toasted, caramel, oily, chemical	MS, LRI, S	1448	ethyl octanoate	x	86	57	57	86	71	71	100	57	29	14	43	43
Floral, undefined	MS *, LRI, S	1612	methyl decanoate	ae	0	0	14	0	14	57	43	29	0	14	0	0

Table 1. Cont.

Odour Attributes (from Sniffers at GC-O Runs)	Identification *	LRI	Identified Compound	Codes	Detection Frequency (%) Data in Grape Spirit Samples											
					61	65	54	60	72	74	1	39	45	49	31	32
Roses, undefined	MS, LRI, S	1684	ethyl succinate	af	0	0	0	14	0	14	0	0	0	43	14	14
Chemical, disinfectant	MS, LRI	1701	ethyl 9-decenoate ‡	ag	86	14	71	57	57	14	0	0	14	0	0	0
Fruity, cooked fruit, honey	MS, LRI, S	1836	2-phenethyl acetate	aj	0	14	14	29	0	43	0	0	0	0	0	0
Jam, cooked fruit	MS, LRI, S	1858	ethyl dodecanoate	ak	29	43	71	43	29	0	14	0	0	14	0	0
Floral, fruity, sweet	MS *, LRI, S	1911	ethyl hydroxycinnamate	al	0	0	14	0	57	29	0	0	0	0	0	0
Floral, herbaceous, fruity	MS, LRI, S	2055	ethyl tetradecanoate	an	0	0	14	0	43	14	0	29	0	14	0	0
Oily, pungent, floral	MS *, LRI, S	2267	ethyl hexadecanoate	ap	14	0	14	43	14	29	0	0	43	0	0	0
Ketones																
Caramel, toffee, butter	MS, LRI, S	978	2,3-butanedione	b	57	43	100	57	57	71	29	71	57	86	0	0
Terpenic alcohols																
Floral, sweetish	MS, LRI, S	1565	linalool	ac	0	43	14	0	14	43	29	57	0	0	0	0
Floral, fruity, sweet	MS, LRI, S	1814	nerol	ai	14	14	14	14	29	14	43	29	43	43	0	0
Alcohols																
Chemical, heavy, foot odour	MS, LRI, S	1119	isobutanol	g	14	29	14	43	86	71	71	43	43	29	14	14
Foot odour, dry fruits, leguminous, chocolate, cheese, herbaceous	MS, LRI, S	1239	3-methyl-1-butanol	m	100	100	100	14	86	100	71	100	71	86	57	100
Oily, pungent, foot odour, herbaceous	MS, LRI, S	1242	2-methyl-1-butanol	n	14	14	0	100	0	14	43	29	29	14	29	0
Burned, foot odour	MS, LRI, S	1279	3-methyl-3-buten-1-ol	r	0	0	0	0	0	0	0	0	0	0	71	43
Herbaceous, fruity, undefined	MS *, LRI, S	1330	3-methyl-1-pentanol	s	14	14	0	43	14	0	0	0	29	0	0	0
Herbaceous, undefined	MS, LRI, S	1375	1-hexanol	t	43	14	43	0	14	29	14	0	14	0	14	14
Herbaceous, fresh grass	MS, LRI, S	1385	cis-3-hexenol	u	71	0	57	71	0	43	0	14	14	29	14	0
Acidified, reminiscent of garlic, undefined	MS, LRI, S	1420	trans-2-hexenol	v	0	29	14	0	14	14	0	43	29	14	0	14
Smoke, herbaceous, earthy	MS, LRI, S	1455	1-octeno-3-ol	z	0	43	0	0	14	43	0	71	0	0	29	57
Jatoba fruit, foot odour, animal feed	MS, LRI, S	1474	1-heptanol	aa	43	0	29	29	14	29	0	43	0	0	0	0
Cooked, undefined	MS *, LRI, S	1512	2-ethyl-1-hexanol	ab	0	29	0	43	43	71	0	0	0	14	14	14
Floral, herbaceous, roses	MS, LRI, S	1939	2-phenylethanol	am	29	14	14	29	43	14	14	43	14	29	0	0
Acids																
Butter, fruity, smoke, vegetable	MS, LRI, S	1600	isobutanoic acid	ad	0	14	43	14	14	0	0	0	14	14	14	0
Foot odour, cheese	MS, LRI, S	1694	3-methylbutanoic acid	ah	43	14	43	29	57	71	0	29	0	0	43	0

Table 1. Cont.

Odour Attributes (from Sniffers at GC-O Runs)	Identification *	LRI	Identified Compound	Codes	Detection Frequency (%) Data in Grape Spirit Samples											
					61	65	54	60	72	74	1	39	45	49	31	32
Undefined and sweetish	MS, LRI, S	2098	octanoic acid	ao	0	0	0	0	0	29	29	43	14	14	0	0
Floral, vegetable, mould, chemical, plastic	MS, LRI, S	2320	decanoic acid	aq	29	0	57	14	43	0	14	43	43	57	0	0

* Identification based on LRI (linear retention index), MS (mass spectrometry with ion trap and TOF detectors), MS* (mass spectrometry with TOF detector) and S (standard). ¥—The only compound for which calculation of the LRI was not possible and for which the LRI value from the literature was used [24].

The majority of the identified compounds (approximately 50%) are esters, which generally exhibit pleasant fruity aromas (Table 1), in agreement with previous publications [2]. This group comprises the largest number of compounds identified in both grape spirits and Port wines. The second largest group consists of alcohols, which exhibit a wide range of sensory descriptors (see Table 1). The third group comprises acids, which typically have unpleasant and heavy aromas (Table 1). Finally, two terpene alcohols and 2,3-butanedione were also identified as odour-active compounds, exhibiting pleasant floral and caramel aromas, respectively.

The results presented in Table 1 were used to perform a PCA (Figure 3) to evaluate whether the compounds could differentiate the various samples and identify which ones contributed most to the observed variability. The analysis showed that the three components explained only 51% of the variability: component 1 accounted for 22%, component 2 for 16%, and component 3 for 13%.

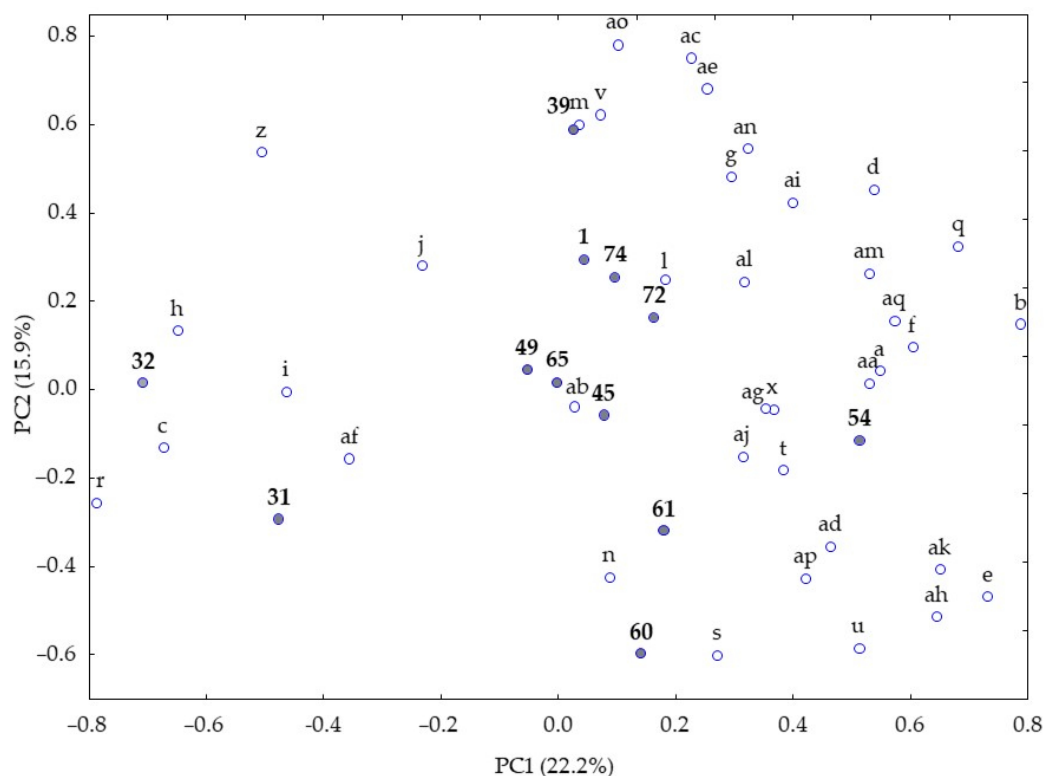


Figure 3. Biplot projection of samples and variables (PC1 × PC2) based on frequency of detection results from all sniffers at GC-O, for all 38 compounds and the 12 spirits. Sample number assignments are described in Table S1.

Figure 3 shows the projection of the variables and samples onto the plane formed by the first two components (PC1 × PC2). The compounds contributing most strongly

to the negative side of Component 1 were c (isobutyl acetate), h (2-methyl butanol acetate), i (isoamyl acetate), r (3-methyl-3-buten-1-ol) and z (1-octen-3-ol). On the positive side, the main contributing compounds were a (ethyl isobutanoate), b (2,3-butanedione), d (ethyl butanoate), e (ethyl 2-methylbutanoate), f (ethyl isovalerate), q (ethyl hexanoate), u (*cis*-3-hexenol), aa (1-heptanol), ad (isobutanoic acid), ah (3-methylbutanoic acid), ak (ethyl dodecanoate), am (2-phenylethanol), and aq (decanoic acid).

For Component 2, the compounds contributing most strongly to the negative side were e (ethyl 2-methylbutanoate), u (*cis*-3-hexenol), ah (3-methylbutanoic acid), ak (ethyl dodecanoate) and s (3-methyl-1-pentanol). In contrast, the compounds contributing most strongly to the positive side were d (ethyl butanoate), g (isobutanol), m (3-methyl-1-butanol), v (*trans*-2-hexenol), z (1-octen-3-ol), ac (linalool), ae (methyl decanoate), an (ethyl tetradecanoate), and ao (octanoic acid).

Sample 54, which exhibited the lowest overall quality, is clearly separated from the other samples, consistent with the sensory analysis results (Figure 1). It appears to be more closely associated with compounds aa (1-heptanol) and t (1-hexanol), which contribute respectively to *heavy* and *herbaceous* aromas. In the same quadrant but further away, samples 60 and 61 appear, most closely associated with the compounds n (2-methyl-1-butanol) and s (3-methyl-1-pentanol). Samples 31 and 32 appear further along the negative side of component 1, more strongly associated with compounds h (2-methyl butanol acetate), c (isobutyl acetate), and r (3-methyl-3-buten-1-ol).

Sample 39, also of low quality (Figure 2), is separated along component 2 and is more closely associated with compounds v (*trans*-2-hexenol) and m (3-methyl-1-butanol) which are related to *vegetal* odour notes.

In contrast, the samples with the highest aroma quality (1, 65, 49, 72, and 74) are located in the central zone of the graph (Figure 3), suggesting that their quality is not linked to a high frequency of any single compound, but rather to a balanced combination of various compounds.

Therefore, a more in-depth study of these compounds specifically, their quantification and threshold determination at alcohol levels similar to those in grape spirit applied at fortification Port wine is necessary to better understand which compounds have the greatest odour impact in grape spirits.

3.3. Orthonasal Odour Thresholds of Identified Odorant Compounds and OAV Calculation

Although many threshold values have been determined in hydroalcoholic solutions with an alcohol content similar to that of wine (10–14% *v/v*), this study aims to understand the potential impact of volatile compounds present in spirits when added to Port wine, which typically has a final alcohol content of around 20% *v/v*. Therefore, thresholds were determined using a 20% *v/v* hydroalcoholic solution, as described in the Materials and Methods Section 2.3.2.

Thresholds were determined for 36 of the 38 identified compounds, as two standards, namely ethyl 9-decenoate and 3-methyl-1-pentanol, were not available. The values obtained are generally higher than those reported in the literature (Table 2), possibly due to the influence of ethanol on sensory perception [25], particularly on threshold values [26]. However, even among studies using similar ethanol concentrations, considerable variability exists (Table 2), likely due to methodological differences, especially the number of panellists, which varies significantly between studies. Notably, for *trans*-2-hexenol, this study presents the first threshold determination in an hydroalcoholic solution.

Table 2. Olfactory detection thresholds determined for 36 compounds in 20% hydroalcoholic solution by a panel of 14 tasters and thresholds values reported in the literature.

Attributes Proposed by the Tasters During the Othonasal Aroma Evaluations	Compound	Ortonasal Threshold at 20% <i>v/v</i> (µg/L)	Threshold(s) Values (µg/L) from the References, Determined at Different Values of Ethanol (EtOH) Content
Fruity, strawberry	ethyl isobutanoate	406	15 (EtOH ~12%) [17,27,28]
Fruity/banana, varnish	isobuthyl acetate	1736	1600 (EtOH ~10%) [29,30]
Fruity and lactic notes	ethyl butanoate	1590	20–400 (EtOH ~12%); 9.5 (EtOH 60%); 800; 32 (EtOH 20%) [17,26,28,29,31,32]
Fruity, strawberry, sweet	ethyl 2-methylbutanoate	12	1–18 (EtOH ~11%) 1.6 (EtOH 35%); 0.2 (EtOH 60%) [17,27,32]
Fruity, banana, strawberry	ethyl isovalerate	24	3–15 (EtOH ~11%), 1.6 (EtOH 60%) [17,27,31,32]
Fruity, banana	2-methylbutanol acetate	570	313 (EtOH 12%) [33]
Fruity, banana	isoamyl acetate	558	30–200 (EtOH ~10%); 245 (EtOH 60%) [17,28,29,31,32,34]
Fruity, stawberry	ethyl pentanoate	53	5.8 (water); 5 (EtOH 20%) [35,36]
Fruity, pinneapple	methyl hexanoate	1049	130 (water) [36]
Fruity	ethyl hexanoate	62	1.5–80 (EtOH ~11%); 30 (EtOH 60%); 27 (EtOH 20%) [17,26–29,31,32]
Fruity, fresh, tropical	ethyl octanoate	199	2–580 (EtOH ~10%); 25 (EtOH 20%) [26–29,31,34]
Floral, sweet	methyl decanoate	475	1200 (EtOH ~12%) [37]
Vegetal/herbaceous	ethyl succinate	441,159	1200–300,000 (EtOH ~12%) [28,29,38]
Roses, floral	2-phenylethyl acetate	1170	108–1800 (EtOH ~10%) [17,28,29,32,34]
Soap, floral	ethyl dodecanoate	757	590 (water) [39]
Floral, sweet, rockrose	ethyl hydrocinnamate	10	1.6–1.9 (EtOH 12%) [27,40]
Fruity, sweet, herbaceous	ethyl tetradecanoate	238,745	494,000 (EtOH 14%) [28]
Fresh, undefined	ethyl hexadecanoate	92,890	2000 (EtOH 11%); 14,000 (EtOH 9.4%) [41,42]
Caramel	2,3-butanedione	56	19.5–100 (EtOH ~11%); 1250 (EtOH 20%); 2.8 (EtOH 60%) [17,32,43,44]
Floral, orange blossom, citrus, rose	linalool	44	15–25.2 (EtOH ~11%); 23 (EtOH 60%); 2 (EtOH 20%) [16,26,27,29,31,32]
Floral, citrus, lemon	nerol	3303	700 (EtOH 10%) [45]
Oily, herbaceous	isobutanol	630,771	40,000; 75,000 (EtOH ~11%) [28,29,34]
Cocoa/chocolate, oily, banana	3-methyl-1-butanol	16,569	6500–56,100 (EtOH 11%); 87,000 (EtOH 20%) [17,26,28,31,32,34,42,43,45]
Oily, varnish	2-methyl-1-butanol	53,267	45,000 (EtOH 60%); 8000 (EtOH 20%) [26,32]
Almond, floral	3-methyl-3-bute-1-ol	6773	547 (water) [35]
Herbaceous	1-hexanol	13,453	1100–41,000 (EtOH ~10%); 2000 (EtOH 20%) [17,26,28,29,32,34,42]
Vegetable/herbaceous	<i>cis</i> -3-hexenol	3941	400–1000 (EtOH 10%) [17,29]
Fruity	<i>trans</i> -2-hexenol	5856	-
Earthy, mushroom, mold/mildew	1-octen-3-ol	36	1.5 (water) [34]

Table 2. Cont.

Attributes Proposed by the Tasters During the Orthonasal Aroma Evaluations	Compound	Ortonasal Threshold at 20% v/v (µg/L)	Threshold(s) Values (µg/L) from the References, Determined at Different Values of Ethanol (EtOH) Content
Citrus, oily	1-heptanol	2482	2500 (wine) [46]
Eucalyptus, herbaceous, pungent	2-ethyl-1-hexanol	64,971	25,482 (water) [34]
Roses, floral	2-phenylethanol	16,024	569–14,000 (EtOH ~11%) [16,17,27,28,31,32,34,42]
Foot odour, cheese	isobutanoic acid	11,699	2300–200,000 (EtOH ~10%) [17,27,28,34]
Cheese, foot odour	3-methyl butanoic acid	1632	0.4–3000 (EtOH ~12%); 33 (water) [16,17,27,28,32,34,39]
Soap, vegetable	octanoic acid	22,697	569–15,000 (EtOH ~11%); 2000 (EtOH 20%) [26–29,31,34]
Plastic, rubber, vomit	decanoic acid	65,624	1000–15,000 (EtOH ~11%) 1000 (EtOH 20%) [17,26–29,32,34]

As expected, the threshold values vary widely among compounds. The lowest thresholds were observed for ethyl 2-methylbutanoate, ethyl isovalerate, ethyl hydrocinnamate, 2,3-butanedione, linalool and 1-octen-3-ol. Most of these compounds are associated with pleasant aromas, except for 1-octen-3-ol, which has an unpleasant odour (Table 2).

Using the calculated threshold values, the odour activity values (OAVs) were determined. Accordingly, the corresponding odour-active compounds were quantified in the 23 samples under study by GC-FID, as detailed in the Section 2.4.3. The detailed results are presented at Supplementary Materials (Tables S2 and S3) and a summary of the results is shown in Table 3.

Table 3. Summary of the ranges of odour compound contents in the 23 grape spirits and the corresponding OAVs.

Code	LRI	Compound Identified	Content (mg/L)		OAV	
			Maximum	Minimum	Maximum	Minimum
		Esters				
a	955	ethyl isobutanoate	2.75	0.22	6.77	0.54
c	1032	isobutyl acetate	0.60	0.08	0.34	0.05
d	1045	ethyl butanoate	4.44	0.46	2.79	0.29
e	1060	ethyl 2-methylbutanoate	1.10	0.08	91.07	6.40
f	1077	ethyl isovalerate	1.73	0.13	71.92	5.21
i	1139	isoamyl acetate	10.41	0.64	18.63	1.15
j	1143	ethyl pentanoate	0.30	0.01	5.74	0.16
l	1192	methyl hexanoate	0.14	0.00	0.13	0.00
q	1254	ethyl hexanoate	10.96	0.87	177.70	14.10
x	1448	ethyl octanoate	25.89	1.52	129.90	7.63
ae	1612	methyl decanoate	0.09	0.00	0.18	0.01
af	1684	ethyl succinate	14.66	1.36	0.03	0.00
aj	1836	2-phenethyl acetate	0.75	0.01	0.64	0.01
ak	1858	ethyl dodecanoate	1.31	0.05	1.73	0.07
al	1911	ethyl hydroxycinnamate	0.11	0.00	11.03	0.00
an	2055	ethyl tetradecanoate	0.48	0.00	0.00	0.00
ap	2267	ethyl hexadecanoate	0.03	0.00	0.00	0.00

Table 3. Cont.

Code	LRI	Compound Identified	Content (mg/L)		OAV	
			Maximum	Minimum	Maximum	Minimum
		Ketones				
b	978	2,3-butanedione	1.98	0.00	35.74	0.00
		Terpenic compounds				
ac	1565	linalool	0.74	0.07	16.73	1.51
ai	1814	nerol	0.03	0.00	0.01	0.00
		Alcohols				
g	1119	isobutanol	420.69	211.16	0.67	0.33
m	1239	3-methyl-1-butanol	2179.06	654.01	131.52	39.47
r	1279	3-methyl-3-buten-1-ol	0.53	0.12	0.08	0.02
t	1375	1-hexanol	12.96	1.15	0.96	0.09
u	1385	<i>cis</i> -3-hexenol	2.76	0.00	0.70	0.00
v	1420	<i>trans</i> -2-hexenol	0.25	0.05	0.04	0.01
z	1455	1-octeno-3-ol	0.22	0.00	6.23	0.00
ab	1512	2-ethyl-1-hexanol	0.90	0.01	0.01	0.00
am	1939	2-phenyl ethanol	46.30	0.66	2.89	0.04
		Acids				
ad	1600	isobutanoic acid	0.32	0.00	0.03	0.00
ah	1694	3-methylbutanoic acid	3.15	0.00	1.93	0.00
ao	2098	octanoic acid	8.99	0.64	0.40	0.03
aq	2320	decanoic acid	4.50	0.24	0.07	0.00

The OAVs shown in Table 3 indicate that several odorants have low values (<1) namely isobutyl acetate, methyl hexanoate, methyl decanoate, ethyl succinate, 2-phenylethyl acetate, ethyl tetradecanoate, ethyl hexadecanoate, nerol, isobutanol, methyl 3-buten-1-ol, 1-hexanol, *cis*-3-hexenol, *trans*-2-hexenol, 2-ethyl-1-hexanol, isobutanoic acid, octanoic acid and decanoic acid, suggesting that they are likely to have little impact on the overall aroma.

The acids associated with unpleasant odours (Table 1) had a low impact on the aroma of the grape spirits, as most exhibited OAVs < 1 (Table 3). The only exception was 3-methylbutanoic acid, which exhibited an OAV > 1, with a maximum value of 1.98 (Table 3), but only in four specific samples, namely samples 45, 49, 60 and 61 (Tables S2 and S3, Supplementary Materials).

Among the alcohols, those with the greatest impact on aroma are 3-methyl-1-butanol, 2-phenylethanol and 1-octen-3-ol. The latter, despite its unpleasant aroma, shows considerable variation among the samples, with the highest impact observed in samples 32, 39, 73, 74 and 77.

The compounds with the greatest impact on aroma and consequently the highest OAVs belong to the ester group. Ethyl hexanoate and ethyl octanoate are particularly prominent, exhibiting OAVs greater than 100 in some samples. Linalool and 2,3-butanedione also exhibit high OAVs. In accordance with previous work [4] the added spirit is the main contributor to the Port wine contents of ethyl octanoate, ethyl hexanoate and linalool.

A PCA was conducted using the odorant compounds with OAVs >1 (see Table 3) and selected results are presented in Figure 4. The three main components explained 65% of the total variability, with Component 1 accounting for 31.5%, Component 2 for 18.9% and Component 3 for 14.6%.

Figure 4 shows the projection (PC1 × PC2) of the samples and odorant compounds on the plane defined by the first two principal components. While most samples are concentrated in the central part of the plot, some separation is evident. Notably, samples 1 and 49 are clearly separated and are associated with high levels of compounds i (isoamyl acetate), ac (linalool), and x (ethyl octanoate), compounds linked to *fruity* and *floral* aromas

(see Table 1). These two samples also clustered together in the dendrogram of the sensory analysis and were associated with higher overall quality scores (Figure 2).

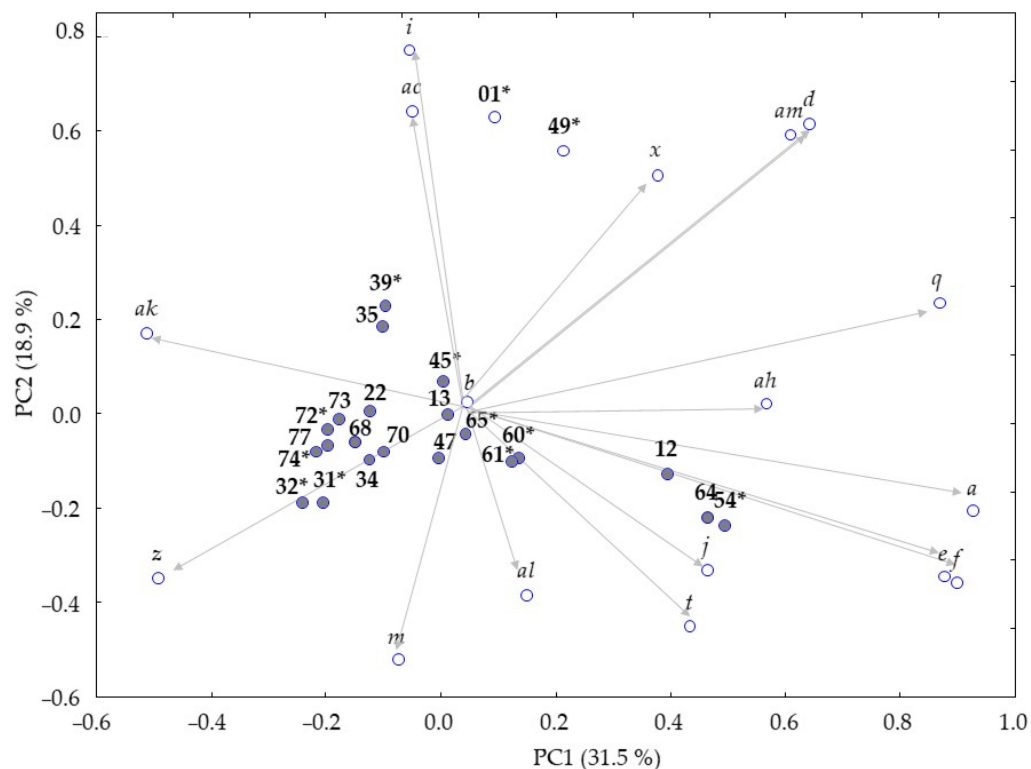


Figure 4. Biplot (PC1 × PC2) constructed using the results of odour compounds with OAV > 1 quantified in the 23 grape spirits samples. Letters correspond to the compounds listed in Table 3. Sample number assignments are described in Table S1. Sample names marked with an asterisk correspond to those selected for GC-O analysis.

In contrast, samples 12, 64, and 54 are positioned separately and are more associated with high levels of ethyl pentanoate (j) and 1-hexanol (t) (Figure 4). These samples exhibit distinct sensory profiles (Figure 1) and differ in their overall quality values (Figure 2).

Given the variability of the presence of different odorants among the different grape spirits samples (Supplementary Materials, Tables S2 and S3) an additional PCA was conducted including only the 12 grape spirit samples evaluated by GC-O. The projection of the samples and the variables onto the plane defined by the first two components is presented in Figure 5.

In the PCA of odorants with OAV > 1 in the 12 samples evaluated by GC-O, the first three principal components explain 70% of the total variability in the data. Specifically, component 1 accounts for 31.5%, component 2 for 24.7%, and component 3 for 13.8% of the variance. In the two-dimensional plane defined by the first two components (Figure 5), a clear separation of samples 1 and 49 is observed. These samples are distinctly positioned and associated with high levels of the compounds ac (linalool), i (isoamyl acetate), x (ethyl octanoate), b (diacetyl), and am (2-phenylethanol), compounds known for their pleasant floral, fruity, and caramel-like aromas, which may help explain their higher overall quality scores (Figure 2).

Other samples with high overall quality scores, specifically samples 72, 74, and 65, are located near the centre of the plot and do not appear strongly associated with any particular compound. Meanwhile, sample 54 is positioned separately and linked to compounds e, f, and a, three esters with pleasant aromatic characteristics, as well as isovaleric acid (ah), which has a notably unpleasant aroma.

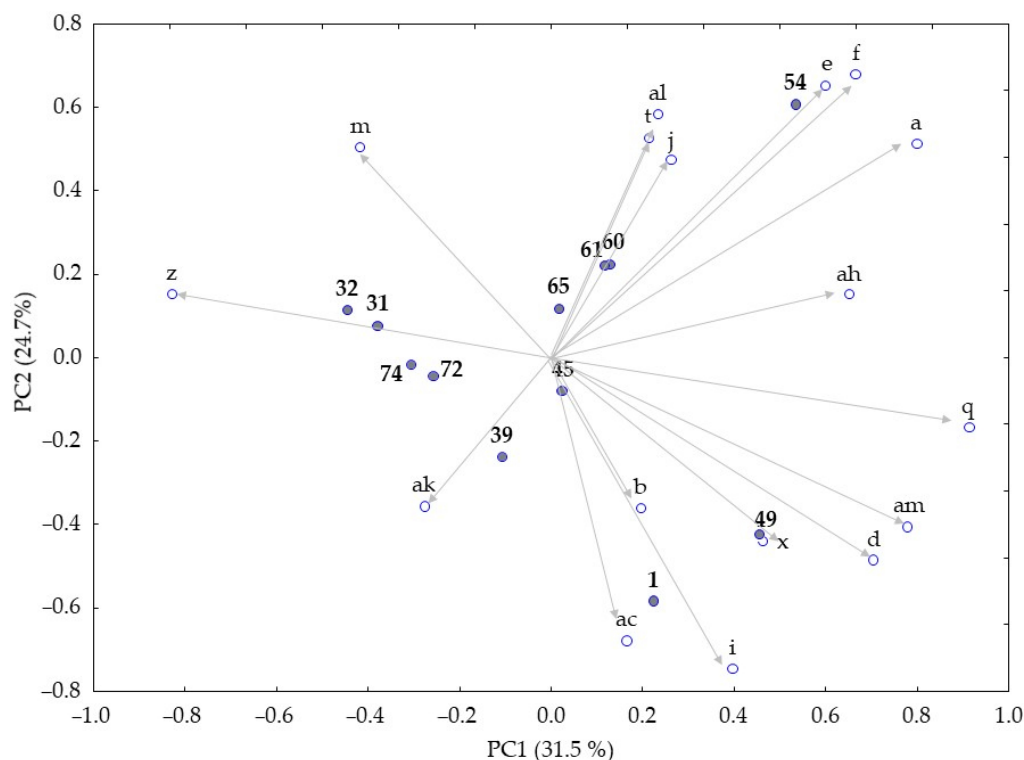


Figure 5. Biplot (PC1 × PC2) constructed using the results of odour compounds with OAV > 1 quantified in the 12 grape spirits samples. Letters correspond to the compounds listed in Table 3. Sample number assignments are described in Table S1.

Samples 32 and 31 also appear in distinct positions within a quadrant where isoamyl alcohol (m) and 1-octen-3-ol (z), both associated with off-notes, are the most influential compounds. This may explain their medium overall quality scores.

These findings highlight a partial mismatch between the sensory analysis and the odourant profile results. This discrepancy likely arises from the fact that the GC-O evaluation was performed on a compound-by-compound basis, without considering potential interactions between volatiles within the spirit matrix. Despite these limitations, the results indicate the importance of certain esters with high OAVs, as well as linalool and 2-phenylethanol, in positively contributing to the quality of spirits intended for Port wine production, whereas 1-octen-3-ol appears to be associated with lower perceived quality. Some of these compounds has been reported to impact the quality of the resulting Port wines [4] while others should therefore be further evaluated, and their quantification should be considered for the characterisation and selection of grape spirits. Further research is required to better elucidate and quantify the implications of the results presented in this study.

4. Conclusions

The work carried out enabled the evaluation of a set of spirit samples intended for use in Port wine production. Sensory analysis of their aroma highlighted the importance of *floral*, *fruity* and *sweet* notes in determining overall quality.

Gas Chromatography-Olfactometry (GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS) revealed the presence of various odour-active compounds from different chemical families, with esters being the most prevalent group. Threshold determinations of these compounds in a 20% hydroalcoholic solution showed a wide range of values. Among them, several esters, diacetyl, and 1-octen-3-ol exhibited the lowest odour thresholds. For many of these compounds, this study provides the first reported threshold values in hydroalcoholic solutions at 20% *v/v*.

Quantification of these compounds enabled the calculation of their corresponding Odour Activity Values (OAVs) in grape spirit samples, revealing that esters exhibited the highest OAVs. Furthermore, the grape spirits studied revealed remarkable variability in their sensory profiles, corresponding to significant differences in the profiles and concentrations of the various odorant compounds analysed.

Although further investigation is warranted, these findings represent valuable insights for the production of Port wines and other fortified wines.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16020941/s1>, Table S1: General information on the sample set of grape wine spirits; Figure S1: Biplot projection of twelve selected grape spirit samples and variables (aroma attributes and overall quality) onto the plane defined by the first and second PCA components. Sample number assignments are described in Table S1; Figure S2: Average aroma profile of the grape spirits samples selected for GC-O analysis. (A) Spirits with a lower overall quality (<12), (B) spirits with an intermediate overall quality (12.0 to 12.5) and (C) spirits with the highest overall quality (13.0 to 14.1). Sample number assignments are described in Table S1; Figure S3: Chromatogram (red) and aromagram (blue) generated by a sniffer from an extract of a grape spirit sample. Compound identification: 1—Ethyl isobutanoate (a); 2—2,3-Butanedione (b); 3—Isobutyl acetate (c); 4—Ethyl butanoate (d); 5—1-Propanol; 6—Ethyl 2-methylbutanoate (e); 7—Ethyl isovalerate (f); 8—Isobutanol (g); 9—2-Methylbutyl acetate (h)+3-Methylbutyl acetate (i); 10—Ethyl pentanoate (j); 11—1-Butanol; 12—Methyl hexanoate (l); 13—2-Methyl-1-butanol (n)+3-methyl-1-butanol (m); 14—Ethyl hexanoate; 15—3-Methyl-3-buten-1-ol (r); i.s.1—5-Methyl-2-hexanol (internal standard); 16—3-Methyl-1-pentanol (s); 17—Ethyl lactate; 18—1-Hexanol (t); 19—trans-3-Hexen-1-ol, 20—cis-3-Hexen-1-ol (u); 21—trans-2-Hexen-1-ol (v); 22—Ethyl octanoate (x); 23—1-Octen-3-ol (z); 24—1-Heptanol (aa); 25—Acetic acid; 26—Furfural; 27—2-Ethyl-1-hexanol (ab); 28—Linalol (ac); 29—Isobutanoic acid (ad); 30—Methyl decanoate (ae) 31—Butanoic acid; 32—Ethyl decanoate; 33—Ethyl succinate (af); 34—3-Methylbutanoic acid (ah); 35—Ethyl 9-decenoate; 36— α -Terpineol; 37—Pentanoic acid; 38—Nerol (ai); 39—2-Phenylethyl acetate (aj); 40—Ethyl dodecanoate (ak); 41—Hexanoic acid; 42—Ethyl hydrocinnamate (al); 43—Benzilic alcohol; 44—2-Phenylethanol (am); 45—Ethyl tetradecanoate (an); 46—Octanoic acid (ao); i.s.2—3,4-Dimethylphenol (internal standard); 47—Ethyl hexadecanoate (ap); 48—Decanoic acid (aq); 49—Dodecanoic acid (ar). The letters presented between brackets correspond to the odour compounds identified in Table 1; Table S2: Content (mg/L) of odour compounds in grape spirit samples (Part I). Sample number assignments are described in Table S1; Table S3: Content (mg/L) of odour compounds in grape spirit samples (Part II). Sample number assignments are described in Table S1.

Author Contributions: Conceptualization, I.C., S.M.R. and F.A.; Methodology, I.C., M.L., S.L. and R.S.; Formal analysis, I.C., R.S., M.L., N.M., S.L. and M.J.C. Investigation, I.C., S.M.R. and F.A.; Resources, R.S., F.A. and I.C.; Data curation, R.S., I.C., S.M.R. and F.A.; Writing—original draft, I.C. Writing—review and editing, I.C., M.L., N.M., S.L., M.J.C., R.S., S.M.R. and F.A. Supervision, I.C., S.M.R. and F.A.; Project administration, F.A., S.M.R. and I.C.; Funding acquisition, I.C., S.M.R. and F.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the project “Q-AD4PUREPORT—Nova Gama de Vinhos do Porto, com base num inovador método de seleção de aguardentes vónicas” (POCI-01-0247-FEDER-039956).

Institutional Review Board Statement: The sensory evaluations conducted in this study were carried out in compliance with the Declaration of Helsinki and were performed in accordance with the ethical guidelines approved by the INIAV executive board (CD-INIAV protocol number CEC-INIAV-Ed. 2-2018, approved on 7 January 2019).

Informed Consent Statement: Informed consent was obtained from all tasters and sniffers who participated in the sensory evaluations carried out across all sessions of the sensory experiments.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would thank, posthumously, to Frank Steve Rogerson for having transmitted his passion for the study of Port wine aroma chemistry, and for the great contribution for the initial idea and promotion of the Q-AD PurePort Project. The authors are also very grateful to all the voluntary tasters of the sensory panel and to all the voluntary sniffers who participated in the GC-O sessions for their commitment and resilience. The authors also thank Otilia Cerveira and Deolinda Mota for their technical support with volatile extraction, GC-FID analysis, and sensory evaluation. The authors acknowledge the R&D unit MED—Mediterranean Institute for Agriculture, Environment and Development (<https://doi.org/10.54499/UID/05183/2025>) and the Associate Laboratory CHANGE—Global Change and Sustainability Institute (<https://doi.org/10.54499/LA/P/0121/2020>).

Conflicts of Interest: Authors Ricardo Silva and Fernando Alves were employed by the company Symington Family Estates, Vinhos S.A. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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