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Comparative Analysis of the Quality of White Wine Prepared with Commercial Wine Yeast and Isolated Yeast Strain from Indigenous *Murcha*

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ABSTRACT
Nepal, has a long tradition of fermented indigenous alcoholic beverages. However, its potential in winemaking remains undiscovered, Nepal's researcher growing interest in viticulture and wine production. This study goal to compare the quality of white wine produced using commercial yeast (CY) and yeast strains (SY1 and SY2) isolated from indigenous murcha, a traditional Himalayan starter culture. Altogether 12 yeast strains were isolated. Screening of fermentative yeast were performed on the basis of effervescence of carbon-dioxide gas in Durham's tube, flavor, turbidity and surface growth in YM broth containing 1.8% sugar. Strain Y1 and Y7 were selected on WYDM media. Strains Y1, Y2 and commercial wine yeast were used as starter culture in wine fermentation. The grape juice (15.4 °Bx) was fermented for 12 days at room temperature. The biochemical characteristics, antioxidant activity, total phenolic content and the sensory analysis of wine were conducted. The methodology involving isolating yeast from murcha, screening fermentative strains, and wine fermentation. Statistical analysis suggested that alcohol content, ester content and total phenolic compounds were significantly different from each other in all wine samples SY1, SY2, CY ($p<0.05$). Results indicated that SY1 and SY2 exhibited higher fermentation activity, producing wines with significantly ($p<0.05$) better alcohol content (7.33–7.51% v/v), lower residual sugar (1.27–1.38), and enhanced phenolic and antioxidant properties compared to CY (7.08% alcohol, 1.45% residual sugar). Sensory evaluation ranked SY2 highest in overall acceptability. Encouraging Nepalese winemakers to adopt and apply murcha-derived yeast strains (SY2) to enhance fermentation efficiency and develop unique regional wine profiles.

KEYWORDS
Murcha; Optimized yeast; Physiochemical analysis; Sensory evaluation; Wine fermentation

1. INTRODUCTION

One of the most traditional and widely used alcoholic beverages worldwide is wine. A practical method for creating novel goods with altered physicochemical and sensory properties particularly taste and nutritional components is fermentation. Alcohol as well as fermentation of lactic and acetic acids is crucial for manufacturing quality [1]. The production of wine is a worldwide industry that brings in billions of dollars annually [2]. The growth of global commerce and technical advancements in winemaking's production, storage, and logistics have increased the industry's economic worth recently [3]. In principle, any fruit with enough sugars would ferment to produce wine. Common examples are cider (apple), perry (pears), cherry wine (cherry), mead (honey), and so on [4].

Yeasts are an important organism in the ancient and intricate process of winemaking [5]. In spontaneous fermentations, indigenous yeasts proliferate in a progressive manner, with alcohol-tolerant *Saccharomyces cerevisiae* strains dominating the latter stages. This species is internationally recognized as the 'wine yeast' and is frequently favored to begin wine fermentations [6]. *Saccharomyces cerevisiae* is commonly employed in modern winemaking to produce high-quality wines. Non-selected *Saccharomyces*

or non-*Saccharomyces* opportunistic yeasts were commonly present during fermentations [7]. Off-flavors include acetic acid, ethyl phenols, and excessive quantities of alcohol. Scientists and winemakers now recognize the potential benefits of non-*Saccharomyces* in winemaking, particularly for fragrance complexity [8], [9], [10], [11].

Murcha is a traditional amylolytic starter used to make ethnic alcoholic drinks in the Himalayan areas of Nepal, India, Bhutan, Tibet, and China. *Murcha* is a Nepali term, the Lepchas name it '*thamik*', the Limbu call it '*khesung*', and the *Bhutias* call it *'phab*'. *Murcha* stays active for several months at room temperature and in a dry environment [12], [13], [14]. Yeasts found in *murcha* include *Saccharomcopsis fibuligera, S. Capsularis, Saccharomyces cerevisiae, S. bayanus, Pichia anomala, P. burtonii*, and *Candida glabrata. Saccharomyces bayanus, Candida glabrata*, and *Pichia anomala* are all engaged in ethanol production. *Sm. fabuligera, S. capsularis*, and *Pichia burtonii* show strong amylolytic activity, indicating that they might be amylolytic yeast, while *S. fibuligera* is the most prevalent yeast in *murcha* [14], [15].

Wine with different varieties have been found in Nepal. This study can be a starting step to find out the possible utilization of *murcha* by using it as the basis of wine making so that it's utility can be upgraded. Studies on *murcha* are limited. Although *murcha* is easily available and prepared locally in Nepal, it is use only for *jandh* and *raksi* preparation in local level. *Murcha* is prepared by natural fermentation so it can give good mouthfeel and flavor in wine as compared to commercial yeast.

The contribution of our scientific work is to prepare and compare quality characteristic of white wine using commercial wine yeast and isolated yeast strain from indigenous *murcha*. Hence, this work may not only create opportunities to develop regionally distinctive wines with unique sensory profiles but also contribute to the global diversification of winemaking practices. By integrating indigenous microbial resources like *murcha* into scientific winemaking, this research could open pathways for sustainable utilization of local biodiversity, enhance cultural heritage preservation, and stimulate economic growth through potential commercialization. Ultimately, the findings may inspire future innovation in functional beverages and strengthen Nepal's positioning in the international wine industry.

2. MATERIALS AND METHODS

2.1. Materials

Grapes (*Vitis vinifera* L.) and cane sugar were purchased from the local market of Kathmandu, Nepal. *Murcha* samples were collected from different places of Kathmandu Valley, such as Bhaktapur, Kritipur, Shivapuri packaging in plastic bags. The *murcha* samples, collected from traditional local producers, were particularly important as they represent the indigenous microbial diversity of Nepal, making this study both scientifically and culturally significant. Commercial wine yeast was provided from Microbiology Lab of National College (NIST) were used during wine preparation. Prior to conducting our research work, the varieties of the samples were tested according to the guidelines and regulations set by the microbiologist of National College of Food Science and Technology, Kathmandu, Nepal. The main raw material for this study was grapes, as they served as the fermentable substrate for wine preparation, while *murcha* acted as the natural fermentation starter containing diverse yeast populations. Grapes were chosen due to their balanced sugar-acid composition suitable for wine making. The grapes and cane sugar were sourced from fresh local harvests in Kathmandu, Nepal, ensuring availability and reflecting typical raw materials used in regional winemaking practices.

2.2. Research Condition

The research was conducted from May 2024 to July 2024 in the research Lab of the National College of Food Science and Technology, Kathmandu, Nepal. Geographically, the experimental site was situated at latitude 27º 41′ 21.86″ N – 27º 41.36′ N and longitude, 85º 19′ 21.60″ E – 85º 19.36′ E with an altitude of 1300 m from sea level. The minimum and maximum temperatures range from -3 and 37 ºC respectively, and the average annual rainfall is about 55 inches. The geographical location of Kathmandu valley, with its moderate temperature, high humidity during monsoon, unique microbial biodiversity, could significantly influence the growth, dominance, and activity of indigenous yeast strains in *murcha.* These environmental factors may impact yeast diversity, fermentation efficiency, and ultimately the sensory quality of the

prepared wine.

2.3. Isolation of Yeast Using Plate Technique

2.3.1. Sample Preparation

Samples (*murcha*) was ground in a sterile mortar and pestle. One gram of grinded *murcha* sample was mixed in 10 mL of sterile diluent. The sample was thoroughly mixed which was the first dilution that was 10^{-1} [16].

2.3.2. Serial Dilution

Using a sterile micropipette, 1 mL of the first dilution, which was regarded as the second dilution, 10^{-2} was moved to the next tube, which contained 9 mL of sterile diluent. Likewise, further dilutions up to 10^{-6} were made.

2.3.3. Plating

Using a sterile micropipette, 0.1 mL of aliquots from chosen dilutions were aseptically removed and placed in a petri plate with sterile YMA (yeast malt agar) medium that had been autoclaved for 15 minutes at 150 lbs of pressure. After that, the plates were left to set and incubated for 48 hours at 30 °C to see if any yeast had grown. On the YMA plate, the yeast's growth was monitored for colony features, or morphological traits. To obtain a pure version of the isolated colony (Figure 1), the identified yeast colony was further sub-cultured using the streak plate procedure. Following a 24–48 hours incubation period at 28–30 °C, the streaked plates were encoded [16].

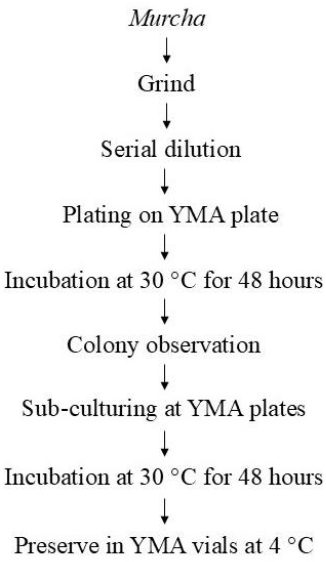

Figure 1. Isolation of yeasts from *murcha*.

2.3.4. Storage

The YMA vials containing the pure yeast colony culture were kept in a refrigerator between 0–4 °C. They were checked for signs of purity at regular intervals. For every presumed colony, a different stock and working sample were used. For confirmation, further analysis of the working sample was conducted. Every two to three months, the pure and chilled yeast isolates were sub-cultured from old YMA vials to brand-new YMA vials.

2.3.5. Selection and Identification of Yeast Strains

The morphological features of the colonies, including color, texture, margin, evaluation, consistency,

and opacity, were used to analyze each of the yeast isolates. Additionally, isolates were analyzed under a microscope using a straightforward staining method to establish the presence of yeast [16].

2.3.6. Screening of Fermentative Yeast

Using generate CO_2, taste, and turbidity as criteria, fermentative yeast was screened. Non-fermentative yeasts were defined as those isolated strains of yeast that are incapable of producing gas (Figure 2) [17].

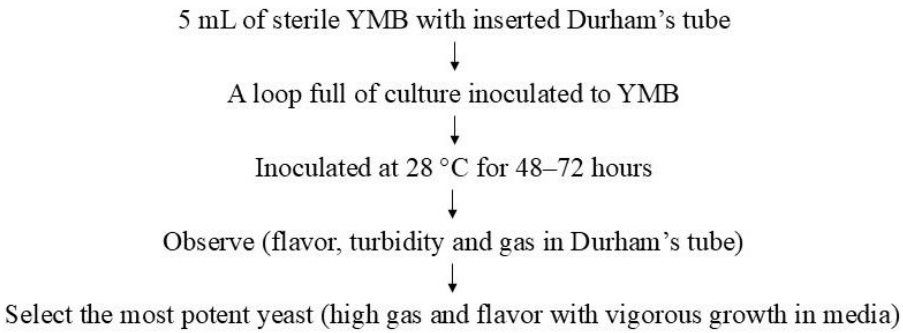

Figure 2. Screening of fermentative yeast.

2.4. Preparation of Wine Differential Media (WYDM)

For wine yeast screening, this wine yeast differential medium works incredibly well. In this medium, wine yeast forms a red colony, while wild yeast forms a pink or colorless colony (Figure 3).

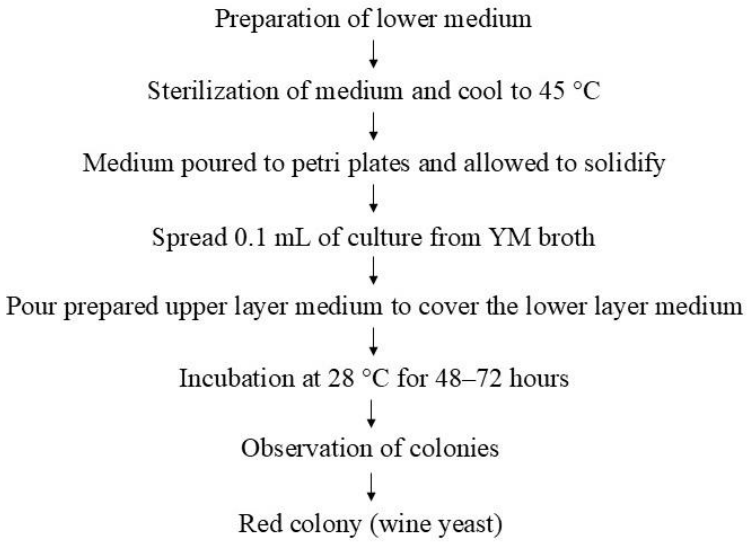

Figure 3. Selection of wine yeast on WTDM.

2.4.1. Preparation of Lower Layer Medium

Following precise weighing, the necessary ingredients for the lower layer medium were dissolved in distilled water. After being autoclave sterilized for 15 minutes at 15 pounds, it was cooled. About 20 mL of it were put onto sterilized petri dishes and let to set. With the use of the sterile bent glass rod, 0.1 mL of the fresh culture was pipetted out, added to the solidified bottom layer media, and evenly distributed [17].

2.4.2. Preparation of Upper Layer Medium

Every component needed for the upper layer medium was precisely weighed and dissolved in distilled water. After all the components were dissolved, it was heated and chilled to 45–40 °C. During the boiling process, the medium turned a faint shade of red. The new culture yeast was spread over the bottom layer of media, which was filled with this top layer medium. After allowing it to firm, it was incubated for 48–72 hours at 28–30 °C to support colony growth. In the same medium, a pink colony denotes wild yeast, whereas a red colony suggests wine yeast [17].

2.5. Processing of Raw Materials

2.5.1. Preparation of Grape Juice

The grapes were carefully destemmed before used. To keep the juice from becoming tainted by bitterness, the stalks were cut off. Before blending and utilizing a juice extractor to obtain the juice, the destemmed grapes were immersed in 100 parts per million sulfuric waters. The settled juice was filtered through muslin cloth to remove the sediments and skins.

2.5.2. Preparation of Starter

In order to activate the isolated yeast that had been kept in nutrient agar, it was inoculated with nutrient broth and incubated at 28 °C for approximately 24 hours. Centrifugation was used to isolate the activated yeast. In order to propagate the yeast, active yeast was then added to a bigger flask that contained sterile grape juice at 15.4 °Bx and was incubated at 28 °C for two days with periodic shaking. The yeast starter was prepared to be added to the fermentation tank after propagation was complete (Figure 4) [17].

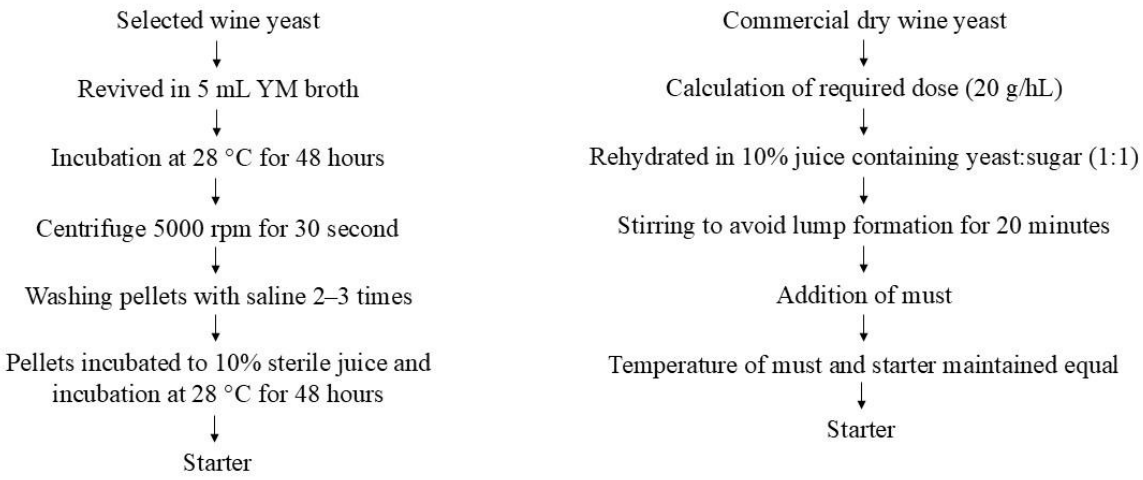

Figure 4. Starter preparation.

2.5.3. Preparation of Must (Crushed Grapes Mixture)

Pulverized sugar was added to the obtained grape juices at 22 °Bx to improve them. After ten minutes of pasteurization at 65 °C, the must was promptly cooled to room temperature. Ammonium sulphate $(NH_4)_2SO_4$ was supplied at a dosage of 0.1% to provide the yeast with nitrogen.

2.5.4. Fermentation

The flow chart for making grape wine is displayed in Figure 5. Must was transferred to conical flasks that were coded as SY1, SY2, and CY and filled to 85% of their content. Two distinct wine yeast strains isolated from *murcha* were used to make SY1 and SY2 wines, while commercial yeast was used to make CY wines. After that, the fermentation was allowed to continue for 12 days at room temperature. While the decreasing sugar was measured every 48 hours, the pH, TSS, and acidity were measured every 24 hours.

Until TSS became consistent, the fermentation process was maintained.

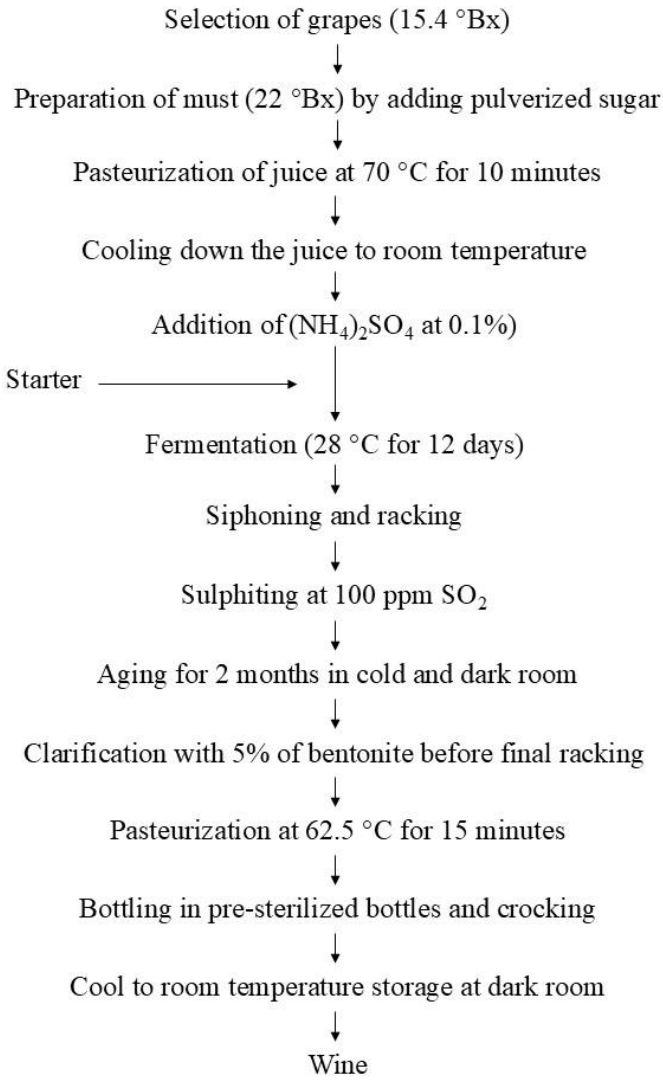

Figure 5. Flow chart of preparation of wine.

2.5.5. Siphoning and Racking

To get rid of the yeast lees, siphoning was done two or three times. Subsequently, to achieve clear free-run wine, the acquired wine was racked for a week in order to sediment any suspended particles.

2.5.6. Sulfiting

By adding the necessary amount of potassium metabisulphite, which would yield 100 ppm of SO_2, sulfiting was carried out before age. Sulfite is the most popular and efficient preservative in winemaking. It protects musts and wine from bacteria that might cause wine spoiling and early oxidation. For maturing wines, it is crucial since it keeps the wine fresh and helps keep its color.

2.5.7. Aging

Bottles were used to mature the wine samples. To achieve the desired changes in flavor and body, aging was carried out for around two months in a chilly, dark environment.

2.5.8. Clarification

Using bentonite, which also adsorbs proteins, the resulting wine was clarified. Warm water was used to make a 5% bentonite solution, which was then added to the wine at a rate of 0.6% and left for a full day.

2.5.9. Bottling, Pasteurization, and Storage

Crown corking was completed when the clear wine was moved to pre-sterilized glass bottles. After 15 minutes of pasteurization at 72.5 °C, the bottled wine was allowed to cool to room temperature. Then, wine was stored on dark place at room temperature.

2.6. Total Soluble Solid (TSS) and Reducing Sugar

A hand refractometer was used to measure total soluble solids using the procedure outlined in [18]. The Lane and Enyon technique were used to calculate the wine's decreasing sugar throughout fermentation. The Berlin Institute approach, was used to determine the reducing sugar of wine samples at the conclusion of fermentation [19].

2.7. pH Determination

The pH values of several wine samples were determined by dipping the pH meter's electrode [19]. pH determination was conducted in triplicates.

2.8. Acidity

The titratable acidity of wine and juice was measured and reported as a percentage of tartaric acid [19]. The acidity value was calculated using equation (1).

$$\%\ Acidity\ (as\ tartaric) = \frac{Normality\ of\ titrant\ \times Volume\ of\ titrant\ \times Eq.wt.of\ acid}{Volume\ of\ sample\ \times 1000} \times 100 \quad (1)$$

2.9. Alcohol Content and Methanol Determination

The pycnometer was used to measure the alcohol concentration of the wine sample. The results were then represented as a percentage of the volume. Kirk and Sawyer (1991) [18] techniques were used to determine the wine sample's methanol content.

2.10. Ester Determination

The ester content of the samples was measured using the Kirk and Sawyer (1991) [18] technique, and the results were reported in g ethyl acetate/100 L of sample alcohol. At 20 °C, the sample was filled to the brim in a 250 mL volumetric flask, and it was then moved to the distillation flask with the help of 20 mL of water. After diluting it, around 250 mL of distillate were gathered in the same flask. Water was then used to make up the volume at 20 °C. A total of 100 mL of distillate and 100 mL of water were added to each flask. Next, the following treatment was applied to both the sample and blank. After adding three drops of phenolphthalein, 0.1M NaOH was used to titrate the mixture. Next, precisely 10 mL of alkali were added. After adding a few glass beads, the mixture was cooked for an hour under reflux. After being quickly chilled, 0.05 M sulfuric acid was used to titrate them. The total esters content was calculated using equation (2).

$$Total\ esters = \frac{800\ \times\ V}{S} \quad (2)$$

Where S is the alcoholic strength of the sample in percent v/v and the difference in titer is V (mL). Total esters value is expressed in g ethyl acetate/100 mL of alcohol in sample.

2.11. Aldehyde Content

A big flask was filled with 300 mL of boiling and cooled water. Using a pipette, 50 mL of the sample's

distillate, which contained around 50% of the original, was added to 10 mL of A (15 g Potassium Metabisulphite ($K_4S_2O_5$) mixed with 70 mL HCl and diluted to a liter with water). After mixing, it was let to stand for 15 minutes. In the stoppered flask, 10 mL of B (200 g sodium phosphate (Na_3PO_4) and 4.5 g EDTA were dissolved in water and diluted to a liter), mixed, and let to stand for an additional 15 minutes. A total of 10 mL of new starch solution (0.2%) and 10 mL of C (250 mL of concentrated HCl diluted to 1 L with water) were added. The solution was brought to a faint blue end point after they were spun to mix and enough iodine (about 0.1 M) was added to completely eliminate any surplus bisulphate. The freed bisulphite was titrated with 0.05 M iodine to get the same faint blue end-point after 10 mL of D (100 g boric acid was combined with 170 g NaOH, water was added to dissolve and dilute it to 1 L) was added [18]. The sample's aldehyde content was determined using equation (3).

$$Total\ aldehide = \frac{Titer \times 2.2}{S} \quad (3)$$

Where S is the alcoholic strength of the sample in percent v/v.

2.12. Total Phenolic Content (TPC)

The Folin-Ciocalteu technique was used to determine the wine's total phenolic content. In a 25 mL volumetric flask with 9 mL of distilled water, 1 mL of extract (the wine sample as is) or a standard solution of gallic acid (100 µg/mL to 1000 µg/mL) was decanted. After adding 1 mL of Folin-Ciocalteu reagent, the mixture was agitated. Ten milliliters of a 7% Na_2CO_3 solution were added after 5 minutes, and the mixture was diluted with distilled water to the appropriate level. The absorbance against a prepared reagent blank (distilled water) was measured at a wavelength of 765 nm during a 90 minutes incubation period at room temperature. The number of total polyphenols was expressed as mg GAE/L. The standard was used to produce a calibration curve. The matching gallic acid content of the samples was ascertained using this curve [20], [21].

2.13. Antioxidant Activity

The DPPH radical scavenging technique was used to assess the antioxidant activity of wine samples [22]. The capacity of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to react with H-donors is the foundation of the DPPH test. When an antioxidant acts on DPPH, diphenyl picryl hydrazine is produced. The scavenging effectiveness was assessed by the degree of stable DPPH decolorization to DPPH-H (reduced from DPPH), which is yellow. In 95% methanol, a DPPH solution (0.004% w/v) was made. The extract was made by mixing the samples with 95% methanol in a 1:9 ratio to get a final volume of 10 mL. After mixing an equal volume of extract with newly made DPPH (0.004% w/v), the tubes were left in the dark for 10 minutes at room temperature. A UV-Vis spectrophotometer was used to measure the absorbance at 517 nm. The blank was used at 95% methanol. The equation (4) was used to determine the extract's scavenging activity against the stable DPPH.

$$Scavenging\ activity\ (\%) = \frac{(A - B)}{A} \times 100 \quad (4)$$

Where A is absorbance of blank and B is absorbance of DPPH and extract combination.

2.14. Sensory Analysis

Using nine-point hedonic assessment, sensory evaluations were conducted for color, smell, taste, mouth feel, and overall acceptability. Ten panelists with bachelor's degrees in food technology from the National College of Food Science and Technology (NCFST) made up the sensory panels. According to the policies of NCFST ethical approval was not required for the study. However, all ethical considerations were followed to ensure participant right and privacy [19], [23].

2.15. Statistical Analysis

The acquired data were reported as the mean and standard deviation of three triplicates. For significance analysis, data were analyzed by two-way ANOVA and post hoc test using IBM SPSS Statistics Version 20 (IBM Corporation, Marlborough, MA, USA) at 5% level of significance [24]. Microsoft Excel LTSC MSO (version 2207), created by Microsoft Corporation in 2021, was used for data documentation, computations, and graph displays.

3. RESULTS AND DISCUSSION

3.1. Isolation of Yeast from *Murcha*

Using the pour plate method, a yeast strain was isolated from a *murcha* sample in YMA medium. A total of twelve yeast colonies were isolated, and the results of the morphological and biochemical analyses of the isolated colonies are displayed in Table 1. Commercial yeast's morphological traits were not noted.

Table 1. Morphological characteristics.

Isolates	Colony characteristics
Y1	Small powdery white smooth, entire margins
Y2	Small, creamy white, smooth texture, entire margins, raised elevation
Y3	Powdery white at surface of the colony, entire margin, raised elevation
Y4	Creamy white, rough appearance, irregular margin
Y5	Creamy white, circular, smooth texture, entire margin, slightly elevated
Y6	Small circular, mucoid, slightly brittle, irregular margin
Y7	Creamy white, raise elevation, irregular margin, smooth
Y8	Small, circular, mucoid, smooth texture, irregular margins, raised elevation
Y9	White colored, creamy, convex elevation, entire margin
Y10	Creamy white, smooth colony, circular, slightly elevated
Y11	White color colony, dry, entire margin, irregular, non-elevated
Y12	Small creamy white, smooth texture, entire margin, raised elevation

3.2. Identification of Yeast

Following the morphological analysis of the colonies, the yeast was identified. Gram staining was done on each isolated colony, and the results showed that they were all gram positive. It was discovered that the isolated colonies had elongated, oval forms. During microscopic investigations, budding was seen in a few of the isolates. The outcome of the observation was displayed in Table 2.

Table 2. Gram staining of yeast isolates.

Isolates	Microscopic observation of gram-stained smears
Y1	Gram positive, oval, budding
Y2	Gram positive, oval, budding
Y3	Gram positive, small oval shaped, transmission
Y4	Gram positive, rod like, transmission
Y5	Gram positive, oval, transmission
Y6	Gram positive, small round, transmission
Y7	Gram positive, oval, budding
Y8	Gram positive, elongated, transmission
Y9	Gram positive, oval, few budding
Y10	Gram positive, round shaped, transmission
Y11	Gram positive, elongated, budding
Y12	Gram positive, elongated, budding

3.3. Screening of Fermentative Yeast

The turbidity of the medium, surface growth, alcoholic odor development, and gas generation were the criteria used to screen fermentative yeasts. The yeast strains Y1, Y2, Y7, and Y10 were discovered to have a high (+++) amount of gas generation. The best isolates were determined to be yeast strains Y1, Y2, Y7, and Y10 based on gas production, smell, and turbidity. The results of the fermentative yeast screening are displayed in Table 3.

Table 3. Screening of fermentative yeast.

Isolate	Gas	Smell	Turbidity	Sediments/Flocculates
Y1	++++	++++	+++	S
Y2	++++	+++	++	S
Y3	+	++	+++	S
Y4	++	+	+	F
Y5	+	+	++	F
Y6	++	++	++	S
Y7	++++	++++	+++	S
Y8	++	++	+++	S
Y9	+++	+++	++	S
Y10	++++	++++	+++	S
Y11	++	++	+	F
Y12	+++	++	++	S

Note: ++++ = very high, +++ = high, ++ = medium, + = very low, S= Sediment, F= Flocculates.

3.4. Differentiation of Wine Yeast from Wild Yeast

The wine yeast was separated from the wild yeast using the WYDM agar medium. Five isolates out of twelve strains were screened for wine yeast, and two isolates were identified as wine yeast by displaying a red colony on WYDM agar media. Y1 and Y7 are the isolates exhibiting red colonies; the remaining isolates are regarded as wild yeast. Additionally, Table 4 displays the results of every observation.

Table 4. Colonies on wine yeast differentiation media (WYDM).

Isolates	Colony characteristics	Inference
Y1	Small red colonies	Wine yeast*
Y2	Pink colonies	Wild yeast
Y7	Red colonies	Wine yeast*
Y9	Pink colonies and some white colonies	Wild yeast
Y10	Pink colonies	Wild yeast

Note: *Indicates wine yeast.

3.5. Different Condition of Must for Wine Fermentation

The physicochemical composition of Grapes (*Vitis vinifera L.)*, was determined and presented in Table 5. The analyzed results were within the ranges revealed by the various researchers [25], [26], [27].

Table 5. Physicochemical composition of grapes juice for fermentation.

Parameters	Grape juice
pH (20 ºC)	3.93 ± 0.01
Acidity (% tartaric acid)	0.47 ± 0.06
Total soluble solids (TSS)	15.40 ± 0.00
Reducing sugar (%)	21.62 ± 0.90
Juice yield (%)	52.60 ± 0.80
Antioxidant activity (%)	65.75 ± 0.40

3.6. Fermentation

The total soluble solids (°Bx) were maintained at 22 °Bx, 0.48% tartaric acid to both the must samples. After that, grape must be fermented for 12 days at room temperature (around 21 °C) using both commercial and isolated wine yeast. While decreasing sugar was measured every other day throughout fermentation, biochemical changes such as °Bx, acidity, and pH were measured every day. The fermentation progress was followed by monitoring the decrease of °Bx value.

3.7. Biological Changes of Wine during Fermentation

3.7.1. Changes in TSS during Fermentation

At room temperature (around 21 °C), the TSS of all the samples SY1, SY2, and CY started out at 22 °Brix and progressively decreased to 7 °Bx, 6.4 °Bx, and 7.2 °Bx, respectively, throughout the course of the 12th days of fermentation (Figure 6). This indicates that samples SY1 and SY2 fermented at a higher pace than sample CY.

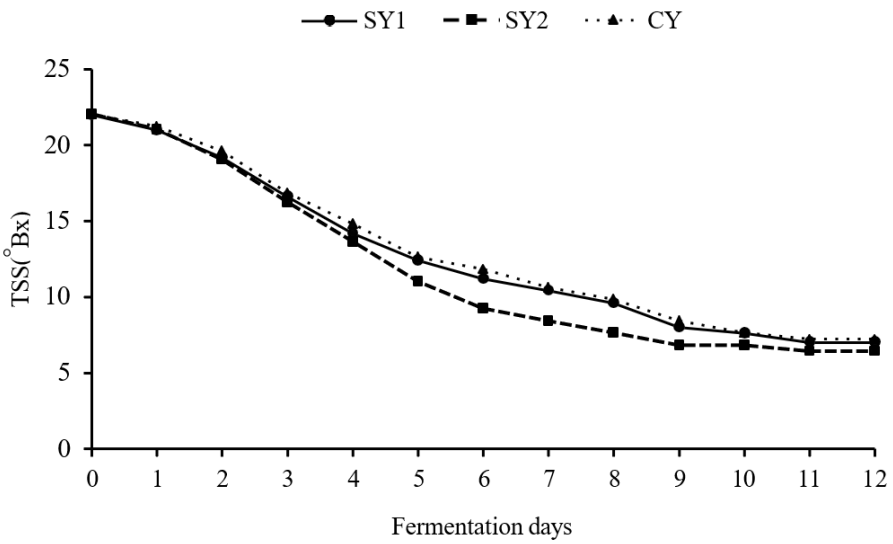

Figure 6. Changes in TSS during fermentation.

In winemaking, measures of soluble solids are frequently used to track the fermentation process. Sugar concentration falls during fermentation as yeast uses it to produce alcohol [28]. The wine samples' sugar content dropped as the °Bx dropped, and their acidity rose as a result of the synthesis of organic acids, which makes the wine sour [29].

3.7.2. Changes in Acidity during Fermentation

Figure 7 indicates that during the 12th days of fermentation at room temperature (around 21 °C), the initial acidity of all the samples, SY1, SY2, and CY, was 0.47 ± 0.005% as tartaric acid. This progressively increased to 0.87 ± 0.005%, 0.84 ± 0.005%, and 0.92 ± 0.005%, respectively. This indicates that compared to CY, samples SY1 and SY2 were less acidic. The titration method was used to calculate the acidity.

The organic acids are either naturally occurring in the fruit or are created by a variety of processes, such as ethanol oxidation, malolactic fermentation, or alcoholic fermentation. Fruit is the source of malic and citric acids, whereas fermentation produces succinic, lactic, and acetic acids [30].

3.7.3. Changes in pH during Fermentation

As the fermentation proceeds, the pH starts to decrease as in the Figure 8. pH was measured with the help of pH meter. The initial pH of the must was 3.93 ± 0.01 and the final was 3.41 ± 0.01, 3.46 ± 0.005 and 3.38 ± 0.01 in sample SY1, SY2 and CY respectively.

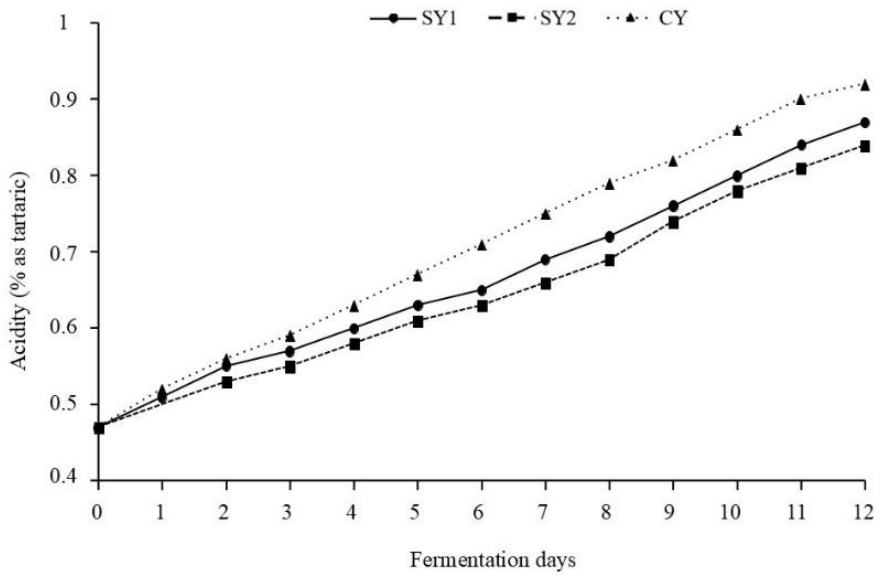

Figure 7. Changes in acidity during fermentation.

During the fermentation, pH was observed to fall due to spike in acid. It is important to keep the pH below 3.8. Wine tends to develop superior sensory qualities, ferment more uniformly, and have a lower chance of malolactic fermentation. White wines in particular need to maintain this low pH. The creation of organic acids, including lactic, acetic, and propionic acid, can reduce pH; Acetic acid is particularly useful in combating molds, yeasts, and bacteria [31]. The fall in pH may also be ascribed due to the formation of carbon dioxide which dissolved in must generating weak acid [32].

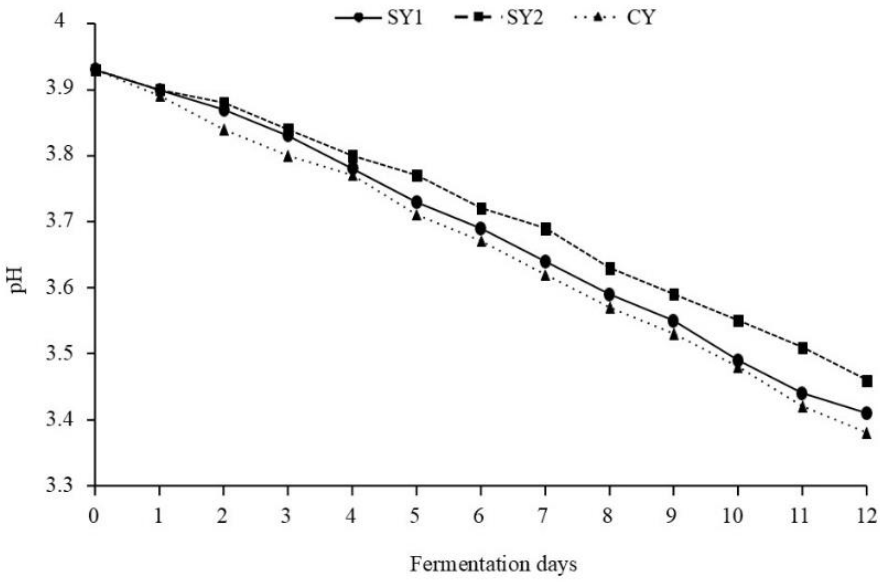

Figure 8. Changes in pH during fermentation.

3.7.4. Changes in Reducing Sugar during Fermentation

The initial reducing sugar in must was 21.62 ± 0.90, as seen in Figure 9. In samples SY1, SY2, and CY, it gradually decreased to 1.38 ± 0.02, 1.27 ± 0.15, and 1.45 ± 0.04 accordingly. Fermentable sugar is converted by yeast during fermentation into ethanol and other useful compounds such higher alcohol,

organic acid, and esters, which causes the amount of sugar to decrease. Fermentable carbohydrates are used as nutrition by yeast. These sugars are ethanol's direct precursors. While sucrose can be fermented after being hydrolyzed chemically or enzymatically to produce glucose and fructose, glucose and fructose can be fermented easily. As much as 16.5–180.0 g/L of sugar are needed to produce 1% of ethanol [33].

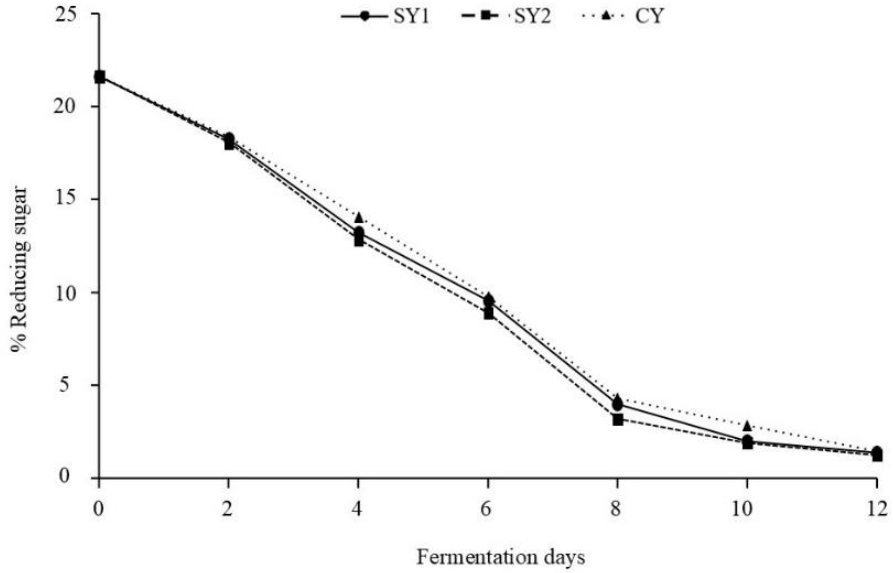

Figure 9. Changes in reducing sugar during fermentation.

3.8. Chemical Properties of Wine

Wine samples SY1, SY2, and CY had final TSS values of 7 ± 0.11, 6.4 ± 0.11, and 7.2 ± 0.11 °Bx, respectively (Table 6). This result explains why the LSD among these wine samples indicates that there was a significant difference between the TSS of samples SY2, SY1, and SY2, CY, but no significant difference ($p>0.05$) between samples SY1 and CY, Statistical analysis (ANOVA) revealed that the same substrate formulation and different starter culture had a significant effect on the TSS of the wine sample ($p<0.05$). Using isolated wine yeast, a 7.5 °Bx TSS peroxide was created from an initial TSS of 19 °Bx [34]. Two pineapple fruit isolates were used to make pineapple wine at temperatures of 9 and 11.6 °Bx [33]. Similarly, commercial wine yeast was used to make the Mead of TSS 7.6–8.4 °Bx from 20 °Bx [35]. An alternative wine Different values of the final TSS of wine were provided by yeast, demonstrating that the ability of yeast to metabolize and use nutrients in a substrate is reliant on it. Unfermentable sugar, pectin, tannins, pigments, acids, and their salts are examples of TSS that yeast is unable to use [36].

Table 6. Biochemical characteristics of grape wine prepared by selected yeast starter.

Parameter	SY1	SY2	CY
Total Soluble Solid (°Bx)	$7.00^{a} \pm 0.11$	$6.40^{b} \pm 0.11$	$7.20^{a} \pm 0.11$
Acidity (% tartaric acid)	$0.870^{a} \pm 0.005$	$0.840^{b} \pm 0.005$	$0.920^{c} \pm 0.005$
pH	$3.41^{a} \pm 0.01$	$3.460^{b} \pm 0.005$	$3.38^{c} \pm 0.01$
Reducing sugar (%)	$1.38^{a} \pm 0.02$	$1.27^{b} \pm 0.15$	$1.45^{c} \pm 0.04$
Alcohol (% v/v)	$7.33^{a} \pm 0.04$	$7.51^{b} \pm 0.04$	$7.08^{c} \pm 0.03$
Methanol (%)	$0.0300^{a} \pm 0.0005$	$0.03^{a} \pm 0.00$	$0.0330^{b} \pm 0.0005$
Aldehyde (mg acetaldehyde/100 L ethanol)	$205.50^{a} \pm 2.46$	$210.50^{a} \pm 1.73$	$194.43^{b} \pm 4.25$
Ester (g ethyl acetate/100 L ethanol)	$82.91^{a} \pm 2.40$	$88.71^{b} \pm 1.31$	$78.50^{c} \pm 2.13$

Note: Values are the Mean ±Standard Deviation (SD) obtained from triplet data. Different letters indicate significant differences in the same column.

According to statistical analysis (ANOVA), the acidity of the wine sample was significantly impacted by both the same substrate formulation and a different starter culture ($p<0.05$) (Table 6). This finding explains why the LSD between these wine samples indicates that the acidity of all three samples differed significantly. A reduction in pH is linked to an increase in acidity, and vice versa. The strain determines the amount of acid that may be produced during fermentation [37].

According to statistical analysis (ANOVA), the pH of the wine sample was significantly impacted by both the same substrate formulation and a different starting culture ($p<0.05$) (Table 6). This finding explains why the LSD between these three wine samples indicates that there was a significant variation in the pH of the three samples. The final pH of white wine was between 3.1 and 3.4. The mean wine's ultimate pH was between 2.9 and 3.75. The wine's pH value, according to this study, fell within the range of the data above [38].

According to statistical analysis (ANOVA), the reducing sugar of the wine sample was significantly impacted by both the same substrate formulation and a different starter culture ($p<0.05$) (Table 6). This finding explains why the LSD between these wine samples indicates that the reducing sugar of the three samples differed significantly. These wine samples' reducing sugar concentration was discovered to be quite comparable to that of wines from other suppliers. The reducing sugar content of honey wine, for instance, was found to be between 0.71 and 0.74%, but the reducing sugar content of bale wine made with wine yeast was 0.25 to 0.4% in accordance with [37], [39].

From Statistical analysis (ANOVA), same substrate formulation and different starter culture had a significant effect on the alcohol content of the wine sample ($p<0.05$) (Table 6), this result explains that the LSD among these wine samples shows that there was a significant difference between the alcohol content of three samples These wine samples were found to have an alcohol content that was somewhat comparable to wines from other sources. For example, honey wine was found to have an alcohol content of 10–11%, while papaya wine, which was made from juice extracted by enzymatic treatment, had an alcohol content of 10 ± 0.47% [40], [41]. Not all of the sugar was used up throughout full fermentation by every yeast strain, meaning that not all of the sugar was transformed into ethanol. A number of 90–95% of TSS is sugar, of which 5% is utilized to create byproducts (such as glycerol, succinic acid, lactic acid, 2,3-butanediol, acetic acid, etc.), 2.5% is used as a carbon source by yeast, and 0.5% is left over as unfermented residual sugars [37].

The LSD among these three wine samples indicates that there was a significant difference between the methanol content of samples SY1, CY, and SY2, CY, but no significant difference between samples SY1 and SY2 (Table 6). This is explained by statistical analysis (ANOVA), which showed that the same substrate formulation and different starter culture had a significant effect on the methanol content of the wine sample ($p<0.05$). Mead was discovered to have a methanol concentration of 0.03–0.032%, while plum wine had a methanol value of 0.0437–0.059%, and bale wine had a methanol content of 0.057–0.08% [37], [42]. The methanol concentration of the study's wine sample was found to be almost equal to the aforementioned results when compared to these values. Methanol, a trace amount of wine, is created when pectin is broken down by enzymes. Methanol is the byproduct of the release of the methyl groups linked to pectin. Methanol production may be impacted by pectolytic enzymes applied after vinification for clarity. The wine's sensory qualities are unaffected. Nonetheless, its significance stems from the body's oxidation to harmful formaldehyde and formic acid [43].

According to statistical analysis, the aldehyde content of the wine sample was significantly affected by the same substrate formulation and a different starter culture ($p<0.05$). This finding explains why the LSD among these three wine samples indicates that there was a significant difference between the aldehyde content of SY1, CY, and SY2, CY, but not between samples SY1 and SY2 (Table 6).

The aldehyde level of bale wine ranged from 633 to 756 mg of acetaldehyde per 100 mL of ethanol, whereas the aldehyde content of Perry wine was 161–206 mg. The perry wine and the data from the analysis above were comparable. White wine has 11–493 mg/L of acetaldehyde, whereas red wine has 4–212 mg/L. But in a highly oxygen-regulated setting, it was recently discovered that lower fermentation temperatures actually increased acetaldehyde levels. This could be because the yeasts used less acetaldehyde in the final

stages of fermentation [44]. A total of 90% of the aldehyde concentration in wine is acetaldehyde, one of the most important sensory carbonyl compounds. When acetaldehyde levels are low, they can give wine a nice perfume, but when they are excessive, they are regarded as a flaw and smell like rotten apples. Wine has a threshold of 100–125 mg/L [45].

According to statistical analysis, the ester content of the wine sample was significantly impacted by both the same substrate formulation and a different starter culture ($p<0.05$) (Table 6). This finding explains why the LSD between these three wine samples indicates that there was a significant difference in the ester content of the three samples. Banana wine has 86.68 g of ethyl acetate per 100 mL of alcohol, whereas bale wine has 59–79 g [46], [47]. Perry wine has an alcohol concentration of 128–187 g ethyl acetate/100 mL of alcohol. The wine sample used in this investigation had an ester concentration that was lower than that of perry wine and comparable to that of banana and hay. During fermentation, yeast reacts with alcohols and acyl CoA molecules to produce esters. The relative quantity of yeast generated by the matching alcohol and acyl CoA determines how many esters are created [45].

3.8.1. Total Phenolic Compound

According to statistical analysis, the phenolic compound of the wine sample was significantly impacted by both the same substrate formulation and a different starter culture ($p<0.05$) (Figure 10). This finding explains why the LSD between these three wine samples indicates that there was a significant difference between the three samples' phenolic compounds. The structurally varied category of chemicals known as phenolic compounds is present in different concentrations. They are crucial in determining the wine's color and taste. They play a crucial part in the aging and maturation of wines and are engaged in the browning processes that occur in grapes and wines. Red wines typically have phenolic contents between 1000 and 3500 mg/L, whilst white wines have phenolic contents between 100 and 250 mg GAE/L [48]. The phenolic concentration of jamun wine was 2200 ± 3 mg GAE /L, but the phenolic content of kiwi fruit wine was 152 ± 0.02 mg GAE/L [49], [50].

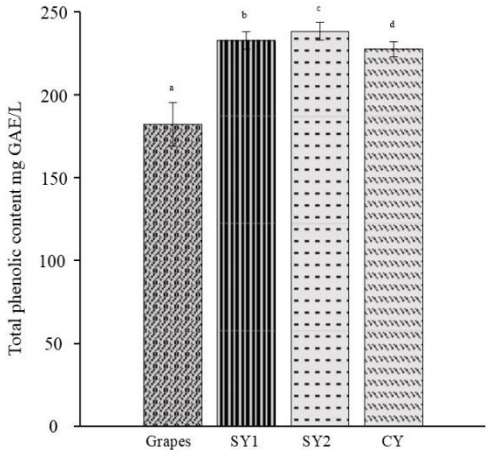

Figure 10. Total phenolic content of grapes, SY1, SY2 and CY.

The primary constituents of phenolic compounds are flavonoids and non-flavonoids. About 85% of all phenols are flavonoids, which include polymers of tannins, anthocyanins, and 3-flavanols, which are monomeric flavonoids or catechins. Phenolic acid families like hydroxycinnamates and hydroxybenzoates are examples of non-flavonoids [51]. Certain polyphenolic wine components, such quercetin and catechin, have been demonstrated to suppress tumor and cancer cells in vitro. Resveratrol and acutissimin A, two other red wine constituents, have drawn a lot of interest lately as potential anti-cancer agents. In addition to its preventive actions against carcinogenesis, resveratrol is a polyphenol that may be beneficial for cardiology. Daily consumption of phenolic compounds is associated with a lower risk of heart disease,

according to several epidemiological research [52], [53].

3.8.2. Antioxidant Activity

Statistical analysis (ANOVA) revealed that both the same substrate formulation and a different starting culture had a significant effect on the antioxidant activity of the wine sample ($p<0.05$) (Figure 11). This discovery clarifies why there was a notable variation in the antioxidant activity of the three wine samples, as shown by the LSD between them. It was discovered that the generated wine's antioxidant activity, measured as a percentage of scavenging activity, was higher than mead's 14.77–21.59% [29], [54]. A number of 52.6 ± 0.02% scavenging activity for apple cider and 48–54% for bale wine. Antioxidant production as a percentage of scavenging activity varied between 60.68 ± 1.07 and 87.58 ± 0.55% in red wines and between 12.7 and 19.05% in white wines [55]. Higher antioxidant activity may be associated with greater polyphenols. Unlike phenols and flavonoids, enzyme-assisted processing is also said to enhance antioxidant activity [56]. As a result, juice that was extracted using enzymes showed a comparatively high level of antioxidant activity. Fruit antioxidant action is thought to be primarily attributed to polyphenols, vitamin C, vitamin E, β-carotene, and lycopene [57]. Antioxidants are substances that prevent oxidation. A chemical process called oxidation can create free radicals, which can start a chain reaction that can kill an organism's cells. Antioxidants like thiols or ascorbic acid (vitamin C) are the end products of such chain reactions. Plants and animals maintain intricate networks of overlapping antioxidants, such as glutathione and internally produced enzymes (like catalase and superoxide dismutase), or dietary antioxidants like vitamin C and vitamin E, to counteract oxidative stress. Because it includes both alcohol and antioxidants, wine is a unique beverage. This cohabitation has important health consequences since wine contains an antioxidant that, once it enters the body through the same processes that our body employs to detoxify ethanol that has been consumed, is different from many other systems [58].

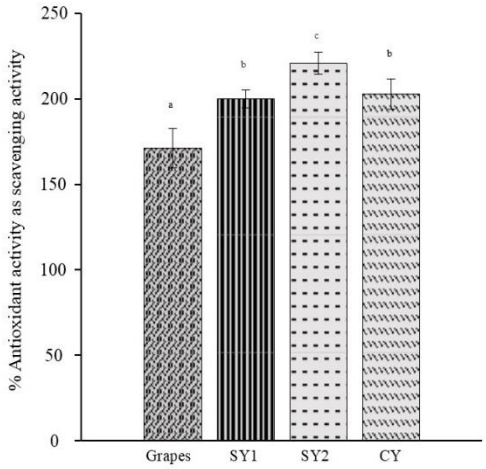

Figure 11. Antioxidant activity of grapes, SY1, SY2, and CY.

3.9. Sensory Evaluation

From statistical analysis (ANOVA), the effect of color-based sensory score and smell on different prepared wine samples SY1 and SY2 showed no significant different ($p>0.05$), whereas CY showed significant different ($p<0.05$) with other samples (Figure 12). The wine's purity, color quality, and quantity are all evaluated, typically prior to tasting. White wine is caused by a phenolic chemical found in grapes, but red wine is determined by its anthocyanin concentration and composition [59]. The chemistry of the entire winemaking process has a direct impact on the wine's smell [60].

From statistical analysis, the effect of taste-based sensory score on different prepared wine samples, shows that there was significant difference between ($p<0.05$) between all of the samples. It's possible that the SY's increased aldehyde content accounts for its preferred flavor. The quantity of sugars (saccharides)

that remain in a finished wine, specifically glucose and fructose, determines how sweet or "dry" it is considered to be in terms of wine taste. Statistical analysis on mouthfeel characteristics showed that there was no significant different between ($p>0.05$) sample SY1 and CY whereas significant different ($p<0.05$) with sample SY2. The phrase "mouth feel" refers to a sensation that is felt as a whole in the mouth and frequently correlates with the term "body." The hydrophilic proline-rich protein-phenol complex is a nice illustration of how it contributes to oral feeling [61]. Additionally, it refers to quick sensations brought on by the free nerve endings of the trigeminal nerve. The propagation of the free nerve ending throughout the oral cavity creates diffuse sensations that are poorly localized. For wine, mouth sensation involves astringency, temperature, prickling, body and burning sensations [60]. From Statistical Analysis (ANOVA), the effect of taste-based sensory score on different prepared wine samples, LSD ($p>0.05$), shows that there was not significant difference between them. The order of superiority can be summarized as; color SY2>SY1>CY; smell SY2>SY1>CY; taste SY2>SY1>CY; mouthfeel after taste SY2>SY1>CY; overall acceptance SY2>SY1>CY.

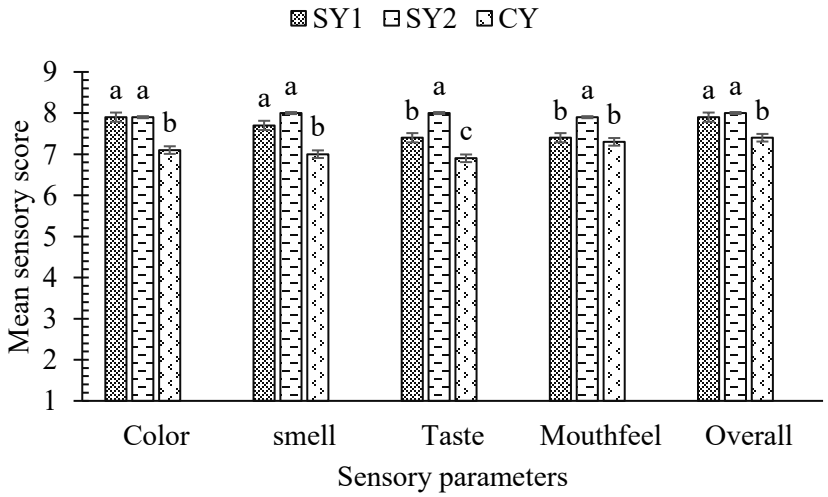

Figure 12. Mean scores of sensory attributes.

3.10. Limitation of Study

The strain used for SY2 wine exhibits the best performance during fermentation and in the sensory assessment. Although isolate colonies were determined to be wine yeast, no further strain type screening was carried out. The isolated yeast colonies' alcohol tolerance level was not assessed. Due to a lack of equipment, it was impossible to identify the taste components of wine.

4. CONCLUSION

Based on our research, In the laboratory, wine fermentation was carried out using both commercial and isolated yeast. Compared to commercial yeast, isolated yeast has increased fermentation activity during fermentation. Commercial yeast-fermented wine and isolated yeast-fermented wine differ significantly ($P<0.05$) in terms of final TSS, acidity, and reducing sugar. Sample SY1 and SY2 had greater levels of alcohol, aldehyde, ester, phenolic compound, and antioxidant activity than CY, indicating that the isolated yeast had a higher fermentation activity than the commercial yeast. Samples SY1 and SY2 are probably more acceptable than sample CY wine, according to sensory analysis of the two isolated strains, the strain utilized for SY2 wine performs the best both during fermentation and in the sensory evaluation of the finished wine. Furthermore, we recommended apple, pear, pineapple, and papaya natural product juices, as well as nectar and flavor concentrate, may be added to the grape must to enhance the wine's flavor, acidity, and other development factors as it matures. It is possible to research the changes in wine's taste character

caused by isolated yeast as it aging. Research might be done on how fermentation impact the antioxidant profile and phenolic compounds of black grapes.

AUTHOR CONTRIBUTION

All author contributed equally to the main contributor to this paper. All authors read and approved the final paper. **Sanjog Sharma**: Writing–original draft, visualization, conceptualization software, methodology, investigation, formal analysis, data curation, resources collection. **Ganga Sangroula**: Writing–review and editing, writing–original draft, visualization, data curation, software, investigation, supervision, validation, resources collection. **Tika Bahadur Karki**: Visualization, methodology, supervision, conceptualization.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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