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Exploring the potential of wine industry by-products as source of additives to improve the quality of aquafeed

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ABSTRACT

The recent growing concern driven by consumer interest in the safety and quality of seafood, has boosted the search for healthy and functional aquafeeds. The current study represents the first approach to assess the potential of volatile composition of the wine industry by products (*e.g.*, grape pomace, grape stems, lees), as additives for improving the quality of fish feeds in terms of organoleptic characteristics (*e.g.*, aroma and flavor) and health benefits. Headspace solid-phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME/GC–MS) was used to establish the volatile profile of wine industry by-products. A total of 153 volatile organic compounds (VOCs), which belong to different chemical families, comprising 36 esters, 31 carbonyl compound, 20 alcohols, 18 terpenoids, 17 acids, 11 furanic compounds, four volatile phenols, two lactones, and 14 miscellaneous, were identified. Esters and terpenoids showed a positive contribution to the aquafeeds aroma with fruity, sweet, green, fresh, and berry notes, whereas some acids (*e.g.*, hexanoic acid) and terpenoids (*e.g.*, limonene) could be used as antimicrobial, antioxidant and antiproliferative agents. Our findings confirmed the potential of wine industry by-products as a rich source of essential compounds to enhance the quality of aquafeeds towards the valorization of winery waste based on the concept of circular economy. Further investigation on the extraction, isolation and purification of VOCs from a natural bio-source will guarantee the safety of the aquafeed and compliance with the requirements of the animal feed industry.

1. Introduction

Aquaculture, the farming of aquatic plants and animals, is the fastest growing food production sector [1], representing approximately half of the total production of human food from aquatic origin since 2012 [2]. Demand for the aquafeed ingredients, particularly fish meal and oil follows the global expansion in production and the development trends of industry, including: (*i*) the continued growth forecasted worldwide for most species and production systems [3]; (*ii*) the production shift towards fed animals compared to others - plants, algae and filter feeders - from about 44% of global production in 2006 [4] to 50% of global production in 2014 [2] and to approximately 70% of global production in 2017 [4]; and (*iii*) the rapid growth occurred in the production of carnivorous fish favored by the economics of global trade and larger scale intensive farming [3]. Considering fish meal and oil resource limitations, as well as increasing global costs, the search for alternative feed ingredients to fill the demand gap is a major challenge in the development of the industry.

The circular economy framework proposes reduction of the food waste by using it as a raw material for new products and applications [5] supporting alternative recycling flows in the food system network [6]. Within the aquaculture sector, the by-products of the fish processing, such as trimmings and offal, are becoming a major source of fish meal and oil for aquafeeds [3]. Moreover, a high value products and functional biomolecules are being developed from processing fish wastes [7] and marine organisms considered pests, wasted as well, such as jellyfish [8], are being used for human consumption and aquaculture livestock

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Agro-industrial waste reuse has been adopted as innovative strategy to improve emerging and sustainable technologies and rise the natural component purchases for industrial benefits [9]. Grape is one of the main fruit crops produced worldwide, being *Vitis vinifera* L. the most common cultivated species, with an annual production of more than 67 million tons [10]. Approximately, 80% of the total harvest grapes are used in winemaking. The wine industry produces millions of tons of residues (*e.g.*, grape pomace, grape stems, lees), which represents a waste management issue both ecologically and economically [10]. In Madeira Island, where the world-famous Madeira wine is produced, 430 hectares of vineyards produce approximately 4.0×10^4 hL of wine per year, resulting in 2150 tonnes of waste and wastewater.

The grape pomace is recovered by the industry for its partial use for tartaric acid extraction or ethanol production [11], and the final solid residue is sometimes cast off as fertilizer [12]. Grape pomace has also been used as the additive in animal feeding, in spite of the presence of polymeric polyphenols (*e.g.*, lignin) reduce the digestibility by inhibition of cellulolytic and proteolytic enzymes, as well as the growth of rumen bacteria [10]. Additionally, the high content of dietary fibers emphasizes the possible nutritional value of grape pomace with a wide range of applications as food ingredients [10]. The grape seed contains a range of compounds, such as phenol, catechins, epicatechins, procyandins and proanthocyanidins, which have antioxidant properties with high potential for feedstuff [13]. Nutritional studies have shown that grape pomace and seed extract used as diet additives promoted a significant increase of growth performance, immune response and even improvements of meat quality in grass carp *Ctenopharyngodon idella* [14] and greenlip abalone *Haliotis laevigata* [15]. Lees are an undervalued by-product of the wine industry due to their scarce current application. They are used for the recovery of tartaric acid (reused to adjust the initial pH of musts before fermentation and must distillation to obtain alcohol) and to remove most undesirable compounds (*e.g.*, mycotoxins, volatile phenols) from wines [16].

In nutrition, volatile organic compounds (VOCs) are responsible for food flavor, antimicrobial [17] and antiproliferative properties [18]. Once the olfactory sense of fish plays an important role in the acceptability of feed [19], the inclusion of specific VOCs may be of interest to improve feed attractiveness in aquaculture. Although the volatile composition of seafood have been studied extensively [20], information related to the volatile composition of raw material used in fish feed is scarce [21,22]. Giogios et al. [21] conducted the screening of volatile composition of several common marine raw materials used in the aquaculture marine industry and all samples were characterized by absence or negligible levels of eight- and nine-carbon alcohols and carbonyl compounds. On the other hand, Mahmoud et al. [22] performed a comprehensive sensory and chemo-analytical characterization of fish feed and 55 of 81 odorants were common to all the samples. Most of these odorants were identified for the first time in fish feeds, namely skatole, indole, (*E,Z,Z*)−2,4,7-tridecatrienal, 4-ethyloctanoic acid, and cresols.

Taken together, there is a growing challenge of developing sustainable and nutritious aquafeeds from natural bio-sources while ensuring that farmed-fish supply meets consumption demands. In this context, the purpose of research is to establish the volatile composition of grape pomace, grape stems and lees of red wine and white wine grapes and to identify VOCs that can potentially enhance the quality of aquafeeds in terms of flavor and bring health benefits to aquaculture livestock. To our best knowledge, this is the first approach that explore wine by-products as source of additives to improve the quality of aquafeed, and simultaneous increase the valorization of winery waste based on the concept of circular economy. This is important mainly for lees that are an undervalued by-product of the wine industry, due to their scarce applications.

2. Material and methods

2.1. Reagents and standards

Identification of the target compounds was performed using VOCs standards obtained from Acros Organics (Geel, Belgium), Fluka (Buchs, Switzerland) and Sigma-Aldrich (Madrid, Spain) with purity higher than 98%. The stock solutions of each VOCs standard were dissolved in ethanol to reach a concentration of 500 mg/L and stored at 4 ºC. Sodium chloride (NaCl, 99.5%) and 4-methyl-2-pentanol (internal standard, IS) were obtained from Sigma-Aldrich (Madrid, Spain), while helium (GC carrier gas) of purity 5.0 was supplied from Air Liquide, Portugal. The glass vials, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber and SPME holder for manual sampling were obtained from Supelco (Bellefonte, PA, USA). The kovat index (KI) was calculated using an alkane series, C_8 to C_{20}, with a concentration of 40 mg/L in *n*-hexane purchased from Fluka (Buchs, Switzerland).

2.2. Wine industry by-products samples

Grape pomaces, grape stems and lees from red and white wine grapes were generously donated by several Madeira wine companies (Madeira Island, Portugal). The *Vitis vinifera* L. grapes were harvested in 2016 at maturity in excellent health and vinified depending on the winery protocol, as illustrated in Fig. 1. The grape clusters were crushed and destemmed using a destemmer-crusher. The crushed grapes were treated with sulfur dioxide (0.2–0.5% total mash) and with selected strains of *Saccharomyces cerevisiae* to start up the fermentation. After 6–8 days of maceration, when alcoholic fermentation was finished, the mash was pressed. Stems come from the destemming procedure, grape pomace from the maceration procedure, whereas wine lees are obtained from the filtration and clarification step. All winemaking by-products were subjected to the HS-SPME/GC–MS within 24 h after their collection.

2.3. Solid-phase microextraction (SPME) procedure

The SPME procedure used in the current study was adopted from the previous research works carried out in our laboratory [23,24]. For each extraction, 4 g of sample, 2 g of NaCl (*salting-out* effect), 5 mL of ultra-pure Milli-Q water and 10 µL of 4-methyl-2-pentanol (IS, 250 µg/L) were putted into a 20 mL glass vial containing a stirring bar (2×0.5 mm, 800 rpm). Afterwards, the vial was capped with a PTFE-faced silicone septum and putted in a thermostatic block at 40 °C and the DVB/CAR/PDMS fiber was manually inserted into the sample vial headspace for 45 min. Afterwards the fiber was withdrawn into the holder needle, removed from the vial and instantly placed into the GC injector port (equipped with a glass liner, 0.75 mm I.D.) at 250 °C during 6 min for thermal desorption of the VOCs. All analyses were carried out in triplicate.

2.4. Gas chromatography-mass spectrometry (GC–MS) conditions

The separation of target analytes were carried out using an Agilent 6890 N (Palo Alto, CA, USA) gas chromatograph system equipped with a BP-20 (30 *m* × 0.25 mm i.d. × 0.25 µm film thickness) fused silica capillary column purchased from SGE (Darmstadt, Germany). Helium was used as carrier gas with a flow rate of 1 mL/min. The temperature of injector, GC-qMS interface and quadrupole detector were 250, 180 and 220 ºC, respectively. The GC oven temperature started at 45 °C (1 min), increased to 100 °C at 2 °C/min and held for 3 min, then ramped to 130 °C at 5 °C/min (5 min), and reaching the final temperature of 220 °C at 7 °C/min (15 min), total runtime of 70.36 min. The MS used was an Agilent 5975 quadrupole inert mass selective detector, that operated in the electron impact (EI) at 70 eV, while keeping the source temperature at 180 ºC. The mass range acquired was from 30 to 300 *m/z*, at the rate 1.9 spectra/s, in full scan mode. The ion extraction chromatogram (IEC) mode was used to obtain the GC peak area of each VOC. Signal acquisition and data processing were performed using the HP ChemStation (Agilent Technologies).

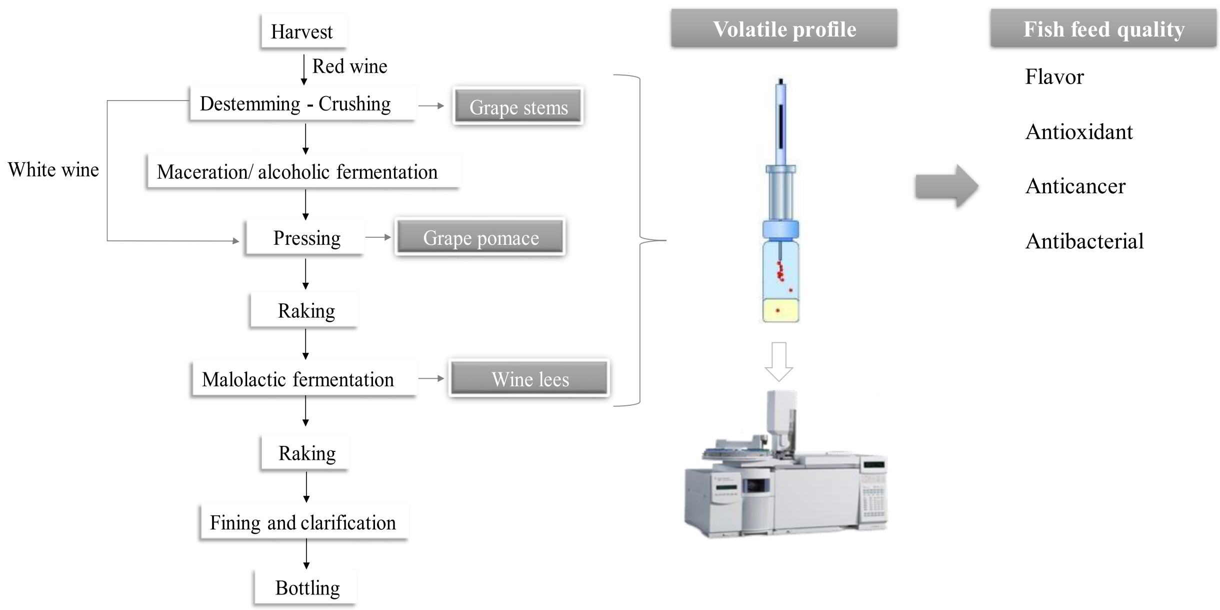

Fig. 1. Schematic winemaking and extraction process.

The VOCs identification was done through the: (*i*) comparison the GC retention times and mass spectra with those of pure standards, when available; (*ii*) all mass spectra were also compared with the data system library (NIST, 2005 software, Mass Spectral Search Program v.2.0d; Nist 2005, Washington, DC); (*iii*) KI values were calculated based on van den Dool and Kratz equation [25], and were compared with values reported in the literature for comparable columns (Rizzolo et al., 2005; Xu, Fan, & Qian, 2007).

The semi-quantification of VOCs was performed using 4-methyl-2-pentanol (IS) addition using the following equation: VOCs concentration = (VOC GC peak area / IS GC peak area) × IS concentration.

2.5. Statistical analysis

Statistical analysis was carried out using Metaboanalyst 4.0, that comprise the data pre-processing to eliminate VOCs with missing values (MV) and normalization (data transformation using data scaling by mean-center and cubic root). Afterwards, the normalized data were processed using the *one-way* ANOVA followed by Tukey's test for post-hoc multiple comparisons of means and multivariate statistical analysis (*e.g.*, principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA)) in order to identify VOCs that can be used to differentiate the wine industry by-products under study and to obtain insights into the separations of among the samples sets. Finally, Pearson´s correlation was done to build the heat map of the wine industry by-products using the VOCs identified with the purpose of recognize clustering patterns.

3. Results and discussion

3.1. Volatile composition of wine industry by-products

A total of 153 VOCs' were tentatively identified in the wine industry by-products samples, from which 32 were identified in all samples (Table 1). The VOCs tentatively identified comprise 36 esters, 31 carbonyl compounds, 20 alcohols, 18 terpenoids, 17 acids, 11 furanic compounds, four volatile phenols, two lactones and 14 miscellaneous. In grape pomace and grape stems the same number of VOCs, 114, were identified, whereas in lees from white and red grapes were identified 67 and 68 VOCs, respectively. The VOCs extracted by HS-SPME technique have also been identified in fish feeds, marine oils, meals, among other products [21,22,28].

The relative concentration of each VOC (µg/L) ± standard deviation grouped by chemical family, and odor descriptor [22,29,30] are listed in Table 1. Fig. 2 shows a typical HS-SPME/GC–MS total ion chromatogram of lees collected after fermentation of white and red grapes. Significant differences in the volatile profile between lees from white and red grapes are clearly observed.

The distribution of VOCs, according to chemical family, is presented in Fig. 3. As can be observed, the esters are dominant in lees compared to grapes by-products, which is in agreement with the literature data; most of the esters are formed during the fermentation [31] and they are present in grapes in a very small amounts.

Short-chain esters (*e.g.*, ethyl hexanoate, ethyl octanoate, ethyl decanoate) are the most important VOCs in a diversity of food samples, such as cheese, wine and apple. Moreover, methyl acetate and ethyl butanoate have been detected in marine oils and fish meals [21]. They have a positive impact on the overall food flavor, but if present at high concentrations they can lead to overly fruity and fermented off-flavor notes [29]. In general, esters impart fruity flavor odors with sensory descriptions ranging from fruity and solvent-like (*e.g.*, ethyl acetate), banana- and pear-like (*e.g.*, isoamyl acetate), rose- and honey-like (*e.g.*, 2-phenylethyl acetate), or apple-like and sweet (*e.g.*, ethyl hexanoate, ethyl octanoate) [29]. Eight from the identified esters, Table 1, namely, ethyl acetate (0.85 ± 0.06–275.62 ± 27.9 µg/L), isobutyl acetate (0.03 ± 2.11E-3–2.10 ± 0.08 µg/L), ethyl hexanoate (0.25 ± 0.02–180.33 ± 10.6 µg/L), hexyl acetate (0.23 ± 0.02–32.09 ± 1.25 µg/L), ethyl 2-hexenoate (0.03 ± 3.10E-3–0.67 ± 0.10 µg/L), ethyl decanoate (0.12 ± 0.02–1842.52 ± 137 µg/L), methyl salicylate (0.14 ± 4.31E-3–9.07 ± 0.47 µg/L), and 2-phenylethyl acetate (0.02 ± 1.52E-3–48.40 ± 4.24 µg/L), are common to all wine industry by-products analyzed.

The alcohols fraction is composed of aliphatic (*e.g.*, 1-propanol, 3-methyl-1-butanol) and aromatic alcohols (*e.g.*, benzyl alcohol, 2-phenylethanol). The total concentration determined for alcohols was 953.16 and 414.15 µg/L for lees obtained from red and white grapes, respectively, whereas for grape pomace and stems, its concentration is much lower 5.89 and 131.89 µg/L, respectively. 3-Methylbutan-1-ol, 1-hexanol and (*Z*)-2-hexen-1-ol are dominant alcohols in grape stems, whereas ethanol, 3-methylbutan-1-ol and 2-phenylethanol are the

Table 1
Relative concentration (μg/L) of volatile organic compounds (VOCs) in wine industry by-products using HS-SPME/GC–MS.

Peak nº	RT (min)[a]	IEC[b]	KI_{cal}[c]	KI_{lit}[d]	chemical families	odor descriptors[e]	Relative concentration (μg/L)[f] ± Standard deviation			
							Grape pomace	Grape stems	Red lees	White Lees
Esters										
1	5.81	43	814	813	Methyl acetate	Blackcurrant, fruity, solvent	8.03 ± 0.81	0.81 ± 0.11	0.34 ± 0.06	–
2	6.67	43	870	874	Ethyl acetate[g]	Fruity, sweet	0.85 ± 0.06	61.17 ± 9.20	275.62 ± 27.9	71.08 ± 8.62
3	7.03	57	892	890	Methyl propionate[g]	Ethereal, fruity, rum	0.10 ± 0.01	0.36 ± 0.03	–	–
11	9.89	43	997	993	Isobutyl acetate	Banana, flowery, fruity, pear	0.03 ± 2.11E-3	0.61 ± 0.07	2.07 ± 0.28	2.10 ± 0.08
13	10.11	74	1003	1009	Methyl 2-methylbutanoate	Blackberry, green apple, sweet	0.05 ± 4.26E-3	–	–	–
15	10.74	71	1019	1022	Ethyl butanoate[g]	Bubble-gum, caramel, fruity	–	0.59 ± 0.04	8.35 ± 1.10	3.16 ± 0.40
20	14.47	70	1101	1102	Isoamyl acetate[g]	Banana, fresh, fruity, sweet	0.07 ± 4.97E-3	–	103 ± 13.9	46.15 ± 1.21
25	17.54	74	1161	1154	Methyl hexanoate[g]	Fruity, solvent	3.09 ± 0.15	0.15 ± 0.01	0.16 ± 0.02	–
32	20.27	88	1207	1212	Ethyl hexanoate[g]	Apple, thinner, sweet	0.25 ± 0.02	3.98 ± 0.32	180.33 ± 10.6	69.24 ± 8.25
37	22.61	43	1247	1251	Hexyl acetate[g]	Acid, citrus, fruity, green, spice	0.23 ± 0.02	16.36 ± 1.09	32.09 ± 1.25	22.13 ± 3.90
44	25.01	43	1283	1276	Hexenyl acetate	–	–	0.85 ± 0.05	1.05 ± 0.09	0.59 ± 0.04
50	27.1	97	1314	1305	Ethyl 2-hexenoate	Fruity, pungent, slightly	0.03 ± 3.10E-3	0.16 ± 0.01	0.67 ± 0.10	0.25 ± 0.03
56	30.06	74	1359	1355	Methyl octanoate[g]	Fruity, green	0.05 ± 0.01	–	0.96 ± 0.11	0.49 ± 0.05
65	33.49	88	1410	1408	Ethyl octanoate[g]	Fruity, must, soap, sweet	–	2.12 ± 0.12	1163.00 ± 162	721.28 ± 52.8
70	34.97	70	1440	1447	Isoamyl hexanoate[g]	–	–	–	3.40 ± 0.38	1.58 ± 0.16
72	35.73	43	1454	1461	Octyl acetate	Floral, fruity	–	–	0.47 ± 0.07	0.22 ± 0.03
78	37.95	43	1495	1504	Propyl octanoate	Brandy, fruity, winey	–	–	0.59 ± 0.08	0.31 ± 0.03
83	38.76	88	1511	1503	Ethyl nonanoate[g]	Fruity, rose, tropical, waxy	–	–	0.67 ± 0.10	0.28 ± 0.01
85	39.59	56	1528	1537	Isobutyl octanoate	–	–	–	0.63 ± 0.09	0.20 ± 0.02
90	41.44	74	1564	1570	Methyl decanoate[g]	Fruity, oily, wine	0.02 ± 2.45E-3	–	1.79 ± 0.16	0.47 ± 0.05
96	43.51	105	1602	1600	Methyl benzoate	Flowery, herbal, honey, lettuce	0.01 ± 7.31E-04	0.16 ± 0.01	–	–
97	43.87	88	1608	1609	Ethyl decanoate[g]	Fruity, pleasant, soap, sweet	0.12 ± 0.02	0.14 ± 0.01	1842.52 ± 137	760.29 ± 74.9
100	45.04	70	1625	1640	Isoamyl octanoate[g]	–	–	–	8.60 ± 1.36	5.72 ± 0.75
102	45.19	101	1627	1640	Diethyl butanedioate	Fruity, winey	–	0.05 ± 2.82E-3	20.11 ± 1.46	0.23 ± 0.02
109	46.16	88	1642	1668	Ethyl 9-decenoate	Floral, fruity, honey, spice	–	–	34.03 ± 3.84	11.27 ± 1.30
113	47.43	71	1660	1664	Ethyl 3-hydroxyhexanoate[g]	Citrus, fruity, green, sweet	–	–	1.52 ± 0.22	0.30 ± 0.02
120	48.46	108	1735	1739	Phenylmethyl acetate	–	–	–	0.97 ± 0.17	0.28 ± 0.03
121	48.54	120	1738	1745	Methyl salicylate	Berry, sweet, wine, wintergreen	0.14 ± 4.31E-3	9.07 ± 0.47	0.19 ± 0.03	0.16 ± 0.01
123	49.71	104	1778	1785	2-Phenylethyl acetate[g]	Floral, fruity, rose, sweet	0.02 ± 1.52E-3	1.12 ± 0.09	47.75 ± 1.74	48.40 ± 4.24
127	50.76	88	1817	1811	Ethyl dodecanoate[g]	Soap, sweet	0.05 ± 1.70E-3	–	460.38 ± 55.8	201.65 ± 27.9
129	51.28	91	1840	1821	Benzyl propanoate	Floral, fruity	–	–	30.48 ± 0.54	11.27 ± 1.33
140	54.96	88	2014	2019	Ethyl tetradecanoate	Ether	–	–	12.92 ± 1.28	7.94 ± 1.10
141	55.31	70	2016	2048	Isoamyl laurate[g]	Fatty, oily, waxy, wine	–	–	2.50 ± 0.27	1.70 ± 0.28
147	58.63	88	2237	2238	Ethyl hexadecanoate	Mild sweet, waxy	–	–	56.86 ± 8.74	19.84 ± 1.73
148	59.11	55	2239	2259	Ethyl 9-hexadecenoate	–	–	–	3.24 ± 0.44	1.40 ± 0.20
149	59.56	120	2242	2239	Hexyl salicylate[g]	Balsamic, dry, floral, hay, waxy	–	0.78 ± 0.06	0.38 ± 0.04	0.79 ± 0.12
Alcohols										
5	7.46	31	911	913	Ethanol[g]	Pungent, sweet	2.91 ± 0.25	10.67 ± 1.36	497.48 ± 11.9	273.46 ± 10.7
14	10.43	31	1011	1005	1-Propanol[g]	–	–	0.49 ± 0.01	39.27 ± 3.91	7.25 ± 0.82
18	12.66	43	1064	1066	2-Methyl-1-propanol[g]	Solvent, alcohol, leek,	–	0.92 ± 0.06	19.43 ± 2.35	2.88 ± 0.35
22	16.58	57	1143	1157	Pentenol	–	0.10 ± 2.95E-3	0.09 ± 0.02	–	–
28	19.1	55	1188	1196	3-Methyl-1-butanol[g]	Balsamic, burnt, fruity, pungent,	0.06 ± 0.01	43.79 ± 4.31	237.37 ± 30.5	56.98 ± 9.64
43	24.78	70	1279	1273	Heptanol[g]	–	–	0.84 ± 0.02	–	–
46	25.41	45	1288	1280	2-Heptanol[g]	–	0.11 ± 0.01	0.74 ± 0.09	–	–
51	27.55	56	1321	1324	1-Hexanol[g]	Green, herbal	0.47 ± 0.05	35.99 ± 6.98	10.04 ± 1.20	2.68 ± 0.38
53	28.87	45	1342	1332	2-Octanol	–	–	–	0.90 ± 0.14	–
54	29.36	67	1349	1346	(*E*)-3-Hexen-1-ol[g]	Grassy, green	–	2.93 ± 0.18	–	–
60	30.84	57	1371	1377	(*Z*)-2-Hexen-1-ol[g]	Grassy, green	–	16.19 ± 1.04	–	–
68	34.65	95	1433	1431	6-Methyl-5-hepten-2-ol	–	0.80 ± 0.11	–	–	–
73	36.08	57	1461	1470	2-Ethyl-1-hexanol[g]	Musty	0.69 ± 0.07	2.38 ± 0.14	–	–
76	36.97	69	1477	1503	3-Ethyl-4-methylpentanol	–	0.29 ± 0.03	5.23 ± 0.72	–	–
84	39.29	56	1522	1526	1-Octanol[g]	Green, herbal	–	0.84 ± 0.05	–	–
89	41.31	71	1561	–	2,6-Dimethylcyclohexanol	–	0.02 ± 3.07E-3	–	–	–
108	46.12	57	1641	1666	4-Tert-butylcyclohexanol	Phenolic	0.06 ± 0.01	–	–	–
115	47.58	55	1705	1719	Undecanol	Mandarin	0.02 ± 1.29E-3	0.14 ± 0.01	–	–

(*continued on next page*)

Table 1 (*continued*)

128	51.14	79	1833	1834	Benzyl alcohol[g]	Blackberry, floral, fruity	0.24 ± 0.02	5.01 ± 0.72	0.39 ± 0.02	0.17 ± 0.01
131	52.19	91	1968	1965	2-Phenylethanol[g]	Floral, fresh	0.12 ± 0.02	5.63 ± 0.38	148.29 ± 9.00	70.72 ± 7.60
Acids										
66	34.17	43	1424	1424	Acetic acid[g]	Sour, vinegar	4.48 ± 0.90	0.50 ± 0.05	8.64 ± 1.31	3.73 ± 0.32
80	38.37	74	1503	1510	Propanoic acid[g]	–	0.23 ± 0.02	0.44 ± 0.04	0.16 ± 0.02	0.19 ± 0.02
86	39.71	43	1530	1533	2-Methylpropanoic acid	Cheese, fatty, phenolic, sweaty	2.69 ± 1.64	–	–	–
94	42.88	60	1591	1600	Butanoic acid[g]	Buttery, rancid, cheese	0.18 ± 0.02	0.24 ± 0.02	0.52 ± 0.06	–
99	44.85	105	1622	1624	Benzoic acid[g]	Balsamic, wine	1.09 ± 1.28	0.48 ± 0.07	0.29 ± 0.04	0.14 ± 0.02
104	45.68	41	1635	1644	Methylbutyric acid	–	0.05 ± 0.01	–	–	–
111	47.34	60	1659	1665	3-Methylbutanoic acid	Cheese, sweaty	0.57 ± 0.08	0.39 ± 0.03	–	–
125	50.41	60	1801	1803	Hexanoic acid[g]	Musty	2.16 ± 0.37	3.49 ± 0.49	11.56 ± 2.03	7.23 ± 0.92
132	52.56	41	1995	1977	(*Z*)-3-Hexenoic acid	Pungent, sweaty, vinegar	0.08 ± 4.87E-3	–	–	–
133	52.73	73	2000	1975	2-Ethylhexanoic acid[g]	–	0.26 ± 0.03	0.17 ± 0.01	–	–
134	53.06	73	2002	1997	(*Z*)-2-Hexenoic acid	Fruity, herbal, sweet, warm	1.44 ± 0.10	0.34 ± 0.02	1.63 ± 2.11E-3	0.23 ± 2.86E-4
139	54.74	60	2013	2011	Octanoic acid[g]	Musty, coriander-like	0.28 ± 0.03	0.36 ± 0.06	42.45 ± 6.94	47.98 ± 4.08
142	56.54	60	2124	2127	Nonanoic acid	Musty	0.05 ± 0.01	0.39 ± 0.04		–
145	58.46	60	2235	2242	Decanoic acid[g]	Coriander-like, plastic	0.05 ± 2.90E-3	0.10 ± 0.01	30.81 ± 3.00	16.26 ± 1.45
150	62.07	105	2456	2427	Undecylic acid	Oily	0.27 ± 0.05	0.56 ± 0.04	1.06 ± 0.16	0.66 ± 0.06
151	63.3	73	2542	2537	Dodecanoic acid[g]	Musty, plastic	–	–	1.11 ± 0.09	1.69 ± 0.07
153	65.56	91	2554	2551	Phenylacetic acid	Bee wax-like, honey	0.34 ± 0.04	–	–	–
Terpenoids										
27	17.99	68	1169	1178	Limonene	Citrus, fruity	0.02 ± 1.41E-3	0.63 ± 0.04	–	1.16 ± 0.42
34	21.67	93	1231	1238	γ-Terpinene	Citrus, green	0.05 ± 0.01	0.99 ± 0.01	–	–
36	22.3	119	1242	1242	Cymene[g]	Oregano	0.02 ± 1.04E-3	0.57 ± 0.05	–	0.65 ± 0.06
38	23.15	119	1255	1248	(*E*)-Ocimene	–	0.03 ± 1.88E-3	0.45 ± 0.04	–	0.22 ± 0.01
64	33.4	59	1408	1421	(*E*)-Linalool oxide	Floral, green, rose, sweet	0.03 ± 2.54E-3	0.28 ± 0.02	–	–
71	35.02	59	1441	1448	(*Z*)-Linalool oxide	Floral, green, rose, sweet	0.01 ± 2.65E-3	0.34 ± 0.02	–	–
82	38.73	93	1510	1490	(*E*)-Limonene oxide	Citrus, fresh	0.56 ± 0.09	0.14 ± 0.01	0.17 ± 0.02	0.18 ± 0.01
91	41.64	93	1568	1570	Isocaryophyllene	Spice, woody	0.01 ± 1.68E-3	0.13 ± 2.45E-3	–	–
92	42.37	137	1582	1581	β-Cyclocitral[g]	Floral, grassy, green, sweet	0.02 ± 2.35E-3	0.13 ± 0.01	–	–
103	45.2	41	1628	1628	(*E*)-β-Farnesene[g]	Woody	0.08 ± 0.01	–	–	–
105	45.94	59	1638	1669	α-Terpineol[g]	Anise, mint, oil	0.06 ± 0.01	–	–	–
107	46.07	161	1640	1638	Alloaromadendrene	Woody	–	0.15 ± 0.01	–	–
110	47.12	161	1655	1661	γ-Muurolene	Woody	–	0.15 ± 0.02	–	–
112	47.41	93	1660	1658	β-Farnesene	Woody	0.06 ± 0.01	0.09 ± 3.85E-3	–	–
117	48.13	93	1724	1725	α-Farnesene[g]	Woody	0.15 ± 0.02	0.09 ± 4.21E-3	0.29 ± 0.04	0.16 ± 0.02
119	48.41	161	1734	1730	α-Curcumene	Herbal	0.02 ± 1.16E-3	–	–	–
122	49.24	41	1762	1783	Isogeraniol[g]	Floral	0.03 ± 0.01	0.08 ± 0.01	–	–
126	50.68	43	1813	1811	Geranyl acetone[g]	Floral, fresh, fruity, green, rose	0.04 ± 2.76E-3	0.58 ± 0.08	–	–
Carbonyl compounds										
4	7.27	44	904	905	2-Methylbutanal[g]	Almond, nutty	0.25 ± 0.02	0.59 ± 0.02	–	–
8	8.73	43	959	949	2-Butanone[g]	Cheesy, ethereal	1.47 ± 0.16	0.55 ± 0.03	1.67 ± 0.25	1.16 ± 0.12
9	8.8	44	962	964	Pentanal[g]	Almond, green, herbal, malty	0.31 ± 0.04	0.14 ± 0.01	–	–
10	9.15	57	973	970	Pentanone[g]	–	0.02 ± 2.29E-3	–	–	–
12	10.01	55	1000	993	Pentenone	–	–	0.63 ± 0.08	–	–
17	12.46	44	1060	1065	Hexanal[g]	Grassy, green	0.38 ± 0.02	1.31 ± 1.67	–	–
24	17.31	43	1157	1160	2-Heptanone[g]	Cheese	0.59 ± 0.11	0.16 ± 3.03E-3	–	–
26	17.65	44	1163	1168	Heptanal[g]	Citrus, potato	–	2.24 ± 0.13	–	–
29	19.32	41	1191	1189	(*Z*)-2-Hexenal[g]	Apple, fruity, green	0.05 ± 0.01	4.87 ± 0.20	–	–
33	21.43	43	1227	1224	3-Octanone[g]	–	0.23 ± 0.02	–	–	–
39	23.31	43	1257	1265	2-Octanone	–	0.13 ± 0.02	0.94 ± 0.16	–	–
40	23.56	45	1261	1268	3-Hydroxy-2-butanone[g]	–	5.96 ± 0.66	0.97 ± 0.14	–	–
41	24.28	55	1272	1272	3-Octenone	–	0.14 ± 0.22	2.41 ± 0.33	–	–
42	24.48	43	1275	1275	1-Hydroxy-2-propanone	–	0.44 ± 0.04	–	–	2.12 ± 0.21
45	25.19	82	1285	1284	2,2,6-Trimethyl cyclohexanone	–	0.03 ± 2.08E-3	0.12 ± 0.01	–	–
47	25.6	83	1291	1299	(*Z*)-2-Heptenal	Almond, mushroom, onion	0.11 ± 0.01	2.83 ± 0.17	–	–
49	26.52	43	1305	1301	6-Methyl-5-hepten-2-one	Pepper, rubber	0.49 ± 0.09	0.54 ± 0.09	–	–
55	29.86	58	1356	1350	2-Nonanone	Green, hot milk	0.06 ± 0.01	0.31 ± 0.02	0.28 ± 0.04	–
57	30.2	57	1361	1367	Nonanal[g]	Citrus, fatty	0.08 ± 2.79E-3	0.43 ± 0.03	–	–
59	30.58	81	1367	1368	(*E*,*E*)-2,4-Hexadienal[g]	–	–	0.80 ± 0.06	–	–
61	31.17	81	1375	1373	(*E*,*E*)-2,4-Heptadienal	Fatty, orange oil	–	0.83 ± 0.15	–	–
62	32.37	55	1392	1402	(*E*)-2-Octenal	Fatty, grassy, soapy	0.09 ± 0.01	2.81 ± 0.16	–	0.56 ± 0.06
69	34.69	81	1434	1451	(*Z*,*Z*)-2,4-Heptadienal	Fatty, orange oil, oily, rancid	–	0.37 ± 0.05	–	–
74	36.51	43	1469	1465	Decanal[g]	Green, floral, soapy	0.06 ± 3.61E-3	10.10 ± 0.76	–	–

(*continued on next page*)

Table 1 (*continued*)

77	37.37	106	1485	1486	Benzaldehyde[g]	Almond, butter sugar, wood	0.10 ± 0.02	6.44 ± 0.61	0.37 ± 0.01	0.14 ± 0.01
79	38.3	43	1501	1510	(*E*)-2-Nonenal[g]	Cardboard-like, fatty	–	0.31 ± 0.02	–	–
81	38.54	41	1506	1534	(*E,E*)-2,4-Nonadienal	Fatty	–	1.27 ± 0.19	–	–
88	40.82	109	1552	1561	6-Methyl-3,5-heptadiene-2-one	–	0.03 ± 8.92E-4	–	–	–
95	43.05	57	1594	1605	4-Tert-butylcyclohexanone	–	0.11 ± 0.01	–	–	–
118	48.23	98	1727	1747	2-Hydroxycyclopent-2-en-1-one	–	0.82 ± 0.06	0.41 ± 0.04	–	–
124	49.86	112	1783	1781	3-Methyl-1,2-cyclopentanedione	–	0.04 ± 4.55E-3	–	–	–
Furanic compounds										
6	8.13	81	938	945	2-Ethyl furan	Pungent, rubber	0.02 ± 1.33E-3	0.35 ± 0.03	–	–
31	19.96	81	1202	1204	2-Pentyl furan	Fruity, sweet	0.15 ± 0.01	1.65 ± 0.12	–	–
67	34.51	96	1431	1437	2-Furfural[g]	Almond, sweet	0.73 ± 0.05	0.17 ± 0.01	0.54 ± 0.08	2.07 ± 0.07
75	36.59	95	1470	1475	1-(2-Furanyl)-ethanone[g]	Smooky, sweet	0.08 ± 0.01	1.06 ± 0.07	–	1.98 ± 0.17
87	39.84	110	1533	1539	5-Methyl-2-furfural[g]	Almond, caramel, sweet	–	0.08 ± 0.01	0.20 ± 0.03	3.29 ± 0.28
98	44.36	98	1615	1623	2-Furanmethanol[g]	Honey, sweet	0.07 ± 0.01	0.37 ± 0.03	–	–
101	45.05	55	1625	–	5-Methyl-2(5H)-furanone	–	0.09 ± 0.01	0.25 ± 0.02	–	–
116	47.79	41	1712	1718	3-Methyl-2(5H)-furanone	–	0.09 ± 0.01	0.11 ± 4.99E-3	–	1.66 ± 0.17
135	53.35	124	2004	2006	2,5-Furandicarboxaldehyde[g]	–	0.09 ± 0.01	0.10 ± 3.36E-3	0.45 ± 0.05	1.48 ± 0.19
138	54.41	85	2011	–	Dihydro-5-pentyl-2(3H)-furanone	–	0.19 ± 4.89E-3	0.14 ± 0.01	–	–
152	63.74	97	2545	2537	5-Hydroxymethyl-2-furfural[g]	Almond, cardboard nutty	1.17 ± 0.16	0.28 ± 0.01	0.73 ± 0.13	3.23 ± 0.47
Lactones										
93	42.66	42	1587	1594	Butyrolactone[g]	Caramel, coconut, cream, peach	0.08 ± 0.01	0.34 ± 0.02	0.57 ± 0.08	0.43 ± 0.04
106	46.02	85	1640	1655	γ-Caprolactone[g]	Fruity, sweet	0.11 ± 0.02	0.06 ± 4.54E-3	–	–
Volatile phenols										
136	53.78	108	2007	2000	*o*-Cresol	Phenolic, medical	0.01 ± 9.65E-4	0.40 ± 0.03	–	–
137	53.82	94	2008	2010	Phenol[g]	Medicinal, phenolic	0.04 ± 3.84E-3	0.35 ± 0.04	0.35 ± 0.05	0.94 ± 0.09
143	56.73	164	2125	2122	Eugenol[g]	Clove	–	0.34 ± 0.06	–	–
144	57.2	135	2128	–	2,3,4,6-Tetramethyl phenol	–	0.02 ± 1.20E-3	–	1.10 ± 0.15	1.87 ± 0.13
Miscellaneous										
7	8.64	57	956	957	2,2,4,6,6-Pentamethyl-heptane	–	0.62 ± 0.07	0.22 ± 0.01	–	0.29 ± 0.03
16	10.9	91	1023	1028	Toluene[g]	Pungent, rubber	0.97 ± 0.08	0.96 ± 0.08	–	–
19	14.22	91	1096	1100	Ethyl benzene	Ethereal, floral	0.39 ± 0.01	0.51 ± 0.01	–	–
21	16.09	174	1134	1140	Dibromomethane	–	0.11 ± 0.01	0.24 ± 0.01	–	–
23	17.16	91	1154	1150	*m*-Xylene[g]	Cold meat fat-like, plastic	0.29 ± 0.01	1.16 ± 0.12	–	–
30	19.5	91	1194	1204	Propyl benzene	–	0.22 ± 0.02	0.94 ± 0.13	–	–
35	21.74	104	1232	1241	Styrene[g]	Aromatic, pungent, roast	0.08 ± 0.01	0.26 ± 0.04	1.25 ± 0.13	0.42 ± 0.04
48	26.33	105	1301	1293	1,2,3-Trimethyl benzene	Aromatic, herbal	0.05 ± 2.38E-3	1.51 ± 0.18	–	–
52	28.36	117	1334	–	Propenyl benzene	–	0.04 ± 0.01	1.32 ± 0.11	–	–
58	30.43	69	1365	1355	2,7-Dimethyl-2,6-octadiene	–	0.06 ± 0.01	0.98 ± 0.12	0.30 ± 0.04	0.15 ± 0.01
63	33.05	173	1401	1407	Tribromomethane	–	6.24 ± 1.10	9.64 ± 1.06	–	–
114	47.48	128	1701	1701	Naphthalene	Medicinal	–	0.45 ± 0.04	0.12 ± 0.02	0.40 ± 0.03
130	52	205	1875	1870	Butylated hydroxytoluene	–	0.02 ± 1.28E-3	0.12 ± 2.44E-3	0.17 ± 0.03	–
146	58.52	43	2236	–	Hydroxydihydromaltol	–	0.86 ± 0.10	–	–	16.33 ± 0.81

- : Not detected.
[a] Retention time (min) determined in a BP-20 capillary column;.
[b] IEC: ion extraction chromatogram, *m/z* used to obtained the GC peak area of each compound;.
[c] Kovats index relative n-alkanes (C_8 to C_{20}) on a BP-20 capillary column;.
[d] Kovats index relative reported in literature for equivalent capillary column [26,27];.
[e] Odor descriptors reported in literature [29,30];.
[f] Mean of three replicates;.
[g] Identification confirmed by comparing mass spectra and retention time with those of authentic standard;.

predominant ones in lees. These alcohols contribute positively to global aroma of many foods and beverages [32], with 3-methyl-1-butanol being associated to fruit notes (*e.g.*, banana), 2-phenylethanol to floral, honey and fragrant aromas, and 1-hexanol and (*Z*)-2-hexen-1-ol to green/herbaceous odors. (*E*)-3-Hexen-1-ol was only detected in grape stems, and its presence was responsible for green odor, similar to freshly cut grass. Carlone et al. [33] showed that green odor (a mixture of (*E*)-3-hexenol and (*Z*)-2-hexenal) may induce a light stress-alleviating effect, although reported only for dogs. 2-Ethylhexan-1-ol and 2-phenylethanol have been reported in fish feeds [22] and various aquaculture fish species [30].

Acids contributed around 2.41% for the total volatile composition of targeted wine industry by-products, with exception of grape pomace where this chemical family accounts 22.19% of total volatile fraction. In general, acids are associated to negative smell impressions [22], since their presence is normally responsible for rancid (*e.g.*, butanoic acid), musty (*e.g.*, hexanoic acid) and sweaty (*e.g.*, 3-methylbutanoic acid) odors. Phenylacetic acid (0.34 ± 0.04 μg/L) only detected in grape pomace, with a sweet honey-like and floral type odor, is an exception. This acid was previously identified as the most potent odorant in fish feeds, contributing positively with honey and bee wax-like notes [22]. From a sensorial point of view, acids do not contribute positively

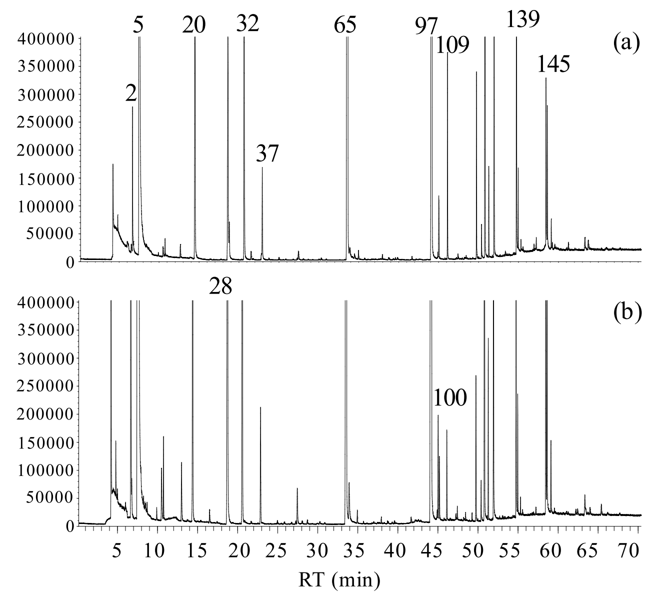

Fig. 2. Total ion chromatograms obtained by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS analysis of lees obtained from (a) white and (b) red *V. vinifera* L. grapes (for identification of peak numbers see Table 1).

for global aroma, but its potential could be explored in terms of antimicrobial properties and nutritional supplements [17]. Octanoic and decanoic acids were already reported in fish feeds [22]. In addition, previous studies have demonstrated antimicrobial properties of acids (*e.g.*, hexanoic acid, octanoic acid, decanoic acids), which fight against various foodborne pathogens, such as *E. coli* O157:H7, *Helicobacter pylori, Listeria monocytogenes, Staphylococcus aureus* [17] and oral microorganisms such as *Streptococcus mutans, Streptococcus gordonii, Streptococcus sanguis, Candida albicans* [34]. Octanoic (42.45 ± 6.94–47.98 ± 4.08 μg/L) and decanoic acids (16.26 ± 1.45–30.81 ± 3.00 μg/L) are the most dominants in lees, whereas in grape pomace and stems the hexanoic acid (2.16 ± 0.37–3.49 ± 0.49 μg/L) presents the highest concentration.

The grape stems (2.10%) seems to be the richest in terpenoids, followed by grape pomace (1.87%) and lees (< 0.1%). From the pool of the identified terpenoids (Table 1), only two: (*E*)-limonene oxide (0.14 ± 0.01–0.56 ± 0.09 μg/L) and α-farnesene (0.09 ± 4.21E-3–0.29 ± 0.04 μg/L) were detected in all wine industry by-products analyzed. From a sensorial point of view, terpenoids showed the positive contribution to aroma, with fruit, floral, woody and citrus notes [29]. On the other hand, previous studies in the field of cancer research and food nutritional sciences reports its cellular antiproliferative properties [18]. Among them, limonene, a dominant VOC in grape stems and white wine lees, has well-validated chemopreventive activity against several types of cancer. Suppression of the growth of pancreas, stomach, colon, skin, and liver cancers in animal models [35] by incubation with limonene has been demonstrated. α-Terpineol, detected only in grape pomace, has been reported as potent anti-inflammatory and antimicrobial agent against periodontopathic and cariogenic bacteria [36]. α-Farnesene, detected in all samples analyzed, showed a significant correlation for anticancer activity [37]. Moreover, limonene, cymene and muurolene were already found in fish flesh and feed [28].

Carbonyl compounds (aldehydes and ketones) represent 18.60 and 13.06% of total volatile profile in grape pomace and stems, respectively, whereas their contribution in lees are lower than 0.16%. Aldehydes are common products resulting from lipid oxidation reactions, which are used as indicator of oxidized flavor. Decanal (10.10 ± 0.76 μg/L), (*Z*)-2-hexenal (4.87 ± 0.20 μg/L) and benzaldehyde (6.44 ± 0.61 μg/L) were the most dominant aldehydes in grape stems and were well know contributors of green and almond oil odor notes, respectively. In recent studies, benzaldehyde has been reported to be one of the most prevalent aldehydes in dry dog foods [38], whereas (*Z*)-2-hexenal has been identified as a light stress-alleviating effect on the domestic dog [33]. (*E*)-2-Nonenal (0.31 ± 0.32 μg/L), only detected in grape stems, has been identified as a potential odorant in fish feeds [22]. On the other hand, 3-hydroxy-2-butanone (acetoin, 5.96 ± 0.66 μg/L) one of the most dominant carbonyl compound in grape pomace, is widely applied in foods, cosmetics, or for asymmetric optically active pharmaceuticals [39].

Furanic compounds are formed by caramelization and Maillard reactions, occurring simultaneously during baking process. However, thermal degradation of amino acids (*e.g.*, serine, threonine, cysteine), thermal oxidation of ascorbic acid and oxidation ofpolyunsaturated fatty acids can contribute to furanic compounds formation as well [40]. 2-Pentyl furan, 2-furfural, 1-(2-furanyl)-ethanone, 5-methyl-2-furfural and 2-furanmethanol are detected in dry dog foods [38,41], where 2-furanmethanol plays an important role in its flavor [38].

There are qualitative and quantitative differences between the VOCs

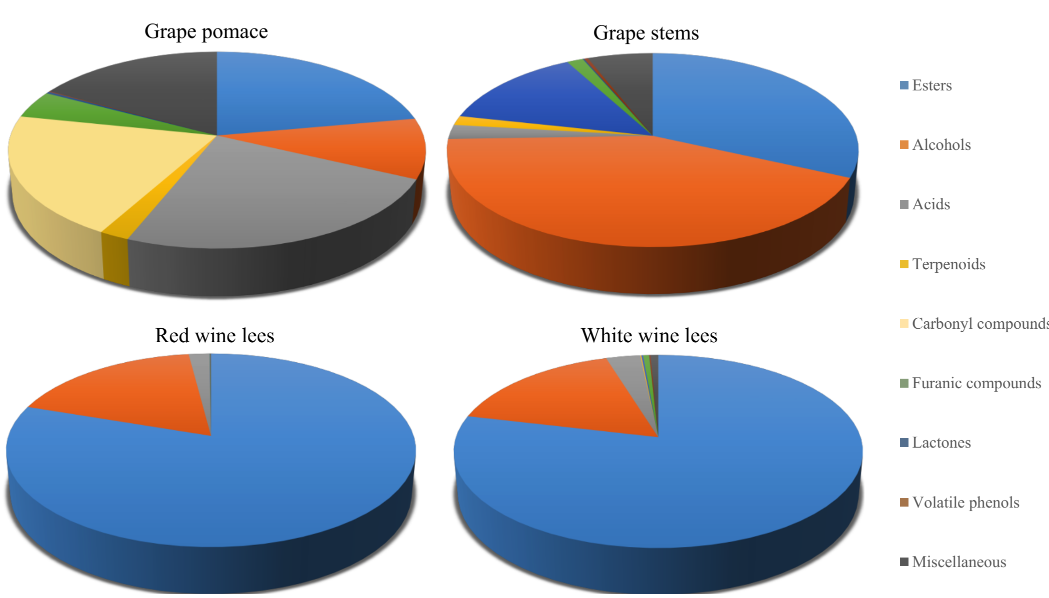

Fig. 3. Total relative concentration (μg/L) of chemical families identified wine industry by-products.

contained in wine industry by-products analyzed, with wine lees having the highest levels of VOCs (2501.59–5357.52 µg/L), whereas the grape by-products presents the highest number of VOCs identified (114 VOCs). In qualitative terms, remarkable differences were observed in number of esters, terpenoids, carbonyl and furanic compounds identified in all wine industry by-products. Moreover, regarding to the acids, 2-methylpropanoic acid, methylbutyric acid, (*Z*)-3-hexenoic acid and phenylacetic acid were detected only in grape pomace samples. Thirty two VOCs were common for all wine industry by-products and the difference was observed in terms of their relative concentration and respective contribution to the total volatile profile.

Principal component analysis (PCA), multivariate pattern recognition procedure, was applied to determine if there were similarities/differences in the volatile composition of wine industry by-products analyzed. The dataset composed by 153 VOCs and 12 samples (four samples × three collection) was submitted to PCA. The PCA score plot and loading plot from wine industry by-products analyzed are showed in Fig. 4a and b, respectively.

The variances of PC1 and PC2 were 87 and 8.5%, respectively, representing 95.5% of the total VOCs variability of data. The grape pomace, projected in PC1 and PC2 negative, are mainly characterized by 1-hydroxy-2-propanone (42), 2-methylpropanoic acid (86) and hydroxydihydromaltol (146), whereas grape stems placed in PC1 negative and PC2 positive by 1-hexanol (51), (*Z*)-2-hexen-1-ol (60) and decanal (74). The white wine lees, PC1 and PC2 positive, is characterized by ethanol (5), ethyl hexanoate (32) and 2-phenylethanol (131), whereas the red wine lees (PC1 positive and PC2 negative) are characterized by ethyl octanoate (65), ethyl decanoate (97) and ethyl dodecanoate (127).

Furthermore, the PLS-DA (Fig. 5) was applied as supervised clustering and 10 differently expressed VOCs were found with VIP score > 1.5: ethyl decanoate (97), ethyl octanoate (65), ethyl dodecanoate (127), ethanol (5), 3-methylbutan-1-ol (28), ethyl hexanoate (32), ethyl acetate (2), 2-phenylethanol (131), isoamyl acetate (20) and 2-phenyethyl acetate (123), which are the most significant VOCs for explaining the discrimination of wine industry by-products.

The *p* values obtained by *one-way* ANOVA with post-hoc Tukey test ($p < 0.05$) showed that from the pool of the 153 VOCs previously identified, the differences in VOCs were statistically significant among the wine industry by-products. Fig. 6 shows the heat map constructed using Pearson´s correlation for the VOCs with VIP scores higher than 1.5. It is possible to observe that these VOCs had higher chromatographic area in wine lees than in grape by-products.

4. Conclusions

The current study evaluated the potential of volatile composition of wine industry by-products using HS-SPME/GC–MS methodology to enhance the quality of fish feeds in terms of organoleptic characteristics (manly aroma and flavor) and health benefits to aquaculture livestock. A total of 153 VOCs, which belong to different chemical families were identified, namely 36 esters, 20 alcohols, 17 acids, 18 terpenoids, 31 carbonyl compound, 11 furanic compounds, two lactones, four volatile phenols and 14 miscellaneous, 32 of which were common to all wine industry by-products analyzed.

From a sensorial point of view, the esters and terpenoids showed a positive contribution to aroma with fruity, sweet, green, fresh, anise and berry notes, since they have low odor threshold (in order of µg/L) and may play an important role as feed attractant. (*E*)-3-Hexen-1-ol and (*Z*)-2-hexenal were responsible for green odor, similar to cut grass. This odor had a light stress-alleviating effect on the domestic dog [33]. On the other hand, from a healthy point of view, acids (*e.g.*, hexanoic acid, octanoic acid, decanoic acid) can be used to provide fish feeds with antimicrobial properties and nutritional supplements, whereas terpenoids (*e.g.*, limonene, α-farnesene) can be used as chemopreventive activity against numerous types of cancer.

Our findings constitute a significant contribution to circular economy, since they confirmed the potential of wine industry by-products as enhancers of quality of fish feeds. This calls for further investigation on the extraction, isolation and purification of VOCs from wine industry by-products, in order to guarantee the safety of the animal feed and compliance with the requirements of the animal feed market. Notwithstanding the above, additional research is needed to evaluate any deterrent effects of the grape by-products for fish feeds

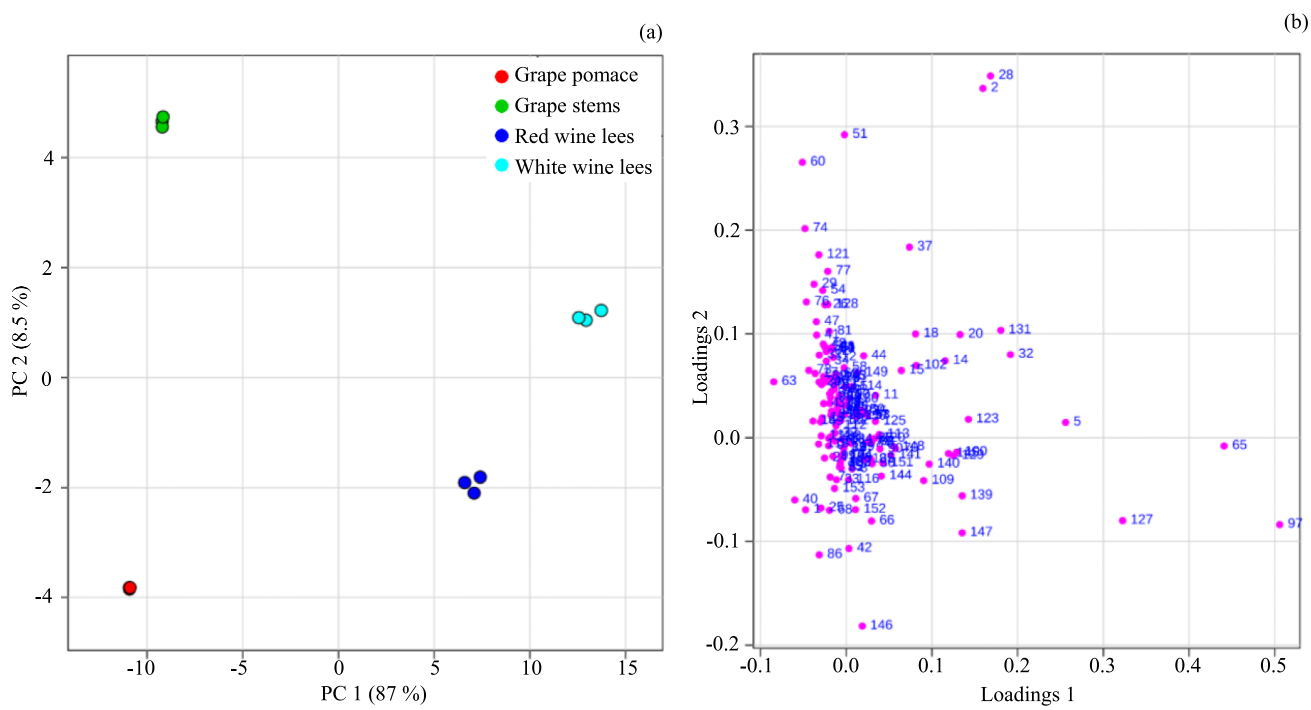

Fig. 4. PCA of the volatile profile of wine industry by-products. (a) PC1 × PC2 score scatter plot and (b) loading weight plot (attribution of the peak number is shown in Table 1).

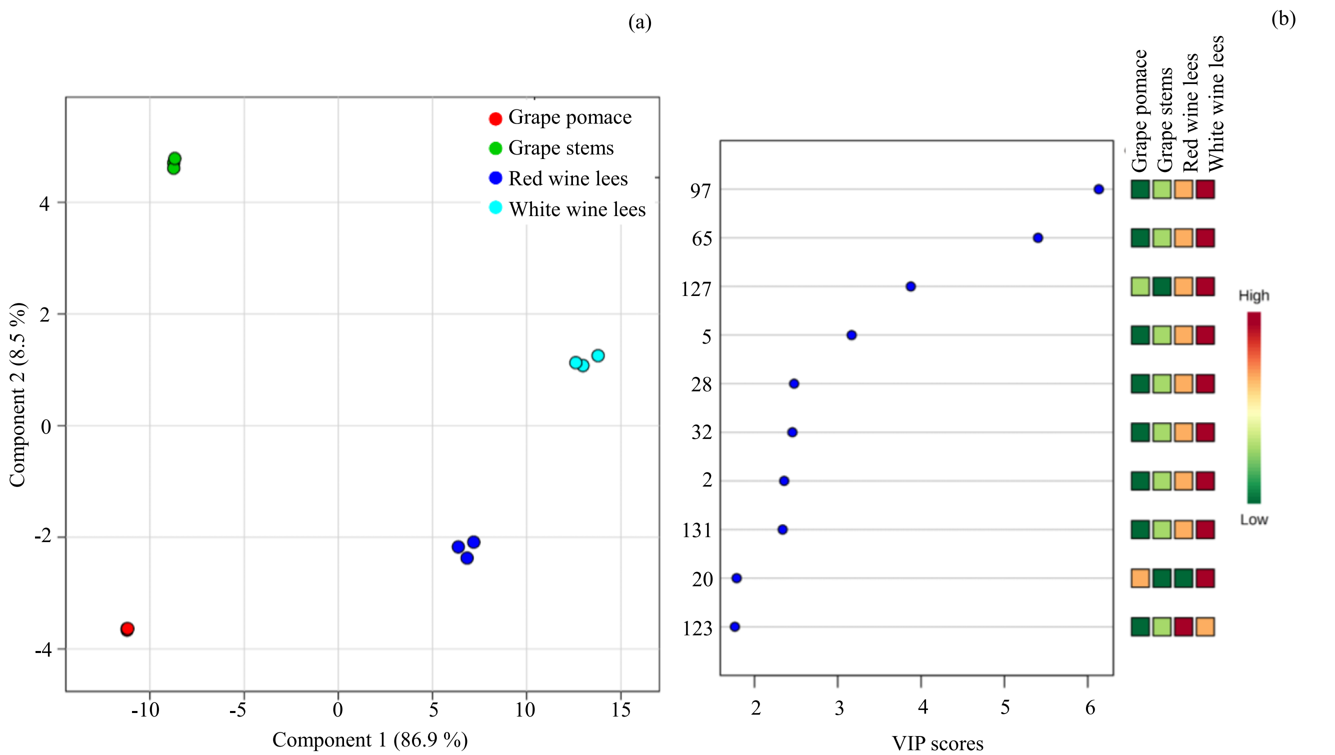

Fig. 5. PLS-DA of the volatile signature of wine industry by-products. (a) Score scatter plot, and (b) VIP scores (attribution of the peak number is shown in Table 1).

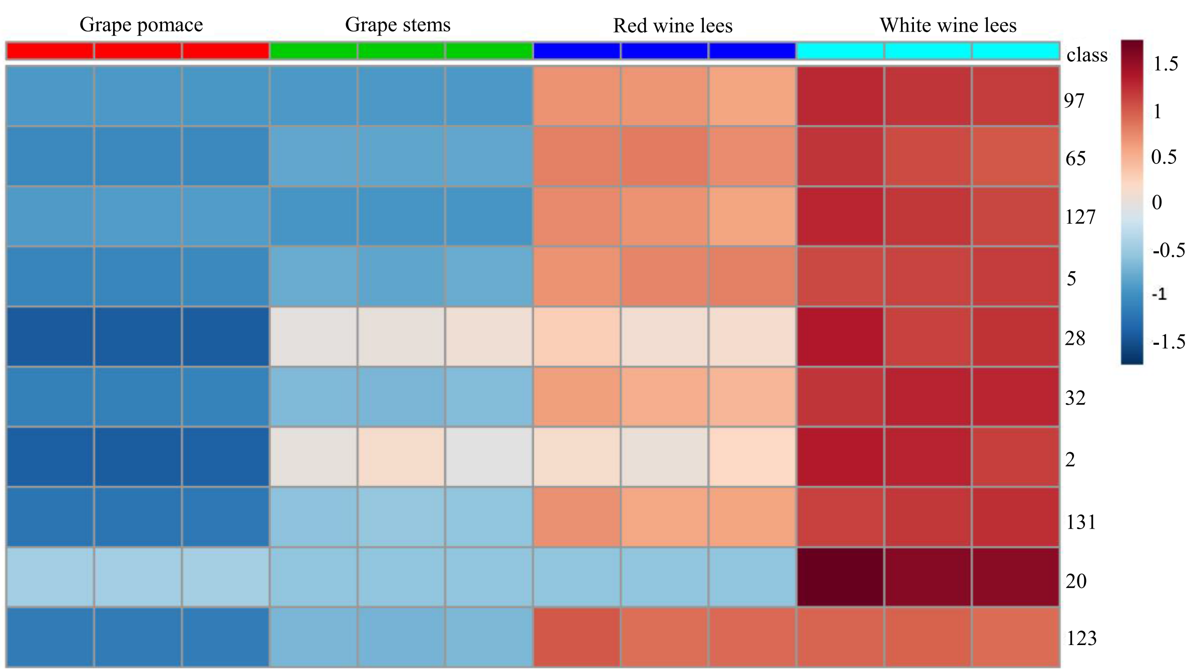

Fig. 6. Hierarchical cluster analysis (HCA). The heat maps of the ten VOCs (VIP values > 1.5), identified in wine industry by-products were generated by an average algorithm and Pearson's distance analysis (attribution of the peak number is shown in Table 1).

and to perform fish trials using the identified by-products in practical diets. Finally, in the case of winery waste, limited studies have been conducted on life cycle analysis regarding full economic costing of the use wine waste as a feed ingredient.

CRediT authorship contribution statement

José S. Câmara: Conceptualization, Formal analysis, Writing - original draft, Supervision. **Sílvia Lourenço:** Conceptualization, Formal analysis, Writing - original draft. **Catarina Silva:** Methodology, Formal analysis, Writing - review & editing. **André Lopes:** Conceptualization, Writing - review & editing. **Carlos Andrade:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition. **Rosa Perestrelo:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

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

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