Influence of Sulfur Dioxide on the Formation of Aldehydes in White Wine

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Relatively little is known about the effects of sulfur dioxide on the general formation of flavors during fermentation. Volatile, saturated aldehydes are an important class of aroma compounds that can impact the sensory characteristics of the final wine. This study quantified the saturated aldehydes C1 through C9 formed during fermentation of white juice with different concentrations of added sulfur dioxide. Results showed that maximum concentrations of acetaldehyde (C2), 2-methyl-1-propanal (isobutanal, i-C4), and 3-methyl-1-butanal (isovaleraldehyde, i-C5) were affected by an increase in sulfur dioxide concentration during fermentation. Changes in aldehyde production patterns were observed for the different sulfur dioxide additions. Final concentration for acetaldehyde (C2) was also affected by an increase in sulfur dioxide concentration. Production patterns of formaldehyde (C1), propanal (C3), pentanal (C5), hexanal (C6), and heptanal (C7) were only slightly affected by the different levels of sulfur dioxide up to 150 mg/L, but when the sulfur dioxide addition reached 200 mg/L, aldehyde production patterns and concentrations were affected. Butanal (C4) concentrations remained constant independent of sulfur dioxide additions, and octanal (C8) and nonanal (C9) were not found in significant amounts.

Key words: Sulfur dioxide, aldehydes, analysis, fermentation, white wine, derivatization, cysteamine, thiazolidines

The saturated, short-chain aldehydes contribute flavors to many alcoholic beverages, including nutty, bruised apples, herbaceous, grassy, green, fatty, fruity, and pungent [3]. Aldehydes can affect wine during aging via their ability to undergo reduction-oxidation reactions. They can also influence the stability of wine color by reacting with sulfites to prevent bleaching and by undergoing polymerization reactions with anthocyanins and with other phenolic compounds [8]. In addition, the aldehydes, especially formaldehyde and acetaldehyde, can react with biological nucleophiles such as proteins, DNA, and cellular membranes. These reactions can have toxic, mutagenic, and carcinogenic effects [4].

Aldehydes are a normal by-product of fermentation; acetaldehyde is an intermediate in the biochemical production of ethanol. Factors such as yeast strain, temperature, pH of must, O2 levels in juice, added SO2 levels, and nutrient availability are among the variables that can influence production of acetaldehyde during fermentation [2]. Among these variables, SO2 addition is a major factor before, during, and after fermentation, since SO2 either inhibits aldehyde dehydrogenase (ADH) so that acetaldehyde is not converted to ethanol [3] or binds directly with acetaldehyde and thereby reduces the amount of acetaldehyde that can be transformed to ethanol [6].

At the pH of wine (pH 3 to 4), the dominant forms of SO2 are the molecular and the bisulfite. The SO2 solution is usually added at the crusher, during settling, or after primary and secondary fermentation [6]. For white wine fermentations, SO2 is generally added before or during fermentation of white juice and is preserved in the white wine as an acetaldehyde bound complex [6]. Acetaldehyde levels in young wines are typically less than 100 mg/L; however, if a finished white wine has unacceptably high levels of aldehydes, possibly arising from oxidative reactions, the addition of SO2, which can react with the aldehydes, may make the wine less aldehydic (sensory thresholds for free acetaldehyde range from 100 to 125 mg/L [8]). Addition of SO2 to red juice can cause temporary color reduction or so-called sulfite bleaching by occupying the C4 position of the anthocyanins. The presence of SO2 can also inhibit polymerization among flavonoid compounds because the sulfate occupies the same C4 position of the flavonoid.

There is a general trend toward reduced use of SO2 in wine processing as a result of consumer preferences and regulatory requirements. However, little is known about the effects of reduced SO2 use on flavor formation during winemaking, particularly in the case of aldehyde production. This is partially due to limits of previously available analytical methods. Among these methods, the standard titration and colorimetric analysis gives a combined measure of total aldehydes, while enzymatic methods only measure acetaldehyde levels [4]. The method used in this study quantifies each saturated, short-chain, volatile aldehydes.
aldehyde of interest in one analysis [3]. In this method, the saturated aldehydes are reacted with a primary amine (cysteamine) to form a thiazolidine. The 5-membered thiazolidine ring compound that is formed is stable and can be readily quantified by gas chromatography with nitrogen phosphorous detection. The method has been previously used to quantify aldehydes in wines and sherry [3].

The objectives of this present study were to use the cysteamine derivatization method to monitor formation of aldehydes C1 through C9 (Table 1) during grape juice fermentations and to evaluate the influence of SO2 on aldehyde production during white wine fermentation. To reach these goals, the method was adapted to handle a large number of samples (minimum 600), and the juice used in the experiment was uniform in all factors influencing the production of aldehydes, except for the different levels of added SO2.

Materials and Methods

Materials. All aldehyde standards were obtained from Aldrich Chemical Co Inc. (Milwaukee, WI). Purities were as follows: formaldehyde ~37%, acetaldehyde 99.5%, propanal 97%, butanal 99%, 2-methyl-1-propanal (isobutanal) 99%, pentanal 99%, 3-methyl-1-butanal (isovaleraldehyde) 97%, hexanal 98%, heptanal 95%, octanal 99%, nonanal 95%, 2,4,5-trimethylthiazole (used as internal standard) 98%, cysteamine (2-aminoethanethiol hydrochloride) 98%. Chloroform (Optima Grade and certified ACS, Fisher Scientific, Fair Lawn, NJ) was the extracting solvent. To break the emulsion in the samples, anhydrous magnesium sulfate (certified from Fisher) was used. To dry the extract, anhydrous sodium sulfate (certified ACS grade from Fisher) was used. Cresol red (2-aminoethanethiol hydrochloride) 98%. Chloroform (Optima Grade and certified ACS, Fisher Scientific, Fair Lawn, NJ) was the extracting solvent. To break the emulsion in the samples, anhydrous magnesium sulfate (certified from Fisher) was used. To dry the extract, anhydrous sodium sulfate (certified ACS grade from Fisher) was used. Cresol red was purchased from Eastman Kodak Co. (Rochester, NY). Absolute ethanol (100%; Gold Shield Chemical Company, Hayward, CA) was used to prepare the stock solutions. A 5% sulfur dioxide (SO2) solution was prepared by dissolving SO2 gas (Complete Welders Supply, Napa, CA) in distilled water as described in Amerine et al. [1] and was added to the juice in variable amounts. 5% NaOH (certified ACS grade from Fisher) was used to adjust pH to above 8.5 for the aldehyde analysis.Clinistest reagent tablets (Bayer Corp, Elkhart, IN) were used to test reducing sugar levels in the finished wines. Ethanol measurements of the finished wine were done by ebulliometry [8]. The free and total SO2 analysis on the juice was done by the Ripper titrametric method using iodine [8]. Concentrated juice was obtained from Canandaigua Wine Company, Madera, CA. Gases for the gas chromatography-nitrogen-phosphorous detection (GC-NPD) were air (ultra zero grade), helium (certified 99.999%), and hydrogen (99.99%). All water additions used distilled and filtered water (Milli-Q-Water Systems, Millipore Corporation, Bedford, MA).

Preparation of solutions. Cysteamine solution: 0.232 grams of cysteamine (2-aminoethanethiol hydrochloride) was dissolved into 100 mL of water to produce a 0.03 M cysteamine solution, and the pH was adjusted to 8.5. The solution should be stable for one to two weeks at room temperature, even longer if refrigerated at 6°C. Internal standard: 100.7 µL 2,4,5-trimethylthiazole was dissolved into 10 mL of 10% ethanol solution to produce a concentration of 10 mg/mL. The thiazole compound is itself stable; however, evaporation of ethanol from the solution can be a problem. The solution should be stored in a tightly sealed vial with a Teflon-lined closure. Cresol red pH indicator: cresol red was dissolved into a 20% ethanol solution at a level of 0.1 g/100 mL. Stock aldehyde solutions: each aldehyde, except acetaldehyde, was first dissolved into absolute ethanol to obtain a concentration of 10 g/L. Aldehydes were then diluted to a final concentration of 1 g/L with a 10% ethanol solution. Acetaldehyde (C2) was first dissolved in absolute ethanol to obtain a concentration of 150 g/L, then diluted with a 10% ethanol solution to a final concentration of 15 g/L. All aldehyde stock solutions were stored in glass bottles with Teflon-lined closures at 6°C.

Standard curves. The C2 aldehyde was diluted from the stock solution with 10% ethanol to obtain a concentration of 1.5, 15, 45, 75, 120, and 270 mg/L. C1 and C3 to C9 aldehydes were diluted from stock solutions with 10% ethanol to obtain 0.01, 0.1, 1.0, 3.0, 5.0, 8.0, and 18 mg/L. Since the concentration of the purchased formaldehyde stock was not exactly known, the spiked concentrations were compared to the peak area ratios from a pure thiazolidine (95%) standard curve. From this curve, the exact formaldehyde concentrations of the C1 standards were determined to be 0.08, 0.8, 2.4, 4.1, 6.5, and 14.6 mg/L. All standards were treated in the same way as the actual samples, derivatized, and analyzed by GC-NPD as described previously. Standard curves were calculated by plotting peak area ratios of analyte and internal standard (IS) versus concentrations of aldehydes and IS. The result-
ing regression equations (Table 2) were used to quantify each targeted aldehyde concentration in the wine samples. The quantitative results were calculated from the average of the two fermentation replicates in each group.

**Preparation of juice/wine samples.** The concentrate consisted of a mixture of Thompson Seedless and French Colombard grapes that were destemmed, crushed, depectinized, dejuiced, and pressed. The resulting juice was stored at -1°C in tanks to settle the solids, then centrifuged and coarse filtered. It was then concentrated in a vacuum pan at ~54°C and the crude concentrate stored at 13°C in tanks. At the onset of this study the concentrate was diluted with water to 23 Brix. Premier Cuvee yeast (32.5 g) was also added to the juice at the same time to ensure sufficient nitrogen content. The juice, used in each of the tanks was critical, so the SO2 addition was then stirred to obtain a uniform solution before approximately 11.4 L was transferred to each of 10 different fermentation culture vessels with ports (Nalgene, Nalge Nunc Int., Rochester, NY). Fermentation vessels were washed and then sprayed with 70% ethanol before the juice was added. Vessels were weighed before and after the transfer of the juice. The experiment was conducted in a room with controlled air temperature, which varied from 16.2 to 18.5°C during the course of the fermentations.

**Sulfur dioxide (SO2) addition and sampling.** Analysis of the juice revealed 3 mg/L free and 12 mg/L total SO2 before any additions. An hour after the transfer of the juice to the carboys, a 5% SO2 solution was added to the juice at the following levels: 0, 50, 100, 150, and 200 mg/L (Table 3). SO2 is usually added before the yeast so that the SO2 concentration is low enough to not inhibit/damage the yeast. In this case uniformity of the juice used in each of the tanks was critical, so the SO2 was added 1 hr after the yeast was added. From the progress of the fermentation, this did not seem to have a significant effect on the progress of the fermentations at the lower levels of the SO2 additions (0 to 150 mg/L). At the highest level of SO2 addition (200 mg/L), however, the fermentations had a longer lag period, which may be a result of this procedure. Fermentation progress was monitored by weight loss (CO2 evolution). Sampling for aldehyde analysis was done every 12 hr by taking 2 x 3 mL of juice from each vessel until the fermentation started. After the onset of the fermentation, sampling (2 x 3 mL) was done approximately every 4 hr. The samples were transferred to 13 x 100 mm Pyrex glass round-bottom tubes that were closed with Teflon-lined screw caps. The glass tubes were then stored at -80°C until analysis. Samples were taken with a glass pipette that was cleaned with ethanol and distilled water after each sample. Before the screw caps on the fermentation vessels were opened, they were sprayed with 70% ethanol. Sampling was stopped when no further significant loss in weight was observed and Clinitest showed <0.25% reducing sugar in the wine. During fermentation the vessels had BioTech mixers (Nalgene) mounted on the top that were programmed to operate at 80 rpm. All stirrers were turned off before sampling and then turned on again after the sampling. Total sampling time for all samples was between 30 and 60 min. The finished wines were settled, racked, and put in cold storage.

**Derivatization and extraction.** To a 3-mL frozen fermentation sample, 30 µL of 2,4,5-trimethylthiazole solution (10 mg/mL) was added, together with 1 mL of cysteamine derivatization solution (0.03 M) and 3 drops of cresol red solution. The pH in the sample was then adjusted to ~8.5 with 5% NaOH (the cresol red indicated the pH in the sample by color, so the pH adjustment was done visually). The solution was left for 1 hr at room temperature to allow for complete derivatization of the aldehydes to their corresponding thiazolidines. If necessary, the sample was then re-adjusted to pH 8.5 with 5% NaOH after the reaction, and 1.5 mL of chloroform was added to extract the derivatized aldehydes. Dried magnesium sulfate was also added to the sample to reduce emulsion problems and was mixed well before the glass tubes were centrifuged at 1300 rpm for 15 min. The clear chloroform extract was removed with a glass pipette and dried with anhydrous sodium sulfate. The extraction and drying procedure was repeated on the sample remaining in the glass tube, and the combined chloroform extracts were transferred to vials and sealed with Teflon caps. The derivation and extraction method is modified from that described by Spaulding and Ebeler [3].

**Instrument conditions.** An aliquot (2 µL) of the clear chloroform extract was analyzed by gas chromatography using a
Hewlett Packard 6890 Series instrument (Hewlett Packard, Avondale, PA). The injector temperature was set to 250°C with a split ratio of 30:1. The column was a DB35 (35% phenyltrimethyl-polysiloxane) 30 m x 0.25 mm i.d. x 0.25 µm phase thickness (J&W Scientific, Folsom, CA). The carrier gas, helium, was set on constant flow with an average velocity of 30 cm/sec. The oven temperature was programmed to start at 80°C with a hold for 1 min. The oven temperature was then increased at a rate of 3.5°C/min to 115°C with no holding time. This was followed by a second increase, at a rate of 15°C/min, to a temperature of 180°C with a hold for 3 min. From there the third increase followed; the temperature was increased by 60°C/min to a final temperature of 220°C with a hold for 6 min. The detector was a nitrogen-phosphorus detector. The detector temperature was set to 260°C, and the output was set to ~30 pA. The hydrogen flow rate for the detector was set to 3 mL/min, and the air-flow rate was set to 60 mL/min. The make-up gas, helium, was set to 20 mL/min. HP ChemStation software was used to process all of the chromatographic data.

Results

Fermentation. All wines reached dryness and the final ethanol content measured 13.7 ± 0.1 vol.%. The color of the juice in fermentation vessels with 200 mg/L added SO2 changed from a uniform, light yellow color to a darker yellow, brown color at the end of the fermentation. There was no noticeable color change in the other fermentation vessels. As seen from the fermentation weight loss ratio graph (Figure 1), all treatments from 0 to 100 mg/L SO2 completed fermentation in 180 hr, or 7.5 days. The fermentations with 150 mg/L added SO2 experienced a slightly longer (~16 hr) lag period than the earlier mentioned fermentations and took slightly longer to complete (196 hr, or 8.2 days). Fermentations with 200 mg/L added SO2 had a different development: the lag period of the yeast was very long (~100 hr) and the rate of weight loss was slow during the entire fermentation. Total fermentation time was approximately 429 hr, or 17.9 days. Slightly greater weight loss occurred also compared to the other SO2 treatments, probably due to increased vaporization.

Formaldehyde (C1). An average of measured formaldehyde in the four first SO2 treatments (0, 50, 100, and 150 mg/L) and measured formaldehyde in the 200 mg/L SO2 treatment is shown in Figure 2. At the beginning of the fermentation, the formaldehyde level was 0.48 ± 0.01 mg/L for all samples. The formaldehyde concentrations remained constant until the fermentations reached approximately 110 hr, when the formaldehyde concentration for all SO2 additions except 200 mg/L rose sharply to ~1.0 mg/L. After reaching this value, the production of formaldehyde remained high until the end of the fermentation. The increase coincided with the fermentations reaching the end of the exponential phase. The formaldehyde concentration in the 200 mg/L SO2 addition fermentation remained constant at ~0.5 mg/L during the entire fermentation.

Acetaldehyde (C2). All SO2 treatments with their corresponding measured acetaldehyde values are shown in Figure 3. The acetaldehyde concentration at the beginning of the fermentation was 9.55 ± 0.15 mg/L for the 0, 50, and 100 mg/L SO2 treatments. For the 150 and 200 mg/L SO2 treatments, the initial concentration was 5.61 ± 1.18 mg/L. As seen in Figure 3, the time required for rapid production of acetaldehyde to be observed increased from ~12 to 60 hr as the SO2 concentration increased. For the 0, 50, and 100 mg/L SO2 treatments, acetaldehyde concentrations increased rapidly to ~68, 100, and 156 mg/L. These levels were reached at the time coinciding with the end of the lag period of the fermentation. During fermenta-

![Figure 1](https://example.com/fig1.png)

**Figure 1** Mean weight loss (fermentation progress) for the white grape juice fermentsations is represented as percent (%) of initial weight for each SO2 level. (n = 2. Where only one value was obtained due to sample preparations/analysis errors, this value was used.)

![Figure 2](https://example.com/fig2.png)

**Figure 2** Formaldehyde levels in white grape juice fermentations shown in black; △ represents average level from the 0, 50, 100, and 150 mg/L SO2 treatments and ○ represents the level from the 200 mg/L SO2 treatment. The fermentation progress is shown in gray; △ represents the average of the 0, 50, 100, and 150 mg/L SO2 treatments and ○ represents the mean from the 200 mg/L SO2 treatment.

The propanal concentration in the 0 to 150 mg/L SO\textsubscript{2} addition treatments are shown in Figure 4. The initial propanal concentration was 0.26 ± 0.01 mg/L for all fermentations. A rapid rise in isobutanal concentration in all fermentations occurred early in the fermentation, but the exact time for the increase depended on SO\textsubscript{2} addition. The delay time before isobutanal began to increase was ~36, 36, 50, and 80 hr for the 0, 50, 100, 150, 200 mg/L SO\textsubscript{2} treatments, respectively. The maximum isobutanal concentration for the 0 to 150 mg/L SO\textsubscript{2} treatment fermentations was ~0.65 mg/L, while for the 200 mg/L SO\textsubscript{2} addition the maximum value was ~0.9 mg/L. After the maximum levels were obtained, a relatively rapid decrease to ~0.5 mg/L for the 0 to 150 mg/L SO\textsubscript{2} treatments occurred and the concentrations then remained near this level. After reaching the maximum, the concentration of the 0 and 50 mg/L SO\textsubscript{2} treatments continued to rise slightly to ~93 and 112 mg/L, respectively. For the 150 and 200 mg/L SO\textsubscript{2} addition fermentations, the maximum acetaldehyde concentrations were higher, ~173 and 290 mg/L, respectively. Near the end of the exponential phase of the fermentation, the acetaldehyde levels for all treatments decreased and then remained nearly constant for the remainder of the fermentation. Final concentrations were higher with increased SO\textsubscript{2} addition, and the relative drop in acetaldehyde concentration after the maximum value had been reached appeared to be smaller as SO\textsubscript{2} levels increased.

**Propanal (C3).** The average measured propanal concentrations in the first four SO\textsubscript{2} treatments (0, 50, 100, and 150 mg/L) and measured propanal concentration in the 200 mg/L SO\textsubscript{2} treatment are shown in Figure 4. The initial propanal concentration for all SO\textsubscript{2} treatments was 0.26 ± 0.01 mg/L. The propanal concentration in the 0 to 150 mg/L SO\textsubscript{2} samples had a slow increase during the lag phase and early exponential phase of the fermentation. The propanal concentration increased to a maximum value of ~0.36 mg/L approximately half way through the exponential phase. The 200 mg/L SO\textsubscript{2} addition fermentation took a longer time to reach the maximum value of ~0.4 mg/L (140 versus ~80 hr). After the maximum concentration had been reached, the propanal concentration in the 0 to 150 mg/L SO\textsubscript{2} addition fermentations decreased fairly rapidly to ~0.28 mg/L, and then remained near this value until the end of the fermentation. The propanal concentration in the 200 mg/L SO\textsubscript{2} addition fermentation slowly decreased to approximately the same final concentration.

**2-Methyl-1-propanal (isobutanal, i-C4).** Only the 0, 150, and 200 mg/L SO\textsubscript{2} treatments with their corresponding measured 2-methyl-1-propanal values are shown in Figure 5 for simplicity. The isobutanal concentration was initially 0.49 ± 0.01 mg/L for all fermentations. A rapid rise in isobutanal concentration in all fermentations occurred early in the fermentation, but the exact time for the increase depended on SO\textsubscript{2} addition. The delay time before isobutanal began to increase was ~36, 36, 50, and 80 hr for the 0, 50, 100, 150, 200 mg/L SO\textsubscript{2} treatments, respectively. The maximum isobutanal concentration for the 0 to 150 mg/L SO\textsubscript{2} treatment fermentations was ~0.65 mg/L, while for the 200 mg/L SO\textsubscript{2} addition the maximum value was ~0.9 mg/L. After the maximum levels were obtained, a relatively rapid decrease to ~0.5 mg/L for the 0 to 150 mg/L SO\textsubscript{2} treatments occurred and the concentrations then remained near this level. After reaching the maximum, the concentration of the 0 and 50 mg/L SO\textsubscript{2} treatments continued to rise slightly to ~93 and 112 mg/L, respectively. For the 150 and 200 mg/L SO\textsubscript{2} addition fermentations, the maximum acetaldehyde concentrations were higher, ~173 and 290 mg/L, respectively. Near the end of the exponential phase of the fermentation, the acetaldehyde levels for all treatments decreased and then remained nearly constant for the remainder of the fermentation. Final concentrations were higher with increased SO\textsubscript{2} addition, and the relative drop in acetaldehyde concentration after the maximum value had been reached appeared to be smaller as SO\textsubscript{2} levels increased.

**Propanal (C3).** The average measured propanal concentrations in the first four SO\textsubscript{2} treatments (0, 50, 100, and 150 mg/L) and measured propanal concentration in the 200 mg/L SO\textsubscript{2} treatment are shown in Figure 4. The initial propanal concentration for all SO\textsubscript{2} treatments was 0.26 ± 0.01 mg/L. The propanal concentration in the 0 to 150 mg/L SO\textsubscript{2} samples had a slow increase during the lag phase and early exponential phase of the fermentation. The propanal concentration increased to a maximum value of ~0.36 mg/L approximately half way through the exponential phase. The 200 mg/L SO\textsubscript{2} addition fermentation took a longer time to reach the maximum value of ~0.4 mg/L (140 versus ~80 hr). After the maximum concentration had been reached, the propanal concentration in the 0 to 150 mg/L SO\textsubscript{2} addition fermentations decreased fairly rapidly to ~0.28 mg/L, and then remained near this value until the end of the fermentation. The propanal concentration in the 200 mg/L SO\textsubscript{2} addition fermentation slowly decreased to approximately the same final concentration.

**2-Methyl-1-propanal (isobutanal, i-C4).** Only the 0, 150, and 200 mg/L SO\textsubscript{2} treatments with their corresponding measured 2-methyl-1-propanal values are shown in Figure 5 for simplicity. The isobutanal concentration was initially 0.49 ± 0.01 mg/L for all fermentations. A rapid rise in isobutanal concentration in all fermentations occurred early in the fermentation, but the exact time for the increase depended on SO\textsubscript{2} addition. The delay time before isobutanal began to increase was ~36, 36, 50, and 80 hr for the 0, 50, 100, 150, 200 mg/L SO\textsubscript{2} treatments, respectively. The maximum isobutanal concentration for the 0 to 150 mg/L SO\textsubscript{2} treatment fermentations was ~0.65 mg/L, while for the 200 mg/L SO\textsubscript{2} addition the maximum value was ~0.9 mg/L. After the maximum levels were obtained, a relatively rapid decrease to ~0.5 mg/L for the 0 to 150 mg/L SO\textsubscript{2} treatments occurred and the concentrations then remained near this level. After reaching the maximum, the concentration of the 0 and 50 mg/L SO\textsubscript{2} treatments continued to rise slightly to ~93 and 112 mg/L, respectively. For the 150 and 200 mg/L SO\textsubscript{2} addition fermentations, the maximum acetaldehyde concentrations were higher, ~173 and 290 mg/L, respectively. Near the end of the exponential phase of the fermentation, the acetaldehyde levels for all treatments decreased and then remained nearly constant for the remainder of the fermentation. Final concentrations were higher with increased SO\textsubscript{2} addition, and the relative drop in acetaldehyde concentration after the maximum value had been reached appeared to be smaller as SO\textsubscript{2} levels increased.
200 mg/L SO₂ treatment had a very slow decrease to ~0.5 mg/L.

**Butanal (C4).** The initial concentration for butanal was 1.06 mg/L, and the concentration did not change with time in any of the fermentations. In the case of butanal then, there was no significant effect on butanal production regardless of the different SO₂ treatments.

**3-Methyl-1-butanal (isovaleraldehyde, i-C5).** Only the 0, 150, and 200 mg/L SO₂ treatments with their corresponding measured isovaleraldehyde concentrations are shown in Figure 6 for simplicity. The starting isovaleraldehyde concentration for all SO₂ treatments was 1.09 ± 0.01 mg/L. The concentration in the 0 mg/L SO₂ addition fermentation then slowly rose to a maximum value of ~1.2 mg/L before the concentration slowly decreased to ~1.1 mg/L, where it remained until the end of the fermentation. The increase in production in the 0 mg/L SO₂ additive fermentation coincided with early fermentation lag phase and reached a maximum half way through the exponential phase of the fermentation. For the 50 to 150 mg/L SO₂ addition fermentations, isovaleraldehyde concentrations rapidly increased to ~1.2, 1.4, and 1.5 mg/L, respectively, followed by a slow decrease in concentration over the course of the fermentation to ~1.1, 1.1, and 1.2 mg/L. The rapid increase in production with these SO₂ treatments coincided with early fermentation lag phase, and the maximum levels of isovaleraldehyde were reached at the end of the lag phase and/or in the beginning of the exponential phase. The 200 mg/L SO₂ addition fermentation had a slow increase in isovaleraldehyde concentration to a maximum of ~1.3 mg/L, which was reached approximately midway through the exponential phase of the fermentation. The concentration decreased slowly at the end of the fermentation to ~1.1 mg/L.

**Pentanal (C5).** The initial pentanal concentration for all treatments was 1.12 ± 0.01 mg/L and remained near this level (1.13 to 1.18 mg/L) for the entire fermentation. There was little effect of SO₂ addition on pentanal production; however, the highest pentanal level at the end of fermentation was observed for the 200 mg/L SO₂ treatment.

**Hexanal (C6).** An average of measured hexanal concentrations in the four first SO₂ treatments (0, 50, 100, and 150 mg/L) and measured hexanal concentration in the 200 mg/L SO₂ treatment are shown in Figure 7. The initial hexanal concentration for all the different SO₂ treatments was 1.47 ± 0.0 mg/L. The control experienced a delay of ~40 hr before an increase in hexanal concentration occurred; this delay coincided with the lag in the fermentation curve. The concentration reached a maximum of ~1.6 mg/L early in the exponential phase of the fermentation, and then decreased again to a value of 1.47 mg/L, where it remained until the fermentation ended. Samples with SO₂ added followed the same general trend, but had a longer delay (40, 40, 60, and 100 hr) in hexanal production with increasing SO₂ levels (50, 100, 150, 200 mg/L SO₂, respectively). The maximum hexanal concentrations were 1.6, 1.6, 1.55, and 1.8 mg/L, and the final values were ~1.5 mg/L for all treatments.

**Heptanal (C7).** An average of measured heptanal concentration in the four first SO₂ treatments (0, 50, 100, and 150 mg/L) and measured heptanal concentration in the 200 mg/L SO₂ treatment are shown in Figure 8. The initial heptanal concentration for the different SO₂ treatments was 1.3 ± 0.04 mg/L. The control had a rapid drop in heptanal concentration after ~24 hr to a minimum of ~1.18 mg/L, before slowly increasing to ~1.22 mg/L when the fermentation ended. The added SO₂ fermentations followed a similar trend, but the delays in heptanal production were ~36, 36, 60, and 92 hr instead of ~24 hr. The final heptanal value for all treatments was 1.21 ± 0.02 mg/L.

**Octanal (C8) and nonanal (C9).** The values for the concentrations for octanal and nonanal were below the limit of detection for all treatments.
quantitation and only occasionally reached measurable concentrations. The spikes in concentration were too randomly placed to develop a clear pattern for these compounds.

**Discussion**

The adapted derivation and extraction procedure used in this study allows individual aldehydes to be monitored simultaneously throughout the fermentation. The method has ~90% recovery for the short-chain aldehydes (C1 to C5) and between 40 and 80% recovery for the long-chain aldehydes (C6 to C9) [4]. The noise seen in the data could be caused by the fact that in most cases the concentrations measured were very close to the limit of quantitation. In addition, aldehydes that are produced and volitized as gases during fermentation were not measured in this analysis. This gas disappearance can vary and can be a main cause of the variability seen in the results.

The final acetaldehyde level in this study was between 19 and 183 mg/L for all SO₂ treatments, compared to a study of German wines of varying quality [7], which reported a range between 6.7 and 66.8 mg/L acetaldehyde (unknown SO₂ addition). The German wines also contained propanal, butanal, pentanal, and hexanal in ranges of 0.06 to 2.27, 0.01 to 1.41, 0.05 to 14.36, and 0 to 0.24 mg/L, respectively. In the current study, the average final concentration level for these compounds was 0.28 ± 0.007, 1.06, 1.15 ± 0.03, and 1.5 mg/L, respectively, which, with the exception of hexanal, are within the ranges reported in the German wines. The German study noted that wines spoiled with lactic acid bacteria had the higher observed levels of acetaldehyde and longer chain aldehydes.

An Austrian study measured acetaldehyde concentrations during fermentation with different amounts of SO₂ added to red mash [6]. When 0 mg/L SO₂ was added to the red mash, acetaldehyde concentrations were initially 12 mg/L, reached a maximum of 100 mg/L, and ended at 15 mg/L acetaldehyde. When 100 mg/L SO₂ was added, similar initial and final concentrations were obtained, but the maximum value was 60 mg/L. When 200 mg/L SO₂ was added, the initial concentration was the same, the highest maximum was 150 mg/L, and the final concentration measured 50 mg/L. In the current study, similar trends of increased maxima and final acetaldehyde levels were observed at the highest SO₂ levels; however, the absolute concentrations were greater than reported by Paul [6]. One reason for the variability obtained may be that juice from concentrate was used in the present study, which may react differently from fresh-pressed juice. It must also be remembered that white and red grape fermentations have different relationships with SO₂, which may explain the concentration differences in the two studies.

Acetaldehyde, 2-methyl-1-propanal, and 3-methyl-1-butanal were most affected by SO₂, both in the higher maximum concentrations formed during fermentation and in the change in production patterns. Final acetaldehyde concentrations also increased as SO₂ increased, while SO₂ had less effect on the final concentrations of 2-methyl-1-propanal and 3-methyl-1-butanal. These three aldehydes appeared to be formed early in the fermentation.

Aldehydes that appeared to be formed later in the fermentation, such as formaldehyde, propanal, pentanal, hexanal, and heptanal, were not as sensitive to the different levels of SO₂. However, all of these later-produced aldehydes did have a slight change in the production pattern at the 200 mg/L SO₂ treatment level. This is most likely associated with the effects of SO₂ on the length of the initial lag phase of the fermentation. It is also possible that early in the fermentation there is sufficient free SO₂ in all treatments to affect the production/pattern of aldehydes; however, for the lower SO₂ treatments, all free SO₂ is eventually bound, resulting in less effect on the aldehydes. In the 200 mg/L SO₂ treatment, the free SO₂ remains high throughout the fermentation; therefore, the effects may persist longer through the fermentation.

Alcohol dehydrogenase (ADH) is the enzyme that reduces the intermediate acetaldehyde to ethanol. There are four species or isozymes of this enzyme in Saccharomyces, and ADH-I is responsible for the conversion of acetaldehyde to ethanol. This enzyme is constitutive, but the concentration or activity is dependent on the yeast strain, yeast growth phase, stage of fermentation, and fermentation temperature. As discussed previously, when SO₂ inhibits the ADH enzyme, acetaldehyde concentration begins to increase. Free SO₂ binds with the produced acetaldehyde in the white grape juice/wine, preventing regeneration of NAD⁺ from NADH through the reduction of acetaldehyde to ethanol. This again leads to an inhibition of the pathway from glyceraldehyde 3-phosphate leading to pyruvate, since the cofactor NAD⁺ is required for this reaction. This may force the use of alternative pathways such as the production of glycerol via glycerophosphate. The switch from acetaldehyde to dihydroxyacetone phosphate as a hydrogen acceptor will continue until acetaldehyde is produced in amounts that will exceed the acetaldehyde level that is bound by the free SO₂. Future studies should measure available or free SO₂ and should include measurements of other metabolic products such as glycerol.
An improved understanding of aldehyde formation may lead to better control of factors that influence the aldehyde levels. More research on sensory properties of the aldehydes is needed in order to relate relative quantitative measurements with the flavor of the final wine. Depending on the desired sensory “picture” of the wines produced, one can introduce different amounts of SO2 to obtain different levels of aldehydes in the wine and begin to optimize the SO2 additions. Future trials are needed to fully evaluate the sensory properties of wines with variable SO2 levels.

Summary

Using a derivation and extraction method, production of short-chain, volatile aldehydes was readily monitored during white grape juice fermentation. Acetaldehyde (C2), 2-methyl-1-propanal (i-C4), and 3-methyl-1-butanal (i-C5) were most affected by different levels of SO2 added to the juice. In general, aldehydes C1 to C7 were found to be affected when the levels of SO2 reached 200 mg/L. Understanding factors that influence aldehyde production is critical for producing wines with optimal sensory and chemical properties.

Literature Cited