Research article

Received: 26 October 2017 Revised: 11 September 2018 Accepted: 14 September 2018 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jib.549

Impact of fining agents on the volatile composition of sparkling mead

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Sparkling mead is obtained by secondary fermentation of the mead involving the addition of starter yeast culture, sucrose, nutrients and fining agents. The aim of this study was to evaluate the effect of different fining agents (tannins vs combined fining agents) on the volatile composition of sparkling mead. Sparkling mead was produced from a base mead using a commercial yeast strain (*Saccharomyces bayanus*) and the volatile compounds were determined by gas chromatography–flame ionisation detection and gas chromatography–mass spectrometry. Thirty six volatile compounds were quantified and the major groups were alcohols (73.2%), acetates (19.1%), carbonyl compounds (5.5%) and ethyl esters (1.2%), represented by 3-methyl-1-butanol, ethyl acetate, acetaldehyde and monoethyl succinate, respectively. The remaining compounds were present at <1%. Eleven volatile compounds exhibited odour activity values >1, with ethyl octanoate and ethyl hexanoate contributing to the aroma of sparkling mead, with fruity, strawberry and sweet notes. The combined fining agents caused a marked decrease in the concentration of volatile compounds compared with tannins. In general, 3-ethoxy-1-propanol, ethyl lactate, ethyl octanoate, diethyl succinate, diethyl malate, monoethyl succinate, 2-methylpropanoic acid, hexanoic acid, octanoic acid, acetaldehyde, acetoin, furfural, benzaldehyde, 5-hydroxymethylfurfural, *trans*-furan linalool oxide, *cis*-furan linalool oxide and 4-oxo-isophorone decreased in concentration. Conversely, 1-propanol and 2-methylpropanoic acid (tannins) and ethyl butyrate (combined fining agents) increased in concentration. The remaining volatile compounds were not affected. Significant differences ($p < 0.05$) were found for 19 volatile compounds independently of the type of fining agents used.

Keywords: mead; sparkling mead; fining agents; volatile compounds; odour activity value

Introduction

Mead is a traditional alcoholic beverage (alcoholic strength, by volume, between 8 and 18%) produced by the fermentation by yeasts of diluted honey. The production of mead has been the focus of numerous studies *(1–19)*. However, few reports consider the volatile profile of mead *(1,4,6,8,9,12,19)* and even fewer consider the production and characterisation of sparkling mead. Sparkling wines require a second fermentation and subsequent carbonation *(20)* following four main methods: classical *champenoise* or 'in bottle'; discontinuous or *charmat*; the transfer process; and the continuous method *(21)*. Despite the differences in approach, all require the addition of a yeast starter culture, sucrose, commercial nutrients and fining agents to the base wine.

Given the characteristics of the base beverage for producing sparkling drinks, the yeasts used for the secondary fermentation must satisfy more criteria than those used for the primary fermentation. In addition to being resistant to ethanol, they must exhibit high flocculation in order to facilitate their elimination from the bottle *(22)*. This is important as it facilitates the removal of the sediment during disgorging and minimises any turbidity in the final product *(22)*. *Saccharomyces bayanus* (EC-1118) has been reported to be a popular commercial strain for the production of sparkling wines *(23,24)*. Various compounds including lipids, amino acids, peptides and volatiles are released during the production of sparkling drinks owing to yeast autolysis *(23,24)*. Proteins, which play a key role during the fermentation process and form part of the final composition of the sparkling drinks, may agglomerate, causing turbidity of the beverage with an associated negative impact on consumer acceptance *(25)*. Therefore, the use of fining agents is important for ensuring the physicochemical stability of the fermented beverages and the prevention of hazes and deposits *(26)*.

Worldwide, common fining agents are sodium bentonite, polyphenolic tannins or mineral agents *(27)*. Bentonite is a clay made of soft phyllosilicate mineral considered to be generally safe *(28)*.

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It has been reported that the addition of bentonite to the base wine prior to secondary fermentation had no effect on wine volatiles *(28)*. However, other reports suggest that bentonite removes some aroma compounds in wine *(25,27,29)*. Other researchers suggest that bentonite is very efficient in protein removal and can also reduce browning. The addition of a fining agent during the fermentation process may promote yeast growth and accelerate fermentation *(30)*. Indeed, Marchal and Jeandet *(27)* reported that a mixed fining agent (including bentonite) may be advantageous in producing wines with less residual sugar and, therefore, a more complete fermentation process. However the same authors note that the use of bentonite for the fining of sparkling wines may produce a final product that has a large bubble size with poor bubble stability.

Recently, Pascoal *et al. (31)* showed that the use of different fining agents had a significant effect on the volatile composition of mead and its organoleptic characteristics. In this context, the aim of the present study was to produce sparkling mead clarified using tannins compared to combined fining agents (bentonite + gelatine + egg albumin) and to assess the impact on volatile compounds in the sparkling mead.

Material and methods

Fining procedures

The agents used for the fining process were combined fining agents (bentonite + gelatine + egg albumin; 150 g/hL) and tannins (30 g/hL) *(31)*. The fining agents were purchased from AEB Group Company (Bioquímica Portuguesa SA).

Mead samples were codified and fining agents – tannins and combined fining agents – were added to the base mead in bottle. To compare the effect of the fining agents in the sparkling mead, the results of previous studies *(31)* were used to plan the experimental design of the current study. In this context, the volatile composition of sparkling meads produced was studied using combined (CFA) and single fining agents (SFA). All of the secondary fermentation assays were performed in duplicate.

It is important to highlight that a control was not considered, as our objective was to verify the behaviour/influence of the selected fining agents on volatile composition during sparkling mead production.

Sparkling mead production

The physicochemical parameters of base mead were previously described Pascoal *et al. (31)*, including final pH (3.4), reducing substances (31.2 g/L), total acidity (expressed as tartaric acid) (5.1 g/L), yeast assimilable nitrogen (46.7 mg/L), volatile acidity (expressed as acetic acid; 1.5 g/L), total sulphur dioxide (32.4 mg/L) and ethanol (11.5%, by volume).

Sparkling mead was produced in bottles (following the traditional method, see Figure 1), to which fining agents were added. The tirage liquor (containing sucrose, 24 g/L), in order to achieve an extra-dry product, with a sugar concentration between 12 and 17 g/L *(33)*. Active dried yeasts (30g/hL) *Saccharomyces cerevisiae* and *Saccharomyces bayanus* (Fermol Reims Champagne, Collection de Levures d'Intérêt Biotechnologique, INRA of Paris Grignon France), were activated according to the manufacturers' instructions. Commercial nutrients (Sal Enovit at 40 g/hL) were also added to the base mead. A bottle stopper and manometer (EN 837-1-WIKA) were installed for pressure control inside the bottle. Bottles – in the horizontal position – were placed in an incubator (Incubated Shaker SIF6000R, Lab Companion, SCANSCI) at 12°C for 60 days. During this time, the bottles were gently mixed at least one a day.

The bottles were aged for 29 days and inclined (12.5%) for 45 days to enable riddling/*remuage*. Once the lees were fully in the neck of the bottle and the product was entirely clear, the sediment could be removed. The neck of the bottle was frozen at about 2 cm below the cork. After freezing, the bottles were put in vertical position and the cork was removed allowing the disgorging of the frozen zone. Finally, the addition of *liqueur d'expédition* (sugar) was added and bottle corked.

Analysis of major volatile compounds

The gas chromatographic analysis of volatile compounds was carried out in a Chrompack CP-9000 gas chromatograph with flame ionisation detection (FID) and a Meta-Wax capillary column (30 m × 0.25 mm; 0.2 μm film thickness, Teknokroma). The carrier gas was helium (1 mL/min). The temperature of the injector and the detector were both 250°C. The oven temperature was initially held at 50°C, for 2 min, then programmed to rise from 50 to 177.5°C, at 5°C/min, then from 177.5 to 230°C at 10°C/min and finally held at 230°C for 15 min. A sample (1 μL), prepared by adding 350 μg of 4-nonanol (internal standard) to 5 mL of mead, was injected in the split mode (15 mL/min). The quantification of volatile compounds was performed with the software Star-Chromatography Workstation version 6.41 (Varian*) (4,31,34)*. Injections were carried out in duplicate.

Extraction and analysis of minor volatile compounds

In a 10 mL tube, 8 mL of sample, 3.5 μg of internal standard (4-nonanol) and a magnetic stirring bar (22.2 mm × 4.8 mm) were added. Extraction was performed by stirring the sample with 400 μL of dichloromethane according to Oliveira *et al. (35)*. After cooling at 0°C for 15 min, the magnetic bar was removed, the organic phase was separated by centrifugation (4000 rpm, 7 min, 4°C) and the extract recovered. The extract was dried over anhydrous sodium sulphate. Extraction of volatiles from each sample were carried out in duplicate.

Volatile compounds were analysed using a gas chromatograph–mass spectrometer (GC–MS; Varian Saturn 2000) with a 1079 injector and an ion-trap mass spectrometer (IT-MS). The sample (1 μL) was injected in splitless mode (30 s) to a Sapiens-Wax MS Teknocroma column (30 m × 0.15 mm; 0.15 μm film thickness). The injector temperature was 250°C and the oven was held at 60°C, for 2 min, then programmed to rise to 234°C at 3°C/min, raised from 234 to 260°C at 5°C/min and finally held 5 min at 260°C. The carrier gas was helium at a constant flow rate of 1.3 mL/min. The detector was set to electronic impact mode (70 eV) with an acquisition range (*m/z*) from 35 to 300. The identification of volatile compounds was performed using the software Star Chromatography Workstation version 6.9.3 (Varian). Volatile compounds were determined, semi-quantitatively, as 4-nonanol equivalents *(4,31,34)*.

Determination of odour activity values

Odour activity values (OAV) were determined in order to evaluate the contribution of the compounds to the aroma of sparkling

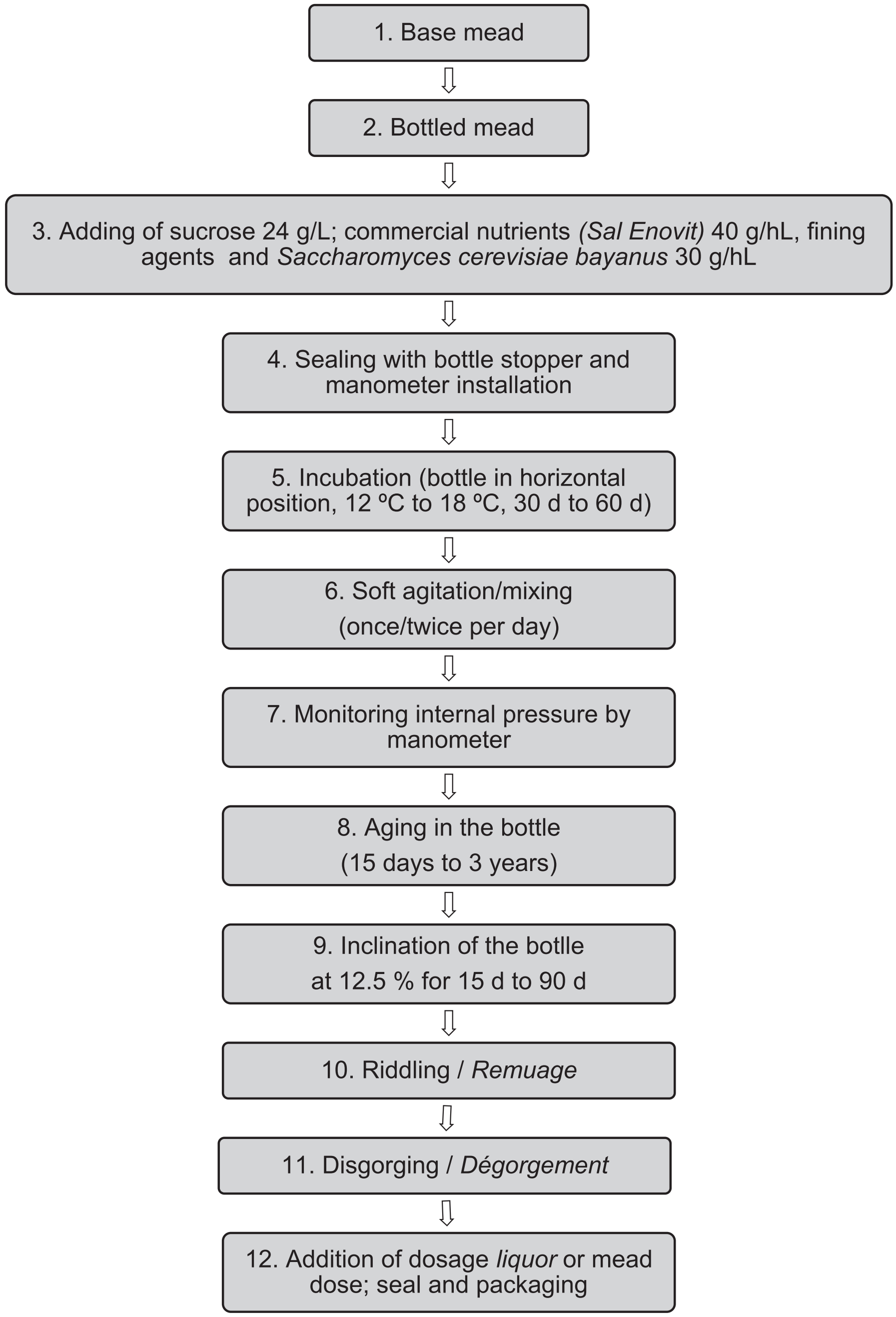

Figure 1. Flow diagram of sparkling mead production by the traditional method adapted from Torresi *et al.* *(12)* and Martínez-Rodríguez and Pueyo *(32)*.

mead. This measure was calculated as the ratio between the concentration of an individual compound and the reported sensory threshold *(34,36)*. Only compounds with OAV > 1 were considered to provide a significant contribution to the aroma of the sparkling mead *(6)*.

Statistical analysis

The analysis of variance (one-way ANOVA) was performed using the general linear model procedure as implemented in the SPSS software, version 20.0 (SPSS Inc.). The sample means were compared using Tukey's test. All statistical tests were performed at a 5% significance level.

Results and discussion

Alcoholic strength and reducing substances

The alcoholic strength (as alcohol by volume) of the base mead for sparkling mead production was 11.5% *(31)*. After secondary fermentation, the abv and reducing substances were 13.1% and 5.2 g/L, respectively. The sparkling mead can be classified as 'extra-dry', in agreement with the EU Regulation no. 1308/2013 *(37)*.

Effects of fining agents on volatile composition

Table 1 presents the volatile composition of mead produced after primary fermentation and in sparkling mead after secondary

Table 1. Mean of concentration ($\overline{C}$) and standard deviation (SD) of major and minor volatile compounds analysed by gas chromatography–flame ionisation detection (GC–FID) and GC–mass spectrometry (MS), respectively, in mead and sparkling mead treated by different fining agents

Identified volatile compounds (μg/L)	Combined fining agents, CFA (bentonite + gelatine + egg albumin)		Single fining agent, SFA (tannins)	
	Mead CFA $\overline{C}$ ± SD	Sparkling CFA $\overline{C}$ ± SD	Mead SFA $\overline{C}$ ± SD	Sparkling SFA $\overline{C}$ ± SD
Alcohols				
1-Propanol*	40,723.0 ± 1,081.4^{ab}	50,335.0 ± 3,572.9^{ab}	25,423.0 ± 6,993.8^{a}	57,456.0 ± 11,038.7^{b}
2-Methyl-1-propanol*	23,028.0 ± 215.0	22,034.0 ± 2,064.1	15,341.0 ± 1,336.8	29,160.0 ± 4,612.3
2-Methyl-1-butanol*	18,152.0 ± 1217.3	17,200.0 ± 1096.9	15,054.0 ± 1,693.3	22,702.0 ± 3,702.7
3-Methyl-1-butanol*	137,143.0 ± 5,738.3	106,457.0 ± 4,578.7	101,719.0 ± 10,161.1	122,916.0 ± 19,567.7
1-Hexanol	5.2 ± 0.9^{ab}	6.5 ± 0.9^{b}	3.0 ± 0.2^{a}	3.6 ± 0.1^{a}
3-Ethoxy-1-propanol	25.2 ± 2.5^{b}	8.6 ± 0.1^{a}	15.8 ± 3.9^{ab}	7.7 ± 3.5^{a}
2-Phenylethanol*	49,568.0 ± 7,580.0	27,696.0 ± 5,087.0	30,813.0 ± 5,388.8	32,841.0 ± 5,055.3
Tyrosol	139.4 ± 15.5^{b}	80.1 ± 10.1^{ab}	65.9 ± 7.9^{a}	84.1 ± 10.4^{ab}
Subtotal	**268,783.8**	**223,817.2**	**188,434.7**	**265,170.4**
Ethyl esters				
Ethyl butyrate	42.6 ± 1.8^{a}	134.6 ± 20.4^{b}	22.6 ± 1.8^{a}	13.3 ± 2.8^{a}
Ethyl hexanoate	241.4 ± 3.6^{b}	219.1 ± 6.1^{ab}	121.1 ± 3.8^{ab}	60.7 ± 15.2^{a}
Ethyl lactate	635.2 ± 1.7^{b}	312.3 ± 1.5^{a}	379.6 ± 67.9^{a}	269.5 ± 43.9^{a}
Ethyl octanoate	107.3 ± 1.5^{c}	101.8 ± 2.7^{c}	58.4 ± 0.5^{b}	4.7 ± 0.8^{a}
Ethyl 3-hydroxy-butanoate	37.1 ± 0.1^{b}	28.2 ± 0.3^{ab}	20.9 ± 3.8^{a}	25.7 ± 4.8^{ab}
Diethyl succinate	2,283.9 ± 25.9^{c}	1,184.9 ± 46.7^{b}	1,205.1 ± 72.2^{b}	578.9 ± 10.2^{a}
Diethyl malate	200.0 ± 2.2^{c}	52.5 ± 3.0^{a}	116.0 ± 21.6^{b}	25.3 ± 2.5^{a}
Monoethyl succinate	5,340.2 ± 117.4^{b}	2,299.5 ± 127.4^{a}	3,398.7 ± 620.8^{a}	2,528.3 ± 111.4^{a}
Subtotal	**8,887.7**	**4,332.9**	**5,322.4**	**3,506.4**
Acetates				
Ethyl acetate*	52,325.0 ± 1,352.5	55,064.0 ± 100.4	60,602.0 ± 3,841.0	73,156.0 ± 10,307.5
Isoamyl acetate	59.2 ± 0.4	105.0 ± 12.7	63.2 ± 3.0	54.2 ± 12.6
2-Phenylethyl acetate	31.0 ± 0.4	33.8 ± 1.2	33.8 ± 2.1	21.9 ± 2.3
Subtotal	**52,415.2**	**55,202.8**	**60,699.0**	**73,232.1**
Volatile fatty acids				
2-Methylpropanoic acid	432.1 ± 20.2^{b}	262.1 ± 30.6^{a}	225.5 ± 3.8^{a}	512.0 ± 7.1^{c}
Butanoic acid	47.8 ± 1.0^{ab}	60.8 ± 2.0^{a}	25.2 ± 2.2^{b}	51.5 ± 11.2^{ab}
Hexanoic acid	969.6 ± 13.6^{b}	671.0 ± 7.3^{a}	660.5 ± 119.6^{a}	632.9 ± 31.1^{a}
Octanoic acid	1,592.6 ± 33.9^{c}	1,605.2 ± 140.2^{c}	1,096.5 ± 103.6^{b}	668.3 ± 14.9^{a}
Decanoic acid	135.3 ± 5.9^{ab}	214.2 ± 23.5^{b}	81.3 ± 9.1^{a}	95.5 ± 20.3^{a}
Phenylacetic acid	269.0 ± 4.2	226.7 ± 31.4	207.0 ± 17.0	187.3 ± 32.3
Subtotal	**3,446.4**	**3,040.0**	**2,296.0**	**2,147.5**
Carbonyl compounds				
Acetaldehyde*	146,937.0 ± 2,568.2^{b}	14,112.0 ± 1,620.9^{a}	160,586.0 ± 18,116.3^{b}	22,446.0 ± 1,737.5^{a}
Acetoin	2,071.8 ± 5.7^{c}	211.9 ± 16.0^{a}	957.4 ± 278.7^{b}	113.8 ± 16.4^{a}
Furfural	217.3 ± 4.1^{b}	46.5 ± 1.3^{a}	146.7 ± 28.2^{b}	26.7 ± 6.0^{a}
Benzaldehyde	33.6 ± 0.9^{b}	1.1 ± 0.2^{a}	53.8 ± 0.9^{c}	5.6 ± 1.4^{a}
5-Hydroxymethylfurfural	605.8 ± 21.7^{b}	172.1 ± 35.6^{a}	426.9 ± 70.7^{b}	89.9 ± 17.3^{a}
Subtotal	**149,865.5**	**14,543.6**	**162,170.8**	**22,682.0**
Terpenes and lactones				
trans-Furan linalool oxide	666.1 ± 10.3^{b}	332.4 ± 0.4^{a}	509.4 ± 50.6^{ab}	373.5 ± 0.8^{a}
cis-Furan linalool oxide	263.3 ± 0.8^{b}	121.6 ± 0.3^{a}	229.7 ± 26.4^{b}	137.2 ± 9.6^{a}
HO-Trienol	158.6 ± 0.6	170.6 ± 10.7	151.6 ± 11.8	150.2 ± 11.0
α-Terpineol	18.8 ± 0.7	10.8 ± 1.4	12.6 ± 2.1	17.6 ± 1.5
Pantolactone	37.2 ± 2.1^{b}	25.5 ± 0.8^{ab}	21.5 ± 2.5^{a}	23.3 ± 0.4^{a}
Subtotal	**1,106.8**	**635.4**	**903.3**	**678.5**
Norisoprenoids				
4-oxo-isophorone	26.2 ± 1.1^{b}	10.1 ± 1.4^{a}	19.3 ± 2.5^{ab}	14.4 ± 1.3^{ab}
Total	**484,568.8**	**301,607.5**	**419,867.0**	**367,454.6**

* Major volatile compounds (quantified by GC–FID).
Means with different superscripts letters ($^{a–c}$) within the same row differ significantly ($p < 0.05$). Lack of a superscript letter indicates no significant difference ($p > 0.05$).
For Mead_CFA and Sparkling_CFA, combined fining agents were added; for Mead_SFA and Sparkling_SFA tannins were added.

fermentation. Studies in sparkling wine reveal that the second fermentation is responsible for the loss of characteristic volatile compounds but also the formation of new aromas *(38,39)*. Our results are consistent with this since, in general, the second fermentation resulted in a reduction (47.2%) of volatile compounds, independently of the fining agents used. Table 1 also presents the mean concentration and standard deviation of the 36 volatile compounds detected by GC–FID and GC–MS in the sparkling mead samples, clarified with CFA or with tannins (SFA).

It was observed that the combined fining agent caused a decrease in the concentration of 15 (41.7%) volatile compounds, while for the tannin fining agent a decrease was observed in 10 (27.8%) compounds. The groups affected by combined fining agent were alcohols (3-ethoxy-1-propanol), esters (ethyl lactate, diethyl succinate, diethyl malate, monoethyl succinate), volatile fatty acids (2-methylpropanoic acid, hexanoic acid), carbonyl compounds (acetaldehyde, acetoin, furfural, benzaldehyde, 5-hydroxymethylfurfural), terpenes (*trans*-furan linalool oxide, *cis*-furan linalool oxide) and norisoprenoids (4-oxo-isophorone). The groups whose concentrations decreased following the use of tannin fining agent were esters (ethyl octanoate, diethyl succinate, diethyl malate), volatile fatty acids (octanoic acid), carbonyl compounds and terpenes (*cis*-furan linalool oxide).

The concentration of carbonyl compounds decreased during sparkling mead production. Similar findings were reported by Madrera *et al. (40)*. These authors found an increase in the concentrations of ethyl acetate, ethyl lactate and ethyl octanoate during aging in contact with lees.

Marchal and Jeandet *(27)* report that, although bentonite treatment is efficient in protein removal and for enhancing wine filterability, it is responsible for the loss of aromatic compounds. In the present study a considerable loss of some volatile compounds (around 41.7%) was found when CFA was added. Previous studies by Armada and Falqué *(29)* found a reduction of about 13% of the total volatile compounds in Albariño musts and wines clarified with bentonite and highlighted that the use of this fining agent would have a negative impact on varietal characteristics of wine.

Although the concentration of some compounds decreased, some did not differ significantly between the base mead and sparkling mead when clarified by the combined fining agents (bentonite + gelatine + egg albumin). These included 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, 2-phenylethanol, tyrosol, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxy-butanoate, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, butanoic acid, octanoic acid, decanoic acid, phenylacetic acid, HO-trienol, α-terpineol and pantolactone. Some of these compounds are important in the aroma of sparkling mead, notably ethyl octanoate and ethyl hexanoate, which are responsible for providing fruity, strawberry and sweet aromas *(38)*.

Aside from the fining agents, there may be other reasons for the decrease of some volatile compounds. For example, Torrens *et al. (38)* reported that, during the secondary fermentation and subsequent aging with lees, acetate and ethyl esters decreased. This is in agreement with the results reported here as ethyl lactate, diethyl succinate, diethyl malate and monoethyl succinate all decreased in concentration.

Thirty six compounds were identified in this work. Three increased in concentration following sparkling mead production: ethyl butyrate treated by the CFA with 1-propanol and 2-methylpropanoic acid in mead treated with tannins. This is in agreement with Torrens *et al. (38)* who reported an increase in ethyl butyrate in young and cava reserve. The remaining compounds accounting for 50% of the total were not affected by fining agents. These included 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, 2-phenylethanol, tyrosol, ethyl hexanoate, ethyl octanoate, ethyl-3-hydroxy-butanoate, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, butanoic acid, decanoic acid, phenylacetic acid, HO-trienol, α-terpineol and pantolactone.

Of all the volatile compounds identified, 44.4% (n = 16) differed significantly ($p < 0.05$) following treatment with the combined fining agents and 33.3% (n = 12) after addition of tannins. The major volatiles in decreasing order were 3-methyl-1-butanol, ethyl acetate, 1-propanol, 2-phenylethanol, 2-methyl-1-propanol, 2-methyl-1-butanol and acetaldehyde. The minor volatile compounds, including monoethyl succinate, hexanoic acid and octanoic acid, were found at >0.6 mg/L.

The alcohols represented 73.2% of total volatile compounds and were the most prevalent group of compounds in the sparkling mead, followed by acetates, carbonyl compounds and esters. The remaining groups, including volatile fatty acids (but excluding octanoic acid, lactones, terpenes and norisoprenoid), were present <1 mg/L.

The alcohol, 3-methyl-1-butanol was the most abundant of the volatile compounds in sparkling mead. This is in agreement with Amerine and Ough *(41)*, who reported that higher alcohols are quantitatively the largest group of aroma compounds in alcoholic beverages. Similar results were obtained by García *et al. (42)* and Pérez-Magariño *et al. (43)* with sparkling wines with isoamyl alcohol, 2-phenylethanol, 1-propanol and isobutanol (2-methyl-1-propanol). Concentrations of 1-propanol from 5 to 27 mg/L have been reported by Garofolo *et al. (44)* as being ideal for sparkling wines. However, the results obtained here are higher than those previously reported. The concentrations of 2-phenylethanol and 1-hexanol were higher than those reported by Torrens *et al. (38)* while those of 2-methyl-1-propanol were higher after treatment with tannins and lower with CFA. Higher concentrations of 1-hexanol, 3-methyl-1-butanol were found by Verzeletti *et al. (45)*, although the concentrations of 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 2-phenylethanol were lower compared with the present study. Higher concentrations of alcohols were also observed by Madrera *et al. (40)* for 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol in sparkling ciders during aging. Also Valles *et al. (46)* found higher concentrations of amyl alcohols and 2-phenylethanol produced by *S. bayanus* than in this work. Methanol is a toxic volatile compound whose content in distillates is controlled by EU regulation *(47)*. The concentration of methanol determined on the sparkling meads produced in this study were very low and in some cases undetectable. According to Tukey's test there were no significant differences between the volatile compounds composition in the extra-dry sparkling meads clarified using combined fining agents vs tannins.

The group of esters (1.2% of total volatile compounds) was represented primarily by monoethyl succinate, followed by diethyl succinate, ethyl lactate, ethyl hexanoate, ethyl butyrate, ethyl octanoate, diethyl malate and ethyl-3-hydroxy-butanoate (Table 1), with concentrations <1 mg/L. This group has been reported to be responsible for the fruit aromas in wines and other fermented beverages *(48)*. Most of these esters are produced by yeasts during fermentation, as secondary products of sugar metabolism. Esters such as monoethyl succinate, diethyl succinate, ethyl lactate and diethyl malate are markers of product aging. Overall esters play an important role in the aroma of sparkling wine *(43)*.

Torrens *et al.* *(38)* found ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl lactate and diethyl succinate at higher concentrations and reported ethyl lactate and diethyl succinate as the most abundant esters in aged Cava. Verzeletti *et al.* *(45)* reported slightly higher concentrations of ethyl butyrate, ethyl octanoate, ethyl hexanoate and diethyl succinate, and Madrera *et al.* *(40)* found higher concentrations of ethyl lactate, ethyl butyrate, ethyl hexanoate and ethyl octanoate in sparkling ciders. Recently Caliari *et al.* *(49)* found ethyl octanoate at comparatively higher concentrations in Moscato Giallo sparkling wines. The results are similar with those reported by Torrens *et al.* *(38)*, Madrera *et al.* *(40)* and Verzeletti *et al.* *(45)* with higher concentrations of ethyl lactate and diethyl succinate in aged Spanish Cava and Brazilian sparkling wine, respectively. The concentrations of monoethyl succinate ranged between 2.3 and 2.5 mg/L, followed by diethyl succinate (0.6–1.2 mg/L) and ethyl lactate (269.5–312.3 μg/L), but had no marked impact on aroma at these concentrations. Statistically significant differences ($p < 0.05$) between sparkling meads treated with the combined fining agents and tannins were noted with ethyl butyrate, ethyl octanoate and diethyl succinate.

The 'acetates' accounted for 19.1% of the total volatile compounds. Ethyl acetate was the major compound ranging from 55.1 to 73.2 mg/L. Of the minor volatile compounds, isoamyl acetate was present at a higher concentration than 2-phenylethyl acetate. Ethyl acetate exhibits a fruity aroma in wines <200 mg/L but above this contributes a solvent like odour *(50)*. The concentration of ethyl acetate obtained here was higher than that observed by Verzeletti *et al.* *(45)* whilst that of 2-phenylethyl acetate was similar. The concentrations of ethyl acetate reported by Madrera *et al.* *(40)* and Valles *et al.* *(46)* were similar to those reported here but isoamyl acetate was higher. Conversely, the results here for ethyl acetate and isoamyl acetate were higher than those reported by Torrens *et al.* *(38)*. No significant differences ($p > 0.05$) were observed for this group of compounds between the two fining treatments.

The volatile fatty acids accounted for 0.8% of total volatile compounds with octanoic acid at 0.7–1.6 mg/L, followed by hexanoic acid, 2-methylpropanoic acid, phenylacetic acid, decanoic acid and butanoic acid (Table 1). These results are similar to those reported by Torrens *et al.* *(38)* in sparkling wines (young Cava), although concentrations of hexanoic acid and octanoic acid were slightly higher. However, the concentrations of decanoic acid, octanoic acid, hexanoic acid and butanoic acid *(43,45,49)* were higher than those found here. Significant differences ($p < 0.05$) between the combined fining agents and tannins were found for 2-methylpropanoic acid, octanoic acid and decanoic acid.

The carbonyl compounds determined in the sparking mead represent 5.5% of the total volatile compounds (Table 1). Acetaldehyde is the most important carbonyl compound and is formed during fermentation as an intermediate from pyruvate *(32)* and may also be formed during storage/aging owing to the oxidative degradation of ethanol. High concentrations of acetaldehyde are an off-flavour in fermented beverages and accordingly it is an important compound for quality control *(47)*. The acceptable limits of acetaldehyde in sparkling beverages are controversial. Indeed, Rizzon *et al.* *(51)* reported that the recommended content of acetaldehyde in sparkling beverages varies between 60 and 70 mg/L while Paiano *et al.* *(52)* reported a range between 34.8 and 254 mg/L in alcoholic beverages. Other researchers reported that the levels of acetaldehyde produced by *S. cerevisiae* and *S. bayanus* may range from 0.5 to 286 mg/L and from 16 to 683 mg/L, respectively *(53)*. The levels of acetaldehyde obtained in the present study in both mead and sparkling mead are consistent with those levels produced by these yeasts. Moreover, the acetaldehyde concentrations obtained in this study are lower than those found in other sparkling beverages *(38,40,42,45)*. Acetaldehyde at low levels gives a pleasant fruity aroma, but at high concentrations possesses a pungent odour *(54)*. The remaining carbonyl compounds (acetoin, 5-hydroxymethylfurfural, furfural and benzaldehyde) were present <1 mg/L. During alcoholic fermentation, *Saccharomyces* produce acetoin from non-detectable amounts to 12 mg/L *(40)* and has been reported to be important in the flavour of foods and beverages *(55)*. No statistical differences in the samples was found for these compounds ($p > 0.05$).

Terpenes (0.2% of total volatile compounds) were represented by *trans*-furan linalool oxide, followed by *cis*-linalool oxide furan, HO-trienol and α-terpineol (Table 1). The concentration of these compounds was <1 mg/L. The concentration of α-terpineol was lower than that reported by Pérez-Magariño *et al.* *(43)*. No statistical differences were found for sparkling meads treated with either fining agent.

The lactones and norisoprenoids were present at very low concentrations and were represented by pantolactone and 4-oxo-isophorone, respectively (Table 1). The concentrations of these compounds did not differ ($p < 0.05$) between sparkling meads treated with the two fining agents.

Volatile compounds impacting on aroma

Table 2 presents the volatile compounds (OAV > 1) which may influence the sparkling mead aroma profile together with odour thresholds and aroma descriptors. From the 36 volatile compounds identified, only 11 are likely to make a significant contribution to the aroma of sparkling mead. Ethyl octanoate and ethyl butyrate showed concentrations below their odour threshold in the sample treated with tannins while the concentrations of the remaining compounds were always above their odour threshold for both fining agents. In general, all of these compounds play an important role in the aroma of the sparkling mead under study.

The group of ethyl esters, represented by ethyl octanoate, was present in a significant percentage (27.3%) followed by ethyl hexanoate, ethyl acetate and ethyl butyrate. Ethyl octanoate had the highest OAV (20.4) with the CFA and ethyl acetate (9.8) in the tannin-treated sparkling mead. These results are in agreement with Caliari *et al.* *(49)*, who found ethyl octanoate to be the major odorant in Moscato Giallo sparkling wines followed by ethyl hexanoate. Ethyl butyrate has also been claimed to contribute to the fruity and sweet notes of sparkling wine *(38)*. The 'alcohols' represented 18.2% of the total volatile compounds with OAV > 1, and were represented by 3-methyl-1-butanol contributing a solvent-like character *(57)*, followed by 2-phenylethanol with floral and rose aroma notes *(38)*. The volatile fatty acids, represented by octanoic acid and hexanoic acid, have been reported as conferring sweat, cheese and acid aromas in sparkling wines and wines *(34,38)*. The acetates were represented by ethyl acetate and isoamyl acetate. Ethyl acetate in wine gives a solvent and fruity aroma *(57)* with isoamyl acetate contributing banana, fruity and sweet aromas *(38)*. The carbonyl compounds and terpenes contributed similarly (9.1%) with acetaldehyde the major carbonyl compound in sparkling mead. However, Garofolo *et al.* *(44)* reported that butyl acetate and phenethyl acetate are of great sensory importance in sparkling wines. A terpene HO–trienol may confer linden aroma *(59)* in the sparkling mead reported here.

Table 2. Odour threshold, odour activity values (OAV) and aroma descriptor of the volatile compounds with more impact on the sparkling mead

Volatile compounds	Odour threshold (μg/L)	*OAV*		Aroma descriptor
		SFA	CFA	
3-Methyl-1-butanol	30,000 *(56)*	4.1	3.5	Solvent *(57)*
2-Phenylethanol	10,000 *(38)*	3.3	2.8	Rose, sweetish *(34)*
Ethyl hexanoate	14 *(56)*	4.3	15.7	Fruity, strawberry, sweet *(38)*
Ethyl octanoate	5 *(56)*	<1	20.4	Fruity, strawberry, sweet *(38)*
Ethyl butyrate	20 *(56)*	<1	6.7	Fruity, sweet *(38)*
Hexanoic acid	420 *(56)*	1.5	1.6	Cheese, acid *(38)*
Octanoic acid	500 *(56)*	1.3	3.2	Sweat, cheese *(38)*
Ethyl acetate	7500 *(36)*	9.8	7.3	Solvent, fruity *(58)*
Isoamyl acetate	30 *(56)*	1.8	3.5	Banana, fruity, sweet *(38)*
Acetaldehyde	10,000 *(36)*	2.2	1.4	Pungent *(57)*
HO-trienol	110 *(59)*	1.4	1.6	Linden *(59)*

Superscript numbers in brackets are the references from which the datat was taken.

Conclusions

In this study, alcohols, represented by 3-methyl-1-butanol, were the most prevalent group of volatile compounds found in sparkling meads (73.2%), followed by acetates (19.1%), carbonyl compounds (5.5%) and ethyl esters (1.2%). From the 36 volatile compounds identified, 15 (41.7%) differed significantly ($p < 0.05$) among mead clarified with combined fining agents and 10 (27.8%) in the product clarified using tannins, whereas the remaining 17 (47.2%) did not differ statistically. The sparkling mead clarified with combined fining agents presented a higher odour activity value (OAV > 4) with ethyl octanoate, ethyl hexanoate, ethyl acetate and ethyl butyrate. Conversely, ethyl acetate, ethyl hexanoate and 3-methyl-1-butanol had a higher odour activity value in the sparkling mead clarified by tannins. These results on the volatile profile suggest that sparkling mead should be produced using different concentrations of CFA.

Acknowledgements

O. Anjos thanks Centro de Estudos Florestais that is a research unit funded by Foundation for Science and Technology (FCT, Portugal; UID/AGR/UI0239/2013). A. Pascoal would like to thank FCT, Portugal, Programa Operacional Pontencial Humano and the European Union for his postdoctoral grant (SFRH/BPD/91380/2012). The authors also are grateful to the Centre of Molecular and Environmental Biology, funded by FCT, UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and by the ERDF through the COMPETE2020 – Programa Operacional Competitividade e Internacionalização.

This study was also supported by FCT under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 – Programa Operacional Regional do Norte.

Conflict of interest

The authors declare that there are no conflicts of interest. They are indebted for the careful and constructive criticisms of the reviewers.

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