

Chemical Analysis of TCA As a Quality Control Tool For Natural Corks



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Background

TCA



Current QC Programs are based on cork soaks and sensory analysis

- Problems with sensory evaluation
- Cork soak procedures?
- How does TCA in the cork soak relate to TCA in bottled wine?

Background

TCA is the single most prevalent wine defect associated with natural cork. TCA can be generated by a variety of means - most commonly by fungal metabolism of chlorophenols.

TCA is an extremely good indicator of all sensory defects in cork. Current literature and experience indicate that significant TCA is present in 70-80% of corks that trained analysts would reject for any sensory reasons.

Current industry practices include a variety of evaluation techniques to screen corks for the TCA. A wide range of soak media, timing, and volume ratios are commonly used. On top of these variations is the subjective value of the sensory evaluation itself. Trained analysts display a wide range of thresholds for recognizing TCA.

Our research shows that a trained panel, using spike samples of dry white wine, demonstrate sensory recognition thresholds for TCA at 6 parts per trillion (ppt). Performance commonly decreases as the sensory background becomes more complex - as with actual cork soaks.

Objectives

Develop a reliable tool for cork quality control programs

- 1. Move from sensory to chemical analysis**
- 2. Investigate how cork soaks work**
- 3. Determine the relationship between soak TCA and TCA in bottled wine**
- 4. Apply methodology to commercial QC practices**

Objectives

The objective of this applied research project was to develop a reliable and commercially viable means to improve the detection and screening of TCA in cork lots.

The first phase of experiment identified new analytical tools. We wanted to replace the current sensory-based method with a chemical procedure. Our goal was to develop a quantitative and non-destructive test while providing improvements in sensitivity and dependability.

The second phase required an investigation of the mechanics of cork soaks. Information was needed about the dynamics of TCA transfer to understand what conditions are required for a representative analysis.

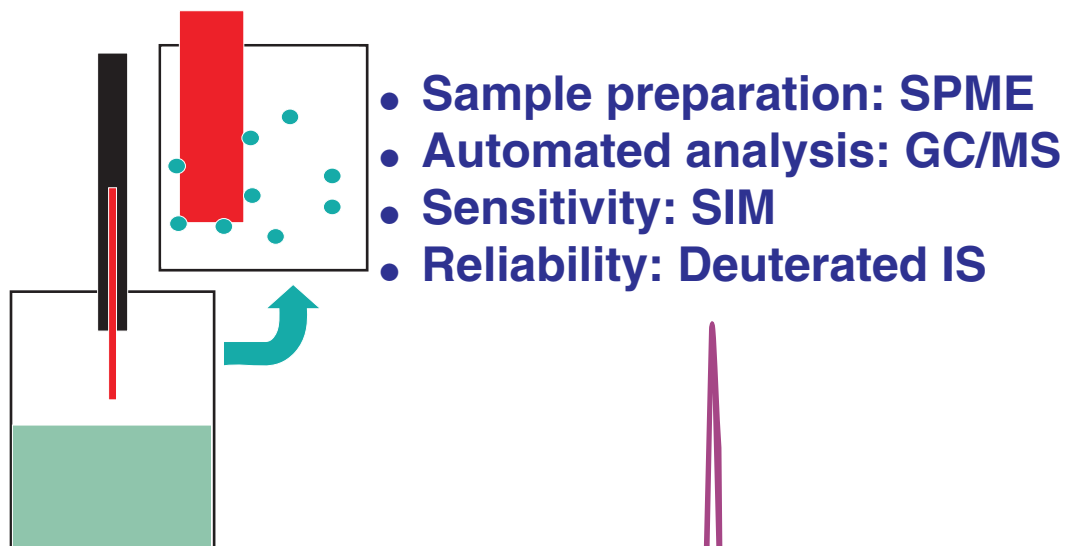
The third phase called for determining the reliability of TCA as measured in cork soaks to act as a predictive indicator of TCA in bottled wine.

The fourth phase, currently underway, calls for applying the experimental methodology into a commercial quality control tool.

Chemical Analysis

Objective: Move from sensory to chemical analysis

Analytical Method



SPME-GC/MS Analysis

- Analytical Limit 1.0 ng/L (ppt)
- Precision (10 ng/L), CV < 5%

Sensory Analysis

- Difference threshold 2 ng/L (ppt)
- Detection threshold 6 ng/L (ppt)

Analytical Methodology

The chemical methodology adopted is based on a combination of solid phase micro-extraction (SPME) and gas chromatography and mass spec analysis (GC/MS).

The SPME technology allows samples to be drawn directly from cork soaks. Methodology drew from previous work by T. J. Evans, Sue Ebler and Christian Butzke of U.C. Davis; Ilona Schneider at the Geisenheim Institute; and Eric Hervé at the Université of Bordeaux (now employed by ETS).

Samples were introduced to GC/MS analysis and measurement utilized Single Ion Monitoring (SIM) - allowing for sensitivity to a level of 1.0 ng/L (ppt).

Samples were calibrated by the use of deuterated internal standard. Precision at levels of 10ppt shows a coefficient of variance less than 5%.

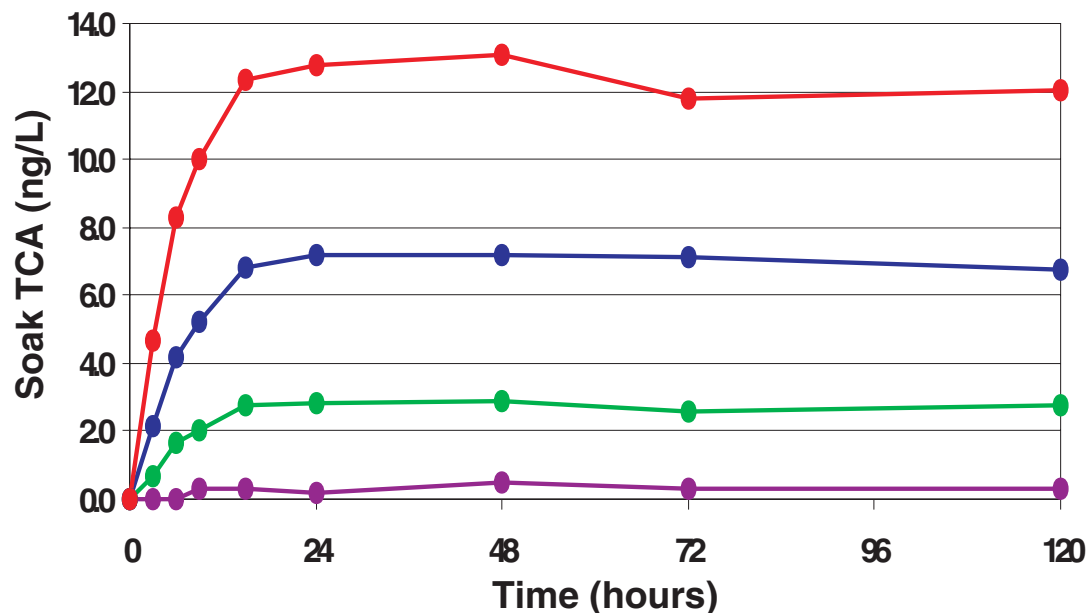
The combination of techniques has created an accurate system for measuring TCA well below the human identification threshold of 6ppt. The system lends itself to automation. To date we have analyzed over 4,000 samples using this technique.

Cork Soaks

Objective: Investigate how cork soaks work

TCA Soak Kinetics

TCA Detected in 100 Cork Soaks
From Four Bales



**24 hr soaks provide reliable data
for TCA at equilibrium.**

Cork Soak Kinetics

Bales of untreated corks were selected from a population of corks had been previously rejected by cork suppliers.

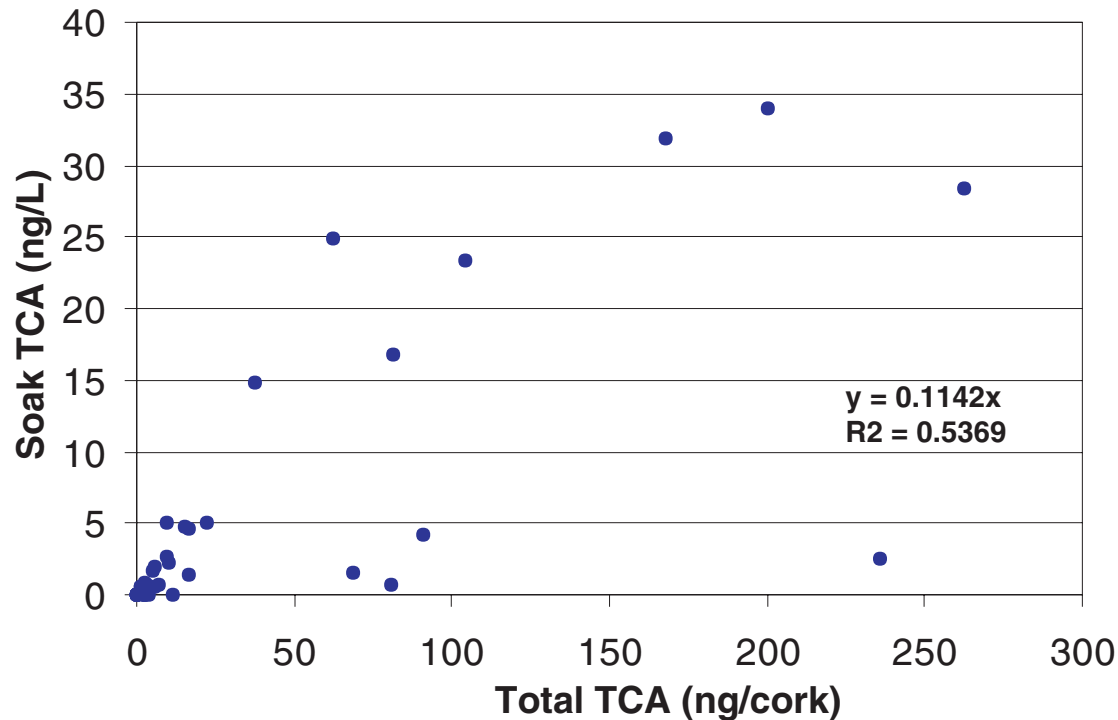
Samples of 100 corks were placed in 4L glass containers. They were soaked in approximately 1.5L of dry white wine (10% alcohol). Analysis was performed over a variety of scheduled periods.

TCA was measured in the soaks at each benchmark to provide a timing curve showing the most efficient time to perform analysis. Results show that a 24-hour period allowed the soak solution to reach equilibrium.

Cork Soaks

Objective: Investigate how cork soaks work

Total vs Soak TCA



- **Poor correlation between total TCA in a cork and that which is found in a cork soak.**
- **Only 0.05% to 2.0% of the total TCA is extracted in the cork soak.**

Correlation to Total TCA

Most previous GC/MS work involving TCA and corks was based on grinding up the cork and measuring total TCA.

Since the SPME methodology is based on cork soaks, and measures only that TCA extracted we looked at the correlation between the two values.

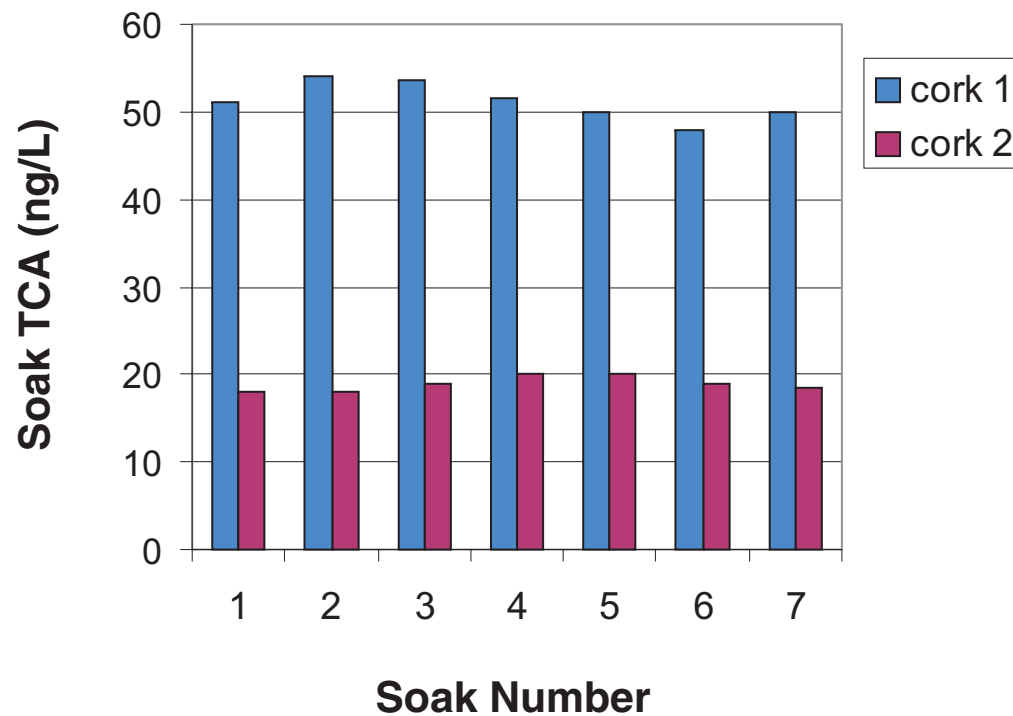
Results showed that there was a poor correlation between total TCA and the amount of TCA extracted during the soak process.

Results also showed that a very small percentage of the total TCA was extracted during the soak. Extraction values ranged from 0.05% to 2.0% of total TCA in the cork.

Cork Soaks

Objective: Investigate how cork soaks work

Repeated Soaks



Replication of Results

When a 24-hour soak is repeated for an individual cork - subsequent soaks of the same cork present identical TCA extraction results.

This test was repeated numerous times with no significant change in the repetition of results even after a dozen soaks.

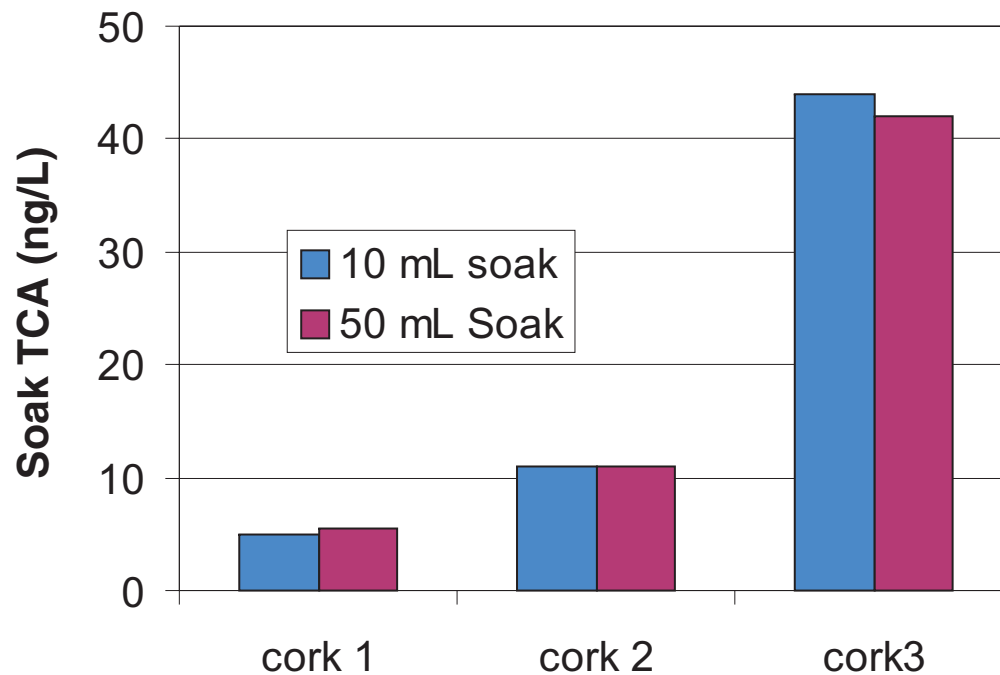
This phenomenon indicates that the characteristic of extracted TCA is related to equilibrium governed by the properties of the individual cork.

- **Repeated soaks of the same cork will result in the same TCA concentration in the soak wine.**

Cork Soaks

Objective: Investigate how cork soaks work

Soak Volume



- Different soak volumes with the same cork have the same TCA concentration.

Soak Volume

Cork soaks routinely present varying levels of sensory volatility based on the ratio of soak solution to each cork. Ten corks soaked in a single flask produce more background aromas than does a single cork with the same quantity of wine. This was not the case with TCA.

When measuring the effect of various cork/wine ratios on the concentration of extracted TCA - no significant difference was seen.

SPME measurements were taken in soaks with 10ml and 50ml of wine per cork. Results showed no significant difference for corks that measured TCA at 5ppt, 10ppt and 41ppt.

Releasable TCA

Defined as the amount of TCA at equilibrium in a wine soak.

A function of:

- **TCA content in the cork**
- **Localization of TCA in the cork**
- **Wine characteristics (EtOH)**

Determination of Releasable TCA in a cork soak does not significantly modify Releasable TCA in that cork

New Term - Releasable TCA

Releasable TCA is defined as the equilibrium value a given cork imparts to a soak solution. The Releasable TCA value of a cork is not modified even after multiple soaks.

It is postulated that the Releasable TCA is determined by a combination of total TCA plus its location in the cork and perhaps other physical properties of the cork that would relate to contact with the soak solution.

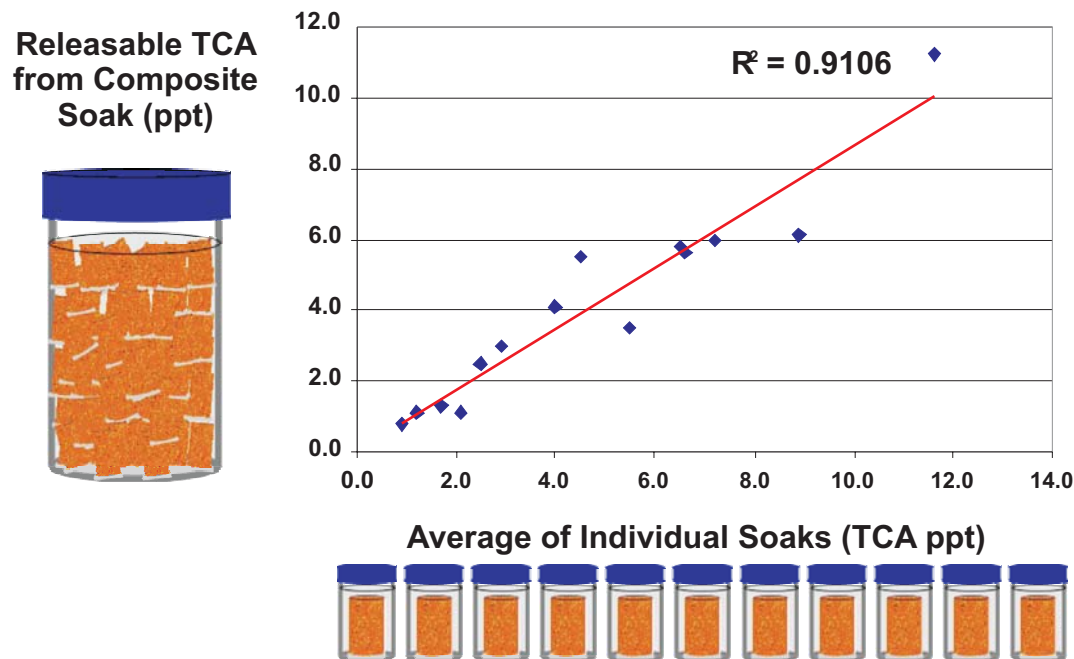
It was also determined that the alcohol level of the soak solution has significant effect on the level of Releasable TCA. Higher alcohol levels create significant increases in TCA extraction levels.

The higher extractions were offset by the fact that TCA is less volatile at higher alcohol levels. The reduction in volatility reduces the relative availability of TCA to sensory detection and the SPME fiber. The net result suggests that detection of TCA is near optimum at a 10% ethanol solution.

Composite Samples

Objective: Determine the accuracy and reliability of composite cork soaks

Individual Cork Soaks vs Composite Soaks



Composite Soaks

Composite soaks of 100 random corks were examined to determine if the releasable TCA measured by the group was representative of the total population. The soaks were made conducted for 24 hours in a wine solution containing 10% ethanol.

The composite soak solution was analyzed for releasable TCA and results were compared to the average releasable TCA measured by individual cork soaks taken from a second sample of 100 random corks from the same Bale.

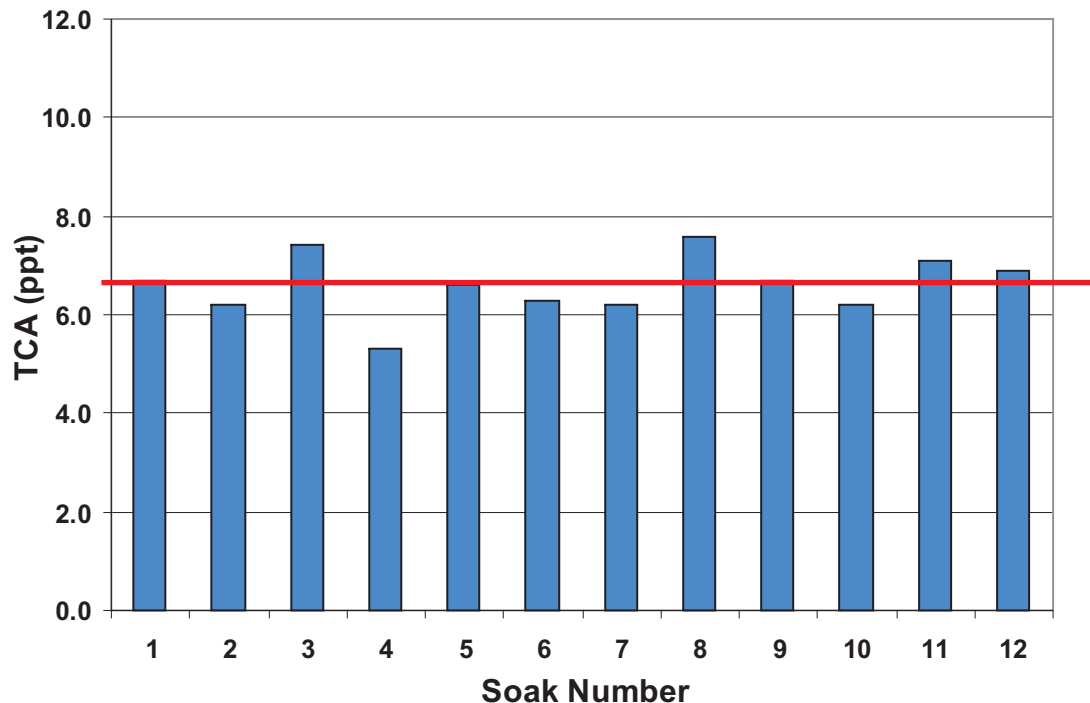
Fourteen Bales were tested. Results indicate a good correlation between the value from the composite soak and the average of individual corks. (R^2 over 90%)

There is a good correlation between composite soaks and the average of the individual corks

Composite Samples

Objective: Determine the accuracy and reliability of composite cork soaks

Repeated Values in Soaks from the Same Bale



There is a good correlation between composite soaks taken from repeated samples from the same Bale

Repetition of Results

To determine the reliability of the composite sampling method - twelve different samples of 100 corks were taken from the same Bale. Each was used for a unique composite soak.

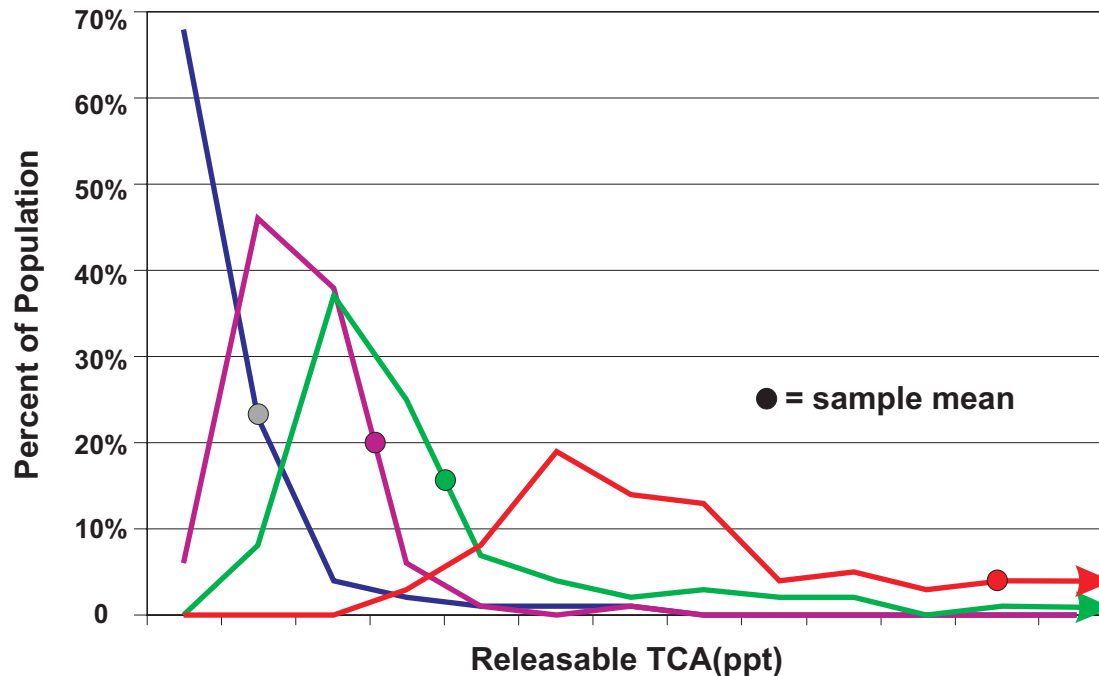
Releasable TCA was measured for each sample and results indicate a high degree of correlation.

The co-efficient of variance between composite soaks was less than 10%.

Composite Samples

Objective: Determine the accuracy and reliability of composite cork soaks

Population Distribution of TCA in Corks within a Bale



Population Distribution

Releasable TCA for 100 individual corks were marked for each of the fourteen bales studied. Results suggest a significant relationship between the releasable TCA of a group soak and population distribution.

In all 14 populations, the population curve displayed a positive skew - with the majority of corks below the composite soak average. Typically, 75% of the population had lower TCA values than the mean.

In the bales we have studied with high group soak TCA concentrations, there were very few corks with low TCA levels. In bales with low group Soak TCA levels, there were very few corks with high TCA levels.

Measuring the TCA content of composite soaks can reveal a likely distribution of high TCA corks within the population

Bottling Experiment

Objective: Determine the relationship between soak TCA and TCA in bottled wine

Methodology

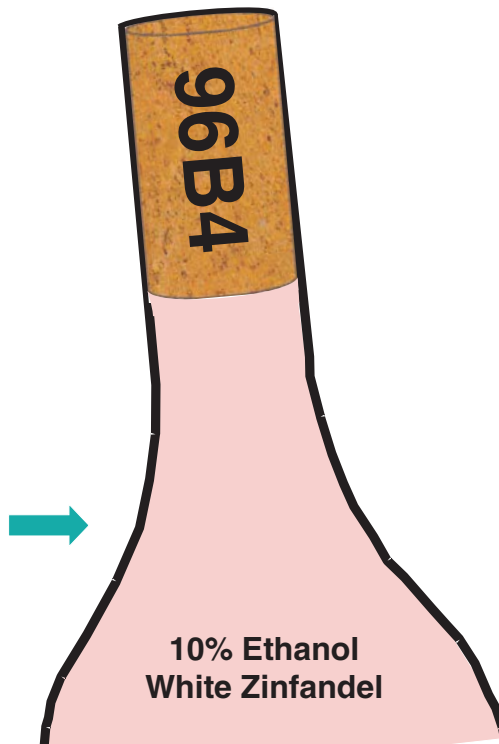
Determination of Releasable TCA



10% Ethanol

Corks dried and coated with Standard Silicone/paraffin

Commercial Bottling and Storage



Bottling Experiment

A bottling experiment was required to determine the relationship between TCA measured in a cork soak and TCA imparted under normal bottling conditions.

A selection of 400 corks was taken from 3 cork bales known to have high incidence of TCA. Individual corks were tested for Releasable TCA - then dried and coded.

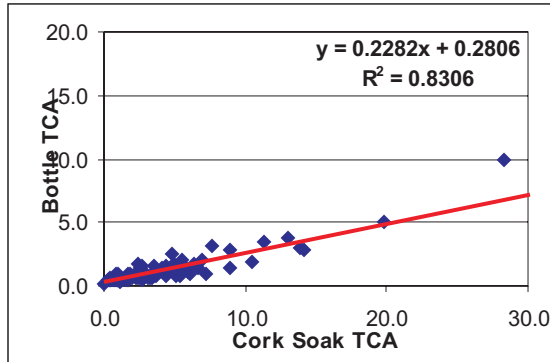
Coded corks were coated with a standard paraffin/silicon mixture and inserted at a commercial bottling line to 750ml bottles of White Zinfandel with an alcohol content of 10%. The wine was tested and found to have no background TCA.

Bottles were stored neck down and wine samples were tested for TCA content at measured intervals of 1, 3, 8 and 14 months.

Bottling Experiment

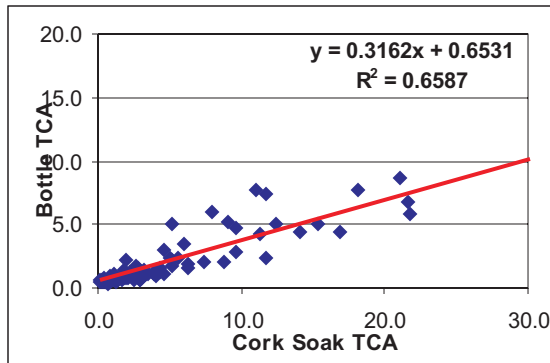
Soaks vs bottled wine

Objective: Determine the relationship between soak TCA and TCA in bottled wine



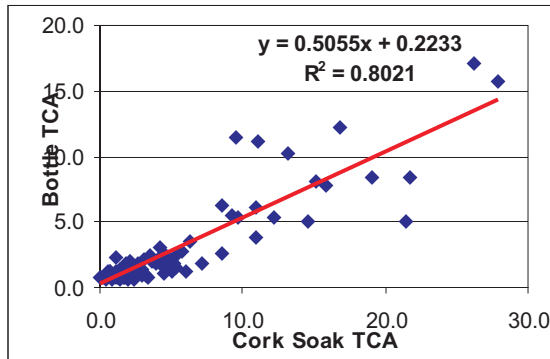
Combined Results After 3 months

- Average Ratio of TCA transfer = 23%
- Correlation $R^2 = .82$



Combined Results After 8 months

- Average Ratio of TCA transfer = 32%
- Correlation $R^2 = .66$



Combined Results After 14 months

- Average Ratio of TCA transfer = 51%
- Correlation $R^2 = .80$

Ratio of TCA Transfer

Analysis of bottled wine for TCA shows a high correlation to the Releasable TCA measured in the individual corks prior to bottling. As an average, the test bottles contained TCA after 14 months at a level that was roughly half the level of Releasable TCA measured by individual cork soaks.

Of the three bales tested, each showed strong individual correlation between Releasable TCA and Bottled TCA. In R squared calculations - the bales ranged for 0.97 to 0.71 over three different measurement points.

Two bales - #15 and #38 were quite similar. Bale #29 showed a higher transfer rate - particularly in the bottles sampled at 8 months.

Transfer Rates By Bale

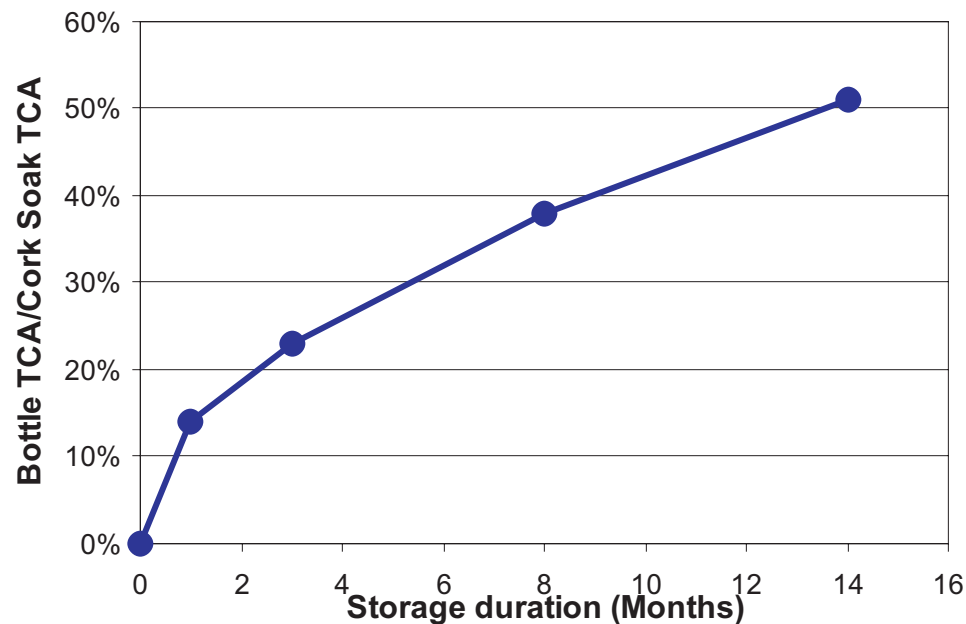
Bale#		3 Mos	8 Mos	14 Mos
15	Transfer	18%	24%	48%
	R square	0.89	0.93	0.88
38	Transfer	21%	29%	47%
	R square	0.82	0.97	0.71
29	Transfer	32%	75%	59%
	R square	0.95	0.83	0.91

Bottling Experiment

Objective: Determine the relationship between TCA in cork soaks and bottled wine

Soaks vs bottled wine

Total Bottles Tested at 14 Months



Bottled wine TCA at 14 months was one half of soak TCA

Rate of TCA Transfer

Unlike a cork soak - which indicates that a TCA equilibrium is reached within 24 hours - TCA in the bottled wine shows a relatively long-term development.

Bottles from combined test bales showed TCA in the bottle at a ratio of slightly less than 15% after 30 days. This ratio increased over time. At the conclusion of the test - bottled wines included TCA at a rate of roughly half of the releasable TCA measured in their respective corks.

Conclusions

- **SPME-GC/MS analysis of TCA is sensitive and reliable**
- **TCA presents an equilibrium in cork soaks**
- **Composite soaks contain TCA equivalent to the average of individual corks**
- **Composite soaks can suggest the population curve of TCA in the sample**
- **Releasable TCA can be used to predict TCA in bottled wine**
- **Determination of Releasable TCA has promise as a cork quality control tool**



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