Removal of Smoke Taint Compounds From Wine using Differential Filtration

White Paper

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Feasibility Study using Differential Filtration at the Bench and Pilot Scale

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Abstract

Differential Filtration (DF) for the removal of smoke taint compounds from wine combines ultrafiltration (UF) and reverse osmosis (RO) to remove both the glycosylated (bound) and free forms of smoke taint compounds. DF represents an important advance over the current use of "RO-only" since using RO alone historically has only removed the free forms of smoke taint.

DF employs three steps within each treatment round.

1. UF protects the colloidal and polyphenolic structure of the wine by separating it from the low color permeate.
2. RO traps the bound smoke taint from the low color permeate.
3. A carbon block array removes the free smoke taint contaminants remaining in the low color permeate.

Two trials using DF at the bench scale (Test Track) reduced guaiacol and syringol, two key markers of smoke taint, by 85% and 91% respectively. Successive runs of DF have shown further reduction of smoke taint markers by a similar order of magnitude. Although cellar scale trials showed a slightly lower reduction of guaiacol (73%) and syringol (55%), the loss of efficiency at the production scale may be attributable to the age of the membrane used. However, runs at both the bench and production scales reduced guaiacol to concentrations below the sensory threshold after two rounds of treatment. It is noteworthy that the bound forms of guaiacol and syringol concentrations were reduced by 83.6% and 77.6% respectively at the Test Track scale; 58.5% and 38.2% respectively at the Cellar scale.

Introduction

Wildfires have impacted the grape growing industry around the world. Smoke from these fires taints the quality of wine grapes as grape skins absorb smoky compounds while they are maturing on the vine. Smoke taint is presumed to involve a myriad of undesirable compounds from combustion of brush, pine and many other unconventional and uncontrolled sources. Once smoke compounds are absorbed into the grape skin, a portion of these molecules become glycosylated, creating a mixture of bound and free forms. The bound forms are odorless but have the potential to dissociate from their glucose moiety and become aromatic. For the winemaker, this means that both the free and bound forms need to be removed prior to bottling.

Differential Filtration provides a mechanical means of treatment without the harsh effects of direct chemical treatment on the wine. To protect the delicate colloidal structure of wine, DF combines two tangential flow filtration technologies with an activated carbon filtration application allowing for more removal of smoke taint contaminants with greater precision.

Method & Apparatus

Guaiacol and syringol are primary chemical smoke taint markers for this feasibility study. Malvidin and other red wine monomeric pigments form chains of polymeric anthocyanins. They are part of an important matrix of macromolecules that include polysaccharides and proteins that are signature components of a wine's true expression of its terroir. The DF treatment process is unique from competitive treatment technologies in that it protects polymerized color and flavors yet allows for the removal of the bound forms of the smoke compounds. Bound forms such as the gentiobiosides of guaiacol and syringol permeate Step 1 but do not permeate the RO membrane due to their size. Untreated they are indiscernable. However they have the potential to hydrolyze and become volatile after the wine is bottled. Using DF, these bound forms are trapped and removed from the system once all processing is complete.

Figure 1: Comparison of Bound and Unbound forms of Guaiacol and Syringol

Guaiacol and syringol are known chemical markers for smoke taint caused by wildfires.

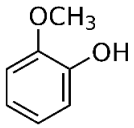
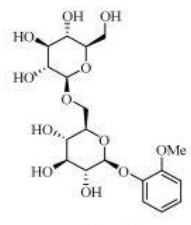
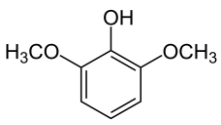
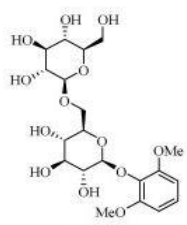
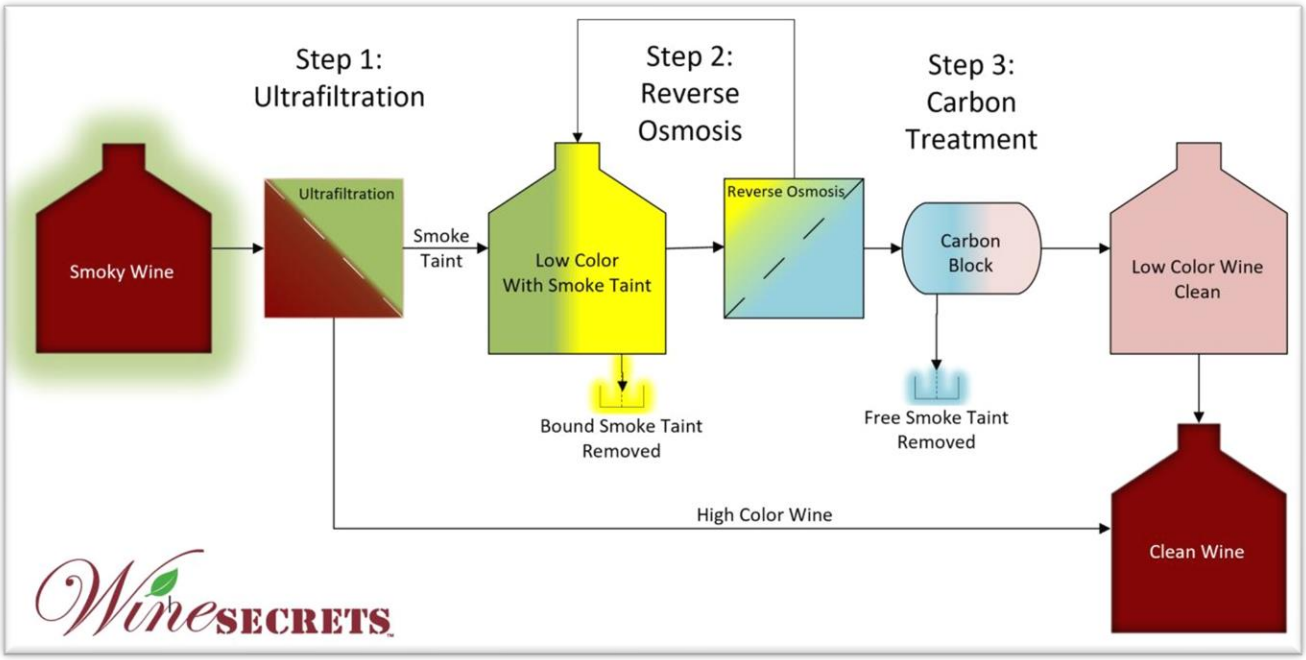
DF Target Molecules	Guaiacol	Guaiacol-β-D-gentiobioside	Syringol	Syringol Gentiobioside
Form	Free	Bound	Free	Bound
Chemical Structure				
MW (Da)	124	448	154	478
Sensory Threshold (ppb)	23 (Red Wine)	non-volatile	570 (10% Ethanol)	non-volatile

Figure 2: The Differential Filtration Process

Differential filtration (DF) has three major steps. Please refer to the flow diagram below in Figure 2 for a graphical presentation of the process.



Step 1 of the Differential Filtration process divides smoke contaminated wine via ultrafiltration into high and low color fractions. The high color fraction, which represents 10 % of the initial volume, retains the wine's polyphenols and colloidal structure. It is kept separate while the low color fraction is processed. This low color fraction represents 90% of the initial wine volume and contains both bound and free smoke taint contaminants. The low color fraction has the appearance of a light rosé wine.

In Step 2, the smoky Low Color fraction is further bifurcated via RO filtration which traps the bound smoke taint molecules in the retentate and permeates the free smoke taint components. The fraction containing the bound forms is discarded once the treatment is complete.

Step 3 begins when the RO permeate is passed through a carbon block array which removes the free smoke taint molecules. Once Step 2 and 3 are complete, the treated low color fraction is then combined with the high color fraction, concluding one round of the DF process.

If the results are not satisfactory to the winemaker, the DF process may be repeated until the desired result is achieved. A "round," represents 100% of the wine's volume and includes all three steps of the DF process.

The data displayed in the Results section of this report represent the most recent iterations of bench and cellar scale trials conducted using a 2017 Cabernet Sauvignon from Atlas Peak, a sub AVA of Napa Valley, during

September 2019 at the Winesecrets facility located in Sebastopol, California. Samples from this trial were submitted to the ETS Labs in St. Helena, the Australian Wine Research Institute, and to a privately owned winery analytical laboratory. The apparatus for both scales is noted below for each round of DF.

Bench Trial Operation

Step 1 – Ultrafiltration: Winesecrets has developed a bench scale Targeted Filtration unit equipped to support a wide variety of crossflow experimentation called Test Track. For Step 1, Test Track was equipped with a Suez PW-1812 ultrafiltration (UF) membrane, rated at 20,000 molecular-weight-cut-off (mwco). Previous in-house studies showed that this membrane allowed the permeation of the glycosylated smoke taint molecules.

Steps 1, 2 and 3: The Test Track Apparatus & Configuration Steps

Scale	Bench
Equipment	Test Track
Step 1: Ultrafilter - 20,000 mwco	Suez PW-1812
Step 2: Reverse Osmosis - 250 mwco	Suez DK-1812
Step 3: Carbon Block	Pentair CBC-5

Wine was introduced into Test Track's feed tank which was then flushed with nitrogen gas to prevent oxidation. The Test Track was flushed with two liters of wine to purge the system of water. The wine was separated by ultrafiltration into a 10% High Color retentate and a 90% Low Color permeate. Each fraction was mixed, sampled and measured for free and bound forms of guaiacol and syringol.

Step 2 – Reverse Osmosis: For the second step of the process, the Test Track was equipped with a Suez DK-1812 molecular reverse osmosis membrane, rated at 250 mwco. This RO membrane is approximately two orders of magnitude more restrictive than the PW-1812 ultrafilter membrane. The smoky, Low Color fraction from Step 1 was concentrated to 10% of its volume through the RO, trapping the bound forms of smoke taint molecules. This 10% retentate returned to the Low Color tank (referred to as the Molecular Trap) and was initially retained then discarded following the conclusion of the trial. The RO permeate fraction, representing 81% of the initial volume, was then passed directly through a Carbon Block Array.

Step 3 – Carbon Block Array: The carbon block array was prepared by flushing it with deionized water for 30 mins and then pumped dry. The RO permeate from Step 2 was introduced into Test Track's feed tank, and one liter of the permeate was used to purge the system of water. The RO permeate was then pumped through the Pentair CBC-5 Carbon Block at a rate of 0.2 l/min. Following passage through the Carbon Blocks, the carbon treated RO permeate was reintroduced to the high color fraction from Step 1 to create the final wine with reduced smoke taint character.

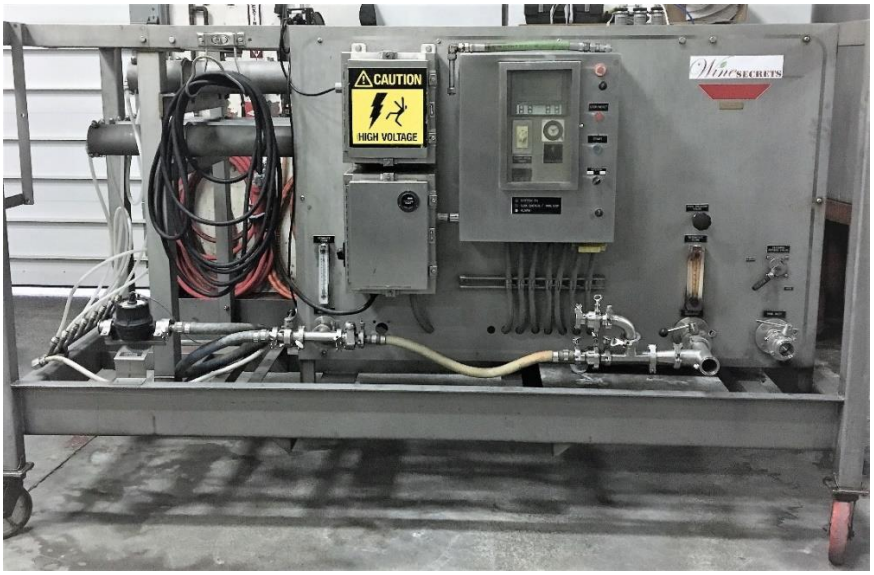
Cellar Trial Operation

Step 1 – Ultrafiltration: A WS 4810 Targeted Filtration System (RO unit) was configured with a single Suez PW-4040 membrane rated at 20,000 mwco. As with the Test Track scale, the wine was filtered into a 10% volume High Color fraction and 90% volume Low Color fraction. Each fraction was mixed and sampled for chemical analysis of the free and bound forms of guaiacol and syringol.

STEP 1: Ultrafiltration Unit

Scale	Pilot
Equipment	WS 4810 Targeted Filtration System
Step 1: Ultrafilter - 20,000 mwco	Suez PW-4040

Step 2 – Reverse Osmosis: A WS 4G10 Targeted Filtration System (RO) was configured to run on 4 housings / 8 elements (not the full complement of 8 housings / 16 elements) and equipped with eight (8) Suez Vinopro 4040 Molecular Reverse Osmosis membranes rated at 250 mwco. As with the Test Track, the Low Color wine fraction from Step 1 was filtered through the RO, trapping the bound forms of smoke taint molecules in the retentate. This 10% retentate recycled to the Low Color tank (referred to as the Molecular Trap) and was discarded following the conclusion of all runs. The RO permeate fraction, representing 90% of the volume, is then passed directly through a Carbon Block Array.



STEP 2: Reverse Osmosis Unit

Scale	Pilot
Equipment	WS 4G10 Targeted Filtration System
Step 2: Reverse Osmosis - 250 mwco	Suez Vinopro Molecular 4040
Step 3: Carbon Block Array	CBC20-BB 0.5 micron block

Step 3 – Carbon Block Array: As permeate from the RO separation was produced, it was passed, in-line, through two Pentek CBC20-BB 0.5-micron block carbon filters set up in parallel and permeate was accumulated in a holding vessel until Step 2 filtration was complete.

STEP 3: Carbon Block

At the end of the RO and carbon filtration process, the remaining RO retentate, referred to as the Molecular Trap, was mixed, and samples were collected. Following passage through the Carbon Blocks, the RO permeate fraction was mixed then samples collected. The carbon-filtered RO permeate was then recombined with the high color fraction from Step 1. All samples collected were analyzed for free and bound guaiacol and syringol.

Once the RO permeate was carbon filtered and recombined with the high color fraction to create the final treated wine, a full round of DF was recorded. For this trial, five full rounds of DF were completed with samples collected after the first, third, and fifth rounds. The efficacy of increasing the number of treatment cycles is presented in Charts 7 and 8 in the Discussion section below.

Sampling Protocol

The initial sample was collected from the wine treatment vessel before the start of processing, and samples were collected at the completion of Round 1 (100%), Round 3 (300%), and Round 5 (500%). Each of these samples was analyzed for the free and bound forms of guaiacol and syringol.

Samples were collected at the beginning of the third and fifth rounds. During each round, samples were taken at the following points within those rounds of the DF process:

- Step 1 Retentate – collected after processing in Step 1
- Step 1 Permeate – collected after processing in Step 1
- Step 2 Retentate – collected after processing in Step 2
- Step 2 Permeate – collected after processing in Step 2

Please note that at both the Test Track and Cellar scales, the retentate "lees" trapped from RO processing were kept until all rounds were complete. These "lees" were only discarded once at the conclusion of processing.

Results

The following tables presented the detailed results of the Test Track and Cellar scale trials.

Table 1: Test Track Trial Data

Test Track	Free Guaiacol (ppb)	Total Guaiacol (ppb)	Bound Guaiacol* (ppb)	Free Syringol (ppb)	Total Syringol (ppb)	Bound Syringol* (ppb)
DFTT-Initial	23.7	47.6	23.9	81.4	197	116
DFTT-100-S1R	32.4	140	108	153	1040	884
DFTT-100-S1P	19.6	27.1	7.47	78.9	132	52.7
DFTT-100-S2R	34.6	46.1	11.5	227	432	205
DFTT-100-S2P	< 1.0	< 1.0	< 1.0	< 10.0	< 10.0	< 10.0
DFTT-300-Initial	< 1	4.28	3.87	< 10.0	29.6	26
DFTT-300-S1R	1.51	39.5	38	17.3	246	228
DFTT-300-S1P	2.53	13	10.5	20.7	96.1	75.4
DFTT-300-S2R	4.95	19.2	14.3	82.3	341	258
DFTT-300-S2P	< 1.0	< 1.0	< 1.0	< 10.0	< 10.0	< 10.0
DFTT-500-Initial	< 1.0	2.27	2.04	< 10.0	15.4	12.9
DFTT-500-S1R	< 1.0	24.7	24	13	143	130
DFTT-500-S1P	< 1.0	9.55	8.97	< 10.0	75.9	70.2
DFTT-500-S2P	< 1.0	< 1.0	< 1.0	< 10.0	< 10.0	< 10.0
DFTT-Final	< 1.0	1.35	1.24	< 10.0	< 10.0	< 10.0

* Bound smoke markers calculated as difference between Total and Free

Key: DFTT=Differential Filtration, Test Track
100=1 full volume treatment
300=3 full volumes treatment
500=5 full volumes treatment
S1R=High color retentate, following hi-low color UF
S1P=Low Color Permeate, prior to RO
S2R=Low color fraction, retentate post RO (Molecular Trap)
S2P=Low color fraction, permeate post carbon block, pre blending

Table 2: Cellar Trial Data

Cellar	Free Guaiacol (ppb)	Total Guaiacol (ppb)	Bound Guaiacol* (ppb)	Free Syringol (ppb)	Total Syringol (ppb)	Bound Syringol* (ppb)
DFCellar-Initial	23.7	47.6	23.9	81.4	197	116
DFCellar-100-S1R	40.7	110	69.3	190	853	663
DFCellar-100-S1P	18.6	32.3	13.7	74.8	161	85.8
DFCellar-100-S2R	24.2	55	30.9	116	337	221
DFCellar-100-S2P	3.08	10.3	7.26	15	61.1	46.1
DFCellar-300-Initial	2.92	12.9	9.93	16.7	88.5	71.8
DFCellar-300-S1R	5.92	59.6	53.7	52.3	601	549
DFCellar-300-S1P	3.16	11.1	7.89	15	74.9	59.8
DFCellar-300-S2R	5.6	28.6	23	28.3	243	215
DFCellar-300-S2P	2.16	9.64	7.48	< 10.0	55.1	55.1
DFCellar-500-Initial	1.61	13.6	12	< 10.0	46.3	46.3
DFCellar-500-S1R	3.38	48.7	45.3	< 10.0	417	417
DFCellar-500-S1P	1.57	7.92	6.35	< 10.0	14.8	14.8
DFCellar-500-S2R	2.75	26.7	23.9	< 10.0	222	222
DFCellar-500-S2P	2.9	7.12	4.22	< 10.0	14.9	14.9
DFCellar-Final	1.62	11.5	9.83	< 10.0	47.7	47.7

* Bound smoke markers calculated as difference between Total and Free

Key: DFCellar=Differential Filtration, Cellar
100=1 full volume treatment
300=3 full volumes treatment
500=5 full volumes treatment
S1R=High color retentate, following hi-low color UF
S1P=Low Color Permeate, prior to RO
S2R=Low color fraction, retentate post RO (Molecular Trap)
S2P=Low color fraction, permeate post carbon block, pre blending

Discussion

This discussion evaluates the feasibility of using Differential Filtration for the purpose of smoke taint remediation in wine. DF solves two problems; it removes the free smoke taint compounds which contribute to the smoky off-aromas in wines made from smoke contaminated grapes. More importantly, it also removes the bound forms which behave as precursors that may dissociate later to create additional smoky off aromas.

Current methods of smoke taint treatment focus on removing only the free molecules below their sensory threshold using RO. Based on literature provided by the Australian Wine Research Institute (AWRI), guaiacol is an indicator of smoke taint in wine. Guaiacol's sensory threshold is 23 ppb in red wine. The threshold for guaiacol has been annotated on the charts below as "← GST" (guaiacol sensory threshold) to help provide context as a target for successful treatment. Syringol, on the other hand, has a much higher sensory threshold and thus less direct aromatic impact. However, according to the AWRI, it is also an indicator of smoke exposure, so levels of syringol provide a sense of the smoke taint levels in general. The results of free smoke taint treatment are displayed in Charts 1 and 2 at the Test Track scale and in Charts 4 and 5 at the Cellar scale.

Regarding the novel treatment of the bound forms of smoke taint, concentrations of bound smoke taint markers were measured in untreated versus treated wine samples to demonstrate DF's efficacy for removing them at the Test Track and Cellar scales in Charts 3 and 6 respectively.

The charts provided in this section were created solely based on the chemical analyses displayed in the results section above.

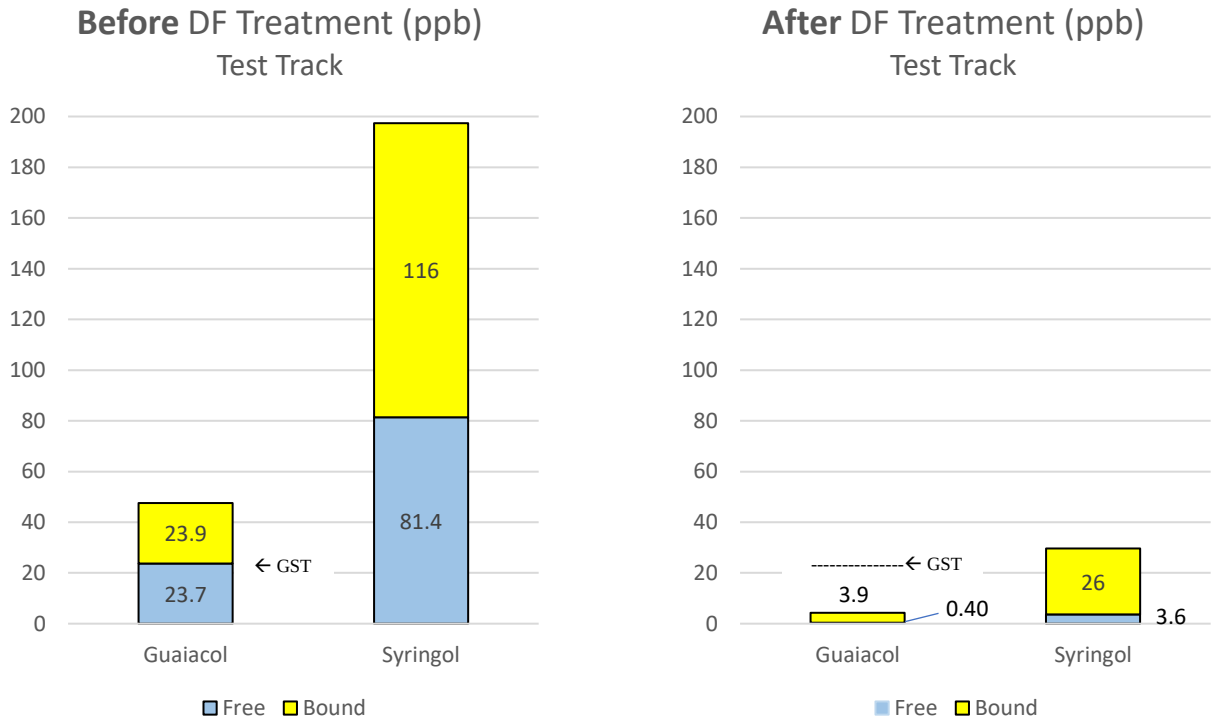
Discussion of Test Track Results

Analysis of the Test Track treatment data after two rounds shows a significant decline in both guaiacol and syringol concentrations: a 91% drop in guaiacol and an 85% drop in syringol. The remaining guaiacol level was also well below the sensory threshold after one round of DF.

Table 3: Test Track Results after Two Rounds of DF

	Untreated wine	Treated Wine (Two Rounds) (DFTT – 300 Initial)	% Removal
Total Guaiacol (ppb)	47.6	4.28	91.0%
Total Syringol (ppb)	197	29.6	85.0%

Charts 1 and 2: The following bar graphs represent the effectiveness of DF treatment by showing smoke taint levels measured as total guaiacol and syringol before and after two rounds of a DF treatment at the Test Track scale.

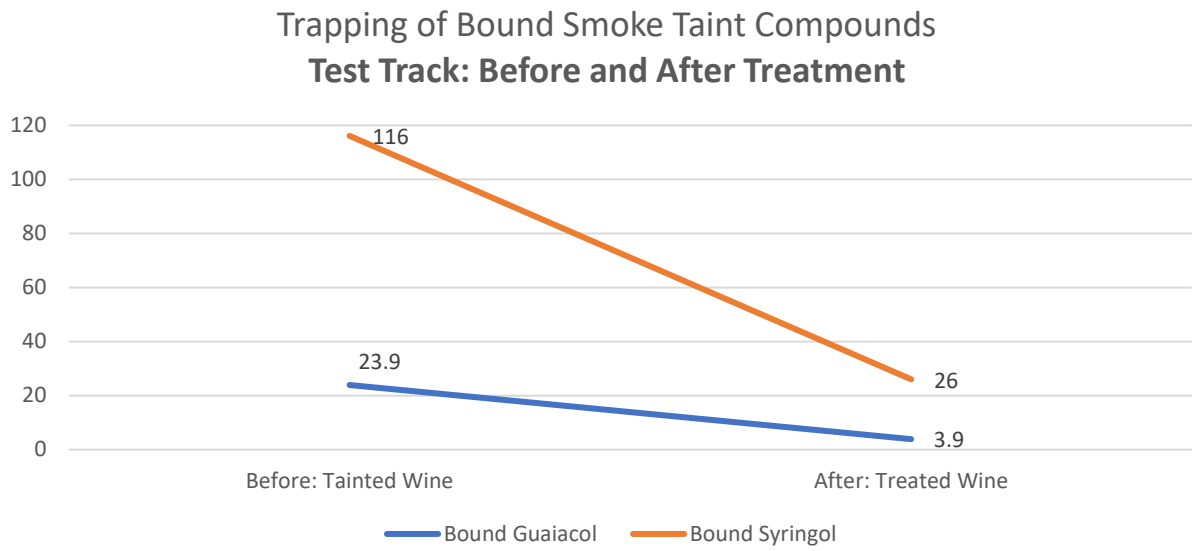


Novel Process for Trapping of Bound Smoke Taint Molecules

DF is the only technology to address the removal of the bound forms of smoke taint compounds by trapping them on the retentate side of the membrane following an ultrafiltration step.

As discussed previously, many existing treatment methods aim to reduce the presence of free smoke taint markers below the sensory threshold, however these methods do not take into account the bound forms of these compounds which can dissociate from their sugar moiety during aging which may cause a resurgence in smokey or off aromas. This is especially problematic if the wine is in bottle and distributed throughout sales channels. This risk is also exacerbated by additional aging time as is the case for ultra-premium red wines such as Cabernet Sauvignon.

Chart 3: The following chart shows the reduction in bound guaiacol and syringol after one treatment (two rounds) at the Test Track scale.



Discussion of Cellar Results

Analysis of the Cellar treatment data after two rounds of DF shows a 73.0% reduction in the concentration of guaiacol and a 55.2% drop in syringol. The guaiacol concentration was below the sensory threshold. While the cellar process did not achieve the same degree of reduction in guaiacol as that achieved by the Test Track, this may have been due to the age of the membranes in service rather than a problem related to process scalability. Further testing will repeat this study with different wines and examine the use of new membranes.

Table 4: Cellar Results after Two Rounds of DF (One Treatment)

	Untreated wine (DF Cellar Initial)	Treated Wine (Two Rounds) (DF Cellar 300 Initial)	% Removal
Total Guaiacol (ppb)	47.6	12.9	73.0%
Total Syringol (ppb)	197	88.5	55.2%

Charts 4 and 5: The following bar charts represent the effectiveness of DF treatment by showing smoke taint levels measured as total guaiacol and syringol before and after two rounds of a DF treatment at Cellar scale.

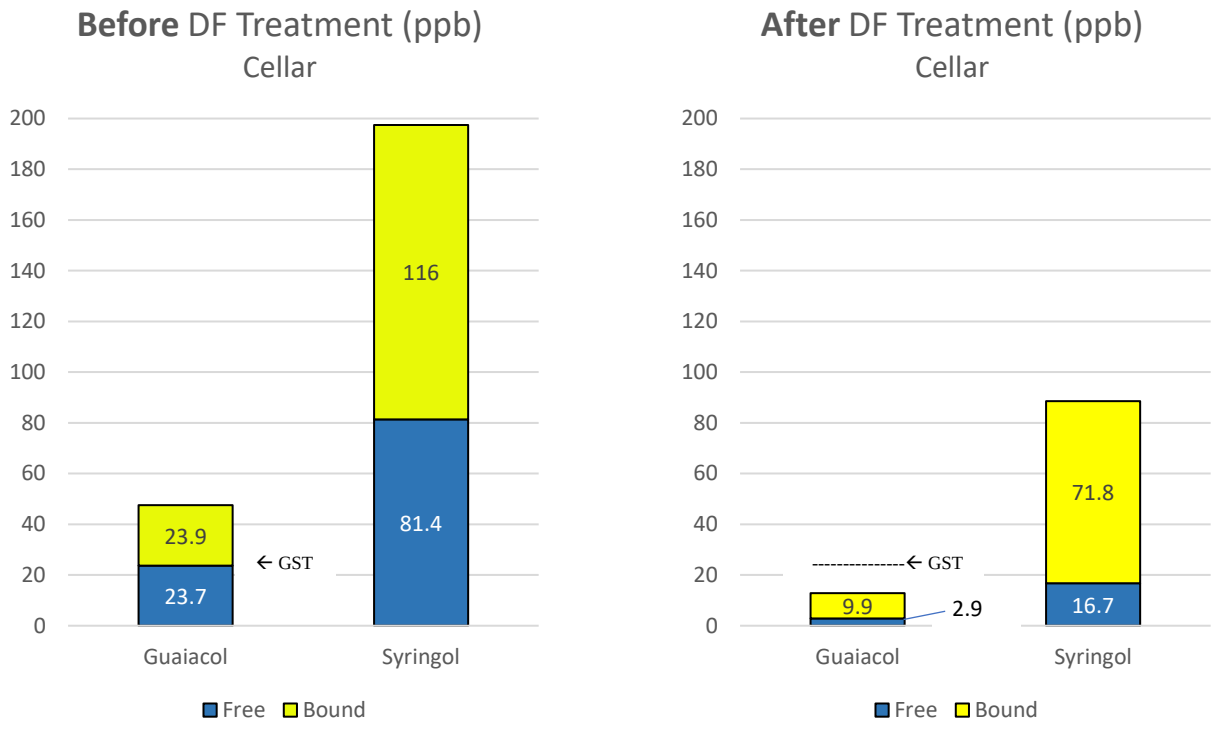
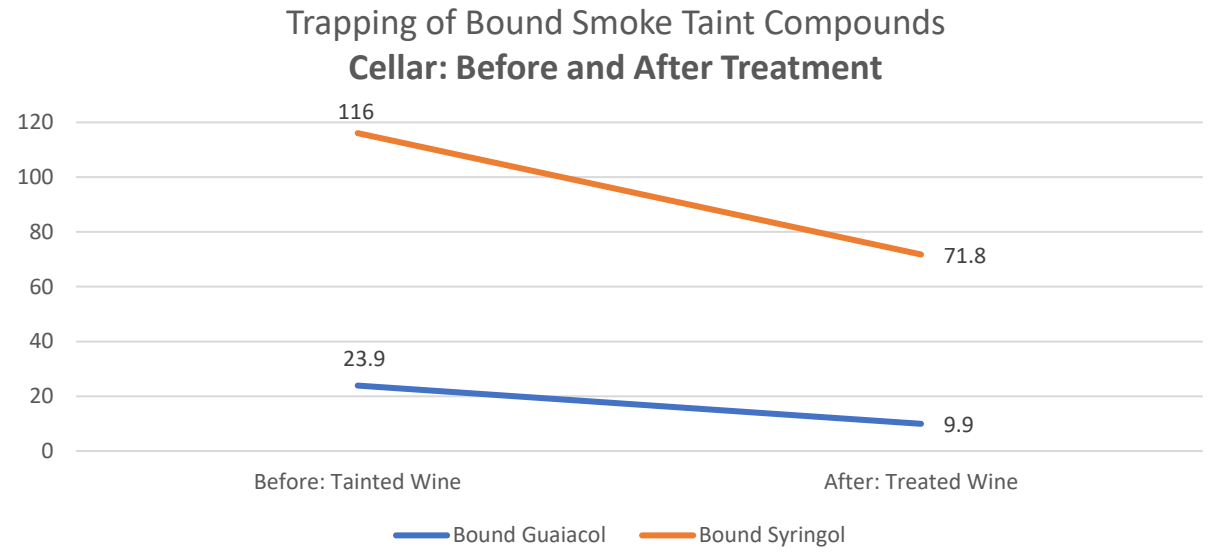


Chart 6: The following chart shows the reduction in bound guaiacol and syringol after one treatment (two rounds) at Cellar scale.



The effect of successive DF treatments on smoke taint markers.

As 90% of the initial wine can be treated during each round of DF, treatment naturally follows a negative logarithmic trend. The first two rounds of treatment remove the greatest portion of the smoke taint markers. Further rounds of treatment show a diminishing return by an order of magnitude. Fortunately, the Test Track and Cellar scale trials of this study both achieved a reduction in guaiacol to a concentration below the sensory threshold. While successive runs may further deplete the concentration of smoke taint markers, the sensory benefit of performing extra cycles of DF is not always perceptible.

Table 5: Test Track treatment efficiency between treatments

Test Track	Total Guaiacol (ppb)	% Decline	Total Syringol (ppb)	% Decline
DFTT-Initial	47.6	-	197	-
DFTT-300-Initial	4.28	91.0%	29.6	85.0%
DFTT-500-Initial	2.27	95.2%	15.4	92.2%
DFTT-Final	1.35	97.2%	10	95.1%

Chart 7: This chart shows the decline in smoke taint markers over the course of five complete runs at the Test Track scale.

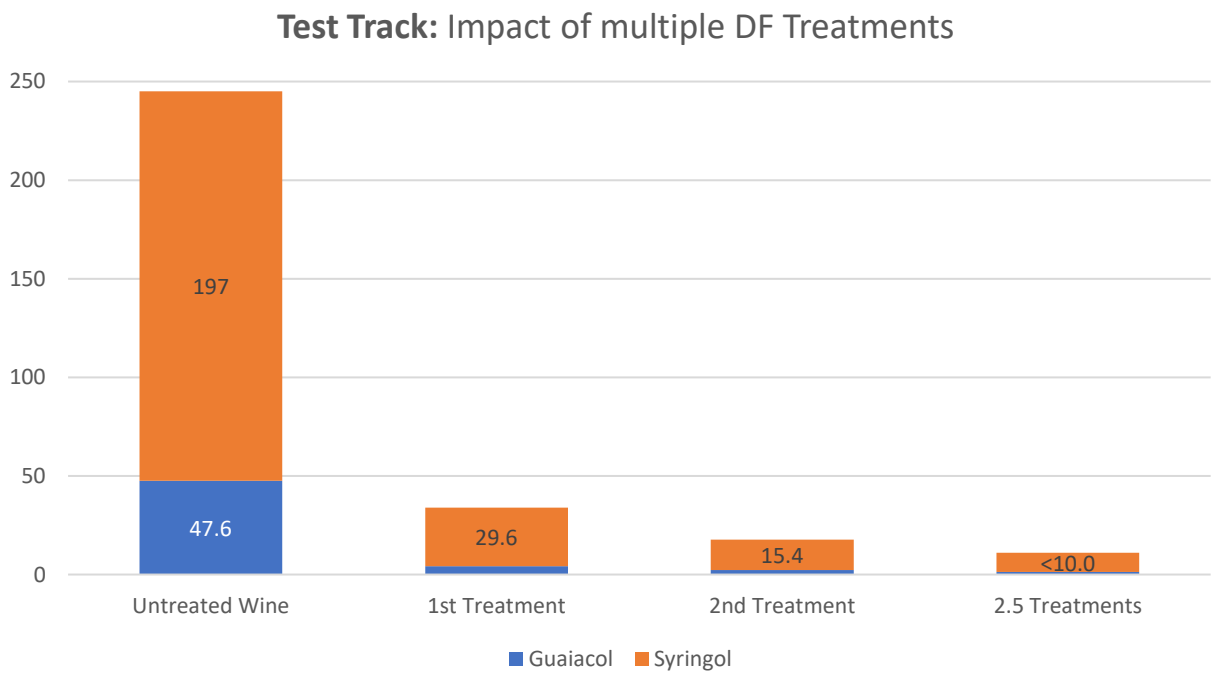
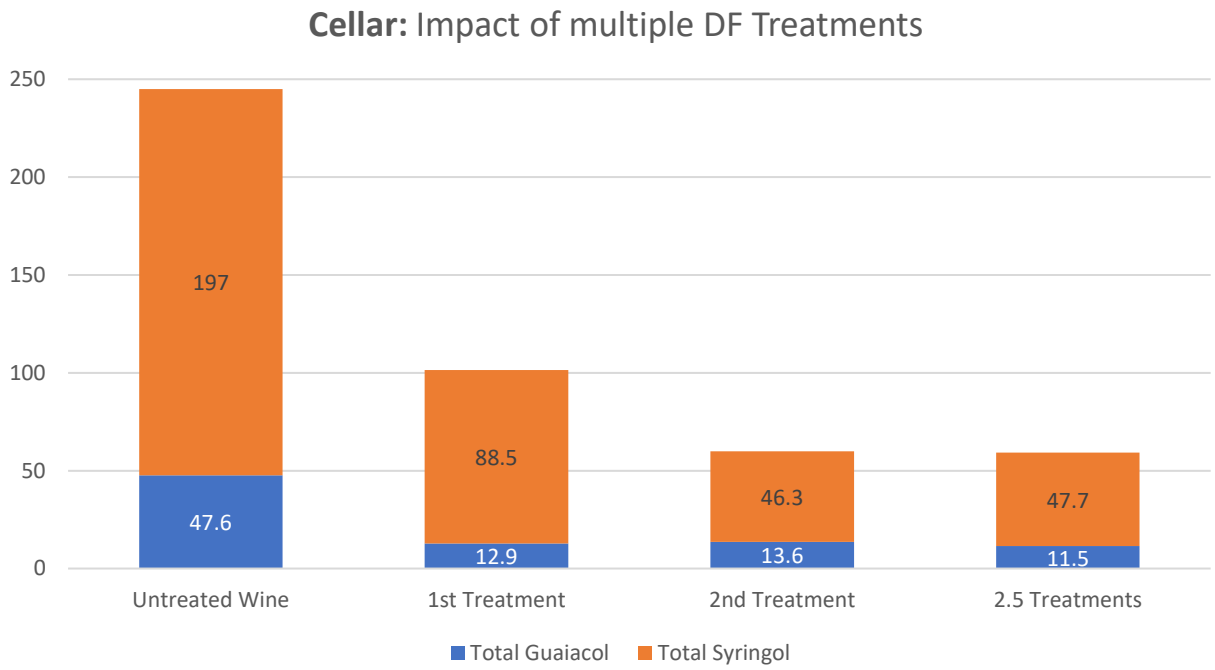


Table 6: Cellar treatment efficiency between treatments

Cellar	Total guaiacol (ppb)	% Decline	Total syringol (ppb)	% Decline
DFCellar-Initial	47.6	-	197	-
DFCellar-300-Initial	12.9	73.0%	88.5	55.2%
DFCellar-500-Initial	13.6	71.3%	46.3	76.6%
DFCellar-Final	11.5	75.9%	47.7	75.8%

Chart 8: This chart shows the decline in smoke taint markers over the course of five complete runs at Cellar scale.



Comparison of Analytical Methods

The above reported analyses were determined by Gas Chromatography Mass Spectrometry (GC-MS) for free volatile phenols. In order to determine total volatile phenol, glycosylated phenols were acid hydrolyzed, cleaving them from the glycoside and releasing them as free volatile phenols. The GC-MS was again run to determine the total volatile phenol content of a wine. Bound smoke markers were calculated as the difference between total and free volatile phenols.

Winesecrets stored initial and final Test Track Differential Filtration samples for future analysis. The advent of the Triple Quadrupole (QQQ) Liquid Chromatograph Mass Spectrometer (LC-MS) method allowed for direct

analysis of glycosylated smoke taint markers. In this case a separate laboratory analyzed the initial and finished wine for free volatile phenols via GC-MS and bound volatile phenols by LC-MS. Total smoke taint markers is calculated as the sum of free and bound species. A comparison of the results is available in Table 7.

Table 7: Comparison of Analytical Methods to Determine Bound Smoke Markers

Test Track	DFTT-Initial GC-MS/Hydrolysis	DFTT-Initial GC-MS/LC-MS	DFTT-Final GC-MS/Hydrolysis	DFTT-Final GC-MS/LC-MS
Free Guaiacol (ppb)	23.7	24.1	<1.0	<1.0
Bound Guaiacol (ppb)	23.9*	22.7	1.24*	<1.0
Total Guaiacol (ppb)	47.6	46.8†	1.35	<2.0†
Free Syringol (ppb)	81.4	74.0	<10.0	<10.0
Bound Syringol (ppb)	116*	148	<10.0*	7.2
Total Syringol (ppb)	197	222†	<10.0	<17.2†

***** value calculated – difference between total and free

† value calculated – sum of bound and free

Review of bound guaiacol results between the two methods indicated very close alignment. Bound Syringol results for treated wine are also relatively close differing by approximately 11%. Bound Syringol results for initial wine differ by 28%. Given the 12 months storage time for the samples before analysis by LC-MS, results in the same order of magnitude is acceptable and verifies the viability of bound marker determination by indirect analysis.

Conclusion

In summary, this feasibility trial shows great promise for removing smoke taint via Differential Filtration. Both bench and production scales of smoke taint removal using DF accomplished the primary goal of reducing the effects of smoke taint in wine below its sensory threshold. DF reduced total guaiacol by 91% and total syringol by 85% after the first run at the Test Track scale, and by 73% and 55% at the Cellar scale respectively. Successive runs have shown to achieve further reduction by the same order of magnitude but with diminishing returns as expected given the function of the technology.

DF also demonstrates its ability to remove the bound smoke taint markers by approximately 80% of their starting concentration measured in the untreated wine at the Test Track scale and by approximately 50% of their concentration at the cellar scale, reducing the perceived risk associated with aging and bottling smoke tainted wine.

It was noted that one source of variance in this experiment was that the membranes used on the Test Track were new, while the membranes used at the Cellar scale were at the end of their lifespan. This may have contributed to the superior performance demonstrated by the Test Track system. Further testing will repeat this application using smoke-tainted wines from various grape varietals and appellations to determine the consistency of this

application from one wine to another. Further studies will also observe how the condition of the membrane affects free and bound guaiacol and syringol rejection rates during RO and carbon treatment.

One of the greatest obstacles for this project was the extreme technical difficulty in accurately measuring the concentration of bound smoke taint species in a wine matrix. The difficulty and resultant cost precluded replication of these trials. Once commercial analysis of the bound species is more readily available, a more rapid pace of research and development is expected.

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