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Assessing the Performance of Film Coatings in Vineyard Smoke Exposure Studies: Findings and Experimental Limitations

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Abstract

Background and goals

Wildfire smoke exposure has become an increasing concern in vineyards worldwide and effective mitigation strategies have yet to enter the market. The aim of this study was to evaluate the barrier functionality of cellulose nanofiber (CNF) films on smoke-exposed grapes.

Methods and key findings

Pinot noir grapes were treated with CNF-based films by spray application and exposed to smoke in custom-made cages. After 6 hr of smoke exposure, half of the grapes within each film treatment were washed prior to vinification. Wine and grape samples were analyzed for volatile phenols by gas chromatography-tandem mass spectrometry and for phenol glycosides by liquid chromatography-tandem mass spectrometry. Film type significantly influenced both volatile phenol and phenol glycoside concentrations, while water treatment effects varied. However, responses were highly variable and effect sizes were small, suggesting limited practical impact on mitigation of smoke-derived compounds.

Conclusions and significance

Film coatings did not meaningfully reduce smoke-derived compounds under the conditions tested, likely due to experimental limitations that increased variability and compromised film performance. These findings are not definitive and indicate the need to evaluate CNF-based films under vineyard-representative smoke exposure conditions.

Key words: cellulose nanofibers, glycosides, guaiacol, Pinot noir, wine

Introduction

The wine industry is increasingly vulnerable to climate change, with drought and limited water access straining vineyards (van Leeuwen et al. 2024). Rising heat and more frequent wildfires have emerged as serious challenges for grapegrowers in particular (Daler 2024 and as reported by Cal Fire statistics: https://www.fire.ca.gov/our-impact/statistics), as smoke exposure can lead to off-flavors in wine that compromise quality and render entire vintages unsellable (McKay et al. 2019, Zakowski et al. 2023). In response, researchers have tested preventative strategies in vineyards to reduce the impact of smoke exposure before grapes enter the vinification process. These strategies include enclosing grape clusters with activated carbon fabric (Wilkinson et al. 2022) and applying treatments such as kaolin sprays (van der Hulst et al. 2019).

Preventative strategies seek to reduce the concentrations of volatile phenols (VPs), which are the compounds partially responsible for smoke taint in wine. VPs form when vegetation burns and lignin undergoes pyrolysis (Lu and Gu 2022); once absorbed by grapes, VPs can produce harsh smoky or ashy flavors (Parker et al. 2012, Fryer and Tomasino 2022). These undesirable smoky characteristics and the limited information on compound concentrations that cause issues have led to conflicts between grapegrowers and wineries. In 2018, a significant number of grape contracts were canceled due to potential smoke exposure (as reported at https://www.sfchronicle.com/wine/article/Wineries-vineyards-clash-over-how-to-handle-13285829.php). In other cases, an estimated 165,000 to 325,000 tons of grapes were left unharvested over concerns about compromised quality (as reported at https://www.winebusiness.com/news/article/249148).

To minimize losses, researchers have tested different barriers to protect grapes from smoke exposure. In one study, woven activated carbon bags were used to enclose whole grape clusters prior to smoke exposure (Wilkinson et al. 2022). The coverings successfully reduced cluster uptake of VPs by 80 to 90%. Despite their effectiveness, activated carbon bags are

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not a viable large-scale solution due to the time and labor required to apply them before wildfire smoke reaches the vineyard. A more feasible approach involves spraying grapes with a protective coating. One study found that applying kaolin to Merlot fruit before smoke exposure reduced VP levels, though its effectiveness depended on coverage quality and application rate (van der Hulst et al. 2019). Similarly, a different study showed that while kaolin can trap VPs, it must be thoroughly washed off after harvest to avoid releasing the VPs into the wine (as reported at https://www.winebusiness.com/news/article/297561).

A promising film spray showed no adverse effects on grape development, and laboratory film studies indicated a reduction in VP uptake into grape tissues (Tran et al. 2023a). Initial work evaluated films composed of cellulose nanofibers, β-cyclodextrin, and chitosan, with chitosan exhibiting the greatest efficacy (Garcia et al. 2026). These results prompted a subsequent study incorporating chitosan with low methoxyl pectin (LMP) (Tran et al. 2023b). However, while VP exposure was conducted under controlled laboratory conditions, evaluation under vineyard smoke exposure has been limited, in part because natural smoke events failed to deliver exposure sufficient to assess film efficacy (Tran et al. 2023a, 2023b). Consequently, enclosed systems have been adopted as an effective approach for simulating wildfire smoke exposure under controlled conditions and generating smoke uptake levels suitable for evaluating mitigation strategies (Kennison et al. 2007, Noestheden et al. 2017).

The primary objective of this study was to evaluate the efficacy of the films used by Tran et al. (2023b) under heavy vineyard smoke exposure conditions. The necessity of film removal by washing prior to winemaking was also assessed. Film formulations were tested in smoke-controlled cages, as no natural smoke events occurred at the research location during the years of study. Smoke-exposed grapes were made into wine and both grapes and wines were analyzed for VPs and phenol glycosides (PG), using gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), respectively. Based on prior research, film coatings are hypothesized to reduce concentrations of smoke-derived phenols in Pinot noir wines. If effective, these film coatings could serve as a preventative viticulture strategy to maintain fruit quality during wildfire events and reduce grape rejection that results from elevated levels of smoke-derived compounds.

Materials and Methods

Chemicals

Chemicals used to make the coating matrix included 2.95% w/w cellulose nanofiber (CNF) with -8.8 ± 1.6 mV zeta potential (University of Maine Processing Development Center), Trappsol food-grade β-cyclodextrin (CTD Inc.), 97% degree of deacetylated chitosan (Primex ehf), glycerol (Thermo Fisher Scientific), potassium sorbate (Thermo Fisher Scientific), glacial acetic acid (Avantor Inc), Span 80 (VWR Chemicals), Tween 80 (VWR Chemicals), and 98% anhydrous magnesium chloride (VWR Chemicals). VPs and their isotope standards were purchased from Sigma-Aldrich and CDN Isotopes, respectively. VP glyconjugates and the internal standard, salicin (≥99%), were purchased from Toronto Research and Sigma-Aldrich, respectively. Isotope-labeled internal standards are generally preferred for glycoside quantification because of their closer chromatographic and matrix-matched behavior with target analytes; however, isotope-labeled standards for PGs were not commercially available at the time of analysis. Salicin was therefore selected as a practical, commercially accessible internal standard alternative (Caffrey et al. 2019). All standard and internal standard information can be found in Supplemental Tables 1 and 2.

Vineyard site and plant material

Vineyard experiments were conducted at Oregon State University's (OSU) Woodhall II Vineyard in Monroe, OR. The vineyard is located on a south-facing slope (elevation range 192 to 229 m asl), with vine rows orientated on a north-south axis. Grapevines were planted in 2006 with 2.1-m row spacing and 1.4-m vine spacing. Pinot noir vines (clone Pommard) were grafted onto 101-14 rootstock, dry-farmed, cane-pruned on a double-Guyot system, and trained onto a vertical shoot-positioned trellis with movable catch wires. For experiment 1, grapes were manually harvested at ~20 Brix on 16 Sept 2021 and combined before they were used in smoke trial 1. Experiment 2 evaluated films in the field, and grapes were harvested at ~21 Brix after smoke trial 2 on 7 Oct 2022.

Experiment 1

Film preparation

The four film formulations investigated were prepared as described (Tran et al. 2023a). Coating suspensions were prepared using 0.5% CNF as the base and consisted of: (1) CNF alone, (2) CNF with 0.5% β-cyclodextrin (BCD), (3) CNF with 1% chitosan in 1% acetic acid (CHI), and (4) CNF with both 0.5% β-cyclodextrin and 1% chitosan (MIX). All suspensions included 0.1% glycerol and 0.2% surfactant (Tween/Span 80, 1:1) to enhance flexibility and adhesion.

Smoke trial 1

One day prior to smoke exposure, clusters (n = 18 per treatment) were hung on rods and treated with experimental films using a 467-g HVLP gravity feed air spray gun (Central Pneumatic). The grapes were sprayed until visually covered, then left to dry for ~5 min. Afterward, the treated grape clusters were removed from the rods and stored overnight in buckets at 4°C.

Smoke trials were held on 20 and 21 Sept 2021. Smoke exposure was conducted using purpose-built cages as described (Tomasino et al. 2023), with a few alterations. Two cages were used each smoke trial day, for a total of four replicates. Cage size differed from the original design, with small enclosures (0.91 × 0.61 × 1.4 m; length × width × height)

constructed using 15-mm nominal size, schedule 40 PVC pipes. The cages were also divided into horizontal sections, with each cage containing five rows and one film treatment assigned per row (Supplemental Figure 1). To eliminate potential bias due to placement within the smoke enclosure, the position of each treatment group was rotated for each replicate. Grape clusters were suspended within each chamber using 38.1-mm 24-gauge galvanized steel wire and 3.17 × 25.4-mm stainless steel S-hooks. Every 30 min for 6 hr, 5 g of barley was ignited inside a 35.6-cm Smokey Joe charcoal grill (Model #10020, Weber). The smoke was distributed throughout the chamber using a Multifan S3-P, AC-powered fan (AC Infinity) positioned at the bottom of the cage. A SidePak AM520 personal aerosol monitor (TSI Incorporated) was used to monitor the smoke particulate concentration, which was maintained at 20 to 50 mg/m³ for particulate matter (PM_1) with a size ≤1.0 µm.

Winemaking

After smoke exposure, each treatment was stored overnight at 4°C in separate 13.2-L buckets (Uline). Each treatment group had ~3.4 kg of grape clusters. Before destemming, half of the grapes for each treatment were gently sprayed in harvest containers with hose water for 30 sec to remove the film coating. Destemmed grapes were crushed then transferred into 3.8-L glass jars with polyethylene-lined caps (Uline) and plastic punch-down discs (Supplemental Figure 2). Prior to alcoholic fermentation, Fermaid K (Lallemand Oenology) was added at a rate of 0.25 g/L, along with 0.115 g/L of diammonium phosphate (Sigma-Aldrich), to reach a yeast assimilable nitrogen of 150 mg/L. The must was inoculated with *Saccharomyces cerevisiae* (Levuline BRG) at a rate of 0.25 g/L. Fermentations were kept at room temperature and punched down once a day. Musts were monitored for residual sugars using a DMA 35 portable density meter (Anton Paar). After fermentations reached dryness (<0.1 g/L), musts were pressed with an applied constant pressure of 0.28 MPa for 5 min using a pneumatic wine press equipped with a VH-SRR cylinder valve pump (Allenair). Potassium metabisulfite (40 mg/L) was added to the pressed wine, which was then placed in a cold room (4°C) to settle for 48 hr. After settling, the wines were racked off lees into high-density polyethylene plastic juice bottles (Uline) and stored at -15°C until further analysis.

Experiment 2

Film preparation

Based on preliminary findings and results from experiment 1, new film formulations were developed for vineyard testing. Films were prepared as described (Tran et al. 2023b). The three film suspensions used for this experiment included CNF with LMP, CNF with chitosan at a 1:2 ratio (CH2), and CNF with chitosan at a 1:4 ratio (CH4).

Smoke trial 2

On 5 Sept 2022, grape clusters were visually covered with liquid film suspensions using a 16-L Jacto PJB-16c

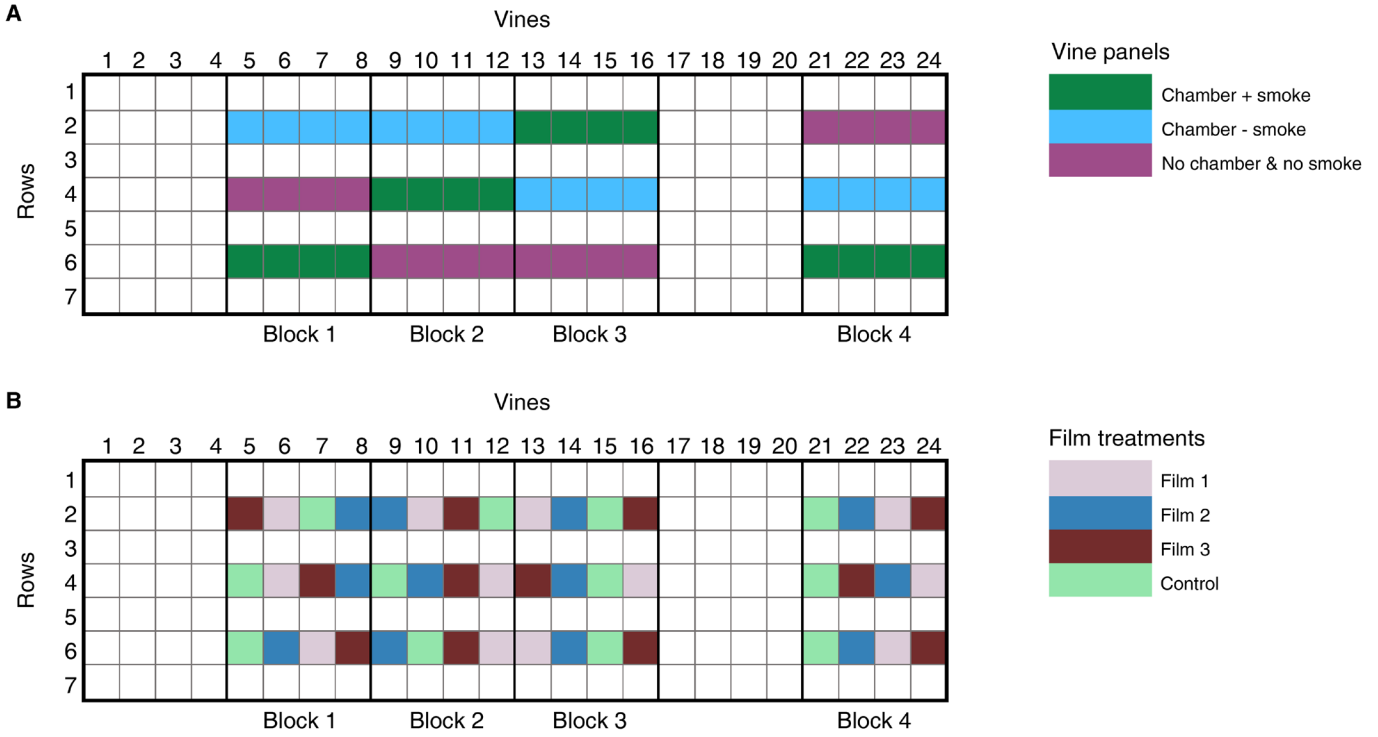

Figure 1 Experiment 2 vineyard field layout of treatment allocation in a randomized complete block design. **A**) Vines enclosed in cages along with exposure to smoke or no-smoke control conditions; **B**) allocation of film treatments (Film 1, cellulose nanofiber [CNF] with low methoxyl pectin [LMP]; Film 2, CNF with chitosan at a 1:2 ratio [CH2]; Film 3, CNF with chitosan at a 1:4 ratio [CH4]; Control, a no-film control) applied to vines within each block.

backpack sprayer (Grupo Jacto). Suspensions were homogenized (Polytron PT10/35 GT Homogenizer, Kinematica) at 10,000 rpm for 3 min before application. The three film treatments were applied to grape clusters in a randomized complete block design with four replicates (Figure 1). Each replicated block consisted of three rows, with four-vine panels per row. Panels within each block were assigned randomly to a smoked or unsmoked treatment. The no-film control and three film treatments were randomized within each panel.

Smoke trials were held 2 wk postveraison on 12 to 15 Sept 2022. Grapevine smoke exposure followed previously described methods (Tomasino et al. 2023), using a longer cage. Four grapevines were enclosed within a 5.5 × 1.4 × 2.2-m (length × width × height) chamber constructed from 40-mm PVC pipes (Supplemental Figure 3). Each chamber contained two 40V 35.6-cm hybrid air cannon fans (Ryobi) to circulate smoke evenly throughout the enclosure. Smoke exposure was conducted on four separate days, taking place in the evening to minimize internal cage temperatures. As in smoke trial 1, smoke was generated every 30 min for 6 hr and maintained at 20 to 50 mg/m^3 for PM_1. However, 10 g of barley was used per smoke generation event, consistent with described parameters (Tomasino et al. 2023).

Winemaking

After harvest, half of the grapes for each treatment were gently shaken in a bucket with water to remove the film coating. One grape cluster was set aside from each treatment group for basic grape chemistry analysis. The remaining grape clusters were destemmed and crushed in 7.6-L Ziploc bags. Approximately 350 g of must per treatment was transferred into separate 600-mL mason jars (Ball Corporation) (Supplemental Figure 4) for microvinification (Sampaio et al. 2007). Prior to inoculation, a 10-mL sample of grape juice was collected from each treatment to determine residual sugar using a DMA 35 density meter. After the musts were inoculated with Lalvin EC118 yeast (Danstar Ferment AG) at 0.3 g/L, the mason jars were placed in a 31°C water bath. Fermentations were pressed at dryness (<0.1 g/L) with a potato ricer (Priority Chef) then stored in 50-mL Falcon tubes (VWR International) at -20°C until further analysis.

Basic chemical analysis

Grape clusters were destemmed and homogenized using a 600-W personal blender (Nutribullet). The homogenate (HMG) was poured into 50-mL Falcon tubes and stored at -10°C until analyzed. Prior to analysis, HMG and wine samples were thawed overnight at 4°C. Five mL of sample was centrifuged at 5000 rpm for 5 min using an Allegra X-22 centrifuge (Beckman Coulter), after which the supernatant was decanted through a fine mesh strainer. This centrifugation and decanting step was repeated three times. The final supernatant was then filtered through a Buchner funnel fitted with Whatman grade 602 H ½ qualitative filter paper (Cytivia). The resulting clarified grape juice and wine were stored at 4°C and analyzed the same day.

Both grape juice and wine samples (750 mL) were analyzed for the following using an automatic oenological analyzer (SPICA, Biosystems) and their corresponding reagent kits (Biosystems): primary amine nitrogen (code 21807), acetic acid (code 23930), malic acid (code 23803), free sulfite (code 21813), polyphenols (code 12815), color (code 12816), and glucose/fructose (code 23800). Samples were also measured for titratable acidity (TA) using 0.1 M sodium hydroxide (NaOH) and the pH titration method (Iland et al. 2013).

Smoke compound analysis

VP sample preparation

Adapted liquid-liquid extraction methods (Liu et al. 2020) were used to isolate volatile compounds from grape juice and wine samples. Briefly, 1 mL of ethyl acetate:hexane (1:1, v/v) containing internal standard was added to 1 mL of sample and vortexed for 30 sec. An aliquot of the upper organic layer was transferred to a 2-mL amber autosampler vial (Microsolv). Vials were stored at -20°C until GC-MS/MS analysis.

PG sample preparation

Grape HMG and wine samples for LC-MS/MS analyses were clarified similarly to basic chemical analysis samples, with the only difference being that samples were centrifuged for 10 min. Clarified grape juice and wine samples were prepared as described (Caffrey et al. 2019), with a few modifications. Briefly, 50 µL of 5-mg/L salicin standard were added to the sample and vortexed for 30 sec. Solid-phase extraction (SPE) was performed using a Resprep QR-24 vacuum manifold (Restek) equipped with polytetrafluoroethylene liners (Restek). Reverse-phase 3-mL cartridges (Strata-X, Phenomenex) were conditioned with 2 mL of acetonitrile and 2 mL of deionized (DI) water. After conditioning, 1 mL of sample was loaded into the cartridge then washed with 1 mL of 0.1 M NaOH and 2 mL of DI water. The PGs were eluted with 2 mL of 40% acetonitrile. A 24-position N-EVAP nitrogen evaporation system (Organomation Associates) was used to dry the extracted sample, with a constant flow rate of 10 L/min at room temperature. The sample residue was reconstituted with 1 mL of MilliQ water, vortexed for 30 sec, and stored in 2-mL amber vials at -20°C until analysis.

VP analysis

Wine and grape samples were sent to the Smoke, Wine, and Grapes (SWAG) Analytical Chemistry Lab at OSU for free phenol analysis. VPs were measured using an 8890 gas chromatograph equipped with a 7000D triple quadrupole mass spectrometer (Agilent Technologies). Compound separation was achieved using an Agilent DB-HeavyWax column (30 m × 0.25 mm × 0.25 µm). Liquid injections were conducted by a PAL RTC 120 autosampler. Previously described GC-MS/MS parameters were used (Caballero et al. 2025). The Agilent MassHunter Quantitative Analysis software (ver. 12) was used for quantitative analysis of VPs. Ion transitions and collision energies used for quantification are listed (Supplemental Table 1).

PG analysis

Grape and wine samples were sent to the SWAG chemistry lab for bound phenol analysis. A 1290 Infinity II liquid chromatography (LC) instrument equipped with an Ultivo triple quadrupole mass spectrometer (Agilent) was used. Compound separation was achieved using an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl (2.1 × 100 mm, 2.7-µm thickness) column. LC-MS/MS parameters were as described (Caballero et al. 2025). Quantification of compounds was performed using the Agilent MassHunter Workstation Quantitative Analysis for QQQ (ver. 10) software. A table with the dynamic multiple reaction monitoring (dMRM) transitions and collision energies for each compound is included (Supplemental Table 2).

Statistical analysis

All statistical analyses were performed in RStudio software (ver. 4.5.1). Two-way multivariate analysis of variance (MANOVA) was conducted to examine the interactive effect of film and wash treatment. The response variables were six VPs and five PGs for the analysis of free and bound phenols, respectively. Assumptions for MANOVA were evaluated prior to analysis. Univariate and multivariate normality were assessed using Shapiro-Wilk tests and Mardia's Skewness and Kurtosis test, respectively. Homogeneity of covariance was assessed using Box's M test and inspection of outliers, using Mahalanobis distance. Because Pillai's Trace is more robust to assumption violations, this test statistic was used to interpret the MANOVA at $\alpha = 0.05$ to test for significance. Partial eta squared (η_p^2) was calculated to estimate the effect size of each independent variable, with values closer to 1 indicating a stronger effect. This measure helps assess the practical significance of the film and wash treatment impact on smoke-derived compounds. Differences in basic chemical composition and chromatic properties were tested using two-way ANOVA, followed by the post-hoc Tukey test for mean comparisons when main effects were significant ($\alpha = 0.05$).

Results

Smoke Trial 1

A two-way MANOVA was conducted to evaluate the combined effects of film type and wash treatment on concentrations of smoke-derived compounds in wines produced from smoke-exposed grapes. An unsmoked control was not included in this trial; therefore, comparisons for Smoke Trial 1 were made among smoke-exposed treatments only. Prior to analysis, the assumption checks indicated the normality assumption was not fully met. Therefore, Pillai's trace was used as the primary MANOVA test statistic and results were interpreted with caution.

Multivariate test results demonstrated significant main effects of film type ($p = 0.02$) and wash treatment ($p < 0.01$) on VP concentrations (Table 1). However, their interaction was not statistically significant ($p = 0.8$). Among wines produced from washed grapes, guaiacol concentrations were greater in all film treatments relative to the no-film control, with the highest concentration of 320 µg/L observed from BCD (Table 2). Similar patterns were observed for 4-methylguaiacol (4MG) and cresols. Concentrations of 4MG were highest in MIX (15.8 µg/L) relative to the control (10.9 µg/L), while *o*-cresol increased from 16.2 µg/L in control wines to 23.4 µg/L in CHI. Syringol was the most abundant VP across all treatments and ranged from 584 µg/L in BCD to 928 µg/L in MIX. In wines produced from unwashed grapes, guaiacol concentrations were greatest in BCD (278 µg/L), while *o*-cresol and *p/m*-cresol were highest in MIX (25.1 and 28.8 µg/L, respectively). Total VP concentrations were generally greater in wines produced from washed grapes than from unwashed grapes.

Similar multivariate patterns were observed for PGs, for which significant main effects of film type ($p < 0.01$) and wash treatment ($p < 0.001$) were detected, but no significant interaction ($p = 0.16$) (Table 1). In washed samples, BCD exhibited higher concentrations of all measured PGs relative to the control, including guaiacol rutinoside (0.52 mg/L) and 4-methylsyringol (4MS) gentiobioside (3.33 mg/L) (Table 2). Other film treatments (CNF, CHI, and MIX) produced concentrations similar to or slightly greater than those of control wines. Among unwashed grapes, PG concentrations were generally comparable across treatments, although MIX showed a modest increase in 4MS gentiobioside (2.77 mg/L) relative to the control (1.69 mg/L). Washed samples resulted in higher total PG concentrations for CNF, BCD, and MIX films compared to unwashed samples. Despite statistically significant effects on both VPs and PGs, η_p^2 values indicated small effect sizes (<0.2) for film, wash treatment, and their interaction (Table 1).

Results from two-way ANOVAs showed basic wine chemistry parameters did not differ significantly, with the exception of total polyphenols. Washed CHI samples exhibited significantly lower polyphenol concentrations (729 mg/L) than unwashed BCD samples (1490 mg/L) (Supplemental Table 3). Comparisons between wash treatments further revealed significant effects on primary amino nitrogen (PAN) and polyphenol concentrations ($p < 0.001$), both of which were lower in washed samples. For instance, PAN concentrations were below 20 mg/L in washed samples and above 35 mg/L in unwashed samples. When comparing chromatic properties, significant treatment effects were not observed, although absorbance and color intensity tended to be greatest in BCD and lowest in CHI (Supplemental Table 4).

Smoke Trial 2

For both grape and wine samples, statistical analyses showed clear differences in smoke-derived compound concentrations between smoked and unsmoked samples (data not shown). Additionally, multivariate assumption checks showed evidence of deviation from normality. Therefore, MANOVA results were interpreted with appropriate caution using Pillai's trace, as it is more robust to assumption violations.

Within smoked grape samples, multivariate analyses revealed significant main effects of film type ($p < 0.001$), wash treatment ($p < 0.01$), and their interaction ($p < 0.01$) on VP concentrations (Supplemental Table 5). For washed grapes, control samples had the highest *o*- and *p*-cresol concentrations (2.63 and 7.68 µg/L, respectively), whereas CH4 showed higher concentrations for most other VPs (Supplemental Table 6). In unwashed grapes, CH2 consistently resulted in the lowest concentrations for several VPs, while *p*-cresol was highest in LMP (8.02 µg/L).

There were significant effects observed for film ($p < 0.001$) and the film × wash interaction for PG concentrations ($p < 0.05$) (Supplemental Table 5). Control samples from washed grapes exhibited higher concentrations of guaiacol gentiobioside (0.94 µg/L) and guaiacol rutinoside (15.3 µg/L) than film-treated grapes (Supplemental Table 6). Among film treatments, LMP generally resulted in the lowest PG concentrations. For unwashed grapes, PG concentrations were highest in control samples, with 4MS gentiobioside as the predominant compound (17.1 µg/L). As observed for washed grapes, LMP generally showed the lowest PG concentrations. Although significant treatment effects were detected, η_p^2 values (listed in Supplemental Table 5) indicate small effect sizes for main factors and their interaction, with the exception of a moderate effect of film type on VP concentrations ($\eta_p^2 = 0.4$).

MANOVA analyses for wines showed film type ($p < 0.05$) and film × wash interaction ($p < 0.01$) significantly affected both VP and PG concentrations (Table 3). Clear differences in smoke-derived compound concentrations were observed between wines produced from smoked and unsmoked samples (data not shown). Within smoked washed samples, guaiacol concentrations were highest in control samples (22.6 µg/L), whereas CH2 wines showed elevated concentrations of cresols and syringol (Table 4). In unwashed samples, CH4 wines contained the highest concentrations of 4MG (1.92 µg/L), *m*/*p*-cresol (3.21 µg/L), and syringol (9.47 µg/L), while LMP generally exhibited lower cresol and 4MS concentrations. For PGs, washed control samples had the highest concentrations

Table 1 Multivariate analysis of variance results for experiment 1 wines, showing the effects of film type, washing treatment, and their interaction on smoke-derived compounds. Reported statistics include Pillai's trace, associated *p* values (α = 0.05), and partial eta squared (η_p^2) as a measure of effect size.

	Volatile phenols			Phenol glycosides		
Effect	**Pillai's trace**	***p***	**η_p^2**	**Pillai's trace**	***p***	**η_p^2**
Film	0.554	0.023	0.139	0.51516	0.008	0.132
Wash	0.245	0.007	0.245	0.29507	<0.001	0.295
Film × wash	0.261	0.804	0.065	0.35956	0.163	0.090

Table 2 Concentrations (µg/L) of volatile phenols (**A**) and phenol glycosides (**B**) in smoke-affected wines from experiment 1. Wines were produced from washed or non-washed Pinot noir grapes subjected to film treatments (CNF, 0.5% cellulose nanofiber; BCD, CNF with 0.5% β-cyclodextrin; CHI, CNF with 1% chitosan in 1% acetic acid; MIX, CNF with both 0.5% β-cyclodextrin and 1% chitosan) and a no-film control. Values are reported as mean ± standard deviation. 4MG, 4-methylguaiacol; 4MS, 4-methylsyringol.

		Compound	Control	CNF	BCD	CHI	MIX
A	Washed	Guaiacol	261 ± 22.5	263 ± 22.4	320 ± 19.0	299 ± 47.0	315 ± 134
		4MG	10.9 ± 3.00	11.2 ± 2.90	14.2 ± 2.30	14.1 ± 5.50	15.8 ± 10.2
		o-Cresol	16.2 ± 7.40	17.5 ± 4.00	18.2 ± 3.40	23.4 ± 12.5	22.8 ± 14.3
		p/*m*-Cresol	21.7 ± 5.70	20.2 ± 4.20	23.0 ± 3.70	24.0 ± 8.80	24.1 ± 12.1
		Syringol	678 ± 450	785 ± 605	584 ± 457	691 ± 440	928 ± 869
		4MS	24.1 ± 16.0	22.8 ± 23.3	21.9 ± 13.4	17.8 ± 9.30	27.6 ± 24.1
	Non-washed	Guaiacol	251 ± 33.9	263 ± 71.7	278 ± 26.6	257 ± 90.2	231 ± 92.1
		4MG	10.9 ± 3.60	13.0 ± 5.10	16.9 ± 4.70	12.6 ± 4.50	13.0 ± 7.70
		o-Cresol	16.8 ± 5.20	19.7 ± 4.30	24.2 ± 3.30	23.4 ± 11.7	25.1 ± 12.4
		p/*m*-Cresol	21.6 ± 5.40	22.6 ± 5.60	27.9 ± 2.60	23.4 ± 8.30	28.8 ± 13.0
		Syringol	636 ± 322	309 ± 133	516 ± 204	330 ± 133	618 ± 226
		4MS	24.4 ± 14.0	13.8 ± 10.4	29.9 ± 12.9	12.9 ± 7.40	22.0 ± 13.6
B	Washed	Guaiacol gentiobioside	0.09 ± 0.02	0.12 ± 0.08	0.17 ± 0.14	0.13 ± 0.08	0.11 ± 0.05
		Phenol rutinoside	0.31 ± 0.05	0.35 ± 0.15	0.67 ± 0.50	0.36 ± 0.12	0.36 ± 0.09
		Guaiacol rutinoside	0.17 ± 0.05	0.22 ± 0.17	0.52 ± 0.50	0.26 ± 0.12	0.26 ± 0.11
		4MS gentiobioside	1.63 ± 0.53	1.46 ± 0.35	3.33 ± 3.03	1.45 ± 0.26	1.47 ± 0.22
		p-Cresol rutinoside	0.08 ± 0.03	0.09 ± 0.04	0.22 ± 0.23	0.12 ± 0.01	0.09 ± 0.05
	Non-washed	Guaiacol gentiobioside	0.09 ± 0.03	0.11 ± 0.06	0.09 ± 0.02	0.09 ± 0.04	0.12 ± 0.08
		Phenol rutinoside	0.34 ± 0.12	0.33 ± 0.10	0.35 ± 0.13	0.30 ± 0.12	0.44 ± 0.27
		Guaiacol rutinoside	0.16 ± 0.08	0.19 ± 0.11	0.20 ± 0.06	0.17 ± 0.07	0.26 ± 0.20
		4MS gentiobioside	1.69 ± 0.42	1.47 ± 0.17	1.27 ± 0.09	1.28 ± 0.44	2.77 ± 2.39
		p-Cresol rutinoside	0.11 ± 0.06	0.11 ± 0.03	0.10 ± 0.02	0.10 ± 0.05	0.14 ± 0.09

of guaiacol glycosides and *p*-cresol rutinoside, whereas chitosan films exhibited the lowest PG concentrations. In contrast, unwashed samples treated with chitosan films showed higher concentrations, with the exception of guaiacol gentiobioside. Although significant differences were observed, effect sizes remained small ($\eta_p^2 < 0.2$) (Table 3).

Significant differences in basic wine chemistry were limited to TA, with the greatest difference observed between CH4 (5.83 μg/L) and LMP (4.92 μg/L) in unwashed samples (Supplemental Table 3). Chromatic measurements showed higher absorbance and color intensity in film-treated samples compared to control wines, with chitosan films generally exhibiting the highest values (Supplemental Table 4).

Discussion

This study evaluated whether the application of protective films could reduce concentrations of smoke-derived compounds in wines made from smoke-exposed grapes. Responses differed between the two smoke trials, precluding a definitive conclusion regarding film efficacy. In Smoke Trial 1, film type significantly affected both VP and PG concentrations, but the observed response was contrary to expectations. Wines produced from no-film control grapes generally exhibited VP concentrations that were comparable to or lower than those from film-covered grapes, regardless of wash treatment. For instance, *o*-cresol concentrations in both washed and unwashed control wines were below 17 μg/L, whereas wines made from film-covered grapes exceeded this concentration (Table 2). In contrast, Smoke Trial 2 showed inconsistent differences across treatments, with control wines exhibiting lower concentrations only for syringol, while levels of PGs were similar to or higher than those in film-treated wines (Table 4).

Although unexpected, similar outcomes have been reported for other surface-applied preventative strategies. Studies evaluating polymer-based anti-transpirant and oil-based horticulture sprays have documented equal or greater concentrations

Table 3 Multivariate analysis of variance results for experiment 2 wines, showing the effects of film type, washing treatment, and their interaction on smoke-derived compounds. Reported statistics include Pillai's trace, associated *p* values (α = 0.05), and partial eta squared (η_p^2) as a measure of effect size.

	Volatile phenols			Phenol glycosides		
Effect	**Pillai's trace**	***p***	**η_p^2**	**Pillai's trace**	***p***	**η_p^2**
Film	0.507	<0.001	0.169	0.117	0.019	0.040
Wash	0.018	0.596	0.018	0.037	0.110	0.037
Film × wash	0.168	0.001	0.056	0.132	0.006	0.044

Table 4 Concentrations (μg/L) of volatile phenols (**A**) and phenol glycosides (**B**) in smoke-affected wines from experiment 2. Wines were produced from washed or non-washed Pinot noir grapes subjected to film treatments (CH4, cellulose nanofiber (CNF) with chitosan at a 1:4 ratio; CH2, CNF with chitosan at a 1:2 ratio; LMP, low methoxyl pectin) and a no-film control. Values are reported as mean ± standard deviation. 4MG, 4-methylguaiacol; 4MS, 4-methylsyringol.

		Compound	Control	CH4	CH2	LMP
A	Washed	Guaiacol	22.6 ± 14.9	19.1 ± 16.8	16.1 ± 13.7	16.5 ± 9.67
		4MG	0.26 ± 0.08	1.17 ± 2.90	0.30 ± 0.20	0.22 ± 0.12
		o-Cresol	0.22 ± 0.30	0.40 ± 0.40	1.07 ± 0.18	0.47 ± 0.47
		p/*m*-Cresol	0.91 ± 0.33	2.74 ± 3.09	2.93 ± 2.88	1.55 ± 2.11
		Syringol	2.86 ± 0.89	5.39 ± 4.23	10.9 ± 6.12	7.09 ± 6.01
		4MS	0.37 ± 0.52	0.49 ± 0.52	0.48 ± 1.03	0.23 ± 0.42
	Non-washed	Guaiacol	22.8 ± 16.5	14.7 ± 10.8	8.97 ± 3.81	21.3 ± 10.7
		4MG	1.26 ± 3.24	1.92 ± 3.79	0.15 ± 0.11	0.27 ± 0.17
		o-Cresol	0.51 ± 0.61	0.67 ± 0.61	0.85 ± 0.31	0.36 ± 0.64
		p/*m*-Cresol	2.22 ± 2.22	3.21 ± 3.68	1.83 ± 0.96	1.28 ± 1.64
		Syringol	5.08 ± 5.63	9.47 ± 11.7	8.69 ± 4.22	5.26 ± 5.92
		4MS	0.24 ± 0.21	0.44 ± 0.39	0.63 ± 1.54	0.14 ± 0.15
B	Washed	Guaiacol gentiobioside	0.37 ± 0.14	0.15 ± 0.08	0.27 ± 0.46	0.20 ± 0.13
		Phenol rutinoside	0.25 ± 0.33	0.11 ± 0.09	0.10 ± 0.06	0.26 ± 0.31
		Guaiacol rutinoside	0.32 ± 0.12	0.10 ± 0.02	0.05 ± 0.03	0.21 ± 0.19
		4MS gentiobioside	0.71 ± 0.46	0.43 ± 0.23	0.49 ± 0.31	1.21 ± 2.22
		p-Cresol rutinoside	0.15 ± 0.17	0.06 ± 0.04	0.07 ± 0.04	0.13 ± 0.23
	Non-washed	Guaiacol gentiobioside	0.15 ± 0.15	0.20 ± 0.17	0.19 ± 0.16	0.27 ± 0.30
		Phenol rutinoside	0.27 ± 0.32	0.27 ± 0.31	0.29 ± 0.31	0.16 ± 0.27
		Guaiacol rutinoside	0.18 ± 0.16	0.26 ± 0.21	0.18 ± 0.16	0.11 ± 0.09
		4MS gentiobioside	0.74 ± 0.60	0.80 ± 0.69	0.89 ± 0.80	0.52 ± 0.52
		p-Cresol rutinoside	0.15 ± 0.18	0.15 ± 0.20	0.15 ± 0.18	0.10 ± 0.10

of VPs and their glycosides in treated grapes compared to untreated controls (Favell et al. 2019, Szeto et al. 2020, Culbert et al. 2021). Kaolin-based products have likewise produced variable responses, with one study reporting modest reductions for a limited number of VPs, while another observed an 80% reduction in guaiacol glyconjugates in specific cultivars (van der Hulst et al. 2019, Szeto et al. 2022). Collectively, these findings highlight the challenge of achieving sufficient barrier protection without inadvertently retaining or concentrating smoke-derived compounds within the grape.

A second objective of this study was to determine whether leaving films on grapes during processing increased smoke taint in the resulting wine. To evaluate the potential release of film-bound VPs during winemaking, grapes were either washed prior to fermentation or left unwashed. Similar to film efficacy, washing effects differed between smoke exposure trials, preventing a definitive conclusion regarding film removal prior to processing. MANOVA results from Smoke Trial 1 indicated a significant main effect of washing on VPs and PGs, but no significant washing effects were observed in Smoke Trial 2 (Tables 1 and 3). The inconsistent response between smoke trials may partially reflect differences in washing methodology. In Smoke Trial 1, grapes received a more vigorous washing treatment via hose spraying, which may have enhanced mechanical removal but resulted in less uniform detachment of the film. In contrast, immersion in water likely facilitated more uniform film removal across berries in Smoke Trial 2, potentially contributing to the absence of significant differences between wash treatments. This interpretation would suggest that film removal before fermentation does not substantially affect VP and PG concentrations in wine, which would be consistent with prior work examining post-smoke grape rinsing. For example, washing smoke-exposed grapes with water, water with 5% ethanol, or milk did not significantly alter guaiacol concentrations in the resulting wine (AWRI 2003).

Although the combined results across smoke exposure trials were inconclusive, the small effect sizes observed in this study suggest that neither CNF-based film type nor grape washing is likely to provide a practically meaningful reduction in smoke-derived compounds. From a winemaking standpoint, these results indicate that smoke taint concerns play a limited role in decisions about leaving films on grapes during processing. A similar conclusion was reached in a previous study employing in-canopy misting during smoke exposure (Szeto et al. 2020). Although there were significant differences in PGs (but not in VPs), sensory analysis indicated no reduction in perceived smoke taint. It should be noted that the Szeto et al. (2020) study was conducted using grapes without film treatments. Therefore, the presence of films could potentially influence sensory perception indirectly through their effects on wine chemistry and color rather than through changes in smoke-derived phenol concentrations.

Wine chemistry parameters also showed inconsistent responses to film and wash treatments across smoke exposure trials, complicating interpretation of treatment effects. No significant differences in chromatic properties were detected in experiment 1, while in experiment 2, only absorbance at 620 nm differed significantly among treatments (Supplemental Table 4). Additional compositional differences further highlighted this complexity. Significant treatment effects were limited to polyphenol concentration in experiment 1 and TA in experiment 2, with lower concentrations observed in washed samples (Supplemental Table 3). Variable responses in wine acidity were also reported in previous studies evaluating similar preventative strategies (Tran et al. 2023a). Significantly higher TA was found in wines produced from uncoated grapes than wines made from grapes coated with films, similar to those evaluated in experiment 1. In contrast, no significant differences in TA were observed among wines produced from grapes treated with the same films used in experiment 2 (Tran et al. 2023b). Although washing did not significantly affect TA in either study, washed samples generally exhibited lower TA values, which is consistent with the present findings.

The presence of residual water after washing may have contributed to slight dilution of TA concentrations. A similar explanation may apply to PAN and polyphenol concentrations in experiment 1. However, the lower concentrations observed in washed samples may also reflect differences in washing intensity, as grapes in experiment 1 were sprayed with hose water rather than dipped in a bucket of water. The more vigorous washing treatment may have removed nitrogen and phenolic compounds from the grapes' surface, contributing to the lower concentrations measured in the resulting wines. Despite these treatment-related differences in wine chemistry, it remains unclear whether the presence of film would result in perceptible sensory differences in wine. Given that these parameters influence mouthfeel, color stability, and overall wine quality, further sensory evaluation is needed to determine whether treatment-induced changes in wine chemistry translate to measurable differences in sensory perception. Overall, these findings suggest that decisions regarding film removal are more likely to influence wine composition and stylistic attributes than concentrations of smoke-derived compounds.

Although film type was identified by multivariate analysis as a significant factor influencing smoke-derived compounds, the interpretation of these results is limited by the high variability observed across treatments. Large standard deviations indicate substantial inconsistency among replicates, thereby reducing confidence in the magnitude and reproducibility of treatment effects. A likely contributor to this variability was the enclosed smoke environment, which resulted in elevated temperature, humidity, and prolonged exposure to smoke particulates. These conditions may have compromised film integrity and barrier performance through

moisture-induced swelling, chemical degradation, and surface contamination by particulate residues.

Water vapor in smoke can be readily absorbed by hydrophilic film components, altering diffusion pathways through changes in microporosity (Paulraj et al. 2018, Kwon et al. 2020, Surat'man et al. 2025). Films with high water vapor permeability, such as CNF and BCD, are more prone to structural disruption under high humidity conditions, as demonstrated in prior water barrier evaluations showing rapid disintegration in water (Tran et al 2023a). A comparable effect was reported in which increased VP concentrations in grapes treated with a biofilm were attributed in part to rain showers that occurred near the time of application (Favell et al. 2021). As with CNF and BCD films evaluated here, the biofilm likely exhibited high water solubility which may have resulted in partial film removal or disruption of polymer chains, thereby compromising film integrity.

Continuous exposure to smoke-derived acids, including formic and acetic acids, may further promote partial hydrolysis of cellulose glycosidic bonds, weakening interfibrillar hydrogen bonding and inducing microcrack formation (Zhang et al. 2020, 2024, Liu et al. 2024). In addition, deposition of smoke particulates (such as tar residues) may alter surface hydrophobicity and destabilize the film matrix over time (Liu et al. 2024, Adekunle et al. 2025). The severity of exposure was further amplified by smoke concentrations that substantially exceeded those typically observed during wildfire events. For context, the highest PM_1 concentrations reported during the 2020 Silverado and Bond fires in California were ~280 and 130 $\mu g/m^3$, respectively, measured by detectors located ~20 km from the source (Fang et al. 2023). In contrast, PM_1 concentrations in this study were at least 100 times greater than these reported values, likely leading to rapid surface saturation and minimizing functional differences among treatments. Collectively, these factors likely reduced the ability to distinguish consistent film performance and should be considered when extrapolating the results of this study to field-scale conditions.

To address these limitations, future evaluations of CNF-based film performance should be conducted under more representative smoke exposure conditions. Smoking these films in open or semi-open environments at concentrations closer to those observed during wildfire events would allow for greater airflow. This reduces humidity and provides continuous air exchange, thereby limiting smoke saturation and acid accumulation. Under such conditions, adsorbed smoke compounds may desorb intermittently, helping to preserve a film barrier through reversible surface interactions (Andersson et al. 2002, Butler and Mulholland 2004).

Two additional methodological limitations should be acknowledged. First, syringol gentiobioside, one of the most abundant and widely reported PG markers of smoke exposure (Hayasaka et al. 2013), was not included among the compounds quantified in this study; its absence limits the ability to fully characterize the glycoside profile associated with smoke exposure and should be considered when interpreting the PG results presented here. Second, Smoke Trial 1 did not include an unsmoked control, precluding direct assessment of the absolute impact of smoke exposure independent of film and wash treatments. This omission does not affect the validity of the relative comparisons among film and wash treatments, which were the primary focus of this study, but it does limit the ability to contextualize treatment effects against an unexposed baseline.

Ideally, films would be evaluated during an actual wildfire smoke event, however, vineyard trials present logistical and methodological challenges. Previous field studies have identified several constraints, including the limited temporal window, which restricts opportunities for fieldwork and timely methodological adjustments; the difficulty of securing vineyard partners willing to expose select vines to smoke without affecting adjacent blocks; and the influence of environmental conditions on the efficacy of proposed smoke-taint mitigation strategies (Favell et al. 2021). These sources of variability are evident in prior work, where field trials conducted during a wildfire event failed to achieve smoke exposure sufficient to clearly differentiate film treatment performance relative to unsmoked control grapes (Tran et al. 2023b). Despite these challenges, further investigation under conditions that better reflect real-world smoke exposure is warranted, as CNF-based films may still hold potential as a preventative strategy when evaluated under appropriate environmental conditions.

Conclusion

This study evaluated whether CNF-based film coatings applied to smoke-exposed grapes could reduce the transfer of smoke-derived compounds into wine. No film treatment consistently reduced concentrations of VPs or PGs across trials. Reductions were compound-specific, with decreases observed sporadically for guaiacol, 4MS, and 4MS gentiobioside. Multivariate analysis indicated significant effects of film treatment on smoke-derived compounds, though these effects were inconsistent and often associated with increased concentrations. Similarly, the effects of washing films prior to processing were not consistent between trials, precluding definitive recommendations. Moreover, the small effect sizes observed suggest that neither film nor wash factor had practical significance. Overall, the CNF-based films were not more effective than previously studied spray-on films. Limitations associated with the smoke exposure approach likely compromised film integrity and contributed to increased data variability. Consequently, these results should not be interpreted as definitive evidence against the use of CNF-based film coatings. To more accurately assess film performance, further research employing a more controlled and representative vineyard smoke exposure approach is warranted.

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CRediT Authorship Contributions

LG, TT, DC: Formal Analysis; LG: Investigation, Writing – Original Draft; DC: Methodology; MP: Conceptualization; MP, ET: Supervision; ET: Funding Acquisition, Project Administration, Resources, Writing – Review & Editing

Conflict of Interest

The authors declare no conflicts of interest.

Supplemental Data

The following supplemental materials are available for this article at ajevonline.org:

Supplemental Table 1 List of volatile phenols with parameters used for the dynamic multiple reaction monitoring (dMRM) method in gas chromatography-tandem mass spectrometry. ISTD, internal standard.

Supplemental Table 2 Liquid chromatography-tandem mass spectrometry parameters and compound information for target phenolic glycosides. Parameters used for the dynamic multiple reaction monitoring (dMRM) method are listed, with quantifier ions shown above qualifier ions. ISTD, internal standard; TRC, Toronto Research Chemicals.

Supplemental Table 3 Concentrations of basic chemical composition of wines produced from smoke-exposed Pinot noir grapes (washed or non-washed, subjected to film treatments [CNF, cellulose nanofiber; BCD, CNF with 0.5% β-cyclodextrin; CHI, CNF with 1% chitosan in 1% acetic acid; MIX, CNF with both 0.5% β-cyclodextrin and 1% chitosan; CH4, CNF with chitosan at a 1:4 ratio; CH2, CNF with chitosan at a 1:2 ratio; LMP, low methoxyl pectin] and a no-film control) in experiment 1 (**A**) and experiment 2 (**B**). Values are reported as mean ± standard deviation. Glu/Fru, glucose/fructose; PAN, primary amino nitrogen; TA, titratable acidity.

Supplemental Table 4 Chromatic properties of wines produced from smoke-exposed Pinot noir grapes (washed or non-washed, subjected to film treatments [CNF, cellulose nanofiber; BCD, CNF with 0.5% β-cyclodextrin; CHI, CNF with 1% chitosan in 1% acetic acid; MIX, CNF with both 0.5% β-cyclodextrin and 1% chitosan; CH4, CNF with chitosan at a 1:4 ratio; CH2, CNF with chitosan at a 1:2 ratio; LMP, low methoxyl pectin] and a no-film control) in experiment 1 (**A**) and experiment 2 (**B**). Absorbance values are at 420, 520, and 620 nm (A420, A520, and A620, respectively). Values are reported as mean ± standard deviation.

Supplemental Table 5 Multivariate analysis of variance results for experiment 2 grapes, showing the effects of film type, washing treatment, and their interaction on smoke-derived compounds. Reported statistics include Pillai's trace, associated *p* values (α = 0.05), and partial eta squared (η_p^2) as a measure of effect size.

Supplemental Table 6 Concentrations (µg/L) of volatile phenols (**A**) and phenol glycosides (**B**) in smoke-exposed grapes from experiment 2. Wines were produced from washed or non-washed Pinot noir grapes subjected to film treatments (CH4, CNF with chitosan at a 1:4 ratio; CH2, CNF with chitosan at a 1:2 ratio; LMP, low methoxyl pectin) and a no-film control. Values are reported as mean ± standard deviation. 4MG, 4-methylguaiacol; 4MS, 4-methylsyringol.

Supplemental Figure 1 Experimental chamber setup for experiment 1 smoke-exposure trials. **A**) Custom-designed smoke chambers, with a representative horizontal arrangement of the no-film control and the four film treatments (Treatment 1, 0.5% cellulose nanofiber [CNF]; Treatment 2, CNF with 0.5% β-cyclodextrin; Treatment 3, CNF with 1% chitosan in 1% acetic acid; Treatment 4, CNF with both 0.5% β-cyclodextrin and 1% chitosan); **B**) Pinot noir grape clusters suspended within the chamber.

Supplemental Figure 2 Small-scale fermentation setup of smoke-exposed Pinot noir grapes from experiment 1. Film-treated grapes were placed in glass jars and maintained in a room under controlled environmental conditions.

Supplemental Figure 3 Vineyard cage structures used for controlled smoke exposure in experiment 2. **A**) Open-frame structure installed over four Pinot noir grapevines and **B**) structure fully covered with greenhouse plastic and connected to a grill used to generate and deliver smoke to the grapevines.

Supplemental Figure 4 Microvinification vessel used for small-scale fermentation in experiment 2. Smoked-exposed and film-treated Pinot noir grapes were placed in a sealed glass jar fitted with an airlock and allowed to ferment under controlled laboratory conditions.

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Data Availability

The data underlying this study are available on request from the corresponding author.

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