

## ORIGINAL ARTICLE

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# Guidelines to optimise the sensory detection of 2,4,6-trichloroanisole in neutral spirit and whisky

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## Abstract

**Why was the work done:** Guidelines are required to optimise the sensory detection of musty/stale taints in alcoholic spirits caused by 2,4,6-trichloroanisole. Early detection of the taint is necessary as the low sensory threshold can result in significant quality issues and masking the musty/stale off-note through blending is difficult.

**How was the work done:** The standard industry practice for 'nosing' is to dilute samples of spirit to 20% ABV to prevent sensory fatigue. It is not known if 20% ABV is the optimal concentration for detecting 2,4,6-trichloroanisole. In this study, the effects of ethanol concentration on the detection of the taint were evaluated using a sensory panel and gas chromatography-mass spectroscopy. The efficacy of nosing versus tasting to detect 2,4,6-trichloroanisole was investigated at different dilutions of distilled spirits. Further, the stability of this off-note at 20% ABV was determined to assess if the taint could be detected by nosing over a working day. Finally, the serving temperature of the sample was assessed in the sensory perception of the taint.

**What are the main findings:** The recommendations to optimise the sensory detection of 2,4,6-trichloroanisole in spirit samples are (i) nose samples at 20% ABV, (ii) as soon as possible after dilution conduct sensory tests and (iii) chill the sample.

**Why is the work important:** The limits of detection for conventional solid phase microextraction with gas chromatography-mass spectroscopy are typically above the threshold of human perception. Therefore, the industry needs to maximise the detection by sensory panellists of contaminated whisky samples to ensure the product does not go to market so as to avoid any reputational damage.

## Keywords:

2,4,6-trichloroanisole, TCA, musty, stale, off note, sensory detection, whisky

## Introduction

Above the threshold concentration, 2,4,6-trichloroanisole (TCA) contributes musty/stale/damp cardboard off-notes in whisky. Research has shown that very low levels of TCA can disrupt olfactory signal transduction, suppressing odour detection in wine (Takeuchi et al. 2013), where sub-threshold concentrations of TCA suppressed desirable flavours. Practical observations at The Scotch Whisky Research Institute (SWRI) support the 'flattening' of the desirable aroma characteristics in whisky spiked with below threshold concentrations of TCA. The TCA taint is linked to contaminated wood-based materials (casks, cardboard packaging materials, pallets, wooden materials used in the construction of warehouses) and, in the case of wine, linked to air pollution in cellars (Chatonnet et al. 2004; Chatonnet et al. 2010; Cravero 2020). The synthesis of TCA is via the O-methylation of its precursor 2,4,6-trichlorophenol (TCP) by fungi and bacteria. TCP is abundant in the environment having been widely used as a biocide (Simpson and Sefton 2007; Zhang et al. 2016) as well as being a by-product of the chlorination of paper pulp (Field and Sierra-Alvarez 2008).

The exceptionally low sensory threshold of TCA makes it a problematic taint for the spirit drinks industry. Whilst there are numerous publications citing sensory thresholds in wine (Tarasov et al. 2022), no data on detection thresholds for TCA in distilled spirits has been reported. Earlier work conducted at SWRI (unpublished) using 217 sensory panellists found an average detection threshold of 4.40 ng/L TCA in 20% ABV neutral ethanol. Given the large number of panellists involved, the range of detection thresholds (0.25 to >10 ng/L) provides a good approximation of the variation in consumer thresholds. Further (unpublished) research into thresholds in whisky, using the SWRI expert panel found TCA detection thresholds of 2 to 33 ng/L depending on the whisky matrix tested. When using conventional solid phase microextraction-gas chromatography-mass spectroscopy (SPME-GC-MS) analysis, instrumental limits of detection are ca.10 ng/L. This highlights the importance of sensory panels in identifying TCA tainted spirit and removing it from the production chain.

Industry practice is to dilute distilled spirits to ca.20% ABV (alcohol by volume) for 'nosing'. This reduces alcohol burn, reduces sensory fatigue and improves the perception of volatile flavours (Jack 2003). The partitioning of flavour congeners from the liquid into the headspace, as measured by GC-MS, is related to alcoholic strength (Conner et al. 1994; Conner et al. 1998). Sensory perception of congeners can be affected by alcohol concentration, depending on the hydrophobic/hydrophilic properties (Karlsson and Friedman 2017; Ickes and Cadwallader 2017, 2018; MacGarry 2023). Further, the complexity of the matrix has been shown to affect the sensory perception of TCA in wines (Mazzoleni and Maggi 2017) presumably due to masking effects. The research reported here looks to assess the impact of ethanol concentration on the ability of sensory panellists to detect TCA in grain neutral spirit, blended and single malt whisky.

In this work, the chosen ABV replicated off-note detection checkpoints: 50% ABV (higher/cask bottling strength); 40% ABV (standard bottling strength); 30% ABV (a possible concentration for sensory analysis) and 20% ABV (recommended strength for nosing). Given that samples diluted for sensory assessment may not be evaluated by panellists until much later in the day, a stability study was conducted to assess whether mustiness was detectable in a spiked sample prepared up to six hours earlier. Finally, given that temperature affects the partitioning of volatile components into the headspace for gas chromatography-mass spectrometry (Agilent 2023), the impact of the temperature on the sensory detection of TCA-spiked samples was assessed.

The aims of this research were to provide guidelines for the spirit industry to optimise the sensory detection of TCA in tainted samples. These include:

- The optimal ethanol concentration for detecting TCA in grain neutral spirit (GNS) and whisky by sensory assessment.
- Is nosing or tasting the better mode of detection of TCA at different ethanol concentrations.
- The stability of TCA in a diluted sample over the course of a working day.
- If sample temperature affects ease of detection of TCA in spiked samples of GNS and whisky.

## Materials and methods

Grain neutral spirit (GNS, 96.4% ABV), blended (40% ABV) or single malt whisky (57.5% ABV) were used as the base spirit. The single malt Scotch whisky was a cask-strength whisky matured in ex-bourbon and ex-sherry casks. The base spirits were nosed by the sensory panel leader prior to spiking with TCA (2,4,6-trichloroanisole) and were found to be free of taints. This could not be confirmed by instrumental analysis as the limit of detection for TCA exceeds the sensitivity of the sensory panel. Food grade TCA capsules were used for experiments requiring panellists to taste samples (Aroxa™; Cara Technology Ltd., Leatherhead, UK). For experiments involving nosing, 99% pure TCA in solid crystal form was used (Sigma-Aldrich).

### TCA-spiked grain neutral spirit

A stock solution was prepared from two Aroxa™ capsules of TCA (each containing 73 ng of TCA), added to 1.9 L of GNS (40% ABV) (TCA was insoluble at higher ethanol concentrations). Samples were adjusted to the required ethanol concentration, each containing 30 ng/L of TCA, by adjusting with GNS and water.

### TCA-spiked single malt whisky

TCA (Aroxa™ flavour capsules) was added to single malt whisky prediluted to the desired strength (50, 40, 30 and 20% ABV) to give a final TCA concentration of 50 ng/L.

### TCA-spiked blended whisky

These samples were used for nosing only. TCA (99% pure) in crystal form (Sigma-Aldrich) was used to spike 20% ABV blended whisky at a TCA concentration of 30 ng/L.

### Sensory panel

Sensory evaluation was performed by an expert panel at SWRI of 22 panellists (employees, over 18 years of age, mixed gender) with expertise in the evaluation of Scotch whisky and other spirits.

Panellists were pre-screened using an odour recognition test with everyday odours to test their ability to recognise and describe aromas. Selected panellists were trained on specific flavours based on the SWRI Scotch Whisky Flavour Wheel. Trainee panellists were exposed to different spirits and sensory techniques during training. Their performance compared to the trained panel average, was tracked across tests and the overall performance of both trainee and trained panellists is regularly assessed using the Whisky Sensory Proficiency Scheme (FlavorActiV™, Watlington, UK). Trainees join the expert panel when they have reached the required level of expertise.

### Samples for sensory analysis

Unless otherwise stated, samples were diluted using distilled water on the day of testing. Samples (20 mL) were presented in blue nosing glasses covered with a watch glass and identified with a 3-digit random code.

### Stability of TCA in diluted samples for sensory analysis

Two samples of 20% ABV blended whisky were prepared for each panellist; one contained 50 ng/L TCA, the other a control without TCA. Panellists nosed samples in the morning and then again in the afternoon. Samples were covered with a watch glass at room temperature.

### Effect of sample temperature on the perception of TCA

To determine the effect of temperature on the perception of TCA, spirit (either GNS or blended whisky) was diluted to 20% ABV and spiked with 50 ng/L of TCA. Samples (20 mL) were dispensed into nosing glasses covered with watch glasses and stored at 5, 20 (ambient) and 40°C for one hour before sensory testing commenced. A control sample (the appropriate spirit without TCA, at 20% ABV) was kept at ambient temperature. Testing of the GNS samples was conducted on a separate day from that for the blended whisky samples to avoid sensory fatigue.

## Quantitative descriptive profiling

To assess the samples, the sensory panel used Quantitative Descriptive Profiling (QDP) conducted in accordance with ISO guidelines (ISO 13299:2016). Only attributes pertinent to each test were assessed so as to minimise exposure to TCA as it is known to cause olfactory fatigue (Cravero et al. 2015). The intensity of each attribute was scored using a 0-3 continuous line scale marked at 0.5 intervals. A monadic design (one sample presented at a time and rated for all attributes) was used. Compusense® cloud based sensory software (Compusense Inc., Guelph, ON, Canada) was used for the design and presentation of the tests. All sensory testing took place at the SWRI sensory laboratory with individual booths and red lighting as a standard protocol. A summary of the practical parameters in each sensory experiment is outlined in [Table 1](#).

## Analysis of sensory data

Mean scores for each attribute were calculated. For experiments with two samples, t-tests were performed. For experiments with more than two

samples, analysis of variance (ANOVA) was used to find any statistically significant ( $p < 0.05$ ) differences between the samples followed by Tukey's post hoc test to determine sample groupings for any significant result. Unistat® 10.0 for Excel (Unistat Ltd., London, UK) was used for all statistical analysis.

## Gas chromatography-mass spectroscopy

To determine the effect of dilution on the amount of TCA available in the headspace, aliquots of the single malt whisky samples for sensory analysis were analysed by solid-phase microextraction (SPME-Arrow) with headspace gas chromatography - mass spectrometry (GC-MS). The instrument used was an Agilent 5975 GC-MS (Agilent Technologies, Inc, USA) with a PAL 3 RTC autosampler (CTC Analytics AG, Switzerland). Five 3 mL replicate samples were taken from 50 ng/L TCA spiked whisky samples at the different dilutions (20, 30, 40 and 50% ABV). The vials were incubated for 5 min at 30°C and an 85µm DVB/CAR/PDMS fibre used to extract volatiles for 15 min. TCA captured by the fibre was desorbed splitless for 10 min in the injector port of the GC. Compound separation used a 60m x 0.32mm DB-WAXETR column (df = 0.5µm) (Agilent Technologies,

**Table 1.**

### Experimental parameters for sensory testing.

Experiment	<sup>1</sup> Matrix	<sup>2</sup> TCA ng/L	Ethanol % ABV	Sensory attributes	Nosing or tasting	Panellists
Effect of ethanol concentration on TCA detection	GNS	30	20, 30, 40, 50	<u>Aroma and Taste:</u> musty, alcohol burn	Both	17
	SMWh	50	20, 30, 40, 50	<u>Aroma and Taste:</u> musty, alcohol burn, sweet, green/grassy, fresh fruit, dried fruit, floral, woody, spicy, nutty.	Both	15
Stability of diluted TCA-spiked samples	BWh	50 (sample) 0 (control)	20	<u>Aroma:</u> overall aroma intensity, musty, alcohol burn, sweet aroma	Nosing	15
Effect of sample temperature on the perception on TCA	GNS	50	20	<u>Aroma:</u> musty, alcohol burn, overall aroma intensity	Nosing	17
	BWh	50	20	<u>Aroma:</u> musty, alcohol burn, overall aroma intensity	Nosing	12

<sup>1</sup> Matrix: GNS - grain neutral spirit; SMWh - single malt whisky; BWh - blended whisky.

<sup>2</sup> A higher concentration of TCA was required for whisky to ensure panellists could detect a musty note.

Inc, USA) with the oven at 40°C for 3 minutes after injection, then increasing to 240°C at 5°C/minute and holding for 6 minutes. The carrier gas was helium at a flow of 1.4 ml/minute. The mass spectrometer was operated in SIM-Scan mode, scanning 35 to 450 amu alternating with the selected ions  $m/z$  195 and 197 (dwell time 100ms) for TCA. Selected ions were integrated separately, and the areas were combined. The relative standard deviation for each strength was 12% or better.

### Stability of TCA in the headspace of diluted samples assessed by GC-MS

Blended whisky (20% ABV) spiked with 50 ng/L TCA was prepared and left in an amber glass bottle at room temperature for 3 days. On analysis, a freshly prepared spiked whisky sample was made and five 3 mL replicate samples of both the 'old' sample and the 'fresh' sample were prepared for GC-MS as detailed above.

## Results and discussion

### The effect of ethanol concentration on the sensory detection of TCA

#### Neutral spirit

Average panel scores for the attributes assessed in TCA-spiked samples of neutral spirit at each dilution together with statistical analysis are reported in Table 2. Ethanol concentration had a significant effect on the perception of musty aroma ( $p \leq 0.001$ )

Table 2.

Panel scores for TCA (30 ng/L) in GNS at different concentrations of ethanol.

	GNS (ABV) with 30 ng/L TCA				p-value
	20%	30%	40%	50%	
Musty aroma	1.65 a	1.25 ab	0.75 b	0.86 b	$\leq 0.001$
A - Alcohol burn	0.54 c	1.07 b	1.55 a	1.73 a	$\leq 0.001$
Musty taste	1.29	1.35	1.21	1.44	0.53
T - Alcohol burn	0.86 c	1.56 b	2.13 a	2.41 a	$\leq 0.001$

A – aroma assessment; T- taste assessment

Results of ANOVA are reported and Tukey's post hoc groupings (a,b,c) where statistically significant differences were found between samples.

and alcohol burn (aroma and taste;  $p \leq 0.001$ ). The scores for musty aroma in GNS spiked with TCA were greater at 20% ABV than at  $\geq 40\%$  ABV. Alcohol burn increased in line with ethanol concentration. Dilution of samples to 20% ABV made it easier for panellists to detect TCA.

### Single malt whisky

Average panel scores for each sample across all attributes together with statistical analysis are reported in Table 3. Perception of musty aroma in whisky was significantly affected by the ethanol concentration with the off-note being easier to detect at 20% ABV. Alcohol burn (aroma and taste), sweet aroma and dried fruit taste were significantly affected by sample concentration. As anticipated, the perception of alcohol burn increased with the concentration of ethanol while the perception of some desirable flavour characteristics (sweet aroma and dried fruit taste) changed.

In both GNS and single malt whisky, stale/musty aromas from TCA were easier to detect when the product was diluted to 20% ABV. Sweet aroma was lower in the 20% ABV whisky sample possibly due to the masking effect of TCA together with potentially more TCA in the headspace. The 'dampening' effect of TCA on olfactory perception has been reported in wine (Tempere et al. 2017). Research on the effect of dilution on sensory perception of whisky congeners (MacGarry 2023) (performed in the absence of TCA) found that the sweet-associated aroma of vanillin was not significantly affected by ethanol concentration.



Table 3.

Panel scores for TCA (50 ng/L) in single malt whisky at different concentrations of ethanol.

Results of ANOVA are shown and Tukey's post hoc groupings (a,b,c) where statistically significant differences were found between samples.

	Single malt whisky (ABV) with 50 ng/L TCA				p-value
	20%	30%	40%	50%	
<b>Aroma attributes</b>					
Musty	0.85 a	0.64 ab	0.29 b	0.49 ab	0.02
Alcohol burn	0.73 c	1.11 bc	1.53 ab	1.78 a	≤0.001
Sweet	0.99 b	0.68 b	1.15 ab	1.63 a	≤0.001
Green/grassy	0.43	0.31	0.42	0.41	0.83
Fresh fruit	0.81	0.75	0.68	0.70	0.88
Dried fruit	0.98	1.11	0.72	0.75	0.10
Floral	0.35	0.23	0.39	0.35	0.46
Woody	0.70	0.77	0.76	0.72	0.94
Spicy	0.68	0.72	0.69	0.49	0.41
Nutty	0.51	0.32	0.50	0.52	0.22
<b>Taste attributes</b>					
Musty	0.55	0.57	0.47	0.57	0.88
Alcohol burn	1.23 c	2.00 b	2.16 ab	2.53 a	≤0.001
Sweet	0.73	0.80	0.77	1.05	0.32
Green/grassy	0.28	0.28	0.35	0.33	0.87
Fresh fruit	0.41	0.59	0.38	0.43	0.31
Dried fruit	0.63 ab	0.71 a	0.41 ab	0.30 b	0.03
Floral	0.13	0.21	0.27	0.22	0.07
Woody	0.83	0.68	0.63	0.61	0.44
Spicy	0.52	0.66	0.63	0.53	0.44
Nutty	0.25	0.31	0.30	0.23	0.73

Given the preferential association of TCA with ethanol rather than water, it was proposed that at higher alcohol concentrations TCA would be detectable in the liquid (rather than headspace) and would possibly suppress the taste of whisky. However, the sensory panel did not find significant differences in intensity of stale/musty taste across the range of ethanol concentrations. The significant difference in perception of dried fruit taste with different ethanol concentrations may suggest suppression of the intensity of desirable flavours due to the increasing presence of TCA in the liquid as sample strength increased. The single malt whisky used was full-bodied and it is possible that the strong flavours masked the effects of TCA and only dried fruit taste was impacted.

### Is nosing or tasting better for the detection of musty off-notes?

The data from the experiment on the effects of ABV on perception of TCA were analysed using a t-test on the scores for 'musty' assessed by nosing versus tasting at each alcohol strength. The results are shown for GNS (Table 2) and whisky (Table 3).

### Neutral spirit

At 20% ABV, TCA was detected better by nose in GNS, whereas at 50% ABV it was detected better by taste. However, the highest score for mustiness was found for the 20% dilution assessed by nose (Figure 1). These results are in accord with the greater solubility of TCA in ethanol.

### Single malt whisky

The method of assessment (nosing or tasting) had no statistically significant effect on the perception of mustiness for the samples at any concentration of whisky (Figure 2). This suggests that compared to GNS the presence of flavour congeners in whisky masked the effect of the TCA.

Nosing was better than tasting for detecting TCA tainted samples of GNS at 20% ABV whereas for the whisky sample there was no significant difference due to sensory mode of assessment. Generally, quality assessments of spirits are made by nosing and it is recommended that this continues with the nosing of diluted spirits to 20% ABV.

Figure 1.

**Average panel scores for mustiness in GNS**

GNS spiked with 30 ng/L TCA. Mustiness determined by nose and taste (t-test).

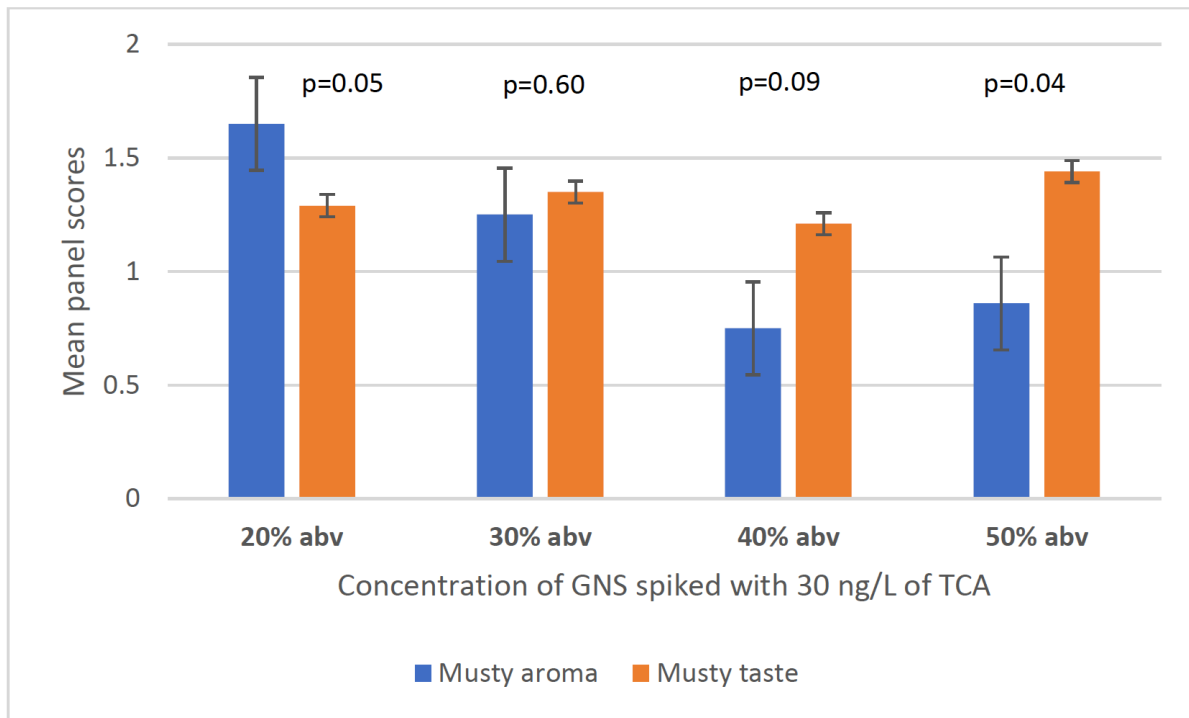
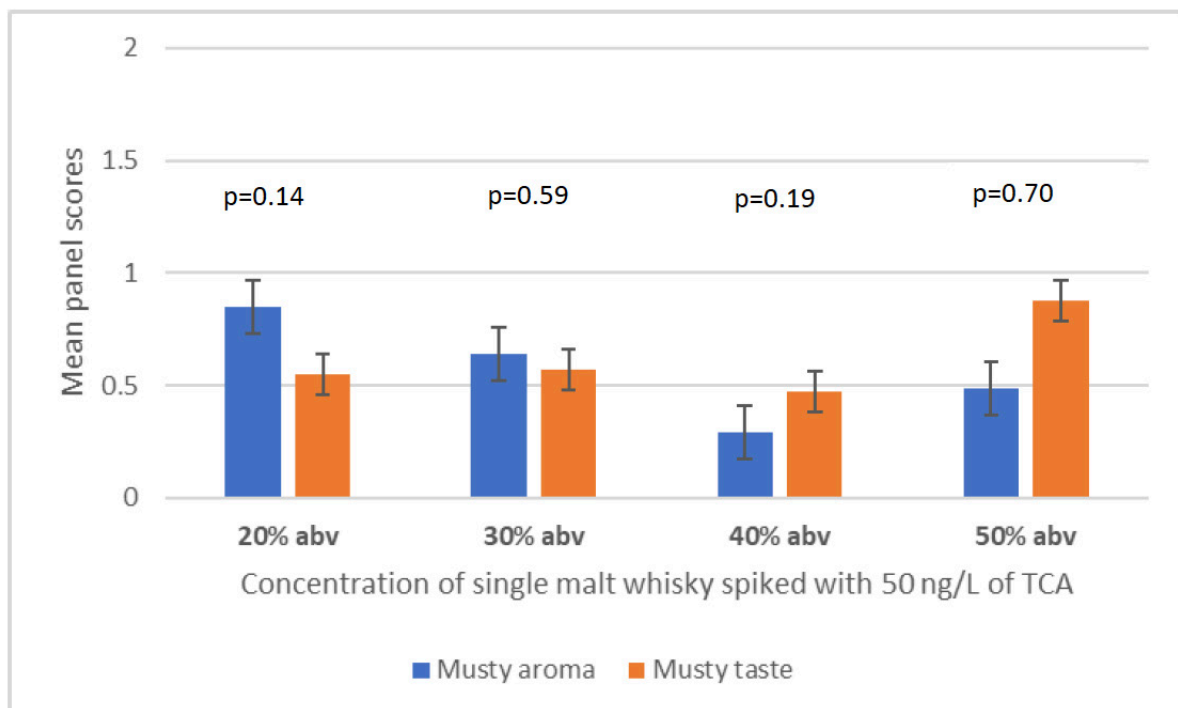


Figure 2.

**Average panel scores for mustiness in single malt whisky**

Whisky spiked with 50 ng/L TCA. Mustiness determined by nose and taste (t-test).



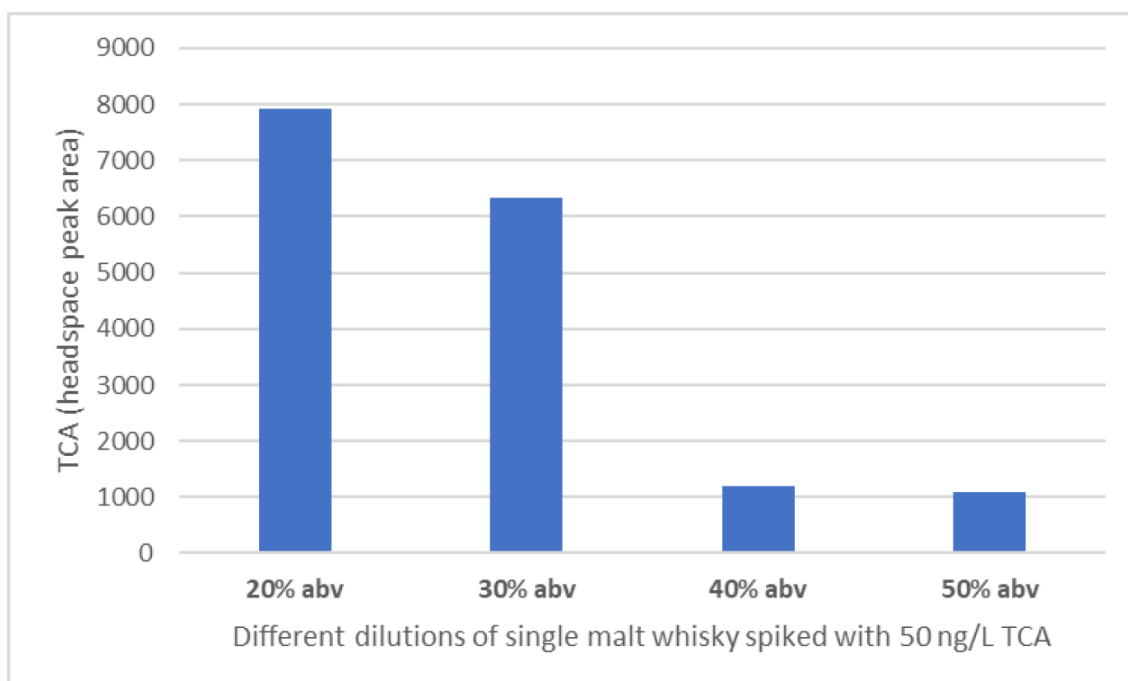
## The effect of ethanol concentration on the partitioning of TCA : headspace analysis by GC-MS

TCA is soluble in ethanol but practically insoluble in water (<https://www.sigmaaldrich.com/GB/en/product/aldrich/235393>). Therefore, as the sample strength increases, the amount of TCA in the headspace would be expected to decrease. The response for each TCA ion (TCA-195 and TCA-197) was integrated separately and the ratio used to confirm the presence of TCA. The sum of these results was used to plot the TCA peak area at increasing ethanol strength (Figure 3).

Headspace TCA concentration diminishes as the ethanol concentration of the whisky increases with a sharp decrease observed between 30-40% ABV (Figure 3). The GC-MS peak areas represent the relative amount of volatile TCA available in the headspace of a nosing glass at each ABV and clearly show that dilution to 20% ABV is optimal for the detection of TCA. The diminished peak area for the samples  $\geq 40\%$  ABV suggests the TCA molecules have aggregated with the ethanol molecules in the liquid and, accordingly, would be less easily detected by nosing.

### Figure 3.

#### GC-MS headspace analysis of single malt whisky spiked with 50 ng/L TCA.



## Stability of TCA in diluted samples

### Sensory

As recorded by Compusense®, the average time for panellists between nosing samples in the morning and afternoon was four hours 56 minutes (range from three hours 18 minutes to six hours 15 minutes). The average panel scores for each sample were calculated across all attributes and ANOVA was conducted to determine any statistically significant differences between the freshly prepared samples and those that were older (Table 4).

Whilst there was a significant difference between the samples for musty and sweet aroma, the difference was between the control and the TCA-spiked 20% ABV blended whisky and not the 'paired' time samples. Therefore, there was no statistically significant difference between the fresh and older samples of TCA-spiked blended whisky.

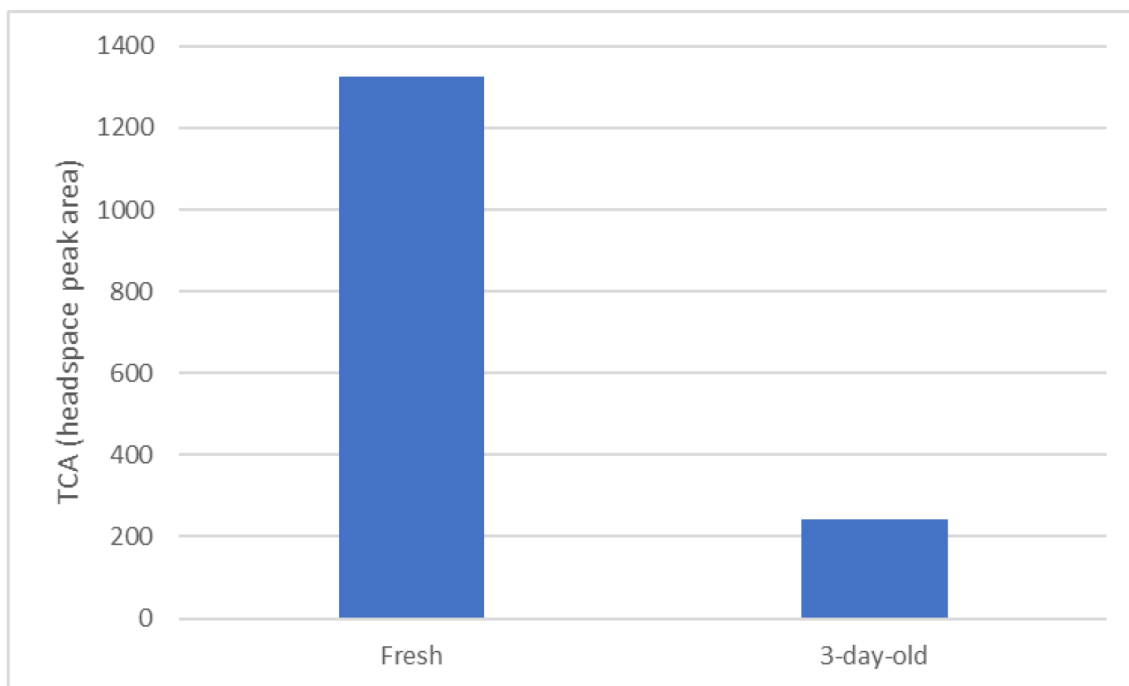
### Headspace analysis by GC-MS

Integration for the TCA headspace peak area was checked and the average of the five replicates of each sample of 20% ABV blended whisky spiked with 50 ng/L TCA reported in Figure 4. Analysis by GC-MS showed a marked decline in headspace TCA after three days at room temperature.



Figure 4.

GC-MS headspace analysis of fresh and three day old blended whisky spiked with 50 ng/L TCA.



Nosing of samples containing TCA at 20% ABV remained stable with respect to aroma for an average of five hours. It is worth noting that individual samples were provided for each panellist and stability may be diminished when nosing samples are shared. Analysis by GC-MS showed a marked decline in available TCA in the headspace of samples three days after preparation (Figure 4). Therefore, it is suggested that samples diluted for nosing are stable for the typical working day, but to maximise the detection of TCA-tainted samples, they should be assessed promptly.

Table 4.

Stability of TCA - panel scores and ANOVA.

Tukey's post hoc groupings (a,b) are shown where statistically significant differences were found between samples.

	Overall aroma intensity	Musty	Alcohol burn	Sweet aroma
TCA fresh	1.01	1.37 a	0.56	0.71 b
TCA old	1.16	1.21 a	0.56	0.63 b
Control fresh	1.37	0.24 b	0.69	1.26 a
Control old	1.34	0.25 b	0.76	0.93 a
p-value	0.15	≤0.001	0.54	0.01

Figure 5.

Average panel scores for GNS spiked with 50 ng/L TCA assessed at different serving temperatures.

ANOVA (p-values) are shown with Tukey's post hoc groupings (a,b,c).

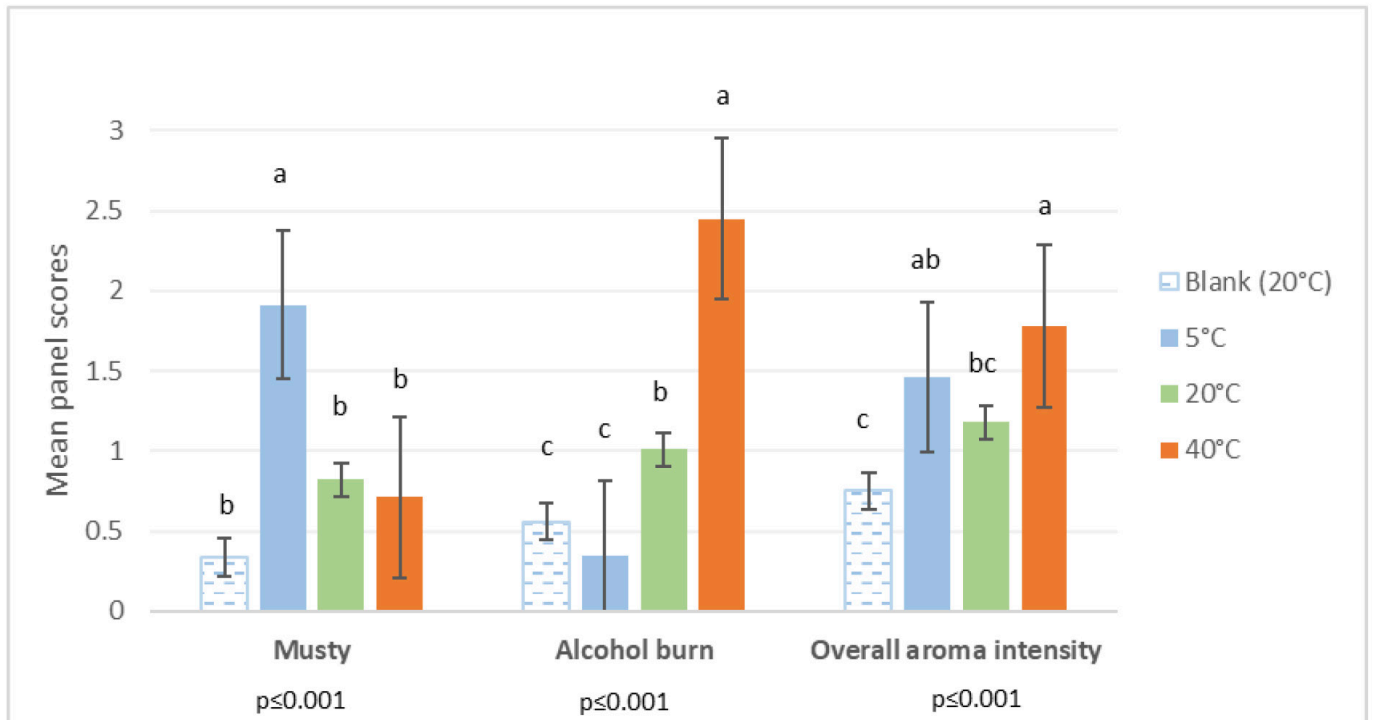
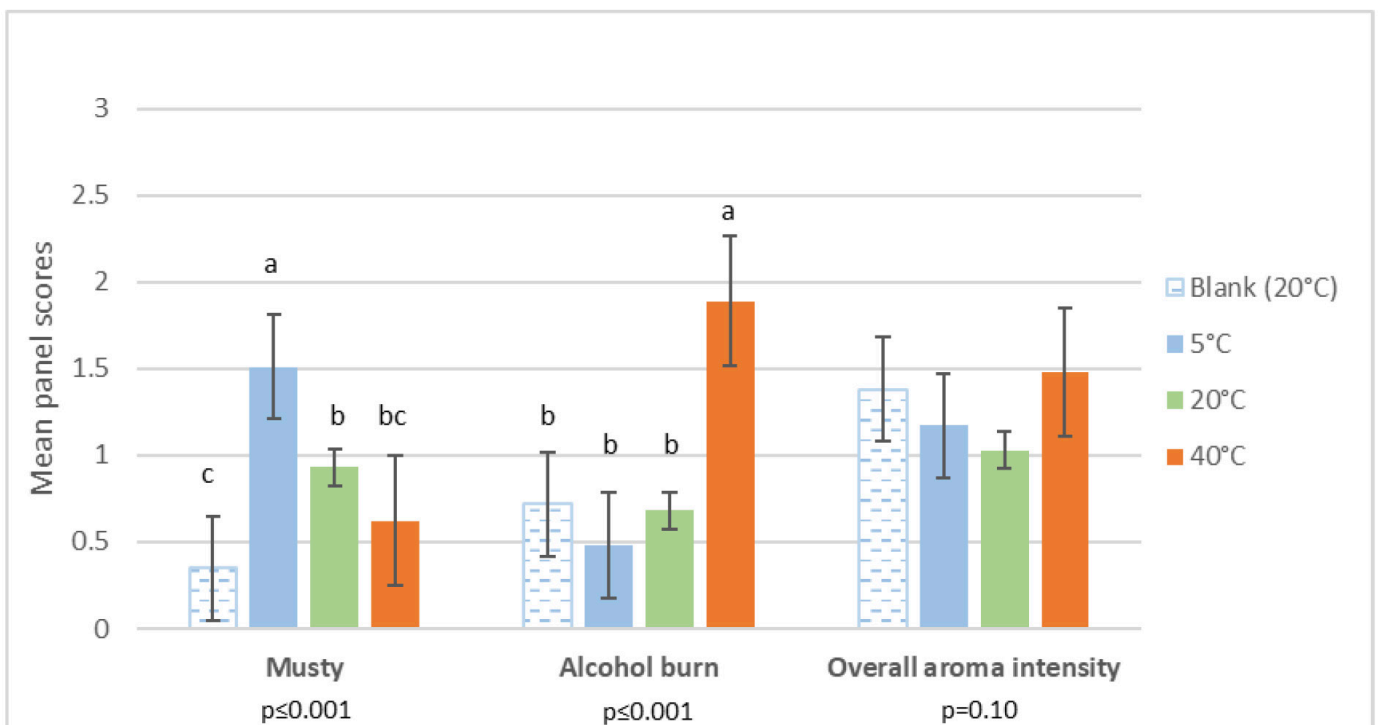


Figure 6.

Average panel scores for whisky spiked with 50 ng/L TCA assessed at different serving temperatures.

Results of ANOVA (p-values) are shown with Tukey's post hoc groupings (a,b,c).



## Effect of sample temperature on the perception of TCA

### Neutral spirit

Average panel scores for the GNS samples and the results of the statistical analysis are shown in [Figure 5](#). There were significant differences between the GNS samples served at different temperatures for musty aroma, alcohol burn and overall aroma intensity ( $p \leq 0.001$ ). Musty aroma was higher in the 5°C sample compared to the other temperatures while alcohol burn and overall aroma intensity were highest at 40°C.

### Blended whisky

Average panel scores and the results of the statistical analysis for the whisky samples are presented in [Figure 6](#). There were statistically significant differences (both at  $p \leq 0.001$ ) for perception of musty aroma and alcohol burn. Perception of musty aroma was significantly higher at 5°C than at ambient temperature (20°C) whereas alcohol burn was highest at 40°C.

TCA was easier to detect in samples of grain neutral spirit and blended whisky when nosed at 5°C compared to room temperature (*ca.* 20°C). Sensory assessment of samples is typically performed at room temperature, but where panel agreement is difficult to achieve, it would be worthwhile to chill samples to improve detection of TCA.

## Conclusions

To optimise the sensory detection of 2,4,6-trichloroanisole tainted whisky or GNS, it is recommended that panellists nose samples at 20% ABV soon after dilution. Flavour congeners mask the sensory perception of mustiness in TCA tainted samples. Conversely, the perception of some desirable whisky characteristics is diminished in spiked samples. However, the temperature of the sample was found to have an effect on the detection of TCA tainted samples with those that had been chilled (5°C) being perceived as being more musty.

Future work will assess the impact of alcohol strength and temperature on the sensory detection of other off notes of importance to the distilled spirits industry.

## Author Contributions

**Irene Baxter:** conceptualisation, methodology, investigation, formal analysis, writing (original draft, reviewing and editing), supervision.

**Augustin Réveillé:** methodology, investigation, project administration, formal analysis, writing (original draft).

**John Conner:** investigation, formal analysis, supervision.

## Conflict of interest

The authors declare there are no conflicts of interest.

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