Investigating the Effect of Grape Variety on the Initial Fermentation Rate of Alcoholic Fermentation by Saccharomyces cerevisiae

Word Count: 3858

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Introduction

Research Question:

How does the type of grape, which are *Vitis vinifera cv. Muscat of Hamburg*, *Vitis vinifera cv. Cardinal* (red table grape), *Vitis vinifera cv. Sultanina* (Thompson seedless), affect the rate of alcoholic fermentation start by *Saccharomyces cerevisiae* as measured by the carbon dioxide production at 300 second intervals from 15 to 60 minutes at constant temperature?

Hypothesis:

Since grape composition differ in the fermentation affecting compounds, I expect these varieties will significantly influence the initial rate of alcoholic fermentation by Saccharomyces cerevisiae. Muscat type grapes often have higher sugar and aromatic compound concentrations, which will lead to more dynamic fermentation (Philipp et al., 2021). Sultanina (Thompson Seedless) grapes generally contain lower phenolic content and moderate sugar, that often results as slower fermentation (Acuña-Avila et al., 2023). Cardinal grapes typically show intermediate chemical properties (Ribéreau-Gayon et al., 2006). Therefore, the predicted order of fermentation rate is Muscat of Hamburg > Cardinal > Sultanina, centering on the generally higher phenolic and sugar levels of darker cultivars compared to the lower sugar white grapes.

Background Information:

Alcoholic fermentation is an anaerobic respiration that is usually conducted by yeasts, such as *Saccharomyces cerevisiae*. In this process, monosaccharides, specifically fructose and glucose, are broken down through glycolysis to form pyruvate (CH_3–CO–COO^-), which is then converted into ethanol and carbon dioxide in the absence of oxygen. This allows the regeneration of NAD^+ from NADH, enabling glycolysis to continue producing ATP (Walker & Stewart, 2016). The general reaction is:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{energy (ATP)}$$

Since *Saccharomyces cerevisiae* is tolerant to ethanol and exhibits efficient fermentative metabolism relative to other types of yeasts, it has been used worldwide in baking, brewing, and winemaking for thousands of years. Its fermentation efficiency, however, depends not only on internal enzyme activity but also on the chemical environment of the substrate. The key factors that play a role in fermentation outcomes of *Saccharomyces cerevisiae* are temperature, oxygen availability, sugar concentration, osmotic stress, pH, ethanol stress and nutrient status. In order to understand the effect of

the grape composition on the initial kinetic profile of fermentation, all these factors must be considered because they can alter the fermentation.

All grape juices are a chemically rich environment that contains sugars, organic acids, minerals, and phenolic compounds. The relative proportions of these vary by grape cultivar and maturity (Ribéreau-Gayon et al., 2006). Since glucose and fructose are substrates of glycolysis, sugar concentration strongly affects fermentation. There is more food to munch thus the yeast will normally produce more CO 2. However, excess sugar may cause osmotic stress to the point of halting or paralyzing the yeast.

The next major impact on the rate of fermentation is the levels of acidity in grape juice. The acidic environment prevents bacteria entry and it is a bit vital to the grape itself, though may also interfere with the enzymes by Saccharomyces cerevisiae. Enzymes in glycolysis tend to be most active when the pH is near neutral hence in case the juice is very acidic, the fermentation process may not be as effective (Walker, 1998).

Phenolics such as tannins, flavonoids, and anthocyanins contribute to grape color and taste, but they also interact with yeast metabolism. Tannins can bind to proteins and potentially inhibit enzyme activity (Waterhouse et al., 2016). Conversely, certain phenolics function as antioxidants, helping protect yeast cells from ethanol-induced stress (Rodriguez-Clemente et al., 2022). Therefore, more phenolic content theoretically leads to higher fermentation rates.

CO_2 release is in scientific context commonly used as a measure of fermentation because it occurs in a 1:1 molar ratio with ethanol production. Gas collection through water displacement is a well-established method that allows quantitative monitoring of fermentation in simple setups (Walker & Stewart, 2016).

Method Development and Planning:

The current work will focus on the early stage of the fermentation process since the influence of the grape juice chemistry on the early metabolic flux (fermentable sugars, pH, phenolics, YAN) is the most significant before the effects of ethanol buildup and nutrient loss bias the results. Measuring CO_2 volume every 300 seconds from 15 to 60 minutes provided a dense, manageable workload, allowing the initial rate to be estimated as the linear slope ($mL \cdot min^{-1}$). The first 15 minutes were skipped because of the artefacts from initial mixing. Gentle shaking during setup increases bubble formation and CO_2 release unrelated to steady metabolic flux. CO_2 was chosen as the readout because it is stoichiometrically equal to ethanol formation in alcoholic fermentation, therefore water displacement gas collection

yields a direct, instrument-light, and quantitative proxy of yeast activity suitable for school laboratories while remaining scientifically valid.

All other non-grape related factors were put under control to isolate the independent variable and directly observe the effect of variety of grape. All flasks were added with 100 mL of filtered juice and 5.0 g of the same active dry S. cerevisiae (single brand), and glassware, stoppers, tubing, and graduated cylinders were the same. With inter-reader bias minimized by recording meniscus readings by a single observer, all of the runs were initiated at the same time in the same room in order that any temperature drift would act equally on samples (systematic rather than cultivar-specific). The pH of every grape must be measured before fermentation to describe the initial acidity. Since pH varies naturally between grape cultivars, it was not controlled but it is measured also with sugar amounts to explain the rate differences. The dry yeast used in the experiment contained sorbitan monostearate (E491) as an emulsifier, which is a stabilizing additive. Since this compound does not interfere with yeast metabolism, it was not considered a variable in this investigation.

I did the experiment five times in order to obtain more accurate and credible data as well as enabling us to generate solid summary statistics. Where the apparatus did not permit running a very large number of replicate runs, I made up by sampling a great deal, and pre-specifying outlier diagnostics- linear-fit residual checks, sensitivity analyses, such that the conclusions we arrived at could not depend upon any single outlier result. Finally, natural juice composition (°Brix, acids, phenolics) was not adjusted, because the research question explicitly asks how cultivar chemistry as encountered modulates early fermentation kinetics any standardization would have removed the very mechanism under investigation.

Methodology:

Variables:

Table 1: Variables and details

Independent variable:	How It is controlled:	Why it is controlled:
The grape variety used as the fermentation substrate	The variety was checked from the product label. (Cardinal, Sultanina, Muscat of Hamburg)	Directly about research question
Dependent variable:		
The volume of CO2 produced (mL) during fermentation.	This was measured by water displacement in inverted graduated cylinders. Gas volumes were recorded at fixed time intervals (300s) using identical equipment for all replicates.	To ensure accurate data
Controlled variables:		
Volume of grape juice	Each flask contained exactly 100mL, measured with a graduated cylinder.	To ensure equal substrate/sugar amount
Mass of yeast (±0.01g)	5.00g of active dry yeast was directly added to each flask from 5.00g packages.	To ensure equal enzyme concentration
Fermentation time (±0.2s)	All flasks were sealed simultaneously and CO2 readings were taken at fixed intervals measured by mobile app ±0.2s. (Every 300s)	To compare at same metabolic stage.
Temperature (±0.5 C°)	All setups were kept in the same laboratory room and monitored with a thermometer ±0.5C°	To ensure consistency
pH and °Brix of juice	The pH and °Brix of each sample was checked with pH meters	To make better conclusion and interpretation of results
Type of equipment	The same size Erlenmeyer flasks, stoppers, tubing, graduated cylinders were used across all replicates.	To control pressure and workspace.
Observer	Measurements were recorded by the same individual to minimize human error.	To prevent bias, and error

While the exact nitrogen nutrient content could not be fully controlled across grape varieties, the initial sugar concentration (°Brix) and pH were measured for each cultivar before fermentation to analyze their correlation with the initial fermentation rate

Materials:

Table 2: Materials and details

Material/Equipment	Detail / Use
Dry yeast	Dr. Oetker, 10g, Saccharomyces cerevisiae, commercial formulation contains sorbitan monostearate (E491) as an emulsifier (listed on the package label) (see in Figure 1)
500±1g of Vitis vinifera cv. Cardinal (red table grape)	Weighed without the stem, only the fruit, prewashed (see in Figure 4)
500±1g of Vitis vinifera cv. Sultanina (seedless white grape, also known as Thompson Seedless)	Weighed without the stem, only the fruit, prewashed (see in Figure 5)
500±1g of Vitis vinifera cv. Muscat of Hamburg (black table grape)	Weighed without the stem, only the fruit, prewashed (see in Figure 3)
3 Erlenmayer flasks	250mL (see in Figure 2)

3 rubber stoppers with one hole	to fit the gas delivery tubes (see in Figure 2)
3 graduated cylinders	250 mL (see in Figure 2)
Alcohol Thermometer	±0.5 °C, graduated in 1 °C divisions (see in Figure 2)
Stopwatch	App on smartphone, display precision ±0.01 s, practical uncertainty ±0.2 s due to human reaction time
Clamps	to hold the cylinders
1 large plastic basin	for water bath
1 medium plastic basin	to wash the grapes and collect the juice (see in Figure 3)
Digital pH meter	±0.01g , *Lab Quest 3* Digital Measuring device
Digital refractometer attachment	±0.2 °Brix, An attachment for *Lab Quest 3,* Used to measure the initial total sugar concentration of every grape juice.
metal strainer	(fine grit) (shown in Figure 6)

See the Figures in Appendices at the end.

Experimental Procedure:

1. Collect ~500 g of each grape variety (Cardinal, Sultanina, Muscat of Hamburg) and squeeze to obtain fresh juice.
2. Filter the juice through a strainer or filter paper to remove pulp and solids.
3. Allocate 300 mL of juice per variety, dividing into three replicates (3 × 100 mL).

4. Add a few drops of grape juice in the pH meter. Additionally place a few drops of each grape juice onto the prism of the optical refractometer to measure the initial sugar content (°Brix). Record both pH and °Brix values in a data table. Repeat for every cultivar.
5. Label 3 Erlenmeyer flasks clearly (Cardinal 1–3, Sultanina 1–3, Muscat 1–3).
6. Measure 100 mL of juice into each flask using a graduated cylinder.
7. Weigh 5.0 g of active dry yeast (*Saccharomyces cerevisiae*) on an analytical balance per flask (3 times).
8. Seal each flask with a rubber stopper with two holes.
9. Fill graduated cylinders (250 mL) completely with water, invert them in the rubber stopper.
    a. When the gas exceeds 250 mL, without letting the gas inside exit with placing your finger on the hole, refill the cylinder with water and keep recording, add the recording 250mL.
10. Place the end of each tube under the inverted cylinder so that CO_2 produced will displace water.
11. Start the experiment simultaneously for all flasks, add the weighed 5.0 g of active dry yeast to the flasks and begin timing.
12. Record the volume of displaced water after 15 minutes (CO_2 collected).
13. Record the volume of displaced water for every 15 minutes, total of 4 times.
14. Monitor and record room temperature with a thermometer to ensure a steady temperature during the experiment.
15. Record all results in a data table with time (s) vs. CO_2 volume (mL) for each replicate.
16. Dispose of all fermented grape juice as laboratory waste; it must not be consumed.
17. Clean and rinse all glassware thoroughly with distilled water.
18. Repeat the steps from 5 to 17 for a total of 5 times.

Safety and Ethics:

The experiment involves only materials that have no risks such as food grade grape juice and *Saccharomyces cerevisiae* yeast. Basic laboratory precautions were followed. Lab coat, gloves and goggles were worn, and all flasks with the inverted cylinders were securely placed to prevent spills or glass breakage. The experiment was performed in a ventilated laboratory. The fermented samples were not consumed. All glassware were then washed with water and detergent that is appropriate for lab glassware. The liquid waste was diluted and safely disposed of according to the school laboratory guidelines and government regulations.

No human and animal subjects were involved in the experiment. The procedure does not generate any environmental impact. Data was collected and reported with honesty. The purpose of the experiment is only educational and scientific. All brand names and references were clearly acknowledged. All data, calculations and analysis files were preserved as an insurance of transparency.

Results:

This section consists of raw data tables of the experiments. The pH measurements from each grape juice are displayed. The carbon dioxide production of each trial is displayed. Linear regression was used to calculate the initial fermentation rate for each trial. The mean rate ±95% confidence interval was determined for each grape variety.

Raw Data:

Table 3: Initial pH and Sugar Content (°Brix) of each grape juice

Grape cultivar	**Initial pH (±0.01)**	**Initial Sugar Concentration (°Brix ±0.2)**
Vitis vinifera cv. Sultanina	4.99	23.2
Vitis vinifera cv. Muscat of Hamburg	5.44	16.3
Vitis vinifera cv. Cardinal	5.17	13.9

Table 4: CO_2 volume (mL) measurements for Sultanina

	Trial Number	CO_2 accumulation in the graduated cylinder (mL)	Volume of Solution (mL)	Temperature of Room (±0.5 °C)
Sultanina 15 min	Trial 1	30± 3	100± 1	25.1
	Trial 2	12± 3	100± 1	25.0
	Trial 3	14± 3	100± 1	25.4
	Trial 4	13± 3	100± 1	25.3
	Trial 5	16± 3	100± 1	25.1
Sultanina 30 min	Trial 1	143± 3	100± 1	25.1
	Trial 2	107± 3	100± 1	25.1
	Trial 3	113± 3	100± 1	25.4
	Trial 4	110± 3	100± 1	25.3
	Trial 5	115± 3	100± 1	25.1
Sultanina 45 min	Trial 1	266± 6	100± 1	25.2
	Trial 2	217± 3	100± 1	25.2
	Trial 3	223± 3	100± 1	25.4
	Trial 4	221± 3	100± 1	25.3
	Trial 5	228± 3	100± 1	25.1
Sultanina 60 min	Trial 1	372± 6	100± 1	25.2
	Trial 2	313± 6	100± 1	25.2
	Trial 3	317± 6	100± 1	25.3
	Trial 4	319± 6	100± 1	25.3
	Trial 5	325± 6	100± 1	25.2

Among the fermentation of Sultanina trials the first trial produced a higher CO_2 accumulation compared to the other two trials. This deviation is attributed to excessive shaking of the flask. This likely mixed additional oxygen and temporarily boosted the yeast metabolism. Consequently Sultanina Trial 1 was treated as an experimental outlier and is excluded from the mean rate calculations.

Table 5: CO_2 volume (mL) measurements for Muscat

	Trial Number	CO_2 accumulation in the graduated cylinder (mL)	Volume of Solution (mL)	Temperature of Room (±0.5 °C)
Muscat 15 min	Trial 1	22± 3	100± 1	25.1
	Trial 2	19± 3	100± 1	25.0
	Trial 3	17± 3	100± 1	25.4
	Trial 4	20± 3	100± 1	25.3
	Trial 5	18± 3	100± 1	25.1
Muscat 30 min	Trial 1	123± 3	100± 1	25.1
	Trial 2	124± 3	100± 1	25.1
	Trial 3	120± 3	100± 1	25.4
	Trial 4	122± 3	100± 1	25.3
	Trial 5	119± 3	100± 1	25.1
Muscat 45 min	Trial 1	238± 3	100± 1	25.2
	Trial 2	236± 3	100± 1	25.2
	Trial 3	241± 3	100± 1	25.4
	Trial 4	240± 3	100± 1	25.3
	Trial 5	236± 3	100± 1	25.1
Muscat 60 min	Trial 1	340± 6	100± 1	25.2
	Trial 2	343± 6	100± 1	25.2
	Trial 3	347± 6	100± 1	25.3
	Trial 4	345± 6	100± 1	25.3
	Trial 5	341± 6	100± 1	25.2

Table 6: CO_2 volume (mL) measurements for Cardinal

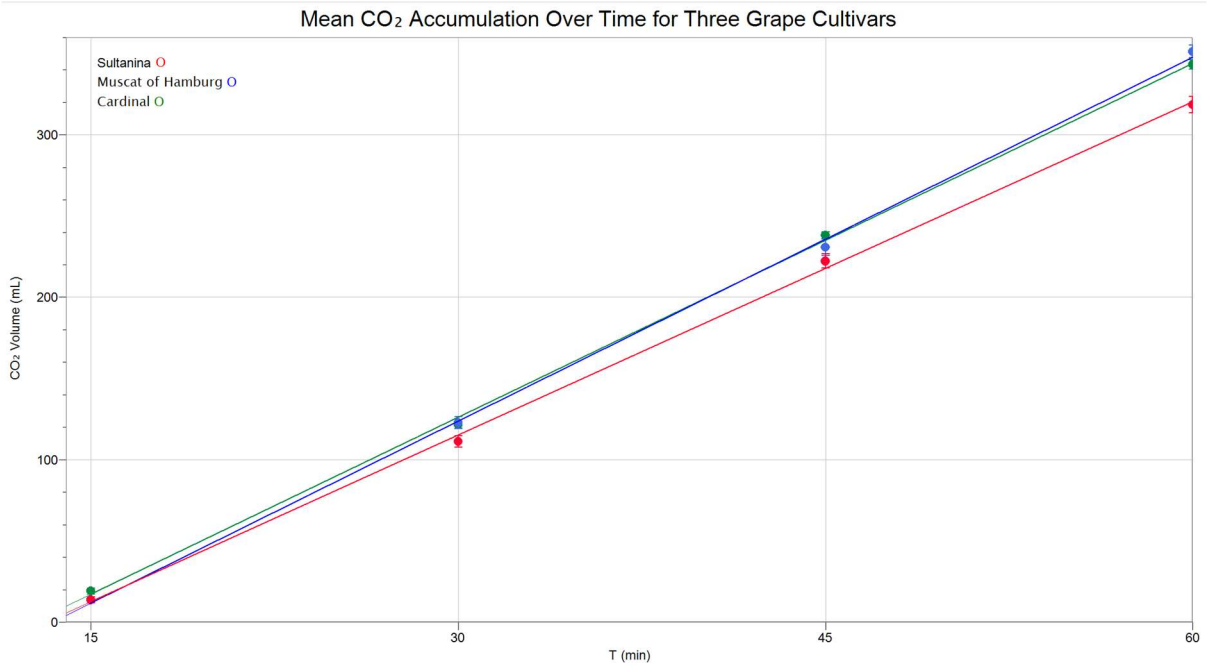
	Trial Number	CO_2 accumulation in the graduated cylinder (mL)	Volume of Solution (mL)	Temperature of Room (±0.5 °C)
Cardinal 15 min	Trial 1	13± 3	100± 1	25.1
	Trial 2	15± 3	100± 1	25.0
	Trial 3	12± 3	100± 1	25.4
	Trial 4	14± 3	100± 1	25.3
	Trial 5	16± 3	100± 1	25.1
Cardinal 30 min	Trial 1	121± 3	100± 1	25.1
	Trial 2	118± 3	100± 1	25.1
	Trial 3	122± 3	100± 1	25.4
	Trial 4	125± 3	100± 1	25.3
	Trial 5	128± 3	100± 1	25.1
Cardinal 45 min	Trial 1	228± 3	100± 1	25.2
	Trial 2	230± 3	100± 1	25.2
	Trial 3	225± 3	100± 1	25.4
	Trial 4	233± 3	100± 1	25.3
	Trial 5	238± 3	100± 1	25.1
Cardinal 60 min	Trial 1	349± 6	100± 1	25.2
	Trial 2	352± 6	100± 1	25.2
	Trial 3	346± 6	100± 1	25.3
	Trial 4	353± 6	100± 1	25.3
	Trial 5	356± 6	100± 1	25.2

Processed Data and Graphical Representations:

Table 5: Mean rate and statistical analysis

Grape	Trials Included	Mean rate(mL/min)	Standard Deviation	95% CI (±)
Sultanina	T2 - T5	6.77	0.08	±0.13
Muscat of Hamburg	T1 - T5	7.20	0.09	±0.12
Cardinal	T1 – T5	7.49	0.054	±0.07

Graph 1: CO_2 volume (mL) vs Time

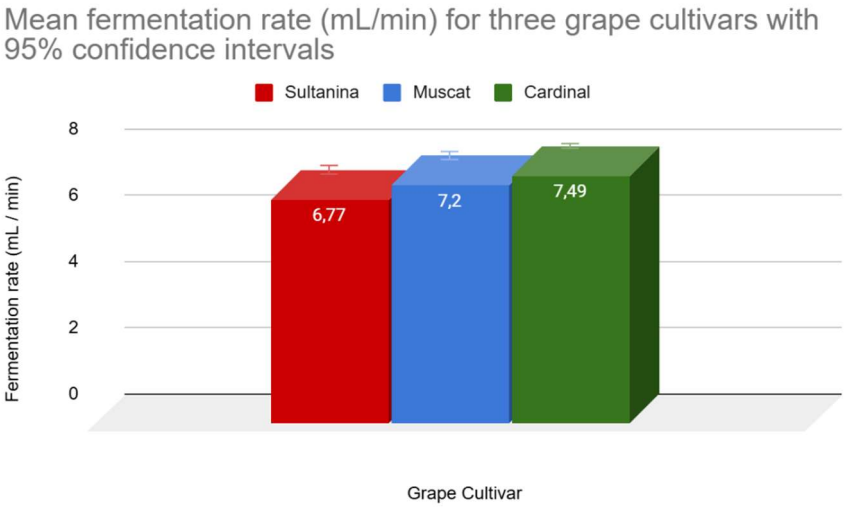


Mean CO_2 volume over 60 minutes for Sultanina, Muscat of Hamburg, Cardinal grape cultivars are given. Error bars represent standard deviation across replicate trials. Linear fits are:

Sultanina R^2= 0.98
Muscat of Hamburg R^2= 0.96
Cardinal R^2= 0.99

CO_2 volume increased linearly from 15 to 60 minutes for all three cultivars (Graph 1). Cardinal consistently showed the highest mean CO_2 accumulation, followed by Muscat of Hamburg while Sultanina displayed lowest. Error bars were relatively small compared to the differences between cultivars, indicating good repeatability and precision.

Graph 2: Fermentation rate of the three grape cultivars



Sample Calculation:

Data from Cardinal Trial 1:

- $t_1 = 15\ min, V_1 = 13\ mL$
- $t_2 = 60\ min, V_1 = 349\ mL$

Formula of Rate:

$$Rate = \frac{\Delta y}{\Delta x} = \frac{V_2 - V_1}{t_2 - t_1}$$

Calculation of Rate:

$$Rate = \frac{349mL - 13mL}{60\text{min} - 15\text{min}} = 7.47\ mL/min$$

**The final values in the table were calculated using the slope in Excel to account for all data points, which is more precise than the two-point method.*

Formula of Mean:

$$\bar{x} = \frac{\sum x}{n}$$

Calculation of Cardinal Mean Rate:

$$Mean = \frac{7.47 + 7.51 + 7.44 + 7.53 + 7.50}{5} = 7.49mL/min$$

Statistical Analysis:

Table 7: One-way ANOVA comparing

Source	SS	df	MS	F	p-value
Between groups	1.733	2	0.8665	152.3	<0.001
Within Groups	T1 - T5	11	0.00569	-	-
Total	1.7956	13	-	-	-

A one-way ANOVA showed a highly significant effect of grape cultivar on initial fermentation rate ($F_{2,11}$ = 152.3, p< 0.001). This indicates that the differences observed between the mean rates in Graph 2 are statistically meaningful.

Table 8: Tukey's post-hoc test comparing cultivars

Comparison	Mean Difference (mL / min)	HSD = 0.131	Is this Significant?
Cardinal – Sultanina	0.7215	0.131	YES
Muscat – Sultanina	0.4275	0.131	YES
Cardinal – Muscat	0.294	0.131	YES

Tukey's HSD test confirmed that all pairwise differences were statistically significant. Cardinal shows faster fermentation than both Muscat and Sultanina. Muscat fermented significantly faster than Sultanina. This indicates that each grape cultivar produced a distinct fermentation rate.

Analysis and Discussion:

Analysis:

The CO2 accumulation increase was linear over the period of 15-60 minutes in all grape cultivars, which has shown that Saccharomyces cerevisiae was in an early exponential fermentation state where availability of sugars and enzyme activity was constant. The coefficient of determination (R2 = 0.96-0.99) is strong enough to confirm little systematic error with small deviations (standard deviation 0.054-0.09 mLmin-1) that can be explained by the natural variability of biology but not the imprecision of measurements.

The extraction of the Sultanina T1 anomalous trial decreased the within-cultivar variance but left the overall ranking unchanged, which indicates strong experimental design. The outlier can be biologically attributed: too much shaking during preparation would have

introduced dissolved oxygen, giving S. cerevisiae an opportunity to undergo aerobic respiration, through the Crabtree effect, temporarily (Bisson, 1999). In high glucose concentrations and aerobic conditions, yeast also has respiro-fermentative metabolism in which both oxidative phosphorylation and fermentation co-exist, partially increasing CO2 production until oxygen exhaustion induced an exclusively anaerobic fermentation.

The highest mean fermentation rate (7.49 mLmin-1) was exhibited by Cardinal, after that, by Muscat of Hamburg (7.20 mLmin-1) and Sultanina (6.77 mLmin-1). The variations were 0.3 to 0.7 mLmin-1 which corresponds to a 4.3-10.6% range. Although these percentages seem insignificant, they indicate statistically significant ($p < 0.001$) and biological values. Such variations play a very important role in industrial winemaking in terms of fermentation time, energy usage, and product properties (Ribereau-Gayon et al., 2006). Reproducibility coefficient of variation between repeat trials was 0.7% (Cardinal) to 1.2% (Sultanina) which implies that there is truly more variation between cultivars biologically than there is in error of measurement.

Discussion:

The first hypothesis was partially negated by the results of the experiment. Although it was right about the fact that Sultanina would have the slowest rate, the hypothesis was wrong in its expectation that Muscat should ferment faster than Cardinal. The realized ranking (Cardinal > Muscat > Sultanina) was contrary to the predictions (Muscat > Cardinal > Sultanina) and needed biological interpretation.

The assumptions of the hypothesis were that darker varieties had more phenolics which gave more fermentation and that Muscat varieties had more sugar content. Nonetheless, the second assumption was directly negated in terms of measured values of °Brix: Sultanina had the highest sugar (23.2 °Brix), then Muscat (16.3 °Brix) and Cardinal (13.9 °Brix). This anti-intuitive connection between the sugar concentration and the rate of fermentation is intricate, and it shows how complicated the process of fermentation is.

The negative relationship between fermentation rate and °Brix indicates that osmotic stress was more important than substrate availability. The Sultanina juice had approximately 232 g/L of dissolved solids at 23.2 °Brix, which generated much osmotic pressure on the other side of the cell membrane of the yeasts and could possibly inhibit water uptake and lower the turgor pressure inside the cell to facilitate optimal metabolism (Bisson, 1999).

S. cerevisiae has a biphasic sugar concentration response. At intermediate temperatures (12-18 °Brix), the rate of fermentation is directly proportional to the amount of substrate and follows Michaelis-Menten kinetics. Nevertheless, beyond about 20 °Brix, the effect of osmotic stress exceeds the effect of substrate benefits, slowing down fermentation rates (Walker, 1998). This threshold effect is the reason why Sultanina, though having the greatest sugar content, had the slowest fermentation. The values of cardinal and Muscat (13.9 and 16.3 °Brix respectively) are in the optimum scale in which there is an abundance of substrate but a low level of osmotic pressure.

In addition, the degree of brix estimates the total dissolved solids but it does not differentiate between glucose and fructose. The hexose affinities of hexose transporters make S. cerevisiae have a preference to use glucose compared to fructose. Had Sultanina contained a more fructose:glucose ratio, this would also serve to explain why Sultanina had a lower rate yet contained a lot of sugar--a limitation that would be discussed below.

Mesured values of pH give further mechanistic information. Sultanina was most acidic with pH 4.99, Muscat was least acidic with pH 5.44 and Cardinal intermediate was 5.17. This is associated with fermentation rate: intermediate pH resultant in highest rate, most acidic in lowest.

This is in line with known physiology of yeast. Phosphofructokinase (PFK) and pyruvate kinase are glycolytic enzymes that are pH-sensitive, and best operate at pH 5.0-5.5 (Walker, 1998). At a pH below 5.0, the concentration of protons becomes excessive and inhibits these enzymes, decreasing glycolytic flux. On the other hand, pH greater than 5.5 affects the proton-motive force across the plasma membrane which is employed by S. cerevisiae to co-transport glucose in the presence of hexose permeases. The pH of 5.17 of Cardinal places it in the ideal position which perhaps can be the reason why it has a better rate despite the lower sugar content.

Moreover, the cells, when at extremely acidic pH (below 5.0), have to utilize extra ATP to regulate cytoplasmic pH (around pH 6.5-7.0 internally) at the expense of biosynthesis. Such a metabolic load might further lower the rate of fermentation in Sultanina. The pH of Muscat of 5.44, though not having direct inhibitory effects on enzymes, might have been unfavorable to maintain the proton gradient, which is required to facilitate effective uptake of glucose.

The grapes with dark skin (Cardinal and Muscat) have more anthocyanins, flavonoids and condensed tannin, and Sultanina have much less phenolics. The hypothesis anticipated the increased level of the phenolic content would augment the fermentation rate by antioxidant defense. Nonetheless, phenolic effects are more understated.

Recent studies show that phenolic compounds have positive and negative impact depending on concentration and molecular structure (Rodriguez-Clemente et al., 2022). Phenolics, such as quercetin and catechin, inhibit yeast cell damaging reactive oxygen species (ROS) and ethanol-induced membrane damage, supporting the cellular viability of cells at the beginning of fermentation at moderate concentrations. This was probably one of the contributing factors to the better performance of Cardinal and Muscat as compared to Sultanina.

Nevertheless, too much phenolic levels are inhibitory. Proteins, such as cell-surface enzymes and membrane transporters, may be precipitated by the condensed tannins, and this may lower the efficiency of glucose uptake. This equilibrium between the modes of protection and precipitation is probably the reason as to why there is a relatively minor difference in Cardinal and Muscat (0.29 mLmin-1) even though both of them had high levels of phenolics. Notably, these findings are supported by Philipp et al. (2021), who showed that grape must composition, such as phenolic profiles, has a significant effect on the initial kinetics of fermentation with S. cerevisiae, and differences by cultivar become apparent in the first 24 hours.

Collectively, the pH, °Brix and phenolic profiles can be used to offer a biological explanation of the hierarchy witnessed. All these factors work together in a collaborative fashion. The optimum pH (5.17) of Cardinal gave the best efficiency of glycolytic enzymes and retained proton-motive force to transport glucose. It had moderate sugar (13.9 °Brix) that did not subject it to osmotic stress. Its anti-oxidant properties were provided with phenolic content with no over-protein precipitation. The elevated PH (5.44) of Muscat was probably responsible to lower the efficiency of glucose uptake even though the substrate was sufficient (16.3 °Brix). Although hexose transporter functionality was inhibited by suboptimal pH conditions, the presence of phenolic content offered protection and thereby decreased the overall rate. Sultanina had tripled disadvantage, i.e. high sugar (23.2 °Brix) osmotic stress, poor pH (4.99) to inhibit enzyme activity and spend more energy to maintain pH homeostasis and low phenolic amount to provide low antioxidant activity.

This multi-factorial description is in line with known physiology of yeast fermentation (Waterhouse et al., 2016) and shows that the rate of fermentation cannot be predicted using individual biochemical measures.

Compared with the published literature, these results are consistent with the current studies of cultivar-specific fermentation kinetics. A study by Ribereau-Gayon et al. (2006) has determined that the grape variety has a big impact to the results of fermentation based

on the composition of the must. This is corroborated by the current study and determines cultivar effects in the key initial stage.

Acuna-Avila et al. (2023) studied the phenol dynamics during fermenting Vitis vinifera, and the authors observed that yeast response to health stress depends on phenolic content. Although their research was on later stages (3-7 days), their current research shows that the effects of phenolics can be observed within the first hour indicating that antioxidant protection is immediate the moment yeast-must contact.

Walker and Stewart (2016) note that the efficacy of fermentation of S. cerevisiae is contingent on the combined influence of various environmental factors. These current findings are highly suggestive of this: not only sugar, not only pH, not only phenolic content could be used as a predictor of the rate of fermentation. The observed ranking was only possible by combined consideration.

The 4.3-10.6% variation is real adaptive differences in response of S. cerevisiae in different chemical environments. The surprising anti-correlation between fermentation rate and sugar content has practical implications: grapes with very high °Brix might actually ferment slower because of the initial osmotic pressure implying that must dilution might have a beneficial effect on fermentations with high sugar content.

It has now been revised that fermentation rate is best determined by optimal correspondence of moderate sugar concentration (not osmotic stress), pH, 5.0-5.3 (optimal enzyme activity and glucose uptake), and phenolic content (antioxidant protection). The cardinal was the most effective in meeting these three criteria, which makes its unexpectedly better results than those of Muscat and actualizes that multi-factorial analysis is vital in forecasting the results of fermentation in grape must.

Evaluation:

Strengths:

The experiment was able to find statistically significant differences in fermentation rates. Five replicates per cultivar, simultaneous processing being standardized and single-observer measurement were used to achieve high reproducibility. High coefficients of determination (0.96 -0.99) and low coefficients of variation (0.7-1.2%), testify to the fact that the observed differences are truly biological, and not due to the technical error. Also, the 15-60 minute range was successful in capturing early kinetics prior to the attainment of confounding variables such as ethanol toxicity or nutrient depletion.

Limitations and Suggestion:

Table 9: Limitations of the experiment and suggestions to fix them

Limitation	Suggestion
The measure of Brix was not able to distinguish between glucose and fructose.	Use High-Performance Liquid Chromatography (HPLC) to quantify specific sugar profiles.
The slow rate in Sultanina indicates osmotic stress and nitrogen deficiency, but without the quantification performed, the mechanism is speculative.	Measure Yeast Assimilable Nitrogen (YAN) prior to fermentation to verify nutrient levels.
The short observation window may not predict full fermentation of 5-14 days.	Extend the monitoring period over several days until CO2 production completely ceases.
The use commercial introduced variables regarding maturity, storage and handling that may have found confused cultivar specific results.	Use vineyard-controlled samples of grapes.

Further Experimentation:

- How does the specific amino acid profile (arginine vs. proline) of different cultivars affect the maximum fermentation rate and biomass yield? This matters because, yeast prefers certain nitrogen sources over others. Researching this would help winemakers determine exactly what supplements to add to a specific grape juice to prevent stuck fermentation
- Do specific anthocyanins in dark-skinned grapes act as antioxidants that protect yeast from oxidative stress, or do they act as inhibitors that slow down sugar transport? This connects to understanding how polyphenols interacts with microorganisms and can lead to the development of "functional beverages" with higher antioxidant bioavailability.

Conclusion:

The results of this investigation demonstrate that grape cultivar has a significant effect on the initial rate of alcoholic fermentation by *Saccharomyces cerevisiae*. Among the three cultivars tested, Cardinal showed the highest mean CO_2 production rate (7.49 $mL \cdot min^{-1}$), followed by Muscat of Hamburg (7.20 $mL \cdot min^{-1}$), while Sultanina exhibited the lowest rate (6.77 $mL \cdot min^{-1}$, with the anomalous trial excluded). A one-way ANOVA confirmed that these differences were statistically significant ($F_{2,11} = 152.3$, $p < 0.001$), and Tukey's post-hoc test verified that each pairwise comparison differed significantly.

These findings support the research question by showing the biochemical differences among grape cultivars which are reflected in their measured pH values and implied sugar and phenolic composition. The grape cultivars produce measurable differences in early fermentation kinetics. Therefore, the type of grape used provides a meaningful determinant of the initial fermentation performance of *S. cerevisiae*.

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Appendices:

Figure 1

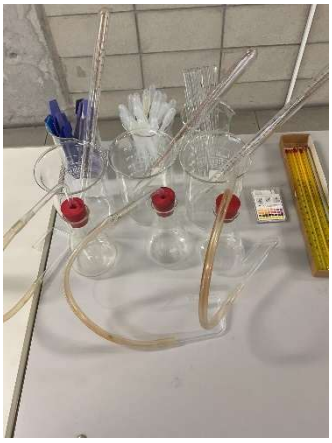

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6