

# Winemaking

## An assessment of the effect of the ROSA treatment on the level of TCA in naturally-contaminated cork granules

### Martin Hall

Director of Food Science Division  
Campden & Chorleywood Food Research Association (CCFRA)  
Chipping Campden, Gloucestershire, GL55 6LD  
United Kingdom

### Nick Byrd

Chromatography Section Manager  
Campden & Chorleywood Food Research Association (CCFRA)

### James Williams

Flavour Research & Taint Investigations Manager  
Campden & Chorleywood Food Research Association (CCFRA)

### Introduction

Cork taint is a term often used to refer to musty off-flavours in wines. Several studies have been conducted to estimate the extent of this problem in commercially-produced wines but there is still considerable debate about the true level. A recent survey organised by the Wine and Spirit Association indicated that on a sample of 13,780 wines sold in the UK the verified incidence of commercially-significant mustiness was 0.7% (Hall, 2002). Many commentators in the wine industry believe the incidence to be much greater than this. However, there is general agreement that mustiness constitutes a serious industrial problem and a practical means to reduce its incidence is urgently required.

The most common causative agent of mustiness in wine has been shown to be 2,4,6-trichloroanisole (TCA) (Buser, 1982) derived from the microbial methylation of the corresponding chlorophenol (Chatonnet, 1994). One route of contamination of wine is by the migration of TCA from contaminated cork material into wine during storage.

Recent years have seen the development and use of a number of alternatives to cork stoppers including synthetic cork substitutes and screw caps. Parallel to this the cork industry has invested significantly in new and improved methods and controls during stopper manufacture to remove as far as is possible the occurrence of TCA contamination. One such new approach, developed by Amorim, a major Portuguese cork producer, is a treatment procedure called ROSA. It has been claimed that the ROSA treatment significantly reduces TCA levels in granulated cork used for stopper manufacture. The purpose of this study was to determine whether the ROSA treatment reduced TCA levels and if so to what extent.

### Approach

The TCA content of a naturally-contaminated batch of granulated cork material was characterised by analysis of 50 sub-samples taken randomly from the granule-intake stage. The batch of cork granules was then passed through the ROSA treatment and collected. A further set of 50 samples was taken from the treated material for TCA analysis. The releasable TCA content of

all samples was determined by extraction into a 10% ethanol solution. The effectiveness of the ROSA treatment, to reduce TCA levels, was determined by a direct comparison of the levels before and after treatment. The statistical significance of any differences was determined by comparison of means and standard deviations.

All sampling, processing and analytical work was conducted by, or under the direct supervision of, an independent representative from Campden & Chorleywood Food Research Association.

### Materials & Methods

#### Materials

#### Sampling

An independent representative of Campden & Chorleywood Food Research Association undertook all sampling.

Fifty duplicated samples of naturally-contaminated cork material were taken randomly from incoming bales of cork granules (6-8mm diameter) prior to treatment. A further set of 50 duplicated samples was taken randomly from the corresponding material emerging from the ROSA-treatment stage. Samples were packed into aluminium cans and assigned random codes to ensure that analysts were not aware of the nature or identity of the sample provided, ie. control or treated.

Twenty-five duplicate samples from each set were analysed on-site in the laboratory of Amorim. The remaining samples were transported directly to CCFRA for analysis.

#### Extraction of TCA from cork granules

TCA binds strongly and probably variably to cork material making it impossible to extract and measure the total amount of TCA present in any sample of cork. In an extraction process equilibrium is established between the matrix and the extractant. Any measurement is, therefore, influenced by the amount of the analyte extractable into the extraction solvent. For this reason the widely accepted concept of releasable TCA (Herve, 1999) was adopted for this investigation. Releasable TCA reflects the ►

amount of TCA taken up by a wine simulant from the cork. This value has been used by the industry as a quality assurance indicator of cork quality and has been shown to be largely independent of the ratio of sample to extractant.

Glass bottles (100ml) were filled with cork granules (approximately 10g). The wine simulant (10% ethanol in water) was poured over the cork granules to fill the bottles. Bottles were sealed with plastic screw caps lined with aluminium foil and held at room temperature (21°C) for 24 hours prior to analysis.

#### Determination of TCA in wine simulant

Wine simulant (10ml) was taken from each bottle using a Gilson pipette and mixed with salt (2g) and a  $4\mu\text{g.l}^{-1}$  solution of TCA- $d_5$  in 96% ethanol (100 $\mu\text{l}$ , final [TCA- $d_5$ ] = 40ng.l $^{-1}$ ) in a 22ml glass headspace vial.

For quantification, calibration standards were prepared with TCA concentrations of 1, 2, 5, 10, 15, 25 and 50ng.l $^{-1}$ . All standards contained TCA- $d_5$  at a concentration of 40ng.l $^{-1}$ . Each standard (10ml) was mixed in a 22ml glass headspace vial with salt (2g).

At CCFRA analyses were carried out on a Varian 3800 gas chromatograph (GC) and Varian Saturn 2000 ion trap mass spectrometer (MS) via a CTC Combi-Pal autosampler.

The vial was sealed with a PTFE lined septum and equilibrated at 75°C for 1 minute with agitation. The headspace of the vial was then sampled for 30 minutes at 75°C (with agitation) using a DVB/Car/PDMS coated SPME fibre. The volatiles adsorbed onto the fibre were analysed by thermal desorption at 260°C in the injector port of a GC/MS for 5 minutes.

GC/MS conditions are outlined in Table 1.

At the Amorim Laboratories TCA assays were conducted under the supervision of a CCFRA representative. TCA was

Table 1. GC/MS conditions.

Column:	30m x 0.25mm fused silica with HP5-MS stationary phase.	
Helium carrier gas flow rate:	1 ml.min $^{-1}$	
Desorption temperature:	260°C	
Column temperature:	1 min at 50°C; then 20°C.min $^{-1}$ to 150°C for 5 minutes; then 20°C.min $^{-1}$ to 320°C for 2 minutes.	
MS analysis mode:	MRM	
TCA acquisition parameters:	Scan time:	0.25 s/scan
	Multiplier offset:	200 volts
	Emission current:	50 $\mu\text{amps}$
	Parent ion mass:	212
	Isolation window:	1.0
	Waveform type:	Non-resonant
	Excitation storage level:	85.0
	Excitation amplitude:	98.0
	Quantifying ions (m/z):	107, 109 & 169
TCA- $d^6$ acquisition parameters:	Scan time:	0.25 s/scan
	Multiplier offset:	200 volts
	Emission current:	50 $\mu\text{amps}$
	Parent ion mass:	217
	Isolation window:	1.0
	Waveform type:	Non-resonant
	Excitation storage level:	85.0
	Excitation amplitude:	98.0
	Quantifying ions (m/z):	108, 110 & 171

extracted using the same procedure as detailed above. Levels of TCA were quantified using gas chromatography with electron capture detection.

#### Results and discussion

Large differences were detected in the levels of releasable TCA present in the extracts from cork granule material before and after the treatment (Table 2). Low standard deviations demonstrated good agreement in results between samples of the same type within each laboratory. Furthermore, similar data was obtained in the two laboratories.

Table 2. Summary of levels of releasable 2,4,6-trichloroanisole (TCA) detected in ROSA treated and untreated cork (ng.l $^{-1}$ )


	Analysis at Amorim		Analysis at CCFRA		Combined	
	Mean	Std Deviation	Mean	Std Deviation	Mean	Std Deviation
Treated	4.2	0.81	3.9	0.68	4.1	0.76
Untreated	20.6	3.3	20.3	6.7	20.4	5.2

The standard deviation of the results typically ranged from 16% to 19% of the means for all data sets, ie. treated and untreated at CCFRA and at Amorim. However, two relatively high levels of TCA (>45ng.l $^{-1}$ ) in the untreated samples at CCFRA increased the standard deviation of this set to around 33% of the mean.

The data arising from the CCFRA laboratories indicated a reduction in the mean TCA levels from 20.3ng.l $^{-1}$  to 3.9ng.l $^{-1}$  (80.8%) compared with the 20.6ng.l $^{-1}$  to 4.2ng.l $^{-1}$  (79.6%) reduction observed from the Amorim laboratory data.


A combination of all results obtained in both laboratories showed a reduction in the levels of releasable TCA in the region of 80% as a result of the ROSA treatment. A student t-test showed this difference to be highly significant ( $P < 0.0001$ ).

This work was directed at determining whether the ROSA treatment reduced the level of TCA in cork material. The results indicate that the releasable TCA is certainly reduced. It is fair to assume that the treatment is likely to have similar effects on other organic compounds found in natural cork material. This would result in a general reduction in the level of organic compounds extracted into wine and reduce the likelihood of other wine defects resulting from such migration.



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**Conclusion**

The results of this study indicate that the ROSA treatment applied to the cork granules tested gave rise to a consistent and significant reduction of around 80% in the levels of releasable TCA.

**References**

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## Shiraz enthusiasts gather in Barossa

What makes Shiraz the leading grape variety in the world at the moment? Why have consumers from New York to Newcastle, California to China, Oxford to Ottawa embraced the rich flavours of this historic grape? Where do the best Shiraz wines come from and what makes them special?

These will be some of the questions answered by hundreds of the world's leading Shiraz lovers at The First International Shiraz Alliance, a celebration of the variety to be held in the Barossa, Australia from July 30 to August 2.

"Winemaker and supplier groups from Europe, the United States, Argentina, Chile, New Zealand and Asia are all registering and we have had strong interest from Australian winemakers," said organiser Peter Fuller.

"We have also been pleased to see the number of trade and consumers who are registering. They are excited about the opportunity to taste Shiraz from all over the world and meet the makers."

The focus of the event will be a two-day masterclass program centred around the theme *The Texture of Flavour*. Featuring 35 Shiraz makers, growers, marketers and media, every delegate will have a chance to taste premium Shiraz from Australia, the United States, France and South Africa.

Sessions will investigate natural influences on wine flavour such as the age of vines, the clones, region and terroir as well as human intervention - fermentation, pressing, alcohol, tannin and oak.

There will also be a chance to investigate blending and the role Grenache, Mataro and other varieties have on Shiraz and the

marketing and future development of the varietal.

A highlight of the program will be a visit by leading Rhone winemaker Michel Chapoutier, who will bring Old World knowledge and experience to the panels. He will be joined by other internationals John Alban and Steve Fennell from the United States and Charles Back and Kevin Arnold from South Africa.

Leading UK wine writer and judge Robert Joseph will moderate the sessions and provide insight and comment.

Prominent Australian Shiraz makers to take part will include Ron Laughton, Jasper Hill; Trevor Mast, Mount Langi Ghiran; John Duval, former Penfolds Grange winemaker; Stephen Henschke of Hill of Grace fame; Robert O'Callaghan, maker of the cult Rockford Basket Press; Pat Carmody, Craiglee; Viv Thompson, Best's Great Western and Clive Otto who will be bringing wines from Margaret River to balance the SA and Victorian contingent.

Barossa legends such as Peter Lehmann, Charlie Melton, Nigel Dolan and Grant Burge will line up alongside McLaren Vale's Chester Osborn and Wirra Wirra's Samantha Connew and the Hunter Valley's Iain Riggs in good natured inter-regional comradeship.

Key Australian wine media will also take part in the sessions including James Halliday, *The Australian*; Max Allen, *The Weekend Australian* and Huon Hooke, *Sydney Morning Herald*.

For program and registration information visit [www.shirazalliance.com](http://www.shirazalliance.com) or email [bridget.laucke@fuller.com.au](mailto:bridget.laucke@fuller.com.au)

## New edition of Rankine 'bible'

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Contact: Peter Cooper  
Business Development Manager

OSMOFLO Pty Ltd  
100 Hayward Avenue  
Torrensville, South Australia 5031

Phone +61 8 8159 8999  
Fax +61 8 8234 7066  
Email [wine@osmoflo.com.au](mailto:wine@osmoflo.com.au)  
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