Peer review

Harris, Hannah, and Thomas Harris. 2026. “Optimizing High-Altitude Peach Fermentation Biochemistry across the Colorado Front Range via 2x3 Mixed Level Full Factorial Design.” *Journal of High School Science* 10 (3): 547–81. https://doi.org/10.64336/001c.169839.

The manuscript appears to conflate three distinct stages:
-environmental effects on peach growth,
-must preparation,
-fermentation physiology.
The causal chain is not convincingly established.

The central biological rationale requires substantial strengthening. The manuscript links Colorado Front Range environmental conditions (high altitude, reduced growing season, increased UV radiation) directly to fermentation management decisions, but several of these causal connections are insufficiently justified.

First, the oxygen argument appears overstated. The manuscript argues that lower atmospheric pressure reduces oxygen dissolution sufficiently to require continuous supplemental aeration during fermentation. While reduced oxygen partial pressure at altitude is physically correct, commercial fruit wine fermentations—including peach wines—are not normally conducted with continuous forced aeration. Oxygen is typically supplied only during yeast rehydration, inoculation, or the early growth phase through mixing or limited aeration, after which fermentation proceeds under largely anaerobic conditions to avoid oxidation. Consequently, the manuscript should explain why the proposed daily aeration regime is necessary specifically for peach fermentation at 5,600 ft and demonstrate that conventional fermentation practices are inadequate under these conditions. Without such evidence, the connection between altitude and the selected aeration protocol appears speculative rather than experimentally justified.

Second, the microbial argument is internally inconsistent. The introduction argues that increased UV radiation alters native microbial communities on peach skins; however, the methods subsequently state that all peaches were scrubbed and sanitized before inoculation with a commercial Saccharomyces cerevisiae strain. Since indigenous microorganisms were intentionally eliminated before fermentation, any altitude-induced differences in native microbiota would no longer contribute to fermentation outcomes. As written, the discussion of altered skin microbiota serves primarily as background and does not appear mechanistically relevant to the experimental system.

Third, the nitrogen hypothesis requires stronger justification. The manuscript argues that Colorado’s shorter growing season reduces yeast assimilable nitrogen (YAN) accumulation in peaches and therefore motivates DAP supplementation. However, nitrogen accumulation is a property of fruit development prior to harvest. Once the peaches are harvested and incorporated into the must, the physiological processes responsible for nitrogen assimilation have already ceased. Fermentation depends on the measured nitrogen content of the must rather than on the environmental mechanism that produced it. Accordingly, the study should directly measure the initial YAN concentration of the experimental peach must instead of estimating it from regional assumptions. Without direct YAN measurements, the proposed connection between growing-season stress and fermentation performance remains indirect and speculative.

The manuscript claims to optimize “high-altitude fermentation,” yet all fermentations were conducted at a single altitude (approximately 5,600 ft). Consequently, the study does not experimentally isolate altitude as a factor; rather, it optimizes aeration and nitrogen supplementation under one specific environmental condition. Demonstrating that these interventions compensate for altitude would require comparison with otherwise identical fermentations performed at lower elevation or under controlled barometric pressure. As currently designed, the study identifies an empirical operating point for one location but does not establish that altitude itself is the causal factor necessitating the proposed interventions.

Overall, the manuscript would be substantially strengthened by distinguishing pre-harvest environmental effects on fruit composition from post-harvest fermentation engineering variables. At present, several environmental stressors introduced in the rationale are either experimentally removed (native microbiota) or only indirectly related to the fermentation process (plant nitrogen assimilation), making the mechanistic narrative less convincing than the statistical optimization itself.

Below are points of concern related to the experiment itself:

1.Experimental scope versus conclusions

The study is presented as an optimization of "high-altitude peach fermentation"; however, altitude itself was never included as an experimental factor. All fermentations were performed at a single elevation (approximately 5,600 ft), and only YAN supplementation and aeration were varied. Consequently, the Design of Experiment optimizes fermentation conditions at one location rather than demonstrating that altitude is the causal factor responsible for the observed effects. Conclusions should therefore be restricted to the experimental factors that were actually manipulated, or alternatively, a comparison with identical fermentations conducted at lower elevation should be incorporated to substantiate altitude-specific claims.

2.Independent variable (YAN) was not experimentally verified

The study treats YAN concentration as one of its principal independent variables; however, the baseline YAN concentration of the peach must was not measured experimentally. Instead, it was estimated from literature values and regional assumptions before diammonium phosphate additions were calculated. As a result, the actual YAN concentrations achieved in the fermentation vessels remain unknown. Since the validity of the factorial design depends on accurate knowledge of factor levels, direct measurement of initial YAN should be performed to confirm that the intended treatment concentrations were actually achieved.

3.Oxygen availability was inferred rather than measured

Similarly, dissolved oxygen—the second principal experimental factor—was never measured. The study assumes oxygen availability based on atmospheric pressure, calculated gas delivery, and estimated gas-liquid transfer efficiency, but no dissolved oxygen measurements were obtained during fermentation. Consequently, the experiment manipulated air delivery rather than directly quantifying oxygen available to the yeast. Measurement of dissolved oxygen would substantially strengthen the mechanistic interpretation of the aeration treatments.

4.Aeration protocol requires stronger biological justification

The aeration factor is defined as daily air delivery volumes (0, 5.5, and 11.0 L/day), yet the manuscript provides limited justification for why these treatment levels represent biologically meaningful interventions. Oxygen requirements for Saccharomyces cerevisiae are largely confined to the early exponential growth phase, whereas prolonged aeration may also increase oxidative degradation of aroma compounds. The manuscript should clearly describe the timing, duration, and biological rationale for the selected aeration schedule and explain why daily aeration throughout the fermentation process was chosen.

5.Limited replication reduces statistical robustness

Each treatment combination was replicated only twice, resulting in twelve total fermentations for the six treatment combinations. Although this design provides sufficient degrees of freedom to perform ANOVA, the limited replication reduces the precision of variance estimates and increases the sensitivity of interaction effects to experimental variability. The statistical conclusions would be considerably strengthened by additional biological replicates.

6.Regression model complexity relative to sample size

The regression models include main effects and interaction terms while being fitted to a relatively small dataset. Although high R^2 values are reported, such values should be interpreted cautiously because models fitted to small datasets may exhibit optimistic goodness-of-fit. Additional model validation, such as prediction error estimates, cross-validation, or external validation experiments, would increase confidence that the observed relationships are robust rather than reflecting overfitting.

7.Lack of ANOVA diagnostic analyses

The manuscript reports ANOVA results but does not present the diagnostic analyses required to verify the underlying statistical assumptions. Residual normality, homogeneity of variance, independence of residuals, and identification of influential observations should be evaluated and presented. Residual-versus-fitted plots, normal probability plots, and residual histograms would substantially strengthen confidence in the reported statistical inferences.

8.Justification of optimization targets

The optimization objective is defined as achieving 11–12.5% ethanol together with 15–30 g/L residual sugar. While these targets may be reasonable, the manuscript does not sufficiently justify why these ranges represent the optimal product profile for Colorado peach wine. These target values should either be supported by published sensory or industry standards or validated experimentally through sensory evaluation.

9.Rich longitudinal dataset was reduced to a single endpoint

One of the strengths of the study is the collection of daily density measurements throughout fermentation. However, these longitudinal data were ultimately reduced to Day-18 endpoint measurements for statistical analysis. As a result, much of the information regarding fermentation kinetics—including lag phase, fermentation rate, time to completion, and maximum fermentation velocity—is not utilized quantitatively. Incorporating kinetic analyses would provide a more complete understanding of treatment effects.

10.Repeated measurements were not statistically modeled

Because each fermentation vessel was measured repeatedly over nineteen days, the observations within a vessel are not statistically independent. Although the authors ultimately analyzed only the terminal measurements, the longitudinal dataset would be well suited for repeated-measures ANOVA or mixed-effects modeling, which would exploit the temporal structure of the data and provide greater statistical power while accounting for within-vessel correlations.

11.Biological interpretation of interaction effects

The manuscript identifies statistically significant interactions between YAN supplementation and aeration; however, these interactions are primarily described from a statistical perspective. Greater biological interpretation would strengthen the discussion by explaining why the response to nitrogen supplementation changes depending on aeration level and how these interactions relate to known yeast physiology and metabolic regulation.

12.Clarification of the experimental unit

The manuscript would benefit from explicitly defining the experimental unit used in the statistical analyses. Each fermentation vessel appears to represent a single biological replicate, whereas repeated daily measurements within a vessel are repeated observations rather than independent replicates. Explicitly stating this distinction would improve the clarity and transparency of the statistical methodology.

13.Claimed optimum occurs at the boundary of the design space

The reported optimum aeration level (11.0 L/day) corresponds to the highest aeration level evaluated in the experiment. Because the optimum lies at the boundary of the experimental design rather than within its interior, it is difficult to conclude whether 11.0 L/day represents a true optimum or simply the best value among those tested. Future work extending the aeration range beyond the current upper limit would help determine whether the response reaches a maximum or continues to improve.

14.Selection of factor ranges requires additional justification

The rationale for selecting YAN concentrations of 150 and 300 mg/L and aeration levels of 0, 5.5, and 11.0 L/day should be described in greater detail. Although these values are derived from engineering calculations and literature estimates, it is unclear whether preliminary experiments or pilot studies were conducted to verify that these ranges encompass the biologically relevant response space. A stronger justification for the selected factor levels would improve confidence that the Design of Experiment adequately explored the optimization landscape.

Reviewer-1

Open response questions

Comments to author

The manuscript appears to conflate three distinct stages:

-environmental effects on peach growth,
-must preparation,
-fermentation physiology.
The causal chain is not convincingly established.

The central biological rationale requires substantial strengthening. The manuscript links Colorado Front Range environmental conditions (high altitude, reduced growing season, increased UV radiation) directly to fermentation management decisions, but several of these causal connections are insufficiently justified.

First, the oxygen argument appears overstated. The manuscript argues that lower atmospheric pressure reduces oxygen dissolution sufficiently to require continuous supplemental aeration during fermentation. While reduced oxygen partial pressure at altitude is physically correct, commercial fruit wine fermentations—including peach wines—are not normally conducted with continuous forced aeration. Oxygen is typically supplied only during yeast rehydration, inoculation, or the early growth phase through mixing or limited aeration, after which fermentation proceeds under largely anaerobic conditions to avoid oxidation. Consequently, the manuscript should explain why the proposed daily aeration regime is necessary specifically for peach fermentation at 5,600 ft and demonstrate that conventional fermentation practices are inadequate under these conditions. Without such evidence, the connection between altitude and the selected aeration protocol appears speculative rather than experimentally justified.

Thank you for the feedback; it is appreciated. Many decisions in this project and analysis and reporting were driven in part by a need to manage the overall scope. One could easily expand the research and analysis to many months of time and many hundreds of pages of writing, which would not be ideal.
Our initial research found that oxygen deficiency at altitude, an "environmental stressor", served as a specific input variable that generated multiple problems for high-altitude primary fermentation operations. Refer to Jackson Hole Winery's short discussion (comingled with a marketing bias) of their primary fermentation problems at https://jacksonholewinery.com/winemaking-2-2, where they speak of two very specific and substantial problems driven by altitude, one being the delayed fermentation and the other being oxygen deficiency. This delay drives heavy operations costs and margin detriment, which they choose to absorb. They resolve their admitted oxygen deficiency problem via "punch downs versus pump overs are done several times a day during the fermentation

process." We chose to pursue a more quantified solution to this precise problem.
We will clarify this in the report per your concern, that a daily aeration regime is warranted, based on the actions of Jackson Hole Winery and others (refer to modified section 3.3).

Second, the microbial argument is internally inconsistent. The introduction argues that increased UV radiation alters native microbial communities on peach skins; however, the methods subsequently state that all peaches were scrubbed and sanitized before inoculation with a commercial Saccharomyces cerevisiae strain. Since indigenous microorganisms were intentionally eliminated before fermentation, any altitude-induced differences in native microbiota would no longer contribute to fermentation outcomes. As written, the discussion of altered skin microbiota serves primarily as background and does not appear mechanistically relevant to the experimental system.
Fully agreed. Three micro-climate stressors were identified as being likely relevant to successful primary peach fermentation. Of those three, the microbial was seen as unique, and removed from the analysis, for two reasons: (1) analyzing, controlling, and deliberately manipulating it would be highly complex, and (2) commercial production simply does not deal with this, they remove the natural diversity and add their choice of commercial yeast strains. Effectively, the Colorado industry, and others, have already removed this variable from their production processes. Based on this knowledge, we chose to keep this climate stressor as a constant.
We were clear in explaining its existence as an altitude-driven stressor (section 3.1) as well as our justification for its withholding from the DoE factors (section 4.1).

Third, the nitrogen hypothesis requires stronger justification. The manuscript argues that Colorado's shorter growing season reduces yeast assimilable nitrogen (YAN) accumulation in peaches and therefore motivates DAP supplementation. However, nitrogen accumulation is a property of fruit development prior to harvest. Once the peaches are harvested and incorporated into the must, the physiological processes responsible for nitrogen assimilation have already ceased. Fermentation depends on the measured nitrogen content of the must rather than on the environmental mechanism that produced it. Accordingly, the study should directly measure the initial YAN concentration of the experimental peach must instead of estimating it from regional assumptions. Without direct YAN measurements, the proposed connection between growing-season stress and fermentation performance remains indirect and speculative.
The experimenters chose industry-utilized estimates for YAN deficiency for two specific reasons: (1) this laboratory equipment would explode the project's budget while (2) providing precision that, as promised by the manufacturers, is only marginally better than the range of industry-standard estimates for the Front Range. To minimize the impact of this admitted imprecision, whether originating from industry-standard estimates or inherent to a measuring device, the experimenters chose factor levels that spanned approximately five times the expected error of the baseline measurement (150mg/L vs 30mg/L or 10mg/L).

The manuscript claims to optimize "high-altitude fermentation," yet all fermentations were conducted at a single altitude (approximately 5,600 ft). Consequently, the study does not experimentally isolate altitude as a factor; rather, it optimizes aeration and nitrogen supplementation under one specific environmental condition. Demonstrating that these interventions compensate for altitude would require comparison with otherwise identical fermentations performed at lower elevation or under controlled barometric pressure. As currently designed, the study identifies an empirical operating point for one location but does not establish that altitude itself is the causal factor necessitating the proposed interventions.
Very interesting and solid argument. The researchers tested at altitude and did not use altitude as a

design variable. Section-1 clarifies this, that the experiment would seek to find solutions to the specific climatic challenges, without any claim to be solving for changes in altitude. The end of section-3 is also clear about this, that the researchers are solving for problems specific to 5,600'. The beginning of section-7 is also clear about this, never claiming or implying that the solution space accommodates moving from 0' to 5,600', but simply finding a strong and successful solution space at 5,600'.
We will clarify this distinction: that the research finds a solution space for 5,600', but not for a solution that specifically accommodates the move from 0' to 5,600'. See the end of section-8. Thank you for the good point.

Overall, the manuscript would be substantially strengthened by distinguishing pre-harvest environmental effects on fruit composition from post-harvest fermentation engineering variables. At present, several environmental stressors introduced in the rationale are either experimentally removed (native microbiota) or only indirectly related to the fermentation process (plant nitrogen assimilation), making the mechanistic narrative less convincing than the statistical optimization itself.
Understood. To contain the scope of the research project, the focus was kept on the primary fermentation process design. Nearly boundless complexity could be studied 'upstream' (in the orchards) and 'downstream' (after primary fermentation). The researchers made a careful and deliberate decision to focus within primary fermentation and within the principle micro-climate stressors.

Below are points of concern related to the experiment itself:

1. Experimental scope versus conclusions

The study is presented as an optimization of "high-altitude peach fermentation"; however, altitude itself was never included as an experimental factor. All fermentations were performed at a single elevation (approximately 5,600 ft), and only YAN supplementation and aeration were varied. Consequently, the Design of Experiment optimizes fermentation conditions at one location rather than demonstrating that altitude is the causal factor responsible for the observed effects. Conclusions should therefore be restricted to the experimental factors that were actually manipulated, or alternatively, a comparison with identical fermentations conducted at lower elevation should be incorporated to substantiate altitude-specific claims.
Fully understood and agreed. As detailed above, the report has been modified to further clarify that this research seeks solution spaces for stresses specifically at 5,600' on Colorado's Front Range and does not seek to compare across altitudes. Conclusions are restricted to the two factors' effects on the two responses.

2. Independent variable (YAN) was not experimentally verified

The study treats YAN concentration as one of its principal independent variables; however, the baseline YAN concentration of the peach must was not measured experimentally. Instead, it was estimated from literature values and regional assumptions before diammonium phosphate additions were calculated. As a result, the actual YAN concentrations achieved in the fermentation vessels remain unknown. Since the validity of the factorial design depends on accurate knowledge of factor levels, direct measurement of initial YAN should be performed to confirm that the intended treatment concentrations were actually achieved.

The biochemistry of peach fermentation is extremely complex. To bound this project into science and mathematics that the primary researcher would fully understand and lead, and to work within reasonable laboratory equipment sourcing constraints, many interesting research avenues were declined. Per previous explanation, multiple industry sources were used to identify sound estimates for YAN deficiency in CFR, and their range of values was not greatly different from the likely real-world accuracy of laboratory YAN measuring equipment, and both were deliberately far below the range of YAN levels selected for this experiment. This level range was selected specifically to minimize the significance of imprecision of YAN baseline.

3. Oxygen availability was inferred rather than measured

Similarly, dissolved oxygen—the second principal experimental factor—was never measured. The study assumes oxygen availability based on atmospheric pressure, calculated gas delivery, and estimated gas-liquid transfer efficiency, but no dissolved oxygen measurements were obtained during fermentation. Consequently, the experiment manipulated air delivery rather than directly quantifying oxygen available to the yeast. Measurement of dissolved oxygen would substantially strengthen the mechanistic interpretation of the aeration treatments.
The researchers agree with your argument. Direct measurement of oxygen absorption, however, is difficult, complex, and expensive: a low-cost Thermo Scientific Orion dissolved oxygen probe is $1,200. As before, to contain this experiment within the primary researcher's capacities, the researchers chose to use common industry estimates of oxygen uptake from micro spargers. The previously referenced Jackson Hole Winery states their use of supplemental aeration in a non-quantified, crude delivery: "punch downs … three times per day during primary fermentation".

4. Aeration protocol requires stronger biological justification

The aeration factor is defined as daily air delivery volumes (0, 5.5, and 11.0 L/day), yet the manuscript provides limited justification for why these treatment levels represent biologically meaningful interventions. Oxygen requirements for Saccharomyces cerevisiae are largely confined to the early exponential growth phase, whereas prolonged aeration may also increase oxidative degradation of aroma compounds. The manuscript should clearly describe the timing, duration, and biological rationale for the selected aeration schedule and explain why daily aeration throughout the fermentation process was chosen.
The origin of 11.0 L/day, the factor's upper level, is detailed and calculated in section 4.4.2, justified as the back-calculation for lost air pressure and oxygen supply versus Saccharomyces cerevisiae's kinetic requirement. The factor's lower level, 0.0 L/day, was chosen as a natural baseline for Front Range peach fermentation. To gain more insight, and more specifically to answer concerns about over-oxygenation, a center-point was used, calculated to 5.5 L/day. Section 4.4.2 was expanded to address timing, duration, and rationale.

5. Limited replication reduces statistical robustness

Each treatment combination was replicated only twice, resulting in twelve total fermentations for the six treatment combinations. Although this design provides sufficient degrees of freedom to perform ANOVA, the limited replication reduces the precision of variance estimates and increases the sensitivity of interaction effects to experimental variability. The statistical conclusions would be considerably strengthened by additional biological replicates.
The researchers fully agree. Two replicates suited the need to bound the potentially broad scope of this experiment but increased the risk of a high error calculation. Had the ANOVA error calculations been far higher, the experimenters could have chosen a third or fourth replicate. At the low calculated levels shown, further replications were not warranted.

6. Regression model complexity relative to sample size

The regression models include main effects and interaction terms while being fitted to a relatively small dataset. Although high R^2 values are reported, such values should be interpreted cautiously because models fitted to small datasets may exhibit optimistic goodness-of-fit. Additional model validation, such as prediction error estimates, cross-validation, or external validation experiments, would increase confidence that the observed relationships are robust rather than reflecting overfitting.
The researchers fully agree, again. The primary researcher learned the difference between model R^2 and R^2(adj) calculations. The report specifically and deliberately utilizes R^2(adj) values in the "Limitations" section, exactly matching your concern.

7. Lack of ANOVA diagnostic analyses

The manuscript reports ANOVA results but does not present the diagnostic analyses required to verify the underlying statistical assumptions. Residual normality, homogeneity of variance, independence of residuals, and identification of influential observations should be evaluated and presented. Residual-versus-fitted plots, normal probability plots, and residual histograms would substantially strengthen confidence in the reported statistical inferences.
The researchers reviewed these analyses with an eye to the concerns you list. We chose not to include this set of secondary analyses due to the secondary nature of the data relative to the high level analyses and the additional length it would put on a report that is already longer than most. The more insightful of these have now been added at Graph-6 and Graph-9, with corresponding analyses added to section 7.1 and section 7.2.

8. Justification of optimization targets

The optimization objective is defined as achieving 11–12.5% ethanol together with 15–30 g/L residual sugar. While these targets may be reasonable, the manuscript does not sufficiently justify why these ranges represent the optimal product profile for Colorado peach wine. These target values should either be supported by published sensory or industry standards or validated experimentally through sensory evaluation.
Proper justification of these targets has been added to section 4.1 and accordingly to the endnotes, using values from commercial products.

9. Rich longitudinal dataset was reduced to a single endpoint

One of the strengths of the study is the collection of daily density measurements throughout fermentation. However, these longitudinal data were ultimately reduced to Day-18 endpoint measurements for statistical analysis. As a result, much of the information regarding fermentation kinetics—including lag phase, fermentation rate, time to completion, and maximum fermentation velocity—is not utilized quantitatively. Incorporating kinetic analyses would provide a more complete understanding of treatment effects.
The researchers fully agree with this argument. This full experiment opened the door to many analyses. The researchers chose to focus on the connection of YAN and Aeration to RS and ethanol, which, of itself, is 25 pages.

10. Repeated measurements were not statistically modeled

Because each fermentation vessel was measured repeatedly over nineteen days, the observations within a vessel are not statistically independent. Although the authors ultimately analyzed only the

terminal measurements, the longitudinal dataset would be well suited for repeated-measures ANOVA or mixed-effects modeling, which would exploit the temporal structure of the data and provide greater statistical power while accounting for within-vessel correlations.
The researchers fully agree with this argument as well. This represents another potentially interesting study.

11. Biological interpretation of interaction effects

The manuscript identifies statistically significant interactions between YAN supplementation and aeration; however, these interactions are primarily described from a statistical perspective. Greater biological interpretation would strengthen the discussion by explaining why the response to nitrogen supplementation changes depending on aeration level and how these interactions relate to known yeast physiology and metabolic regulation.
The researchers chose to keep the project's focus closer to the core interactions and their mathematical relations. Delving into greater biological interpretations would have tremendously expanded the scope.

12. Clarification of the experimental unit

The manuscript would benefit from explicitly defining the experimental unit used in the statistical analyses. Each fermentation vessel appears to represent a single biological replicate, whereas repeated daily measurements within a vessel are repeated observations rather than independent replicates. Explicitly stating this distinction would improve the clarity and transparency of the statistical methodology.
Section-6 has been modified to clarify this. The researchers can understand the potential confusion of the dependent series of density measurements within each trial/vessel versus the independence of measurements between trials/vessels. Thank you.

13. Claimed optimum occurs at the boundary of the design space

The reported optimum aeration level (11.0 L/day) corresponds to the highest aeration level evaluated in the experiment. Because the optimum lies at the boundary of the experimental design rather than within its interior, it is difficult to conclude whether 11.0 L/day represents a true optimum or simply the best value among those tested. Future work extending the aeration range beyond the current upper limit would help determine whether the response reaches a maximum or continues to improve.
The researchers fully agree. The majority of two-level DOEs find optimizations at solution space corners. Any following DOE would wrap its factors around the first's optimization point.

14. Selection of factor ranges requires additional justification

The rationale for selecting YAN concentrations of 150 and 300 mg/L and aeration levels of 0, 5.5, and 11.0 L/day should be described in greater detail. Although these values are derived from engineering calculations and literature estimates, it is unclear whether preliminary experiments or pilot studies were conducted to verify that these ranges encompass the biologically relevant response space. A stronger justification for the selected factor levels would improve confidence that the Design of Experiment adequately explored the optimization landscape.
The researchers did not conduct pilot studies, instead using industry experience and environmental calculations as elaborated in Section 4.4.1 and 4.4.2.

Thank you for your feedback; it’s a privilege to read such thoughtful insights and see this work from a different perspective.

Reviewer-2

Open response questions

Comments to author

Please verify that all links to the references point to the correct source.

The National Laboratory of the Rockies changed their domain as well as the underlying datasets for their calculations. New, separate endnotes have been made. Minor data updates to match NLR database changes have also been made in section 3.1.

The authors must disclose and acknowledge any assistance received in the preparation of this manuscript, including but not limited to editorial, technical, analytical, or writing support. All such contributions must be clearly stated in the Acknowledgments section.

11.3 is added to clarify third-party contributions.

Include enough recent references along with foundational ones. Ensure references directly support your claims. Avoid “padding.” Use credible sources (peer-reviewed journals, reputable books, official reports).

Revised references include recent professional industry trade group publications from the Colorado Wine Industry Development Board (2022 & 2024) and the Australian Wine Research Institute (2017) as well as the just-updated National Laboratory of the Rockies’ radiation data. Multiple peer-reviewed journals are also included: Thomasen E. and Sablayrolles, J.-M.

All assumptions (including implicit assumptions) must be explicitly stated and clearly justified. The authors should explain why each assumption is reasonable and discuss its impact on the results and conclusions.

Section 3.2 was updated to justify the comparative climate assumption, with endnotes added.

Section 3.3 was updated to explicitly underpin the risks of nitrogen deficiency, with peer-reviewed references endnoted.

Section 4.1 was updated with reference to the peer-reviewed professional journal from which that knowledge was gained.

Avoid overreaching conclusions that extend beyond what is supported by the data and analysis.

Section 8 was updated to remove the broad assumption of this research on final product quality. The researchers can only draw conclusions about successful primary fermentation, and not about broader final product quality parameters.

The last paragraph of section 10 was narrowed to focus exclusively on RS and ethanol, and to avoid broader conclusions.

Verify that you have used past perfect tence and third person throughout the manuscript wherever applicable.

All first-person usage was removed, and past perfect conjugations were utilized as applicable.

Thank You for attempting to address my comments. Deficiencies remain, but I wil try to mitigate them by granting you the following assumptions and scope adjustment:

-Estimated YAN is an acceptable substitute for direct YAN measurement.
-Calculated oxygen delivery is an acceptable substitute for dissolved oxygen measurement.
-You did not find the global optimum (although you set out to do so), but rather, identified the best-performing treatment among the tested combinations.
-The conclusions are limited to fermentation at approximately 5,600 ft, not generalized to all altitudes.
If you include these explicitly in the paper, then I think the paper becomes scientifically coherent. A detailed comments section follows:
1.Please move most of the figures (retain one representative figure) to supplementary file(s). Likewise, present all rudimentary calculations in a supplementary file. Also present all raw data that you have not used for analysis or drawing conclusions in a supplemtary file. The manuscript should only contain relevant analysis-ready information used drawing conclusions.
2.Tone down claims that cannot be justified by your engineering optimization study. For example “globally ideal solution”, “optimization”…. What you have actually done is an engineering design study: “Which combination of DAP supplementation and supplemental aeration produces approximately 12% ABV and 15–30 g/L residual sugar under one specific fermentation setup conducted at 5,600 ft?”. Everything else is speculation or motivation. Please frame the manuscript consistent with its scope.
3.Present engineering calculations as factor selection - not validation. Currently, the manuscript reads almost as if : engineering calculation → therefore 11.05 L/day is correct. A better interpretation is “engineering calculation → therefore 11.05 L/day is a reasonable upper factor level to test experimentally.” Explicitly state that the justification for using engineering calculations

rather than actual measurement was that the levels were farther apart than the engineering versus actual, carry-through error (plus cost etc.).
4.You cannot prove that “11.05 L/day fully restores lost atmospheric pressure” or imply that the oxygen deficit has been quantitatively compensated. Please modify language accordingly.
5.Replace 'asymmetic DoE" (this is not common terminology) with something like 2X3 mixed level full factorial desgn.
6.Remove all ‘upstream’ and ‘downstrem’ factor discussion or introduction that has nothing to do with primary fermentation - or explicity designate such verbiage (as having nothing to do with primary fermentation). All you need to say is “Commercial Colorado wineries routinely encounter fermentation challenges at 5,600 ft. Regardless of the underlying mechanism, we sought to optimize two controllable engineering variables (YAN supplementation and aeration).”
7.What happens if a new peach cultivar is discovered capable of assimilating YAN despite the shorter growing season? Since you do not measure starting YAN, you may un-necessarily then be adding costs and effort. The manuscript would benefit from explicitly acknowledging this limitation. A statement such as: “The recommended YAN supplementation strategy assumes peach cultivars exhibiting the characteristic low-YAN profile currently observed in Colorado Front Range production. Future cultivars with substantially different native YAN concentrations would require recalibration of the supplementation protocol.” A similar sitution could arise for yeast. Please include “Because yeast oxygen requirements vary among strains, the optimal aeration strategy identified here is expected to be strain-dependent. Future work should evaluate whether low-oxygen-tolerant or genetically improved Saccharomyces strains require reduced aeration under otherwise identical high-altitude fermentation conditions.”

Open response questions

Comments to author

Thank You for attempting to address my comments. Deficiencies remain, but I wil try to mitigate them by granting you the following assumptions and scope adjustment:

-Estimated YAN is an acceptable substitute for direct YAN measurement (It would greatly help if you could provide literature references, if you have not already done so).
-Calculated oxygen delivery is an acceptable substitute for dissolved oxygen measurement (agan, literature references will help here).
-You did not find the global optimum (although you set out to do so), but rather, identified the best-performing treatment among the tested combinations.
The researchers did not set up to find a global optimum The Abstract clarifies that the researchers sought “*an optimization space* that resolves these unique climatic stressors”. The Results section is also clear on this, that the researchers’ objective was “… to identify solutions to Front Range environmental stresses”.

-The conclusions are limited to fermentation at approximately 5,600 ft, not generalized to all altitudes.
Agreed. Altitude was never presented or used as an input variable. To clarify that this analysis is focused solely on Front Range altitudes of 5,600’, the authors have expanded the middle of the Abstract section, immediately after the above quotation, per your concern.

If you include these explicitly in the paper, then I think the paper becomes scientifically coherent [thank you] - although nowhere as robust as if you had actually measured these attributes. A detailed comments section follows:

1. Please move most of the figures (retain one representative figure) to supplementary file(s). Likewise, present all rudimentary calculations in a supplementary file. Also present all raw data that you have not used for analysis or drawing conclusions in a supplemtary file. The manuscript should only contain relevant analysis-ready information used drawing conclusions.
   All figures/graphs/tables but for "Figure-1" have been removed. All were uploaded on 7/28. Rudimentary calculations have been copied to a supplementary file and uploaded.
2. Tone down claims that cannot be justified by your engineering optimization study. For example "globally ideal solution", "optimization"…. What you have actually done is an engineering design study: "Which combination of DAP supplementation and supplemental aeration produces approximately 12% ABV and 15–30 g/L residual sugar under one specific fermentation setup conducted at 5,600 ft?". Everything else is speculation or motivation. Please frame the manuscript consistent with its scope.
   "Globally ideal solution" removed and reframing added. Abstract modified to narrow the presentation toward solutions to the environmental challenges that modify specific inputs to deliver specific targeted outputs. The "Limitations" section is also modified to clarify the exclusive focus of nitrogen and oxygen to the model.
3. Present engineering calculations as factor selection - not validation. Currently, the manuscript reads almost as if : engineering calculation → therefore 11.05 L/day is correct. A better interpretation is "engineering calculation → therefore 11.05 L/day is a reasonable upper factor level to test experimentally." Explicitly state that the justification for using engineering calculations rather than actual measurement was that the levels were farther apart than the engineering versus actual, carry-through error (plus cost etc.).
   This has been updated at both the beginning and end of Section 4.4.1, and at the end of 4.4.2.
4. You cannot prove that "11.05 L/day fully restores lost atmospheric pressure" or imply that the oxygen deficit has been quantitatively compensated. Please modify language accordingly.
   Agreed & updated.
5. Replace 'asymmetic DoE" (this is not common terminology) with something like 2X3 mixed level full factorial desgn.
   Agreed & done.
6. Remove all 'upstream' and 'downstrem' factor discussion or introduction that has nothing to do with primary fermentation - or explicity designate such verbiage (as having nothing to do with primary fermentation). All you need to say is "Commercial Colorado wineries routinely encounter fermentation challenges at 5,600 ft. Regardless of the underlying mechanism, we sought to optimize two controllable engineering variables (YAN supplementation and aeration)."
   Abstract modified accordingly. Discussion modified accordingly. Limitations modified accordingly.
7. What happens if a new peach cultivar is discovered capable of assimilating YAN despite the shorter growing season? Since you do not measure starting YAN, you may un-necessarily then be adding costs and effort. The manuscript would benefit from explicitly acknowledging this limitation. A statement such as: "The recommended YAN supplementation strategy assumes peach cultivars exhibiting the characteristic low-YAN profile currently observed in Colorado Front Range production. Future cultivars with substantially different native YAN concentrations would require recalibration of the

supplementation protocol.” A similar sitution could arise for yeast. Please include “Because yeast oxygen requirements vary among strains, the optimal aeration strategy identified here is expected to be strain-dependent. Future work should evaluate whether low-oxygen-tolerant or genetically improved Saccharomyces strains require reduced aeration under otherwise identical high-altitude fermentation conditions.”

Both considerations were added to the Limitations section.