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Optimizing high-altitude peach fermentation biochemistry across the Colorado front range via 2x3 mixed level full factorial design

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Abstract
Colorado produces ~ 15,000 tons of peaches annually, yet peach wine production on the Colorado Front Range is challenged by a high-altitude microclimate characterized by elevated solar radiation, a shortened growing season, and reduced atmospheric pressure that decreases oxygen mass transfer during fermentation. This study employed a 2 × 3 mixed-level full-factorial Design of Experiments (DOE) to optimize primary fermentation by evaluating two YAN levels (150 and 300 mg/L) and three aeration rates (0.0, 5.5, and 11.0 L/day). To minimize variation associated with indigenous microorganisms, peach musts were inoculated with a standardized *Saccharomyces cerevisiae* strain following fruit sanitization. Day-18 residual sugar and ethanol concentration were analyzed using analysis of variance (ANOVA), factorial regression modeling, and multi-response treatment evaluation. Both regression models demonstrated excellent performance, explaining 96.95% of the variation in residual sugar ($p < 0.001$) and 90.26% of the variation in ethanol concentration ($p = 0.005$). Significant YAN × aeration interactions were identified for both response variables, demonstrating that nitrogen supplementation and oxygen availability should be optimized jointly rather than independently. Multi-response evaluation identified 150 mg/L YAN and 11.0 L/day aeration as the best-performing tested treatment combination, producing an off-dry to semi-sweet wine with 12.3% (v/v) ethanol and 23.9 g/L residual sugar. These findings provide an evidence-based process optimization strategy for high-altitude peach wine fermentation and demonstrate the value of factorial experimental design for optimizing interacting fermentation variables.

Keywords
Yeast assimilable nitrogen, High-altitude fermentation, Peach wine, Fruit wine fermentation, Aeration, Fermentation kinetics, Design of experiments, Factorial design, Residual sugar, Ethanol production

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1. Introduction

Colorado ranks fourth in peach production nationally and has aggressively expanded its fruit oenology sector and associated agrotourism (1). The state's non-grape commercial wine industry expanded nearly twentyfold since the early 1990's, with the majority of that growth centered on the Front Range (1). This pomology, and peach or stone-fruit in particular, are challenged by the Front Range's unique micro-climate. This high-altitude, high solar radiation, short growing season creates three primary obstacles to stone-fruit viniculture:

[1] The Front Range receives, on average, 5.62 [kWh/(m^2xday)] of solar radiation (2). This is ~ 14% more than California's primary growing region [4.93 kWh/(m^2xday)] in the Central Valley (3) and ~ 6% more than Georgia's primary growing region [5.32 kWh/(m^2xday)] in the central plains (4). This comparatively high solar radiation may alter fruit composition, surface microbial communities, or other physicochemical and biological characteristics of the fruit that subsequently influence fermentation performance and outcomes.

[2] The Front Range suffers from a shorter growing season than its peach-producing competitors (California, Georgia, and South Carolina rank 1st, 2nd, and 3rd by weight, with Colorado in 4th place). The Front Range has only three months in which the average daily low temperature is above 10 ºC (5), while Fresno and Macon have six and seven months respectively (6,7). The Front Range also has a shorter growing season than major competing peach-producing regions. Because inadequate YAN can impair yeast growth and fermentation performance, YAN availability was treated as an experimentally controllable process variable in this study (8,9).

[3] The Front Range is a high-altitude micro-climate, between 5,000 ft and 6,000 ft above sea level. This research was performed at 5,600 ft ASL, where air pressure is 0.812 atm (82.3 kPa), and natural oxygen absorption into the must is therefore expected to be reduced by ~18%. High-altitude commercial winemaking has similarly been reported to occur under ~ 18% lower oxygen availability, with slower primary fermentation than at lower elevation (10). In contrast, this study applied carefully controlled and quantified aeration, enabling rigorous statistical analysis and greater confidence in defining the effective process solution space.

Over the past two decades, production of peach and other stone-fruit wines has expanded across the United States, with Colorado participating in this growing sector. The growth of small-scale wineries has contributed to regional economic activity, while expanding agritourism has provided additional opportunities for direct-to-consumer sales and improved producer margins. Despite this growth, a notable gap remains in the peer-reviewed literature regarding how concurrent abiotic constraints associated with high-altitude fermentation can be systematically mitigated through process engineering to preserve fermentation performance and final product quality. This study uses a Design of Experiments (DoE) framework to quantify the individual and interactive effects of yeast-assimilable nitrogen supplementation and controlled mechanical aeration as a multivariable mitigation strategy for optimizing high-altitude fermentation kinetics. The approach enables identification of operating

conditions that compensate for altitude-associated constraints while providing a statistically defined process solution space.

2. Methods

2.1 Research design

This research established a framework to optimize the interactive effects of key environmental constraints on high-altitude primary fermentation kinetics.

Yeast-assimilable nitrogen (YAN) is an important determinant of fermentation performance because yeast requires accessible nitrogen sources for biomass formation and metabolic activity (8, 9,11). Because native YAN was not directly measured in the experimental peach must, this study treated YAN supplementation as an experimentally controlled process variable rather than assuming a measured regional YAN deficiency.

Yeast similarly exhibits an absolute requirement for dissolved oxygen during the early exponential growth phase to drive biosynthesis. Yeast requires oxygen during early growth for biosynthetic processes, and inadequate oxygen and nutrient availability can contribute to sluggish or stuck alcoholic fermentation (9,12,13). Aeration treatment levels were designed to compensate for the ~ 18.6% reduction in oxygen partial pressure and associated oxygen mass-transfer driving force estimated for the Front Range experimental altitude.

Wild microflorae exist naturally on all raw agricultural inputs. Attempting to track and control this diversity through primary fermentation would have introduced unmanageable experimental noise. Because industrial production relies heavily on single-strain yeasts, peach skins were cleaned to reduce wild microflorae before introduction into the musts. All trials were inoculated with standardized commercial *Saccharomyces cerevisiae* (Lalvin-71B), an industry standard for stone-fruit fermentations.

These environmental boundaries and process management constraints dictated the deployment of a 2x3 mixed level full-factorial Design of Experiment (DoE). Two levels were chosen for YAN: a low, baseline setting of 150 mg/L (coded as -1) and a high-supplementation setting of 300 mg/L (coded as +1). Three levels were chosen for aeration: a low control of 0.0 L/day (coded as -1), a mid-tier configuration at 5.525 L/day (coded as 0), and an upper operational limit of 11.050 L/day (coded as +1).

Crossing these factor levels generated six unique treatment combinations. To strengthen the Analysis of Variance and enable the isolation of pure experimental error, the six-treatment matrix was fully replicated, for a total of 12 distinct fermentation trials.

The specific objective of this research was to manipulate these two independent inputs to achieve a target window for two simultaneous dependent variables: final ethanol yield and residual sugar concentration. Representative commercial peach wines are marketed at approximately 12% ABV (14,15). Based on these commercial examples, an experimental target range of 11.0–12.5% ABV was selected.

A residual sugar range of 15–30 g/L was selected to represent an off-dry to semi-sweet product target **(**16**)**. Thus, the multi-response optimization parameters for this DoE model were constrained to a target ethanol concentration of 11.0%-12.5% v/v and a residual sugar concentration of 15.0-30.0 g/L.

2.2 Material selection

Redhaven peaches are commonly utilized for Colorado peach wine production due to their environmental suitability. This experiment utilized 48 lbs of Redhaven peaches. Acid blend, pectic enzyme, tannin, yeast energizer, metabisulfite, cane sugar, Lalvin-71B winemakers' yeast (in 5 g packs) were commercially sourced.

2.3 Test equipment Selection

Commercially sourced fermentation supplies consisted of 2-gallon fermentation vessels, fermentation airlocks, polyethylene catheters, 250 mL syringes, aerator pumps (Aquaneat AP-204) tuned to deliver 5.5 L/day and 11.1 L/day and a Hydrometer model (SOLIGT, triple scale hydrometer). Representative images of the set-up and equipment are shown in Appendix A.

2.4 Calculations

YAN, supplemental aeration modeling, barometric, fluid density response, ethanol, and residual sugar calculations are presented in Appendix B.

2.5 Set up

All vessels were sanitized. Two penetrations were fabricated through each lid, and a third penetration was fabricated through eight of the twelve lids. Airlocks were installed in the first penetration to permit out-gassing while preventing atmospheric contamination during the experiment. In the second penetration a polyethylene catheter was permanently installed to enable daily sample draws without the need to open the vessels. Vessels were never shaken during the entire duration of the experiment. All catheters were installed to a depth of 2" above the vessel floor.

Of the 12 trials, four would receive 11 L/day of supplemental aeration, four would receive 5.5 L/day of supplementation aeration, and four would not receive any supplemental aeration.

Two commercial aerators (Aquaneat AP-204) were sourced, each with four output ports. Silicone tubing of equal lengths was attached to each manifold port. Flow rates through each individual tube were measured, and the tube lengths were tuned to equalize the four rates on each of the two aerators, in units of s/L. For the first aerator, this flow rate was transformed into the necessary number of seconds (352 s) to provide 11.05 L of air to the (+1) aeration treatment vessels. For the second aerator, this flow rate was transformed into the necessary number of seconds (190 s) to provide 5.525 L of air to the center-level (coded 0) aeration treatment vessels.

Through four of the lids with a third penetration, a stainless-steel micro-sparger was inserted, connected to the first aerator, and set to 352 s of air injection per day. These were configured to be trials for which aeration would be at its (+1) level. Through the other four lids with a third penetration, an identical micro-sparger was inserted, connected to the second aerator, which

was set to 190 s of air injection per day. These were configured to be trials for which aerations would be at its coded (0) level. Air was drawn from the room and was not filtered.

All raw materials were scrubbed to reduce surface-associated environmental molds and bacteria. Residual bioburden after this step was not measured. All were pitted and chopped into 0.23”x0.23” batonnets by a commercial food processor. Samples were blended into 12 homogenous 2.50 lb batches and loaded into the trial vessels. Each vessel then received identical material matrix to ensure that treatments and trials were identical before the application of any treatment differentiation.The standardized peach must formulation used for all experimental fermentations is provided below.

Peach must formulation

Qty	Ingredients
7 pints	water
2.00 lb	Cane Sugar
1.5 tsp	Acid Blend (40% malic, 20% citric, 40% tartaric)
1.0 tsp	Pectic Enzyme
0.25 tsp	Tannin (grape, desiccated and pulverized)
0.25 tsp	Yeast Energizer (detailed in Appendix B.1, YAN Calculations)
1 tablet	Campden (providing 0.44g potassium metabisulfite, $K_2S_2O_5$ as a baseline sterilizer)
5.0 g	Yeast, Lalvin-71B (*Saccharomyces cerevisiae*)

To the half of treatments assigned the (-1) YAN level, 0.602 g of DAP were added, and to the half of treatments assigned the (+1) YAN level , 3.280 g of DAP were added (see calculations in Appendix B).

Initial density measurements were recorded for each of the 12 vessels, and subsequent density measurements were recorded for each vessel, each day, for the duration of the experiment. A sample volume of 200ml was drawn through the catheter for each measurement for a particular vessel. The sample was returned through the same catheter after measurement. Airlock CO_2 outgassing served as a nonquantitative representation of the rate of fermentation. Progressive declines in must density enabled quantitative calculations of the rate of fermentation (including the lag time) as well as of the evolution of residual sugar and ethanol concentrations.

2.6 Statistical analysis

Minitab Statistical Software (Version 22; Minitab, LLC, State College, PA, USA) was utilized to analyze the DoE with day-18 residual sugar (g/L) and day-18 ethanol (%v/v) as the sole responses. Statistical significance was evaluated using a predefined $\alpha = 0.10$ threshold, with results between $p = 0.05$ and $p = 0.10$ interpreted cautiously as weaker statistical evidence. For simplicity, the factors’ levels were labeled (150,300) (coded as -1 and +1 for the DoE) for YAN and (0.0,5.5,11.0) (coded as -1, 0

and +1 for the DoE) for aeration. The resultant ANOVAs (with R^2 values), regressions, main effects plots, and interaction plots for residual sugar and ethanol were then calculated and presented by the software. Significant figures for the software calculated coefficients and statistics do not represent measurement precision.

Units are deliberately presented throughout this paper in the forms most commonly used in industrial fermentation processes, rather than being converted to a single uniform system of measurement.

3. Results

3.1 Daily fermentation results

Nineteen daily measurements (day 0 through day 18) were obtained from each of the 12 fermentation vessels, producing 228 specific-gravity measurements in total (Table 1). Initial specific gravity was highly similar across vessels, ranging from 1.079 to 1.081, providing a consistent starting point from which subsequent fermentation progression could be compared. Specific gravity decreased in every vessel over the 18-day observation period, although both the onset and rate of decline differed among treatments. By day 18, specific gravity ranged from 0.982 to 0.996.

Table 1. Daily specific gravity profiles across fermentation vessels

Density	Vessel 01	Vessel 02	Vessel 03	Vessel 04	Vessel 05	Vessel 06	Vessel 07	Vessel 08	Vessel 09	Vessel 10	Vessel 11	Vessel 12
Day-0	1.081	1.081	1.080	1.079	1.080	1.081	1.081	1.080	1.079	1.081	1.080	1.079
Day-1	1.081	1.080	1.080	1.079	1.081	1.057	1.083	1.079	1.078	1.082	1.078	1.052
Day-2	1.081	1.080	1.067	1.069	1.067	1.035	1.080	1.079	1.065	1.068	1.053	1.031
Day-3	1.078	1.069	1.053	1.056	1.031	1.006	1.081	1.069	1.055	1.052	1.032	1.003
Day-4	1.070	1.055	1.042	1.042	1.016	0.985	1.071	1.050	1.040	1.042	1.010	0.988
Day-5	1.063	1.037	1.026	1.022	0.983	0.984	1.058	1.042	1.022	1.025	0.987	0.983
Day-6	1.048	1.025	1.011	1.018	0.989	0.982	1.051	1.027	1.014	1.015	0.986	0.983
Day-7	1.042	1.010	1.004	1.007	0.984	0.982	1.037	1.021	1.003	1.006	0.986	0.983
Day-8	1.024	1.005	0.988	0.999	0.983	0.984	1.030	1.004	0.992	0.993	0.983	0.983
Day-9	1.018	0.990	0.990	0.999	0.983	0.984	1.021	0.994	0.991	0.993	0.984	0.983
Day-10	1.012	0.993	0.988	0.998	0.985	0.983	1.016	0.992	0.990	0.992	0.985	0.982
Day-11	1.008	0.992	0.989	0.997	0.984	0.982	0.999	0.990	0.989	0.993	0.985	0.983
Day-12	0.995	0.990	0.989	0.996	0.985	0.982	0.996	0.993	0.988	0.994	0.984	0.984
Day-13	0.993	0.991	0.988	0.996	0.984	0.983	0.996	0.991	0.989	0.992	0.984	0.982
Day-14	0.992	0.991	0.989	0.995	0.985	0.983	0.993	0.989	0.989	0.993	0.984	0.984
Day-15	0.992	0.990	0.988	0.997	0.985	0.984	0.995	0.988	0.990	0.994	0.985	0.983
Day-16	0.991	0.989	0.990	0.996	0.984	0.982	0.995	0.989	0.988	0.994	0.982	0.983
Day-17	0.991	0.988	0.989	0.997	0.983	0.983	0.994	0.988	0.987	0.994	0.985	0.982
Day-18	0.993	0.987	0.990	0.996	0.984	0.982	0.993	0.989	0.988	0.993	0.984	0.982

The temporal profiles in Figure 1 show that the principal differences among treatments occurred during the early and intermediate phases of fermentation rather than at initiation. Some vessels exhibited rapid decreases in specific gravity beginning within the first several days, whereas others showed a delayed and more gradual decline. The most rapidly progressing

fermentations approached their terminal specific-gravity values by ~ days 5–7, while the slowest continued to show appreciable decreases through ~ days 10–12. Thereafter, the trajectories generally plateaued, with relatively small changes in specific gravity through day 18. Thus, although all vessels underwent substantial fermentation, the daily measurements demonstrate clear differences in fermentation kinetics among the experimental conditions.

The residual-sugar trajectories derived from these density measurements closely mirrored the specific-gravity profiles (Figure 2). All vessels began with similar calculated residual-sugar concentrations of ~ 234–240 g/L. Residual sugar subsequently decreased in every vessel, but the timing and magnitude of sugar depletion varied substantially. By day 18, residual sugar ranged from 8.8 to 38.8 g/L (Table 2a), representing an ~ fourfold range in terminal residual-sugar concentration despite the similar starting conditions. The graphical trajectories further show that much of this separation among vessels was established during the first ~ 10 days of fermentation and was largely retained after the curves approached their terminal plateaus.

Ethanol production exhibited the reciprocal temporal pattern (Figure 3). All vessels began at 0% ethanol, after which ethanol concentration increased as residual sugar declined. The fastest increases in ethanol corresponded temporally to the steepest decreases in specific gravity and residual sugar, whereas vessels with slower declines in these parameters exhibited correspondingly slower ethanol accumulation. Most ethanol trajectories approached a plateau by ~ days 7–12, depending on treatment. By day 18, ethanol concentrations ranged from 11.4% to 13.4% (v/v) across the 12 vessels (Table 2b).

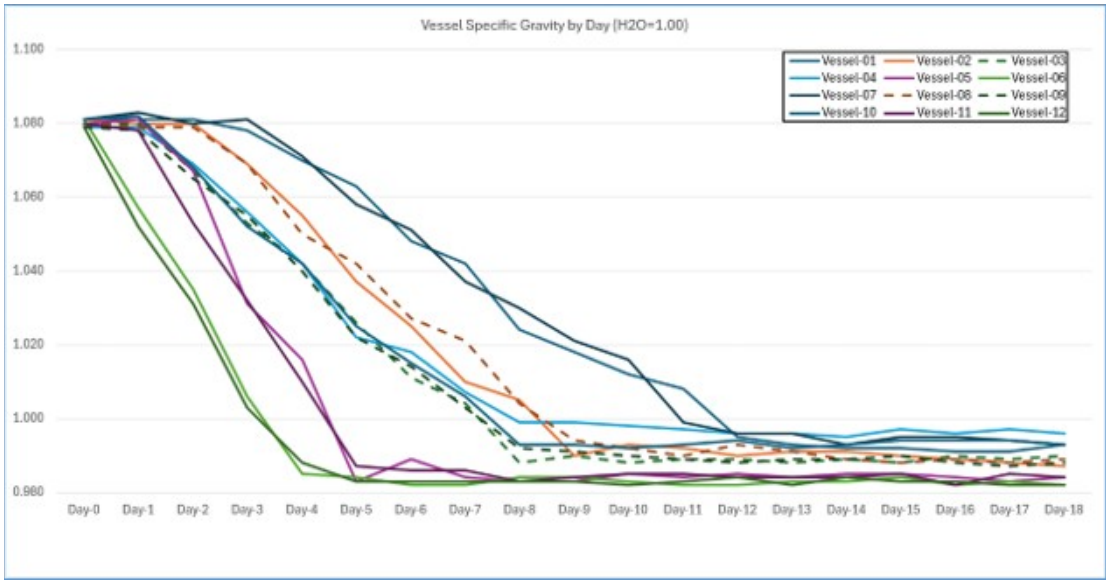

Figure 1. Temporal profiles of specific gravity across fermentation treatments

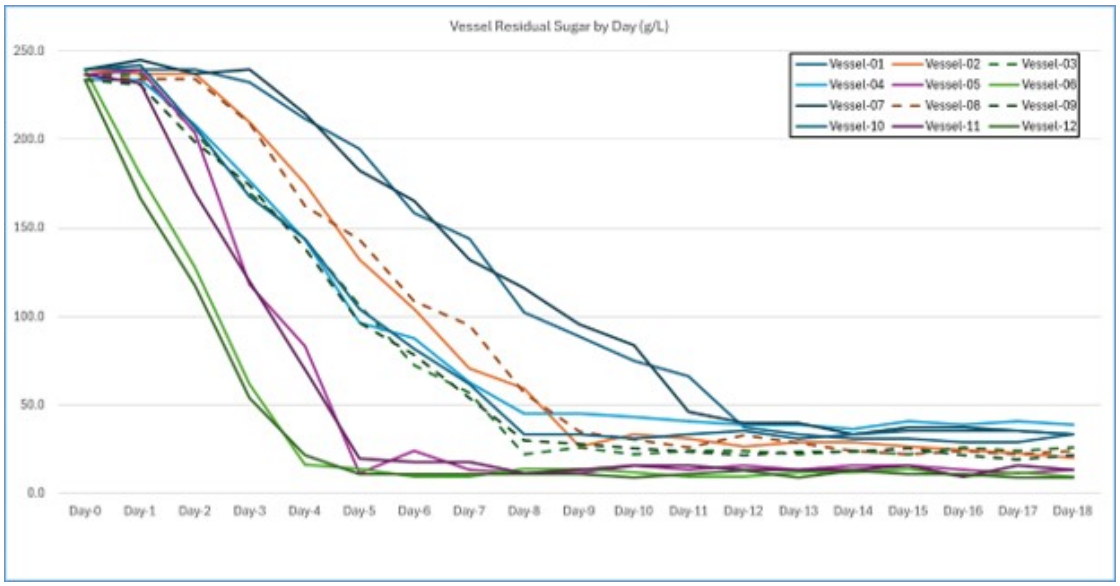

Figure 2. Temporal profiles of residual sugar depletion across fermentation treatments

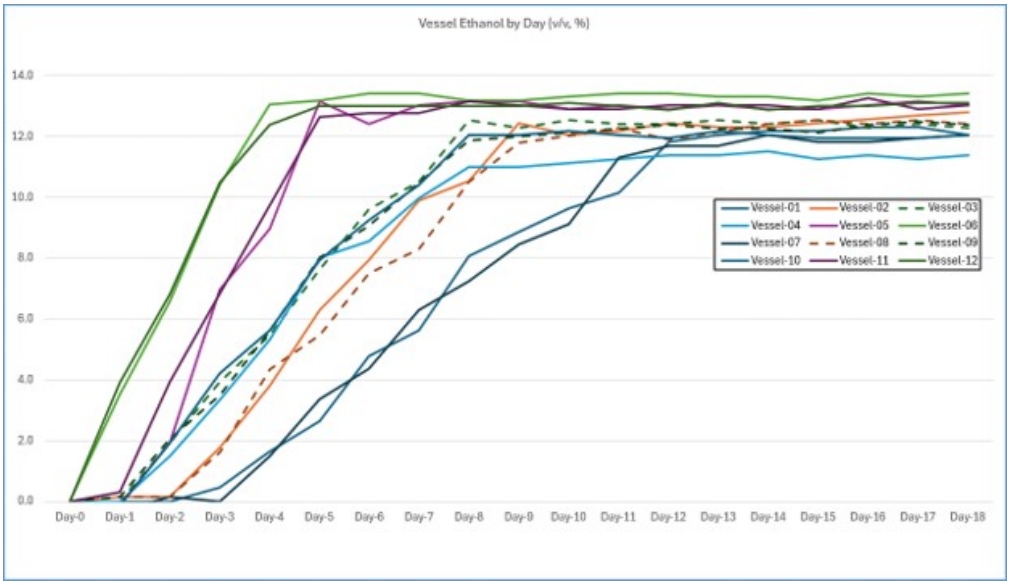

Figure 3. Temporal profiles of ethanol production across fermentation treatments

Taken together, Figures 1–3 demonstrate internally consistent progression of the three fermentation measures: decreasing specific gravity was accompanied by decreasing residual sugar and increasing ethanol concentration. The treatment trajectories separated primarily during

the active phase of fermentation and subsequently converged toward treatment-specific plateaus. Importantly, the final products did not converge to a single common endpoint. Instead, measurable differences remained at day 18 in both residual sugar and ethanol concentration, providing the terminal response values subsequently used for the factorial analysis.

Table 2. Residual sugar & ethanol by vessel, day-0 vs day-18

Table 2a. Residual sugar (g/L)

Day	Vessel ID											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0	239.8	239.8	236.7	233.7	236.7	239.8	239.8	236.7	233.7	239.8	236.7	233.7
18	33.2	20.3	26.3	38.8	13.5	9.7	33.2	24.1	21.5	33.2	13.5	8.8

Table 2b. Ethanol (%v/v)

Day	Vessel ID											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	12.1	12.8	12.3	11.4	13.0	13.4	12.1	12.4	12.4	12.1	13.0	13.1

3.2 Results specific to residual sugar concentrations

The general factorial regression model for day-18 residual sugar (RS) was statistically significant ($F = 38.17$, $p < 0.001$; Table 3).

Table 3. Regression model: RS vs YAN, Aeration

Table 3a. Factor information

Factor	Levels	Values
YAN	2	150, 300
Aeration	3	0.0, 5.5, 11.0

Table 3b. Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1107.60	221.521	38.17	0.000
Linear	3	950.22	316.740	54.57	0.000
YAN	1	140.77	140.767	24.25	0.003
Aeration	2	809.45	404.726	69.73	0.000
2-Way Interactions	2	157.38	78.692	13.56	0.006
YAN*Aeration	2	157.38	78.692	13.56	0.006
Error	6	34.82	5.804		
Total	11	1142.43			

The model explained 96.95% of the observed variation in RS, with an adjusted R^2 of 94.41% and a predicted R^2 of 87.81%. Both experimental factors exhibited significant main effects. YAN significantly affected RS concentration ($F = 24.25$, $p = 0.003$), while aeration produced a larger effect within the tested design space ($F = 69.73$, $p < 0.001$). The YAN × aeration interaction was also significant ($F = 13.56$, $p = 0.006$), indicating that the effect of YAN on terminal RS depended on the level of aeration.

Table 3c. Model summary

S	R^2	R^2(adj)	R^2(pred)
2.40918	96.95%	94.41%	87.81%

The regression coefficients further characterized the magnitude and direction of these effects (Table 4). The model constant was 23.008 g/L. The fitted coefficients for YAN were +3.425 g/L at 150 mg/L and −3.425 g/L at 300 mg/L ($p = 0.003$). The corresponding aeration coefficients were +11.592 g/L at 0 L/day, −5.158 g/L at 5.5 L/day, and −6.433 g/L at 11.0 L/day ($p \leq 0.002$). The larger magnitude of the aeration coefficients was consistent with the larger aeration F-value observed in the ANOVA.

Table 4. Regression model: RS vs YAN, Aeration

Table 4a. Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	23.008	0.695	33.08	0.000	
YAN					
150	3.425	0.695	4.92	0.003	1.00
300	-3.425	0.695	-4.92	0.003	*
Aeration					
0.0	11.592	0.984	11.79	0.000	1.33
5.5	-5.158	0.984	-5.24	0.002	1.33
11.0	-6.433	0.984	-6.54	0.001	*
YAN*Aeration					
150 0.0	-4.825	0.984	-4.91	0.003	1.33
150 5.5	0.925	0.984	0.94	0.383	1.33
150 11.0	3.900	0.984	3.97	0.007	*
300 0.0	4.825	0.984	4.91	0.007	*
300 5.5	-0.925	0.984	-0.94	0.383	*
300 11.0	-3.900	0.984	-3.97	0.007	*

The regression coefficients also characterized the significant YAN × aeration interaction. At 0 L/day aeration, the interaction coefficients were −4.825 for 150 mg/L YAN ($p = 0.003$) and

+4.825 for 300 mg/L YAN (p = 0.007). At 11.0 L/day, the corresponding coefficients reversed direction, to +3.900 and −3.900, respectively (p = 0.007). In contrast, at the intermediate aeration level of 5.5 L/day, the interaction coefficients were substantially smaller (+0.925 and −0.925) and were not statistically significant (p = 0.383). Thus, both the magnitude and direction of the YAN effect varied across aeration levels, consistent with the significant interaction identified by the overall ANOVA.

The complete fitted regression equation, incorporating the YAN, aeration, and YAN × aeration terms, is presented in Table 4b. The factor structure comprised two YAN levels (150 and 300 mg/L), three aeration levels (0, 5.5, and 11.0 L/day), and the resulting six YAN × aeration treatment combinations (Table 4c).

Table 4b. Regression equation

Component	Equation
Residual Sugar	**23.008** + 3.425 YAN_150 − 3.425 YAN_300 + 11.592 Aeration_0.0 − 5.158 Aeration_5.5 − 6.433 Aeration_11.0 − 4.825 YAN*Aeration_150 0.0 + 0.925 YAN*Aeration_150 5.5 + 3.900 YAN*Aeration_150 11.0 + 4.825 YAN*Aeration_300 0.0 − 0.925 YAN*Aeration_300 5.5 − 3.900 YAN*Aeration_300 11.0

Table 4c. Factor information

Factor	Levels used in model
YAN	150, 300
Aeration	0.0, 5.5, 11.0
Interaction	YAN × Aeration (six treatment combinations)

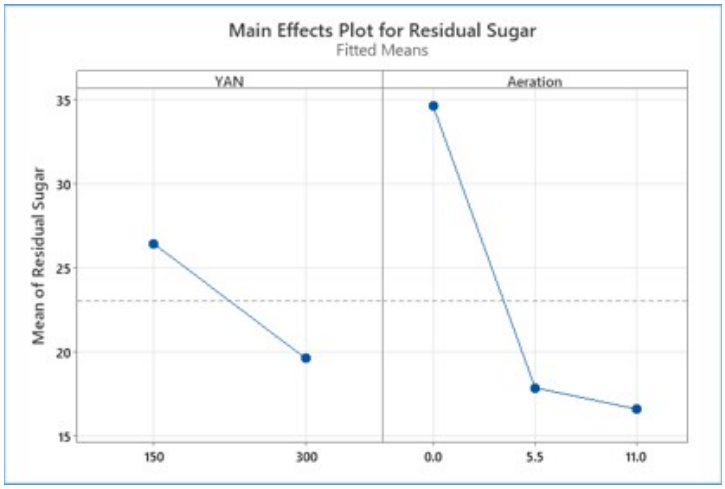

Figure 4. Main effects plot: RS vs YAN, Aeration

The main-effects plot (Figure 4) shows that mean RS decreased as YAN increased from 150 to 300 mg/L. A larger change was observed across aeration levels: mean RS was highest

without supplemental aeration and decreased markedly at 5.5 L/day, with a smaller additional decrease at 11.0 L/day. Mean RS at 0 L/day was above the predefined target range of 15–30 g/L, whereas the mean responses at 5.5 and 11.0 L/day were within the target range, at ~ 17.85 and 16.58 g/L, respectively.

The interaction plot (Figure 5) demonstrates that the effect of YAN was dependent on aeration level. At 0 L/day aeration, both YAN levels produced mean RS concentrations above the target range, with increasing YAN associated with a modest increase in RS. In contrast, at 5.5 and 11.0 L/day aeration, increasing YAN from 150 to 300 mg/L decreased terminal RS. Consequently, the direction of the YAN effect changed according to aeration level, consistent with the significant YAN × aeration term identified by ANOVA.

The regression coefficients further characterized this interaction (Table 4). Interaction coefficients were statistically significant at the 0 L/day aeration level ($p = 0.003–0.007$) and at 11.0 L/day ($p = 0.007$), whereas the interaction coefficients at 5.5 L/day were not significant ($p = 0.383$). The interaction coefficients also changed sign across the aeration levels, further demonstrating that the relationship between YAN and RS was not constant across the experimental design space.

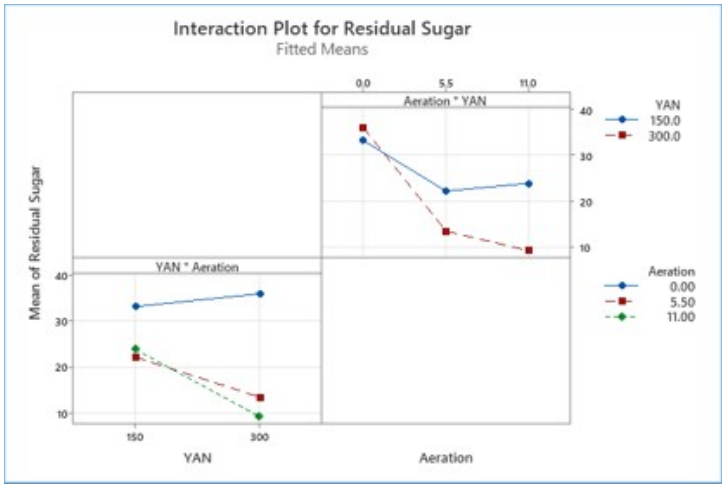

Figure 5. Interaction plot: RS vs YAN, Aeration

The residual diagnostics (Figure 6) showed no prominent systematic pattern in residuals versus fitted values or observation order, while the normal probability plot was broadly consistent with the assumed error distribution. Together with the model statistics, these diagnostics indicate that no major violation of the evaluated regression assumptions was apparent within this dataset. Given the limited replication of each treatment combination, however, these diagnostic results should be interpreted as supporting the adequacy of the fitted model rather than as independently establishing model robustness.

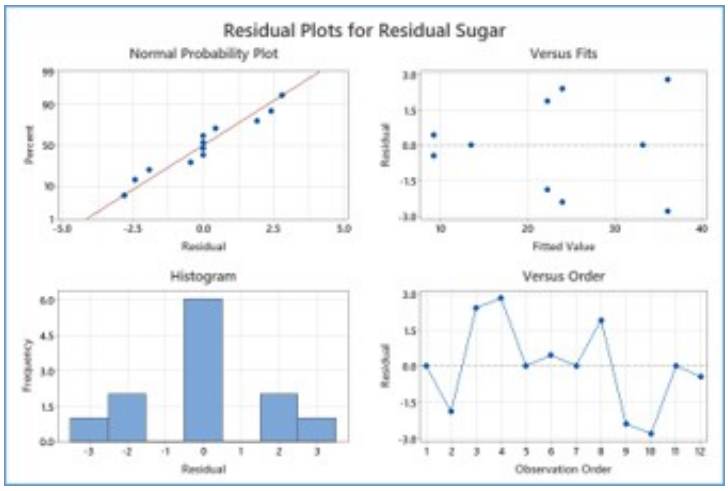

Figure 6. Residual plots for RS

Overall, the RS results demonstrate three features of the experimental response: both YAN and aeration were associated with terminal RS concentration; aeration accounted for the larger main-effect signal within the tested factor levels; and, importantly, the effect of YAN depended significantly on aeration level. Thus, the RS response cannot be adequately characterized from the independent main effects of YAN and aeration alone.

3.3 Results specific to ethanol percent

The general factorial regression model for day-18 ethanol concentration was statistically significant ($F = 11.11$, $p = 0.005$; Table 5). The model explained 90.26% of the observed variation in ethanol concentration, with an adjusted R^2 of 82.14% and a predicted R^2 of 61.02%. Both experimental factors exhibited effects within the study's predefined significance threshold ($\alpha = 0.10$). YAN was associated with ethanol concentration ($F = 5.57$, $p = 0.056$), although this effect would not meet a conventional $\alpha = 0.05$ threshold, while aeration produced a stronger effect within the tested design space ($F = 18.23$, $p = 0.003$). The YAN × aeration interaction was also statistically significant ($F = 6.77$, $p = 0.029$), indicating that the effect of YAN on terminal ethanol concentration depended on the level of aeration.

Table 5. Regression model: Ethanol vs YAN, Aeration

Table 5a. General factorial regression: Ethanol versus YAN, Aeration

Factor	Levels	Values
YAN	2	150, 300
Aeration	3	0.0, 5.5, 11.0

Table 5b. Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	3.3956	0.67913	11.11	0.005
Linear	3	2.5683	0.85609	14.01	0.004
YAN	1	0.3401	0.34010	5.57	0.056
Aeration	2	2.2282	1.11409	18.23	0.003
2-Way Interactions	2	0.8274	0.41368	6.77	0.029
YAN*Aeration	2	0.8274	0.41368	6.77	0.029
Error	6	0.3666	0.06110		
Total	11	3.7622			

Table 5c. Model summary

S	R^2	R^2(adj)	R^2(pred)
0.247187	90.26%	82.14%	61.02%

The regression coefficients further characterized the magnitude and direction of these effects (Table 6). The model constant was 12.5032% (v/v). The fitted coefficients for YAN were −0.1683 percentage points at 150 mg/L and +0.1683 percentage points at 300 mg/L ($p = 0.056$). The corresponding aeration coefficients were −0.609 percentage points at 0 L/day ($p = 0.001$), +0.313 percentage points at 5.5 L/day ($p = 0.021$), and +0.296 percentage points at 11.0 L/day ($p = 0.026$). The larger F-value for aeration relative to YAN in the ANOVA was consistent with the stronger statistical evidence for an aeration main effect.

The regression coefficients also characterized the significant YAN × aeration interaction. At 0 L/day aeration, the interaction coefficients were +0.339 for 150 mg/L YAN and −0.339 for 300 mg/L YAN ($p = 0.015$). At 11.0 L/day, the corresponding coefficients reversed direction, to −0.300 and +0.300, respectively ($p = 0.025$). In contrast, at the intermediate aeration level of 5.5 L/day, the interaction coefficients were substantially smaller (−0.039 and +0.039) and were not statistically significant ($p = 0.712$). Thus, both the magnitude and direction of the YAN effect varied across aeration levels, consistent with the significant interaction identified by the overall ANOVA.

The complete fitted regression equation, incorporating the YAN, aeration, and YAN × aeration terms, is presented in Table 6b. The factor structure comprised two YAN levels (150 and 300 mg/L), three aeration levels (0, 5.5, and 11.0 L/day), and the resulting six YAN × aeration treatment combinations (Table 6c).

The general factorial regression model for ethanol incorporated the same two experimental factors used for the residual sugar analysis, consisting of two YAN levels (150 and 300 mg/L) and three aeration levels (0.0, 5.5, and 11.0 L/day), thereby generating six treatment combinations for analysis (Table 5a). The corresponding ANOVA demonstrated that the overall model was statistically significant ($F = 11.11$, $p = 0.005$). Aeration exhibited a significant main effect on ethanol production (F

= 18.23, p = 0.003), whereas the main effect of YAN was weaker (F = 5.57, p = 0.056), although still below the study's predefined significance threshold (α = 0.10). The YAN × Aeration interaction was also statistically significant (F = 6.77, p = 0.029), indicating that the effect of YAN on ethanol production differed across aeration levels.

Table 6. Regression model: Ethanol vs YAN, Aeration

Table 6a. Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	12.5032	0.0714	175.22	0.000	
YAN					
150	-0.1683	0.0714	-2.36	0.056	1.00
300	0.1683	0.0714	2.36	0.056	*
Aeration					
0.0	-0.609	0.101	-6.04	0.001	1.33
5.5	0.313	0.101	3.10	0.021	1.33
11.0	0.296	0.101	2.94	0.026	*
YAN*Aeration					
150 0.0	0.339	0.101	3.36	0.015	1.33
150 5.5	-0.039	0.101	-0.39	0.712	1.33
150 11.0	-0.300	0.101	-2.98	0.025	*
300 0.0	-0.339	0.101	-3.36	0.015	*
300 5.5	0.039	0.101	0.39	0.712	*
300 11.0	0.300	0.101	2.98	0.025	*

Table 6b. Regression equation

Response	Regression equation
Ethanol	**12.5032** − 0.1683 YAN_150 + 0.1683 YAN_300 − 0.609 Aeration_0.0 + 0.313 Aeration_5.5 + 0.296 Aeration_11.0 + 0.339 YAN*Aeration_150 0.0 − 0.039 YAN*Aeration_150 5.5 − 0.300 YAN*Aeration_150 11.0 − 0.339 YAN*Aeration_300 0.0 + 0.039 YAN*Aeration_300 5.5 + 0.300 YAN*Aeration_300 11.0

Table 6c. Factor information

Factor	Levels used in model
YAN	150, 300
Aeration	0.0, 5.5, 11.0
Interaction	YAN × Aeration (six treatment combinations)

The main-effects plot (Figure 7) shows that mean ethanol concentration increased as YAN increased from 150 to 300 mg/L, from ~ 12.3% to 12.7% (v/v). A larger change was observed across aeration levels: mean ethanol concentration increased markedly from ~ 11.9% without supplemental aeration to ~ 12.8% at 5.5 L/day, with little additional change at 11.0

L/day. Relative to the predefined ethanol target range of 11.0–12.5% (v/v), the mean response at 150 mg/L YAN was within the target range, whereas the mean response at 300 mg/L YAN was slightly above its upper limit. Similarly, the mean response at 0 L/day aeration was within the target range, while the mean responses at 5.5 and 11.0 L/day were slightly above the upper target limit.

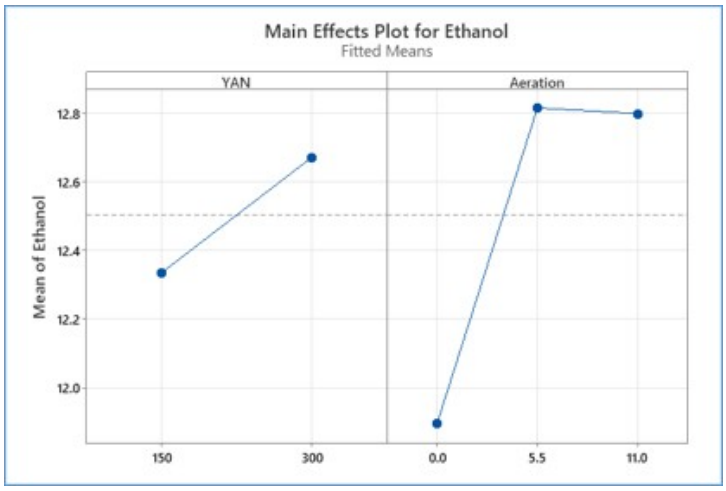

Figure 7. Main effects plot: Ethanol vs YAN, Aeration

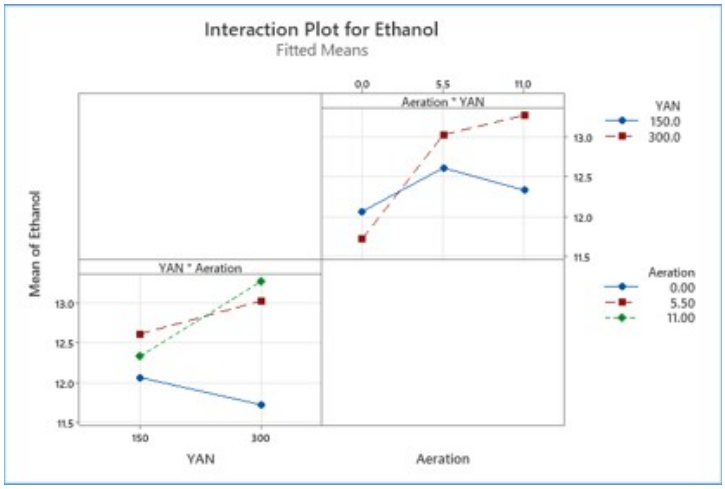

Figure 8. Interaction plot: Ethanol vs YAN, Aeration

The interaction plot (Figure 8) demonstrates that the effect of YAN on ethanol concentration was dependent on aeration level. At 0 L/day aeration, increasing YAN from 150 to 300 mg/L was associated with a decrease in mean ethanol concentration. At 5.5 L/day, the difference between the two YAN levels was comparatively small. At 11.0 L/day, however, increasing YAN

from 150 to 300 mg/L was associated with an increase in mean ethanol concentration. Consequently, the direction and magnitude of the YAN effect changed across aeration levels, consistent with the significant YAN × aeration interaction identified by ANOVA ($F = 6.77$, $p = 0.029$).

The regression coefficients further characterized this interaction (Table 6). Interaction coefficients were statistically significant at 0 L/day aeration ($p = 0.015$) and 11.0 L/day ($p = 0.025$), whereas the interaction coefficients at 5.5 L/day were not statistically significant ($p = 0.712$). The interaction coefficients also reversed sign between the low and high aeration levels, further demonstrating that the relationship between YAN and terminal ethanol concentration was not constant across the experimental design space.

The residual diagnostics (Figure 9) showed no prominent systematic pattern in residuals versus fitted values or observation order, while the normal probability plot was broadly consistent with the assumed error distribution. Together with the model statistics, these diagnostics indicate that no major violation of the evaluated regression assumptions was apparent within this dataset. Given the limited replication of each treatment combination, however, these diagnostic results should be interpreted as supporting the adequacy of the fitted model rather than as independently establishing model robustness. The lower predicted R^2 (61.02%) relative to the model R^2 (90.26%) and adjusted R^2 (82.14%) also indicates that the model's predictive performance is less strong than its fit to the observed experimental data.

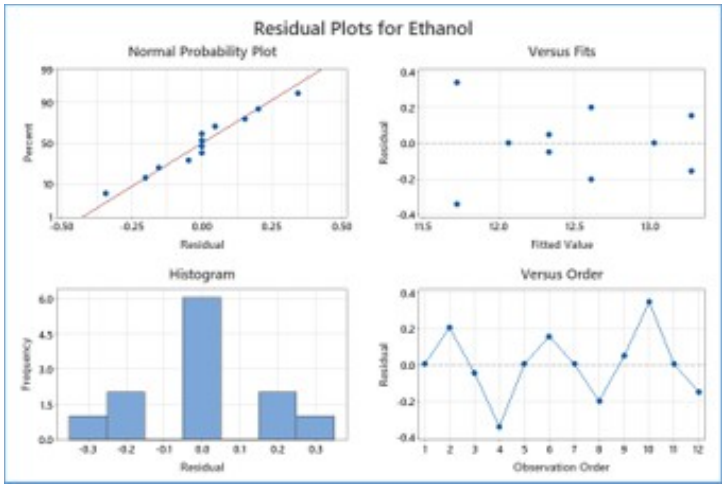

Figure 9. Residual plots for ethanol

Overall, the ethanol results demonstrate three features of the experimental response: both YAN and aeration were associated with terminal ethanol concentration, although the evidence for the YAN main effect was weaker; aeration accounted for the larger main-effect signal within the tested factor levels; and, importantly, the effect of YAN depended significantly on aeration level. Thus, as with residual sugar, the ethanol response cannot be adequately

characterized from the independent main effects of YAN and aeration alone, and their interaction must be considered when evaluating fermentation outcomes.

3.4 Combined response optimization

These interaction plots illuminate a key finding of this research: within the six tested treatment combinations, simultaneous satisfaction of both response targets occurred under a restricted set of conditions. The following table highlights the treatments that drove ideal average responses. The only treatment that satisfied both response targets was #3, with YAN=150 mg/L and Aeration=11.05 L/day.

Table 7. Multi-response classification of treatment combinations

Treatment	Trials (2 Replicates)	Treatment combination	Residual sugar (15–30 g/L)	Ethanol (11.0–12.5%)
#1	(1, 7)	(150, 0.0)	**FAIL**	**PASS**
#2	(2, 8)	(150, 5.5)	**PASS**	**FAIL**
#3	(3, 9)	(150, 11.0)	**PASS**	**PASS**
#4	(4, 10)	(300, 0.0)	**FAIL**	**PASS**
#5	(5, 11)	(300, 5.5)	**FAIL**	**FAIL**
#6	(6, 12)	(300, 11.0)	**FAIL**	**FAIL**

Table 7 reconfirms the preceding conclusions, showing that only treatment #3 achieved dual ideal responses on both of its trials (vessels 03 & 09).

Table 8. Final fermentation responses by experimental vessel

Parameter	Vessel ID											
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Target YAN (mg/L)	150	150	150	300	300	300	150	150	150	300	300	300
Aeration (L/day)	0.0	5.5	11.0	0.0	5.5	11.0	0.0	5.5	11.0	0.0	5.5	11.0
Day-18 Residual Sugar (g/L)	33.2	20.3	26.3	38.8	13.5	9.7	33.2	24.1	21.5	33.2	13.5	8.8
Day-18 Ethanol (% v/v)	12.1	12.8	12.3	11.4	13.0	13.4	12.1	12.4	12.4	12.1	13.0	13.1

Table 8 presents the vessel-level day-18 responses underlying the treatment classifications in Table 7. The replicate structure shows that residual sugar exhibited greater within-treatment variability than ethanol concentration. For example, the two replicates of treatment #2 (vessels 2 and 8; 150 mg/L YAN, 5.5 L/day aeration) produced residual-sugar concentrations of 20.3 and 24.1 g/L and ethanol concentrations of 12.8% and 12.4% (v/v), respectively. In contrast, treatment #3 (vessels 3 and 9; 150 mg/L YAN, 11.0 L/day aeration) produced 26.3 and 21.5 g/L residual sugar and 12.3% and 12.4% (v/v) ethanol, with both replicates remaining within both predefined target ranges. Thus, the vessel-level results confirm that treatment #3 was the only tested condition for which both independent replicates simultaneously satisfied both product criteria.

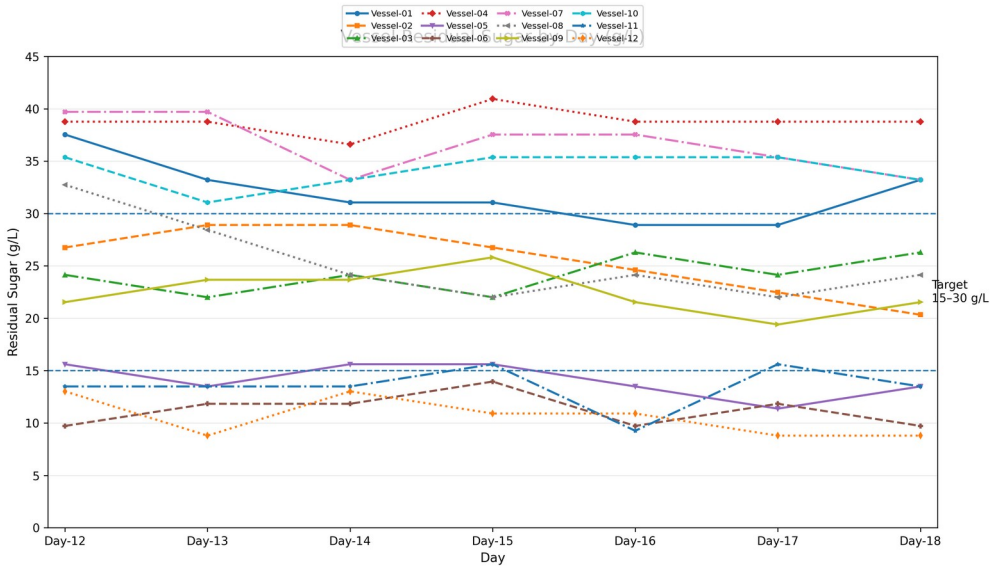

Figure 10. Day-18 residual sugar relative to the target range across treatments

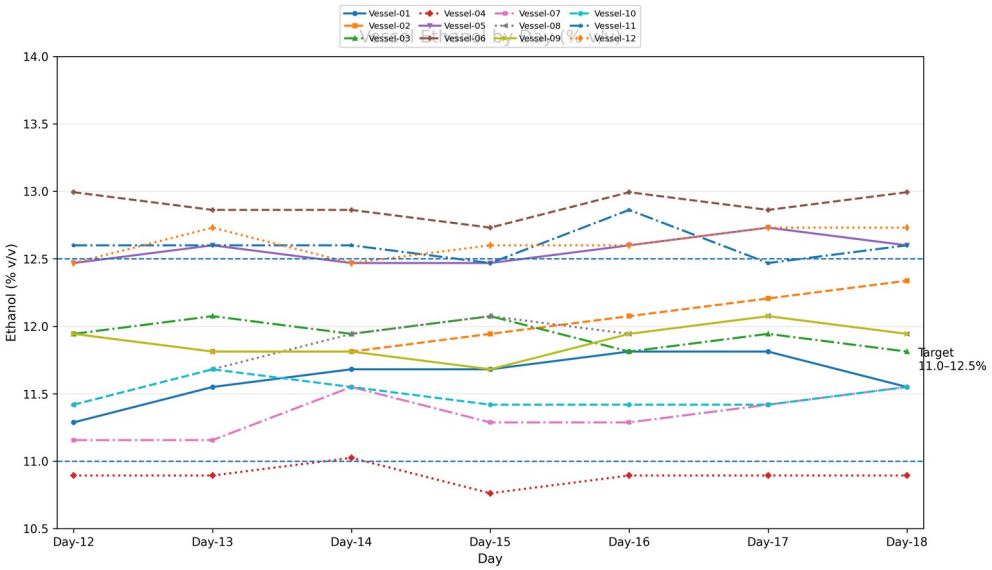

Figure 11. Day-18 ethanol concentration relative to the target range across treatments

Figures 10 and 11 highlight the terminal phase of primary fermentation and the predefined residual-sugar and ethanol target ranges. Vessels 3, 8, and 9 individually satisfied both response criteria. Vessels 3 and 9 were the two replicates of treatment #3 (150 mg/L YAN, 11.0 L/day aeration), making treatment #3 the only treatment for which both replicates

simultaneously achieved both target responses. Vessel 8 also individually achieved both targets; however, its treatment replicate, vessel 2, exceeded the upper ethanol target.

4. Discussion

This study evaluated whether controlled supplementation of yeast-assimilable nitrogen (YAN) and mechanical aeration could mitigate two biochemical constraints relevant to primary peach-wine fermentation under Colorado Front Range conditions. The factorial design demonstrated that both process variables influenced fermentation outcomes, but, importantly, their effects were not independent. Rather than identifying a simple condition in which increasing either YAN or aeration consistently improved fermentation, the results revealed a factorial response pattern in which the appropriate level of one factor depended on the level of the other. This interaction was evident for both residual sugar (RS) and ethanol and is central to interpretation of the experiment.

The daily fermentation profiles provide the first indication of this treatment dependence. All 12 vessels began at similar specific gravity and calculated residual-sugar concentrations, yet their fermentation trajectories subsequently separated during the active phase of fermentation. Decreasing specific gravity was accompanied by decreasing RS and increasing ethanol, as expected, but the timing and extent of these changes varied among treatments. Some fermentations approached their terminal values within ~ the first week, whereas others continued to change into the second week. Importantly, these trajectories did not ultimately converge to a common endpoint: measurable differences in both RS and ethanol remained at day 18. Thus, the effects observed in the factorial analysis represent persistent differences in fermentation completion rather than merely transient differences in early fermentation rate.

Residual sugar was particularly responsive to the experimental factors. The RS model explained 96.95% of the observed variation, with an adjusted R^2 of 94.41% and predicted R^2 of 87.81%. Aeration generated the larger main-effect signal ($F = 69.73$, $p < 0.001$), while YAN also significantly affected the response ($F = 24.25$, $p = 0.003$). These findings indicate that oxygen availability was a major determinant of the extent of sugar utilization under the experimental conditions. Fermentations receiving no supplemental aeration generally retained substantially more sugar at day 18, whereas the intermediate and high aeration treatments produced much lower terminal RS concentrations.

However, the main effects alone do not adequately describe the RS response. The YAN × aeration interaction was also significant ($F = 13.56$, $p = 0.006$). At 0 L/day aeration, increasing YAN did not reduce terminal RS; mean RS instead remained above the predefined 15–30 g/L target range. In contrast, under intermediate and high aeration, increasing YAN was associated with substantially greater sugar depletion. The direction of the YAN effect therefore changed according to oxygen availability. This finding is important from a process-engineering perspective because it indicates that additional nitrogen cannot be assumed to improve fermentation simply

because nitrogen is a necessary yeast nutrient. Its observed effect depends upon the accompanying aeration condition.

The ethanol response exhibited a related but less pronounced pattern. The ethanol model remained statistically significant ($F = 11.11$, $p = 0.005$) and explained 90.26% of the observed variation, although its adjusted R^2 (82.14%) and particularly its predicted R^2 (61.02%) were lower than those of the RS model. Aeration again produced the stronger main-effect signal ($F = 18.23$, $p = 0.003$), whereas the YAN main effect was weaker ($F = 5.57$, $p = 0.056$) and met the study's predefined $\alpha = 0.10$ threshold but not the more conventional $\alpha = 0.05$ threshold. Consequently, the evidence for an independent YAN effect on terminal ethanol should be interpreted more cautiously than the corresponding evidence for RS.

As with RS, however, the ethanol response cannot be understood solely from the main effects. The YAN × aeration interaction was significant ($F = 6.77$, $p = 0.029$). At zero supplemental aeration, increasing YAN was associated with lower mean ethanol, whereas at high aeration the direction of this relationship reversed. At intermediate aeration, the difference between YAN levels was comparatively small. The significant interaction therefore independently reinforces the conclusion obtained from the RS analysis: YAN and aeration behave as interacting process variables rather than as two independent inputs whose effects can simply be added together. The interaction was statistically stronger for RS than for ethanol, with interaction F-values of 13.56 and 6.77, respectively.

The differing behavior of the two response variables is also important when defining an operational optimum. Maximizing fermentation activity is not equivalent to optimizing the desired wine product. The experimental objective was not to minimize RS or maximize ethanol independently, but to achieve both predefined product specifications simultaneously: 15–30 g/L RS and 11.0–12.5% (v/v) ethanol. The highest YAN and aeration combination produced extensive sugar depletion and comparatively high ethanol, but these responses exceeded the intended product boundaries. Conversely, eliminating supplemental aeration preserved ethanol within the desired range in some treatments but generally left excessive RS. The useful operating region therefore lies between incomplete fermentation and excessive fermentation relative to the selected product specifications.

This distinction explains why the optimal treatment was not the treatment with the maximum amount of both process inputs. Of the six factorial treatment combinations, the combination of 150 mg/L YAN and 11.05 L/day aeration was the only treatment for which both replicate trials simultaneously satisfied the predefined RS and ethanol target ranges. The two replicate vessels produced day-18 RS values of 26.3 and 21.5 g/L and ethanol concentrations of 12.3% and 12.4% (v/v), respectively. Their mean response was therefore ~ 23.9 g/L RS and 12.35% ethanol, placing both product characteristics within the targeted off-dry to semi-sweet operating region. The experimental optimum should consequently be interpreted as a multi-response process

optimum, rather than as the condition producing either the lowest residual sugar or highest ethanol concentration.

The factorial results further show why optimization based on a single response could lead to an undesirable process recommendation. For example, increasing both YAN and aeration promoted more complete sugar utilization, but the 300 mg/L YAN treatments at 5.5 and 11.0 L/day produced terminal RS concentrations below the desired range while ethanol concentrations exceeded its upper target. Conversely, combinations producing ethanol concentrations comfortably within the desired interval did not necessarily produce acceptable RS. Product optimization therefore requires simultaneous consideration of both responses. This is particularly relevant to fruit-wine production, where complete substrate conversion is not necessarily the desired endpoint when residual sweetness is itself a product characteristic.

The results also provide a quantitative basis for supplemental aeration under the conditions examined. The experiment was performed at ~ 5,600 ft above sea level, where the manuscript estimates atmospheric pressure at ~ 0.812 atm and correspondingly reduced natural oxygen transfer into the must. The strong aeration effect observed for both RS and ethanol is consistent with supplemental aeration functioning as an effective controllable process variable under these conditions. Importantly, however, the experiment does not demonstrate that the same aeration requirement applies universally across elevations, fermenter geometries, batch sizes, peach cultivars, or commercial production systems. Rather, it establishes an experimentally defined solution space for the Front Range conditions represented by this study.

Similarly, the findings support controlled YAN supplementation but do not support maximizing nitrogen addition. The 150 mg/L YAN condition was sufficient to achieve the desired dual response when paired with high aeration, whereas increasing YAN to 300 mg/L under the same aeration condition moved both responses beyond the selected product targets. This result has practical significance because it demonstrates that the process objective can potentially be achieved with comparatively modest nitrogen supplementation when the accompanying oxygen environment is appropriately controlled. The value of YAN supplementation therefore lies not simply in supplying more nitrogen, but in selecting a dose appropriate to the overall fermentation operating condition.

Taken together, the results demonstrate that high-altitude peach fermentation can be approached as a multivariable process-engineering problem rather than as a series of independently corrected environmental deficiencies. Under the conditions evaluated here, neither supplemental nitrogen nor aeration had a universally beneficial direction of effect across the entire experimental space. Instead, the desired product emerged from a specific combination of the two variables. The experimentally identified condition of 150 mg/L YAN with 11.05 L/day supplemental aeration achieved the targeted balance between fermentation completion and retention of residual sweetness while maintaining ethanol

within the predefined commercial range. The significant interactions for both RS and ethanol provide statistical evidence that the two process inputs should be optimized jointly rather than independently.

These findings therefore address the study's principal engineering objective: controlled aeration can compensate for reduced oxygen availability under the tested Front Range fermentation conditions, while controlled YAN supplementation can provide sufficient accessible nitrogen to support fermentation. More importantly, the factorial design demonstrates that these interventions must be considered together.

4.1 Limitations

Several limitations define the scope within which these findings should be interpreted. First, the full factorial design contained six treatment combinations with two replicate fermentation vessels per combination. Replication enabled estimation of experimental error and identification of statistically significant YAN, aeration, and YAN × aeration effects; however, two replicates per treatment provide limited power for characterizing biological variability and identifying occasional fermentation failures or outlier behavior. The relatively strong model fits and low residual error observed in this experiment support the internal consistency of the results, but they do not eliminate the uncertainty associated with limited replication. In particular, the present experiment is better suited to identifying major factor and interaction effects than to precisely estimating treatment-to-treatment reproducibility.

A second limitation is the inherent biological heterogeneity of the raw material. Although experimental procedures were standardized, peaches are not chemically uniform substrates. Individual fruit may differ in maturity, soluble solids, acidity, micronutrient composition, native nitrogen content, phenolic composition, and other characteristics relevant to fermentation. Homogenization and standardized must preparation reduce, but cannot completely eliminate, this source of variation. Consequently, some vessel-to-vessel variation may reflect biological variation in the starting material rather than the imposed experimental factors alone.

The experiment also exclusively utilized the Redhaven peach cultivar. Cultivars may differ in sugar concentration, acidity, nitrogen availability, aromatic composition, and other physicochemical properties that influence fermentation. The identified operating region therefore should not be assumed to transfer directly to other peach cultivars or other stone fruits without experimental validation. Similarly, the fruit used in this experiment represents a particular agricultural source and growing environment; variation among harvest years, orchards, fruit maturity, soil conditions, irrigation practices, and seasonal climate could alter must composition and potentially shift the optimal YAN and aeration requirements.

Importantly, altitude itself was not an experimental factor in the DoE. All fermentations were performed under Front Range conditions, and the experiment therefore demonstrates how YAN supplementation and aeration affect fermentation within that

environment rather than directly demonstrating that altitude caused the observed fermentation behavior. Reduced atmospheric pressure provides a mechanistic rationale for investigating supplemental aeration, but the present design did not include otherwise identical fermentations performed at lower altitude. Consequently, the study identifies a process solution space applicable to the tested Front Range conditions but does not quantify how the optimum changes as a function of elevation. A multi-altitude experiment using identical must, vessels, yeast, temperature, YAN, and aeration conditions would be required to directly establish an altitude-dependent effect.

Similarly, dissolved oxygen was not directly measured within the must. Aeration was experimentally controlled as an air-flow input, but air delivery is not equivalent to dissolved-oxygen availability. Actual oxygen transfer depends on factors including bubble size, diffuser characteristics, vessel geometry, liquid depth, agitation, temperature, must composition, and oxygen consumption by yeast. Thus, the results establish relationships between the applied aeration rates and fermentation outcomes rather than defining a specific dissolved-oxygen concentration or oxygen-transfer coefficient. Direct measurement of dissolved oxygen and, ideally, volumetric oxygen mass-transfer coefficients (kLa) would improve the scalability and mechanistic interpretation of future experiments.

The study similarly manipulated YAN according to calculated supplementation levels rather than repeatedly measuring the concentration and depletion of individual assimilable nitrogen species throughout fermentation. The experiment therefore establishes an empirical relationship between the imposed YAN treatment and fermentation performance but does not directly determine the rate at which nitrogen was assimilated by the yeast. Direct measurement of YAN before and during fermentation would be particularly valuable for evaluating the proposed physiological relationship between nitrogen availability and oxygen-supported assimilation.

Residual sugar and ethanol concentrations represent additional measurement limitations. Both responses were derived from specific-gravity measurements using established calculation relationships rather than quantified by direct analytical methods. Hydrometer measurements are practical and appropriate for process monitoring, but density is simultaneously influenced by declining sugar concentration and increasing ethanol concentration, as well as temperature and other dissolved constituents. Calculated RS and ethanol should therefore be regarded as estimates of the corresponding product characteristics. Future validation using direct analytical techniques, such as enzymatic sugar assays, refractometry corrected for ethanol, high-performance liquid chromatography for sugars, or direct ethanol measurement, would provide independent confirmation of the modeled endpoints.

The experimental factor space was also intentionally limited. Only two YAN levels (150 and 300 mg/L) and three aeration levels (0, 5.5, and 11.05 L/day) were evaluated. The factorial

design can therefore characterize responses within these tested conditions but cannot establish whether a superior operating point exists between the selected levels or immediately beyond them. In particular, identification of 150 mg/L YAN and 11.05 L/day aeration as the preferred treatment should not be interpreted as establishing a mathematically exact global optimum. Rather, it represents the best-performing tested treatment combination relative to the predefined RS and ethanol specifications. A subsequent response-surface experiment using additional intermediate factor levels surrounding this region would allow more precise localization of the optimum.

Relatedly, the preferred operating condition was identified and evaluated using the same experimental dataset. An independent confirmation experiment was not performed at the predicted optimum. Although both replicate vessels at 150 mg/L YAN and 11.05 L/day aeration achieved the predefined product targets, additional independent batches are required to determine how reliably this operating condition reproduces the desired outcome. Such confirmation runs would be particularly important before extrapolating the process to commercial production.

Scale is another limitation. The experiment employed small fermentation vessels under controlled experimental conditions, whereas commercial fermenters differ substantially in volume, geometry, hydrostatic pressure, mixing, heat transfer, gas dispersion, and oxygen-transfer efficiency. An aeration rate expressed simply as L/day therefore cannot necessarily be transferred proportionally from the experimental vessels to commercial tanks. Scale-up should instead consider aeration relative to fermentation volume and, ultimately, oxygen-transfer performance. The present aeration rate should consequently be regarded as an experimentally effective condition for this system rather than a directly transferable commercial specification.

Several fermentation variables were deliberately controlled or excluded to preserve the statistical power of the two-factor DoE. A single commercial *Saccharomyces cerevisiae* strain (Lalvin 71B) was used, fermentation conditions were standardized, and indigenous microbial variability was intentionally suppressed. These choices strengthen attribution of the observed responses to YAN and aeration but limit generalizability. Different yeast strains may differ substantially in nitrogen requirements, oxygen demand, ethanol tolerance, fermentation kinetics, and aroma production. Likewise, temperature, pH, initial soluble solids, micronutrients, inoculation density, agitation, and fermentation vessel characteristics were not independently varied. The identified YAN × aeration response therefore applies to the controlled biological and process background used in this experiment.

Finally, the optimization criteria were restricted primarily to terminal RS and ethanol concentration. These are important commercial parameters but do not alone define wine quality. Supplemental aeration and nutrient addition could also influence volatile aroma compounds, oxidation, acidity, phenolics, color, mouthfeel, sensory characteristics, fermentation duration, microbial stability, and shelf stability. No formal

sensory panel or direct chemical analysis of aroma and flavor compounds was conducted. Consequently, the designation of an operating condition as optimal refers specifically to its ability to satisfy the predefined RS and ethanol targets, rather than demonstrating superior overall sensory quality or consumer preference.

Taken together, these limitations do not negate the factorial relationships observed in the present study but define their appropriate scope. The experiment provides evidence that YAN and aeration interact in determining peach-fermentation outcomes under the tested Front Range conditions and identifies a promising operating region within the investigated factor space. Further replication, direct chemical measurements, intermediate factor levels, independent confirmation runs, additional cultivars and harvests, scale-up studies, and controlled comparisons across elevations will be necessary to determine the robustness, mechanistic basis, and commercial generalizability of the proposed process conditions.

4.2 Perspectives

The present study was deliberately constrained to the kinetics and terminal outcomes of active primary fermentation. Certain secondary biochemical responses and additional fermentation metrics were observed but intentionally excluded from the primary analysis in order to preserve the focus of the original Design of Experiments. These downstream outcomes should be evaluated independently in subsequent work.

The most immediate next step is to refine the operating region identified in the present study. The current 2 × 3 factorial design established significant YAN × aeration interactions and identified 150 mg/L YAN with 11.05 L/day aeration as the most favorable tested combination for simultaneously achieving the predefined residual-sugar and ethanol targets. A follow-up response-surface study using additional intermediate YAN and aeration levels around this region could determine whether comparable or improved outcomes can be achieved with lower nutrient supplementation or reduced aeration.

Future studies should also incorporate fermentation kinetics as explicit response variables. The daily specific-gravity trajectories suggested treatment-dependent differences in lag phase, fermentation rate, and time to terminal density. More frequent measurements could quantify lag duration, maximum fermentation rate, time to target residual sugar, and total fermentation duration. These outcomes could then be optimized alongside terminal residual sugar and ethanol rather than treating fermentation completion alone as the principal objective.

Greater biological replication would further allow fermentation reproducibility to become an explicit response variable. The current study was designed primarily to compare treatment means rather than treatment-specific variances. Increasing the number of independent vessels per treatment would permit direct assessment of whether particular YAN–aeration combinations produce more consistent fermentation outcomes. This could expand optimization from

maximizing average performance toward balancing product quality, fermentation duration, resource use, and batch-to-batch robustness.

The role of altitude itself should also be isolated experimentally. Because all fermentations in the present study were conducted under Front Range conditions, the study identifies an operating solution for this environment but does not establish how the optimum changes with elevation. Repeating an identical factorial experiment at multiple elevations, or under controlled atmospheric pressures, could determine whether aeration requirements vary systematically with barometric pressure. This could ultimately allow aeration requirements to be modeled as a function of altitude rather than specified only for one location.

Generalizability should be tested across additional peach cultivars, harvest years, orchards, and yeast strains. The present study used Redhaven peaches and Lalvin 71B *Saccharomyces cerevisiae*. Because fruit composition and yeast nutrient requirements can vary, the magnitude and location of the YAN–aeration optimum may differ under other biological conditions. Replication across these backgrounds would distinguish broadly applicable process relationships from cultivar- or strain-specific effects.

Future work should also directly validate the principal chemical responses and broaden the definition of product quality. Residual sugar and ethanol were derived from density measurements in the present study; independent chemical measurements could confirm these estimates. Additional outcomes such as volatile aroma compounds, acidity, phenolics, oxidation, color, mouthfeel, and sensory preference could then be incorporated into subsequent optimization models. This would determine whether operating conditions that achieve desirable ethanol and residual-sugar values also preserve the sensory characteristics expected of high-quality peach wine.

Finally, commercial translation will require scale-up studies. The aeration rates identified here are specific to the experimental vessel volume and geometry and cannot be assumed to transfer directly to commercial tanks. Pilot-scale experiments should therefore characterize air delivery relative to fermentation volume and, ideally, oxygen-transfer capacity. Combining these engineering measurements with fermentation time, nutrient consumption, energy requirements, reproducibility, and product-quality responses could support a more complete technical and economic optimization of the process.

Taken together, these directions extend the present work from identification of an effective two-factor operating region toward a more general high-altitude fermentation-control framework. The most important next steps are to refine and independently validate the identified solution space, directly test the physiological basis of the YAN × aeration interaction, and determine whether the relationship remains robust across biological materials, elevations, and production scales.

5. Conclusion

This study demonstrates that YAN

supplementation and aeration act as interacting rather than independent process variables during primary peach fermentation under the tested Colorado Front Range conditions. Aeration produced the stronger main-effect signal for both residual sugar and ethanol, while significant YAN × aeration interactions showed that the effect of nitrogen supplementation depended on the accompanying aeration level. Within the tested design space, 150 mg/L YAN combined with 11.05 L/day supplemental aeration provided the most favorable simultaneous outcome, producing ~ 23.9 g/L residual sugar and 12.35% (v/v) ethanol, both within the predefined product targets. The optimum therefore represented a balance between the two process inputs rather than their simultaneous maximization.

Collectively, these findings support a process-engineering approach in which nitrogen availability and aeration are optimized jointly to achieve desired fermentation outcomes. More broadly, they suggest that successful fermentation may depend not simply on supplying sufficient nutrients or oxygen independently, but on matching nutrient availability to the yeast's oxygen-supported assimilation capacity.

Regulatory and safety considerations

This project utilized a strict division of labor to ensure full compliance with statutes regarding minors and ethanol production. H. Harris served as the project's principal researcher and investigator, responsible for factor selection and base DoE model selection, equipment preparation, pre-fermentation must preparation (0.0% ABV), data entry, and primary statistical analysis. Yeast addition, the monitoring of fermentation kinetics, and all physical handling of solutions exceeding 0.5% ABV were performed exclusively by an adult laboratory technician.

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Appendix A. Representative figures

Figure A1. Vessel preparation for (3) aeration levels

Figure A2. Sample access port preparation

Figure A3. Must preparation

Figure A4. Must preparation

Figure A5. *Left-Right:* YAN-low, YAN-high, YAN-low, YAN-high; *Back-Front:* Aeration-high, Aeration-medium, Aeration-low

Figure A6. Densimeter response

Figure A7. Densimeter response

Appendix B. Calculations

B.1 YAN calculations including environmental stress accommodation
YAN concentrations around 150–300 mg/L encompass commonly used values for supporting wine-yeast fermentation, although requirements depend on yeast strain and fermentation conditions (9,11,19). These values were therefore selected as the lower and upper YAN levels for the DoE.

[A] The experimental must formulation included 1.8 g/must of yeast energizer per gallon of must. With 3.785 L/gallon, the concentration of raw energizer is calculated as $\frac{1.800\ \ g}{3.785\ \ L} = 0.4755\ \ g/L$. Because the specific energizer formulation contains 5% YAN by mass, it provides a baseline contribution $(0.4755\ \ g/L)(0.05) = 0.0238\ \ g/L\ \ YAN = 23.80\ \ mg/L\ \ YAN$

[B] Native YAN was not directly measured in the experimental peach must. For the purpose of calculating supplementation quantities, a nominal native-YAN concentration of 92.5 mg/L was therefore used as a design assumption. Consequently, the calculated 150 and 300 mg/L treatment levels represent nominal target inputs rather than analytically verified starting YAN concentrations.

[C] Combining the native peach must baseline (92.5 mg/L) and the fixed yeast energizer contribution (23.80 mg/L) established an unamended starting concentration of 116.3 mg/L. This presents a structural deficit relative to the target design specifications of 150 mg/L and 300 mg/L. The net required supplemental nitrogen masses required to construct the Low (coded as -1) and High (coded as +1) experimental levels were hence defined as,
$\Delta YAN_{Low} = 150.0 \ \ mg/L \ - 116.3 \ \ mg/L = 33.7 \ \ mg/L$ and
$\Delta YAN_{High} = 300.0 \ \ mg/L \ - 116.3 \ \ mg/L = 183.7 \ \ mg/L$

[D] Industrial-grade diammonium phosphate, $(NH_4)_2HPO_4$ (DAP), was selected as the nitrogen provisioning agent. Upon dissolution, DAP dissociates to yield bioavailable ammonium ions as, $(NH_4)_2HPO_4 \rightarrow 2NH_4^+ + HPO_4^{2-}$. Pure DAP contains 21.2% assimilable nitrogen by mass. Therefore, the raw DAP mass concentration required to satisfy the ΔYAN deficit was calculated as, $DAP_{Low} = \frac{33.7 \frac{mg}{L}}{0.212} = 159.0 \ \ mg/L$ and $DAP_{High} = \frac{183.7 \frac{mg}{L}}{0.212} = 866.5 \ \ mg/L$.
Scaled to 1.0-gallon experimental vessel volumes, the final required additions were,
$Mass \ \ DAP_{Low(-1)} = \left(159.0 \frac{mg}{L}\right)(3.785 \ \ L) = 601.8 \ \ mg = 0.602 \ \ g/gal$ and
$Mass \ \ DAP_{High(+1)} = \left(866.5 \frac{mg}{L}\right)(3.785 \ \ L) = 3{,}280.1 \ \ mg = 3.280 \ \ g/gal$. These additions are based on input calculations *in lieu* of direct calculations of the must. This is justified principally on the large spread between the factor levels relative to measurement system error and, secondarily, on project financial management.

B.2 Supplemental aeration modeling and barometric calculations
Reduced barometric pressure at high altitudes proportionately depresses the concentration of dissolved oxygen in the liquid must. This environmental mass-transfer limitation may therefore decrease yeast propagation during the experimental growth phase, which can decrease the fermentation rate or altogether stop it. Conversely, excessive aeration can induce destructive oxidation, degrading the volatile compounds responsible for the wine's final sensory profile. To mitigate this, a multi-level aeration profile was engineered by calculating the elevation-induced oxygen deficit relative to ideal baseline fermentation requirements.

At sea level, Earth's atmosphere is 101.35 kPa with 20.95% oxygen. The effective sea level oxygen pressure is given by, $P_{O_2} = (101.35 \ \ kPa)(0.2095) = 21.23 \ \ kPa$ On Colorado's Front Range (5,600 ft ASL), barometric pressure decreases to 82.46 kPa. At the same 20.95% oxygen, the local oxygen partial pressure decreases to, $P^1_{O_2} = (82.46 \ \ kPa)(0.2095) = 17.28 \ \ kPa$. This represents a climate-induced reduction of 18.6% in the driving force for oxygen mass transfer.

Oxygen availability can materially affect wine-yeast fermentation, and oxygen demand varies among enological yeast strains. For exploratory treatment sizing, a nominal 10% gas-to-liquid mass-transfer efficiency was assumed, and 2.50 g O_2/gal was selected as a deliberately conservative upper oxygen-delivery target. At sea level density, air contains 0.278 g/L of oxygen. At 5,600 ft ASL, this drops 18.6% to 0.226 g/L. The total volume of alpine air required to satisfy the 2.50 g/gal upper requirement was calculated as,

$$Volumetric\ \ Air\ \ Requirement = \left(\frac{2.500\ \ g/gal}{0.226\ \ g/L} \right) = 11.052\ \ L/gal$$

An upper experimental aeration condition of 11.05 L air/day was selected to provide an intentionally conservative oxygen-delivery condition (2.5 g/gal) under the reduced atmospheric pressure of the experimental site. This treatment level should therefore be interpreted as an experimentally selected upper aeration condition rather than as a direct stoichiometric requirement for yeast oxygen demand. To maintain design symmetry for this three-level factor (coded as -1, 0, +1), the daily aeration targets were established at exactly 0.0 L/gal, 5.525 L/gal, and 11.05 L/gal. For the nominal 1-gallon experimental fermentation volume, the three daily aeration treatments were established at 0.0, 5.525, and 11.05 L of air per day. These correspond nominally to 0.0, 5.525, and 11.05 $L \cdot gal^{-1} \cdot day^{-1}$ and are reported throughout the manuscript as 0.0, 5.5, and 11.0 L/day.These aeration targets were provided by two calibrated aerators through tuned silicone tubing at 10:00am daily. The 11.05 L/gal represented the high end of environmental offset, the 0.0 L/gal represented the natural Front Range environmental condition, and the 5.5 L/gal midpoint provided insight into potential model non-linearity. As with the YAN additions, these additions were based on input calculations, for the same reasons.

B.3 Fluid density response, ethanol, and residual sugar calculation

Fluid density was the fundamental response measurement of this experiment. Manual densitometry using a calibrated glass hydrometer (0.001 precision) was performed at the same time daily, from day-0 through day-18, for each of the 12 trials (vessels).

Baseline sugar concentrations (g/L) were calculated for each trial on day-0 using the following formula, $Sugar = [\,261.30(SG_0 - 1) + 1.016\,](SG_0)$ (eq. 1). Subsequent residual sugar concentrations (g/L) were calculated on day-1 through day-18, for every vessel, using the following formula,

$$Residual\ \ Sugar = [\,47.243(SG_0 - 1) + 214.057(SG_1 - 1) + 1.016\,](SG_1) \quad \text{(eq. 2)}$$

Concentrations of biochemically produced ethanol (%v/v) were calculated on day-1 through day-18, using the following formula, $Ethanol = \left[\frac{76.08(SG_0 - SG_1)}{1.775 - SG_0} \right] \left(\frac{SG_1}{0.794} \right)$ (eq. 3).