



Optimization of Reducing Sugars in Ber Wine

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Introduction and Data Description

Introduction

- **Post-harvest Loss Problem:**
 - Tropical fruits like **Ber (*Ziziphus mauritiana*)** have a **very short shelf life**, causing major **post-harvest losses** during peak seasons.
- **Why Ber Fruit?**
 - Ber thrives in marginal, saline, drought-prone soils, grows with low cultivation cost, and **offers high economic returns to farmers**.
- **Nutritional Richness:**
 - Ber is high in Vitamin C, minerals (Ca, P, Fe), and antioxidants. Provides **health benefits**: immune system support, liver protection, and anticancer properties.
- **Opportunity for Value Addition:**
 - Converting surplus Ber into wine can reduce wastage, enhance farmer income, and deliver a nutritious, value-added product.
- **Importance of Reducing Sugars:**
 - Reducing sugars are essential for yeast fermentation, wine flavor, and achieving higher alcohol yield.
- **Study Objective:** Evaluate acid(Sulfuric acid), alkali(Potassium Hydroxide), and enzyme(Pectinase) pretreatments at different time intervals and concentrations to optimize reducing sugar release for Ber wine production.

Data Source and Experiment Description

The dataset used in this study was collected by an independent researcher affiliated with our project team (Department of Agricultural Engineering at the University of Agricultural Sciences, Raichur, India, 2016-2017).

Production of wine from Ber fruit

Collection of ber fruits



Cleaning, deseeding, and crushing



Juice extraction



Pretreatment of Juice at **Concentrations**

(Acid, Alkali, Enzyme at 0.5%, 1.0%, 1.5%)



Monitoring Reducing Sugars (**Time**)

(at every 4, 8 and 12 h interval)



Selection of best sample for fermentation process

(based on maximum reducing sugars content)

Key Traits Evaluated:

1. Fixed Factors:

1. **Pretreatments:** Treatments used to improve reducing sugars output (Acid, Alkali, Enzyme)
2. **Concentration:** % concentration of reducing sugar treatments (0.5%, 1.0%, 1.5%)
3. **Time:** Hours passed after pre-treatment (4h, 8h, 12h)

2. Response:

1. **Reducing sugars:** Amount of reducing sugars present after pre-treatment

What's interesting about this Data

- Captures a controlled, multifactorial fermentation experiment using Ber fruit, a drought-tolerant tropical crop.
- Shows a wide dynamic range of reducing sugar release depending on pretreatment, concentration, and time.
- Reveals complex interaction effects rather than simple additive responses.
- Offers a statistical opportunity to model main effects and interactions.
- Supports food science innovation by promoting sustainable, high-quality alcohol production from underutilized tropical fruits.





Statistical Model and Assumptions

Model

3 x 3 x 3 Full Factorial Model with **fixed effects** was used to analyze data.

Predictors:

1. Pre-treatment: 3 levels, fixed: Acid, Alkali, and Enzymes
2. Concentrations: 3 levels, fixed: 0.5%, 1.0%, 1.5%
3. Time Interval: 3 levels, fixed, 4, 8, 12 hours

Replications: 3 replications performed for each of the twenty-seven treatments.

Response: Reducing sugars measured after time has elapsed.

Effects Model: $y_{ijkl} = \mu + \tau_i + \gamma_j + \delta_k + (\tau\gamma)_{ij} + (\tau\delta)_{ik} + (\gamma\delta)_{jk} + (\tau\gamma\delta)_{ijk} + e_{ijkl}$

- y_{ijkl} is the response variable for the l -th observation in the i -th level of factor τ , the j -th level of factor γ , and the k -th level of factor δ .
- μ is the overall mean.
- τ_i is the effect of the i -th level of factor τ (Pretreat).
- γ_j is the effect of the j -th level of factor γ (Conc).
- δ_k is the effect of the k -th level of factor δ (Time)

Hypotheses and Assumptions

Main Research Questions:

- What is the effect of our factors on the mean reducing sugar (RS) content?
- Do interactions exist between the factors?

Main Effects Hypothesis (for each factor):

H_0 : The mean RS is the same across all levels.

H_1 : At least one factor level produces a different mean RS content.

Two-Way Interactions Hypothesis:

H_0 : There are no 2-way interactions between the factors on RS content.

H_1 : There is an interaction between any two levels on RS content.

Three-Way Interaction Hypothesis:

H_0 : There is no 3-way interaction between the factors on RS content.

H_1 : 3-way interaction exists between the factors on RS content.

Assumptions:

- Equal Variance: Brown Forsythe p-value > 0.05 so variances are equal.
- Normality: Shapiro-Wilk p-value > 0.05. Residuals are normally distributed.



Prelim Analysis

Normality Condition

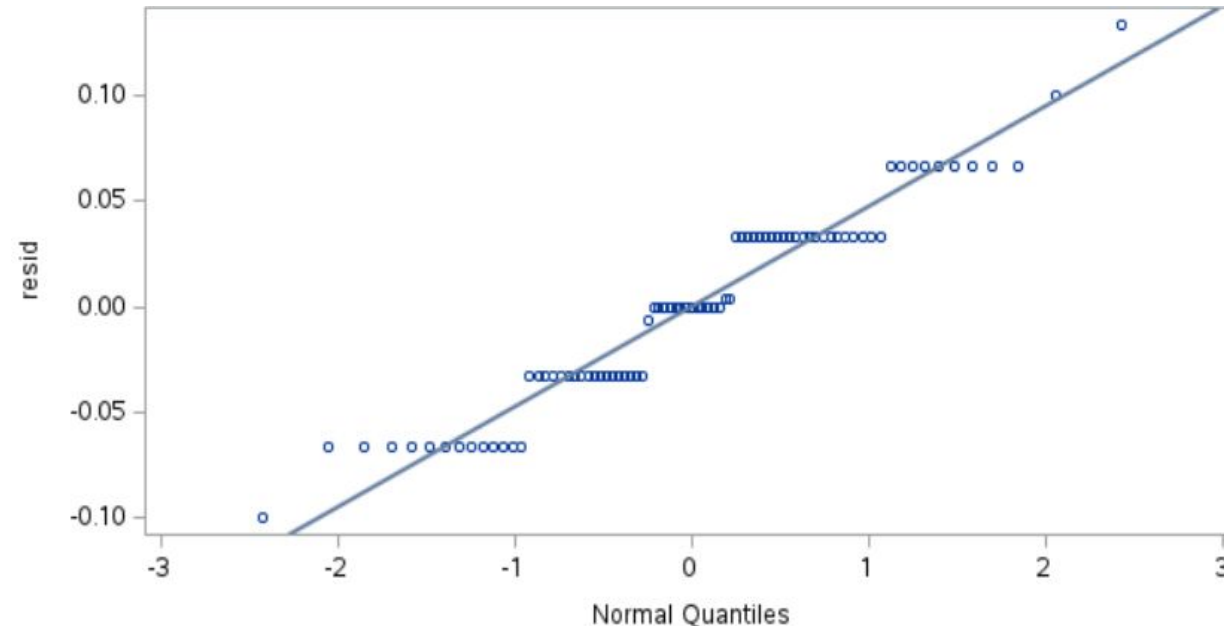
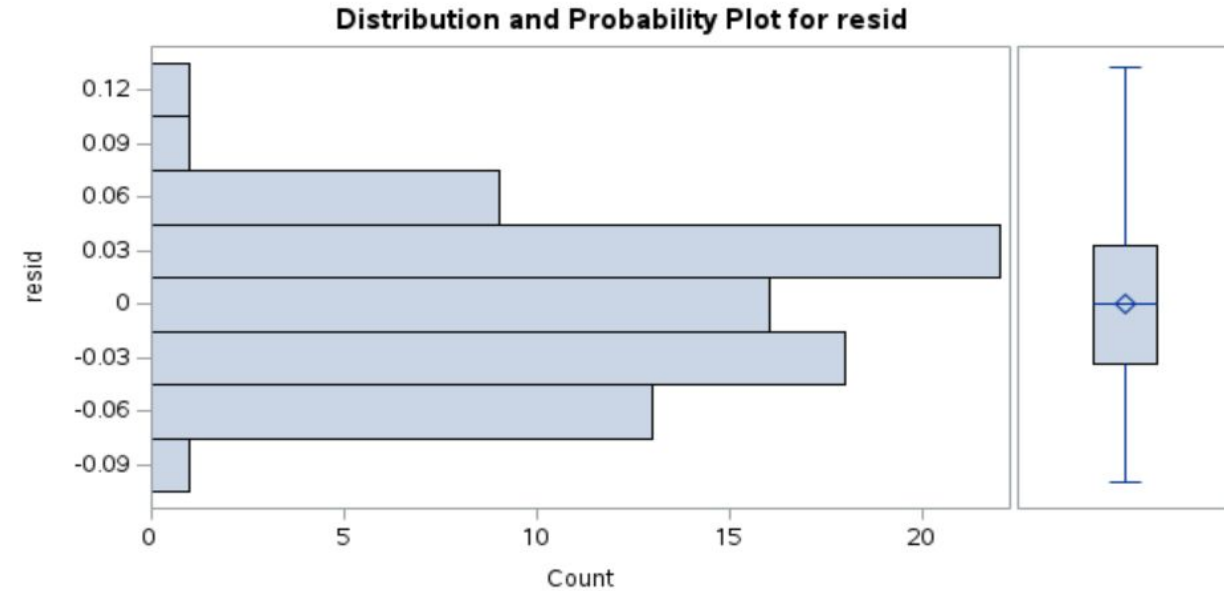
Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.940982	Pr < W	0.0010

Shapiro-Wilk

- p-value: 0.001
- W Statistic: 0.940982

Although p-value < 0.05 , indicating violation of normality, the data satisfy other key assumptions:

- equal sample sizes across groups
- total sample size $N > 30$



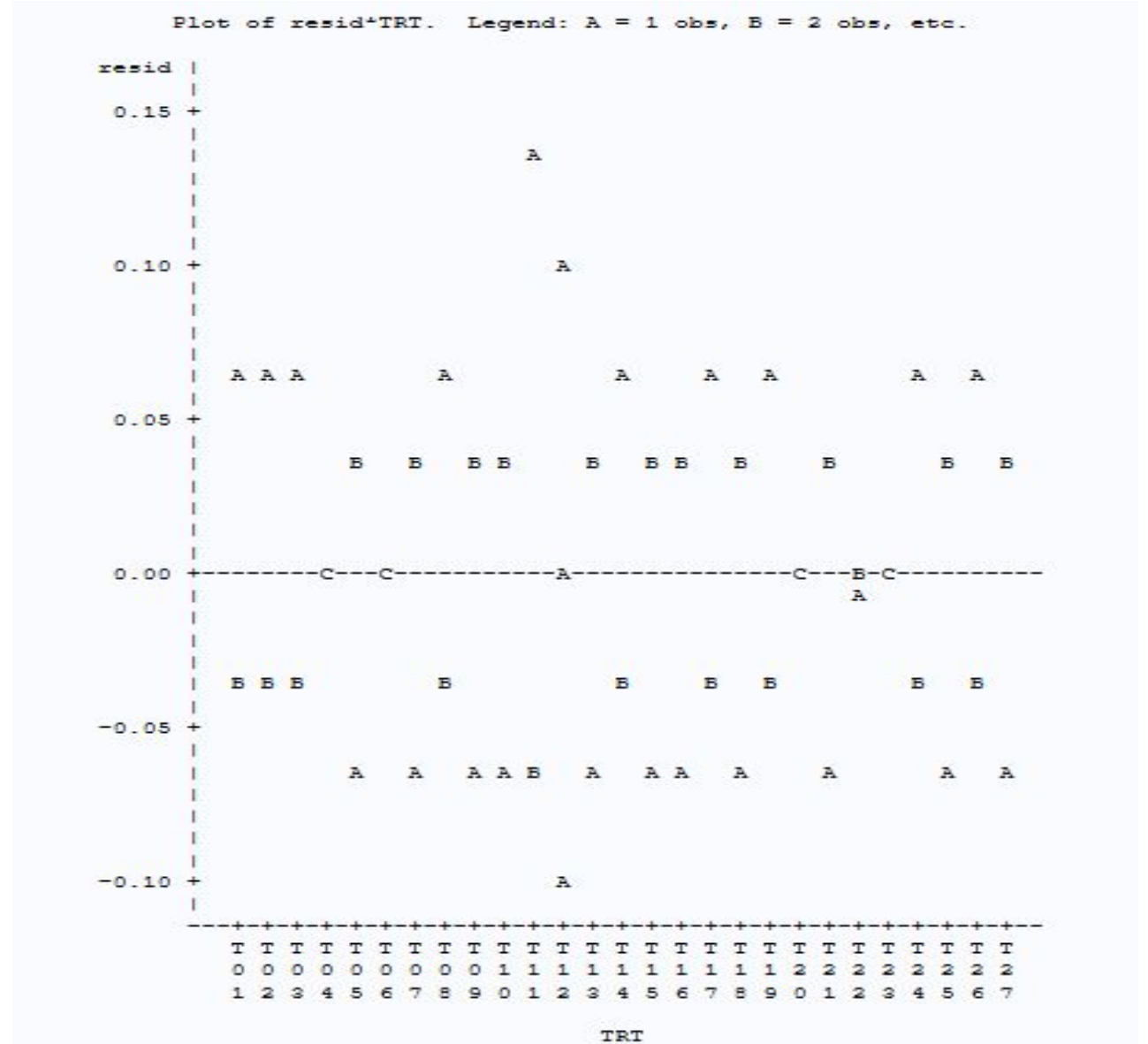
Equal Variance Condition

Brown and Forsythe's Test for Homogeneity of RS Variance					
ANOVA of Absolute Deviations from Group Medians					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TRT	26	0.0217	0.000833	0.27	0.9997
Error	54	0.1667	0.00309		

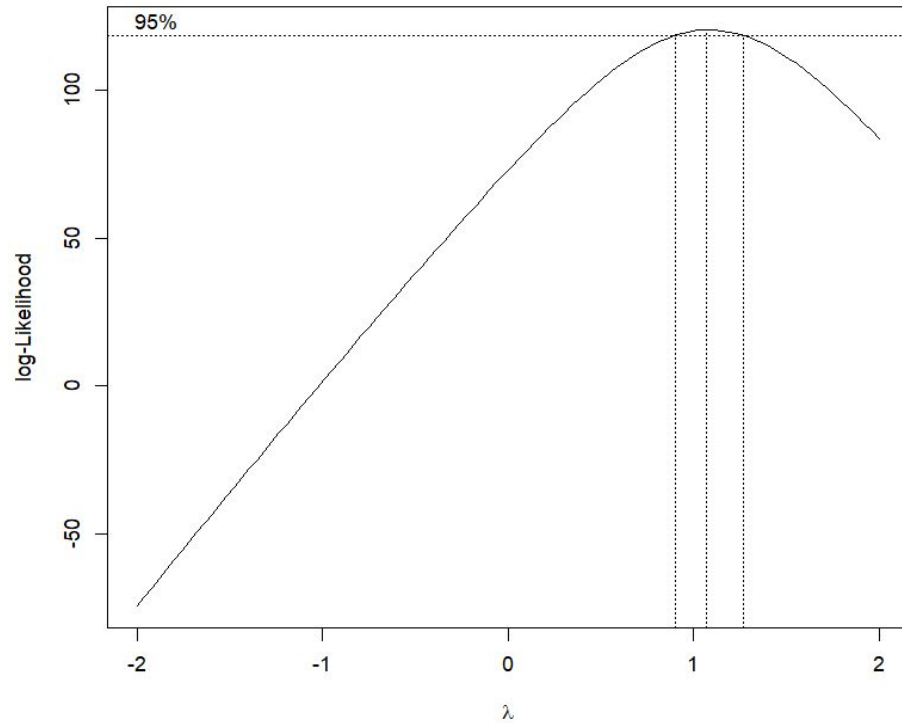
Brown-Forsythe-Levene

- p-value: 0.9997

The Brown-Forsythe-Levene
p-value > 0.05. Therefore, our
data meets the condition of
equal variances.



Normalization Attempts



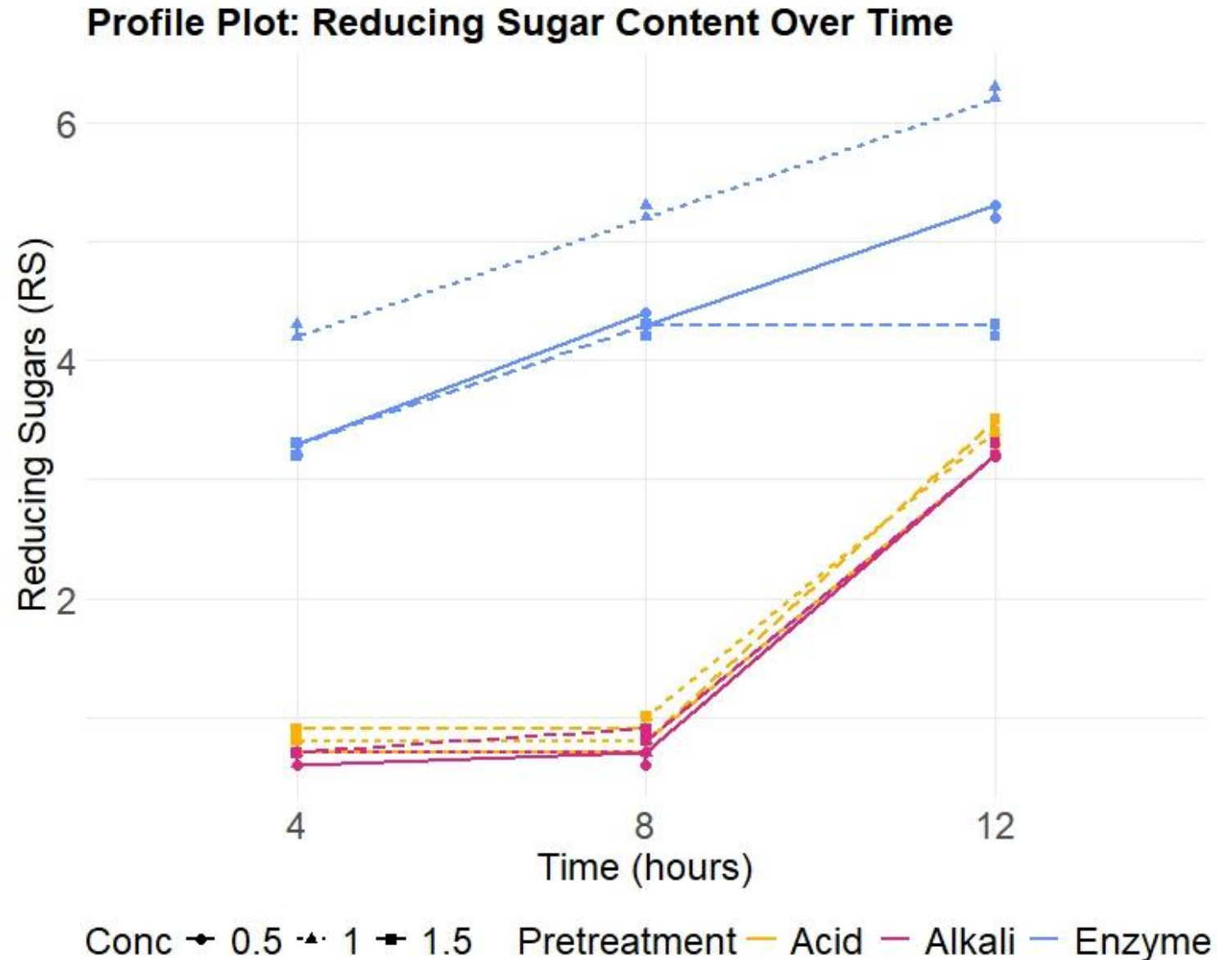
- **Log Transformation**
 - Residuals further deviated from normality
- **Box-Cox Transformation**
 - $\lambda = 1.072 \approx 1$, suggests little to no need for transformation.
- **Kruskal-Wallis**
 - Not applicable due to the design having more than one factor

Interactions

The profile plot suggests that interactions exist in our data.

The way concentration affects reducing sugar content is not uniform across the different treatment types, and changes over time.

Need to statistically confirm these interactions with ANOVA.





Analysis

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	26	250.9427728	9.6516451	2894.42	<.0001
Error	54	0.1800667	0.0033346		
Corrected Total	80	251.1228395			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Pretreat	2	150.9766691	75.4883346	22638.1	<.0001
Conc	2	2.5778543	1.2889272	386.53	<.0001
Pretreat*Conc	4	5.7313383	1.4328346	429.69	<.0001
Time	2	80.0041506	40.0020753	11996.2	<.0001
Pretreat*Time	4	10.3394864	2.5848716	775.17	<.0001
Conc*Time	4	0.4689679	0.1172420	35.16	<.0001
Pretreat*Conc*Time	8	0.8443062	0.1055383	31.65	<.0001

- All main effects are highly significant ($p < 0.0001$):
- Pretreatment explains the most variation in RS ($F = 22\,638$), followed by Time ($F = 11,996$) then Concentration ($F = 387$).
- All two-way interactions are significant.
- Significant three-way interaction: tells us that which treatment works best changes with both concentration and incubation time.

Post-hoc analysis: Multiple Comparison Groupings

Groupings based on $\alpha = 0.00185$ (adjusted using $\alpha/M = 0.05/27$, where $M = 3 * 3 * (3 \text{ choose } 2)$)

Pretreatment (all Concentrations & Time): {Acid, Alkali}, {Enzyme}

Concentration:			Time:		
Level, P	Levels, T	Groupings, C	Level, P	Levels, C	Groupings, T
Acid	4h, 8h, 12h	{1.0%, 1.5%}, {0.5%}	Acid	0.5%, 1.0%	{4h}, {8h}, {12h}
Alkali	4h & 8h	{0.5%, 1.0%, 1.5%}	Acid	1.50%	{4h, 8h}, {12h}
Alkali	12h	{1.0% , 1.5% }, { 0.5%}	Alkali	0.5%, 1.0%	{4h, 8h}, {12h}
Enzyme	4h & 8h	{0.5%, 1.5%}, {1.0%}	Alkali	1.50%	{4h}, {8h}, {12h}
Enzyme	12h	{0.5%}, {1.0%}, {1.5%}	Enzyme	0.5%, 1.0%	{4h}, {8h}, {12h}
			Enzyme	1.50%	{4h}, {8h, 12h}

Post-hoc analysis: Multiple Comparison Groupings

The GLM Procedure
Least Squares Means

Pretreat	Conc	Time	RS LSMEAN	Standard Error	Pr > t	LSMEAN Number
ACID	0.5	12	3.2333333	0.0333395	<.0001	1
ACID	0.5	4	0.6333333	0.0333395	<.0001	2
ACID	0.5	8	0.7666667	0.0333395	<.0001	3
ACID	1.0	12	3.4000000	0.0333395	<.0001	4
ACID	1.0	4	0.7333333	0.0333395	<.0001	5
ACID	1.0	8	0.8666667	0.0333395	<.0001	6
ACID	1.5	12	3.4666667	0.0333395	<.0001	7
ACID	1.5	4	0.8333333	0.0333395	<.0001	8
ACID	1.5	8	0.9000000	0.0333395	<.0001	9
ALKALI	0.5	12	3.1966667	0.0333395	<.0001	10
ALKALI	0.5	4	0.6000000	0.0333395	<.0001	11
ALKALI	0.5	8	0.6666667	0.0333395	<.0001	12
ALKALI	1.0	12	3.2000000	0.0333395	<.0001	13
ALKALI	1.0	4	0.6666667	0.0333395	<.0001	14
ALKALI	1.0	8	0.7333333	0.0333395	<.0001	15
ALKALI	1.5	12	3.2333333	0.0333395	<.0001	16
ALKALI	1.5	4	0.7000000	0.0333395	<.0001	17
ALKALI	1.5	8	0.8666667	0.0333395	<.0001	18
ENZYME	0.5	12	5.2666667	0.0333395	<.0001	19
ENZYME	0.5	4	3.2666667	0.0333395	<.0001	20
ENZYME	0.5	8	4.3666667	0.0333395	<.0001	21
ENZYME	1.0	12	6.2333333	0.0333395	<.0001	22
ENZYME	1.0	4	4.2333333	0.0333395	<.0001	23
ENZYME	1.0	8	5.2333333	0.0333395	<.0001	24
ENZYME	1.5	12	4.2666667	0.0333395	<.0001	25
ENZYME	1.5	4	3.2666667	0.0333395	<.0001	26
ENZYME	1.5	8	4.2666667	0.0333395	<.0001	27

Least Squares Means for effect Pretreat*Conc*Time Pr > t for H0: LSmean(i)=LSmean(j)																											
Dependent Variable: RS																											
i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1		<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.4402	<.0001	<.0001	0.4826	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	0.4826	<.0001
2	<.0001		0.0066	<.0001	0.0385	<.0001	<.0001	<.0001	<.0001	<.0001	0.4826	0.4826	<.0001	0.4826	0.0385	<.0001	0.1631	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.0066		<.0001	0.4826	0.0385	<.0001	0.1631	0.0066	<.0001	0.0008	0.0385	<.0001	0.0385	0.4826	<.0001	0.1631	0.0385	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	0.0008	<.0001	<.0001		<.0001	<.0001	0.1631	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001
5	<.0001	0.0385	0.4826	<.0001		0.0066	<.0001	0.0385	0.0008	<.0001	0.0066	0.1631	<.0001	0.1631	1.0000	<.0001	0.4826	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.0385	<.0001	0.0066		<.0001	0.4826	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001	0.0008	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7	<.0001	<.0001	<.0001	0.1631	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	<.0001	0.1631	<.0001	0.0385	0.4826	<.0001		0.1631	<.0001	<.0001	0.0008	<.0001	0.0008	0.0385	<.0001	0.0066	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
9	<.0001	<.0001	0.0066	<.0001	0.0008	0.4826	<.0001	0.1631		<.0001	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
10	0.4402	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	0.9439	<.0001	<.0001	0.4402	<.0001	<.0001	<.0001	<.0001	0.1435	<.0001	<.0001	<.0001	<.0001	<.0001	0.1435
11	<.0001	0.4826	0.0008	<.0001	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001		0.1631	<.0001	0.1631	0.0066	<.0001	0.0385	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
12	<.0001	0.4826	0.0385	<.0001	0.1631	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	0.1631	<.0001	1.0000	0.1631	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9439	<.0001	<.0001		<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	0.1631	<.0001	<.0001	<.0001	<.0001	<.0001	0.1631	<.0001
14	<.0001	0.4826	0.0385	<.0001	0.1631	<.0001	<.0001	0.0008	<.0001	<.0001	0.1631	1.0000	<.0001		0.1631	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	0.0385	0.4826	<.0001	1.0000	0.0066	<.0001	0.0385	0.0008	<.0001	0.0066	0.1631	<.0001	0.1631		<.0001	0.4826	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	1.0000	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.4402	<.0001	<.0001	0.4826	<.0001	<.0001		<.0001	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	0.4826	<.0001
17	<.0001	0.1631	0.1631	<.0001	0.4826	0.0008	<.0001	0.0066	<.0001	<.0001	0.0385	0.4826	<.0001	0.4826	0.4826	<.0001		0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	0.0385	<.0001	0.0066	1.0000	<.0001	0.4826	0.4826	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001	0.0008		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001
20	0.4826	<.0001	<.0001	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	0.1435	<.0001	<.0001	0.1631	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.0066	<.0001	0.0385	<.0001	0.0385
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001		<.0001	0.4826	<.0001	0.4826
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001
25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0385	<.0001	0.4826	<.0001		<.0001	1.0000
26	0.4826	<.0001	<.0001	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	0.1435	<.0001	<.0001	0.1631	<.0001	<.0001	0.4826	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
27	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0385	<.0001	0.4826	<.0001	1.0000	<.0001		

$\alpha = 0.00185$

Colored boxes on the table show comparisons of the three incubation times for each Pretreatment*Concentration combination.

Post hoc analysis: Hsu's Best Procedure

Hsu's procedure was run to determine the best treatment out of the 27 treatments, based on highest RS value.

Treatment	Mean	Best_Diff	t_stat	Significant	Best
12.1.Enzyme	6.2333333	0.0000000	0.00000	FALSE	TRUE
12.0.5.Enzyme	5.2666667	0.9666667	20.50230	TRUE	FALSE
8.1.Enzyme	5.2333333	1.0000000	21.20928	TRUE	FALSE
8.0.5.Enzyme	4.3666667	1.8666667	39.59065	TRUE	FALSE
8.1.5.Enzyme	4.2666667	1.9666667	41.71158	TRUE	FALSE
12.1.5.Enzyme	4.2666667	1.9666667	41.71158	TRUE	FALSE
4.1.Enzyme	4.2333333	2.0000000	42.41855	TRUE	FALSE
12.1.5.Acid	3.4666667	2.7666667	58.67900	TRUE	FALSE
12.1.Acid	3.4000000	2.8333333	60.09295	TRUE	FALSE
4.0.5.Enzyme	3.2666667	2.9666667	62.92085	TRUE	FALSE
4.1.5.Enzyme	3.2666667	2.9666667	62.92085	TRUE	FALSE
12.0.5.Acid	3.2333333	3.0000000	63.62783	TRUE	FALSE
12.1.5.Alkali	3.2333333	3.0000000	63.62783	TRUE	FALSE
12.1.Alkali	3.2000000	3.0333333	64.33480	TRUE	FALSE
12.0.5.Alkali	3.1966667	3.0366667	64.40550	TRUE	FALSE
8.1.5.Acid	0.9000000	5.3333333	113.11614	TRUE	FALSE
8.1.Acid	0.8666667	5.3666667	113.82312	TRUE	FALSE
8.1.5.Alkali	0.8666667	5.3666667	113.82312	TRUE	FALSE
4.1.5.Acid	0.8333333	5.4000000	114.53009	TRUE	FALSE
8.0.5.Acid	0.7666667	5.4666667	115.94404	TRUE	FALSE
4.1.Acid	0.7333333	5.5000000	116.65102	TRUE	FALSE
8.1.Alkali	0.7333333	5.5000000	116.65102	TRUE	FALSE
4.1.5.Alkali	0.7000000	5.5333333	117.35799	TRUE	FALSE
8.0.5.Alkali	0.6666667	5.5666667	118.06497	TRUE	FALSE
4.1.Alkali	0.6666667	5.5666667	118.06497	TRUE	FALSE
4.0.5.Acid	0.6333333	5.6000000	118.77195	TRUE	FALSE
4.0.5.Alkali	0.6000000	5.6333333	119.47892	TRUE	FALSE

Best treatment ("best" = highest mean):
Enzyme x 1% concentration x 12 hours
incubation
Critical value: 3.0169

Summary of Findings

- All main effects and interactions (two-way and three-way) were **highly significant** ($p < 0.0001$), influencing the release of reducing sugars.
- **Significant two-way and three-way interactions** indicated that the best approach to treating the ber juice is highly dependent on the combination of pretreatment type, concentration, incubation time.
- Pretreatment was the most influential factor explaining variation in reducing sugar (RS) levels, as evidenced by its higher Sum of Squares (SS) relative to Time and Concentration in the ANOVA results.
- **Post-hoc groupings revealed:**
 - Enzyme pretreatment consistently outperformed Acid and Alkali.
 - 1.0% concentration** and **12 h incubation** achieved the highest RS yield

Practical Application: The high reducing sugar enables efficient Ber wine production and offers a solution to reduce post-harvest losses while boosting farmer income through value-added processing.

References

- Course Handouts, Three-Way ANOVA and Experimental Design, STATISTICS 642 – Methods of Statistics II, Instructor: Derya Akleman, Ph.D, Texas A&M University, Spring 2025.
- Rachana, M. N. (2017). *Studies on Production Technology of Wine from Ber (Ziziphus mauritiana) Fruit (cv. Umran)* (Master's thesis, University of Agricultural Sciences, Raichur, India). Major Advisor: Dr. Ramappa, K. T.



Questions?