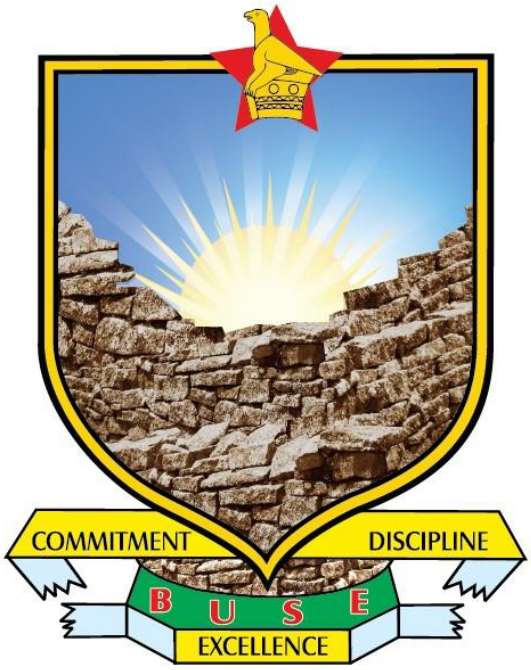
Bindura University of Science Education



Faculty of Science

Biological Sciences Department

OPTIMIZATION OF 5% ABV CIDER PRODUCTION FROM *SCLEROCARYA BIRREA*

By

ASSAN MARUFU

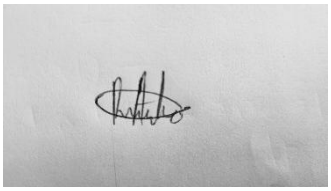
B223304B

A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honors Degree in Biotechnology.

JUNE 2026

Approval form

The undersigned certify that they have read the dissertation titled 'Optimization of 5% ABV cider production from *Sclerocarya Birrae'* and confirm that it is suitable for submission to the Biological Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 22/06/2026

Signature of Supervisor:  Date: 22/06/2026

Signature of Chairperson of Department:  Date: 25 August 2026

Declaration

I, Assan Marufu (B223304B), declare that this research herein is my own work and has not been plagiarized from another source (s) without acknowledgement of the concerned author(s) either electronically or otherwise.



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Supervisor

I, Siamayuwa C., declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date:22/06/2026

Dedication

I would like to dedicate this piece of work to my mother and father who have shown me great, profound and benevolent concern for my education and life. This work would have never been a success without them.

Acknowledgements

It is my pleasure to appreciate the people who put their time and effort in my research project as a student. Firstly, I would like to thank God for his wisdom and guidance until the completion of my research project and for the gift of life. I would like to express my heartfelt gratitude to my supervisor Mr. Siamayuwa who nurtured me to the best of his knowledge and experience during this project study. My special thanks also go to my supervisors during industrial attachment period Mr. Machinya and Mrs. Dondo and I also give thanks to National Biotechnology Authority for giving me access to the laboratory equipment. I would also like to express my heartfelt gratitude to my parents and fellow colleagues for the unwavering support they gave me. Lastly, I would also like to acknowledge my friends Kelly Marimo, Mirabell and Flame Tapfuma for putting their efforts during the writing of my dissertation.

List of abbreviations

ABV -Alcohol by volume

TSS -Total soluble sugars

NBA -National biotechnology authority

ºBx -Degree Brix

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Abstract

The marula tree *(Sclerocarya birrea subspecies caffra)* is a popular fruit-bearing tree native to central and southern Africa. The fruits of the marula tree have various uses, including being processed into drinks, jams, and other products. High alcohol consumption can impact job performance, leading to absenteeism, and workplace accidents. The increasing prevalence of alcohol consumption among young adults and the people in our communities, presents significant public health challenges. The aim of this study was to produce a 5% alcohol by cider from *S. birrae* using the yeast strain *Saccharomyces cerevisiae* dv10 ex-bayanus, specifically targeting health-conscious consumers who seek lower alcohol options. This beverage could serve as a safer alternative to higher-alcohol products, potentially reducing the incidence of binge drinking and its associated harms. The experiments were conducted at National Biotechnology Authority laboratories in Rutenga Zimbabwe. The fruits were received from local farmers and they were graded by hand picking. The ripe ones were then washed with 5% potassium hypochlorite to kill and wash away wild yeasts. The fruits were then pulped using the pulping machine, pulp was collected and loaded into 20L fermentation vats labeled A, B, C and D. The bioreactor D was the control experiment. In each vat 3kgs of pulp was added followed by 12L of water. Different amounts of sucrose were added to each fermentation vat. All the fermentation vats were then pitched using the yeast strain *S. cerevisiae* DV10 ex-bayanus. Fermentation was allowed to occur under anaerobic conditions and on day 10 and 11 fermentation in all vats was stopped. The brews were harvested and filtered. Two bioreactors A and B reached the targeted alcohol content of 5%. Sensory properties like aroma, flavor, and aftertaste were then evaluated by the trained panelists and the scores showed that the cider samples from the bioreactor A scored higher than samples from the bioreactor B, the commercial cider sample scored higher than marula cider samples. Samples from the bioreactor A scored the same with the commercial cider in some of the attributes. The alcohol content was measured using the digital refractometer and it was found to be 5%. Finally, the formulation for marula cider production was developed. After the study it was concluded that *S. cerevisiae* DV10 is a good yeast strain in cider production.

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CHAPTER 1

1.1 INTRODUCTION.

There is an increasing demand in the beverage industry for innovative and health conscious products especially in the case of alcohol consumption (Keric & Stafford, 2019). As consumer choices move towards beverages with decreased alcohol content, a special opportunity to satisfy this need is provided by making a 5% alcohol cider from marula fruit. Many younger adults and health-conscious drinkers are cutting back on heavy alcohol intake, choosing lighter drinks that let them socialize without getting intoxicated or dealing with harsh hangover the next day. The marula fruit contains up to eight times more vitamin C than an orange along with antioxidants. The marula tree (*Sclerocarya birrea*), which originates in Africa, is famous for the fruity taste, and it also holds social meaning and health advantages (Lekhuleni, Shabalala, & Maluleke, 2024). Marula fruit contains a large amount of vitamin C, and it has a unique flavor profile that is fruity, and aromatic which makes it stand out from apples or grapes typically used in cider.

This project aimed to explore the production of a 5% alcoholic beverage that harnesses the good characteristics such as nutritional benefits and unique flavor profile of the marula fruit. The project also tested how to make 5% ABV marula cider with consistent quality. By targeting consumers seeking moderate drinking options, the beverage could provide a flavorful option to traditional higher-alcohol drinks. The project involved research on optimal fermentation processes and flavor enhancement to capture consumer preferences.

This approach supports rural economic development in regions where marula trees naturally grow, turning a traditional resource into a modern business opportunity. Using marula fruit in the beverage industry can promote sustainable agricultural practices by local farmers and communities involved in marula cultivation. This also aims to create a product that people would buy and improve economic development in regions where marula trees grow (Mabhaudhi et al., 2019).

1.2 Problem statement.

Many people are consuming too much alcohol, which can then cause problems at workplaces, such as job absenteeism and even accidents. The marula cider can be a choice for people who want to drink less-alcoholic drinks while being health conscious. Patrick & Schulenberg (2010) found that about 50% of students aged 18 to 25 years drink alcohol in large portions and end up engaging in binge drinking behaviors. This is bad because it can lead to health problems like alcohol use disorder, getting hurt and having trouble at college institutions, injuries may also occur due to binge drinking activities. Low-alcohol beverages allow individuals to enjoy drinks over a longer period without getting over drunk, promoting more prolonged social interactions (Kostadinov & Bartram, 2025). They provide options for those who prefer lighter drinks or cannot consume beverages with higher alcohol content. Lower alcohol levels typically result in less impairment, making it safer for activities like driving or operating machinery. Consuming lower alcohol beverages can reduce the risk of liver disease, addiction, and other health problems associated with excessive alcohol consumption (Roerecke et al., 2019).

1.3 Justification.

The project aimed to develop a 5% ABV cider from natural ingredients, targeting consumers who prefer beverages with health benefits and low alcohol content. This cider could serve as an option to the beverages with higher alcohol content such as spirits. The low alcohol beverage could also serve to reduce binge drinking and subsequent bad behaviors. Research shows that drinking alcohol moderately is part of a balanced life style. According to the research by Malinauskas et al., (2006), the dietary guidelines recommend that adults should limit their alcohol consumption to 2 drinks per day for men and one drink for women.

Nevertheless, the market for low-alcohol beverages is still underexplored, especially in fruit based products (Codjia et al., 2025). Due to the unique flavor profile of marula fruit, the proposed beverage could meet consumer preferences for natural and flavorful products while promoting good drinking habits.

1.4Aim and objectives.

1.4.1General aim: aim of this project is to produce a 5% alcohol cider from marula *(S. birrea)* fruits.

1.4.2 Specific objectives.

The specific objectives of the study are to:

- Formulate a marula cider recipe that achieves a 5% alcohol content. Research question: Can *S. birrea* be fermented to produce a cider?
- Conduct a sensory evaluation of the cider using standardized descriptive sensory analysis methods. Research question: What are the sensory properties of marula cider?
- To determine the alcohol content of the marula cider using a digital refractometer. Research question: How accurate can a digital refractometer measure the alcohol content of marula cider?

CHAPTER 2: Literature review

2.1 Introduction to Marula Fruit

Sclerocarya birrea subp, *Caffra* (also known as *Mapfura* in Shona and *Umgamu* in Ndebele) is an indigenous tree of Central and Southern Africa (Figure 1). The tree belongs to the Anacardiaceae family and class Magnoliopsida a and it is drought resistant and available in Namibia, Botswana, Zambia, Swaziland, Zimbabwe, South Africa extending to Ethiopia and Sudan (Wynberg et al., 2002). In Zimbabwe, the tree grows abundantly in different regions such as the Middle and Lowveld regions. These regions cover the provinces of Masvingo, Matebeleland North and South and parts of Midlands and Mashonaland Central.



Figure 1:Marula fruit tree in a field at a homestead in Rutenga. (Adapted from: Machinya, 2024).

It bears fruits that are fleshy, pale green turn yellow when ripe, and highly valued by animals and people for their nutritional and medicinal properties (Figure 2) (Wynberg et al., 2002). Marula fruit is rich in vitamin C, antioxidants, and essential nutrients (Hiwilepo-van Hal et al., 2014). It has been used in traditional medicines and beverages, particularly in the production of alcoholic drinks such as amarula liqueur in South Africa.



Figure 2:Ripened marula fruit captured at the Mwenezi marula value Addition Plant.
(Adapted from: Machinya, 2024).

2.2 Importance of Exploring Alternative Fruits, Such as Marula, in Cider Production.

Marula fruit *(S. birrea)* is characterized by a sweet and scented taste and vitamin C rich content. While traditionally used to make local alcoholic drinks, such as mukumbi, it can still be used to make cider but has not been adequately evaluated (Maluleke, 2019).

Harnessing marula fruit for cider making will contribute to increased variety in the beverage industry, improve local economies through employment creation and the use of locally available resources, reducing fruit imports, and create unique flavors that would appeal to the growing artisanal drink market.

The use of marula fruit contributes to sustainability because it involves the use of locally grown crops and reduces dependency on fruit imports (Fajinmi et al., 2025).

2.3 Nutritional and Phytochemical Properties of Marula Fruits

Marula fruit contains various nutrients such as vitamin C and vitamin A, it also contains ascorbic acid ranging from 62 mg per 100g up to more than 400 mg per 100g of fruit, and higher in vitamin C than other fruits (Hiwilepo-van Hal et al., 2014). Marula fruit is also rich in essential minerals such as calcium, iron, and zinc, which are physiologically important. It also contains fats and fiber, with a fat content estimated to be 51.67%,

and protein with essential amino acids such as glutamic acid and arginine. Due to its nutrients, the fruit is beneficial to local populations and industries (Mfini et al., 2025).

Marula fruit contains phytochemicals such as polyphenols and flavonoids with therapeutic and preventive qualities. However, detailed characterization of these phytochemicals remains elusive (Mfini et al., 2025). Because of its components that have properties that are beneficial to health, marula is considered a functional food source. Its nutritional and phytochemical properties make it a valuable resource for health and industrial applications (Mashau et al., 2022).

2.4 Traditional Uses of Marula in Local Cultures.

Marula is culturally significant and is used in several ways by the surrounding communities. It can be eaten raw, juiced, and even made into beer, and enjoys wide popularity among communities in Masvingo region. The fruit is consumed fresh, and is also used to make juice and beer, which are popular among local communities (Mokganya, M.G, 2019).

Marula is used traditionally to treat illnesses such as diarrhea, fever, and skin diseases. The bark and leaves of the plant have been used to dress wounds and treat skin infections (Semenya & Maroyi, 2012).

Because of its moisturizing properties and antioxidant contents, marula is used to treat skin illnesses (Mashau et al., 2021). Marula trees have cultural significance that goes beyond practical usage, as they are considered a sacred plant and have played a significant part in traditional religious practices (Sinthumule & Mzamani, 2019).

2.5 Fermentation Basics

2.5.1 Explanation of Fermentation Processes in Alcoholic Beverage Production

Fermentation is a biological process involving the conversion of sugars to ethanol and carbon dioxide by microorganisms such as yeast (Figure 3) (Dunford, 2012.). Fermentation in the production of alcoholic drinks is a crucial step determining the taste and aroma of the drink.

First, the substrate is prepared. This stage involves the use of fruit juice, grain mash, and other liquids containing sugars. Then a particular strain of yeast capable of fermenting sugars to ethanol is added to the prepared substrate. Yeast consumes sugars and produces ethanol and carbon dioxide as byproducts.

During fermentation, various biochemical reactions take place, including glycolysis, in which glucose is converted into pyruvate and then into ethanol and carbon dioxide through the action of enzymes such as pyruvate decarboxylase and alcohol dehydrogenase (Mbaye, 2023).

The fermentation process is influenced by factors such as temperature, pH, oxygen levels, and yeast strain. Temperature affects yeast growth and metabolism, with optimal temperatures ranging from 15°C to 30°C for most yeast strains (Lin et al., 2012) .

The time required for fermentation also depends on the drink type, yeast selection, and conditions provided. Beer fermentation usually takes 7-14 days while wine fermentation can take 1-3 weeks or more (Cosme et al., 2020).

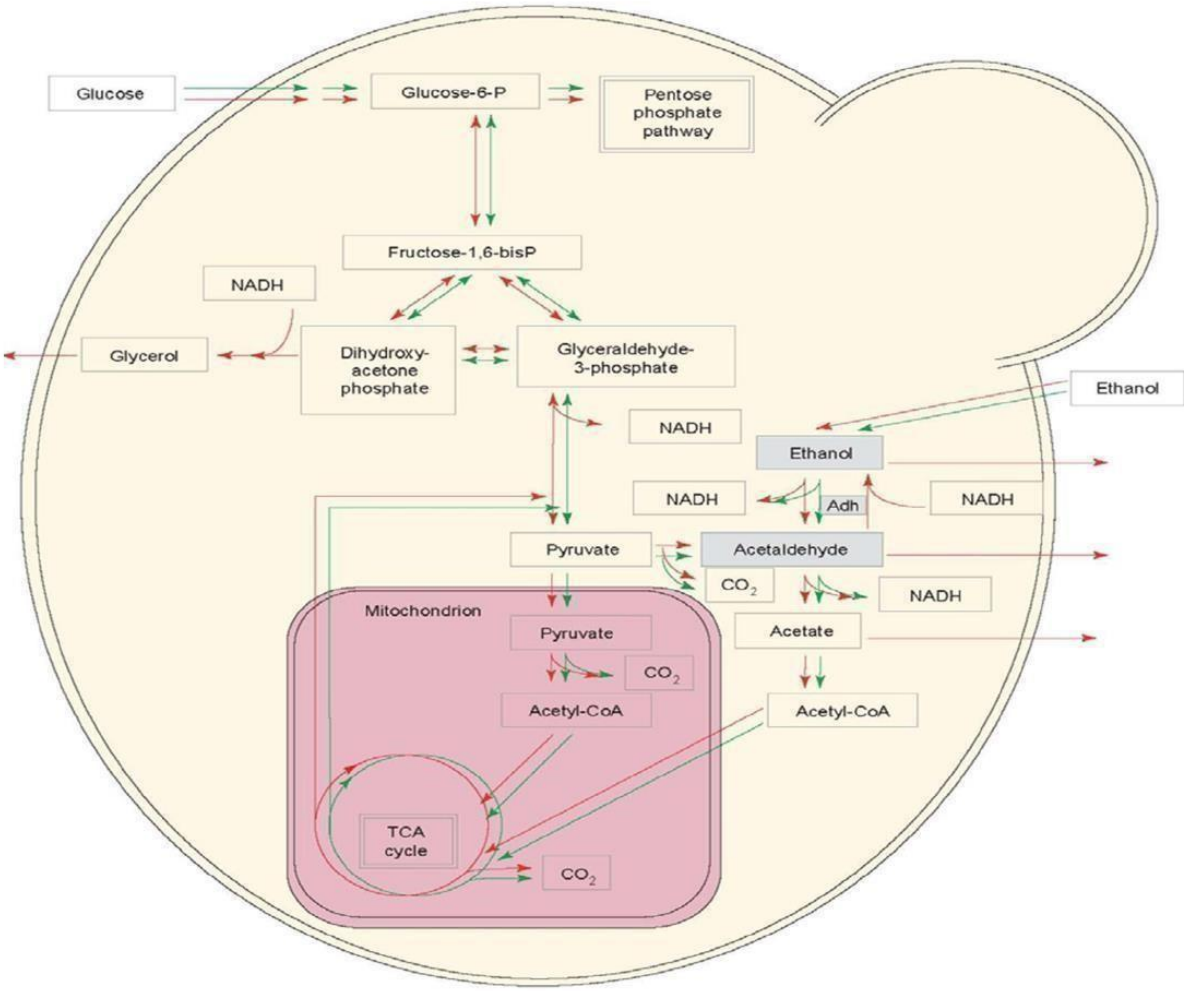


Figure 3: The yeast alcoholic fermentation pathway showing pathways involved in glucose and ethanol assimilation under aerobic conditions.

(Adapted from: Patrascu *et al.*, 2009 in Motlhanka, 2020).

2.5.2 Role of Yeast in the Fermentation of Sugars into Alcohol

Yeast is an important part of sugar fermentation as it converts sugar into ethanol and carbon dioxide via a series of biochemical processes (figure 4). The process begins with the uptake of sugars from the surrounding medium by yeast followed by breakdown of these sugars to produce pyruvate via glycolysis. Pyruvate undergoes decarboxylation to produce acetaldehyde, which is then reduced to produce ethanol (Walker & Stewart, 2016). The process of fermentation stimulates certain biochemical processes in yeast cells, making it possible for the cells to synthesize enzymes responsible for converting sugar into ethanol. The yeast cell membrane, including its transport system, plays an important role in controlling sugar absorption and release

of the products of fermentation. There are many conditions under which fermentation takes place such as appropriate temperature between 15°C and 30°C and pH ranging between 4.5 and 6.5 (Emmanuel, 2018).

Not all yeasts have equal capability of fermentation. Different species of yeasts provide different flavors and aromas depending on what kind of end product is sought after. For example, beer production uses ale yeast while lager production utilizes lager yeast (Meier-Dörnberg et al., 2017).

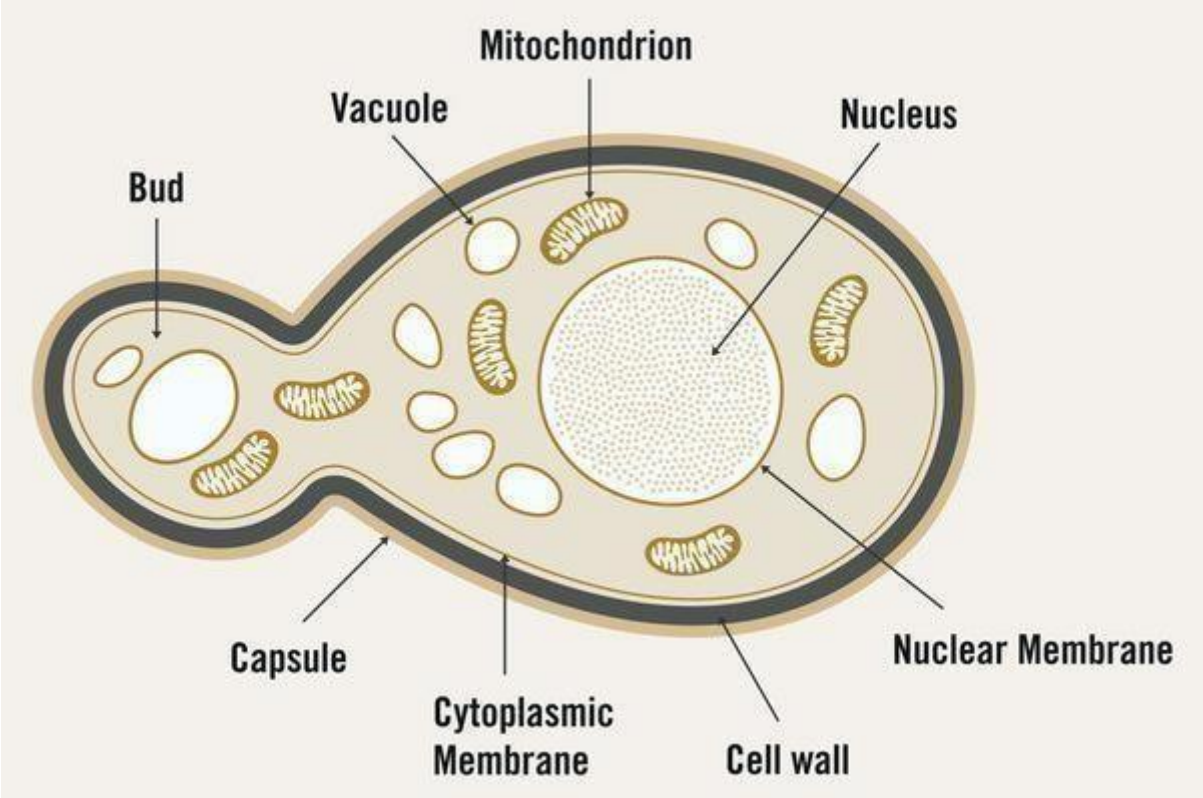


Figure 4: shows the structure a yeast cell (Adapted from: https://www.edinburghwhiskyacademy.com).

2.5.3 Overview of Yeast Strains Suitable for Cider Production

Yeast strains suitable for cider production are selected based on their ability to handle the characteristics of a fruit juice such as acidity and sugar content. These yeast strains are tolerant of acidic environments, with optimal pH ranges between 3.0 and 4.5 (Al Daccache et al., 2020).

One of the widely used strains of yeast in cidermaking is *S. cerevisiae* that can undergo fermentation within a wide temperature range and provide good flavor combinations (Cosme et al., 2020). In addition, *S. bayanus*

is used for its ability to withstand low temperatures and produce smooth and delicate flavors (Naumov et al., 2011). Non-*Saccharomyces* yeast strains, such as *Metschnikowima pulcherrima* and *Torulaspora delbrueckii*, are being analyzed for their potential to contribute unique flavor profiles to cider (Granchi et al., 2025). The yeast strain choice depends on factors like cider style, fruit variety, and fermentation conditions. When selecting yeast strains, cider makers are considering factors like temperature tolerance, sugar utilization, and flavor compound production to achieve the desired cider characteristics (M. Way, 2023). Cider production is a multi-step process that involves harvesting,

pressing, fermentation, and maturation. The process begins with collecting fruits which are then washed to remove microbes found on the skin of the fruits and crushed to extract juice (Codjia et al., 2025).

2.5.4Beer fermentation.

Yeasts play a crucial role in the production of beer, with *S. cerevisiae* being the primary microorganism responsible for converting the sugars in wort into ethanol and other compounds that contribute to the characteristic flavors and aromas of beer (Lodolo et al., 2008). Differences between ale and lager yeasts, such as *S. cerevisiae* and *S. pastorianus*, can significantly impact the final beer flavor profile. Factors such as temperature, oxygen availability, and wort composition can all affect yeast performance and the resulting beer characteristics (Gibson et al., 2017). There is a growing trend in the use of non-conventional or wild yeasts strains for the production of specialty beer, which can enhance the flavor profile of the beer (Zapryanova et al., 2025).

Following primary fermentation, the beer undergoes a conditioning phase and during this phase the flavors and aroma develop and the carbonation builds, which can occur in the bioreactor or in bottles. Throughout the process, fermentation also significantly influences the beer's overall flavor, aroma, and mouthfeel, making it a key component of brewing (Lodolo et al., 2008).

2.5.5 Wine fermentation.

Saccharomyces cerevisiae is the primary yeast responsible for alcoholic fermentation, converting the sugars in marula juice into ethanol and other metabolites that contribute to the wine's aroma, flavor, and mouthfeel. The marula fruit, native to southern Africa, is rich in sugars and has a tart flavor, making it suitable for fermentation (Ramoba, 2024.).

The process starts with collecting ripe marula fruits, which are then pulped to produce the fruit pulp. This pulp, or must, contains natural sugars, acids, and aromatic compounds that contribute to the wine's full flavor profile. Because marula fruits harbor wild yeasts, wine fermentation can occur spontaneously without inoculation of a yeast strain (Mapheto, 2024).

Various non-*Saccharomyces* yeasts may also play a crucial role during the initial fermentation stages. These yeasts, such as *Hanseniaspora* spp and *Kloeckera* spp, can contribute to the complexity of the wine by producing various volatile compounds such as phenols and flavonoids which then enhance its aroma and flavor profile (Martin et al., 2018). Their presence can result in development of more diverse range of flavors, setting marula wine apart from more common fruit wines like grape wines. The fermentation process can last from several days to a few weeks, depending on the environmental conditions and the specific yeast strains involved. When the fermentation process is over, the wine is then filtered and can undergo further aging or conditioning to fully develop the flavor profile (Wang et al., 2016).

2.5.6 Cider Production Process.

Cider production involves harvesting, pressing, fermentation, and maturation. The process starts by gathering fruits, this include, sweet, sharp, and bitter varieties, which are then washed to remove impurities and unwanted wild yeasts and bacteria, they are then crushed to extract juice. The juice is then fermented using yeast, either naturally occurring or inoculated, which converts the sugars into alcohol and carbon dioxide. The fermentation process can occur for several weeks to months, depending on factors like temperature, yeast strain, desired alcohol content and flavor profile (Wang et al., 2016). After fermentation, the cider is racked leaving behind sediments hence improving clarity of the product. Some ciders may undergo secondary

fermentation when bottled with yeast and sugar, allowing it to mature and develop complexity, this can result in alcohol content increase of the cider. Cider is then matured for a period of time ranging from a few months to several years, allowing the full development of flavor profile. Finally, the cider is packaged and distributed to market places (Natić et al., 2024).

2.5.7 Conditions for Fermenting Marula Juice

Fermenting marula juice requires specific conditions to ensure optimal yeast growth, sugar conversion, and flavor development. The fermentation conditions include:

Temperature: The ideal temperature range for fermenting marula juice using *S. cerevisiae* is 20°C to 30°C (Ramoba, 2024.). This temperature range allows for optimal yeast catalytic activity. If the temperature goes above or below (Figure 5) recommended range the yeast cells will not carry their biological function properly and there will be no reactions in the bioreactor which means there will be no fermentation, the deactivation of yeast cells will cause some discrepancies in the fermentation process among the fermentation vats. If the temperature goes below the range, the reactions will occur at a slow rate resulting in increased fermentation time (Legodi et al., 2022). When the temperature is increased above its ideal level, denaturation takes place. This results in the loss of the quaternary, tertiary, and secondary structure, which will alter the structure of the cell and may result in cell death (Lepock, 2005).

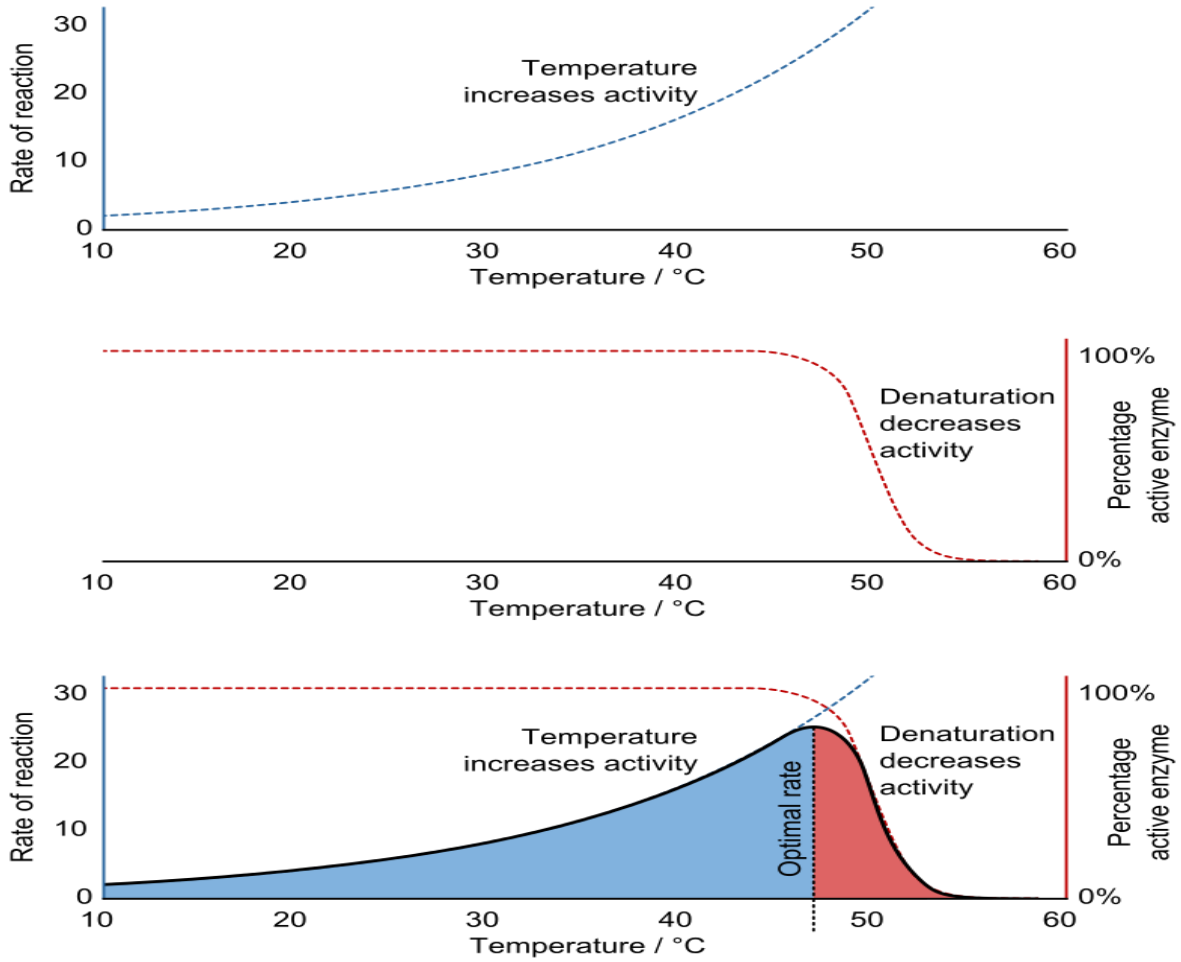


Figure 5: shows effects of temperature on fermentation rate.

(Extracted from: https://commons.wikimedia)

The ideal pH values that can be used for the fermentation of marula juice using *S. cerevisiae* are between 3.0 and 4.5 (Legodi et al., 2022). This acidic environment inhibits the growth of other yeasts such as *Brettanomyces* species, hence allowing the brewer's yeast to grow and carry out their biological functions properly (Deak, 2006). Deviations from the pH range can lead to an increase in bacteria growth. The level of pH affects the shape of proteins, if the pH is increased this affects the shape of the proteins by disrupting the bonds in the protein. When the pH is lower (acidic) the fermentation rate at which fermentation occurs increases (Infantes et al., 2011).

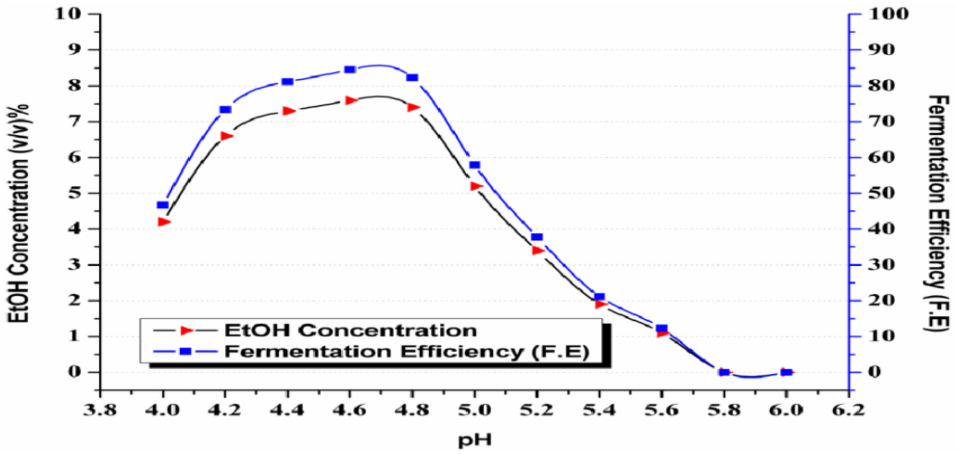


Figure 6: shows the effect of pH fermentation rate.

(Extracted from: https://www.researchgate).

Yeast Strain: The choice of yeast strain becomes vital during the fermentation of marula juice. The use of some wild strains of yeast can result in inconsistent fermentation, which will affect the fermentation rate and possibly lead to stuck fermentation (Christ et al., 2015) . This poses problems during the cider-making process, leading to spoilage and ultimately affecting the taste of the product. *S. cerevisiae* is a typical yeast strain for fermenting marula juice (Ngwenya et al., 2023).

Oxygen Levels: Oxygen content is critical for yeast reproduction and fermentation. However, an excess of oxygen often leads to bitter taste and off flavor formation, thereby causing contamination of the beverage probably by air borne microbes, as well as changing the beverage's color due to oxidation (Ngwenya et al., 2023).

2.5.8 Clarification and Aging: Techniques for Clarifying and Aging Cider for Flavor Development.

Clarification and aging are important steps in developing the flavor and character of the final beverage product. Clarification techniques include racking, fining, and filtration, which help remove sediment and microbial biomass hence improve clarity.

During racking the beverage is transferred to another tank or vessel, leaving behind sediment and micro-biomass allowing the cider to clarify. Fining agents like gelatin and bentonite aggregate the sediments including microbial biomass hence removing impurities and improving clarity of the drink. Filtration using

sheets with small pore sizes improves clarity of the cider. The choice of clarification technique depends on the desired level of clarity and flavor profile. Aging cider allows full development of the flavor profile. Cider can be aged in oak barrels, stainless steel vessels, or bottles (secondary fermentation), each enhancing development of different characteristics in the cider. Bottle aging can allow the cider to develop complex flavors, aromas and color through slow oxidation. The length of aging time depends on the desired flavor profile, with some ciders aged for a few months and others for several years. Proper aging techniques can result in balanced flavor profile (Seriš et al., 2024).

2.5.9 Challenges in Marula Cider Production.

Making marula cider has several problems that affect quality and consistency of the final product. One of the primary challenges is the variability in marula fruit quality, which can be affected by factors like climate, soil, and harvesting practices. The shelf life of marula fruit is short; it spoils quickly after harvest. Spoiled fruit leads to poor juice quality and altered perception of the final product. Also yeast selection and fermentation control is challenging because marula fruit harbors wild yeasts that will cause variations during fermentations, marula juice may require specific yeast strains and fermentation conditions to produce a high-quality cider. Contamination and spoilage could also occur during production, particularly if proper sanitation and hygiene practices are not carried out properly. Scaling up the production process is costly and challenging due to specific conditions needed for fermenting marula juice (Legodi et al., 2022).

2.6 Sensory properties of Marula Cider.

Overview of Sensory Characteristics Expected in Marula Cider

The sensory analysis involves the use of a hedonic scale (table 1) to measure how much panelists like it. Based on the past studies marula beverages must have a sensory profile which include fruity, floral, and slightly sweet notes (Ngwenya et al., 2023). The aroma is often described as intense and fruity, with characteristics of tropical fruit, citrus, and stone fruit. The flavor profile of marula cider can include notes of ripe fruit, as well as a slightly sweet and tangy taste. The mouthfeel is often described as smooth and refreshing (Ngwenya et al., 2023). The color of marula cider can be light yellow and golden, depending on method of production and

the level of oxidation, whereby high levels of oxidation will result in dark brown color. Marula cider is also likely to have a distinctive sensory profile due to the exceptional nature of the marula fruit (Maluleke, 2019).

Table 1: 9-point hedonic scale.

Scale	Description
9	Liked extremely (LE)
8	Liked very much (LVM)
7	Liked moderately (LM)
6	Liked (L)
5	Neither liked nor disliked (NLD)
4	Disliked slightly (DS)
3	Disliked moderately (DM)
2	Disliked very much (DVM)
1	Disliked extremely (DE)

2.7 Health Benefits and Nutritional Aspects.

2.7.1 Potential Health Benefits of Consuming Marula Cider.

The marula fruit has many health benefits because of its high level of nutrients such as vitamins, minerals, and antioxidants. It contains different types of nutrients such as vitamin C, potassium, and dietary fiber. The fruit also contains some phytochemicals which act as antioxidants such as flavonoids, phenolic acids, and ascorbic acid (Hiwilepo-van Hal et al., 2014). The fruit is known for having anti-inflammatory effects which helps in

reducing the risks of heart diseases, diabetes, and cancer. The antioxidants play a role in lowering oxidative stress. The health benefits of marula fruit include enhancing the immune system thereby reducing inflammation and improving digestion (Molelekoa, 2021).

CHAPTER 3: MATERIALS AND METHODS.

3.1 Study site

The experiments were conducted at National Biotechnology Authority laboratories in Rutenga Zimbabwe.



Figure 7: illustration of the location of National Biotechnology Authority Laboratories in Rutenga.

3.2 Marula fruit preparation

Marula fruits were collected from the surrounding villages in Rutenga. Fruits were collected from different trees by convenience sampling. After the fruits were received they were then graded by hand picking to separate the ripe fruits, unripe fruits and rotten fruits (Figure 8).



Figure 8: Fruit grading by hand picking.

The fruits were washed using 5% potassium hypochlorite (KOCI) before juicing to remove the unwanted microbes. There are some wild yeast strains which are found on the skin of marula fruits which must be washed away because they can significantly impact fermentation by altering fermentation speed (Ngwenya et al., 2023). After the fruits have been washed, they were pulped using the pulping machine which separates the marula stones and peels from the pulp juice.

3.3 Pulp loading

The fermentation tanks were labelled bioreactor A to bioreactor D. Water and pulp juice were mixed in 4:1 ratio, respectively to reduce the viscosity of the pulp juice, Fermaid O (yeast nutrient) and yeast were mixed in ratios 1:1, so the amount of yeast added to the juice was equivalent to the amount of yeast nutrient added. To each tank, 3kgs of marula pulp and 12L water was added to make the total wort of 15L in each tank (figure 3.1). To tank A 3kgs of sugar was added, 2kgs to tank B, 1kgs to tank C and no sugar was added which was our control experiment tank D. The tanks had slightly different volumes depending on the amount of sugar that was added to each tank.



Figure 9: Marula pulp and water in 20L bioreactors.

3.4 Brix adjustment

First, the natural brix of the marula juice was measured using Milwaukee digital refractometer (Model MA 871) (figure 3.2). Due to low levels of total soluble sugars (TSS) in marula, we chose to increase the level of sugar by adding brown sugar to the pulp, except the control experiment. Brix adjustment was done gradually using sucrose which is our table sugar. After brix was adjusted the initial brix of the tanks A, B, C and D were 23, 17, 10, and 3 respectively.



Figure 10: Digital refractometer used to measure brix content.
(Adapted from: https://milwaukeeinstruments.eu/digital-brix-refractometer).

3.5 Pitching

Yeast activation: the yeasts strain used for the experiment was *S. cerevisiae ex-bayanus DV10*. 6g of yeast was put in 60ml of mast. The mast was heated to the temperature range of 35-40 ºC, then mixed with the yeast (figure 11).



Figure 11: yeast activation in plastic containers.

After 20 minutes activated yeast was poured into the tank, this was done for each tank. So, after pitching the tanks were then closed, fermentation was to occur under anaerobic conditions (figure 3.6). The fermentation parameters (pH, brix, temperature and potential alcohol) were recorded daily (figure 3.7).



Figure 12: Closed bioreactors after pitching.



Figure 13: Parameter reading.

3.6 Maturity.

The cider was bottled and left for maturity for one month before sensory evaluation was done, for the full development of the flavor profile.



Figure 14: shows produced ciders in glass bottles.

3.7 Data analysis.

Two-way ANOVA was done using the Panelcheck V1.4.2 software. The Product Effect, Assessor Effect, and Assessor Product interaction was analyzed using Panelcheck V1.4.2 software.

CHAPTER 4: RESULTS

The results show how the fermentation parameters (pH, temperature, brix, ABV) were changing daily. pH was decreasing, temperature increased and brix content decreased, hence increasing alcohol content due to the microbial activity of the yeasts. The fermentation was stopped on day 11 when the desired alcohol content was reached.

Table 2: The results of the bioreactor A.

Day	pH	temperature	Brix(°BX)	ABV= [(initial brix X 0.6 - 1) - (current brix X 0.6 - 1)]
1	4.4	23	23	0
2	4.4	24.3	22	0.6
3	4	24.4	19.6	2
4	3.9	24.7	19	2.4
5	3.9	24.1	18.9	2.46
6	3.9	23.2	16.3	4
7	3.9	23.4	15.6	4.4
8	3.9	23.7	15.5	4.5
9	3.9	23.2	15.2	4.7
10	3.9	23.3	14.8	4.9
11	3.8	24.2	14.3	5.2

The results are presented in a tabular form as illustrated below. The table is showing how the fermentation parameters (pH, temperature, brix and ABV) were gradually changing with days due to the microbial activity in the fermentation vats. The fermentation was stopped on day 10 when the desired alcohol content was reached.

Table 3: The results of the bioreactor B.

Day	pH	temperature	Brix(°BX)	ABV= [(initial brix X 0.6 - 1) - (current brix X 0.6 - 1)]
1	5	25	17	0
2	4.4	24.3	15.8	0.7
3	4.1	24.1	15.3	1
4	4	24.4	14.4	1.55
5	3.9	23.4	12	3
6	3.9	22.7	12	3
7	3.9	23.3	11.1	3.5
8	3.8	23.2	10.3	4
9	3.8	23.3	9.7	4.4
10	3.8	23.4	8.75	4.95

The results of the bioreactor C are presented in a tabular form as shown below. The table shows how the fermentation parameters (pH, temperature, brix and ABV) were gradually changing with days through the process. The fermentation stopped spontaneously on day 8.

Table 4: The results of the bioreactor C.

Day	pH	temperature	Brix(°BX)	ABV= [(initial brix X 0.6 - 1) - (current brix X 0.6 - 1)]
1	4.5	23	10	0
2	4.3	24.3	9.8	0.1
3	4	24.4	9.1	0.45
4	3.9	24.6	7	0.5
5	3.9	23.2	5.9	2.05
6	3.9	23.4	5	2.5
7	3.9	23.3	4.2	2.9
8	3.9	23.5	4	3
9	3.9	23.6	4	3
10	3.9	23.3	4	3
11	3.9	23	4	3

The results of the control experiment are shown in the table below. The parameters were recorded daily and the table shows how the parameters were changing daily as the fermentation process was progressing. No sugar and yeast were added to this bioreactor. All changes in fermentation parameters were due to the yeast activity.

Table 5: The results of the bioreactor D.

Day	pH	temperature	Brix(°BX)	ABV= [(initial brix X 0.6 - 1) - (current brix X 0.6 - 1)]
1	5	25	3	0
2	4.5	24.4	2.5	0.25
3	4.4	23	2	0.5
4	4.4	23.4	2	0.5
5	4.4	23	2	0.5
6	4.3	22.7	2	0.5
7	4.3	22.5	2	0.5
8	4.1	23	2	0.5
9	4.1	23.6	2	0.5
10	4.1	23.3	2	0.5

4.1 Sensory evaluation.

The two samples of the marula cider were given to the available five trained panelists at NBA aged between 29 and 37, three females and two males. Also, six random staff members at NBA were given the samples and they were assisted to fill the sensory evaluation forms according to their mouth feel using a hedonic scale. The sensory attributes of the produced cider were compared to those of the commercial cider (P3). The spider plot (Figure 14) shows scores of each of the 9 sensory attributes. P1 and P2 denotes the produced marula ciders.



Figure 15: shows the two marula cider samples.

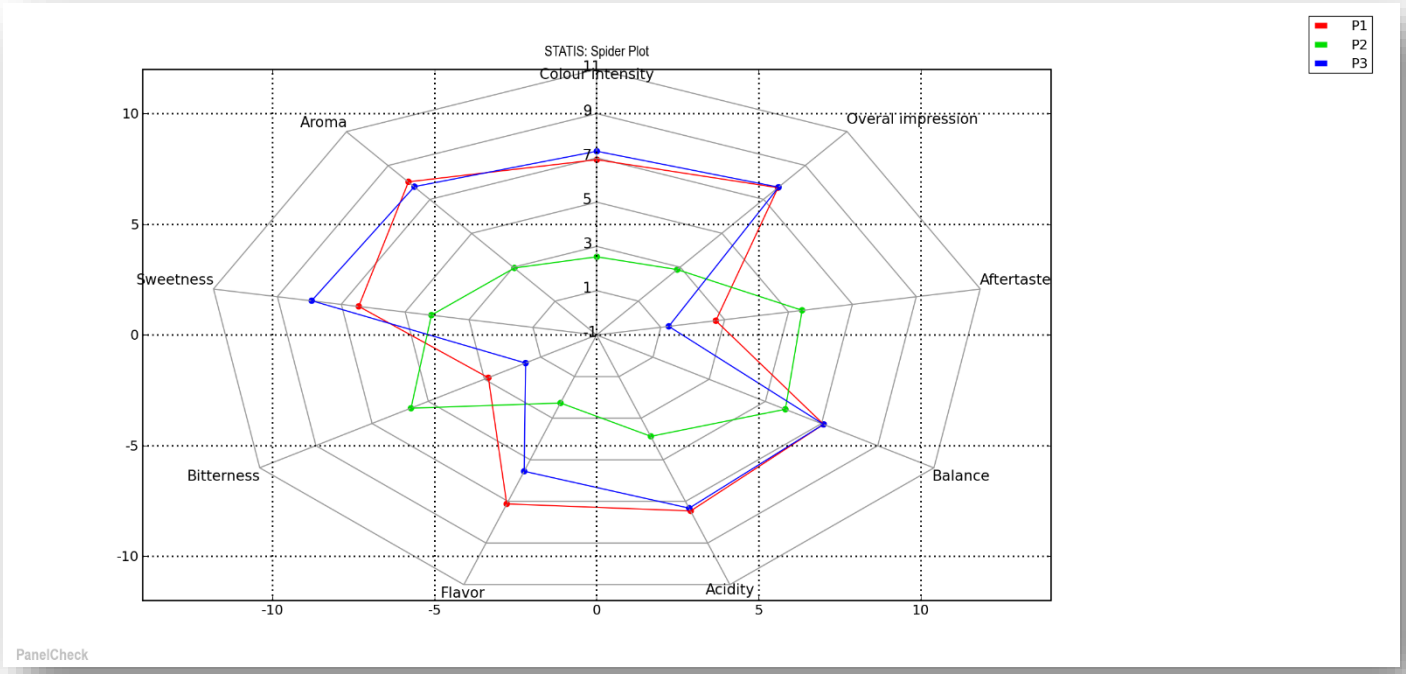


Figure 16: sensory evaluation of cider styles 1-3. Sensory attributes were color intensity, aroma, sweetness, flavor, acidity, bitterness, fruitiness, and aftertaste.

Table 6: shows the average scores of the sensory attributes.

Average data:									
Samples	Color intensity	Aroma	Sweetness	Bitterness	Flavor	Acidity	Balance	Aftertaste	Overall impression
P1	6.8	5.59	4.59	4.56	3.41	3.91	3.99	3.75	4.98
P2	3.43	3.62	4.31	3.8	3.02	4.53	4.25	4.27	3.77
P3	7.06	7.28	7.62	6.46	5.11	6.95	5.76	6.06	7.47

The biplot show how 3 cider samples (P1, P2, P3) scored on the sensory attributes. It is from PCA (Principal Component Analysis) of the panel data. PC1 explains 83.9% of the variation. PC2 explains 16,1%.

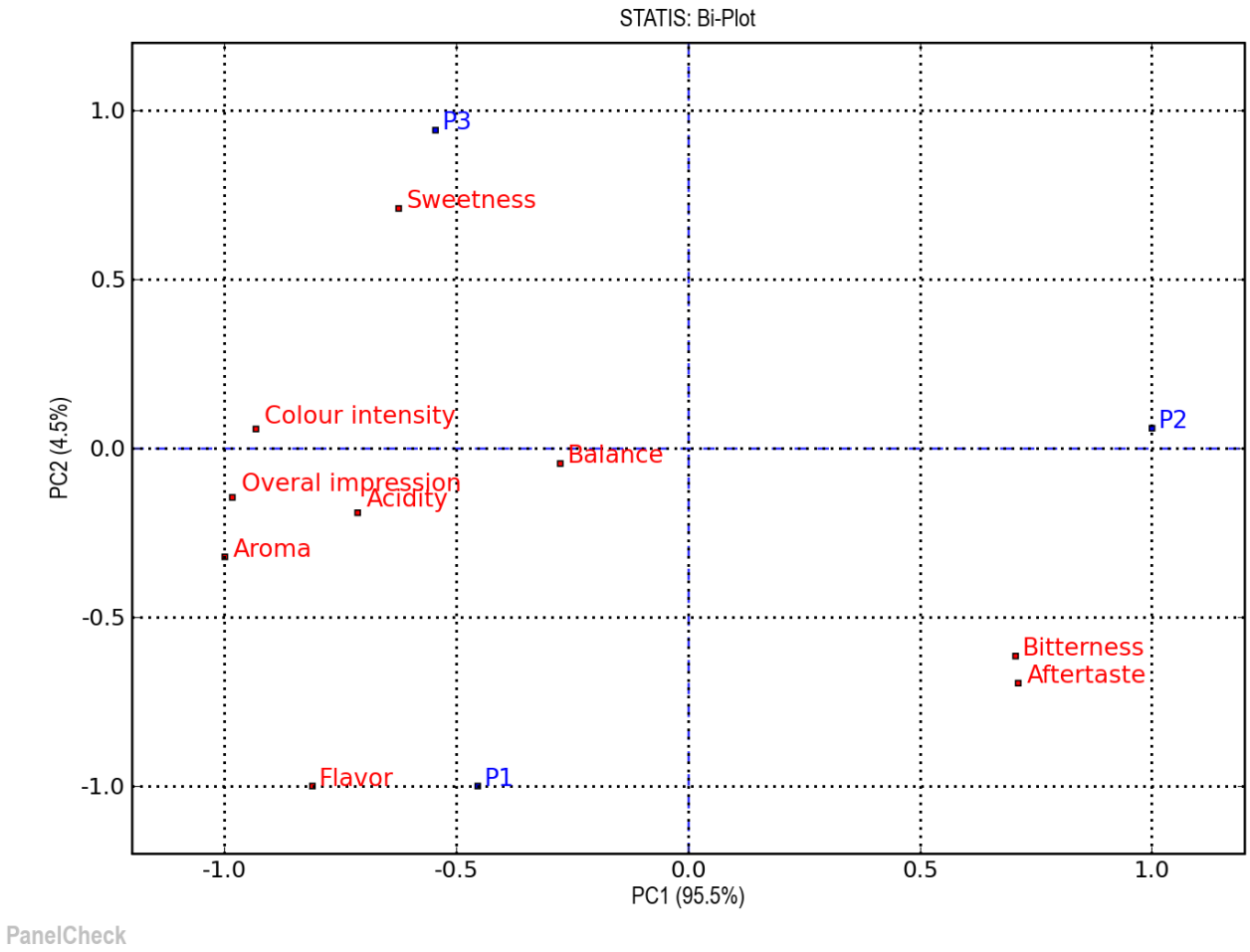


Figure 17: shows the biplot for the sensory attributes.

CHAPTER 5: DISCUSSION

The bioreactors A, B, and C had an average initial pH of 5.0 and an average final pH of 3.8. That significant drop in pH indicated the production of organic acids (like acetic or lactic acid) during fermentation, which is typical as yeast converts sugars into alcohol (Mendes Ferreira & Mendes-Faia, 2020). A pH drop to 3.8 is generally favorable for a cider, according to a study by Natić et al., (2024), a cider has an average pH of 3.6 to 4.0.

On average all the bioreactors had an average initial temperature of around 23°C and the temperatures gradually increased to around 24.3°C by day 7 and 8, this temperature is suitable for yeast activity (Goubet et al., 2002). According to the research carried out by Torija et al., (2003) *S. cerevisiae* typically ferments well within 20°C-30°C (Torija, 2003). A steady temperature helps maintain a steady fermentation. The brix readings showed a steady decrease in all three bioreactors, suggesting sugar consumption by yeasts. The drop of sugar content suggested conversion of sugars to alcohol by the yeasts (Da Silva et al., 2022). The control experiment showed no significant alcohol production due to early exhaustion of the sugars which then caused the death of the yeasts in the fermentation vat.

According to the research carried out by Maluleke (2019), the traditional alcoholic beverage Mukumbi produced from marula fruits has the pH range of 3 to 4.0 and the non-alcoholic Mukumbi has the average pH range of 4.1 to 5. The alcohol content of the alcoholic Mukumbi was found to range from 4% ABV to 7% ABV while for the non-alcoholic is 0.5% ABV. Mukumbi is a traditional beverage produced around villages in Zimbabwe and in South Africa (Shokane et al., 2016).

The pH of Marula wine produced by NBA ranges from 3 to 4.0 and its final brix ranges from 11 to 12 °Bx. In comparison with the produced marula cider, they lie in the same pH range. The pH influences the overall physiological characteristics of the alcoholic beverage such as taste, color and biological stability according the research conducted by Wicklund et al., (2020).

Most importantly, pH also strongly influences sanitation of the product, an attribute important for increasing shelf life of the brew (De Lima et al., 2022).

Commercial ciders alcohol content ranges from 3% ABV to 8% ABV according to the study by Beech, (1972) which aligns with the produced Marula cider. In comparison with the marula wine produced by NBA they all show the golden color which is a common color for marula beverages, but the colors may slightly differ from one brew to another due to different levels of oxidation. According the study by Picinelli et al., (2000), the apple ciders ranged from 5% ABV to 7% ABV which is in line with the produced marula cider.

The alcohol content obtained in this study was found to be higher than those of other traditionally fermented beverages, such as sorghum beer which has a low alcohol content of 2.4 % (Maluleke, 2019). The alcoholic content obtained in this study is also higher than what was obtained by (Machinya, 2024). The traditionally produced marula wine in Zimbabwe was also previously reported to have a low alcohol content of 2% after 4 days of fermentation (Dlamini, and Dube, 2008).

For any fruit juice that is fermented, the alcohol percentage reached is dependent on the levels of fermentable sugars in the juice, and on the characteristics of the yeasts present. The sugar concentrations obtained in this study were found to be higher compared to those obtained by (Maluleke, 2019), this is because of different initial TSS and the duration of the fermentations. The mono-saccharides are co-fermented by microbes such as *S. cerevisiae, L. brevis* and *P. plantarum* amongst others. These bacteria produce numerous compounds such as ethanol, ethyl acetate, isovaleric acid, iso-butanol and other vital volatile organic compounds (Motlhanka, 2021).

According to the sensory evaluation done on the Pink lady cider produced from apple fruit (Way et al., 2022), the sensory scores of the produced marula cider showed attributes of a good cider since marula and apple fruits have some similar sensory attributes. At some levels, microbiological processes can produce an unpleasant off-flavor (Smith, 2016). In production, increased levels of some substances (such as acetic acid, acrolein, allyl alcohol, and 2-butanol) are indicators of raw material deterioration, harmful microbial impacts during or after the fermentation process (Motlhanka, 2021.). Despite the substrate perhaps having a microbial infection, no odor or flavor that was predominately acidic was found. The aftertaste only lasted for a few seconds which matched what Beech, (1972); Picinelli et al., (2000) reported for quality apple cider.

The PCA biplot (figure 15) comparing marula cider samples with the commercial cider was generated using Panelcheck V.1.4.2. P1 and P2 are marula cider samples, while P3 is a commercial cider. P3 and P1 were associated with high scores for overall impression, aroma, color intensity, balance, and acidity and indicating they were the most preferred in terms of these attributes. P2 scored high in aftertaste and bitterness. P1 scored high in terms of flavor, suggesting that it was the most preferred one in terms of flavor. P3 scored high in terms of sweetness. The spider plot (figure 14) clearly shows that P1 and P3 scored almost the same in most of the attributes, and p2 scored high in bitterness and aftertaste, and it was the least preferred in rest of the attributes. P1 was associated with more attributes than P2 suggesting that P1 is the most preferred marula cider than P2.

Each bioreactor yielded about 13 L of the cider after filtration. The yield did not come out as exactly 15 L because each bioreactor contained 3kgs of marula pulp which was then removed during harvesting. After harvesting first filtration was done using the muslin cloth. The filter sheets were not used because they would absorb the cider and significantly reduce the amount of yield. Gelatin was also added to each vat containing the cider. Gelatin enhances clarification by binding particles like proteins, tannins, or other impurities, making them clump together.

These clumps then settle to the bottom, making the cider clearer. After all the sediments were removed and the actual color of the cider became clear. The cider from the bioreactors A and B appeared to be golden and pale yellow, respectively. The colors of the samples from the bioreactors A and B were different due to different oxidation levels and also due to different TSS. The sample from the bioreactor A tested sweeter and the sample from bioreactor B was a little bitter. This was due to different TSS.

As fermentation was progressing all the four bioreactors were fermenting at different rates. This was due to different daily weather conditions (atmospheric conditions). These weather conditions affect the fermentation reactions significantly (Torija, 2003). That was one of the challenges during the conduction of fermentation experiments due to unavailability of fermentation chambers.

Weather conditions also affected microbial dynamics during fermentation, this can be avoided by repurposing an old fridge as a fermentation chamber. On a hot day the fermentation rates were high which implies that

yeasts can function to the optimum in warm temperatures and on a cold day the yeast do not function to the optimum which results in reduced fermentation rates. Some strains of wild yeasts also affect fermentation to some extent.

Some variations in fermentation rates were due to unidentified microbes found in ZINWA water because of poor treatment. ZINWA water may also contain heavy metal ions like lead and zinc which can also affect the functionality of yeasts in fermentation through inhibition. Unavailability of clean water is a serious challenge for brewing experiments and other experiments, as a solution to this problem brewing companies should have their own water purification systems at sites. As an alternative distilled water can be used for fermentation experiments. These microbes, alternate fermentation reactions by inhibiting the yeasts from properly performing their role in fermentation processes. ZINWA water sometimes contain unpleasant odor which then affects aroma and flavor of the final product. It is also not advisable to use borehole water for brewing because it may contain harmful ions such as lead, sulfates, and phosphonates which pose a threat to human health and also provides unfavorable fermentation conditions. Lead ions pose a threat to human brain damage.

The latter flora can generate negative molecules like sulfur compounds under specific conditions. During the cider making process, spoilage yeast, such as *Brettanomyces*, can develop as well, forming negative impact compounds like volatile phenols. These compounds can also interact with macro constituents like phenols, sugar, and alcohol. These different phenomena may change the final perception of the product (Smith, 2016).

The diversity of fermenting yeasts among Marula cider samples could be one of the main reasons of the discrepancies of amounts of ethanol accumulated. Species from the genus *Saccharomyces* produce higher amounts of ethanol as compared to other genera and their non *Saccharomyces* counterpart (Da Silva Fernandes et al., 2022). In addition, the brewing of Marula cider, which is carried out in non-standard conditions such as the use of uncontrolled and partially aerobic fermenting Vats could be another factor. This is because oxygen partial pressures determine the alcoholic fermentation patterns (Brandam et al., 2013). It was observed during conduction of experiments that fermentation occurs fast in conditions with high sugar contents which results in high ethanol levels. This is clearly shown by comparing the rates of fermentation of the bioreactors A and B, ethanol was produced at a faster rate in bioreactor A than in bioreactor B. This suggests that yeasts are highly active and can function to the optimum in conditions with high sugar contents.

This is an important step because it makes it possible to have a clear product without turbidity and deposits at the end of the overall process. This stabilizes the cider and eliminates haziness due to action of proteins or tannins. This is also an important step for elimination of microorganisms and ensuring better microbial stability in the final product. Clarification was done by settling and addition of gelatin. This helps improve filtration and stabilizes the final product by eliminating tannins. Other flocculants, such as bentonites or silica gel, can be used for clarification.

After final filtration the cider is bottled and capped by a capping machine. The second fermentation can spontaneously occur after bottling and which may result in a slight increase in alcohol content. During maturation step after fermentation, other yeasts, such as *Brettanomyces,* immediately grow, which have a negative influence on the aromatic quality of the cider (Kemp et al., 2015).

CHAPER 6.0: CONCLUSIONS AND RECOMMENDATIONS.

6.1 CONCLUSION(S)

Fermentation remains one of the preferred methods for food preparation which has health, cultural and economic significance and it is used throughout the world. In this study *S. cerevisiae* DV10 ex-bayanus showed robust fermentation activity in cider production than wild yeasts harbored by marula fruits by utilizing sucrose to the desired levels, *S. cerevisiae* also improves the sensory properties of the cider. Marula fruit beverages harbor intense aroma and flavor which promotes consumer acceptance of the cider. The ciders golden color is also another pull factor for consumers. We are hoping to scale up the production of the cider in the future as a way of promoting residents in areas with marula fruits by and the economy of the country.

6.2 RECOMMENDATION(S)

Future studies should focus on the reactions that produce fruity aroma via technological flora. Studies should also focus on avoiding spoilage flora that negatively impact overall flavor as these different phenomena may change the final perception of the product. The focus should be more on optimization of the fermentation standards for the production of the marula beverages and exploitation of different yeast strains in cider production. In the next studies we are looking forward on the production of non-alcoholic marula juice, and the co-culturing of *S. cerevisiae* with other yeast strains. On going efforts should be directed towards the exploration of other indigenous fruits in the production of other beverages, alcoholic and non-alcoholic.

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APPENDICES.

Appendix A: Fruit grading by hand picking.



Appendix B: Marula pulp loaded into 20L fermentation vats.



Appendix C: digital refractometer used for brix measurement.



Appendix D: Activation of saccharomyces cerevisiae.



Appendix E: Closed fermentation vats after pitching.



Appendix F and G: Student reading fermentation parameters.





Appendix H: 9-point sensory evaluation form for the cider.

Cider taste panel for

Name of taster.....

Date of taste.....

Parameter	1	2	3	4	5	6	7	8	9
Clarity									
Aroma-alcoholic									
fruitiness									
Aftertaste									
sweetness									
bitterness									
Acidity									
quality									
Overall impression									

Comments

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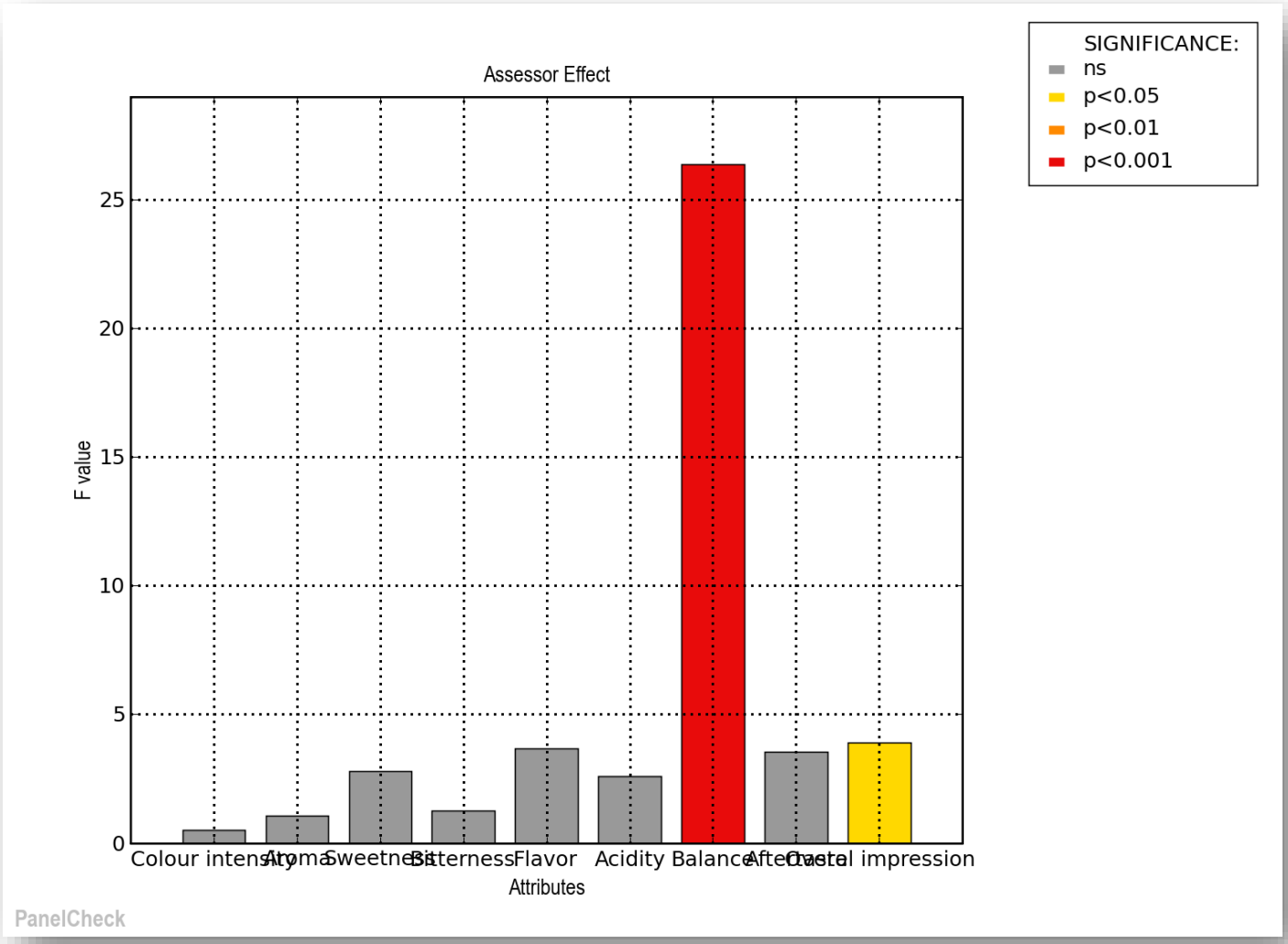
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APPENDIX I: ANOVA output summary from Panelcheck V1.4.2

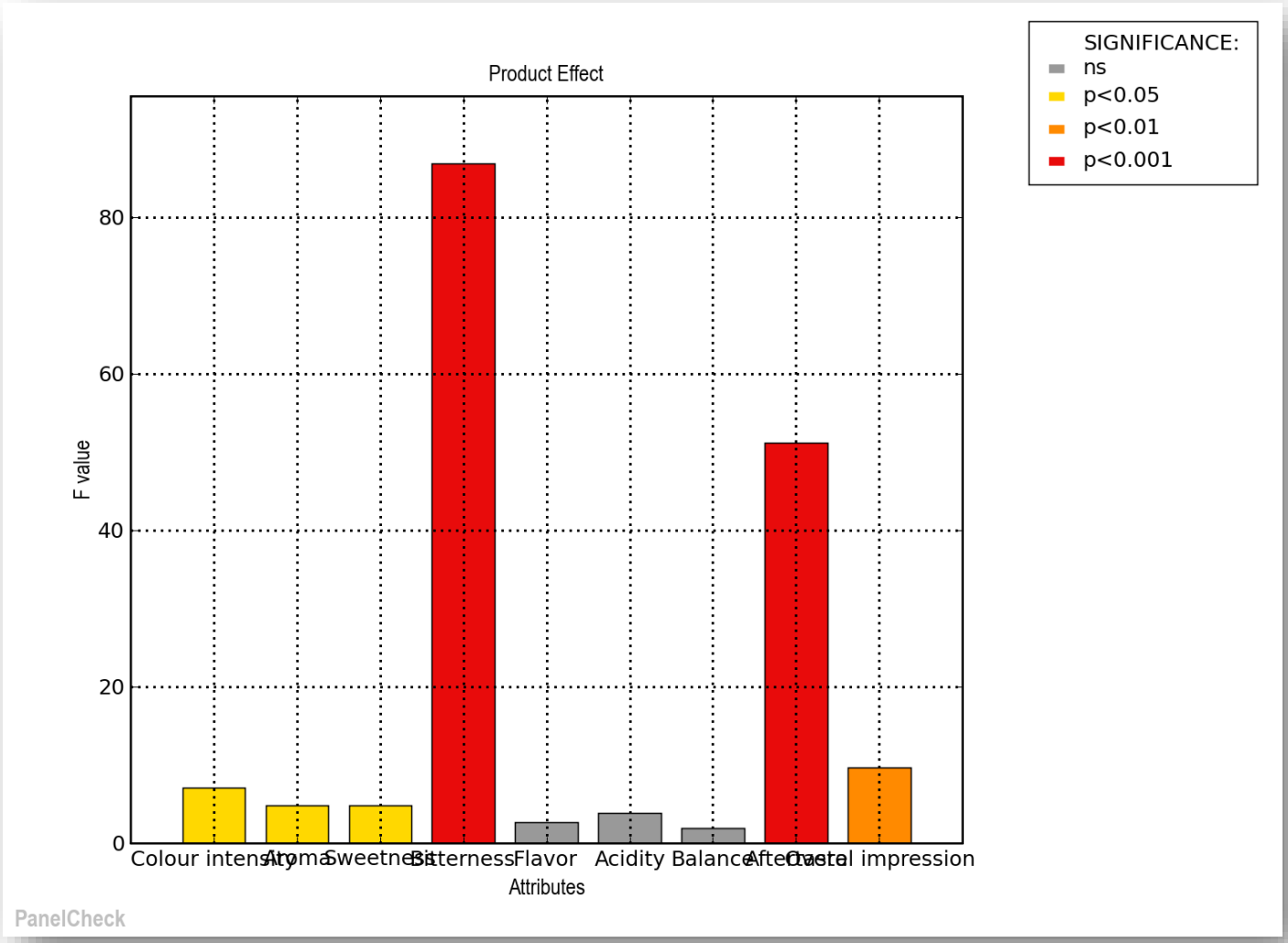
ANOVA Output summary from the Panelcheck V1.4.2. software for the Product Effect, Assessor Effect, and Assessor product Effect evaluated for each cider sample.

	1	2	3	4	5	6	7	8	9	10
1										
2		Colour intensity	Aroma	Sweetness	Bitterness	Flavor	Acidity	Balance	Aftertaste	Overal impressio
3										
4	P1	7.100	6.800	6.300	2.600	6.400	6.700	6.200	2.800	6.900
5	P2	3.600	3.900	4.600	5.700	3.600	4.600	5.000	5.600	3.700
6	P3	7.200	6.900	7.200	1.400	5.300	6.600	6.200	1.500	7.000
7										
8	Assessor Effect	0.50	1.06	2.78	1.26	3.66	2.61	26.35	3.53	3.89
9	p-values	0.740	0.435	0.102	0.359	0.056	0.116	0.000	0.061	0.048
10										
11	Product Effect (	7.06	4.78	4.79	86.88	2.67	3.84	1.88	51.15	9.65
12	p-values	0.017	0.043	0.043	0.000	0.129	0.068	0.214	0.000	0.007
13										
14	Assessor*Produ	3.03	7.01	1.58	2.43	5.45	2.34	19.12	2.34	1.66
15	p-values	0.031	0.001	0.211	0.066	0.002	0.075	0.000	0.074	0.190
16										
17	LSD	2.52	2.54	1.97	0.78	2.81	1.97	1.65	0.96	1.97
18	Bonferroni LSD	3.29	3.32	2.57	1.02	3.68	2.58	2.15	1.25	2.58

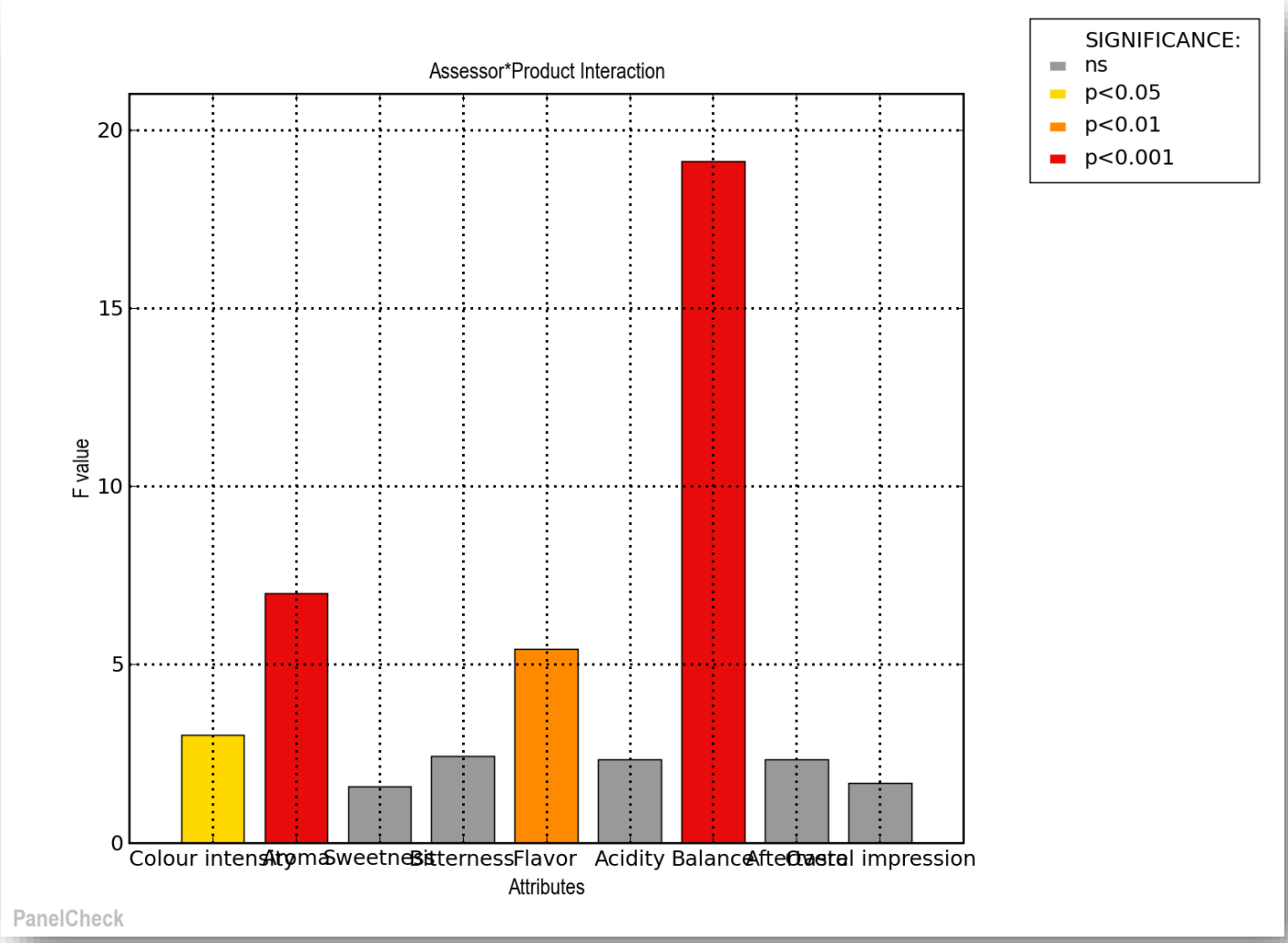
ANOVA output for Assessor Effect $p > 0.05$ for color intensity, Aroma, sweetness, bitterness, flavor, aftertaste, and acidity indicated no significant difference on how the assessors ranked the product, $p < 0.05$ for balance, and overal impression indicated significant difference on how the assessors ranked the product.



ANOVA output for Product Effect $p < 0.05$ for color intensity, aroma, sweetness, bitterness, and aftertaste indicated significant difference on average for these attributes, $p > 0.05$ for flavor, acidity, and balance indicated no significant difference between these attributes.



ANOVA output for Assessor product interaction $p > 0.05$ for sweetness, bitterness, acidity, aftertaste, and overal impression indicated sigficant difference on products, which means panelists disagreed on the pattern of product ranking ; $p < 0.05$ for aroma, color intensity, flavor and balance indicated no significant difference, which means panelists agreed on the pattern of ranking pattern.



Overall impression differed significantly among products ($p = 0.007$). P3 and P1 were rated significantly higher than P2. The mean scores for P3 and P1 were rated significantly higher than P2. The mean scores for P3 and P1 were 7.00 and 6.90 respectively, while P2 scored 3.70. No significant difference was found between P3 and P1.

Below is the ANOVA output summary for two marula cider samples P1 and P2

1	2	3	4	5	6	7	8	9	10
	Colour intensity	Aroma	Sweetness	Bitterness	Flavor	Acidity	Balance	Aftertaste	Overal impressic
P1	7.100	6.800	6.300	2.600	6.400	6.700	6.200	2.800	6.900
P2	3.600	3.900	4.600	5.700	3.600	4.600	5.000	5.600	3.700
Assessor Effect	0.41	0.30	5.30	0.87	1.19	1.40	11.07	6.39	1.74
p-values	0.795	0.863	0.068	0.550	0.436	0.377	0.019	0.050	0.303
Product Effect (	6.81	4.13	7.92	60.06	4.09	4.06	1.88	68.17	9.18
p-values	0.059	0.112	0.048	0.001	0.113	0.114	0.242	0.001	0.039
Assessor*Produ	10.59	8.85	0.64	3.20	5.04	2.65	38.25	1.15	1.86
p-values	0.001	0.003	0.646	0.062	0.017	0.097	0.000	0.388	0.194
LSD	3.72	3.96	1.68	1.11	3.84	2.89	2.43	0.94	2.93
Bonferroni LSD	3.72	3.96	1.68	1.11	3.84	2.89	2.43	0.94	2.93

The ANOVA output Product Effect $p > 0.05$ for sweetness, bitterness, aftertaste, and overal impression which suggested that there was no significant difference between the attributes of the product.

The ANOVA output for Assessor effect, $p < 0.05$ for balance and aftertaste indicated no significant difference on how the assessors ranked the product based on its attributes, $p > 0.05$ for color intensity, aroma, sweetness, bitterness, flavor, acidity, and overal impression.

Assessor Product interaction ANOVA output, $p < 0.05$ for color intensity, aroma, flavor, and balance indicated no significant difference, which suggests that panelists agreed on their ranking patterns of the product; $p > 0.05$ for sweetness, bitterness, acidity, aftertaste, and overall impression, indicated significant difference which implies that the panelists diagreed on how they ranked the product.