Laboratory preparation of fruit, vegetable wine and physicochemical study comparison
Sudhakar Kancharla¹, Prachetha Kolli², Dr.K.Venkata Gopaiah³.*

1. Director Clinical Laboratory, Devansh Lab Werks, 234 Aquarius Drive, Homewood, Alabama, USA-3520.
2. Scientist, Microgen Health Inc, 14225, Sullyfield Cir Suite E, Chantilly, VA, USA-2015.
3. Associate Professor,St. Mary’s College of Pharmacy, Chebrolu, Guntur-A.P-522 212-India

Abstract
Study mainly focused on the process monitoring of homemade wine during its fermentation period. The experimental investigation was aimed to study the variation in each Parameter during the fermentation period. The final analysis of wine of various parameters-alcohol content, pH specific gravity were conducted. These studies were compared with the Apple, Beetroot, Wine. The study concludes that pH showed a decreasing trend and then attains minimize then increases. The sugar concentration of wine decreases with increasing in the number of days. It has been studied that as the number of day’s passes, the specific gravity and volume percentage of alcohol also increases gradually. The Titrable acidity of wine showed a fluctuating trend as the number of days passes. Apple showed a pH range of 4.6 - 2.4, viscosity showed the thickness of the wine 0.17 specific gravity ranges from 1.9 - 1.09 and alcohol content was 14.7. Beetroot showed a pH range of 5.4 - 2.1, viscosity showed the thickness of the wine 1.2 - 0.12, specific gravity ranges from 1.93 - 1.06 and alcohol content was 12.3. The results of process monitoring and final analysis the physicochemical parameters both wines having approximately equal values. But beetroot wine having low alcohol content so it would be more potent then apple wine.

Keywords: Apple, Beetroot, Microbial Study.
DOI: https://doi.org/10.46796/ijpc.vi.139

Introduction
Home wine making is an enjoyable educational and satisfying hobby wine making recipe make the process easy and simple instructions ensure success the basic steps are easy to learn and practice the traditional home made wine base ingredient is the apple because it naturally contains the correct mixture of sugar, moisture, tannin, and nutrient requisite for fermentation and preservation, and it even carries wine can be made from almost in any non toxic plant are plant part if additional ingredient are supplied in the correct amount so the process of making wine from various types of fruits, vegetables and species is no more complicated then making wine from apple, beetroot and it is a good preservation method it needs extra preparation steps and some adjustments in sugar content, acid levels etc.. Fermentation can extract valuable components from the raw materials used for production yeast is the magical ingredient that turns fruit juices into wine. In spontaneous fermentation, the 1st stage in variably being dominated by the alcohol-tolerant strains of saccharomyces
cerevisiae. This species is universal known as the wine yeast and is widely preferred for initiating wine fermentation. The alcohol content of home made wines is only above 7-8% which makes it consumable for person of any age group. Thus, wine stimulate the release of digestive enzyme, which digests not only the alcohol but the many other nutrients found in wine the proper dosage, or a moderate intake of wine, in addition to effect in cholesterol levels favorably, decrees the tendency of blood to clot and assists in dissolving clot, all important in protecting against heart disease. Research also indicates that moderate wine drinking may reduce the tendency of arteries to constrict during stress, lower blood pressure, and increase coronary artery diameter and blood flow. More recently, wine has been identified has a dependable sources of quercetin a potent anti carcinogen, and of many flavonoids and other poly phenol anti oxidants. Considering the importance and medicinal value of wine from some special raw material, it was very interesting to conduct the production of wine in a batch reactor setup in the laboratory. We select apple, beetroot for our study apple, beetroot is one of the useful fruit, vegetable. It is consumed as a fresh fruit, vegetable or in the form of food product like preservative. The fruit, vegetable forms an important constituent of many ayurvedic preparations such as ashyvantrandash triphala and is recorded as “one of the best rejuvenating” herbs preparation of wine using fruit of apple and vegetable of beetroot would be useful for importing healthful properties to the wine[1,24].

Fermentation
Fermentation is the term used by microbiologist to describe any process for the production of a product by means of the mass culture of a microorganism.
The product in either is:
1. The cell itself: referred to as biomass production
2. A microorganism own metabolite: referred to as a product from a natural or genetically improved strain.
3. A microorganism foreign product: referred to as a product from recombinant DNA technology or genetically engineer strain, i.e. recombinant strain [2,23].

Classification of microorganisms
The kingdom protista comprises unicellular organisms capable of self duplication or off directing their own replication. Prokaryotes do not possess a true nucleus or a nuclear membrane, where as eukaryotes having nucleus enclosed with in a distinct nuclear membrane. The non cellular pretists do not undergo self replication, instead their direct their reproduction with in another cell termed the host. Cyanobacteria (blue-green algae) have been classified as a separate group of micro organisms, although they are frequently considered to be included with other bacteria. Fungi may be sub divided in to lower fungi as well as slime moulds and higher fungi which comprises yeast’s. Yeast’s are free leaving, single cells, unlike fungi, which they closely resemble. Protozoa are free leaving, minute organism, which although not generally employed for bio technological processes have been included for completeness. Myxomycota, commonly known as slime moulds (or slime fungi) are widely used as research organisms. Viruses can be recorded as intracellular parasites. The criteria used for the classification of microorganism s include morphology, reproductive mechanisms , pigment presence, means of motility, physiology and structural features [3].

Microbial activity
Microorganisms in the process of self replication produce numerous complex macromolecules from about 100 different monomer units. In the biochemical pathway to achieve a bacterial cell uses well over 1000 different enzymes and a eukaryotic cell may employ twice as many. The biochemical metabolism can be divided in to two broad classes: the anabolic pathway synthesis the complex molecules and their intermediate processes. The catabolic pathway supply the energy needed for the anabolic processes these two divergent activities is closely linked. Microorganisms that carry out their metabolism using oxygen are referred to as aerobic microorganisms’. Some microorganism. Some microorganisms can be substitute nitrate, others sulphate or ferric ion, for oxygen and thus grow in the absence of oxygen. These microorganisms are referred to as anaerobic. Microorganism can be classified according to the lowest temperatures at which significant growth occurs. Growth of yeast is optimal in the region of 20-30c for mesophiles species. In general a shift in the incubation temperature from the optimum to a lower temperature results in a temperature dependent reduction of metabolic activity. An increase in the incubation temperature can cause a reduction in both the biomass concentration and viability due to a temperature and exposure- dependent decrease in enzyme activity. Many microorganism display an optimum PH for growth at around 7, with the majority
favoring the PH range 5-8. However, there are exceptions including acetic acid bacteria, thiobacilli and urea decomposing bacteria. In addition numerous algae live in natural waters above pH10 [4,5].

**Batch fermentation**

Batch fermentation can be considered to be a closed system. t=0 the sterilized nutrient solution in the fermentor is inoculated with microorganisms and incubation is allowed to proceed. In the course of the entire fermentation, nothing is added, except oxygen (in case of aerobic microorganisms), an antifoam agent, and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells. After the inoculation of a sterile nutrient solution with microorganism and cultivation under a lag phase physicochemical equilibration between microorganism and the environment following inoculation with very little growth LOG PHASE By the end of the log phase cells have adapted to the new conditions of growth. Growth of the cell mass can now be described quantitatively as a doubling of cell number per unit time for bacteria and yeast’s, or a doubling of biomass per unit time for filamentous organisms as fungi. By plotting the number of cells or biomass against time on a semi logarithmic graph a straight-line results, hence the term log phase. Although the cells alter the medium through uptake of substrates and excretion of metabolic products, the growth remains constant during the log phase. Growth rate is independent of substrate concentration as long as excess substrate is present [6].

**Stationary phase**

As soon as the substrate is metabolized or toxic substance have been formed, growth slows down or is completely stopped. The biomass increases only gradually or remains constant during this stationary phase, although the composition of cells may change. Due to lysis, new substrates are released which then may serve as energy sources for the slow growth of survivors. The various metabolites formed in the stationary phase are often of great biotechnological interest [7].

**Death phase**

In this phase the energy reverse of the cells is exhausted. A straight line may be obtained when a semi logarithmic plot is made of survivors versus time, indicating that the cells are drying at an dependent on the microorganism and the process used.

The fermentation is usually interrupted at the end of the log phase or before the death phase begins.

**Fed batch fermentation**

In the conventional batch process just described all of the substrate is added at the beginning of the fermentation. An enhancement of the closed batch process is the fed batch fermentation. In the fed-batch process, substrate is added in increment as the fermentation progresses. In the fed-batch method the critical elements of the nutrient solution are added in small concentrations at the beginning of the fermentation and these substances continue to be added in small doses during production phase. [8]

**Continuous fermentation**

In continuous fermentation, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and added to the bioreactor continually and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. In the case of homogeneously state, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted. [9]

**Fermentor system:**

A microbial fermentation can be viewed as a three-phase system, involving liquid-solid, as-solid, gas-liquid reactions. The liquid phase contains dissolved nutrients, dissolved substrates and dissolved metabolites. The solid phase consists of individual cells, pellets, insoluble substrates, or precipitated metabolic products. The gases phase provides a reservoir for oxygen supply and for co2 removal.

**String and mixing**

The transfer of energy, nutrients, substrate and metabolite within the bioreactor must be brought about by a suitable mixing device. The efficiency of any one nutrient may be crucial to the efficiency of the whole fermentation. For the three phases, the stirring of a bioreactor brings about the following; [10]

- Dispersion of air in the nutrient solution
- Homogenization to equalize the temperature and the concentration of nutrients throughout the fermentation
- Suspension of microorganisms and solid nutrients Dispersion of immiscible liquids

**Overcome**

1. resistance within the gas film to the phase boundary
2. penetration of the phase boundary between gas bubble and liquid
3 transfer from the phase boundary to the liquid
4 movement within the nutrient solution
5 transfer to the surface of the cell [11]

For fermentation carried out with single celled organisms such as bacteria and yeast, the resistance in the phase boundary between the gas bubble and the liquid is the most important factor controlling the rate of transfer. Microbial cells near gas bubbles may absorb oxygen directly through the phase boundary and the rate of gas transfer to such cells is increased. In cellagglomerates or pellets, the 02 transfer within the agglomerate can become the limiting factor. The mass transfer of oxygen into liquid can be characterized by the oxygen transfer rate (OTR) or by the volumetric oxygen transfer coefficient (kLa). These values have been thoroughly examined as a critical parameter for bioreactor function. The oxygen transfer rate and the volumetric oxygen transfer coefficient are dependent on the following

**Parameters**

vessel geometry: diameter, capacity
1. Mixing properties: power, impeller configuration and size, baffles.
2. Aeration system: sparger rate, geometry, location.
3. The nutrient solution: composition, density, viscosity.
4. The microorganism: morphology, concentration
5. The antifoam agent used.
6. The temperature.

**Sterilization:** [12]

In virtually all fermentation processes, it is mandatory to have contamination free seed cultures at all stages, from the preliminary culture to the fermentor. A fermentor can be sterilized either by destroying the microorganisms with some lethal agent such as heat, radiation, or a chemical, or by removing the viable microorganisms by a physical procedure such as filtration.

- During fermentation the following points must be observed to ensure sterility:
  - sterility of the culture media
  - sterility of incoming and outgoing air
  - appropriate construction of the bioreactor for sterilization and for prevention of contamination during fermentation

**Sterilization of fermentation air:** [14]

Most fermentations are operated under high aeration and the air supplied to the fermentor must be sterilized. The number of particles and microorganisms in air varies greatly depending on the location, air movement, and previous treatment of the air. On the average, outdoor air has 10–2,000 particles per m³ and 5-2,000 microorganisms per m³. Of these, 50% are fungus spores and 40% are Gram-
negative bacteria. Fermentors generally works with aeration rates of 0.5-2 v/vair volume / liquid volume per minute). Themethods available for sterilizing gases include filtration, gas injection (ozone), gas scrubbing, radiation (UV) and heat. Of these, only filtration and heat are practical.

**Appropriate construction of the fermentor [15]**

There should be a minimum number of openings in the fermentor to favor maintenance of sterility. Small openings must be made leak proof with 0-rings, larger openings with flat gaskets. Whenever a movable shaft penetrates the fermentor wall, special problems of sterility maintenance should be solved.

**Fermentation processes**

An overall scheme of a fermentation process can be described as follows:

- **Stage 1**: inoculums preservation
- **Stage 2**: inoculums build-up
- **Stage 3**: fermentor culture

**Stage 1: inoculums preservation [16]**

- The objective of preservation is to maintain strains as long as possible without cell division. The optimal method of preservation must be worked out for each strain. The following three techniques are most commonly used: Storage at low temperatures (2-6 degrees Celsius)
- Frozen storage (-18, -80 or -196 degrees Celsius)

**Lyophilization**

Storage at 2-6 degrees Celsius is the least secure, there is a relatively high risk of contamination and reverse mutation through frequent transfer. The frozen storage is the most common and frozen cultures may be kept for several years. The proportion of survivors is critical because up to 95% of the microorganisms are generally killed during freezing and subsequent thawing. The best method of strain preservation is Lyophilization (freeze-drying).

**Stage 2: growth of inoculum [17,18]**

The preserved culture is initially revived by growth in aerlenmeyer flask on a biological shaker or on a solid medium (if spore formation is needed). In order to obtain sufficient inoculums for small fermentors, a second series of shake cultures is usually made in more flasks. Out from lyophilized strains the growth of inoculums takes around 4-10 days, out from frozen cultures the growth of inoculums takes 4-48 hours for bacteria and 1-7 days for fungi. Finally out of refrigerated cultures the growth of inoculums takes 4-24 hours for bacteria and 1-5 days for fungi.

**Stage 3: fermentor culture [19,20]**

The nutrient media for production must be optimized not only in the ingredients used but also how the medium is prepared and sterilized, pH value before and after sterilization.

The most important parameters during the fermentation are:

- Temperature
- Aeration
- Stirring
- Process management

The process management is concerned with:

- Setting up the initial process conditions
- Monitoring to ascertain whether the process is following the required course.
- Facilitating manual adjustments to the process variables.
- Deciding when to terminate the process and/or to transfer or harvest the product.
- Calculating the mass and thermal balances, rates of reaction, kinetics and yields.
- Providing information for statistical records on consistency and for archival purposes.
- Monitoring contamination and process hygiene

**Plant profile of apple:** [21]

**Scientific classification:**

- Kingdom: Plantae
- Clade: Tracheophytes
- Angiosperms Clade: Eudicots Clade: Rosids Order: Rosales
- Family: Rosaceae
- Genus: Malus
- Species: M. domestica

**Binomial name:** Malus domestica

**Synonyms:** Malus communis Desf. Malus pumila Mil. M. frutescens Medik. M. paradisiaca (L.) Medikus M. sylvestris Mil.

**Pyrus malus L.**

**Pyrus malus var. paradisiaca L.** Pyrus dioica Moench

**Plant profile of beetroot:** [22]

**Scientific classification:**

- Kingdom: Plantae
- Clade: Tracheophytes
- Angiosperms Clade: Caryophyllales
- Family: Amaranthaceae
- Genus: Beta (plant)
- Species: B. vulgaris
Binomial name: Beta vulgaris L.
Synonyms: Betacida L. crispa Tratt.
Beta esculenta Salisb. (nom. illeg.) Beta sulcata Gasp.
Beta vulgaris subsp. esculenta Cout

Expients
Saccharomyces cerevisiae: is a species of yeast. It has been instrumental to winemaking, baking, and brewing since ancient times. It is believed to have been originally isolated from the skin of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like the model bacterium. It is the microorganism behind the most common type of fermentation at round to avoid, 5-10 µm in diameter. It reproduces by a division process known as budding. Many proteins important in human biology were first discovered by studying their homolog’s in yeast, these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes. S. cerevisiae currently the only yeast cell known to have Berkeley bodies present, which are involved in particular secretory pathways. Antibodies against S. cerevisiae are found in 60-70% of patients with crohn’s disease and 10-15% of patients with ulcerative colitis (and 8% of healthy controls).

Sugar solution
Sugars in wine are at the heart of what makes wine making possible. During the process of fermentation, sugars from wine grapes are broken down and converted by yeast into alcohol (ethanol) and carbon dioxide. Grapes accumulated sugars as they grow on the hydrolyzed (separated) by the enzyme invertase into glucose and fructose. By the time of harvest, between 15 and 25% of the grape will be composed of simple sugars. Both glucose and fructose are six-carbonsugars but three-, four-, five- and carbon sugars are also present in the grape. Not all sugars are fermentable with sugars like the five-carbon arabinose, rhamnose and xylose still being present in the wine after fermentation. Very high sugars content will effectively kill the yeast once a certain (high) alcohol content is reached. For these reasons, no wine is ever fermented completely “dry” (meaning without any residual sugar). Sugar’s role in dictating the final alcohol content of the wine (and such its resulting body and “mouth-feel”) sometimes encourage winemakers to add sugar (usually sucrose) during winemaking in a process known as capitalization solely in order to boost the alcohol content-capitalization does not increase the Sweetness of wine.

Methodology
1. We gather and search the review and research articles related to tissue culture, fermentation, isolation of bacteria then after we decided to go through fermentation of wine.
2. Then that we collecting the requirements and chemicals for the fermentation of wine from fruit, vegetable.
3. After we are going to collect the fruits and vegetables and the sterile them through the washing and drying.
4. Crushing the fruits and grind them thoroughly
5. Then prepare the sugar syrup then inoculating yeast.
6. Allow the container to room temperature in a dark place.

Daily Monitoring pH was measured using digital pH meter. The total sugars were estimated in terms of glucose by Nelson Somogyi method. Estimation of titratable acids was done by titrimetric method using 0.1 N NaOH in terms of tartaric acid. Biomass was determined by dry weight method in g/mL. Alcohol percentage was calculated using specific gravity method. Specific gravity was also determined.

Final Analysis of Wine Tannin content was estimated by Folins
Denis method in mg/100ml. Phenol content was determined by Folins Lowry method in mg/100ml. Free and total SO2 was done by Ripper method in g/L. Total suspended solids was calculated in Degree Brix. Final analysis of all parameters such as pH, alcohol content specific gravity, sugar content, titratable acidity, and biomass were conducted using the methods described in daily analysis.

Analysis of Commercial Wine and Its Comparison
Estimate parameters such as pH, alcohol content specific gravity, sugar content, titratable acidity, Biomass, tannin content, phenol content, free and total SO2 and total suspended solids of the commercially available wine were conducted. The parameters of the homemade wine were compared with that of the commercially available wine.

Process Monitoring (Daily) Daily analysis of homemade wine (fermented medium) has been conducted. Various parameters such as pH, Titratable acidity, specific gravity, alcohol content, sugar concentration, viscosity, and bio mass concentration etc of each batch were determined day by day during the course of fermentation. Results are shown in table. parameters monitoring during fermentation:

- Variation in pH
- Sugar concentration
- Specific gravity
- Alcohol percentage
- Viscosity

**PH**
Variation in PH in the fermentation medium during the course of process was as shown in the figure. pH showed a decrease trend then attains minimum then increases. The initial PH of apple was 4.6 which decrease to 3.42 on the 8th day and decreased to 3.18 on 14th day, in 2.4 on 24th day. The initial PH of beetroot was 5.4 which decrease to 3.6 on the 18th day and decreased to 2.9 on 23rd day, 2.1 in 24th day.

**Substrate (sugar) concentration**
The sugar concentration of wine decreases as the fermentation days passed because of the utilization of substrate. The sugar concentration lies between 25 mg/100gm to 10mg/100ml. In case of SG, the initial sugar concentration was 21.78 mg/100ml. Apple wine was decreased to 14.20 on the 24th day. For G, the sugar concentration started from 21.78 mg/100ml and decreased to 14.20 mg/100ml on 24th day. Beetroot wine was decreased to 5.25 on the 24th day. For G, the sugar concentration started from 23.86 mg/100ml and decreased to 5.25 mg/100ml on 24th day.

**Specific gravity**
Estimation of specific gravity of SG, and G, Has been conducted. It has been studied that as the number of day’s increases, the specific gravity decreases gradually. In apple wine specific gravity ranges from 1.092 to 1.9. Specific for 5 G on 1st day was 1.9 and shows a trend to decreases to 1.13 on 14th day. For G1, the specific gravity starts from 1.92 and decreases to 1.09 on 24th day.

In beetroot wine specific gravity ranges from 1.93-1.08 in 1st day 1.93 and it shows a trend to decreases to 1.35 on 18th day, the specific gravity decreases to 1.08 in 24th day.

**Alcohol Percentage**
By studying the alcohol content in volume percentage of SG and G it can be concluded that the alcohol volume percentage increased as the number of day’s increases. The initial alcohol percentage was zero for all winesamples – SG and G. Apple wine has get Final alcohol content was 14.07% G was 10.3% on 24th day. Beetroot wine has get Final alcohol content was 12.03% G was 10.3% on 24th day.

**Viscosity**
Estimation of the viscosity has been conducted by the number of day’s increases, the specific gravity decreases gradually. In apple wine viscosity was gradually decreases from 1.34-0.17, in 8th day 0.84, in 24th day the final value is 0.17 Beetroot wine gradually decreases from 1.2-0.12 In 18th day the value is 0.7 in 24th day s value of viscosity.

**Analyses of Wine**
Alcohol percentage, tannin content, phenol content, free and total SO2, pH, specific gravity, viscosity titratable acidity and total suspended solid were estimated. Final analysis of wine was conducted after the fermentation period (i.e. After 15 days).

**Methodology for apple winemaking**
- Collected the fresh apples and thoroughly washed it under the water.
- Dry the apples and cut into a small pieces and GRIND it like juice.
- The juice was poured into a glass jar and inoculate yeast in the container allow the container closed for half an hour.
- Add the sugar solution & flavoring agents, wheat in it.
- Closed with aluminum foil with covered plate of the jar for completing the fermentation process.
- After completion of the fermentation process filter the solution from extract.

**Methodology for beetroot wine making**
- Collect the fresh beetroots and remove the peel on it.
- Cut into a small pieces and BOILED with water in this process added the flavoring agents (clove, cinnamon).
- Filter the solution and then add the sugar and
dissolved it.
- After half an hour add the yeast on it and close the container with aluminum foil tightly.
- Place it on a constant temperature.
- After completion of the fermentation process can’t filter the solution.

---

**Results & Discussion: Daily Monitoring of Apple**

| S. no | Days | pH  | Alcohol percentage | Specific gravity | Sugar concentration (mg/ml) | Viscosity |
|-------|------|-----|--------------------|------------------|-------------------------------|-----------|
| 1     | 1    | 4.6 | 0                  | 1.9              | 21.78                         | 1.34      |
| 2     | 4    | 3.72| 0.9                | 1.7              | 19.92                         | 1.05      |
| 3     | 6    | 3.57| 2.06               | 1.49             | 18.14                         | 0.98      |
| 4     | 8    | 3.42| 3.53               | 1.43             | 17.16                         | 0.84      |
| 5     | 11   | 3.38| 5.93               | 1.32             | 16.64                         | 0.66      |
| 6     | 12   | 3.27| 6.85               | 1.23             | 15.54                         | 0.53      |
| 7     | 13   | 3.22| 7.4                | 1.19             | 14.85                         | 0.48      |
| 8     | 14   | 3.18| 10.6               | 1.13             | 14.12                         | 0.35      |
| 9     | 24   | 2.6 | 14.7               | 1.09             | 14.20                         | 0.17      |

**Results & Discussion: Daily Monitoring of Beetroot**

| S. no | Days | pH  | Alcohol percentage | Specific gravity | Sugar concentration (mg/ml) | Viscosity |
|-------|------|-----|--------------------|------------------|-------------------------------|-----------|
| 1     | 1    | 5.4 | 0                  | 1.93             | 23.86                         | 1.2       |
| 2     | 15   | 4.5 | 1.02               | 1.46             | 18.18                         | 0.9       |
| 3     | 16   | 3.75| 2.06               | 1.39             | 17.28                         | 0.8       |
| 4     | 18   | 3.6 | 4.03               | 1.35             | 16.68                         | 0.7       |
| 5     | 20   | 3.3 | 5.82               | 1.32             | 15.58                         | 0.6       |
| 6     | 21   | 3.23| 6.67               | 1.28             | 14.28                         | 0.4       |
| 7     | 22   | 3.10| 8.2                | 1.09             | 14.06                         | 0.2       |
| 8     | 23   | 2.9 | 9.08               | 1.08             | 9.26                          | 0.19      |
| 9     | 24   | 2.1 | 12.3               | 1.08             | 5.23                          | 0/12      |

---

**Estimation of Alcohol & Sugar Concentration Profile for 24 Days.**

**Estimation of PH, Specific Gravity & Viscosity for 24 Days.**
Summary & Conclusion
Wine is one of the functional fermented foods that have many health benefits. Commercially, wine is produced by the fermentation of yeast which involves the conversion of sugar to alcohol. Wine can act as a nutrient supplement for seasonal fruits and vegetables throughout the year. Using fruits and vegetables having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely. Apple and beetroot, which are known for its high medicinal and nutritional value are used as the substrate here. Fermentation is carried out with Saccharomyces cerevisiae commonly known as baker’s yeast. Daily monitoring was done to study the composition and characteristics of the wine. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. During the fermentation period the wines were analyzed for pH, titratable acidity, specific gravity, viscosity biomass content, alcohol and reducing sugar on a daily basis. pH show a decrease trend then attains minima and then increased. As the fermentation days produced, the specific gravity increased and the alcoholpercentageincreasedgradually.

Author Contribution
All authors contributed equally.

References
1. Spilling, Michael; Wong, Winnie (2008). Cultures of The World Georgia. p. 128. ISBN 978-0-7614-3033-9.
2. "Unearthing Georgia's wine heritage".
3. "Georgian wines: older and wiser". Financial Times.
4. Ellsworth, Amy (18 July 2012). "7,000 Year-old Wine Jar". University of Pennsylvania Museum of Archaeology and Anthropology.
5. Tondo, Lorenzo (30 August 2017). "Traces of 6,000-year-old wine discovered in Sicilian cave". The Guardian.
6. Johnson, H. (1989). Vintage: The Story of Wine. Simon & Schuster. pp. 11–6. ISBN 978-0-671-79182-7.
7. "Isis & Osiris". University of Chicago.
8. https://www.haaretz.com/archaeology/earliest-wine-in-world-found-in-8-000-year-
9. Keys, David (28 December 2003). "Now that's what you call a real vintage: professor unearths 8,000-year-old wine". The Independent.
10. Spilling, Michael; Wong, Winnie (2008). Cultures of The World Georgia. p. 128. ISBN 978-0-7614-3033-9.
11. "Evidence of ancient wine found in Georgia a vintage quaffed some 6,000 years BC". Euronews. 21 May 2015. Retrieved 24 May 2015.
12. Georgia’s Giant Clay Pots Hold An 8,000-Year-Old Secret To Great Wine, NPR.
13. Berkowitz, Mark (1996). "World’s Earliest Wine". Archaeology. Archaeological Institute of America. 49(5).
14. Castro-Sowinski, Susana (17 November 2016). Microbial Models: From Environmental to Industrial Sustainability. Springer. p. 42. ISBN 9789811025556.
15. Hames, Gina (2010). Alcohol in World History. Routledge. p. 17. ISBN 978-1-317-54870-6.
16. Prehistoric China – The Wonders That Were Jiahu The World’s Earliest.
17. Fermented Beverage. Professor Patrick McGovern the Scientific Director of the Biomolecular Archaeology Project for Cuisine, Fermented Beverages, and Health at the University of Pennsylvania Museum in Philadelphia. Retrieved on 3 January 2017.
18. "BAC per Drink tables".
19. "Effects At Specific B.A.C.Levels".
20. "wine-serving-size". American Institute for Cancer Research. Retrieved 13 December 2016.
21. "Georgia is cradle of wine, confirms international study". Agenda.ge. Retrieved 21 November 2019.
22. "Georgia made 'world's oldest wine". BBC News. 13 November 2017.
23. McGovern, Patrick; Jalabadze, Mindia; et al. (28 November 2017). 'Early Neolithic wine of Georgia in the South Caucasus'. Proceedings of the National Academy of Sciences. 114 (48): E10309–E10318. doi:10.1073/pnas.1714728114. PMC 5715782. PMID 29133421.
24. Abigail Tucker. "The Beer Archaeologist". Smithsonian.