Effects of Ethanol and Long-Chain Ethyl Ester Concentrations on Volatile Partitioning in a Whisky Model System

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ABSTRACT: Ethanolic atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was used to analyze the headspace concentrations of a test set of 14 whisky volatile compounds above a series of aqueous ethanolic solutions differing in alcohol content (5−40% ABV) and with regard to concentration of ethyl hexadecanoate (0−500 mg/L). The latter was selected to represent the long-chain ethyl esters found at various concentrations in new-make spirit. Headspace ion intensities were modeled against ethanol and ethyl hexadecanoate concentrations as factors. A separate model was prepared for each compound. Not surprisingly, ethanol content in the range of 5−40% ABV had a significant effect ($P < 0.0001$) on headspace volatile concentrations of all volatile compounds, whereas the ethyl hexadecanoate concentration had a selective effect of reducing headspace concentrations of the more hydrophobic compounds ($\log P > 2.5$). This finding is discussed in terms of the "structuring" effects of ethyl hexadecanoate when present above critical micelle concentration, leading to the selective incorporation of hydrophobic volatile compounds into the interior of micelle-like structures. Data presented illustrate that dilution of whiskies to 23% ABV for "nosing" in the presence of long-chain ethyl esters is likely to change the balance of volatile compounds in the headspace and thus the perceived aroma character.

KEYWORDS: alcoholic beverage, APCI-MS, agglomerate formation, whisky aroma, flavor partitioning

■ INTRODUCTION

A blended whisky can contain between 20 and 50 different malt whiskies.[1] Each of these contributes its own distinctive aroma character and together the blend creates a rounded overall flavor. Therefore, a new-make spirit may be prized for, and traded on the basis of, the specific flavor notes it imparts within a blend. For the aroma of a new-make spirit to be evaluated, the distillate is diluted to 23% alcohol by volume (ABV) to reduce pungency.[2,3] The perceived aroma of a whisky sample "nosed" in this way is a consequence of the volatile aroma chemicals it contains and their partition into the gas phase. From a physicochemical perspective whisky is a complex system containing a range of components that influence both the static (equilibrium) and dynamic partitioning of aroma. The addition of water (and hence the dilution of ethanol content) has a direct impact on the solubility of aroma compounds,[4] many of which will be less soluble at lower alcohol strengths and thus will partition into the headspace more efficiently. This is one reason malt whisky is sometimes served diluted with water or ice to fully appreciate its flavor. However, for sparingly soluble amphiphilic compounds such as long-chain fatty acid ethyl esters, lowering the solubility by reducing ethanol content can induce "structuring" of the solution due to the formation of agglomerates.[5−7] These structures comprise surface active molecules such as long-chain alcohols, aldehydes, and esters.[7] They have the ability to incorporate other hydrophobic compounds and thus to lower their concentrations in the headspace, potentially altering perceived aroma.[8] Another way in which agglomeration of ethyl esters can occur is by adding them at a concentration sufficient to saturate the surface of the solution (due to their amphiphilic nature ethyl esters act as surfactants, arranging themselves on the surface of an aqueous solution).

In this paper we consider the impacts on volatile partitioning of two surface active components: ethanol itself, present at a range of concentrations, and ethyl hexadecanoate (a representative agglomerate forming surface active compound) in model spirit solutions. A test set of 14 whisky volatile compounds (selected to cover a range of physicochemical properties in terms of compound volatility and hydrophobicity/hydrophilicity) were used in the study. Our interest in this area stemmed from research into the origins of nutty and cereal flavor characters in malt whisky. The test set of chemicals was selected in part because of our interest in the behavior of heterocyclic, relatively hydrophilic compounds, which were hypothesized to be involved in imparting nutty/cereal characters to new-make spirit. Others were added to display the noted range in physicochemical properties, and we incorporated into this a series of ethyl esters of increasing carbon chain length to enable the progressive impact of increasing molecular size and hydrophobicity to be monitored in a series of compounds that are similar in terms of their functional group chemistry. There was no intention to create a model whisky aroma, as has been reported by other workers using aroma recombination and omission studies.[9] We had observed that one major analytical difference between new-make spirits with distinctive aromas, but very similar volatile compound compositions, was in their content of long-chain

Table 1. APCI-MS Headspace Ion Intensities for One Group of Six Volatiles Measured Simultaneously above 20 Solutions of Various Ethanol (5−40% ABV) and Ethyl Hexadecanoate (0−500 μg/mL) Concentrations[a]

sample	C16 ethyl ester concn (μg/mL)	EtOH (% v/v)	pyrazine m/z 81	furfural m/z 97	benzaldehyde m/z 107	2,5-dimethylpyrazine m/z 109	2-acetylthiazole m/z 128	ethyl hexanoate m/z 145
1	0	5	1.02×10^6	3.97×10^6	6.89×10^6	2.82×10^5	1.13×10^6	2.55×10^7
2	500	5	1.44×10^6	6.80×10^6	1.99×10^7	6.20×10^5	2.23×10^6	1.33×10^8
3	250	5	1.46×10^6	6.65×10^6	2.32×10^7	7.50×10^5	2.50×10^6	1.34×10^8
4	0	5	1.34×10^6	6.31×10^6	1.83×10^7	5.90×10^5	2.13×10^6	1.32×10^8
5	500	5	1.13×10^6	5.07×10^6	1.16×10^7	4.25×10^5	1.58×10^6	6.76×10^7
6	0	5	9.38×10^5	3.70×10^6	6.56×10^6	2.55×10^5	1.02×10^6	2.62×10^7
7	250	15	1.47×10^6	6.96×10^6	2.54×10^7	9.05×10^5	2.81×10^6	1.34×10^8
8	500	23	1.06×10^6	4.27×10^6	7.90×10^6	3.46×10^5	1.26×10^6	2.40×10^7
9	0	23	9.85×10^5	3.86×10^6	6.90×10^6	3.08×10^5	1.09×10^6	2.51×10^7
10	250	23	1.51×10^6	6.99×10^6	2.60×10^7	9.28×10^5	2.91×10^6	1.33×10^8
11	250	23	1.49×10^6	6.53×10^6	2.45×10^7	9.00×10^5	2.73×10^6	1.33×10^8
12	250	23	1.44×10^6	6.65×10^6	2.46×10^7	8.92×10^5	2.72×10^6	1.33×10^8
13	250	23	1.33×10^6	6.22×10^6	1.75×10^7	5.94×10^5	2.16×10^6	1.28×10^8
14	125	32	1.34×10^6	6.24×10^6	1.82×10^7	5.91×10^5	2.19×10^6	1.32×10^8
15	375	32	9.79×10^5	4.02×10^6	6.88×10^6	2.89×10^5	1.12×10^6	2.56×10^7
16	0	40	1.29×10^6	5.79×10^6	1.31×10^7	4.15×10^5	1.61×10^6	7.66×10^7
17	500	40	1.69×10^6	7.37×10^6	2.75×10^7	1.04×10^6	3.19×10^6	1.34×10^8
18	250	40	1.40×10^6	6.51×10^6	1.84×10^7	6.07×10^5	2.22×10^6	1.26×10^8
19	0	40	1.34×10^6	6.18×10^6	1.79×10^7	5.65×10^5	2.14×10^6	1.34×10^8
20	500	40	1.46×10^6	6.55×10^6	2.47×10^7	9.13×10^5	2.83×10^6	1.34×10^8

[a]Data are the mean of three replicate measurements ± SD.

ethyl esters.[10] Furthermore, due to the analytical difficulties of sensitively analyzing volatile chemicals in the presence of very high ethanol concentrations, there is a paucity of data in the scientific literature dealing with the partition behavior of volatile compounds at ethanol concentrations relevant to distilled spirits.[11,12] In our laboratories we have devised an adaptation of atmospheric pressure chemical ionization−mass spectrometry (APCI-MS),[13,14] known as ethanolic APCI-MS,[4,15,16] in which ethanol is introduced into the ionization source via the makeup gas in controlled amounts to compensate for differences in percentage alcohol by volume (%ABV) between samples. In this way the source ethanol concentration is held constant across all samples. This is necessary because in high ethanolic systems ethanol acts as a charge transfer reagent in APCI, and thus the ionization of volatile compounds would otherwise vary according to the accompanying ethanol concentration in the gas phase. This factor was not taken into account in the one prior published study using APCI-MS at ethanol concentrations relevant to distilled spirits.[17]

■ MATERIALS AND METHODS

Chemicals. Authentic compounds (>97% purity) were obtained from Sigma-Aldrich (Poole, Dorset, UK): ethyl-L-lactate, pyrazine, 2-furanmethanol, 2-furaldehyde (furfural), 2-methylpyrazine, 2,5-dimethylpyrazine, 2-acetylthiazole, benzaldehyde, isoamyl acetate, 2-phenylethyl acetate, ethyl hexanoate, ethyl octanoate, β-damascenone. Ethyl hexadecanoate was obtained from Alfa Aesar (VWR, Lutterworth, Leicestershire, UK).

New-make spirit samples were sourced from industry and provided by the Scotch Whisky Research Institute, Riccarton, Edinburgh, UK.

Quantitation of Ethyl Esters in New-Make Spirit. New-make spirit (100 mL) was spiked with an internal standard (2-acetylthiazole; 10 μg/mL), diluted with water (400 mL), and twice extracted with dichloromethane (200 mL) using a 1 L separation funnel. The two dichloromethane fractions were combined and concentrated to 1 mL under a stream of nitrogen while being heated in a 40 °C water bath.

Three replicate extractions were performed on two separate spirit samples.

Solvent extracts were analyzed using a ThermoScientific Trace GC Ultra with a DSQ II mass spectrometer and an AS 3000 Autosampler (Thermo Electron Corp.). Compounds were separated on a Zebron WAX column (30 m × 0.25 mm i.d., 1.0 μm film thickness) starting at an oven temperature of 40 °C (1 min hold) followed by a ramp to 250 °C at 8 °C min⁻¹. The carrier gas flow rate was 1.5 mL/min, and the injection (1 μL) was splitless. The mass spectrometer was operated in full scan mode over the range m/z 35−250.

Ethanolic APCI: Sample Preparation. Aqueous ethanolic solutions (5−40% ABV) containing ethyl hexadecanoate (0−500 mg/L) were prepared according to the experimental design shown in Table 1. Each compound was added at a concentration of 50 μg/mL.

Ethanolic APCI-MS Analysis of Whisky Volatiles. Headspace congener concentrations were analyzed using a Platform LCZ APCI mass spectrometer fitted with an MS Nose interface (Micromass, Manchester, UK) and with a modified source designed to operate at high and differing ethanol concentrations as described previously.[4] The flow of ethanolic vapor to the source was controlled using a mass flow meter (Aalborg, Orangeburg, NY, USA) and was adjusted depending on the ethanol concentration in the sample.

All analyses were performed in selected ion mode, whereby the protonated ion ([M + H]⁺) of each volatile compound was specifically monitored to increase the sensitivity of measurement. Volatile compounds were thus analyzed in small predetermined groups. It had previously been ascertained that there was no interference between compounds with respect to the monitored ions. The full experimental design of 20 samples (as in Table 1) was repeated for each of these groups. Sampling flow rate (set to 2 mL/min) was measured using a VeriFlow 500 gas flow meter (Humonics Inc., Folsom, CA, USA). The heated transfer line (Hillesheim, Waghausel, Germany) was maintained at 170 °C.

The order of sample analysis was fully randomized across the experimental design. Each of the 20 aqueous ethanolic solutions was sampled into the APCI-MS for 2 min, with a 1 min gap between each to let the signal return to baseline and for the ethanol makeup gas flow to be adjusted in accordance with the ethanol concentration of the next sample. An external standard of ethyl nonanoate (50 μg/mL in

5% ethanol v/v) was sampled at the beginning, middle, and end of the sequence.

Experimental Design and Statistical Analysis of APCI Intensity Data. A D-optimal response surface design was used (Design Expert software v. 6.02, Stat-Ease, Minneapolis, MN, USA) as it employs an algorithm that considerably reduces the number of design points required while minimizing the variance associated with the estimation of coefficients in the models fitted to data. The D-optimal experimental design consisted of 20 solutions of various ethanol and ethyl hexadecanoate concentrations (Table 1). For each experiment (group of volatile compounds) the randomized sample sequence was repeated in three blocks. The mean headspace ion concentrations for each compound were normalized to the external standard and modeled using Design Expert software (v.6.02, Stat-Ease) to identify whether the factors (ethanol content and/or ethyl hexadecanoate concentration) had significant impacts upon the headspace ion concentrations. A separate model was prepared for each individual compound.

■ RESULTS AND DISCUSSION

Ethanolic APCI-MS was used to analyze the headspace concentrations of a test set of 14 whisky volatile compounds above a series of aqueous ethanolic solutions differing in alcohol content (5−40% ABV) and with regard to the concentration of ethyl hexadecanoate (0−500 μg/mL).

Quantitation of Ethyl Esters in New-Make Spirit. The concentration range of ethyl hexadecanoate investigated in the model system was set with regard to the total levels of long-chain ethyl esters analyzed in new-make spirit samples (Figure 1). The ester levels in these samples were typical of those found
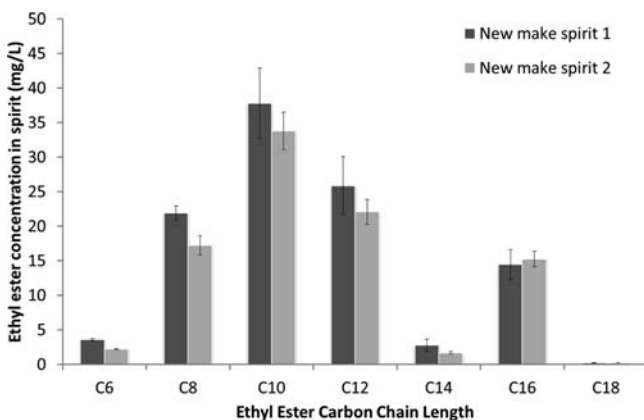

Figure 1. Ethyl ester concentrations in two new-make whisky spirit samples. Data are the mean ± SD of three replicate measurements.

in newly distilled Scotch malt spirits (John Conner, SWRI; personal communication). For the two samples analyzed, total long-chain ethyl ester concentrations (C-8 and greater) were of the order of 100 μg/mL. Hence, the concentration range of ethyl hexadecanoate selected for the model probably exceeded the range typically found in new-make spirit samples, to improve the chances of determining a clear physicochemical effect at higher levels. However, because whisky agglomerates are reported to contain a mixture of surface active components,[7] this may not have been altogether unrealistic.

Ethyl esters are formed intracellularly by yeast during fermentation from medium-chain fatty acids and ethanol. The levels of ethyl esters produced by yeast are governed primarily by the strain used, the conditions of the fermentation, and the composition of the medium.[18] The aliphatic chain length of ethyl esters in spirits can extend up to 18 carbons (C18).[19] Selected physicochemical and odor properties of long-chain ethyl esters commonly found in whisky are presented in Table 2. It is widely known that some of these hydrophobic fatty acid ethyl esters form a significant part of the headspace of distilled beverages. In particular, ethyl octanoate, decanoate, and dodecanoate were found to be quantitatively significant in whisky headspace,[20] making a major contribution to whisky aroma.[12] These findings were in accordance with the current data for new-make spirit samples (Figure 1).

Influence of Ethanol and Ethyl Hexadecanoate Concentrations on Volatile Partitioning Behavior. The mean headspace ion concentrations monitored for each compound (Table 1 illustrates one data set for a group of six volatile compounds) were modeled across the 20-point D-optimal design space using ethanol and ethyl hexadecanoate concentrations as factors. A summary of the modeling data is provided in Table 3, which lists volatile compounds in order of ascending hydrophobicity (log P). Perhaps unsurprisingly, ethanol content over the range of 5−40% ABV had a significant impact upon the headspace ion concentrations of all compounds investigated ($P < 0.0001$), with all 14 showing a reduction in headspace concentration at higher ethanol concentrations. The impacts of ethyl hexadecanoate could be split into two discernible trends; the C16 ester concentration was a significant factor in models derived for relatively hydrophobic compounds (log $P > 2.5$), the partition of which into the headspace was decreased at higher C16 ester concentrations. This would be consistent with increased solubility of hydrophobic compounds due to their incorporation into hydrophobic structures ('agglomerates') formed at higher levels of ethyl hexadecanoate. However, a further group of compounds (2-methylpyrazine, furfural, and 2-phenylethyl acetate) showed the opposite effect, such that there was a moderate "salting out" effect of increasing C16 ester concentrations. This implies that the solubility of these

Table 2. Selected Physicochemical and Odor Properties of the Predominant Long-Chain Fatty Acid Ethyl Esters Found in Whisky[a]

ethyl ester	MW	carbon chain length	organoleptics (flavornet.org)	log P ($K_{o/w}$)	vapor pressure (mmHg, 25 °C)
hexanoate	144	6	apple peel, fruit	2.83	1.8×10
octanoate	172	8	fruit, fat	3.81	2.1×10^{-1}
decanoate	200	10	grape	4.96	3.1×10^{-2}
dodecanoate	228	12	leaf	5.78	8.7×10^{-3}
tetradecanoate	256	14	ether	6.76	2.5×10^{-3}
hexadecanoate	284	16	wax	7.74	2.7×10^{-4}

[a]Log P = log of the oil water partition coefficient. A high log P value represents a greater degree of hydrophobicity. Log P and vapor pressure values were estimated from group contribution using molecular modeling software EPISuite (U.S. EPA 2000-2007).

Table 3. Summary of Modeling Data Showing the Significance (*P* Value) of Each of the Factors %ABV and Ethyl Hexadecanoate Concentration in Models Predicting Headspace Ion Intensities of Individual Compounds across the Design Space[a]

		factor significance (*P* value)				
volatile compound	log *P*	ethanol concn	C16 ethyl ester concn	interaction between C16 and EtOH	model *R*²	interaction effect
ethyl ʟ-lactate	−0.18	**<0.0001**	0.40	0.69	0.61	
pyrazine	−0.06	**<0.0001**	0.92	0.68	0.85	
2-methylpyrazine	0.49	**<0.0001**	**0.018**	**0.0014**	0.82	salting out effect at low EtOH
2-acetylthiazole	0.67	**<0.0001**	0.91	0.70	0.87	
furfural	0.83	**<0.0001**	**0.0034**	**0.0054**	0.82	salting out effect at low EtOH
2,5-dimethylpyrazine	1.03	**<0.0001**	0.79	0.92	0.94	
benzaldehyde	1.71	**<0.0001**	0.83	0.18	0.92	
ethyl butyrate	1.85	**<0.0001**	0.54	0.77	0.96	
isoamyl acetate	2.26	**<0.0001**	0.90	0.31	0.91	
2-phenylethyl acetate	2.57	**<0.0001**	**0.014**	0.23	0.96	salting out effect at low EtOH
ethyl hexanoate	2.83	**<0.0001**	0.50	0.46	0.78	
ethyl octanoate	3.81	**<0.0001**	**<0.0001**	**<0.0001**	0.91	HS reduced on addition of C16
β-damascenone	4.21	**< 0.0001**	**<0.0001**	**<0.0001**	0.92	HS reduced on addition of C16
ethyl decanoate	4.79	**<0.0001**	**<0.0001**	**<0.0001**	0.81	HS reduced on addition of C16

[a]Significant interaction terms are indicated, together with the overall model fit *R*². Aroma compounds are shown in order of increasing hydrophobicity (log *P* values). Significant effects ($P < 0.05$) are indicated in bold type. The concentration of each compound in solution was 50 mg/L.
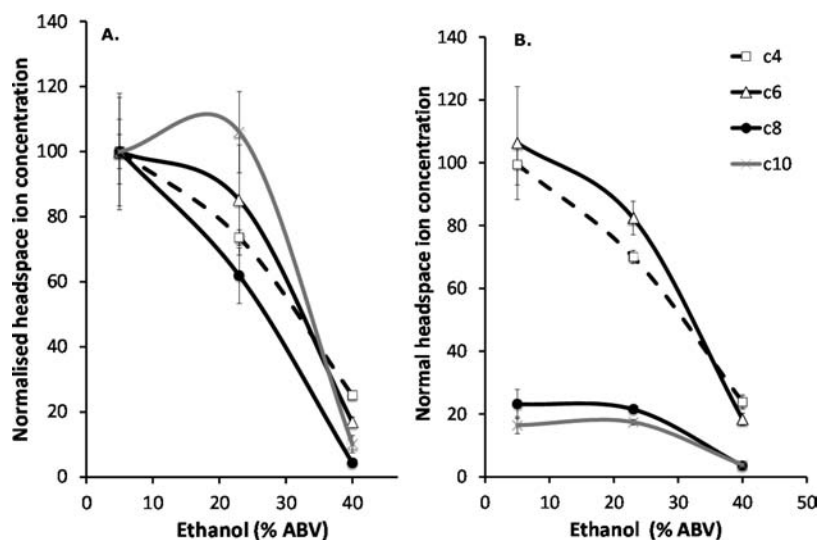

Figure 2. Effect of ethanol concentration on the APCI headspace ion intensities of the ethyl ester series (C4, C6, C8, and C10) with (A) no ethyl hexadecanoate and (B) 500 μg/mL ethyl hexadecanoate. Data have been normalized against the 5% ABV sample with no ethyl hexadecanoate for each volatile and are the mean ± SD of at least three replicate measurements. Smoothed curves represent the model fitted to all data points and not just the points represented in this "slice" of the model.

compounds was decreased by the increasing presence of ethyl hexadecanoate. It is relatively easy to think of explanations for this behavior in terms of changes in solution structure, which presumably decrease the solvating power of ethanol toward these molecules, being as the effect was more pronounced at low ethanol concentrations. It is harder to explain why specifically these three compounds showed a weak but significant effect, whereas others of a similar physicochemical nature did not. However, we confirm that the experiments were repeated on separate occasions and that the findings were reproducible.

Although Table 3 indicates whether each factor had a significant impact on volatile partitioning across the design space, it provides little insight regarding the magnitude or directionality of the effects observed. We shall illustrate these trends further by considering the ethyl ester series (C4, C6, C8,

and C10), which encompass a suitable range of compound hydrophobicities and volatilities. Figure 2 depicts the impact of ethanol on the partitioning behavior of this ethyl ester series with no added ethyl hexadecanoate (Figure 2A) and at the highest concentration of ethyl hexadecanoate (500 μg/mL; Figure 2B). Data have been normalized against the monitored headspace concentration for each series at 5% ABV and 0 μg/mL ethyl hexadecanoate. The solvating effects of ethanol on the ethyl ester homologous series can clearly be observed (Figure 2A) with headspace concentrations at 40% ABV being less than a fourth of those at 5% ABV and with the magnitude of effect increasing with ethyl ester chain length. It is notable that at 23% ABV the mean headspace concentration of ethyl decanoate increased slightly relative to that observed at 5% ABV. This trend is likely due to the observed "structuring" of ethanol–water mixtures as the %ABV increases.[11,21] Aqueous solutions
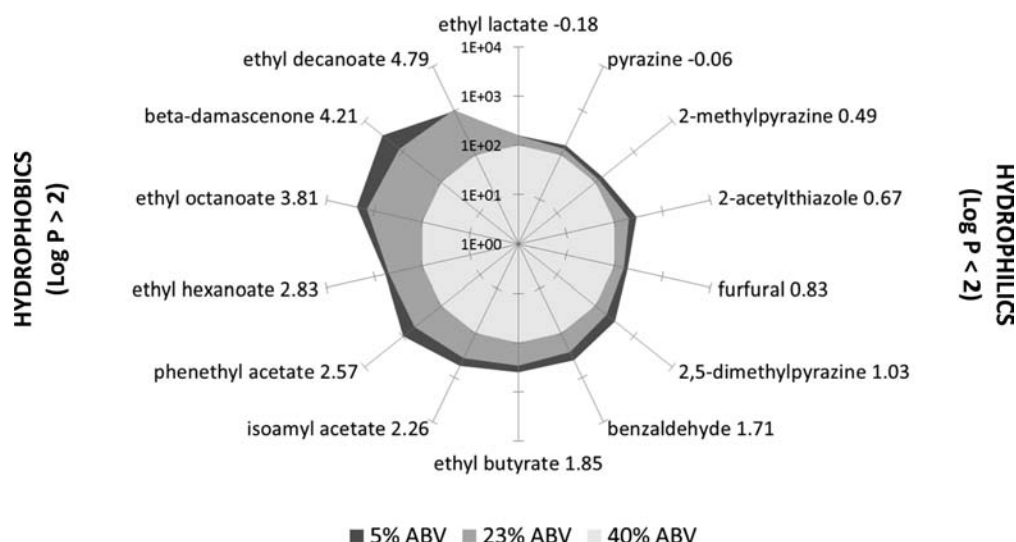
Figure 3. Effect of ethanol concentration on the partition behavior of all 14 volatiles. Data have been normalized against the measured intensity at 0 μg/mL ethyl hexadecanoate and 40% ABV for each volatile and are plotted on a logarithmic scale. Log P values for each compound are shown adjacent to the name.

containing up to 15% ABV have been shown to contain ethanol monodispersed in water; however, between 20 and 57% ABV there is a progressive aggregation of ethanol molecules to form "pseudomicelles", which initially (at 23% ABV) lowers the solvating power of ethanol toward the longer chain length esters such as ethyl decanoate.[11] At 40% ABV, the present results indicated that there was sufficient ethanol in the system to effectively solubilize each of the ethyl esters in the series investigated. This is consistent with the observation by Conner et al.[11] that, for ethyl esters, the change from ethanol-rich pseudomicelles to an ethanolic solution had little effect on headspace partitioning behavior. In other words, by 40% ABV the solution is rich in structured ethanol and is behaving similarly to when it becomes the continuous phase (above 57% ABV ethanol−water mixtures are water monodispersed in ethanol[21]).

In the presence of ethyl hexadecanoate at 500 μg/L (Figure 2B), there was a divergence of behavior between the C4 and C6 ethyl esters, which were largely unaffected (relative to Figure 2A), and the C8 and C10 esters, the headspace intensities of which were dramatically reduced. This effect was most apparent at low to intermediate ethanol concentrations and would certainly be evident at the typical whisky "nosing" concentration (23% ABV).

Ethanol alone had a significant impact on the partitioning of all 14 volatile compounds (Table 3). To illustrate how this would affect the balance of headspace volatile compounds as whisky samples are diluted, we plotted the relative headspace concentrations of the 14 compounds (sorted in order of ascending log P) at 5, 23, and 40% ABV (Figure 3). The data are normalized against the headspace concentration at 40% ABV (=100) in each case and hence increase in all cases as ethanol concentration is lowered. However, there is clearly an exaggerated impact on the headspace concentrations of hydrophobic compounds at lower ethanol concentrations (note that the scale in Figure 3 is logarithmic because of this "orders of magnitude" effect), which would affect perceived aroma. This illustration also makes clear that dilution from 40 to 23% ABV had a marked effect on volatile headspace concentrations (represented by the midgray band), whereas

dilution from 23 to 5% ABV had a progressive but lesser impact (the dark gray band). Note that the data in Figure 3 are for samples containing no ethyl hexadecanoate.

Figure 4 illustrates the impact of varying C-16 ester concentrations on the headspace ion intensities of the ethyl ester series at ethanol concentrations of 5, 23, and 40% v/v (Figure 4A−C, respectively). The data have been normalized against the headspace ion concentrations with no added C16 ester in each case, to facilitate comparison. Once again, at both 5 and 23% ABV, increasing C16 ethyl ester concentrations lowered the air−liquid partition coefficients of the relatively hydrophobic (C8 and C10) ethyl esters. A concentration of >250 μg/L ethyl hexadecanoate was sufficient to reduce the headspace concentrations of these compounds to less than half of those in the absence of ethyl hexadecanoate. By comparison, the partitioning of the C4 and C6 ethyl esters was not significantly affected by the concentration of ethyl hexadecanoate in the system. Figure 4 shows that as the ethanol concentration was increased to 40% ABV, the impact of ethanol progressively outweighed that of ethyl hexadecanoate on the C8 and C10 ethyl esters. Ethyl octanoate in particular showed only a small decline in headspace concentration with increasing ethyl hexadecanoate at 40% ABV.

Figure 5 illustrates the trends in headspace concentration across the design space for the three further volatiles having headspace concentrations that were significantly affected by C16 ethyl ester concentrations, namely, furfural and 2-methylpyrazine (a moderate salting-out effect of increasing ethyl hexadecanoate at low ethanol concentrations) and β-damascenone (solubility increased in the aqueous phase by increasing ethyl hexadecanoate concentration, particularly at 5% ABV). Headspace concentrations of β-damascenone at 23 and 40% ABV were close to the detection limits using APCI-MS and hence the greater error bars associated with these measurements in Figures 5B and 4C. The moderate salting-out effect at low ethanol concentrations observed with furfural and 2-methylpyrazine may be a consequence of their moderate hydrophobicity and planar nature of the molecules. The latter aspect is hypothesized to disrupt the ordering of ester chains in the agglomerates, making their incorporation less likely.
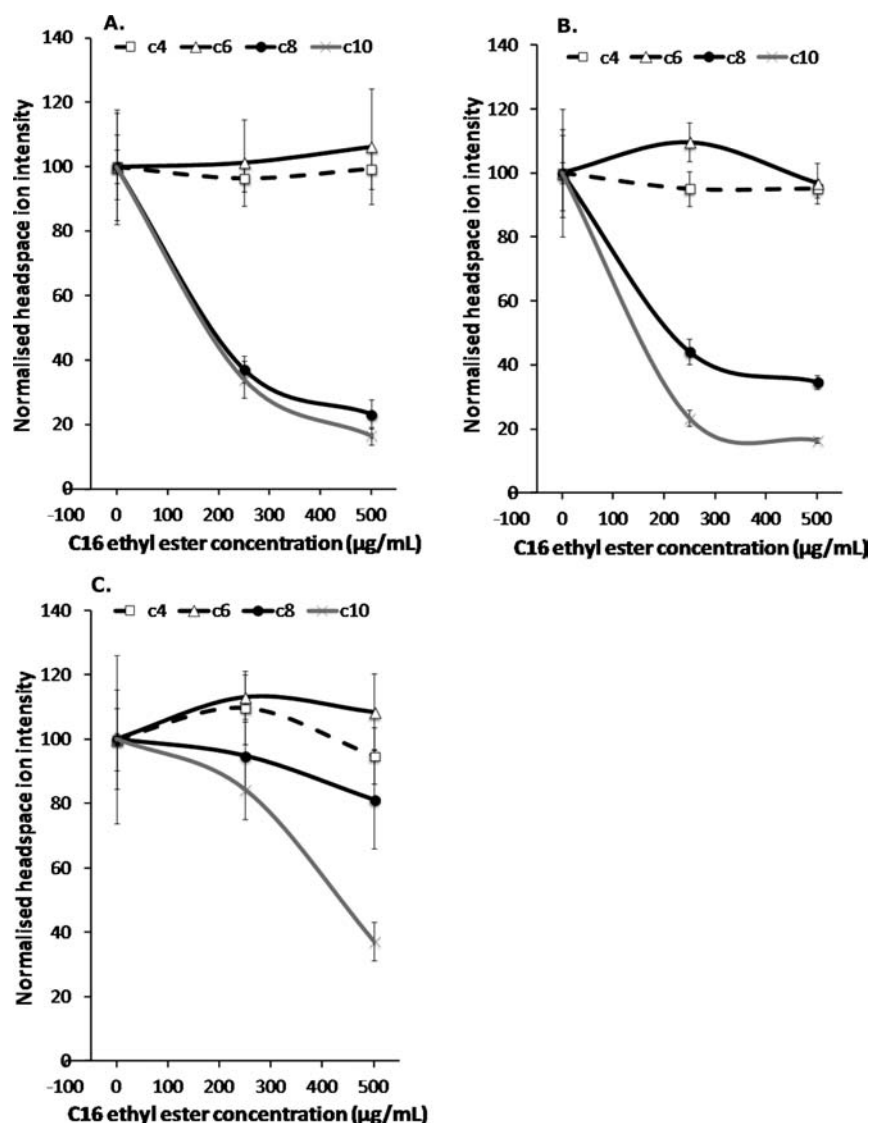
Figure 4. Effect of increasing ethyl hexadecanoate concentration on ethyl ester headspace ion intensities at (A) 5% ABV, (B) 23% ABV, and (C) 40% ABV. Data have been normalized against the measured intensity at 0 μg/mL ethyl hexadecanoate for each volatile and level of ethanol and are the mean ± SD of at least three replicate measurements. Smoothed curves represent the model fitted to all data points and not just the points represented in this "slice" of the model.

Overall, the results reported here demonstrate that the aroma balance of whisky, between its hydrophilic and hydrophobic components, can be influenced both by the ethanol content (Figure 3) and also by the concentration of long-chain ethyl esters, which have the potential to form agglomerates when present above the critical micelle concentration (CMC). These agglomerates form a reservoir for small hydrophobic volatile compounds and reduce their headspace concentrations. Furthermore, there was evidence of interactions between the two factors (Figure 4), such that the impact of ethyl hexadecanoate concentration was more pronounced at 5 and 23% ABV than at 40% ABV. It is hypothesized that as whisky is diluted from 40 to 23% ABV for nosing purposes, the CMC of ethyl ester agglomerate formation is also reduced and hence that under these conditions the aroma balance is more sensitive to long-chain ethyl ester concentrations in the spirit. Distillates high in such esters might thus be expected to have a nosed aroma that (relatively speaking) emphasizes the aroma characters of the polar hydrophilic volatiles present.

In conclusion, aqueous concentrations of ethanol (5−40% ABV) and ethyl hexadecanoate (0−500 μg/mL) significantly affected the partitioning behavior of a test set of 14 whisky volatile compounds in aqueous model systems. These effects can be interpreted in terms of the physical chemistry of ethanolic solutions and the potential for agglomerate formation by long-chain fatty acid ethyl esters in spirit samples, which selectively diminishes headspace concentrations of the more hydrophobic compounds. When spirit is diluted to 23% ABV for nosing, spirits with ethyl esters present at higher concentrations are more likely to form agglomerates. These structures can incorporate small hydrophobic flavor compounds, thus lowering their headspace concentrations and changing the balance of the nosed aroma toward more polar, hydrophilic compounds. In whisky it is anticipated that micelle formation would incorporate long-chain ethyl esters of various chain lengths (as well as surface active alcohols and aldehydes) and thus that the total concentration of these species, as
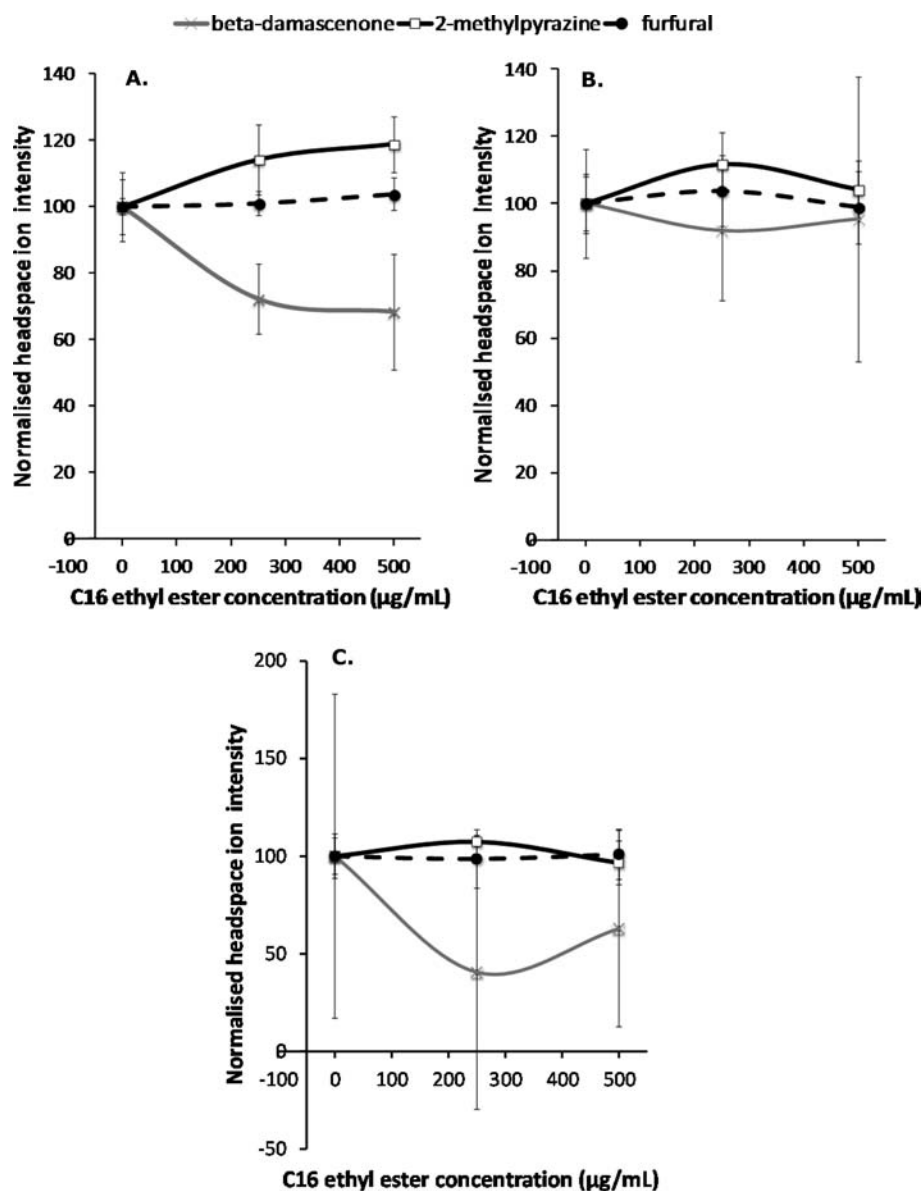
Figure 5. Impacts of ethyl hexadecanoate concentration on the partition behavior of 2-methylpyrazine, furfural, and β-damascenone at (A) 5% ABV, (B) 23% ABV, and (C) 40% ABV. Data are the mean ± SD of at least three replicate measurements. Headspace concentrations of β-damascenone at 23 and 40% ABV were close to the detection limits using APCI-MS and hence the greater error bars associated with these measurements in panels B and C. Smoothed curves represent the model fitted to all data points and not just the points represented in this "slice" of the model.

influenced by the distilling process, would be a significant factor determining aroma balance.

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Notes
The authors declare no competing financial interest.

ABBREVIATIONS USED

ABV, alcohol by volume; APCI-MS, atmospheric pressure chemical ionization mass spectrometry/spectrometer; CMC, critical micelle concentration; GC-MS, gas chromatography–mass spectrometry; log P, log of the octanol–water partition coefficient; SIR, selected ion response.

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