



**ORIGINAL RESEARCH ARTICLE**

# *Agaricus bisporus* chitosan influences the concentrations of caftaric acid and furan-derived compounds in Pinot noir juice and base wine

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## ABSTRACT

Chitosan is a fining agent used in winemaking, although its use in juice and wine beyond fining has been limited until now. Therefore, this study's first aim was to determine if chitosan derived from *Agaricus bisporus* (button mushrooms) could reduce caffeic and caftaric acid concentrations in Pinot noir grape juice (Study A). The second aim was to determine if chitosan, when added to base wine, could influence the synthesis of furan-derived compounds during storage (Study B). In Study A, Pinot noir grape juice was stored at 10 °C for 18 hours after the following treatments: control (no addition), bentonite/activated charcoal (BAC), low molecular weight (< 3 kDa; LMW) chitosan, med. MW (250 kDa; MMW) chitosan, and high MW (422 kDa; HMW) chitosan (all 1 g/L additions). Caftaric acid was decreased, and total amino acid concentration was increased in the LMW chitosan-treated juice, while the estimated total hydroxycinnamic acid content, turbidity, and browning were decreased in the MMW chitosan-treated juice compared to the control. In Study B, Pinot noir base wine destined for sparkling wine was stored at 15 and 30 °C for 90 days with the following treatments: control (no addition), LMW chitosan, MMW chitosan, and HMW chitosan (all 1 g/L additions). The three chitosan treatments stored at 30 °C had increased furfural, homofuraneol, and 5-methylfurfural formation in the base wine compared to the control. At 15 °C, furfural and homofuraneol had greater concentrations in all chitosan-treated wines after 90 days of storage. Our results demonstrate the potential of mushroom-derived chitosan to remove caftaric acid from grape juice and suggest that chitosan can influence the synthesis of furan-derived compounds in wine after short-term storage.

**KEYWORDS:** Base wine, caftaric acid, chitosan, grape juice, furan-derived aroma compounds

## INTRODUCTION

Wine grapes destined for the traditional method of sparkling wine production are typically hand-harvested and whole bunch pressed to minimise the extraction of phenolic compounds into the juice (Charnock *et al.*, 2022). The excessive accumulation of these compounds may be unavoidable in some years to attain appropriate ripeness levels for sparkling wine production (Chamkha *et al.*, 2003). In these years, the removal of phenolic compounds from grape juice is possible through fining agents such as polyvinyl-polypyrrolidone (PVPP) or activated carbon (Spagna *et al.*, 2000). More recently, crustacean- and fungi-derived chitosan have been proposed as sustainable alternatives to these fining agents (Vendramin *et al.*, 2021).

Chitosan is a naturally synthesised biopolymer derived from crustacean exoskeletons and the cell walls/root structures (mycelium) of fungi (such as *Aspergillus niger* and *Agaricus bisporus*) (Silva *et al.*, 2020). Formed via the deacetylation of chitin, it is one of the most abundant biopolymers on Earth (Vendramin *et al.*, 2021). Chitosan molecules are chemically defined by their molecular weight (MW) and degree of deacetylation (DD), which refers to the proportion of the monomer units that are *N*-deacetylated (Abd El-Hack *et al.*, 2020). In 2009, the International Organization of Vine and Wine (OIV) authorised the use of fungal chitosan (derived from *A. niger*) in the winemaking process to improve flocculation of suspended solids and proteins, thereby decreasing protein haze and turbidity (Castro Marín *et al.*, 2020a). In addition to the treatment of protein haze, Spagna *et al.* (1996) revealed that chitosan could remove phenolic compounds (flavanols, proanthocyanidins, and hydroxycinnamic acids (HCAs)) from white wines. Nevertheless, the mechanism/s involved, specifically which compounds interact with chitosan, have not, to our knowledge, been elucidated. Therefore, it is necessary to determine the effect of chitosan on specific phenolic compounds in grape juice and wine.

In sparkling wine, caffeic acid and its tartaric acid ester, caftaric acid, are the HCAs typically found at the highest concentrations (Bosch-Fusté *et al.*, 2009; Chamkha *et al.*, 2003; Pozo-Bayo *et al.*, 2003; Serra-Cayuela *et al.*, 2013). The removal of these phenolic compounds from wine (Spagna *et al.*, 2000) and model wine (Chinnici *et al.*, 2014) by crustacean-derived chitosan has previously been investigated, but not its ability to reduce specific phenolic compounds in grape juice. At the juice stage, polyphenol oxidase (PPO) is yet to be inactivated by ethanol, thereby exposing the juice to the risk of developing browning via enzymatic oxidation (Li *et al.*, 2008).

The impact of chitosan on wine aroma and flavour, positive and/or negative, is important to establish with respect to its overall contribution to wine quality (Castro Marín *et al.*, 2020a). For instance, Castro Marín *et al.* (2020b); Castro Marín *et al.*, 2021) reported that the addition of chitosan (*A. niger*) to sparkling wine prior to the second alcoholic fermentation elicited a higher concentration of furfuryl alcohol, decanoic acid, and acetovanillone (aroma-active compounds) compared

to the control wine. Nunes *et al.* (2016) reported that chitosan films present in Encruzado red wine enhanced the aroma contributions of furfural and benzaldehyde, known products of the Maillard Reaction (MR). The MR is a non-enzymatic set of chemical reactions categorised by the initial reaction of a reducing sugar with an amino acid, protein and/or peptide, generating many flavour compounds (Medeiros *et al.*, 2022). Some of these compounds, such as 2-furfurylthiol and furfural, have been found in aged wines, as well as those that have been heated (Medeiros *et al.*, 2022; Tominaga *et al.*, 2000). The ability of mushroom-derived chitosan to influence the formation of furan-derived aroma compounds in wine, such as furfural, is currently unknown.

The presence of Maillard reaction (MR)-associated products has been reported in a variety of wine styles (Charnock *et al.*, 2022; Le Menn *et al.*, 2017; Medeiros *et al.*, 2022; Pereira *et al.*, 2014; Sawyer *et al.*, 2022). Of particular interest are their contributions to aged sparkling wine aroma and flavour (Tominaga *et al.*, 2003). Studies that targeted aged sparkling wines elucidated many of the aroma compounds responsible for these empyreumatic compounds, such as 5-methylfurfural and 2-furanmethanethiol (Le Menn *et al.*, 2017; Tominaga *et al.*, 2000). Sawyer *et al.* (2022) recently studied the influence of base wine composition on the oxidative character of sparkling wine after 6, 12, and 24 months of storage on or off yeast lees. They reported that the length of time the wines aged, and not yeast lees contact, was the main factor that affected aroma synthesis, particularly for the furanone compound homofuraneol (Sawyer *et al.*, 2022).

The main challenge in assessing the contributions of age-related compounds in older wines is the length of time required for their synthesis (Escudero *et al.*, 2000; Le Menn *et al.*, 2017). Studies that have relied on analysing aged wines have often included only limited information on the winemaking and storage conditions that inform their conclusions. To address this and determine concentrations of aroma compounds that change during ageing, researchers have used heating techniques to accelerate wine ageing (Bosch-Fusté *et al.*, 2009; Elcoroaristizabal *et al.*, 2016; Pereira *et al.*, 2014). This accelerated ageing allows for rapid analysis of compounds contributing to the aroma of aged wine, as well as browning and HCAs (Pickering *et al.*, 1999).

The aims of this study were two-fold: to determine (Study A) the ability of mushroom-derived chitosan to reduce the concentration of caffeic and caftaric acid in Pinot noir grape juice and (Study B) to establish if mushroom-derived chitosan added to sparkling base wine influences the synthesis of furan-derived compounds during storage.

## MATERIALS AND METHODS

### 1. Chemicals and Standards

Potassium metabisulphite (KMS) and potassium bitartrate (cream of tartar) were purchased from Vines to Vintages (Jordan, Ontario, Canada). IOC-2007 yeast, GoFerm®

yeast metabolite, Most-Rein bentonite/ activated-charcoal (product ID 31-15040, 20 kg), and Scottzyme® KS enzyme were purchased from Scott Laboratories Ltd (Niagara-on-the-Lake, Ontario, Canada). < 3 kDa (LMW), > 98 % DD chitosan mushroom oligosaccharide (CAS 9012-76-4, ≥ 99 %), 250 kDa (MMW), 98 % DD mushroom chitosan (CAS 9012-76-4, ≥ 99 %), and 422 kDa (HMW), > 98 % DD mushroom chitosan (CAS 9012-76-4, ≥ 99 %) were purchased from ChitoLytic Inc., (St. John's, Newfoundland, Canada). Milli-Q water was obtained from Millipore (Saint-Quentin-en-Yvelines, France).

## 2. Study A: Influence of chitosan on caffeic and caffeic acid concentrations in Pinot noir grape juice

### 2.1. Experimental Design

250 kg of Pinot noir grapes (clone 667) were hand-harvested from a vineyard in Niagara-on-the-Lake, Ontario. Healthy whole bunches were stored overnight at 10 °C in a temperature-controlled room without sulfur dioxide (SO<sub>2</sub>) additions. Berries were whole bunch pressed (20 L hydraulic bladder press) 22 hours later to 1.5 bar (1.48 ATM) pressure into a 200 L stainless steel (Criveller Company, Niagara Falls, Canada) to ensure homogeneity of the juice. The juice was then divided into 15 × 11.5 L clear glass carboys and gassed with carbon dioxide (CO<sub>2</sub>). Juice samples were collected for laboratory analysis before treatment additions. SO<sub>2</sub> was not added to the juice to prevent potential interference with the chitosan (Castro Marin *et al.*, 2021). Treatments were added directly to carboys in triplicate according to the following treatments: No addition (control), 1 g/L bentonite/activated-charcoal (BAC) slurry made to manufacturer recommendations, 1 g/L < 3 kDa (low MW – LMW) 98 % DD chitosan, 1 g/L 250 kDa (med. MW – MMW) 98 % DD chitosan, and 1 g/L 422 kDa (high MW – HMW) 98 % DD chitosan.

Juices were placed in a refrigerated room and cold-settled at 10 °C for 18 hours before racking. Juice samples were taken after treatment additions prior to inoculation for laboratory analyses. Each replicate was separately inoculated with IOC-2007 yeast (Scott Laboratories Ltd., Niagara-on-the-Lake, Ontario, Canada) after being chaptalised from 15 to 18 °Brix using granulated cane sugar (Redpath Sugar Ltd. Toronto, Ontario, Canada). Fermentation took six days, 50 mg/L of SO<sub>2</sub> was added to each replicate once they reached dryness (< 3 g/L RS), and wines were kept at 4 °C to settle for six days. After settling, the wines were racked off yeast lees. 4 mL/hL of Scottzyme® KS pectinase enzyme (Scott Laboratories Ltd., Niagara-on-the-Lake, Ontario, Canada) was added to each wine to clarify them due to excess pectin haze. Pectin was not treated prior to fermentation to prevent potential interference with chitosan. The wines settled for three days at 15 °C before cold stabilisation (–2 °C) with 4 g/L of potassium bitartrate (Vines to Vintages, Jordan, ON, Canada) and coarse filtration (6 µm, Buon Vino Super Jet, Buon Vino Manufacturing Inc., Cambridge, Ontario, Canada), and were then stored with CO<sub>2</sub> in the headspace until the use of the control wine in Study B six weeks later.

## 3. Study B: Influence of chitosan on furan-derived compound formation in base wine during storage

### 3.1. Experimental Design

The wine used in Study B was the Pinot noir base wine made in Study A (control) that did not undergo any juice treatments. The study B base wine treatments were made in triplicate in screwcap bottles (200 mL narrow mouth HDPE plastic screw-cap bottles (VWR® International, Radnor, Pennsylvania, USA)) by the addition of *A. bisporus*-derived chitosan (200 mg), which remained in the base wine during storage. Treatments were as follows: No addition (control), 1 g/L < 3 kDa (LMW) 98 % DD chitosan, 1 g/L 250 kDa (MMW) 98 % DD chitosan, and 1 g/L 422 kDa (HMW) 98 % DD chitosan. The concentration of chitosan (1 g/L) was chosen because it is the maximum recommended dose for chitosan in winemaking set by the OIV (International Organisation of Vine and Wine, 2015). The chemical analysis of the wine was carried out prior to chitosan additions (Table 1). Bottles were gassed with argon gas before cap closure to prevent oxidation and kept for 90 days at 30 °C (moderate accelerated ageing, per Medeiros *et al.*, (2022)) or 15 °C (approximate cellar temperature) in an insulated, dark temperature-controlled room. After the storage period, duplicate laboratory analysis of each replicate wine was conducted consisting of the following parameters: total HCA estimation (A.U. at λ<sub>320nm</sub>), brown pigmentation (A.U. at λ<sub>420nm</sub>), caffeic acid concentration (mg/L), and nine furan-derived compounds (µg/L).

## 4. Standard Juice and Wine Chemical Analyses

Titrate acidity (TA g/L tartaric acid eq.) and pH were determined by auto-titrator (Hanna Instruments® HI 84502 Woonsocket, Rhode Island, USA). Free and total SO<sub>2</sub> was determined by the aspiration method (Iland *et al.*, 2013) using a Sartorius Biotrate digital burette (Model LH-723082) and Isotemp condenser (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). Ethanol (% v/v) was analysed according to Nurgel *et al.* (2004) by gas chromatography (Agilent 6890 model) coupled with a flame ionisation detector (GC-FID). Acetic acid (g/L), L-malic acid (g/L), yeast assimilable nitrogen (YAN mg N/L), and residual sugars (mg/L D-glucose & D-fructose) were determined by enzymatic kits: K-ACET 02/17; L-LMALL 06/07; K-PANOPA 08/14; K-AMIAR 12/12; and K-FRUGL 05/17, respectively (Megazyme International Ltd, Wicklow, Ireland). °Brix and temperature (°C) were determined by a glass hydrometer and mercury thermometer, respectively. Turbidity (NTU) was measured with a turbidity meter (Hanna Instruments® HI 98703 Woonsocket, Rhode Island, USA), while dissolved oxygen (DO mg/L) was determined by a DO meter (Hanna Instruments® HI 9146 Woonsocket, Rhode Island, USA). Total HCA estimation and degree of browning were analysed according to Iland *et al.* (2013) using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies Canada Inc., Mississauga, ON).

**TABLE 1.** Chemical analysis of the base wine used in Study B.

Chemical Parameters	Pinot noir Base Wine
pH	2.75 ± 0.01
Titrateable acidity (TA g/L)	9.1 ± 0.0
Fructose (g/L)	3.5 ± 0.1
Glucose (g/L)	0.3 ± 0.1
Malic acid (g/L)	3.3 ± 0.0
Acetic acid (g/L)	0.42 ± 0.00
YAN (mg N/L)	7.2 ± 0.2
Alcohol (% v/v)	9.7 ± 0.0
Free SO <sub>2</sub> (ppm)	2 ± 1
Total SO <sub>2</sub> (ppm)	60 ± 3
Total HCA Estimate (A.U)	0.13 ± 0.00
Brown Pigmentation (A.U)	0.08 ± 0.00

± indicates the standard deviation between sample means (n = 2).

**TABLE 2.** Retention times (min), quantifying ions (m/z), qualifying ions (m/z), regression coefficient (R<sup>2</sup>), calibration range (µg/L), LODs, and LOQs of six furan-derived compounds analysed in Study B.

Compound	Retention Time (min)	Quantifying Ion (m/z)	Qualifying Ion(s) (m/z)	Regression Coefficient (R <sup>2</sup> )	Calibration Range (µg/L)	LOD (µg/L)	LOQ (µg/L)
Furfural-d <sub>4</sub>	24.0	100	70, 99	-	-	-	-
Furfural	24.0	96	67, 95	0.9999	2.5–300	0.76	2.5
Ethyl-2-furoate	41.2	95	112, 140	0.9978	1.76–75	0.53	1.76
Homofuraneol	24.0	97	101	0.9995	1.59–300	0.48	1.59
5-Methyl furfural	35.2	110	53	0.9988	4.9–300	1.47	4.9
Furfuryl ethyl ether	27.2	81	98, 126	0.9999	1.09–300	0.33	1.09
2-Acetylfuran	30.3	110	95	0.9995	1.03–300	0.31	1.03

## 5. Liquid Chromatography-UV Diode Array Detection (LC-UV/DAD) and Headspace Solid Phase Micro-Extraction-Gas-Chromatography/Mass Spectrometry (HS-SPME-GC/MS) Analyses

LC-UV/DAD and HS-SPME-GC/MS methods for quantifying caffeic/caftaric acid and furan-derived compounds, respectively, are detailed in Medeiros *et al.* (2022), including the chemicals and reagents used for both methods. Limits of detection (LOD) were calculated by adding the Limit of Blank (LoB) to the standard deviation of the 1 µg/L standard multiplied by 1.645 (LOD = LoB + 1.645<sub>SD 1 µg/L sample</sub>) (Table 2). Limits of quantification (LOQ) were calculated as the LOD multiplied by 3.3 (Armbruster and Pry, 2008).

## 6. Statistical Analyses

The statistical software used was XLSTAT (2021.1.1, Addinsoft, Paris, France), and all data were analysed using the Shapiro–Wilk test for normality to determine which model would be applied to analyse the variance between sample means. Standard chemical parameters were analysed

pre- and post-treatment by two-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test to separate sample means for normally distributed data at  $\alpha = 0.05$ . For non-normally distributed data, the Kruskal–Wallis (KW) test followed by the Conover–Iman procedure was used at  $\alpha = 0.05$ . Post-treatment data, as well as total HCA estimation, degree of browning, LC-UV/DAD, and HS-SPME-GC/MS data, were analysed by one-way ANOVA with Tukey's HSD<sub>0.05</sub> test for normally distributed data, while the KW test followed by the Conover–Iman procedure was used for non-normally distributed data, at  $\alpha = 0.05$ .

## RESULTS

### 1. Study A: Influence of chitosan on caffeic and caftaric acid concentrations in Pinot noir grape juice

Standard chemical analysis of the Pinot noir grape juice pre- and post-treatment addition can be found in Table 3. Post-fermentation standard wine chemical analyses, total

**TABLE 3.** Chemical composition of Pinot noir juice analysed pre- and post-treatment addition.

Parameter	Control		BAC		LMW Chitosan		MMW Chitosan		HMW Chitosan		Significance (Treatment* Timepoint)
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Brix (°)	15.1 ± 0.0	15.1 ± 0.1	15.1 ± 0.2	15.1 ± 0.1	15.1 ± 0.0	15.0 ± 0.1	15.1 ± 0.1	15.0 ± 0.0	15.2 ± 0.1	15.1 ± 0.1	NS
pH	3.14 ± 0.02a	3.07 ± 0.00cd	3.11 ± 0.02b	3.05 ± 0.02d	3.09 ± 0.00bc	3.09 ± 0.01bc	3.09 ± 0.01bc	3.07 ± 0.00cd	3.10 ± 0.01bc	3.07 ± 0.01cd	***
Titratable Acidity (TA g/l)	9.7 ± 0.7a	9.2 ± 0.3ab	9.6 ± 0.1a	8.6 ± 0.2b	9.2 ± 0.2ab	9.2 ± 0.4ab	9.2 ± 0.2ab	8.5 ± 0.2b	9.3 ± 0.3ab	8.9 ± 0.3ab	*
Malic Acid (g/l)	4.7 ± 0.1a	4.7 ± 0.3ab	4.6 ± 0.1ab	4.5 ± 0.0ab	4.6 ± 0.0ab	4.5 ± 0.1ab	4.6 ± 0.1ab	4.4 ± 0.1b	4.6 ± 0.1ab	4.5 ± 0.2ab	*
Acetic Acid (g/l)	0.01 ± 0.00cd	0.02 ± 0.01bc	0.02 ± 0.01bc	0.02 ± 0.00bcd	0.02 ± 0.00bc	0.05 ± 0.01a	0.02 ± 0.00b	0.01 ± 0.00d	0.02 ± 0.01bcd	0.02 ± 0.01bcd	***
Ammonia (mg N/l)	83.5 ± 10.0	76.8 ± 1.7	77.9 ± 4.6	77.6 ± 3.0	80.9 ± 2.0	80.1 ± 0.3	77.9 ± 2.5	78.6 ± 0.6	76.8 ± 3.7	78.4 ± 1.1	NS
Amino Acids (mg N/l)	83.0 ± 3.3b	79.7 ± 2.9b	78.9 ± 3.8b	58.3 ± 2.4c	81.7 ± 4.0b	115.5 ± 3.7a	76.8 ± 3.4b	79.2 ± 1.7b	79.2 ± 4.5b	78.1 ± 1.2b	***
Total YAN (mg N/l)	166.5 ± 11.9b	156.6 ± 1.8b	156.8 ± 2.6b	135.9 ± 2.9c	162.6 ± 4.8b	195.6 ± 4.0a	154.7 ± 2.7b	157.8 ± 1.5b	155.9 ± 5.5b	156.1 ± 1.2b	***

± represents the standard deviation of the means (n = 6), and multiple comparisons of treatment means were carried out using two-way ANOVA with interactions, followed by Tukey's HSD (honestly significant difference) test, using treatment and the time points of analysis as independent variables. Different letters represent means that were separated based on the model applied. Significance: NS = p > 0.05, \* = p < 0.05, \*\*\* = p < 0.001.

**TABLE 4.** Chemical composition of the Pinot noir base wine in Study A.

Parameter	Control	BAC	LMW Chitosan	MMW Chitosan	HMW Chitosan	Significance
pH	2.96 ± 0.00ab	2.93 ± 0.02b	2.96 ± 0.03a	2.94 ± 0.01ab	2.94 ± 0.01ab	*
Titrateable Acidity (TA g/L)	8.4 ± 0.1b	8.4 ± 0.1b	8.6 ± 0.1ab	8.4 ± 0.3b	8.8 ± 0.3a	*
Glucose (g/L)	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	NS
Fructose (g/L)	3.2 ± 0.6a	1.3 ± 0.4c	1.5 ± 0.4c	2.0 ± 0.2bc	2.4 ± 0.3b	***
Total Residual Sugar (g/L)	3.3 ± 0.6a	1.4 ± 0.4c	1.6 ± 0.3c	2.1 ± 0.2bc	2.5 ± 0.3b	***
Malic Acid (g/L)	4.0 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	NS
Acetic Acid (g/L)	0.33 ± 0.00b	0.29 ± 0.01c	0.37 ± 0.01a	0.32 ± 0.01bc	0.31 ± 0.02bc	**
Ammonia (mg N/L)	ND	ND	ND	ND	ND	NS
Amino Acids (mg N/L)	7.0 ± 0.5b	7.8 ± 0.5b	41.5 ± 3.3a	9.1 ± 0.7b	8.0 ± 1.5b	***
Total YAN (mg N/L)	7.0 ± 0.5b	7.8 ± 0.5b	41.5 ± 3.3a	9.1 ± 0.7b	8.0 ± 1.5b	***
Alcohol (% v/v)	9.8 ± 0.0	10.0 ± 0.0	9.9 ± 0.2	9.9 ± 0.1	9.8 ± 0.1	NS
Free SO <sub>2</sub> (mg/L)	9 ± 1	9 ± 0	9 ± 0	9 ± 0	10 ± 1	NS
Total SO <sub>2</sub> (mg/L)	62 ± 1	66 ± 2	63 ± 2	63 ± 1	63 ± 1	NS
Dissolved Oxygen (mg/L)	4.9 ± 0.5a	4.8 ± 0.2ab	4.5 ± 0.1bc	4.3 ± 0.1c	4.4 ± 0.0c	*
Turbidity (NTU)	11.9 ± 1.3a	11.9 ± 0.3a	12.7 ± 0.6a	9.2 ± 0.3b	12.0 ± 0.9a	***
Total HCA Estimate (A.U)	0.4 ± 0.1a	0.4 ± 0.0a	-	0.3 ± 0.0b	0.4 ± 0.0a	**
Brown Pigmentation (A.U)	0.21 ± 0.03a	0.23 ± 0.02a	-	0.17 ± 0.01b	0.20 ± 0.00a	**

± represents the standard deviation of the means ( $n = 6$ ), and multiple comparisons of treatment means were carried out via one-way ANOVA followed by Tukey's HSD test at  $p < 0.05$ . Different letters represent means that were separated based on the model applied. Significance: NS =  $p > 0.05$ , \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ . - signifies no data collected for this treatment due to colour interference with the spectroscopic method.

HCA estimation, brown pigmentation, and caffeic and caftaric acid concentrations data are presented in Table 4.

### 1.1. Juice Chemical Composition

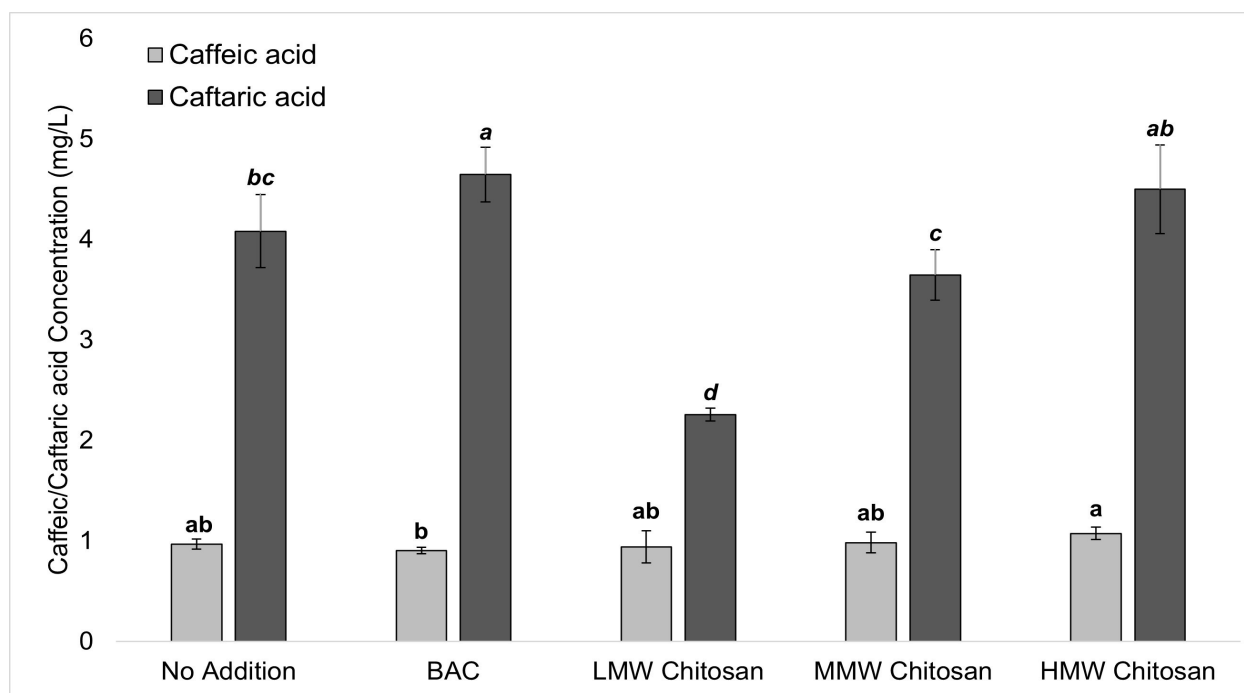
Juice pH in the control and BAC samples decreased after treatment ( $p < 0.001$ ) but not in any chitosan treatments ( $p > 0.05$ ). TA decreased by 1.0 g/L after the BAC treatment was applied to the juice ( $p < 0.05$ ), but no difference in the chitosan-treated juices nor the control was observed. The acetic acid concentration was decreased in the MMW chitosan treatment by 0.01 g/L compared to the pre-treatment juice ( $p < 0.05$ ) but was increased in the LMW chitosan treatment by 0.03 g/L ( $p < 0.001$ ), and the higher acetic acid

concentration persisted post-fermentation compared to the other treatments ( $p < 0.001$ ).

In the LMW chitosan-treated juice, YAN increased by 35 mg N/L post-treatment ( $p < 0.001$ ). This increase was due to a higher concentration of amino acids in the juice and was higher than the other treatments ( $p < 0.001$ ). However, the BAC treatment had decreased amino acid levels by 20 mg N/L ( $p < 0.01$ ) compared to the control.

### 1.2. Base Wine Chemical Composition

Wine pH and TA differences were observed between treatments, though the differences were small (Table 4). The control retained the highest concentration of fructose relative to the other treatments, followed by the HMW and



**FIGURE 1.** Caffeic and caftaric acid concentrations (mg/L) in base wine after juice treatments and fermentation.

Error bars represent the standard deviation of sample means ( $n = 6$ ). Multiple comparison of treatment means was carried out via the KW test followed by the Conover-Iman procedure for caffeic acid data (bold), and a one-way ANOVA was performed followed by Tukey's HSD test for caftaric acid data (bold/italics). Different letters represent means that were separated based on the model applied at  $p < 0.05$ .

MMW chitosan wines, while the LMW chitosan and BAC wines had the lowest fructose. The LMW chitosan wine contained a higher concentration of acetic acid ( $p < 0.01$ ) and amino acids ( $p < 0.001$ ) compared to the other treatments. Turbidity and DO measurements differed between treatments—the control had higher DO compared to the three chitosan treatments. The MMW chitosan wine had the lowest turbidity compared to the other treatments.

### 1.3. Caffeic and Caftaric Acids in Base Wine

The LMW chitosan wine contained 2.3 mg/L caftaric acid, lower than the other treatments ( $p < 0.0001$ ) (Figure 1). Caftaric acid concentration in the MW chitosan-treated wine was 3.7 mg/L, which did not differ from the control but was lower than for the BAC wine ( $p < 0.0001$ ).

## 2. Study B: Influence of chitosan on furan-derived compound formation in base wine during storage

### 2.1. Total HCA Estimation, Degree of Browning, and Caffeic Acid Following 90 Days of Storage

MMW and HMW chitosan-treated wines had a lower estimated HCA content compared to the control (Table 5). Under the 15 °C storage condition of these two chitosan treatments, the HCA content was estimated at zero, while some HCAs were estimated to be present in the MMW (0.08 A.U) and HMW (0.09 A.U) chitosan-treated wines at 30 °C. The LMW chitosan imparted a visible orange hue to the wine, which prevented spectroscopic analysis. For brown

colouration, the MMW and HMW chitosan wines were again found to have reduced absorbance values compared to the control. Under the 15 °C storage condition, only the MMW chitosan wine was lower than the control. Overall, a higher degree of browning was seen in the wines stored at 30 °C compared to 15 °C, though no visible browning was discernable to the naked eye. The caffeic acid variability due to treatments was minimal under both 30 °C and 15 °C storage, and only the HMW chitosan treatment was higher than the control ( $p < 0.05$ ).

### 2.2. Furan-Derived Compounds

Six of the nine compounds analysed were determined at concentrations greater than their LOQ in wines stored at 30 °C (furfural, homofuraneol, ethyl-2-furoate, 5-methylfurfural, furfuryl ethyl ether, and 2-acetylfuran) but at 15 °C, only furfural, homofuraneol, and ethyl-2-furoate were quantified (Table 6).

Furfural, homofuraneol, and ethyl-2-furoate were found at higher concentrations in wines stored at 30 °C than at 15 °C. Furfural and homofuraneol concentrations increased in the chitosan-treated wine, with the greatest increase observed in the HMW chitosan wines for both compounds. Furfural concentrations in the MMW chitosan wine at 15 °C were indistinguishable from the LMW chitosan but 2 µg/L lower than the HMW chitosan ( $p < 0.01$ ). However, at 30 °C, the MMW chitosan wine's furfural concentration was 209 µg/L, higher than the LMW chitosan ( $p < 0.0001$ ) but like HMW chitosan.

**TABLE 5.** Total HCA estimation, brown pigmentation, and caffeic acid concentrations of base wines stored at 15 and 30 °C for 90 days.

Treatment	Total HCA Estimation (A.U)		Brown Pigmentation (A.U)		Caffeic Acid Concentration (mg/L)	
	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C
Control	0.13 ± 0.01a	0.28 ± 0.01a	0.08 ± 0.01a	0.15 ± 0.00a	1.6 ± 0.1b	1.6 ± 0.1b
LMW Chitosan	-	-	-	-	1.7 ± 0.1ab	1.6 ± 0.1ab
MMW Chitosan	0.00 ± 0.00b	0.08 ± 0.01b	0.06 ± 0.01b	0.11 ± 0.00b	1.7 ± 0.2ab	1.6 ± 0.1ab
HMW Chitosan	0.00 ± 0.00b	0.09 ± 0.01b	0.07 ± 0.01ab	0.12 ± 0.00b	1.7 ± 0.1a	1.7 ± 0.1a
Significance	***	***	*	***	*	*

± represents the standard deviation of the means (n = 6). Multiple comparison of treatment means was carried out via one-way ANOVA followed by Tukey's HSD test for normal data, and the KW test followed by the Conover–Iman procedure for non-normal data. Different letters represent means that were separated based on the model applied. Significance: \* = p < 0.05, \*\*\* = p < 0.001. - signifies no data collected for this treatment due to colour interference with the spectroscopic method.

**TABLE 6.** Concentrations (µg/L) of furan-derived compounds in Pinot noir base wine after 90 days of storage at 15 °C and 30 °C.

Treatment	Furfural		Homofuraneol		Ethyl-2-furoate		5-Methyl furfural	Furfuryl ethyl ether	2-Acetyl furan
	15 °C	30 °C	15 °C	30 °C	15 °C	30 °C	30 °C	30 °C	30 °C
Control	6 ± 0c	91 ± 3c	4 ± 1c	89 ± 3c	2.9 ± 0a	10 ± 1a	9 ± 0c	1.6 ± 0b	2.8 ± 0c
LMW Chitosan	26 ± 0b	691 ± 9b	23 ± 1b	701 ± 15b	2.9 ± 0a	11 ± 0a	23 ± 1b	2.1 ± 0a	4.9 ± 0a
MMW Chitosan	26 ± 1b	899 ± 48a	23 ± 1ab	889 ± 48a	2.6 ± 0b	9 ± 0b	34 ± 2a	1.6 ± 0b	3.2 ± 0b
HMW Chitosan	28 ± 3a	955 ± 60a	24 ± 1a	930 ± 68a	2.5 ± 0b	9 ± 1b	37 ± 5a	1.6 ± 0b	3.0 ± 0b
Significance	**	***	*	**	*	**	***	**	*

± represents the standard deviation of the means (n = 6). Multiple comparison of treatment means was carried out via one-way ANOVA followed by Tukey's HSD test for normally distributed data, while the Kruskal–Wallis test followed by the Conover–Iman procedure was performed for non-normally distributed data, at p < 0.05. Different letters represent means that were separated based on the model applied. Significance: NS = p > 0.05, \* = p < 0.05, \*\* = p < 0.01, \*\*\* = p < 0.001. 5-Methylfurfural, furfuryl ethyl ether, and 2-acetyl furan concentrations could not be quantified at 15 °C due to being below the LOQ of the method.

For homofuraneol, a similar pattern was noted between treatments: LMW and HMW chitosan wines were different at both 15 °C (p < 0.05) and 30 °C (p < 0.0001) temperatures. At 15 °C, the MMW chitosan wine contained a similar concentration of homofuraneol compared to both the LMW and HMW chitosan wines. At 30 °C, the MMW chitosan wine was 188 µg/L greater than the LMW chitosan treatment (p < 0.001) but similar to the HMW chitosan treatment.

The control and LMW chitosan wines contained higher concentrations of ethyl-2-furoate compared to the MMW and HMW chitosan treatments, regardless of the temperature condition. At 15 °C, the MMW chitosan wine had lower concentrations of ethyl-2-furoate compared to the control (p < 0.01) and LMW chitosan (p < 0.01). At 30 °C, the MMW chitosan wine had lower ethyl-2-furoate concentrations again

compared to the control (p < 0.001) and LMW chitosan treatment (p < 0.0001). These results were similar for the HMW and the MMW chitosan wines.

5-Methylfurfural concentrations were impacted by the chitosan treatments, with the highest amounts found in the MMW and HMW chitosan wines. The LMW chitosan wine contained lower concentrations compared to the MMW chitosan (p < 0.0001) and the HMW chitosan (p < 0.0001) but was still higher than the control (p < 0.0001).

The concentrations of furfuryl ethyl ether in the control, MMW, and HMW chitosan wines did not differ, while the LMW wine contained a higher amount compared to the control, MMW and HMW chitosan (p < 0.01). 2-acetyl furan concentrations followed a similar trend; LMW chitosan wine



contained the highest concentration compared to the MMW, HMW chitosan, and control ( $p < 0.0001$ ). The MMW and HMW chitosan wines were indistinguishable, though they were both higher in concentration than the control ( $p < 0.05$ ).

## DISCUSSION

### 1. Study A: Influence of chitosan on caffeic and caftaric acid concentrations on Pinot noir grape juice

#### 1.1. Impact of Chitosan on Grape Juice

In contrast to the study by Castro Marín *et al.* (2020b), acetic acid concentrations in our study were higher in the LMW chitosan wines compared to the control but only by 0.1 g/L. However, the increased amino acid content in the LMW chitosan-treated wine is in agreement with Castro Marín *et al.* (2020b), who found elevated protein content (38 mg/L) compared to the control (26 mg/L) after 12 months ageing the wine on lees with 250 mg/L of *A. niger*-derived chitosan (80–90 % DD, 10–30 kDa). Those differences are smaller than in our study, which is notable given the shorter contact time of chitosan with juice (18 hours vs. 12 months). Castro Marín *et al.* (2020b) attributed the increase in total protein content to the greater speed of yeast autolysis caused by polar interactions of the chitosan with the yeast cell walls, favouring lysis and amino acid release. The chitosan treatments in our study were carried out on un-inoculated juice, so the sole source of proteins available for release via cell lysis was grape proteins, yeast present on skins/stems, and bacteria. These could have been responsible for the increased amino acid content observed in the LMW chitosan treatment. The assessment of chitosan as a clarification agent by Spagna *et al.* (1996) included measurements of wine turbidity and reported a correlation between the reduction in brown pigmentation formed and the turbidity of the wine. The MMW chitosan lowered the turbidity in the wine compared to the control, in agreement with Spagna *et al.* (1996).

Total estimated HCA levels in the wines decreased in the MMW chitosan treatment compared to the control, as previously reported by Spagna *et al.* (1996), who found a 40 % reduction in HCAs in Italian wines treated with 0.4–4.0 g/L of chitosan (60–78 % DD, 190–250 kDa). However, only one of the treatments in our study (HMW) reduced the total estimated HCA content. This is in contrast to caftaric acid concentration determined via LC-UV/DAD, in which only LMW chitosan-treated juice reduced caftaric acid. Adsorption of other HCAs or spectroscopically relevant compounds in the  $\lambda_{320\text{nm}}$  range could potentially account for this discrepancy.

#### 1.2. Caffeic and Caftaric Acids in Base Wine

As reported in previous studies by Castro Marín *et al.* (2020b) and Spagna *et al.* (1996), our chitosan-treated wines had lower concentrations of caftaric acid compared to the control and BAC wines, especially the LMW chitosan, which had the lowest concentration of caftaric acid compared to the

control. Chien *et al.* (2007) also determined that in apple juice, the lower MW of the chitosan (12 kDa), the greater its ability to scavenge hydrogen peroxide and exhibit adsorptive properties compared to higher MW (318 kDa) chitosan when DD was the same (98.5 %). These results indicate that lower MW chitosan, which at  $\text{pH} < 6.5$  is water-soluble (Tian *et al.*, 2015), is likely to interact with phenolic constituents in wine better than higher MW chitosan. This may be due to decreased cation repulsion of adjacent chitosan polymer subunits, resulting in a better ionic attraction between the chitosan oligomers and negatively charged small particles (Chien *et al.*, 2007).

In contrast, Castro Marín *et al.* (2020b) determined that a low MW *A. niger*-derived chitosan (10–30 kDa, 80–90 % DD) did not influence the concentration of caftaric acid during a second alcoholic fermentation in a bottle, nor during 12-months ageing on lees. Caftaric acid concentrations in Pinot gris/Pignoletto sparkling wine in their study were within 1–2 mg/L of those determined in our study, though no variation in HCA concentration from chitosan addition was reported. Given the similar chemical parameters and *A. niger* source of the chitosan products used in both studies, this discrepancy could be explained by the rate of chitosan addition; 1 g/L, compared to 250 mg/L in Castro Marín *et al.* (2020b). Our finding agrees with Spagna *et al.* (2000), who tested 0.4 g/L and 0.8 g/L concentrations of shellfish-derived chitosan on Trebbiano and Albana wines. Under a short (< 2 hr) contact time, the authors found that the 0.4 g/L chitosan decreased total HCAs by 20 %, while the 0.8 g/L chitosan treatment decreased HCAs by 40 %, which is comparable to the 54 % decrease in caftaric acid observed from the LMW chitosan treatment in our study. This improved adsorption at higher concentrations requires further exploration to establish the appropriate chitosan dosage for wine for optimal HCA removal.

Concerning caffeic acid, our results align with those of Castro Marín *et al.* (2020b), who reported that 250 mg/L of *A. niger*-derived chitosan did not affect caffeic acid concentrations during 12-month ageing on lees. In fact, an increase in caffeic acid concentration was recorded for the HMW chitosan-treated wine compared to the BAC-treated wine and control. However, these differences in caffeic acid concentration were extremely small (< 0.1 mg/L), and, therefore, these results should be taken with caution.

### 2. Study B: Influence of chitosan on furan-derived compound formation in base wine during storage

#### 2.1. Total HCA Estimation, Degree of Browning, and Caffeic Acid Following 90 Days of Storage

Compared to the control wine, both MMW and HMW chitosan-treated wines had lower HCA content after 90 days of storage under both temperature conditions, with the concentration in 15 °C chitosan wines below the 1.4 A.U limit for HCA estimation (Iland *et al.*, 2013). This result was expected based on results in Study A, where the MMW chitosan treatment reduced total HCAs compared to the

control wine. Additionally, an increase in HCAs was found in the wines stored at 30 °C compared to those kept at 15 °C, which agrees with the findings of Medeiros *et al.* (2022), where the control wine stored at the lower temperature had lower estimated HCA content compared to the wines kept at 30 °C.

Caffeic acid content from the control wine in Study A was used to make treatments in Study B, which had increased slightly over the time period, likely due to the hydrolysis of caftaric acid into caffeic acid (Vallverdú-Queralt *et al.*, 2015). These concentrations align with previous results by Ferreira-Lima *et al.* (2013), who reported caffeic acid concentrations in the range of 2.25–2.75 mg/L in Goethe white wines over the course of four months of ageing at 25 °C.

## 2.2. Furan-derived Compounds

### 2.2.1. Furfural

Furfural is a volatile furan-derived compound that contributes a sweet, bready, and fruity aroma in wine (Nunes *et al.*, 2016). It accumulates over time in aged sparkling wines (Jeandet *et al.*, 2015; Tominaga *et al.*, 2003) and wines subjected to heating (Pereira *et al.*, 2010). Furfural was found at higher concentrations in chitosan-treated wines compared to the control. In wines stored at 30 °C, the furfural concentration amongst treated wines was 849 µg/L, almost 10× higher than the concentration in the control wine. In comparison, Tominaga *et al.* (2003) found that the furfural concentration of bottle-aged Champagne in a 10-year-old Louis Roederer Champagne was just below 1000 µg/L, compared to the 955 µg/L achieved by the addition of 1 g/L HMW chitosan in just 90 days at 30 °C in our study. The influence of chitosan on furfural formation was less at 15 °C. Despite the lower temperature, furfural concentration was higher in all of the chitosan-treated wines.

The odour detection threshold (ODT) is the lowest concentration that an odour can be reliably detected (Czerny *et al.*, 2008). The ODT of furfural is 14 mg/L (in 11 % (v/v) aqueous ethanol, with 7 g/L glycerol, and 5 g/L tartaric acid at pH 3.4) according to Ferreira *et al.* (2000). This is higher than the concentrations in our study; thus, it is unlikely that furfural would directly affect the sensory characteristics of the wines at this stage of production.

These results are in accordance with two separate experiments performed on model wine solutions containing arabinose (a pentose sugar), in which chitosan increased furfural concentrations (Nunes *et al.*, 2016). Rocha *et al.* (2021) also treated a model wine with chitosan, generating almost 3000 µg/L of furfural in just over 100 days at room temperature under magnetic stirring. These studies demonstrate additional factors of surface area–contact and agitation on chitosan reactivity, aspects that were not covered in our study.

The formation of furfural under wine-simulated conditions indicates that arabinose, and possibly other pentose sugars, contribute preferentially to furfural formation rather than

fructose or glucose (Rocha *et al.*, 2021). Chitosan-based reactivity with pentose sugars is also supported by a measured decrease in galactose, mannose, and arabinose determined in Albariño white wines treated with 1 g/L of *A. niger*-derived chitosan (No Brett Inside from Lallemand) (Arenas *et al.*, 2021). Similar to chitosan adsorbing HCAs and other phenolic compounds via electrostatic interactions caused by -NH<sub>3</sub><sup>+</sup> residues on the polymer backbone, Arenas *et al.* (2021) postulated that this chemical behaviour could be responsible for the reduction in pentose sugars observed in their experiment. It is possible that via these electrostatic interactions, the dehydration of hexose and pentose sugars may be enhanced. This is supported by the established understanding that acid-hydrolysis of sugars is the most likely contributing pathway to furfural formation in wine due to the low pH environment (Charnock *et al.*, 2022; Pereira *et al.*, 2010). However, the mechanism behind furfural synthesis in wine treated with chitosan or stored with chitosan immersed in it has not been elucidated in our study.

### 2.1.2. Homofuraneol

Homofuraneol (2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone), also referred to as ethyl-furoate, has been reported in several studies as a compound with a relatively low ODT (10 µg/L) (Kotseridis *et al.*, 2000; Czerny *et al.*, 2008; López *et al.*, 2003; Roscher *et al.*, 1997). Homofuraneol has been associated with aged Champagne and bestows a caramel aroma on wine (Escudero *et al.*, 2000). In our study, it was the only furan-derived compound found at concentrations higher than its ODT, and the treatments increased its concentration and exceeded the ODT value. At 15 °C, the chitosan-treated wines increased their concentrations above 10 µg/L, while the control (4 µg/L) remained below this value. Therefore, the addition of chitosan may have had an actual impact on the aroma of the Pinot noir base wine after 90 days of ageing at 15 °C, though this is based solely on the quantification of a single compound exceeding its determined ODT and is not representative of a true sensory evaluation. No studies could be found that reported the influence of chitosan on homofuraneol synthesis, although the influence of the chitosan treatments on homofuraneol concentrations was large in our study. The mechanism by which homofuraneol is synthesised in wine has not yet been elucidated. However, Escudero *et al.* (2000) reported that homofuraneol was previously identified as a component of the MR via a pathway involving acetaldehyde. Given the similar concentrations obtained between furfural and homofuraneol in our study, we suggest a potential link in their formation is possible. If homofuraneol is the reaction product of acetaldehyde and a sugar (arabinose or xylose), it is possible that the aldol condensation of these compounds might yield a chemical similar in structure. The tautomerisation and cyclisation could then take place in a similar manner to furfural or 5-hydroxymethylfurfural (another furan-derivative found in aged wines), yielding the product homofuraneol (Wang *et al.*, 2019). As this would require the loss of -CH<sub>2</sub>O in the same way as the furfural mechanism, this could explain the increase in homofuraneol synthesis in the chitosan-treated

wines. The exact mechanism of homofuraneol formation in wine is outside the scope of this study, and it requires further investigation.

### 2.1.3. Ethyl-2-furoate

Associated with vanilla and burnt aromas, ethyl-2-furoate is potentially a relevant aroma compound in aged wines (Mariscal *et al.*, 2016; Pereira *et al.*, 2014). Ethyl-2-furoate concentrations did not increase much at 30 °C compared to 15 °C throughout the 90 days of storage, in contrast to a previous study that used Chardonnay base wines (Medeiros *et al.*, 2022). However, results from this study align with those of Pereira *et al.* (2014), who subjected a dry Tinta Negra wine to 45 °C storage conditions for 3 months and found the wine to contain 12 µg/L of ethyl-2-furoate, a concentration comparable to the 10 µg/L observed in the control wine in our study. Whether ethyl-2-furoate at this concentration contributes to aged wine odour cannot be established, given the lack of information on an ODT in existent literature.

### 2.1.4. 5-Methylfurfural

5-Methylfurfural correlates with wine ageing, contributing to almond and caramel aromas (Burin *et al.*, 2013; Le Menn *et al.*, 2017; Pereira *et al.*, 2014). The ODT of 5-methylfurfural has been reported as 16 mg/L (in 14 % EtOH (v/v) solution (Moreno *et al.*, 2005), which is greater than the concentrations found in our base wines. A similar trend to that of furfural and homofuraneol formation was observed for 5-methylfurfural at 30 °C. The MMW and HMW chitosan-treated wines had greater concentrations of 5-methylfurfural compared to the LMW chitosan and control wines. No relationship between chitosan and 5-methylfurfural formation could be established from previous studies, although Castro Marín *et al.* (2020b) mentioned that by treating their Pinot gris/Pignoletto wine with 250 mg/L of *A. niger*-derived chitosan (80–90 % DD, 10–30 kDa), furfural, HMF, and furfuryl alcohol were found at higher concentrations. Given the chemical similarities between these three compounds and 5-methylfurfural, one could anticipate that it would have been affected alongside them; however, 5-methylfurfural was not among the compounds determined. Therefore, it is likely this is the first time an association between chitosan and 5-methylfurfural formation in wine has been reported. Additionally, although the concentrations of 5-methylfurfural and furfural were below their ODT, they could still increase during the cellar ageing of the final wine.

### 2.1.5. Furfuryl ethyl ether

Furfuryl ethyl ether has been previously associated with ageing in wine and beer (Spillman *et al.*, 1998; Vanderhaegen *et al.*, 2004). It was quantified at 2 µg/L for all wine samples, and an increase was observed in wines treated with LMW chitosan compared to the other treatments and control. Spillman *et al.* (1998) determined the ODT of furfuryl ethyl ether in white wine as 430 µg/L, higher than the concentrations in our study. However, the concentrations of furfuryl ethyl ether were in accordance with those found by Vanderhaegen

*et al.* (2004) in beer kept at 20 °C for 100 days, during which time < 10 µg/L of furfuryl ethyl ether was detected. Further research into the mechanism and factors surrounding the formation of furfuryl ethyl ether is necessary to determine its potential contribution to sparkling wine aroma.

### 2.1.6. 2-Acetylfuran

2-Acetylfuran (also known as 2-furyl methyl ketone) has previously been identified in wine, although its contributions to wine aroma and flavour have yet to be established (Burin *et al.*, 2013; Le Menn *et al.*, 2017). 2-Acetylfuran concentrations were higher in wine treated with LMW chitosan compared to the control and the other wines. Similar to 5-methylfurfural, no association between 2-acetylfuran and chitosan could be established from previous studies, and so this is likely the first time its influence on 2-acetylfuran in wine has been described. 2-Acetylfuran has previously been quantified at concentrations similar to those in our study. Burin *et al.* (2013) found 2-acetylfuran concentrations in Alsatian and Bordeaux white wines to be 1–6 and 2–21 µg/L, respectively, while Le Menn *et al.* (2017) reported concentrations ranging from 3–15 µg/L in Champagne wines. The aroma contribution of 2-acetylfuran at these concentrations has not been established since its ODT in wine is yet to be determined.

## 3. Limitations of the study and further recommendations

Only Pinot noir grape juice was tested, which limits the scope of our findings on the applications of chitosan to how it impacts solely this grape variety. Fully elucidating the efficacy of mushroom-derived chitosan in winemaking requires data across a range of grape varieties from multiple stages of winemaking and should include organoleptic characterisation of the treated wine. Only two of the six HCAs present in grape juice were evaluated in determining chitosan's adsorbent capabilities, but this was justified since caffeic and caftaric acid are found at the highest concentrations in grape juice. The adsorption of the other HCAs and various phenolic compounds by chitosan may have also occurred. Additionally, this study only used a single chitosan source (*A. bisporus*), although many other sources, such as shellfish, fungi, and insects, also exist (Vendramin *et al.*, 2021).

Concerning Study B, alternative and multiple concentrations of chitosan addition would help establish the bounds of efficacy in winemaking and help with making recommendations on its application. Here, we only used the maximum OIV recommended dose. Testing chitosan sourced from crustacean or *A. niger* in addition to the *A. bisporus*-derived chitosan under the same conditions would help determine the relative effects of chitosan source on the chemical interactions in wine. Finally, we encourage further research to elucidate the impact of chitosan treatment of juice and wine on other aroma compound groups. Further research into chitosan's ability to adsorb undesirable compounds and increase the generation of desirable ones will further inform its application in winemaking. Further consideration of the impact of chitosan on wine aroma and flavour development,

such as during extended lees ageing, should be given in future research.

## CONCLUSION

Study A showed that the addition of mushroom-derived chitosan to Pinot noir juice as a pre-fermentation fining agent could be a viable strategy for reducing the concentration of caftaric acid, which can contribute to non-enzymatic browning of wine. Additionally, turbidity and browning of the wine were reduced by the addition of chitosan when compared to the control and BAC treatment. Study B determined the positive effect of chitosan on furfural, homofuraneol, and 5-methylfurfural formation in Pinot noir base wine for the first time. This effect was higher in wines that were kept at 30 °C for the 90-day storage period to simulate accelerated ageing. The addition of 1 g/L HMW chitosan to base wine stored for this duration resulted in furfural formation comparable to that observed in a 10-year-old Champagne (Tominaga *et al.*, 2003). Additionally, it is possible that while the concentrations of furfural and 5-methylfurfural are currently too low to influence wine aroma and flavour based on their ODTs, they could continue to increase during cellar ageing, and, therefore, the chitosan treatments of base wine could accelerate “ageing” of the sparkling wines in the cellar. The implementation of chitosan as a winemaking tool requires further understanding of factors such as contact time, temperature, chitosan source, and chemical parameters (MW) and how these factors impact wine quality.

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