PREPARATION, OPTIMIZATION AND QUALITY EVALUATION OF PLUM (*Prunus cerasifera*)- WHITE GRAPE (*Vitis vinifera*) WINE

by

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Preparation, Optimization and Quality Analysis of Plum (*Prunus cerasifera*)-White Grape (*Vitis vinifera*) Wine

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Preparation, Optimization and Quality Evaluation of White Grapes-Plum Wine* presented by Gaurav khadka has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology.

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Abstract

Plums is rich in a variety of vitamins, and minerals, including calcium, which ensures blood clots normally. They also supply potassium and vitamin C as well as being rich in protective polyphenols all of which are associated with cardiovascular risk factors. Plum (*prunus cerasifera*) and grape (*Vitis vinifera*) wine were prepared using different proportions of plum and grape juice (0%, 25%, 33.3%, 50%, 66.7%, 75%, and 100%) using wine yeast (*S. cerevisiae*) at the rate 1 g/L of the must. 100% grape juice in the must was taken as a control sample. The pH and total soluble solids (TSS) of the must were maintained at 3.9±0.1 and 25°Bx respectively and fermentation was carried out for 24 days at 25-30°C. The prepared wine was analyzed for TSS, pH, acidity, reducing sugar, alcohol contents, total phenolics, aldehyde, esters, tannin, methanol contents and antioxidants properties. Sensory attributes (color, flavor, smell, taste, clarity, and overall acceptability) of wine were evaluated using a 9-points hedonic scale rating test. The sensory score data were analyzed by ANOVA using GenStat 12 Edition, 2014 at 5% level of significance.

Plum: grape juice in the ratio of 33.3:66.7% was found to be best, based on a 9-point hedonic rating test. The means sensory score showed that there was significant difference among all the products with respect to color, flavor, smell, taste, clarity, and overall acceptability of the product. Variation in juice content of plum and grape of must significantly ($p<0.05$) affect the wine quality. From sensory evaluation the best sample (plum: grape juice ratio 33.3:66.7%) had 7.5°Bx TSS, 3.89 pH, 0.03% volatile acidity (as acetic acid), 10.7%(v/v) alcohol content, 272.93(mg GAE/100 ml) total phenolic, 49.36(g/100 L) total esters, 0.24 (g/100 L alc.) total aldehyde, 83.34% (DPPH inhibition) and 0.9 (g/L) methanol. Hence this wine had potential for commercialization to be made within the means of common people.

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List of Abbreviations

Abbreviation	Full form
ADY	Active dry yeast
ATP	Adenosine triphosphate
EMP	Embden-Meyerhof glycolytic pathway
FOS	Fructooligosacharides
KMS	Potassium metabisulfite
LSD	Least significance difference
ANOVA	Analysis of variance
MLF	Malo-lactic fermentation
NADH	Nicotinamide adenine dinucleotide
TSS	Total soluble solid
LDL	Low density lipoprotein
CVD	Cardiovascular diseases
FAO	Food and agricultural organization
MOALD	Ministries of agriculture and livestock development

Part I

Introduction

1.1 General introduction

The phrase 'wine' refers to a product formed through alcoholic fermentation of different fruits especially grapes or grape juice by *Saccharomyces cerevisiae*, followed by aging having alcohol percentages ranges from 8-16% abv. However, wines are also made from the fermentation of various berries, fruits, and honey, and the resulting wine is usually named by the substrate utilized. The bioactive compound found in red wine are phenolic acid, anthocyanins, flavonoids, antioxidants etc. Wine is one of God's most beautiful gifts to man, and its history is nearly romantic. The earliest testament in the Bible mentions wine, although there is clear proof of its usage in China and Egypt around 2000 and 3000 B.C., respectively (Andrew, 1980). The oldest documented winemaking practices emerge on the archaeological record as far back as 6,000 years ago in diverse locations throughout the world. Wine has been giving pleasure to people for over 8000 years. Indeed, it may have been significant in human life even before bread is being made (Cavalieri *et al.*, 2003).

Various raw materials have been employed in the making of wine, either for flavor or to enrich the wine with major chemical elements (Gubhaju, 2006). Any fruit that contains enough fermentable carbohydrates can be used to make wine. The grape (*Vitis vinifera or, less often, v. rotundifolia*) is a commercially important plant. Although wine is manufactured on a small commercial basis from fruits like strawberry, gooseberry, and peach. Cider, made by fermenting apple juice, is not technically a wine, but it has a similar process and, together with the less prevalent Perry (Varnam and Sutherland, 1994). Different herb-infused wines were also popular across the world. Ginger wine, for example, is an alcoholic beverage produced from a fermented mixture of crushed ginger *(Zingiber officinale)* and raisins fermented by the yeast *Saccharomyces cerevisiae*. In Europe, it is a popular beverage (Rai, 2012).

White grape (*Vitis vinifera*) is a tasty and healthy fruit with a lengthy agricultural history that was still one of the world's most produced fruits (Restani *et al.*, 2021). The yearly grape output is around 75 million tons, with Europe producing the most (about

41%), followed by Asia (29%) and the Americas (21%) (Unusan, 2020). Grape's health advantages are mostly related to its high concentration of bioactive components, particularly polyphenols, which primarily consist of proanthocyanins, anthocyanins, flavanols, phenolic acids, and stilbenes (Yang and Xiao, 2013). Polyphenol concentration varies greatly depending on grape fractions. Grape seed has the highest concentration of polyphenols, mostly proanthocyanins (Benbouguerra *et al.*, 2020). Grape skin includes a high concentration of anthocyanins, but grape seed contains essentially no anthocyanins (Zhao *et al.*, 2020). The grape is remarkable in that it is both a significant worldwide horticultural crop and has ancient historical ties to the formation of human civilization. The principal product, wine, is revered as divine, a drink of the gods: This beverage is dedicated to Dionysus and Bacchus. Other Mediterranean cultures thought that "the wine" sprung from the blood of humanity who had battled against the gods (McGovern, 2013). Grape quality is one of the factors with the largest influence on wine quality. Its composition depends on the variety, climatic conditions, soil, and cultivation techniques used (Peterlunger *et al.*, 2002)**.**

Grapes are consumed as a fresh fruit and have several food industries uses. Many grape-derived goods, such as wine, grape juice, grape jam, and raisins, were created and sold (Kandylis *et al.*, 2021). Winemaking is the most common use of grapes, accounting for over half of all grape yields (Unusan, 2020). Furthermore, with the widespread manufacture of grape products, byproducts (such as grape pomace and grape seed) are created in significant quantities, potentially causing severe environmental difficulties and representing a waste of resources (Averilla *et al.*, 2019). The usage of these byproducts is gaining popularity. Numerous investigations have revealed that grape byproducts have significant application potential in the food business. Some grape seed-derived products, in particular, grape seed extract capsules and grape seed oil, have been commercially sold (Chen *et al.*, 2020).

Plum (*Prunus cerasifera*) are one of the world's most significant stone fruit crops. Plums also contain some well-known stone fruits, such as apricot, cherry, and peach. There are about 2000 types of plums, although only a handful are commercially important (Birwal *et al.*, 2017). The widely dispersed *prunus cerasifera* (cherry plum) originated in west Asia, was endemic to southeast Europe, and is well recognized as a common plum; yet its exact origin was unknown. Plum helps in prevention of heart diseases, lung and oral cancer, lower blood sugar, blood pressure, improve memory capacity, boost bone health, regulates the digestive system etc. It is the parent of the diploid European plum and is cross-fertile with American and Asian species, making it a fundamental species since it can breed with many other species (Gull *et al.*, 2022). Plums are important source of compounds influencing human health and preventing the occurrence of many diseases (Stacewicz-Sapuntzakis *et al.*, 2001).

The cherry plum is also known as the myrobalan plum (from the Greek terms Myron, which means plant juice, and balanos, which means nut). It is native to Europe and is known as the Stanley prune in the northeast and far west of the United States. ‘Thundercloud’, ‘Krauter Vesuvius’, and ‘Newport’ are some frequent variants. Some writers consider it a cultivated variety of *P. ceresifera,* macrocarpa and a wild variant known as *P. divaricate*, which is more widespread in the Caucasian region but native from Macedonia to northern Persia (Faust and Suranyi, 1998).

In the cherry plum, the number of chromosomes is generally (x=8) 2n=16 (2x), but it can also be 3x (2n=24), 4x (2n=32), or 6x (2n=48) (Watkins, 1976).

1.2 Statement of the problem

Cherry plum is a seasonal fruit mainly found in summer season and it is generally consumed as fresh fruits. In Nepal, plum is being commercially produced in various parts of the country but still they aren’t being efficiently utilized in the market. Its shelf-life is not more than one week. People do not have any idea about its alternative use by making different products like jam, wine, juice etc. Plums are rich in a variety of vitamins, and minerals, including calcium, which ensures blood clots normally. They also supply potassium and vitamin C as well as being rich in protective polyphenols all of which are associated with cardiovascular risk factors. Plums and prunes are high in antioxidants, which assist in reducing inflammation and protect your cells from free radical damage. They are especially high in polyphenol antioxidants, which are beneficial to bone health and may help lower the risk of heart disease and diabetes. Despite its potential importance, the cherry plum (*Prunus cerasifera)* is an underused fruit in Nepal. This fruit, which is sometimes disregarded, poses a fascinating challenge in the country's agricultural and economic backdrop. The problem is defined as the underutilized potential of cherry plum as a valuable resource for both local consumption and prospective commercialization.

Despite being well-suited to specific climatic conditions in Nepal, the cherry plum remains somewhat disadvantaged, receiving little attention and cultivation.

This study may be valuable in determining the potential usage of plum by employing it as a substitute supply of juice required for wine production, hence minimizing spoiling caused by its underutilization. The work primarily focuses on must composition optimization in terms of juice recipe in must. Grapes are costly, and the consumption of wine is growing. Plum has positive medicinal aspects and a catchy color. So new product modification and commercialization of low-cost wine can be done. As a result, the research can help growers develop plum-grapes wine on a small scale, allowing them to create a value-added product from the fruit. This research can also be used in the winemaking industries of Nepal in creating a completely new product with higher quality color, scent, taste, mouth feel, and appearance are all examples of high quality. The outcomes obtained in this work may serve as a springboard for additional research into producing high-quality plum-grapes wine. Furthermore, fermentation is one of the food preservation strategies which delivers distinctive new dishes with flavor, body, look, and texture. It also gives more nutritious nutrients than unfermented meals. As a result of this work may indicate fermentation as a method of maintaining quality of the product without spoilage.

1.3 Objective

1.3.1 General objective

The general objective of this dissertation is to prepare, optimize and evaluate plum-white grape wine using different proportions of plum and white grapes juice.

1.3.2 Specific objective

The specific objectives of the study are as follows:

1. To prepare wine using different proportions of plum juice and white grape juice.
2. To select the best wine based on sensory evaluation.
3. To carry out physiochemical analysis of the best formulated wine.

1.4 Significance of the study

This study may be valuable in determining the potential usage of plum as an alternative supply of juice required for wine production, hence reducing spoiling as it is highly perishable and is a seasonal fruit which can be produced once a year. The work primarily focuses on must composition optimization in terms of juice recipe in must. Grapes are costly, and the consumption of wine is growing. Plum is less expensive than grapes, and plum provides therapeutic benefits as well as a pleasing hue (Nakatani *et al.*, 2000). As a result, new product development and marketing of low-cost wine were possible. As a result, the research can help growers develop plum-white grape wine on a small scale, allowing them to create a value-added product from the fruit.

The post-harvest losses of plum are found to be 20-50% yearly according to B. Pokhrel (2021). So, this research can also assist Nepal's winery businesses create entire new products with higher quality in terms of color, fragrance, taste, mouth feel, and look. The findings of this study might pave the way for additional research into producing high-quality plum-grape wine. Furthermore, fermentation is a food preservation technology that produces different new meals with features such as flavor, body, look, and texture, as well as more nutritious foods than their unfermented counterparts. As a result, this investigation may indicate fermentation as one of the good preservation ways to decrease deterioration.

1.5 Limitations of the study

1. A temperature control apparatus was not available in the laboratory, so the fermentation was carried out in ambient conditions.
2. The fermentation was carried out at the same temperature, and pH adjustment with the same base (sod. Bicarbonate). As a result, no optimization of temperature, relative humidity, pH, base was performed.
3. Due to time and technical restrictions, the prepared plum-white grape wine was not adequately matured.
4. Single strains of yeasts (*Saccharomyces cerevisiae*) were used.
5. Clarifying agents were not used.

Part II

Literature review

2.1 Historical background of alcoholic beverage

Alcoholic drinks are said to have originated approximately 6000 years ago in Egypt and Mesopotamia. Different civilizations produced several forms of alcoholic beverages around the world. Alcoholic beverage manufacturing and consumption is one of Man’s first activities. In the past, winemaking is a significant economic activity (Varnam and Sutherland, 1994). The utilization of wheat, rye, millet, rice, oats, barley, potatoes, or grapes in early fermentation processes opened the path for today's technology (Jones, 1995).

Despite this early use of microbiology, microbes’ potential to drive biochemical changes is established some years later. Gay Lussac discovered alcoholic fermentation in 1810, although yeast is not recognized as the causal organism at the time. Schwan established in 1835 that yeast could make alcohol and carbon dioxide when placed in a sugar-containing solution. He called yeast Zuckerpilz, which means sugar-fungus, and therefore the name Saccharomyces. The *Saccharomyces* group is mostly responsible for the production of alcoholic drinks (Prescott and Dunn, 2004).

The anaerobic yeast cells converted glucose to alcohol, and the researchers also established that fermentation could be carried out using cell free yeast juice, which led to the discovery of the involvement of enzymes in fermentation. The enzyme is given the name “Zymase” by him. Pioneering studies eventually exposed the truth that alcoholic fermentation is anaerobic due to the existence of an enzyme complex known as Zymase, which is made accessible by yeasts. People began cultivating valuable yeasts and utilizing them for the manufacturing of diverse alcoholic drinks after realizing the role of yeasts in fermentation. Yeast is now used in the manufacturing of alcoholic drinks in a variety of forms and flavors all over the world. Starting materials are often sweet compounds that must be hydrolyzed to simple sugars prior to fermentation (Buglass *et al.*, 2011).

A wide variety of alcoholic beverages have emerged throughout the years, but in most cases, they may be classified into one of three groups based on ingredient and method of manufacture: beer, wine, or distilled spirit (Varnam and Sutherland, 1994).

The history of alcoholic beverages in Nepal extends back to prehistoric times. These technologies are created by ethnic groups when celebrating numerous festivals and marriage settlements. Homebrewing expertise has been passed down through generations, yet they were unaware of the vast dimensions of microbial biochemistry or their intricate processes. In fact, they still don't completely understand the process of fermentation (Gubhaju, 2006).

2.2 History of wine making

Wine and winemaking have a history as old as civilization itself. Viticulture, or grape-growing, originated in Georgia some 9000 years ago and moved to the Middle East through the Tigris and Euphrates rivers to Mesopotamia, and then to Persia. There are several stories about how wine is found, one of which involves a mythological Persian monarch named Jamsheed. Grapes are preserved in jars at his court for eating out of season. One jar is thrown out because the juice had lost its taste, and the grapes are poisoned. A damsel from the king's hareem is experiencing anxious headaches and attempted suicide with the so-called poison. She slept off and awoke feeling renewed and invigorated. She informed everyone what she had done and about the miracle cure, and a large quantity of wine was prepared, and his court drank it. In a nutshell, that’s it. Someone put wild grapes in a container somewhere in Asia Minor, probably in contemporary Anatolia or Georgia, and they are crushed by their own weight. The juice that resulted began to ferment, and a new drink was found that would provide tremendous joy to an untold number of people. Wine is also traced back to pre-historic times in the major civilizations of ancient Greece and Rome, with comparable traditions concerning its discovery (Sandler and Pinder, 2002).

2.3 Wine industry and market in Nepal

After water and beer, wine is considered the third most drunk liquid (Poudel, 2023). Commercial wine production in Nepal has a very short history (Karki, 2019). Although certain varieties of traditional wines have been produced since time immemorial. Within the last several years, there has been a significant shift in Nepal's wine-drinking culture (L. Pokhrel, 2018).

Many young people in Nepal prefer wine to beer or other alcoholic beverages. Wine consumption is predicted to have increased by 150% since 2007, and it continues to rise. In

Nepal, drinking wine has grown fashionable. Most Nepalese consumers are unaware of the social and health benefits of drinking wine; instead, they drink to demonstrate their acceptance of Western culture, modernizations, and greater social standing. As we all know, wine is a 'culture' that has social, cultural, and health advantages when used properly. Wine is and still is used as a gift to the deity in many faiths. In 2009, it is predicted that about 450000 liters of wine are drank in Nepal (Karki, 2019).

The nation produces more than 50 different types of wine. Brands such as Hinwa, Dandaghare, and Divine had a considerable market share, while newly launched Black Stone and Moon Dance were battling to garner fans in a short period of time, and consumers of Nepali wines has risen dramatically. Five years ago, no one would had looked at a Nepal-made wine bottle, but now there are roughly 100,000 bottles of Nepal wines on the market (monthly) (Nepal, 2014). The taste and great quality of Nepali wines have enticed customers to buy them.

The following were some of the most well-known Nepalese wine brands.

I. **Dadaghare**

The wine produced in Pokhara, *Dadaghare*, is regarded as the first Nepali wine. It is popular not just with locals but also with visitors from other countries. The wine, which comes in four flavors: *Aangan, Pidi, Majheri*, and *Aati,* is made with various fruits, herbal fruits, and honey and is completely chemical free.

II. **Hinwa**

Hinwa, one of the most popular wines, is produced by Makalu wine businesses in Sankhuwasabha. This wine is created in 1995 using wild fruits such as raspberries, Himalayan barberry, and saffron.

III. **Nettlange**

Nettlange, produced by *Sakaro* Beverages, is a popular Nepali wine in the local market made from nettles (*Sishno*) and oranges.

IV. **Grapple**

Made from black grapes that are imported from India, and apples from Mustang, Grapple is manufactured by Sakaro Beverages.

V. **Divine**

Divine wine, which debuted in 2010, is one of the market's fastest selling brands. Shree Mahakali wine company produces the wine, which is prepared from grapes, spices, tea, and numerous other fruits (Rijal, 2016).

2.4 Classification of wine and chemical composition of some wine

Wines can be classified on various bases viz., (i) Color, (ii) Effervescence, (iii) Relative Sweetness, (iv) Alcohol content, and (v) The system used by Wine Advisory Board, USA. However, the basic groups of wines are most easily distinguishable for the consumer. They were (i) Table wines, (ii) Sparkling wines, and (iii) Fortified wines. A summary of the classifications of wines based on different characteristics is shown in Table 2.1. and composition of some wine is given in Table 2.2.

Table 2.1 Classification of wine

Basis of classification	Class/type	Description	Example
Color	Red wine	Contain the red coloring matter of skin, pulp, and seeds	Burgundy
	White wine	Do not contain the red coloring matter of skin, pulp, and seeds	Rhine wine
	Pink wine	Low concentration of red coloring matter is maintained	Rose
Relative sweetness	Sweet wine	Contain up to 7% sugar	Sherry (sweet)
	Dry wine	Contains less than 0.12% sugar	Sherry (dry)
Alcohol content	Natural	Contains 8.5 – 16% alcohol by volume (% abv)	Tables wine
	Fortified	Contains 17 – 21% abv	Sherry
Effervescence	Still	Does not contain CO_2	Chianti
	Sparkling	Contains CO_2 (natural or added)	Champagne
Wine Advisory Board, USA	Dessert wine	Contains sugar; taken after meal	Sherry (sweet)
	Appetizer wine	Dry; fortified; taken before meal	Sweet (dry)
	Sparkling wine	Contain CO_2	Champagne
	Red table wine	Natural; red in color	Chianti
	White table wine	Natural; pale yellow to straw color	Rhine wine

Note: The categorization listed above has a lot of overlapping wine kinds.

A Red Table wine, for example, might be sweet, effervescent, fortified, or natural all at the same time. A fortified wine can also be sweet, effervescent, red, or white (Rai, 2012).

Table 2.2 Composition of wine

Parameters	Port	Sherry	Claret	Burgundy	Champagne
Specific gravity	0.995-1.050	0.992-1.015	0.995-1.001	0.995-1.001	1.040-1.055
Alcohol (gm/100ml)	13.5-20.0	13.5-20.5	7.5-12.5	7.5-12.5	10.0-14.0
% Total solid	3.3-13.0	2.0-9.6	2.0-3.5	2.0-3.5	9.5-18.0
%Free volatile acid (as acetic acid)	0.05-0.10	0.15-0.23	0.09-0.15	0.2-0.35	0.03-0.20
% Fixed acid (as acetic acid)	0.35-0.55	0.25-0.50	0.30-0.50	0.30-0.60	0.30-0.45
% Ash	0.25-0.35	0.35-0.55	0.20-0.30	0.20-0.40	0.25-0.45
% Sugar	2.5-12.5	2.0-7.0	0.0-0.7	0.03-0.55	8.5-16

Source: Egan *et al.* (1981)

2.5 Chemical composition of wine

2.5.1 Alcohol

2.5.1.1 Ethanol

There are many other types of alcohol, but when winemakers use the phrase loosely, it generally refers to the drinkable alcohol known as ethyl alcohol or ethanol, which is a major element in alcoholic drinks of all kinds. Since ancient times, sugar fermentation has been used to produce ethanol. This technology is still used to produce all drinking ethanol and more than half of industrial ethanol. The basic ingredient is simple sugars. Zymase, a yeast enzyme, converts simple carbohydrates to ethanol and carbon dioxide. The concentration of ethanol generated by fermentation ranges from a few percent to roughly 14%. Ethanol kills the zymase enzyme and causes fermentation to cease at roughly 14%.

Ethanol has a melting point of -114.1°C, a boiling point of 78.5°C, and a density of 0.789 g/ml. It easily combines with water in any proportion, and when amounts are combined, volume is reduced. It is a colorless, transparent liquid that is combustible. It is an excellent solvent for essential oils, ester, tannins, different organic acids, and other organic compounds. It burns well in air, allowing for oxidation, and then produces a blue smokeless flame while emitting water and CO_2 (Shakhashiri, 2009).

Wine contains a variety of alcohols. Ethanol is the most significant of them. Although some ethanol is created in grape cells during carbonic maceration, yeast fermentation is the principal source of ethanol in wine. Ethanol is essential for the stability, aging, and sensory functions of wine. The inhibiting impact of ethanol, along with the wine's acidity, permits wine to remain stable for years in the absence of air. Ethanol has several impacts on flavor and mouthfeel. It directly contributes to the impression of sweetness. It indirectly affects the acidity perception, making acidic wines look less sour and more balanced. In high amounts, alcohol causes a burning sensation and may contribute to the impression of weight (body), particularly in dry wines. Ethanol can also raise the bitterness intensity, decrease tannin astringency, and impact the volatility of aromatic molecules. It is a solvent for 11 many volatile chemicals created during fermentation and developed during aging in oak cooperage, in addition to assisting to dissolve color and tannin extraction from grapes (Jackson, 2014).

2.5.1.2 Methanol

Methanol is present in wine, although in tiny levels. Methanol has no sensory or physiological implications in its usual range (0.1-0.2 g/L). Few of the more than 160 esters detected in wine relate to methanol. Methanol's health problems stem from its conversion to formaldehyde and formic acid. Both have a negative impact on the central nervous system. The optic nerve is one of the primary targets of formaldehyde poisoning, resulting in blindness. Methanol, on the other hand, never accumulates to hazardous quantities in wine, at least not under normal winemaking conditions. The minimal quantity of methanol detected in wine was virtually entirely due to the demethylation of pectin. Methanol was formed from these methyl groups. As a result, methanol content is a partial function of required pectin content. Grapes, unlike other fruits, have a low pectin level. As a result, wine has the lowest methanol concentration of any fermented fruit beverage. Pectolytic enzymes, which are added to juice or wine as a clarifying aid, might, however, mistakenly increase the methanol concentration. Adding distilled alcohol to wine may boost the methanol concentration somewhat (Jackson, 2014).

2.5.1.3 Higher alcohols

Higher alcohols or fuel oil are alcohols containing more than two carbon atoms. They typically contribute around half of the aromatic ingredients of wine, excluding ethanol. The aliphatic alcohols n-propanol, iso-butanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-mthyl-1-butanol), and aromatic alcohols hexanol and 2-phenethyl alcohol are the main higher alcohols generated by yeast. The higher alcohols content in wine should be 80-540 mg/L, and the concentration of higher alcohols below 300 mg/L adds to the ideal scent of wine, however when their level reaches 400 mg/L, these components are viewed as a negative influence in generating the aroma (Usansa, 2003).

Higher alcohols are vital as the immediate precursors of more taste active esters, hence higher alcohol formation must be regulated to guarantee that ester synthesis is managed. Yeast produces higher alcohols as secondary metabolites of amino acid metabolism. The problem is compounded by the fact that yeast cells may synthesize greater alcohols from different pathways rather than from 12 amino acids. Again, as with esters, yeast strain is the most critical component. Conditions that support enhanced yeast growth, such as excessive aeration or oxygenation, promote higher alcohol formation; however, this can be mitigated by using a top pressure during fermentation (Baxter and Hughes, 2001).

2.5.2 Esters

There are several esters that contribute to the flavor of wine. Ester is crucial in the production of wine's sensory qualities. They are created during the wine fermentation and aging processes from acids and alcohols. There are several types of alcohol as well as acids in wines, the number of potential esters was enormous. There are two types of ester in wine which are formed by enzymatic esterification during the fermentation step and chemical esterification as a result of long-term aging (Usansa, 2003). Ester biosynthesis was primarily determined by fruit ripeness, yeast type, must aeration, fermentation method, and temperature. Their concentration in young wines ranges from 25 to 300 mg/L. The bulk of esters were generated at the start of fermentation, and their concentration fluctuates relatively little during wine maturity. Isoamyl acetate (banana scent), 2-phenylethyl acetate (rose aroma), and ethyl acetate (strong, sweet aroma) are among the most important wine esters in terms of bouquet (Clarke and Bakker, 2004).

2.5.3 Aldehydes

Acetaldehyde is of particular significance due to its role as a direct precursor to ethanol. It has a strong ‘grassy’ flavor and scent. Acetaldehyde is created in the early to mid-stages of fermentation and thereafter decreases to a low level. In rare cases, it can accumulate in amounts over the taste threshold of 10-20 ppm during fermentation. The use of low quality pitching yeast, excessive must oxygenation, excessive fermentation temperature, and excessive pitching rates are the primary reasons of elevated acetaldehyde concentrations in wine (Briggs *et al.*, 2004).

In general, the aldehyde concentration of white and red wines is comparable. However, the aldehyde level is low, which may be explained by the fact that sulfur dioxide added to wine combines with aldehydes to generate a-hydroxysufphonic acids, which reduces the free aldehyde content. Aldehydes can also be chemically linked to ethanol and higher alcohols as acetals. In various nations, white and red wines include 1-propanol (11-125 mg/L), 2-methyl-1 propanol (15-174 mg/L), 2-methyl-1-butanol (12-311 mg/L), and 3- 13 methyl-1-butanol (iso-pentanol; 49-180 mg/L). Aldehydes also have a role in color, avoiding bleaching by interacting with sulfites and, more crucially, by aiding in the binding of anthocyanins to tannins and maintaining color. Finally, because of their involvement in tannin polymerization processes, aldehydes has a role in texture (Frivik and Ebeler, 2003).

2.6 Research works in wine

Ancin *et al.* (1996) studied the influence of pre-fermentation clarifying on the higher alcohol level of wines. He stated that the pH, reducing sugars (g/L), total acidity (g/L as tartaric), and volatile acidity (g/L as acetic) levels of rose and white wines were 3.11, 0.98, 4.94, and 0.4, respectively; and 3.34, 1.68, 3.88, and 0.26. Similarly, the ethyl alcohol (%, v/v) and higher alcohol (mg/L) levels of rose and white wines are 12.75 and 204, 10.5 and 258 respectively.

Furthermore, grape varietal and maturity influence the concentration of higher alcohols, owing to the presence of qualitative and quantitative variances in the makeup of necessary amino acids. Furthermore, the presence of substantial quantities of insoluble particles in musts during fermentation results in wines with greater levels of alcohols and esters than wines created with cleared musts.

Vilanova *et al.* (2007) studied on aromatic components in wines generated during fermentation. They stated that the ethanol (%, v/v), total acidity (g/L as tartaric acid), volatile acidity (g/L as acetic acid), reducing sugar (g/L), total phenolics (g/L as gallic acid) in Caino Longo, Caino Tinto, and Caino Bravo wines were 9.46, 9.30, 0.30, 0.70, and 35.81; 9.16, 9.10, 0.30, 0.70, and 36.19; and 7.86, 10.30, 0.30, 1.00, 55.66 respectively. Similarly, the concentrations of methanol (mg/L) and ethyl acetate (mg/L) in Caino Longo, Caino Tinto, and Caino Bravo wines scored 88.69, and 28.51; 135.62 and 22.88; and 214.87 and 58.90 respectively.

Reddy and Reddy (2009) studied on wine production by using a new yeast biocatalyst generated by immobilization on watermelon (Citrullus vulgaris) rind pieces, as well as the characterization of volatile components. He stated that the ethanol concentration was 4 g/L, and the amounts of ethyl acetate and methanol are less than 100 mg/L.

Higher alcohol concentrations in New York wines were investigated, and it was discovered that the higher alcohol contents in red and white wines were 339 and 188 mg/L, respectively.

2.7 Wine yeast

Wine yeast belongs to the *Saccharomyces cerevisiae* genus. This comes from the Greek terms *Sakchar,* which means sugar and *mykes* fungu*s* which refers to the high sugar fermenting characteristics of genus in general. Despite Hansen's classification of them as a distinct species, they had ellipsoidal cells rather than circular or ovate cells like brewery and bread yeasts. Hansen gave them the name *S. ellipsideus.* According to Dutch school nomenclature, these 17 yeasts are categorized as a variation of *Saccharomyces cerevisiae* and hence termed *S. cerevisiae* var. *ellipsideus*. However, in general publications, they are briefly described as ellipsoidal yeasts or real wine yeasts (Raut, 2014).

Wines can be made utilizing either the grapes' natural yeast flora (spontaneous fermentation) or pure cultures (culture yeasts). Many manufacturers still rely on spontaneous fermentation, which may yield wine of exceptional aroma quality because of the interplay of various yeast species. Each yeast strain will provide a distinct taste to the wine. However, the yeast profile was variable, and spontaneous fermentation can sometimes result in failure. Most yeast strains do not create a great volume of wine, and a few strains produce unwanted organic compounds like as organic acids, H_2S, higher alcohols, and so on, which might impair the flavor (Rai, 2012). Nowadays, they must have been partially 'sterilized' using sulfur dioxide, bisulfate, or metabisulphite, which kills most bacteria in the must but leaves wine yeasts. The must is then infected with yeast. The yeast utilized is *Saccharomyces cerevisiae* var. *ellipsoideus* (also known as *S. cerevisiae*, *S. ellipsoideus*, and *S. vini.)* Other yeasts utilized for unique wines include *S. fermentati, S. oyiformis*, and *S. bayanus* (Okafor, 2007). Excellent wine yeast imparts a vinous or fruity flavor, ferments sugar to a low concentration, generating 14-18% alcohol, and is distinguished by remaining suspended during fermentation and then followed by agglomeration to produce a coarse granular material that settles fast and was not readily disturbed during the racking process (Pederson, 1971). In general, good wine yeast should possess the following characteristics (Okafor, 2007).

a) High alcohol tolerance, i.e., the yeast should continue to ferment despite rising alcohol concentration, producing bolder, drier wines with up to 16% alcohol (v/v), or even up to 18% (v/v) if the yeast is supplied with little quantities of sugar on a regular basis.
b) A high degree of agglutination, or the tendency of the yeast to flocculate into little lumps that form a cohesive sediment after fermentation ends, making racking easy and the wine clean.
c) Fermentation capability that is consistent and persistent; this results in higher-quality wines than when fermentation fails after a stormy start.
d) There are no disagreeable tastes produced by dead or dying cells.
e) For fermentation, growth occurs at relatively high acidity, i.e., low pH of grape juice or must.
f) Osmo tolerance, i.e., yeast should be able to tolerate high osmotic pressure caused by high sugar content in must composition.
g) SO_2 tolerance, i.e., when SO_2 in the form of sulfite was used for partial sterilization of must, yeast should not be impacted by the sulfite.

There were two reasons why starters are used. One method is to begin alcoholic fermentation as soon as possible after harvest. Indeed, in certain situations, particularly at the start of the winemaking process, the yeast population is too low (less than 10^4 CFU/ml). Multiplication up to and including 10^6 and much more it takes several days, especially if the weather is cold. Other microorganisms, such as yeasts with oxidative metabolism and acetic acid bacteria that use the presence of oxygen to create volatile acidity and a variety of other abnormalities, can develop during this time. Thus, inoculation with starters at 10^6 CFU/ml inhibits the development of such germs. The winemaker's second motivation for using yeast starters is to improve the last phase of alcoholic fermentation. Indeed, grape musts are so high in sugar yet low in critical nutrients that yeast cannot survive long enough to ferment all the sugars. One of the most serious issues in winemaking was stuck fermentation. As a result, the use of chosen yeast starters allows for improved process control as well as effect on the sensory and sanitary quality of wine (Lonvaud, 2002).

2.8 Alcoholic fermentation

The anaerobic transformation of carbohydrates, primarily glucose and fructose, into ethanol and carbon dioxide in the presence of nitrogen compounds is known as alcoholic fermentation. Fruit juices have the greatest sugar content of all the substrates used to produce ethanol by fermentation. As a result, the ethanol level is among the highest ever recorded, and the significance of substrate and ethanol inhibition. This procedure, which is carried out by yeast and bacteria can be summarized by this overall reaction:

$$\underset{\text{Glucose}}{C_{12}H_{12}O_6} \xrightarrow{\text{yeast}} \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

Fortunately, alcoholic fermentation is a far more difficult process. At the same time as this overall reaction occurs, several additional biological, chemical, and physicochemical reactions occur, allowing grape juice to be converted into wine. Other chemicals formed during alcoholic fermentation, in addition to ethanol, include higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin, and 2, 3-butanediol. Simultaneously, some compounds of grapes juice are also transformed by yeast metabolism. Wine would have little organoleptic appeal if these additional compounds are not produced (Zamora, 2009).

2.8.1 Biochemistry of alcohol fermentation by yeast

Saccharomyces metabolizes glucose and fructose to pyruvate via the glycolytic pathway in wine. One molecule of glucose or fructose produces two molecules of ethanol and two molecules of carbon dioxide. The common term for the enzyme found in yeast was Indeed, yeast has several enzymes, including invertase, which was required to divide the cell sucrose is broken down into its constituent sugars (glucose and fructose). The mechanism of the metabolic pathway from glucose and fructose to ethyl alcohol is well understood; the conversion occurs predominantly through the Embden-Meyerhof glycolytic pathway oxidation to pyruvate, then to acetaldehyde and ethyl alcohol. Yeast cells require a consistent supply of ATP (adenosine triphosphate) together with the reducing power of NADH (nicotinamide adenine dinucleotide). Succinates, glycerol, acetoin, diacetyl, acetic, and succinic acids are formed because of metabolic intermediates. Notably, the synthesis of alcohol during fermentation aids in the physical extraction of various chemicals (for example, Terpenes) from grape cells, which appear in fermented wine (Clarke and Bakker, 2004).

The bacterium employs the EMP route, which produces 2 ATP per mole of glucose converted to ethanol + CO_2. The ultimate product, ethanol, was a major metabolite. The essential goal in industrial fermentation is to sustain the Crabtree effect throughout the fermentation. Figure 2.1 depicts a shortened version of the metabolic process for ethanol production.

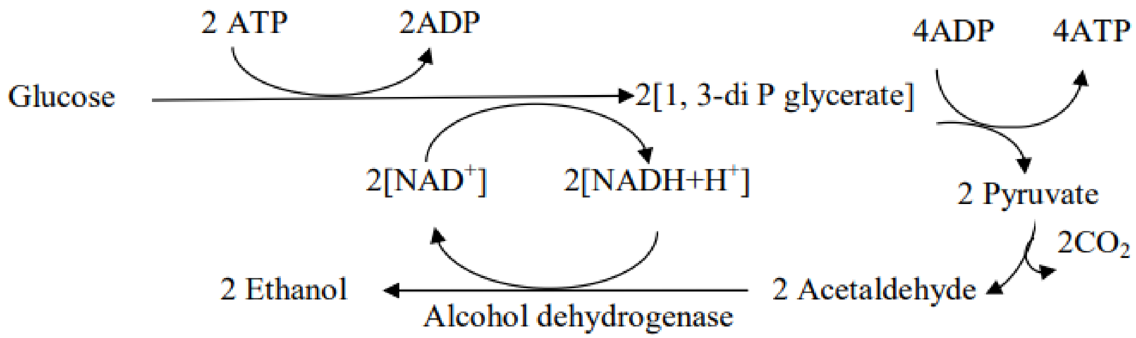


Fig. 2.1 Simplified pathway of alcohol synthesis by yeast

Source: Rai (2012)

2.8.2 Stoichiometry

Ethyl alcohol is the byproduct of the alcoholic fermentation of sugar by the enzyme *zymase* in yeast. One molecule of glucose produces two molecules of ethyl alcohol and carbon dioxide during alcoholic fermentation.

2.8.3 Malo-lactic fermentation

Malolactic fermentation (MLF) in wine is the enzymatic conversion of malic acid to lactic acid, a secondary process that typically occurs after primary (alcoholic) fermentation but may potentially occur concurrently. However, this conversion of malic acid to lactic acid is not a real fermentation (Costantini *et al.*, 2009). MLF is caused by the metabolic activity of specific lactic acid bacteria and leads to the conversion of malic acid to lactic acid. The bacteria may also influence the wine's taste and fragrance. Although spontaneous MLF can develop owing to bacteria naturally present in musts and wines, customized starting cultures of bacteria are increasingly often utilized since they allow for more control over the process and more consistent outcomes (Osborne, 2010). MLF is mostly carried out by *Oenococcus oeni,* a species that can survive wine's low pH (<3.5), high ethanol (>10 vol%), and high SO_2 levels (50 mg/L). More resistant *Lactobacillus, Leuconostoc*, and *Pediococcus* bacteria can also develop in wine and contribute to MLF; particularly if the wine pH surpasses 3.5. Malo-lactic fermentation should be avoided in wines with low acidity: wine quality down if the acid levels become to loo as well uncontrolled MLF also presents a risk of wine spoilage by compounds that can produce off-flavors (including acetic acid, volatile phenols and mousiness) or that may be hazardous to human health (Costantini *et al.*, 2009).

Malo-lactic fermentation can readily be avoided by early racking, cold storage, and keeping SO_2 levels at 100 ppm or above. If such fermentation is desired, it can be aided by keeping the wine on the *lees* (yeast sediments) for extended periods of time at higher temperatures. This storage leads yeast cells to lyse, releasing amino acids and other resources required for the 'contaminant' lactic acid bacteria to proliferate. This fermentation is especially beneficial if the wine's titratable acidity had to be lowered. Malolactic fermentation has a significant impact on wine quality. It is a natural method of lowering acidity in wine (Rai, 2009).

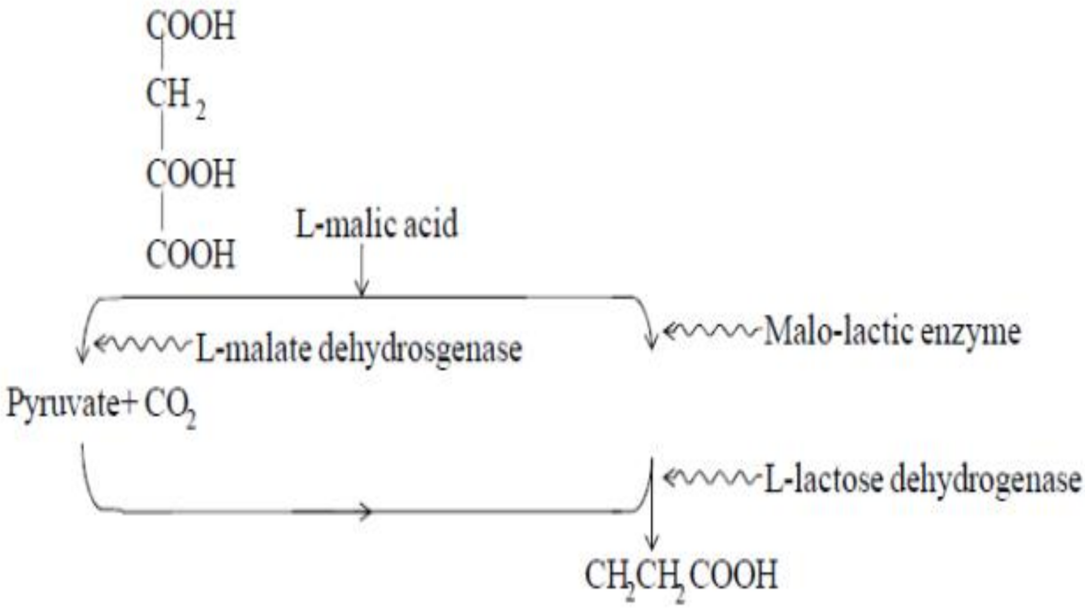


Fig. 2.2 The malolactic pathway

Source: Rai (2012)

2.9 General cultural condition for alcoholic fermentation

The term "cultural condition" refers to the environment of yeast, i.e. fermentative medium, on which yeast propagation and final wine quality are heavily reliant (Reed, 2004)**.** Following are the few parameters, which determine cultural condition of the fermentative media.

2.9.1 pH

The pH of wine is important not just for its flavor but also for almost every other characteristic of the wine. Flavor, fragrance, color, tartrate precipitation, carbon dioxide absorption, malolactic fermentation, stability, agility, and fermentation rate might all be affected by pH of the must. Furthermore, the pH of wine can impact several chemical processes. The optimal pH for wine production varies depending on the kind of fruit and the type of wine that should be produced; a pH range of 2.8 to 4 covers most wines (Butzke, 2010). The content of glycerin increases during fermentation at higher pH levels, whereas log phase had a considerable influence at lower pH levels (Reed, 2004). Higher than 4.0 are generally avoided as spoilage is more likely to occur above this level. Many wine makers keep wine pH below 3.65 (Rotter, 2008). Many preservatives, such as sulfur dioxide and sorbic acid, work better when the pH is low. The most typical way to modify the pH of must is to add acids such as malic, citric, and tartaric acid because it is a stronger acid, tartaric acid is the most commonly prescribed acid for must modifications than malic and citric acid and less vulnerable to microbial degradation during fermentation as well as the alcoholic and malolactic fermentations (Butzke, 2010). Wine is a very buffering drink. This indicates that the pH reduction associated with a given quantity of titratable acid (added acidity) is not directly proportional. Furthermore, because each wine was buffered somewhat differently, the change in pH for a given titratable acidity increase/decrease is unique to each specific wine. However, adding 0.5-1 g/L acid as tartaric tends to lower the pH by roughly 0.1 unit on average (Rotter, 2008).

2.9.2 Temperature

Temperature has a significant impact on fermentation. Above 38°C, the yeast will almost surely be destroyed; below that temperature, it will ferment extremely slowly. In general, the temperature of primary fermentation should be 20°C, the temperature of secondary fermentation should be 15°C, and the temperature of finished wine storage should be 10°C (Berry, 1996). The optimal fermentation temperature is determined by the kind of wine produced. The temperature for white wine is 10-15°C, while red wine is 20-30°C. If the fermentation is carried out at a higher temperature, it may become trapped. Low temperatures, on the other hand, may cause fermentation to begin later. The loss of alcohol and scent compounds occurs at high temperatures. In addition, a substantial number of byproducts such as glycerol and acetaldehyde may be generated. An imbalance of these components might have a negative impact on wine quality. It has been found that the generation of higher alcohol reduces with increasing temperature. The benefit of the lower temperature are the fresher and fruitier the flavor of wine, lower the ethanol losses and a lower risk of creating volatile acidity (Reed, 2004).

2.9.3 Sugar concentration

The 'must' has a high sugar concentration, which causes high osmotic pressure, which has a detrimental effect on yeast cells since both yeast growth and fermentation activity are reduced. In terms of total soluble solids, the optimal sugar concentration is 20-25°Bx. The tolerance of greater sugar concentrations varies by yeast species (Reed, 2004).

2.10 General method of wine preparation

The skin of the grapes contains wild yeast and other microorganisms, which flow into the delicious pulp (known as must) when the fruit is crushed. These are eliminated by introducing the appropriate amount of Sulphur dioxide (or KMS). If the sugar concentration is low, sucrose is added to the appropriate strength, and tartaric acid is added to correct the pH to 2.8 to 4. Following that, a pure culture of actively developing yeast (*S. ellipsoideus*) is introduced into the must. The temperature and time of fermentation are determined by whether the wine is dry or sweet. Fermentation takes 4 to 10 days on average. When fermentation is finished, the clear wine is drained from the yeast sediment into barrels (racking) and aged. Secondary fermentation occurs currently, and the wine loses its rough and harsh taste and softens. Clarification occurs naturally throughout this maturation phase. It can also be accomplished by fining and filtering. Following that, the wine is bottled and left to develop for a number of year, depending on the quality sought (Mmegwa, 1987). Figure 2.3 depicts a simplified flowsheet for wine processing.

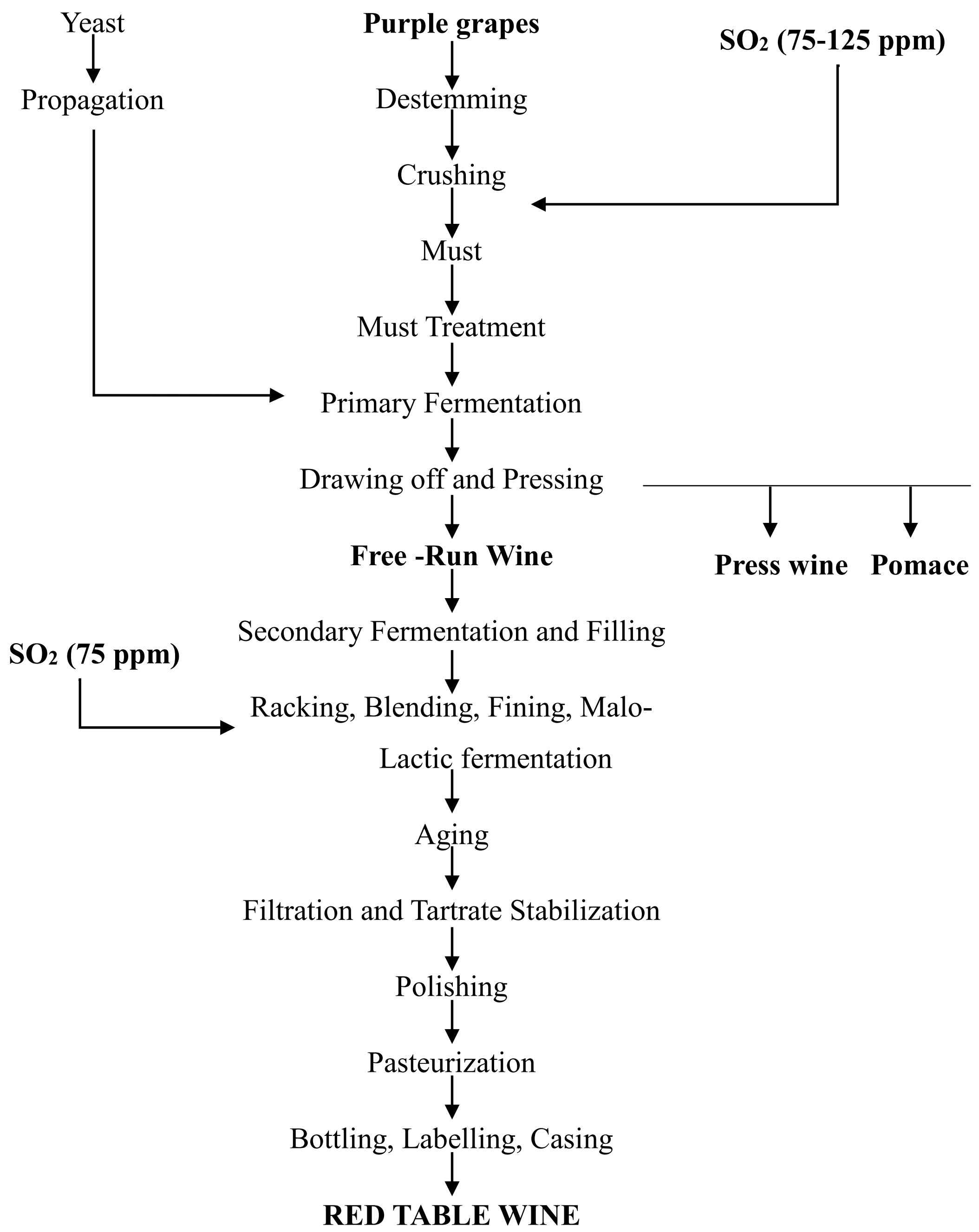


Fig. 2.3 Flow chart of red table wine preparation

Source: Rai (2012)

2.10.1 Selection of raw materials

As a substrate, any acceptable raw material is utilized. Fruit juices, as opposed to cereals, are a more easily used substrate by yeasts for alcoholic fermentation. The latter is also a good medium for yeast growth (Varnam and Sutherland, 1994). When selecting appropriate raw materials for fermentation, the following requirements should be met (Reed, 2004).

- It should be easily accessible.
- It should be an excellent carbon and nitrogen source.
- It should have an enough amount of fermentable sugar.
- It should not contain any harmful compounds and should not have an unpleasant odor or flavor.
- It must be clean, solid, and mature.

2.10.2 Crushing and blending

This is done to remove the juice from the fruit. After the stems that support the fruits have been removed, selected ripe grapes are crushed to release the 'must' juice. These stalks contain tannins that, if left in the must, would give the wine a harsh flavor. The skin contains most of the components that give wine its fragrance and color. Purple grape peels are used in the manufacturing of red wines to provide color (Okafor, 2007).

White grapes are collected from vineyards and transported to the winery, where they are fed through a destemmed crusher equipment. There are three types of crushers that are often used: There are three types of rollers: rollers, disintegrators, and girolles. The latter is more commonly used (Rai, 2009). It has been recommended that the procedure be simple gentle. When the blending and crushing equipment is made of mild steel or cast iron, Iron creates "ferric cause-cloudiness" in wine; iron will really react with the tannin in the juice combines to generate a ferric-tannin complex. Bronze equipment is also utilized, but it is not as common. Copper and tin breakdown from bronze equipment will influence the hue. The crushing machine is typically made of stainless steel. Water may be added at any time. blending/crushing to ensure smooth functioning (Prescott and Dunn, 2004).

Must is the grape juice used for wine fermentation. Consistent wine quality necessitates consistent must quality. If the must does not satisfy the criterion, grape juice concentrate, sugar, acid, and other ingredients must be added to make up the difference. This process of standardizing the must is known as amelioration (Rai, 2009). Following method can be used as per requirement:

Chaptalization: Chaptalization is another word for the addition of just sugar. Sugar addition is thought to make inferior wine and is outlawed in several places. Grapes grown in colder climates sometimes lack sufficient sugars to make a balanced wine. Chaptalization, or the addition of sucrose to the must or juice during the early stages of fermentation, can help with this. Instead of sugar, concentrated grapes must be utilized in some countries.

Gallization: Gallization is a phrase that refers to the addition of water and sugar prior to fermentation to raise the alcohol level, total volume, and acidity.

Acidification: This may be required if the pH of the must is too high, indicating that the acidity is too low. The most common approach is to add tartaric acid, malic acid, or citric acid, or a combination of these acids known as acid blend.

De-acidification: If the pH of the must is too low, this may be essential. It is not authorized in the European Union's warmer areas. There are several materials that may be used, including Calcium carbonate ($CaCO_3$), potassium bicarbonate ($KHCO_3$), and potassium carbonate (K_2CO_3) (Grainger and Tattersall, 2005).

2.10.3 Must sulfiting

Sulfur dioxide (SO_2) has been used as an antibacterial and antioxidant ingredient in winemaking for thousands of years. It is quite successful in these functions, is widely available, and is reasonably inexpensive and simple to use. The primary function of sulfur dioxide is to prevent microbial infection of the juice, hence preventing undesired or spontaneous fermentations by yeasts other than those intended by the winemaker and infections by undesirable bacteria (e.g., *Acetobacter, lactobacillus*). Sulfites in wine come in three varieties. Microbes are inhibited by molecular sulfur dioxide and bisulfite. The sulfite ion (SO_2^{2-}) is primarily in charge of preventing oxidation (Ritchie, 2010). SO_2 is introduced prior to fermentation to prevent air from oxidizing the juice and turning it to vinegar. The air contains bacteria, mostly *Acetobacter*, which are alive in the presence of oxygen. These *Acetobacter* are unable to turn alcohol into vinegar because SO_2 consumes oxygen from the must to allow the wine yeast to convert the fruit sugar into alcohol in an anaerobic environment. SO_2 also produces a layer on the surface of the juice, preventing air from entering the liquid (Andrew, 1980).

Sulfur dioxide can combine with molecules other than oxygen found in musts, such as anthocyanin and acetaldehyde (acetaldehyde having negative organoleptic qualities), to generate 'bound' SO_2, which cannot prevent microbial spoilage or oxidation. As a result, when we add sulfur dioxide to juice or wine, not all of it will be accessible to preserve the wine (depending on how it is distributed throughout the different forms), complicating the decision on how much to apply. In fact, we must estimate how much will be in the bound form to ensure that there is enough molecular SO_2 (Ritchie, 2010). Potassium metabisulfite (KMS) is the most often utilized SO_2 source. In general, SO_2 is rarely employed at concentrations more than 150 ppm. Moldy grapes, on the other hand, may require 200 ppm. Higher SO_2 concentrations cause fermentation to be delayed (occasionally for up to two months) (Rai, 2012).

2.10.4 Yeast

Wine yeasts are members of the *Saccharomyces* genus and so have a high individual relevance (Austin, 1968). A high-quality wine yeast should contain the following characteristics (Varnam and Sutherland, 1994).

i. The use of flocculation and the lowering of H_2S generation.
ii. Higher alcohol production has been reduced.
iii. Fermentation efficiency has been improved.
iv. There is less foaming.
v. Resistance to homicidal activity.

2.10.4.1 Yeast nutrition

Proper nutrients are required for yeast development in culture medium. As a result, the culture medium utilized must include all the necessary components for growth in proportions identical to those found in yeast biomass. Table 2.3 shows the elemental need (as well as the supply) for yeast nutrition.

2.10.4.2 Pitch development

The use of active dry yeast (ADY) in winemaking has expanded significantly in the last 20 years or more. In many wineries, it has superseded the conventional usage of yeast starters. A juice is heavily sulfited (10 g/hl) to eliminate spoilage yeasts while encouraging the development of wine yeasts. It is then injected into a newly filled fermenter with a concentration of 1-3% after many days of spontaneous fermentation. Before preparing the must, a suitable quantity of pitch is generated. The development medium should have a low sugar content to retain the ‘Pasteur effect’. Pitching is done when the pitch culture is at its peak of development. After pitching, vigorous agitation is used to assist spread the culture and aid in their early development (Grainger and Tattersall, 2005).

Table 2.3 Elemental requirement and source for yeast nutrition

Element	Major source
Carbon	Sugar
Hydrogen	Water, organic compound
Sulphur	Na_2SO_4, $Na_2S_2O_3$ and organic sulfur compound
Oxygen	Water, dissolved oxygen, organic compound
Magnesium	$MgCl_2$
Nitrogen	Inorganic source: NH_4Cl, $(NH_4)_2SO_4$
Sodium	NaCl
Phosphorus	KH_2PO_4, Na_2HPO_4
Calcium	$CaCl_2$
Iron	$FeCl_3$, $FeSO_4$

2.10.5 Fermentation

The soul (heart) of winemaking is fermentation because all the favorable responses occur during this stage, so most winemakers pay close attention to it. Fermentation is the process of converting natural sugar to ethyl alcohol by introducing wine yeast (officially known as *S. ellipsoidues*) to fresh juice. CO_2 is emitted concurrently in this process, causing fermentation to be furious at first and gradually slow. The yeast used is 1-3% of the juice volume. In most cases, total alcoholic fermentation takes 14 days. The fermentation process is divided into three phases.

- A preliminary stage during which yeast cells proliferate.
- An extremely active stage characterized by bubbling and a significant rise in temperature.
- Fermentation is quiet and can go on for a long period at a slower and slower rate.

Fermentation period can range from 2 to 20 days depending on a variety of factors such as the kind and condition of the grapes, the type of wine being created, and the meteorological conditions, climatic condition among others. The fermentation process is very dependent on temperature (Karki, 2019). Red wine requires a greater temperature for fermentation than white wine. The ideal temperature is thought to be 21.1-27.4°C (Johnson and Peterson, 1974). Wine aroma and fragrance are likely to be harmed at temperatures exceeding 90°F (32.2°C). Heat-tolerant bacteria are also encouraged to create acid, mannitol, and off-flavor (Karki, 2019).

Johnson and Peterson (1974) reported the normal total sugar level of 19-24%, alcoholic fermentation progresses fast and to completion with alcohol tolerant yeast strains, generating around 10-12.5% alcohol (by volume). If the sugar amount exceeds 24%, the high sugar content may hinder fermentation, resulting in delayed and perhaps incomplete fermentation. Under appropriate simulation conditions, 16-18% alcohol may be obtained. It is widely accepted that methanol is not created by alcoholic fermentation, such as glycine, but rather from the hydrolysis of naturally existing pectin. When ammonium phosphate is introduced before fermentation, the number of higher alcohols generated is reduced. Higher alcohols may play a favorable effect in sensory quality at extremely low concentrations (Amerine *et al.*, 1980).

Guymon *et al.* (1961) shows that higher alcohol production is favored by oxidative conditions during fermentation. According to Wang *et al.* (2001), low temperature, high tartaric concentration, and SO_2 addition all promote glycerol formation. Most of the glycerol is formed during the early stages of fermentation. Because of its sweet taste and oiliness, most enologists believe that glycerol is of significant sensory value. Acetaldehyde is a naturally occurring byproduct of alcoholic fermentation. Acetaldehyde combines with ethyl alcohol to create acetal, a chemical with a strong aldehyde-like odor that is present in very small amounts in wines (Amerine *et al.*, 1980).

The tartaric, malic, and citric acids contained in the must are present in the resultant wines, albeit in lower concentrations. They are vital wine ingredients not just for their acid flavor, but also because they preserve the wine from spoiling, keep the color, and are occasionally attacked by bacteria. Malic acid disappears to the range of 10 to 30% during alcoholic fermentation. Alcoholic fermentation produces succinic acid. Lactic acid is a weak acid with a little odor. It is a continuous byproduct of alcoholic fermentation, with concentrations ranging from 0.04 to 0.75 g/L (Amerine *et al.*, 1980).

The end of fermentation is indicated by a clear liquid, a vinous flavor and aroma, and a decline in temperature, and may be confirmed by monitoring degrees Brix° (residual sugar) (L. Pokhrel, 2018).

2.10.6 Racking

After fermentation is complete, the wine must be removed from the dead cells because they can induce yeast autolysis and, at low redox potential, the creation of H_2S, which gives off tastes and aromas to the wine. Racking is the act of moving juice or wine from one container to another while leaving any sediment behind. Racking, or siphoning, is a key element in making clear, stable wine (Grainger and Tattersall, 2005). The advantages of racking are:

i. It aids in CO_2 removal.
ii. It increases the O/R potential, which slows the generation of H_2S.
iii. It clears the wine.

Normally, wine should be racked within a month following fermentation completion. Racking often results in a 2-3% loss of wine in lees (Rai, 2009).

2.10.7 Fining and filtration

Fining is the process of making murky wine clear. After the coarse sediment has been removed by racking or centrifugation, the wine has additional lighter materials floating in it known as colloids. If they are not removed, the wine will seem ‘hazy’ and a deposit will form. These colloids can be removed by adding other colloids of opposite charge. For example, egg whites, gelatin, isinglass, and bentonite. Fining may also be used to eliminate excess tannin from a wine, improving its flavor. The chemical PVPP (polyvinyl polypyridine) absorbs phenolic compounds. This can be used during the fining process to remove color from white wines and avoid browning (Grainger and Tattersall, 2005). However, before to use, the fining agents must be checked for dose optimization since over fining might result in permanently hazy wine (Rai, 2009).

Filtration is the process of removing solid particles and can occur at several stages of winemaking, such as must or lees filtration. However, one of its primary uses is in the preparation for bottling. There are three types of filtrations that may be utilized at various stages of the winemaking process.

Earth filtration

This filtering process is used for first rough filtration and may remove considerable amounts of "gummy solids" from grapes, which consist of dead yeast cells and other debris. The filtering process is divided into two steps. To begin, kieselguhr, a coarse grade earth frequently employed as the filter medium, is placed on a supporting screen within a filter tank. To create the filter bed, a combination of water and kieselguhr might be employed. This is referred to as precoating. Second, more earth is combined with wine to create a slurry that is used to renew the filtration surface through which the wine travels on a constant basis. The wine is filtered, and the depth of the bed steadily grows (Grainger and Tattersall, 2005).

Sheet filtration (plate and frame filter)

A frame holds a series of precisely designed perforated steel plates. Filter medium sheets (cloth or paper) are strung between the plates, which are subsequently pushed together using screw or hydraulic means. The filter sheets are available in a variety of porosity levels. Filter aids such as hyflosupercel, diatomaceous earth, and others are used to make the filtration process easier. Wine is pumped between pairs of plates, where it passes through the filter sheets and into a hollow in the plates before exiting the system. Yeast cells and other debris are caught in the filter media's fibers (Grainger and Tattersall, 2005).

Membrane filtration

Microfiltration membranes are typically tubular in shape for use with wine. Although pre-filtration is not needed, clarifying and stabilizing chemicals such as bentonite are required to ensure an adequate product flow. The microfiltration system has a somewhat high initial cost; however, this is countered by its operational efficiency, dependability, and adaptability. Maintenance and cleaning expenses are likewise minimal (Varnam and Sutherland, 1994). The membrane acts as a molecular sieve, allowing water, ethanol, taste compounds, chosen macromolecules, and other dissolved species to pass through while retaining suspended material such as colloids and microbial cells. They also significantly lower the number of bacteria. The method is not recommended for full-bodied red wines since it might diminish body and character (Grainger and Tattersall, 2005).

2.10.8 Stabilization of wine

Stabilization can be done after the wine has been bottled to prevent tartrate crystals from developing. The tartrates are either potassium or calcium salts of tartaric acid, and the crystal is also known as wine diamonds. They are completely safe. They can be found on the cork or as sediment in the bottle and might cause customers to be concerned. To prevent tartrate crystal formation in the bottle, the wine is refrigerated to -4°C, or colder in the case of liqueur (fortified) wines. After around 8 days, the crystals will have formed, and the wine may be bottled. The entire process of removal takes just 24 h or so (Grainger and Tattersall, 2005).

2.10.9 Maturing and ageing of wine

This is one of the most interesting and crucial processes in winemaking, as well as one of the most difficult. Newly fermented wine is hazy, harsh in flavor and odor, and lacks the agreeable aroma that emerges later in its life (Rai, 2009). Maturation is the period and accompanying changes that occur in a wine between alcoholic fermentation and bottling when the wine is still in bulk storage in the production facility. The period in a wine's existence following bottling and before consumption should be referred to as bottle ageing', but for the sake of this discussion, it will simply be referred to as 'ageing' (Buglass *et al.*, 2011).

Wines may taste harsh and sour immediately after fermentation. A maturing phase is necessary. Depending on the kind of wine being created, this stage might last anywhere from 2 to 24 months or longer, and could include operations such as malolactic fermentation, oak coopering, racking, ageing in tanks or barrels, fining, and filtering (Buglass *et al.*, 2011). Wine aging increases taste and fragrance through oxidation and ester production. These esters of higher acids generated during aging give the well-aged wine its ultimate appealing fragrance (Clarke and Bakker, 2004).

2.10.10 Bottling

After filtering and clarifying, the wine is passed to storage tanks until bottling. Glass bottles are universally used for high-quality wine. For this reason, bottles are cleaned, dried with hot air, and cooled. The traditional method of sealing the bottle is with a cork, which is protected against dehydration and mold development by a lead foil or, in recent years, a plastic outer cap. To prevent oxidation, wine is bottled in an inert environment (CO_2 and/or nitrogen). Before bottling, additions may be used to stabilize the wine against microbiological and chemical degradation; SO_2 and sorbic acid are the most regularly utilized (Varnam and Sutherland, 1994).

2.10.11 Pasteurization

Pasteurization is the technique of killing microbes in wine to stop fermentation and enhance shelf life. Wine pasteurization is often done for shorter periods of time or at lower temperatures than milk pasteurization. This is the case probably because of the wine's low pH and ethanol concentration, both of which significantly lower the yeast and bacterium heat resistance. And approximately 10 min at 60°C should be enough for a wine with 11% ethanol. Flash pasteurization at 80°C normally takes only a few seconds, and hot bottling of wine at temperatures ranging from 55 to 70°C is also possible. Sulfur dioxides reduce still further the requirement for heating. High temperatures significantly increase the amount of free SO_2 in wine. Although pasteurization kills most microorganisms, it does not inactivate *Bacillus* endospores. These bacteria can cause wine deterioration in rare instances. Pasteurization lowers the quality of some wines while improving the quality of others. Pasteurization kills the enzymes but degrades the product's quality. Membrane filters have mostly replaced pasteurization in most instances due to the complexity of determining the most acceptable time and temperature conditions for pasteurization. Filters also cause little physical or chemical changes in the taste properties of wine. Membrane filters having particle sizes of 0.45 m or smaller are commonly used (Jackson, 2014).

2.10.12 Storage of wine

Wine storage is an important concern for wine that is preserved for long-term aging. Temperature, light, and humidity are some of the most direct influences on the condition of a wine. The ideal storage temperature for wine is 11°C, although anything between 5°C and 18°C would suffice for most kinds of wine. Most wines should be stored horizontally so that the cork remains wet and hence completely inflated and airtight. Sparkling wines and wines sealed with a screw top cap are exceptions to this rule. Wines should also be stored in vibration-free circumstances, but this is only important for sparkling wines and mature wines with sediment over a lengthy period of time (Stevenson, 2005).

2.10.13 Yield

The potential conversion of 180 g of sugar into 88 g of carbon dioxide and 92 g of ethanol results in a weight-based ethanol yield of 51.1%. This proportion may vary based on the size of the inoculums, fermentation temperature, and nutrient availability (Usansa, 2003). Under certain simulation conditions, 16-18% alcohol may be obtained, however in commercial operation, 13-15% is the maximum (Johnson and Peterson, 1974).

2.11 Wine analysis

With the advancement of technology and growing governmental control, analytical procedures have been increasingly significant in the history of wine manufacturing. Wine analysis is done for a variety of purposes, including quality control, spoilage reduction and process improvement, blending, export certification, and worldwide legal requirements (Fugelsang, 1996).

2.11.1 Physical and chemical analysis

All wines should be submitted to suitable analysis during production and storage to fulfill regulatory agency requirements and to provide the winemaker with information to appropriately manage operations (Fugelsang, 1996).

Additional analysis is frequently required for experimental wines to acquire more thorough information and examine the precise impacts of the experimental settings. Experiments are pointless unless analytical procedures are available to analyze the results. Planning for this analysis, as well as the effort and duration involved, should occur prior to the start of the studies. Some analysis can be performed at leisure on the finished wine, while others must be performed at specified times, or the experiment would be ruined. Interim samples can sometimes be promptly frozen and saved for subsequent analysis as a group. Other times, this is not feasible due to experimental or logical constraints (Boulton *et al.*, 2013). The components and must can be broken into classes and are given in Table 2.4

According to Amerine *et al.* (1980), the several parameters viz. alcohol by volume,(%), alcohol, glycerol, ash, total acids, volatile acids, reducing sugars, proteins, tannins and specific gravity of various wines were analyzed. According to Pearson (1976), Specific gravity, alcohol (g/100, total solids, free volatile acids (as acetic acid), fixed acid (as acetic acid), ash, and sugar were the analytical characteristics of distinct wines.

The criteria such as pH, TSS, alcohol concentration, acidity, reducing sugar, aldehydes, esters, specific gravity, total sugars, ash, methanol, and higher alcohols were largely investigated in several dissertations connected to wine held at Central Campus of Technology, Hattisaar, Dharan (Raut, 2014).

Table 2.4 Component of wine

Components	Composition
Soluble solids	Sugar extract, glucose and fructose
Acidity	Total volatile, pH and individual acids
Alcohols	Ethanol, methanol, fusel oils and glycerol
Carbonyl compound	Acetaldehyde and HMF diacetyl
Esters	Ethyl acetate and methyl anthranilate (labruscana)
Nitrogen compounds	NH_3, amino acids, Amine's and proteins
Phenolic compounds	Total phenolic fractions including anthocyanins
Chemical additions	SO_2, sorbic and benzoic acids illegals
Other	Common and trace metals, oxygen, CO_2, fluoride

Source: Fugelsang (1996)

2.11.2 Sensory evaluation

2.11.2.1 Development of sensory evaluation

Sensory tests have, of course, been carried out for as long as humans have been judging the goodness and badness of food, drink, weapons, shelters, and anything else that may be used and ingested. The development of trading prompted more formal sensory testing. A buyer would evaluate a tiny sample of a shipload, expecting that a section would reflect the total. Sellers began to base their prices on an assessment of the quality of items. With the passage of time, ceremonial methods for grading wine, tea, coffee, butter, fish, and meat evolved, some of which are still in use today. In the early 1900s, grading gave rise to the professional taster and adviser to the burgeoning industries of foods, drinks, and cosmetics. A literature arose that utilized the term "organoleptic testing" to refer to the ostensibly objective measuring of sensory qualities. Testing was frequently subjective, tasters were insufficient, and interpretations were susceptible to bias. Scientists created sensory testing as an organized, structured, and 34 defined process just recently, and they continue to develop new procedures and enhance current ones (Meilgaard *et al.*, 1999).

Sensory evaluation is an integrated, multidimensional measure with three significant advantages: it detects the presence of significant differences, quickly identifies, and quantifies important sensory characteristics, and identifies specific problems that other analytical procedures cannot detect. Economic interests are served by the methodologies that have been established. Sensory testing may determine the value of a product or even its acceptance. Sensory testing compares several courses to see which one provides the most value for money. Sensory approaches are mostly used in quality control, product development, and research. The basic goal of sensory testing is to conduct accurate and reliable tests that give evidence for making sensible judgments (Meilgaard *et al.*, 1999).

2.11.2.2 Sensory evaluation of wine and importance

Sensory evaluation, defined by the Institute of Food Technologists as 'a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as perceived by the senses of sight, smell, taste, touch, and hearing,' has become a popular research tool in the food and beverage industries.

Product formulation alterations may result in desirable or undesired changes in the product, which must be examined, analyzed, and interpreted in a meaningful way. It is difficult to analyze sensory responses and make rational and reasonable decisions without the correct sensory assessment tools. Even the most advanced chemical analysis cannot now, and probably never will, pinpoint the nuanced nuances that distinguish one wine from another in the eyes of discerning consumers. That is exactly as it should be. As a result, in addition to chemical and physical procedures, sensory analysis is nearly always required when comparing wines. This is true for commercial wines, but it is especially true for experimental wines (Savits, 2014).

Wine is an extremely complex beverage, containing hundreds of volatile chemicals. The chemicals may be produced by the grape itself, during the crushing and enzyme action process, during fermentation, and during the maturation stage. As a result, the sensory qualities of a given wine are reliant on chemical and physical factors related to the unique matrix or composition (Savits, 2014).

Regardless of what wine writers and some winemakers say, one person's view on a wine's sensory character and quality is far from decisive. That is not to argue that one tester cannot be superior to another in terms of innate talent, concentrated effort, and so on. The amount of experience and/or comparative memory. In terms of sensory assessment, a panel of tasters is required for one or more wines. This panel should be as sensitive as possible; however, each individual is inconsistent, prejudiced, or unobservant on some levels.as a result of the necessity for panels and statistical analysis of testing findings (Lesschaeve, 2007).

The following is a list of the sequence and technique of wine sensory evaluation.

Color: Wine color performance is an important sensory quality property that influences customer buying intent. Anthocyanins are the most important contributors to fruit wine color (Li *et al.*, 2022)**.** After blooming, plum ripening is characterized by a progressively changing red coloring, both in skin and flesh; this is related to anthocyanin production, which is important for fruit quality and customer preference (Fang *et al.*, 2016). To begin, examine each sample at 30° to 45° on a bright white backdrop. The wine's purity (absence of haze), color (shade or tint), and depth (intensity or quantity of pigment), viscosity (resistance to flow), and effervescence (particularly sparkling wines) should then be recorded individually.

Odor: Sniff each at the lip of the glass before swirling, then examine and note the kind and strength of the aroma. Swirl the glass to encourage the release of fragrant elements from the wine, then sniff it first on the lips and then deeper into the bowl. Now investigate and document the kind and intensity of aroma.

In-mouth sensations: Fill mouth with a little (6 to 10 ml) sample. Move the wine around in mouth, coating the whole surface of your tongue and palate. Note where different taste sensations (sweet, acid, bitter) were felt, when they were initially noticed, and how long they lasted and how their perception and intensity altered. Then, focus on the tactile (mouth feel) astringency, prickling, body temperature, and heat. Take note of this perception, and how they interact with one another (Cheynier and Sarni-Manchado, 2010).

Taste and clarity: Taste in wine is due to fruits juice, sugars, and acids. A good taste makes wine more delicious and refreshing. Sourness is due to organic acids and related to pH of the must. Also astringency perception due to organic acids increases as the pH decreases but does not depend on the concentration or nature of the acid (L. Pokhrel, 2018). Clarity is described in terms of wine's reflective quality. A pronounced haziness may signify spoilage, while brilliant, clear, or dull wines are generally sound (Houtman and Du Plessis, 1981). Contrast these experiences with prior ones. Take note of their personality and experiences**.**

Overall quality: After studying the sensory aspects separately, focus shifts to the integration of their impacts on the wine's overall quality, and lastly, provide an overall judgment of the wine's complexity, subtlety, elegance, power, and balance (Jackson, 2014).

2.12 Color of wine

The color of red wine is first produced from anthocyanin pigments. The fermentation of grapes for wine has a significant impact on the color of the result. The final color may be altered by the SO_2, and alcohol concentration achieved during screening (L. Pokhrel, 2018). Between 3 and 6% alcohol, the most color is removed, and the amount of color extracted rises with increasing SO_2 level up to 250 ppm. The color stability of wines during age was superior at higher SO_2 levels. L. Pokhrel (2018) noted that non-fermented wines fortified with alcohol exhibited much higher color retention throughout age than fermented wines. The color balance between anthocyanogens and anthocyanins is influenced by wine production procedures such as SO_2 levels and alcohol concentration. Colors are frequently associated with specific wines by testers. Young, dry white wines range in hue from practically colorless to light straw. Ascorbic acid is a powerful oxygen scavenger, interacting with O_2 (which would otherwise react with phenolic to induce browning) approximately 1700 times faster than SO_2 (Somers and Evans, 1977).

2.13 Side effects of wines

Various than the consequences of outright abuse or excessive intake, there are various negative impacts of wine drinking. These negative consequences include those caused by allergies and side effects in general, even when exposed to tiny amounts of wine. Many people believe that Sulphur dioxide (SO_2) is the primary cause of wine allergies (Randolph and Moss, 1982).

Sulphur dioxide is listed as 220 ppm on the wine bottle's rear label and has been used as a food preservative since Roman times over 2,000 years ago. It always amuses the author when individuals state they are allergic to wine because of the SO_2; yet, when the author asks whether they consume dried fruit, such as dried apricots, they respond it is OK - oblivious to the fact that dried fruit, for example, includes a lot of SO_2 as a preservative! Most of these people, like most wine consumers, are sensitive to histamines or tannins, both of which come from the skin of the grape and are hence more prevalent in red wines than white wines. However, allergy is idiosyncratic, which means that it is up to the person to determine what they are allergic to. As a result, the ancient adage ‘one man's food is another man's poison’ applies.

In theory, one may be allergic to any of the hundreds of components in wine, and Dr. Theron G Randolph has proposed that alcoholism is a severe kind of food addiction in which the patient is hooked to components other than alcohol (Randolph and Moss, 1982).

2.14 Wine defects and spoilage

Wine, like beer, contains defects produced by non-microbial sources as well as deterioration caused by bacteria. Metals or their salts, enzymes, and agents used to color the wine are examples of defects. Certain molds peroxidase and oxidizing enzymes can cause white wines to become brown and red wines to turn crimson. Gelatin, which is used to clarify wines, can induce cloudiness. Microorganism's primary job in winemaking is to convert grape sugars to alcohol, lower wine acidity, and add fragrance and flavor. They can also produce a slew of undesirable wine spoiling issues, lowering wine quality and value. Wine making procedures contain many stages where microbial deterioration is likely to occur, resulting in a change in the wine's quality and sanitary condition. This may leave the wine unpalatable, as spoilage can include bitterness and off taste, as well as esthetic issues including turbidity, viscosity, sediment, and film development. Yeasts, acetic acid bacteria, and lactic acid bacteria are the most common microorganisms related to wine deterioration (Mojsov *et al.*, 2011).

2.14.1 Wine defect caused by yeast

Yeast has an important role in the deterioration of drinks, particularly those with high acidity and low water activity. Yeasts produce refermentation, ester formation, hydrogen sulfide and volatile Sulphur compounds, volatile acidity, the creation of volatile phenols, mousiness, film formation, deacidification, and the formation of ethyl carbamate in wine (Mojsov *et al.*, 2011).

When the yeast *Schizosaccharomyces* pombe grows in bottled wine and forms a sediment at the bottom of the bottle, it has been linked to wine deterioration. One of the most common wine spoilage yeasts is *Zygosaccharomyces bailli,* which re-ferments juice or wine during storage. The yeasts *Hansenula anomala, Kloekera apiculata*, and *Hanseniaspora uvarum* have been linked to ester taint in defective wines, which coincides with high levels of acetic acid. These three species are linked to grape juice and cause spoiling during the early stages of alcoholic fermentation. Wines with a “Bretty character” are easily identified by olfactory flaws ranging from medical scents to farmyard odors and even spicy clove aromas (Mojsov *et al.*, 2011).

2.14.2 Wine defect caused by bacteria

Bacteria are part of wine's natural microbial ecology and play a vital role in winemaking by decreasing acidity and contributing to fragrance and taste. They can produce a slew of undesirable wine spoiling issues, lowering wine quality and value. The primary bacteria families identified in grape must and wine are lactic acid and acetic acid bacteria (Mojsov *et al.*, 2011).

Aerobic acetic acid bacteria, mainly *Acetobacter aceti* or *Gluconobacter oxydans*, oxidize alcohol in wine to acetic acid in the presence of air, a process known as acetification. They may also oxidize glucose in the must to gluconic acid, imparting a mousy or sweet-sour flavor to the must. When the higher levels of sugar in must or wine are fermented by lactic acid bacteria, varying quantities of CO_2, ethanol, volatile acid, and mannitol are produced depending on the species. Wines that have undergone these alterations are considered to have a lactic acid character (Prescott and Dunn, 2004). Lactobacilli development causes milky cloudiness, increases lactic and acetic acid, and creates CO_2. It occasionally imparts a mousy or other undesirable flavor and degrades the color of the wine (Mojsov *et al.*, 2011).

2.14.3 Prevention of wine spoilage

Winemaking procedures contain several phases where microbial deterioration is possible. The first stage involves the fruit to be processed as well as the equipment to be employed. It is necessary to try to limit the number of microorganisms in the juice and on the equipment. This is accomplished by processing the pulp using food hygiene techniques and according to the hazard analysis critical control point (HACCP) protocol. The second stage of microbial deterioration may develop during fermentation because the fruit juice includes both natural flora of the fruit and flora harbored by the wine cellar and its equipment at this time. Sulphur dioxide has traditionally been used to control undesired microorganisms during winemaking, where it is often put to bins of wine. However, because filtering is normally performed before bottling, it is not utilized to eliminate germs during the winemaking process (Mojsov *et al.*, 2011).

2.15 Nutritional aspects and health benefits of wine

The increased usage of distilled alcoholic beverages, along with religious and political conservatism, resulted in a reaction against all alcoholic beverages. From a scientific viewpoint, researchers have paid far more attention to the non-nutritional features of wine than to what components, other than alcohol, may contain of practical value to the customer. Now, research efforts are mostly focused on better understanding components of processing such as flavor, fragrance, keeping characteristics, better ways to employ, chemistry and biochemistry, and so on (Douglas and Considine, 2012).

According to Louis Pasteur, wine is the "healthiest and most health-giving of drinks". Wine has a lengthy history of usage as a medicinal or as a carrier for drugs. It dates to the ancient Egyptians. Wine was often utilized in herbal infusions in ancient Greek and Roman civilization (Jackson, 2009).

According to Mmegwa (1987) beer and wine have certain nutrients found in the original malted barley and fruit juice used in their proportions, and their energy value is inherently higher than that of distilled liquor; 100 ml of wine has around 80 Kcal. The caloric value of wine is derived mostly from the rapidly digested ethanol component. Alcohol is not digestible and can be absorbed straight through the gut membrane. Wine has historically served as a key source of metabolic energy for the adult population in rural viticulture areas. Wine was a food item in those areas (Jackson, 2009).

Wine includes trace amounts of numerous vitamins, most notably vitamin B including B_1 (thiamine), B_2 (riboflavin), and B_{12} (cobalamin). They found that white wines had more riboflavin, and that the mineral contents of red wine typically outweighed those of white wine, particularly in terms of potassium, sodium, phosphorus, magnesium, iron, strontium, manganese, zinc, copper, barium, and hence total ash. Red wines had somewhat lower calcium and aluminum levels. When wines are consumed in conjunction with a healthy and balanced diet, their thiamine, riboflavin pantothenate, niacin, and vitamin B_6 content contribute to overall nutrition. Although wine, particularly red wine, contains soluble dietary fiber (Jackson, 2009).

Moderate wine drinking (250-300 ml/day) offers unquestionable health advantages, which is becoming increasingly evident. Multiple epidemiological studies imply that moderate alcohol use, particularly wine, is related with a lower risk of death from any cause. This is shown by a U-shaped curve, with higher mortality linked with both excessive alcohol use and abstention. Wine also offers several secondary benefits for meal digestion. Wine promotes a healthy appetite by stimulating the production of stomach juices (Jackson, 2014).

2.16 Wine raw materials

Various fruits are used as raw ingredients to make wine. The phrase 'wine' refers to a product manufactured by alcoholic fermentation of grapes or grape juice, followed by an aging process. Wines are also made by the fermentation of different berries, fruits, and honey. These are identified by the material from which they were created. For example, Perry (pear wine) is made from pear juice, Cider is made from apple juice, and Basi is made from banana juice (Jones, 1995). Plum juice has also been used for wine making by some authors (shrestha, 2015) although literature regarding plum wine is still scarce.

2.16.1 White grape (*Vitis vinifera*)

2.16.1.1 Introduction

A wide variety of therapeutic plants may be found all over the world. Many weeds in our environment are very effective medicinal plants that can assist in the treatment of a variety of significant health issues (Parihar and Sharma, 2021). *Vitis vinifera* is a well-known grape species of the Vitaceae family, belonging to the genus Vitis. There are seedless and non-seedless *Vitis vinifera* varieties in addition to red, black, and white types. The *Vitis vinifera* species exceeds all other species by 90 percent, making it easier to find grapes that originated in Western Asia and southern Europe (Filocamo *et al.*, 2015). White grapes are a very significant agricultural commodity. As a result, one of the most beneficial kinds of agriculture is viticulture, or grape farming. There are around 10,000 distinct grape types worldwide. Phytochemical compounds can be found in the root, stem, cane, leaf, seed, fruits, pomace, and skin. Among the major molecules found are phenolic compounds, aromatic acids, flavonoids, proanthocyanins, and stilbenes (Felhi *et al.*, 2016). White grapes are high in nutrients such as minerals, proteins, carbs, lipids, fiber, vitamin C, and sugar, as well as bioactive substances6-8. Grapes have been proven to have traditional applications in Pakistan, Italy, and Turkey, including laxatives, carminatives, colds and flu, anemia, wound care, allergies, and bronchitis medicine (Hayta *et al.*, 2014). Many studies have shown that grape bioactive compounds have antioxidant, anticancer, antibacterial, antifungal, anti-inflammatory, anti-acne, anti-aging, antihypertensive, protective effect, anti-asthma, antiplatelet, anticataract, anti-obesity, anticholinergic, anti-sunburn, anti-hyperpigmentation, wound-healing properties, and antiviral properties: - Viral infections are caused by harmful viruses spreading throughout the body (Chaudhary *et al.*, 2021). The taxonomic classification is shown below.

Taxonomic classification of white grapes

Kingdom	Plantae
Sub Kingdom	Tracheobionta
Division	Magnoliophyte
Class	Magnoliopsida
Sub Class	Rosidae
Order	Vitales
Family	Vitaceae
Genus	Vitis
Species	Vinifera

Source: Walker *et al.* (2019)

2.16.1.2 Historical background of white grape

White grapes (*Vitis* species) are one of the most significant plant species farmed worldwide, accounting for around 9 million acres. *Vitis vinifera L.* is by far the most widely farmed species. Domestication of *V. vinifera* has place a long time ago, as demonstrated by 5000-year-old seeds discovered in Jericho. Domesticated grapes are said to have originated in the Middle East (Alleweldt *et al.*, 1991). Viticulture extended from its ancestral hub throughout the Mediterranean Basin to the Far East and was eventually introduced to the New World, mainly by settlers from the Mediterranean winegrowing countries.

In ancient civilizations, grapes were a popular agricultural produce. The wine produced was probably the major reason for their appeal. As a result, it's simple to see why the deity of wine and vineyards (Taskesenlioglu *et al.*, 2022). Grapes have a lengthy history and have been consumed by humans for over 6000 years. They have been utilized for millennia in a variety of ways, including directly as food (table grapes), in the creation of juice and wine (wine grapes), and as dried grapes with a longer shelf life (raisin grapes). Grape processing, particularly for wine production, generates a huge quantity of waste, including grape pomace, grape seeds, wine lees, and so on. However, these wastes still include grape components, particularly phenolic compounds, which may give health advantages beyond nourishment. Furthermore, not only does wine production generate a huge quantity of trash, but so do vineyard techniques (vine trimming) (Kandylis, 2021).

2.16.1.3 Grape production

Grapes cover 75,866 square kilometers of the world's land area, according to the Food and Agriculture Organization (FAO). Around 71% of global grape output is utilized for wine, 27% for fresh fruit, and 2% for dried fruit. A part of grape production is used to make grape juice, which is then reconstituted for fruits canned "with no added sugar" and "100% natural". Vineyard land is expanding at a rate of around 2% each year. There are no credible statistics on grape production by variety. Grapes have a significant role in horticulture production (Creasy and Creasy, 2018). It is a highly significant fruit that provides more money to farmers than other crops. Given its significance, the current investigation has been ongoing since May of 2020. The world was the study's universe. The primary goal was to examine global grape production. The top 10 nations in the globe are China, France, the United States, South Africa, Italy, Chile, Iran, Turkey, Spain, and Argentina. In 2017, China produced 13,083,000 tons, while South Africa produced 2,032,582 tons. China's output was at the top, while South Africa's production was ranked tenth in the globe.

Similarly, grapes have various critical components that play an important part in health issues. Wine is made from grapes, which provides additional cash to European countries. It requires relatively little water until it reaches maturity. In the world, there are two sorts of grapes: table grapes and non-table grapes. It also helps with diabetes and blood pressure, and it plays an important part in cardiac diseases. Because more individuals utilize grapes in France, the country has less cardiac issues than other countries (Alston and Sambucci, 2019).

Grape production in Nepal: Grape (*Vitis vinifera*) is a widely grown fruit crop that may be grown in tropical, subtropical, and temperate climates (Nimbolkar *et al.*, 2016). It is a member of the Vitaceae family and originated in Western Asia and Europe (Wium, 2008). *Vinifera* and *Muscadinia* are two common grape varieties that are farmed, with *Vinifera* being the commercially produced variety that originated in central Asia (Atreya *et al.*, 2020). Vineyard appropriateness is commonly determined by topography (elevation, slope, aspect), soil (texture, pH, and drainage), and climatic factors (daily maximum and minimum temperature

Table 2.5 Grapes production of Nepal in the fiscal years 2077/2078 (2020/2021)

Region	Total Area (ha)	Production Area (ha)	Production (Mt)	Yield (Mt/ha)
Eastern	10	3.5	28	8
Central	3	1.5	15	10
Western	5	3	27	9
Mid-Western	0.5	0.1	1	10
Far Western	1.5	0.4	5	12.5
Total	20	8.5	76	9.9

Source: (Atreya *et al.*, 2020)

2.16.1.4 Nutritional value of grapes

Historically, Grapes, like a variety of other foods, have been utilized for medical purposes. However, mounting research has assisted the scientific community in beginning to appreciate the possible health benefits of grapes. Human study data give scientific evidence for grapes and grape products significance in the prevention and advancement of certain illnesses (Vislocky and Fernandez, 2013).

According to the American Dietetic Association and American Diabetes Association, a serving of fresh grapes is one-half cup (equivalent to 65 g) and 1 serving of grape juice is one-third cup (equivalent to 80 mL). One serving of fresh grapes provides approximately 45 calories, 12 g of carbohydrate, 2 g of fiber, 2 g of protein, and a negligible amount of fat (Yadav *et al.*, 2009). According to the few published research, regular consumption of grape products improves blood lipids in hemodialysis patients and healthy persons. In hemodialysis patients, 100 mL of concentrated red grape juice daily for 14 days lowered total cholesterol, LDL (low-density lipoproteins), and apolipoprotein B-100 levels while increasing high-density lipoprotein cholesterol (Vislocky and Fernandez, 2013). In addition to the protective effects mentioned above, other research is being conducted in the early stages to investigate a variety of grape properties that may be relevant to future research in the areas of cardiovascular benefits (such as anticlotting effects, nitric oxide production, and decreased homocysteine levels), protection against the development and progression of certain cancers, protection against Alzheimer's disease, enhanced cognitive and motor function, and protection against oxidative stress. Grapes may also have antiviral properties, according to new research. Furthermore, grape products have been found to be efficiently included into healthy diets of persons without deleterious effects on glucose and insulin levels in diabetics and without weight gain in non-diabetics (Leifert and Abeywardena, 2008). The nutritional composition of grapes (*Vitis vinifera*) per 100g, which is given in Table 2.6.

Table 2.6 Nutritional composition of white grapes per 100g

Constituents	Amount
Moisture	84-88 g
Carbohydrate	18.1 g
Protein	0.72 g
Fat	0.16 g
Fiber	1 g
Sugars	15 g
Vitamin C	3.68 mg
Vitamin K	13.4 mcg
Riboflavin (Vit.B_2)	1.50 mg
Vitamin A	4.6 mcg
Sodium	2 mg
Magnesium	70.44 mg
Calcium	44.69 mg
Iron	5.05 mg
Zinc	2.05 mg

Source: Felhi *et al.* (2016)

2.16.1.5 Health benefits of white grapes

Grapes, the world's biggest single fruit crop, are high in phenolic acids, flavonoids, and resveratrol, all of which have been linked to improved health. Many epidemiological studies, animal studies, and cell culture evidence indicate grapes' health advantages in the prevention of CVD (cardiovascular diseases) and certain cancer (Katiyar, 2008). A case-control research found that increasing grape intake was associated with a lower risk of cancer (Zheng *et al.*, 1993). The data from Chaves *et al.* (2009) demonstrated that a modest intake of fresh grapes (1.25 cups) resulted in significant improvement in brachial artery flow mediated dilation within three hours of consumption compared to the consumption of sugar solution ($p < 0.05$), which was the control. Furthermore, chronic intake of fresh grapes can improve an individual's performance, which supports epidemiological data of the health benefits of grapes.

A two-week dietary intervention research with 25 healthy volunteers was done to explore the effects of apple and grape juice intake on body antioxidant status (Yuan *et al.*, 2011). The results demonstrated that drinking apple and grape juice boosted plasma total antioxidant capacity while decreasing malondialdehyde content. Concurrently, fruit juice consumption increased erythrocyte glutathione peroxidase and catalase activities while having no effect on superoxide dismutase, indicating that drinking apple and grape juice together can promote antioxidant status in the body. Furthermore, grapes not only have a low mean glycemic index and glycemic load, but their phenolics have been shown to have potential for lowering hyperglycemia, improving -cell function, and protecting against -cell loss, implying that grapes may have potential health benefits for Type II diabetics (Zunino, 2009). The anti-inflammatory action of grape phenolics may be responsible for some of the chemo preventive and cardioprotective benefits (Jang *et al.*, 1997).

2.16.2 Plum (*Prunus cerasifera*)

2.16.2.1 Introduction

Plums and prunes are among the fruits of the Rosaceae family, which also includes cherries, apples, peaches, pears, and different berries. The genus prunus, which includes all real plums, is included in the Rosaceae family. There are several varieties of plums, but the two most well-known are European (*prunus cerasifera*) and Japanese (*prunus salicina Lindl*) plums (El-Sharkawy *et al.*, 2016).

Plums (*Prunus cerasifera*) have the potential to be a fresh market and processing crop in many places where they may be picked between cherry and apple seasons. Only approximately half of the plums are consumed fresh; the rest are processed (Chang *et al.*, 1994). Plums are low in calories, in addition to simple sugars, proteins, fats, vitamins, and minerals. There are several identified phytochemicals, including polyphenols, carotenoids, triterpenes, and unstable combinations. Consuming plums has been shown to have several health benefits, including higher levels of antioxidants and antiallergic properties (Igwe and Charlton, 2016). Red plum juice is popular worldwide due to its attractive color, taste, and nutritious value. The nutritional benefit of red plums is mostly due to its phenolic components, which include flavonoids and phenolic acid, which reduce the risk of oxidative damage and help prevent some malignancies (Kim *et al.*, 2003). The taxonomic classification is shown below

Taxonomic classification of plum

Kingdom	Plantae
Sub Kingdom	Viridiplantae
Division	Tracheophyte
Class	Magnoliopsida
Order	Rosales
Family	Rosaceae
Genus	*Prunus*
Species	*p. cerasifera*

Source: Gull *et al.* (2022)

2.16.2.2 Historical background of plum

Various plum species have developed and been domesticated independently across Europe, Asia, and North America. Because *Prunus Cerasifera* and *Prunus Spinosa* originated in western and central Asia, astrological evidence implies that *Prunus domestica* originated in

Europe. The *Prunocerasus* species, including *P. americana*, evolved in North America, whereas *P. Salicina* evolved in China (Milosevic and Milosevic, 2018). The Persian Empire advanced into the Near East and the Mediterranean in line with the commercial routes and historical events that brought it into touch with the fourth century. The initial incursion into Europe would be through Greece and Italy, maybe via Iran or Armenia. The species also moved from the Southern Mediterranean to Spain via the Arabs in the later part of the 7th century. Finally, these fruit trees spread from Europe to North America, Mexico, and South Africa in the 16th and 17th centuries (Salazar *et al.*, 2022).

2.16.2.3 Plum production

A look at the present situation of the world's plum production, market, and trade. China is the world's greatest producer of 7 plums and sloes, with a yearly turnover of USD 10 billion. Romania, Serbia, Chile, and the United States are the next most important countries (Gull *et al.*, 2022).

Plum production in Nepal: The total area, production area, production, and yield of plum in Fiscal Year 2078/79(2021/22) are provided by the Ministries of Agriculture and Livestock Development (MOALD) of all seven provinces, with Bagmati province producing the most and Madesh province producing none, which is given in Table 2.7.

Table 2.7 Plum production of Nepal in Fiscal Year 2078/79(2021/2022)

Province	Total Area (Ha)	Production Area (Ha)	Production (Mt)	Yield (Mt/Ha)
Koshi	468	318	1952	6.14
Madesh	-	-	-	-
Bagmati	264	231	1696	7.34
Gandaki	94	69	486	7.04
Lumbini	355	270	1297	4.80
Karnali	368	260	1420	5.45
Sudurpaschim	163	107	722	6.77
Average production	1712	1255	7573	6.03

Source: MOALD (2022)

2.16.2.4 Nutritional value of plum

The plum, like all fruits, has a low starch content but a high sugar content. The quinic acid and malic acid present in plums are responsible for the fruit's notably acidic taste. Plum is also a rich source of vitamin C as well. Catechin, caffeic acid, chlorogenic acid, rutin, and phenolic acid are the phenolic chemicals that give plum its characteristic astringent effects. Plum has a lot of antioxidants (Gunduz and Saracoglu, 2012). The Nutritional composition of plum (*prunus cerasifera*) per 100g is given in Table 2.8.

Table 2.8 Nutritional composition of plum fruit per 100g

Constituents	Amount
Energy	52 K.cal
Carbohydrate	11.1 g
Moisture	86.9 g
Protein	0.7 g
Fat	0.5 g
Fiber	0.4 g
Calcium	10 mg
Potassium	12 mg
Iron	0.6 mg
Magnesium	7 mg
Zinc	0.1 mg
Vitamin C	9.5 g
Thiamine (Vit.B_1)	0.028 mg
Riboflavin (Vit.B_2)	0.026 mg
Niacin	0.417 mg

Source: Gull *et al.* (2022)

2.16.2.5 Health benefits of plums

Fruit juices are an essential component of a normal person's diet. They are regarded as excellent sources of micronutrients such as vitamins, minerals, and phytochemicals, which have beneficial impacts on one's diet and overall health (Shahbaz *et al.*, 2018). The benefits of plums consumption are discussed below:

1. Plums include isatin, sorbitol, and dietary fiber, which help to keep the digestive tract in control and treat constipation.
2. Plums contain vitamin C, which scavenges free radicals and protects against infectious illnesses.
3. Its low beta-carotene level protects against lung and oral cancer.
4. There are carotenoids present, including cryptoxanthin, lutein, and zeaxanthin.
5. Plums include minerals such as iron, potassium, and fluoride, which promote healthy biological activity.
6. Plums contain modest levels of B-complex vitamins, which help in the metabolism of carbohydrates, proteins, and fats.
7. According to research, plums can help prevent macular degeneration, heart disease, and neurological damage (Birwal *et al.*, 2017).

PART III

Materials and methods

3.1 Material

3.1.1 Raw materials collection

3.1.1.1 Collection of white grapes and plum

Fresh, mature, and ripe white grapes and plum of good quality were collected from Dharan local market. The collected samples were properly packed and taken to the college for further examination.

3.1.1.2 Sodium bicarbonate

The sodium bicarbonate was added to adjust pH of must. It was obtained from the laboratory of the Central Campus Technology.

3.1.1.3 Yeast

Wine yeast was brought from DMC college, Dharan. Wine yeast, *Saccharomyces cerevisiae* (SC 22) manufactured in Canada was used for wine preparation.

3.1.2 Chemicals and apparatus required

The chemicals, glassware, and equipment used for my project were of lab-grade quality and collected from the laboratory of Central Campus of Technology, Dharan and local market of Dharan. The major chemicals, apparatus and equipment required were listed in Appendix A.

3.2 Methods

The total project was based on preparation of plum incorporated grapes wine with varying the composition of plum and grapes juice at constant pH and TSS (°Bx) and analysis of optimized wine.

3.2.1 Experimental procedure

The composition of plum and white grapes juice was prepared by using mixture design in Design of Expert (DOE).

3.2.1.1 Selection of raw materials

Fresh, matured, and good quality plum and white grapes were bought from the local market of Dharan.

3.2.1.2 Washing and disinfecting the raw materials

Plum and white grapes were washed with plenty of potable water to remove the dust, soil, and other unwanted materials. The damaged plum and grapes were segregated from good ones. After washing, the raw materials were submerged in a solution of Sodium hypochlorite and water with the concentration of 200 ppm. This process helps to remove the microorganism content which is still present on the surface of the grapes and plums. And then, wash again with distilled water. Sodium hypochlorite is one of the most common, effective, economical and easy to use disinfectants available (Fukuzaki, 2006).

3.2.1.3 Destemming and seed removal

The grapes were destemmed manually by hand, and they were submerged in KMS solution of 100 ppm to control microbial contamination and wash with distilled water before juice extraction. And the seed of plum were also removed manually by hand. The care should be taken during seed removal to prevent loss of flesh with seed (Bhutani and Joshi, 1995).

3.2.1.4 Extraction of juice and oxidation control

Screw press juice extractor was used for extraction of grapes and plum juice. Thus, extracted juice was susceptible to oxidation hence, immediate action had to be taken to prevent juice from oxidation. According to L. Pokhrel (2018) for this purpose juice was collected in the vessel containing calculated amount of antioxidant i.e. citric acid (0.15g/kg of juice weight) and preservative KMS (150 ppm SO_2 by weight of juice). As a result, the juice came into direct touch with antioxidants, which kept juice free from oxidation, and KMS also regulated microbial loads. For complete separation of juice from solid particle the pulp was recycled twice.

3.2.1.5 Straining of juice

The juice extracted by the screw press juice extractor contains a substantial amount of suspended insoluble material, which must be removed. For this reason, the juice was allowed to filter through a double-folded muslin cloth before being analyzed for TSS, pH, acidity, total sugar, and reducing sugar concentration.

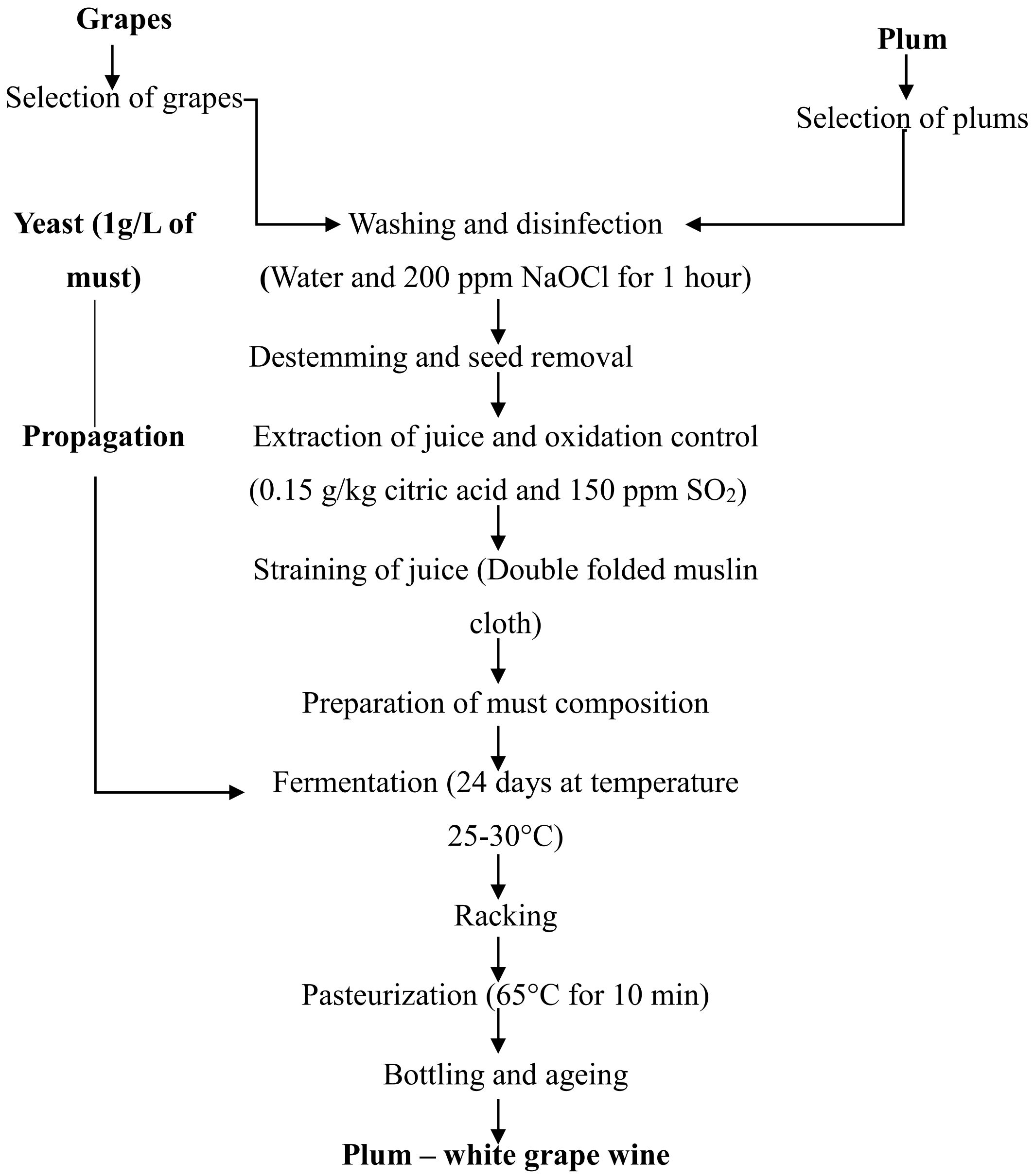
3.2.1.6 Preparation of must composition

After analysis of grapes and plum juice different compositions of must or sample were prepared. The grapes and plum were varied according to the ratio 100:0 as sample A, 75:25 as sample B, 66.7:33.3 as sample C, 50:50 as sample D, 33.3:66.7 as sample E, 25:75 as sample F and 0:100 as sample G respectively. The pH of the raw sample was 3.36 and the final pH of the must was adjusted to 3.9±0.1 by addition of sodium bicarbonate. The final TSS of 25°Bx was maintained by addition of table sugar. The sample prepared for the experiment was presented below Table 3.1.

Table 3.1 Preparation of must

	A	B	C	D	E	F	G
Grapes (%)	100	75	66.7	50	33.3	25	0
Plum (%)	0	25	33.3	50	66.7	75	100
pH	3.9±0.1	3.9±0.1	3.9±0.1	3.9±0.1	3.9±0.1	3.9±0.1	3.9±0.1
TSS(^{0}Bx)	25	25	25	25	25	25	25

Source :Jackson (2014)



Source: Rai (2009)

Fig. 3.1 Flowsheet for preparation of plum – white grape wine

3.2.1.7 Pitching

For pitching, baker's yeast was utilized. It was activated with a moderately heated sugar and water solution, and all musts were pitched at a rate of 1 g per liter. The general flow sheet for procedure is given in above Fig. 3.1.

3.2.1.8 Fermentation

After pitching, the must was stored in plastic bottle for fermentation. The primary fermentation was finished after 5 days of pitching, when the rate of CO_2 evolution increased. The bottle necks were then firmly sealed with cotton plugs for secondary fermentation. The exact process followed in this study was given in Fig. 3.1. It was important to generate an anaerobic environment within the jars during secondary fermentation to improve product quality. The process of fermentation was followed by measuring the drop in degree brix. The fermentation was deemed to be finished when the degree brix dropped below 10°Bx. It takes 24 days from the time of pitching.

3.2.1.9 Racking, pasteurization, and bottling

After fermentation, clear wine was separated from the sediment known as ‘lees’. This was accomplished by inserting a SO_2 treated food grade silicon tube rubber pipe into a sterile glass wine bottle, and the wine was pasteurized by heating the bottle of wine in boiling water for 10 minutes to keep the temperature of the wine at 65°C, and then cooling to room temperature. The cooled wines were racked and placed into pre-sterilized bottles, which were maintained at room temperature until further analysis was required.

3.2.2 Analytical procedure

Even though several authors have detailed various methods and parameters for analyzing juice, must, and wine, only those parameters and associated procedures that were practical in the laboratory were determined in this study. The test was conducted in triplicates. TSS, pH, acidity, and reducing sugar were all measured in juice. The must was evaluated for TSS and pH, and sensory analysis was performed on the made wine based on the following parameters: appearance, odor, in-mouth sensation, finish, and overall acceptability to select the best product. The chemical composition and properties like TSS, pH, total acidity, specific gravity, alcohol content, aldehyde, antioxidant, and tannin content were analyzed for optimized (best product).

3.2.2.1 Total soluble solids (TSS)

The hand refractometer was used for the determination of TSS of the juice, must and wine according to (Ranganna, 1986).

3.2.2.2 pH

The digital pH meter of Labtronic ^{MT} (Deluxe pH meter) of model LT-10 provided by Central Campus of Technology, Nepal and standardized with standard buffers at 25°C was used to measured pH juice, must and wine (Ranganna, 1986).

3.2.2.3 Total acidity and volatile acidity

The total acidity was determined using the following method of Ranganna (1986). The volatile acidity of wine was determined using following the method Jacobson (2006).

3.2.2.4 Total reducing sugar

The reducing sugar of grapes, plum and prepared wine were determined as per Lane and Eynon method as described as K. C and Rai (2007).

3.2.2.5 Specific gravity

Specific gravity was determined as per following the method AOAC (2005). Briefly, 150 ml wine neutralized using 1N NaOH and poured in distillation flasks. Then it was distilled to get 50 ml of distillate. Then the distillate gently poured in a specific gravity bottle. Finally specific gravity was determined.

3.2.2.6 Alcohol content

Alcohol content was determined by using specific gravity method as per AOAC (2005). By using specific gravity chart and the values obtained were expressed in percentage (v/v).

3.2.2.7 Tannin content

Colorimetric determination method was used to determined tannin content of wine as per K. C and Rai (2007). And the values were expressed in mg/ml.

3.2.2.8 Total ester content

The total ester content of a wine sample was determined using the titrimetric method as described by (FSSAI, 2012). In brief, 100 ml of the distillate was neutralized with 0.1 M NaOH and 10 ml of 0.1 M NaOH solution was added to it. It was refluxed for 1 hour using glass beads, cooled, and titrated with 0.05 M H_2SO_4 solution. Similarly, a blank was also run using 100 ml distilled water instead of the distillate.

Total ester (g ethyl acetate/100 L alc. = 880× V/S

Where, V= Blank titer – Sample titer, ml

S= Alcohol content in the distillate, % (v/v)

3.2.2.9 Total aldehyde content

The total aldehyde content of samples were determined as per FSSAI (2012). In a 250 ml iodine flask, 50 ml of distillate (determined by specific gravity) was placed. After taking 10 ml of sodium bisulfite (0.05N) solution, the flask was placed in a dark room for 30 minutes with intermittent shaking. 25 ml of standard iodine solution (0.05N) was added and surplus iodine was back titrated against standard sodium thiosulphate solution (0.05N) using starch indicator (1%) to bright green end point. The difference in titer value in milliliters of sodium thiosulphate gives equivalent aldehyde content. The values were expressed in gram per 100 liter of absolute alcohol as acetaldehyde.

Aldehyde expressed acetaldehyde (g/100 L of abs. alcohol) = $V \times 0.0011 \times 100 \times 1000 \times 2 / V_1$

V= difference in titer of blank and sample in ml of sodium thiosulphate solution

V_1=alcohol % by volume.

3.2.2.10 Antioxidant activity

The antioxidant activity of wine was determined by DPPH method as per Vignoli *et al.* (2011). Briefly, Whatman No. 41 filter paper was used to filter the wine. One ml of the filtered wine was diluted to 10 ml with distilled water. One ml of the diluted wine was taken in a test tube and 4 ml of 0.004% methanolic solution of DPPH was added. Then the test tube was incubated at room temperature (28°C) for 30 min in the dark and absorbance was measured at 517 nm using a UV-vis spectrophotometer. Similarly, blanks were also run using methanol instead of the sample. The DPPH scavenging activity was calculated as follows:

DPPH scavenging activity (%) = (Blank absorbance - Sample absorbance) ×100/Blank absorbance

3.2.2.11 Total phenolic content (TPC)

The total phenolic content was determined by using the method as per Sadasivam and Manickam (1996). . Briefly, Whatman 41 filter paper was used to filter the wine. 1 ml of the filtered wine was diluted to 10 ml with distilled water. One ml of the diluted wine was pipetted into a test tube and 2 ml of distilled water and 0.5 ml of Folin-ciocalteau reagents were added. After 3 min, 2 ml of sodium carbonate solution (20%) was added, mixed thoroughly, and incubated at room temperature for 1h after which the absorbance was measured at 650 nm against a reagent blank. Total phenolic content in the wine was calculated from the standard curve prepared using different concentrations of gallic acid and the result was expressed as mg gallic acid equivalent (GAE)/100 ml wine.

3.2.2.12 Methanol content

Methanol content was determined by chromotropic acid colorimetric method as per (AOAC, 2005). Briefly, 2 ml of $KMnO_4$ solution (3 g $KMnO_4$ dissolved in a mixture of 15 ml H_3PO_3 and 85 ml distilled water) was pipetted into a 50 ml volumetric flask, chilled in ice bath. 1 ml of the distillate sample was added to the flask and stand for 30 min in ice bath. The excess of $KMnO_4$ solution was decolorized with 2% sodium sulfite solution and 1 ml of chromotropic acid solution (5% aqueous solution) was added. Then 15 ml of conc. H_2SO_4 was slowly added with swirling and placed in hot water bath maintained at 70°C for 15 min and cooled. The volume was made up to 50 ml, and the absorbance was read at 575 nm against a reagent blank containing 5.5% ethanol treated similarly. Standard methanol solution (0.025% by volume in 5.5% ethanol) was also treated simultaneously in the same manner, and the absorbance recorded. Methanol content in the wine was calculated as follows:

Methanol content (%, v/v) = Sample absorbance × 0.025/ Standard absorbance

3.2.3 Sensory evaluation

Sensory evaluation of the wines was carried out by using 9-points hedonic scale (1= Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much, 9 = Like extremely) as per Frost and Noble (2002) with slightly modification. Semi-trained panelists were requested to evaluate the samples in terms of taste, color, smell, flavor, clarity, and overall acceptability.

3.2.4 Data analysis

The experiment was carried out in triplicates and all measurements were made triplicate. A two-way ANOVA was used to examine the data using IBM SPSS Statistics, 20 (IMB Corporation, Marlborough, MA, USA) and the treatment means were by Tukey test at 5% level of significance. Microsoft corporation LTSC MSO (version 2207) developed by Microsoft Corporation (2021) GenStat 5 Release 12.1 software package were used for data documentation, calculation, and graph plot.

Part IV

Result and discussion

The plums were bought from Dharan, Sunsari and different physical and chemical analysis were carried out where moisture content of plum 85% and TSS and pH of plum juice were found to be 10°Bx and 3.02 respectively. Grapes were collected from the market of Dharan, and physical and chemical analysis were carried out where moisture content of grapes was 86% and TSS and pH of grapes were found to be 14°Bx and 3.74 respectively. Different proportions of plum juice and grapes juice were blended for preparation and optimization of the plum-grapes wine. In this experiment, plum-grapes wine was prepared from seven musts using different proportions of plum juice content (0%, 25%, 33.3%, 50%, 66.7%, 75% and 100%) and pH was maintaining constant 3.9 with the help of sodium bicarbonate and TSS was maintained at 25°Bx with addition of table sugar. The fermentation was carried out at room temperature around 25-30°C using wine yeast then pasteurized the product at 60°C for 10 minutes. The best product was determined by using sensory analysis and the chemical analysis was carried out for control (0% plum juice) and best product (66.7:33.3).

4.1 Physical and chemical analysis of plum (*Prunus cerasifera*)

The physical properties and chemical composition of plum juice are given in Table 4.1 and Table 4.2 respectively.

Table 4.1. Physical properties of plum

Parameter	Value*
Diameter (cm)	2.5 ± (0.2)
Length (cm)	5.5 ± (0.3)
Weight (g)	24.5 ± 1.4)

Table 4.2 Chemical composition of plum juice

Parameter	Value*
Moisture (%)	86 ± 0.03
TSS (°Bx)	10 ± 0.07
Total acidity (% as malic acid)	0.96 ± 0.02
pH	3 ± 0.02
Juice yield (% total fresh weight)	50.6 ± 0.14
Reducing sugar (% as dextrose)	0.7 ± 0.13
Total sugar (%)	8.6 ± 0.2

*Values are the means of three replications. Figures in the parentheses are the standard deviations.

The result presented that moisture content was 86%, TSS 10°Bx, acidity 0.96, pH 3, reducing sugar (% as dextrose) 4.1, respectively result were similar with Gull *et al.* (2022) and L. Pokhrel (2018).

4.2 Physical and chemical analysis of white grape (*Vitis vinifera*) juice

The physical properties and chemical composition of white grape and juice are given in Table 4.3 and Table 4.4

Table 4.3. Physical properties of grapes

Parameter	Value*
Diameter (cm)	0.8 ± 0.04
Length (cm)	1.6 ± 0.3
Weight (g)	8.6 ± 1.2

Table 4.4 Chemical composition of grape juice

Parameter	Value*
Moisture (%)	86.5 ± 0.05
TSS (°Bx)	14 ± 0.01
pH	3.73 ± 0.02
Total acidity (as malic acid)	0.67 ± 0.02
Reducing sugar (as g/L)	146 ± 0.08
Juice yield (%)	52.8 ± 0.04
Total sugar (%)	15.5 ± 0.07

* Values are the means of three replications. Figures in the parentheses are the standard deviations.

Above parameters for grape juice resemble to the data obtained from Creasy and Creasy (2018).

4.3 Effect of fermentation days on the TSS (°Bx) of must

The trend of fermentation days on the TSS (^{0}Bx) of the must was presented in Fig. 4.1.

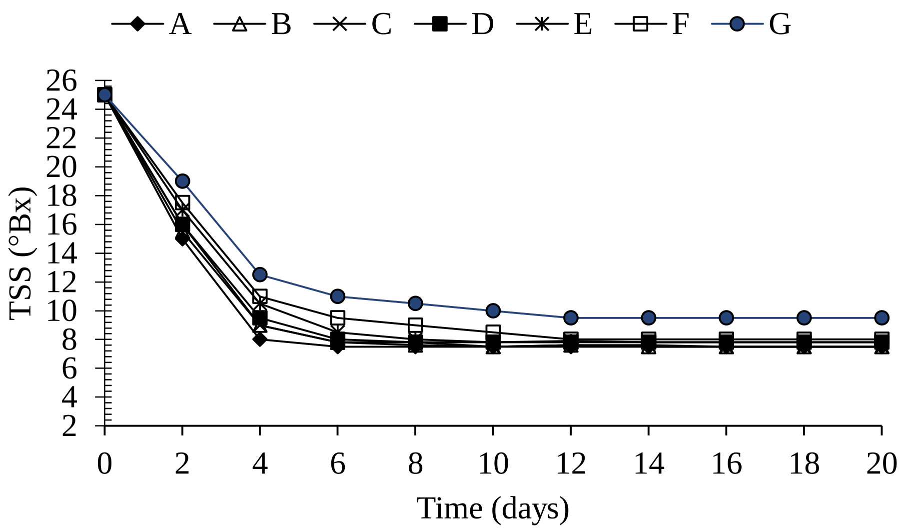


Fig. 4.1. Trend of fermentation days on the TSS(^{0}Bx) of must

It was observed that TSS decreases from 25 to 7.5°Bx in samples (A, B, and C), 25 to 7.8°Bx in samples (D, and E,), 25 to 8°Bx in sample F and 25 to 9.5°Bx in sample G from first day of analysis to 16 days and remained steady till the fermentation has been completed. According to L. Pokhrel (2018) the total soluble solids (TSS) content of the must samples was found to furiously decrease up to 4 days, gradual decreases and remain steady till fermentation complete.

According to the above trend line, TSS was decreased at a significantly different rate among all samples. The rate of TSS decreased was maximum in sample A and least in sample G. It was found that variation in decreasing rate of TSS was due to the variation in presence of natural sugar in which grape juice contained higher percentage of natural sugar than plum juice as revealed by Joshi *et al.* (2005).

4.4 Effect of juice percentage on sensory parameters of wine

The sensory analysis was carried out in the room of the lab building of Central Campus of Technology. The panelists were semi-trained in sensory of wine. They were experienced faculties of Central Campus of Technology, and some semi trained panelists but who were familiar with alcoholic beverages. The sensory evaluation of all seven samples were performed based on a 9-point hedonic rating test.

The parameters for sensory analysis were color, flavor, smell, taste, and overall acceptability of product. The obtained data from sensory analysis was analyzed using two-way ANOVA (Appendix B) at 5% level of significance to study the significance difference among formulation made and among panelists. However, there was no significant difference in all panelist's ability to judge the specified parameters of the product. In sensory analysis, the significant difference in the product based on specified parameters was in the sense of liked or disliked of the product as judged by the panelists but not in sense of physiochemical characteristics of the product.

4.4.1 Color

The mean sensory score for color of seven samples A, B, C, D, E, F, and G were found to 5.7, 6.7, 7.5, 6.8, 6.4, 5.9, and 5.7 respectively. The statistical analysis showed that there was significant effect ($p<0.05$) of plum juice variation on color at 5% level of significance.

Statistical analysis shows that control sample A was not significantly different ($p>0.05$) with samples E, F and G while significantly different ($p<0.05$) with samples B, C and D.

The maximum and minimum mean sensory score for color among seven samples were found to be sample C (7.5) and sample G (5.7) respectively. Statistically the mean sensory score was found to be highest for sample C which was 33.3% plum juice which was significantly higher than all other samples. The mean score for sample with plum juice 33.3% was found to have higher values while that for sample with plum juice percentage (0%, 25%, 50%, 66.7%, 75%, and100%) have lower values. This indicates that panelists preferred wine with plum juice with a percentage of 33.3% in terms of color. The best sample with 33.3% plum juice was of red-violet color.

According to Jackson (2009) analysis of color include absence of haze, color hue (shade or tint) and depth (intensity or amount of pigment), viscosity (resistance to flow) and effervescence (notably sparkling wines). According to Li *et al.* (2022) plum was composed of different pigment such as coloring principle anthocyanin, which is responsible for color. The effect of plum juice percentage of must on color of product was shown in Fig. 4.2.

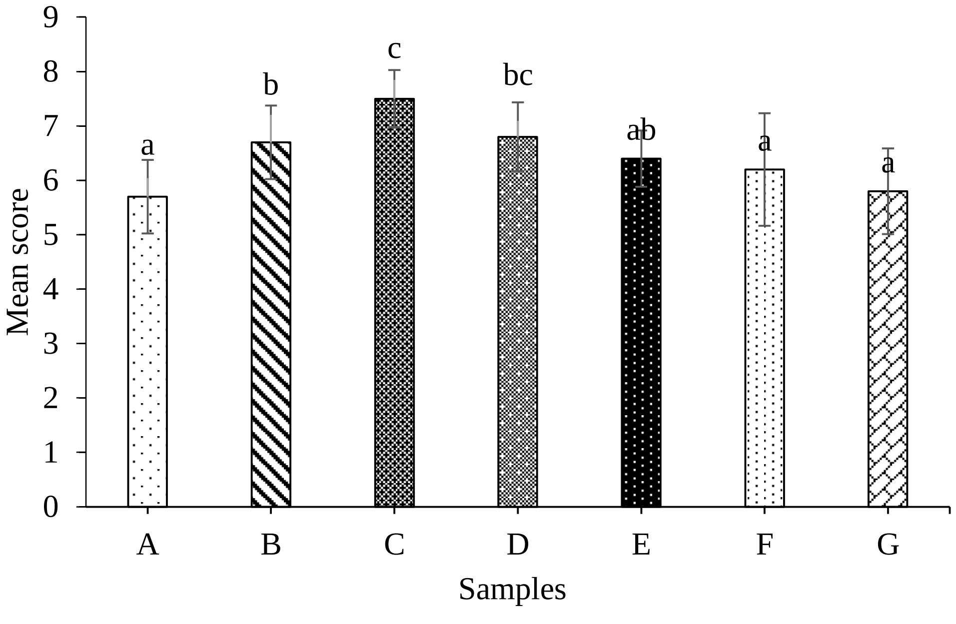


Fig. 4.2 Mean sensory scores for color of plum-grape wine samples

Jackson (2014) illustrated the amount of pigment effects the color of the product. Juice content will effect on color intensity of wine, higher percentage juice will have higher amount of pigments. According to Gull *et al.* (2022) the increment of juice to certain percentage results wine developing good color and after that continues increasing of plum juice percentage results wine of deep brown color and also sensory score shows that panelists preferred wine with plum juice 33.3% hence color intensity of wine with 33.3% plum juice and 66.7% grape juice will have higher values which supports above results.

The wine samples were subjected to sensory evaluation and the results were shown in Fig. 4.1. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by 10 panelists.

4.4.2 Flavor

The sensory score for flavor of seven sample A, B, C, D, E, F, and G were 6.9, 6.7, 7.2, 6.5, 5.9, 5.5, and 5.2 respectively. The statistical analysis showed that there was significant effect ($p<0.05$) of plum juice percentage variation on flavor at 5% level of significance. Statistical analysis shows that control sample A was significantly different ($p<0.05$) with samples E, F, and G while not significantly different ($p>0.05$) with samples B, C, and D respectively.

The maximum and minimum mean sensory score for flavor among seven samples were found to be sample C (7.2) and sample G (5.2) respectively. The mean score was found to be highest for sample C which was 33.3% plum juice which was significantly different from all other samples. This graph shows that first increasing plum juice percentage flavor also increased but on increasing above 33.3% flavor was again scored less thus 33.3% plum juice gave optimum flavor to the wine. Increased plum juice percentage contributed strong acid flavor to the wine.

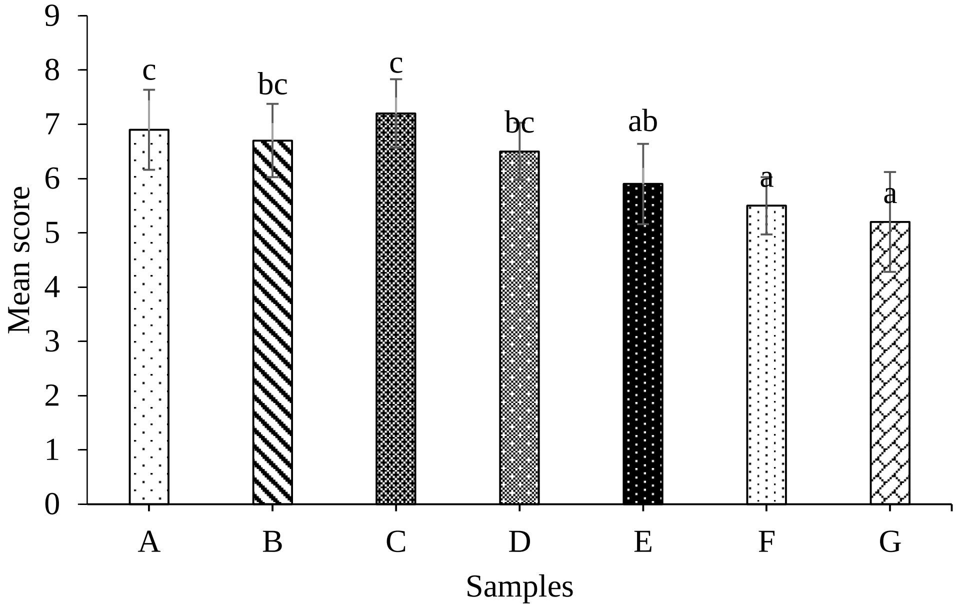


Fig. 4.3 Mean sensory scores for flavor of plum-grape wine samples

The wine samples were subjected to sensory evaluation and the results were shown in Fig. 4.3. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by 10 panelists.

4.4.3 Smell

The mean sensory scores for seven samples A, B, C, D, E, F, and G were be 6.7, 7.1, 7.4, 6.4, 5.7, 5.5, and 5.8 respectively. The statistical analysis showed that there was significant effect ($p<0.05$) of plum juice percentage variation on smell at 5% level of significance such that control sample A was not significantly different ($p>0.05$) with samples B, C, and D while significantly different ($p<0.05$) with samples E, F, and G respectively.

The maximum and minimum mean sensory score for smell among seven samples were found to be (sample C (7.4) and sample F (5.5) respectively. The mean sensory score was found to be highest for sample C which was of 33.3% plum juice which was significantly different from all other samples. The mean score for sample with plum juice percentage (33.3%) was found to have higher values while that for samples with lower and higher plum juice percentage (0%, 25%, 50%, 66.7%, 75%, and 100%) have lower values. This graph shows that the first increasing plum juice percentage smell also increased but on increasing above 33.3% smell was again scored less thus 33.3% plum juice gave optimum smell to the wine. According to Jackson (2009) an increased plum juice percentage contributed astringent smell to the wine and wine having plum juice higher than 33.3% have lower smell values which supports the above results.

According to Mmegwa (1987) illustrated that smell was also important factor that affect the organoleptic properties of wine. This indicates that panelists preferred wine with lower plum juice percentage in terms of smell.

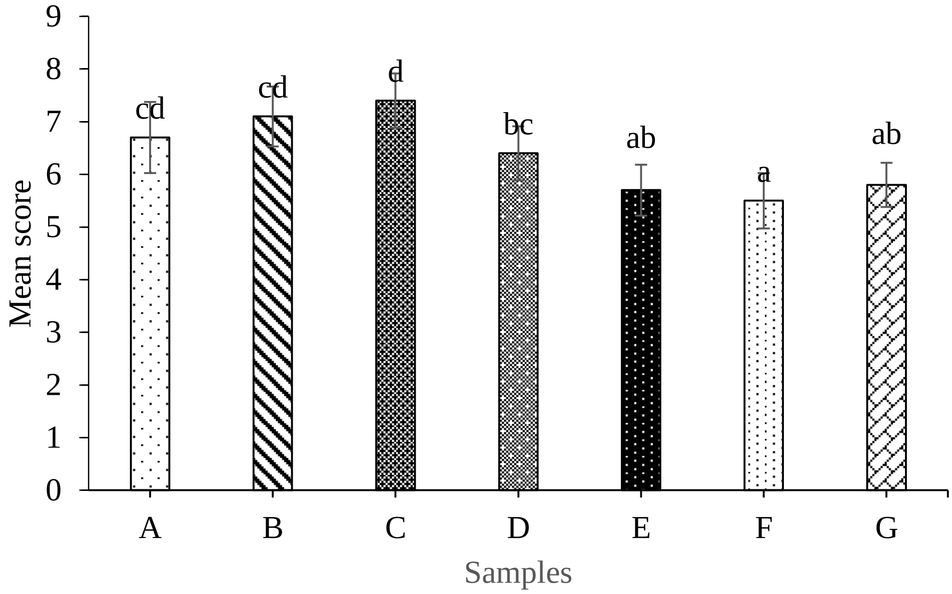


Fig. 4.4. Mean sensory scores for smell of plum-grape samples

The wine samples were subjected for sensory evaluation and the results were shown in Fig. 4.4. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by 10 panelists.

4.4.4 Taste

The mean sensory scores for taste of seven samples A, B, C, D, E, F, and G were be 7.0, 6.7, 7.4, 6.4, 5.8, 5.5, and 5.8 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of plum juice percentage variation on taste at 5% level of significance. The statistical results shows that there was not significantly different ($p>0.05$)

with samples B, C, and D while significantly different ($p<0.05$) with samples E, F, and G respectively.

The maximum and minimum mean sensory scores for taste among seven samples were found to be sample C (7.4) and sample F (5.5) respectively. The mean sensory score was found to be highest for sample C which was of 33.3% plum juice which was significantly different from all other samples. The mean score for samples with plum juice percentage 33.3% was found to have higher values while that of remaining samples have lower values. This indicates that panelists preferred wine with lower plum juice percentage in terms of taste also. The wine sample which was proven to be best from the sensory analysis was found to have moderate sweetness, slight hint of sourness and acceptable amount of astringency.

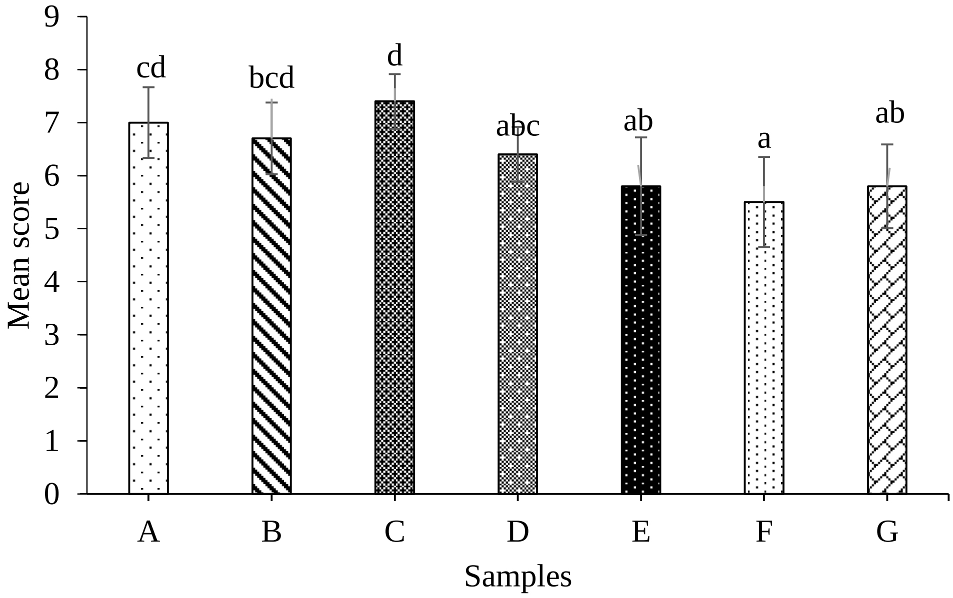


Fig. 4.5. Mean sensory score for taste of plum-grape wine samples

According to Cheynier and Sarni-Manchado (2010) the major taste qualities in wine are sweetness, sourness, and bitterness contributed by sugars, organic acids and ethanol, respectively and are major sensory attributes of red wines while white wines are usually not astringent but can be perceived as sour or bitter. Sourness is due to organic acids and related to pH in the wines. Also, astringency perception due to organic acids increases as the pH decreases but did not depend on the concentration or nature of the acid. Similar results were obtained by L. Pokhrel (2018) in preparation of *yacon* wine.

The wine samples were subjected for sensory evaluation and the results were shown in Fig. 4.5. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by 10 panelists.

4.4.5 Clarity

The mean sensory scores for overall acceptability of seven samples A, B, C, D, E, F, and G were be 7.7, 7.6, 7.5, 6.3, 5.5, 5.3, and 5.25 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of plum juice percentage variation on clarity at 5% level of significance. Statistical analysis shows that there was significantly different $P<0.05$) with samples E, F, and G while not significantly different ($p>0.05$) with samples B, C, and D respectively.

The maximum and minimum mean sensory scores for taste among seven samples were found to be sample A (7.7) and sample G (5.25) respectively. The mean sensory score was found to be highest for sample A which was 100% grape content and 0% plum juice which was significantly different from other samples.

According to Alleweldt *et al.* (1991) the grape juice contain natural sugar in higher amount and juice obtained was clear. According to Amerine *et al.* (1980) the clarity of juice directly affect the clarity of wine. As the percentage of plum juice increases the cloudiness of wines also increases. The mean sensory scores given by panelists decrease with an increase in plum juice percentage which supports the above result. In terms of clarity the sensory panelists preferred sample A which was of 100% grape juice.

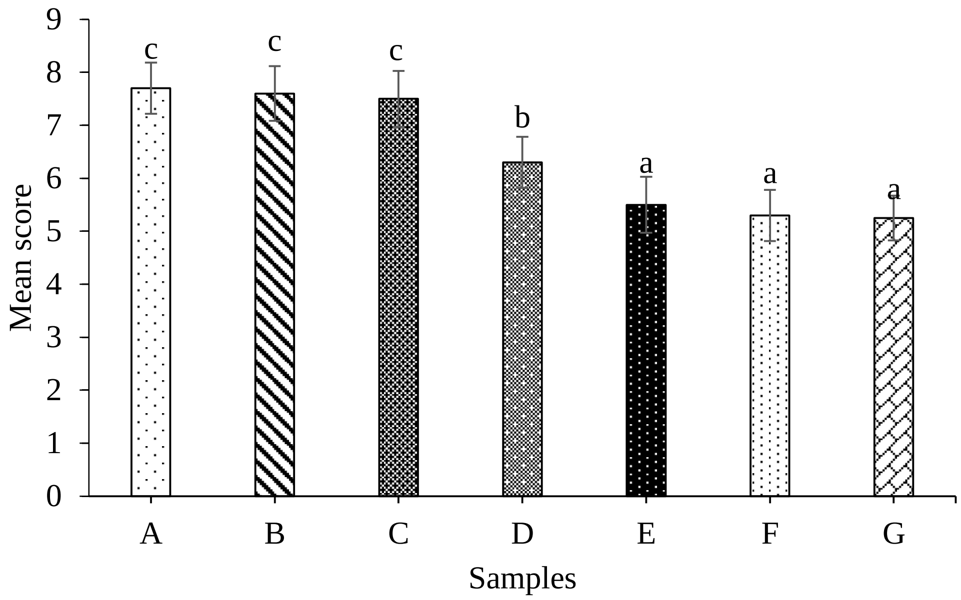


Fig. 4.6. Mean sensory scores for clarity of plum-grape wine samples

The wine samples were subjected for sensory evaluation and the results were shown in Fig. 4.6. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance.

4.4.6 Overall acceptability

The mean sensory score for overall acceptability of seven samples A, B, C, D, E, F, and G were be 7.2, 7.15, 7.3, 6.6, 5.95, 5.45 and 5.6 respectively. The statistical analysis showed that there was significant effect ($p<0.05$) of juice percentage on overall acceptance at 5% level of significance. Statistical analysis shows that there was not significantly different ($p>0.05$) with samples B, C, and D while significantly different with samples E, F, and G respectively.

The maximum and minimum mean sensory score for overall acceptance among seven samples were found to be sample C (7.3) and sample F (5.45) respectively. The mean sensory score was found to be highest for sample C which was 33.3% plum juice and 66.7% grape juice which was significantly different from other samples.

According to Butzke (2010) the pH of wine was crucial to every aspect of the wine. The juice content could affect flavor, aroma, color, tartrate precipitation, carbon dioxide absorption, malolactic fermentation, stability, and fermentation rate. Similar result was obtained by L. Pokhrel (2018) and Karki (2019) in optimization of *yacon* wine fermentation.

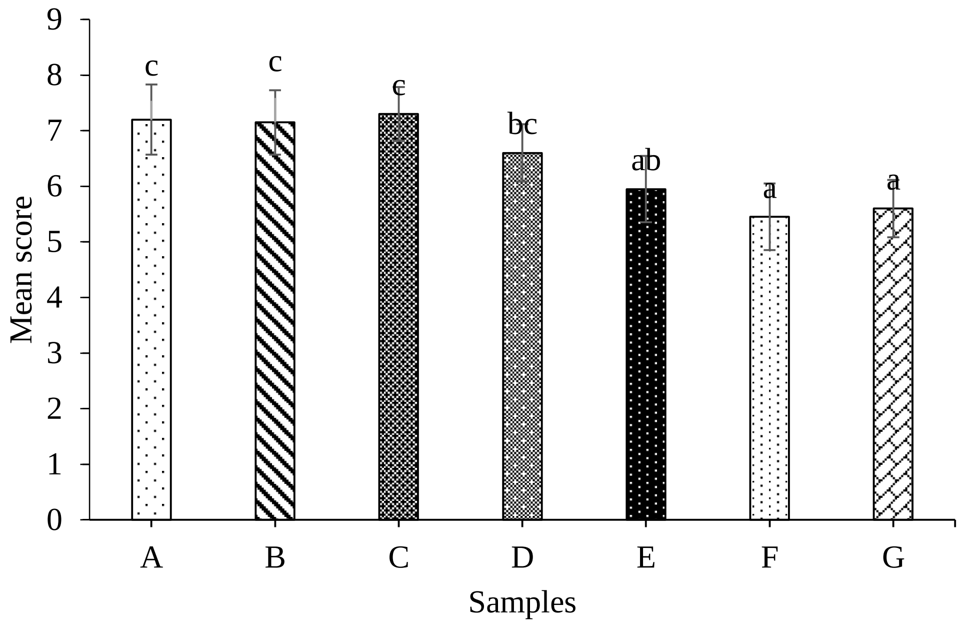


Fig. 4.7. Mean sensory score for overall acceptability of plum-grape wine samples

Even though there was no significant difference (p>0.05) between sample A, B, and C, sample C was selected as best based on mean sensory score value as per color, flavor, and taste. The wine samples were subjected to sensory evaluation and the results were shown in Fig. 4.7. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by 10 panelists.

4.5 Chemical composition

The chemical composition of best formulated plum-grape wine i.e. 33.3% plum juice and 66.7% grape juice in must and grape wine (control) i.e. 100% grape juice in must were given in Table 4.5

Table 4.5 Chemical composition of control and best formulated plum-white grape wine

Characteristics	Control A*	Best formulated*
TSS (°Bx)	7.5 (0.00)[a]	7.5 (0.05)[a]
Acidity		
a) Total acidity (% as lactic acid)	0.52 (0.010)[a]	0.50 (0.005)[a]
b) Fixed acidity (% as lactic acid)	0.49 (0.010)[a]	0.47 (0.005)[b]
c) Volatile acidity (% as acetic acid)	0.02 (0.001)[a]	0.04 (0.002)[a]
pH	3.84 (0.020)[a]	3.89 (0.005)[a]
Total reducing sugar as dextrose (mg/100ml)	0.629 (0.004)[a]	0.539 (0.002)[b]
Total phenolic (mg GAE/100 ml)	365.49 (0.07)[a]	272.93 (0.03)[b]
Alcohol (% v/v)	11.5 (0.00)[a]	10.7 (0.05)[b]
Ester (g/100 L abs. alcohol)	35.24 (2.54)[a]	49.36 (2.13)[b]
Total aldehyde (g/100 L abs. alcohol)	0.19 (0.02)[a]	0.24 (0.35)[a]
Tannin (mg tannic acid/ L)	17 (2.03)[a]	19 (2.4)[a]
Antioxidant activity (% DPPH inhibition)	79.66 (1.78)[a]	83.34 (0.44)[b]
Methanol (g/L)	0.7 (0.03)[a]	0.9 (0.025)[b]

*All results are presented as mean value of three measurements together with the standard deviation (±s).

* 1ml of wine was diluted to 10 ml with distilled water and used for determination.

The TSS of control wine sample was found to be 7.5°Bx while that of best formulated sample was 7.5°Bx. The TSS was not significantly different (p>0.05) among control and best formulated wine sample and the value was similar to those reported by Karki (2019) and L. Pokhrel (2018). The pH of grape wine (3.84) was not significantly different (p>0.05) than best formulated plum-white grape wine (3.89). The pH values of white grape wine and best formulated sample wine found in this study were slightly higher than those reported by in rose wine (3.0) and white wine (3.33) as revealed by Ancin *et al.* (1996).

Control wine samples had the highest total acidity (0.52 as lactic acid), while that of best sample wine had total acidity (0.50) which was not significantly different (p>0.05) among two samples. The fixed acidity in both wines were lower than those reported by Egan *et al.* (1981). Volatile acidity was similar in wines made from white grape and plum. Similar results of volatile acidity were also reported by Egan *et al.* (1981) range from (0.03-0.2%).

The reducing sugar of control wine sample (0.629%) and best formulated (0.539%) was significantly different (p<0.05) and higher than that of dry white wine (0.134%) and dry red wine (0.146%) as reported by Amerine *et al.* (1980). The total phenolic content (mg GAE/100 ml) was maximum in control wine sample (365.49) than the best formulated wine (272.93) which was significantly different (p<0.05). The results of total phenolic reported by Vilanova *et al.* (2007) was significantly lower. The alcohol content in control wine (11.5 v/v) was significantly different (p<0.05) than that of best formulated wine (10.7 v/v) which was higher than those reported by Amerine *et al.* (1980). The aldehyde content was not significantly different (p>0.05) among control and best formulated wines sample and within the range of (200-500 ppm) was recommended as good for quality of wine as reported by Rai (2009). Total ester contents (g ethyl acetate/100 L alc.) between control wine (35.24) and best formulated wine (49.36) were significantly different (p<0.05) among two sample and was lower than those reported by Briggs *et al.* (2004). The higher acetaldehyde in wine was due to the use of poor quality pitching yeast, excessive oxygenation, and high fermentation temperature Briggs *et al.* (2004). The tannin content (mg tannic acid/ L) was not significantly different (p>0.05) in control wine (17) and best formulated plum-grape wine (19) which was lower than those reported by Amerine *et al.* (1980). The antioxidant activity was significantly different (p<0.05) from each other, with the highest value being for best formulated wine (83.34%) and lowest being for control wine (79.66%). The methanol content of the best formulated wine (0.9 g/L) was significantly different (p<0.05) than control wine (0.7 g/L) due to higher pectin content in plum.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results and discussion, the following conclusions were drawn:

1. It was observed that t-test of chemical composition of control sample and best formulated sample shows significant different ($p<0.05$) in total reducing sugar, total phenolic, alcohol content, esters content, antioxidant activity and methanol content while other remaining parameters was not significantly different ($p>0.05$).
2. In comparison with the wine made of all formulations, wine made from formulation having 66.7% white grape juice and 33.3% plum juice seems to be the best.
3. Plum-grape wine (i.e. best formulated) has better antioxidants property.
4. Plum-white grape wine can be produced at a cost within the means of common people plum that resembles a good quality red violet wine.

   Consequently, plum-white grape wine holds a lot of promise from the commercial point of view.

5.2 Recommendations

Based on the present study, the following recommendations have been made:

1. Plum-white grape wine can be prepared with varying TSS, temperature and acid used.
2. Study on changes during ageing of plum-white grape wine in terms of sensory and chemical properties can be carried out.
3. Study on the distillate of plum-white grape wine can be carried out.
4. Study on the quality of plum-white grape wine using different yeast can be carried out.
5. study on the quality of plum-white grape wine using different clarifying agent can be carried out.

Part VI

Summary

In this study, plum was taken from Dharan's market. And other essential materials grape was bought from the local market of Dharan, and other materials (sodium bicarbonate, sugar, and yeast) and other chemicals and apparatus were available from local market of Dharan and Campus laboratory. First, plum, and white grape were subjected to preliminary operation sorting, destemming, washing with plenty of potable water and disinfecting them with 200 ppm sodium hypochlorite solution. After these plum and white grapes were subjected to screw press juice extractor for extraction of plum and white grapes juice. Plum juice is susceptible to oxidation hence to preserve it calculated amount of preservative such as KMS was added. Physiochemical analysis of white grape juice showed 86.5% moisture, 14°Bx TSS, 3.73 pH, and 0.67 acidity (% as malic acid). After that, fermentation was carried out in seven different mashes where plum juice was varied to 0%, 25%, 33.3%, 50%, 66.7%, 75% and 100% and TSS 25°Bx keeping pH 3.9 ± 0.1 without varying to other parameters for 24 days.

Seven different wines were subjected to sensory analysis and data obtained from this was analyzed by two-way ANOVA at 5% level of significance to study the difference among all types. There was a significant difference in terms of color, flavor, smell, taste, clarity, and overall acceptability of all types. From the sensory evaluation, wine made from 66.7% grape juice and 33.3% plum juice obtained the highest score among other formulations. An independent t-test was carried out to compare the chemical analysis of control sample wine and best formulated wine. The cost of the best formulated product was calculated and found to be Rs. 366 per bottle (750 ml) of wine made from 66.7% grape juice and 33.3% plum juice.

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Appendices

Appendix A

A.1 Chemical used:

- Potassium metabisulfite (KMS)
- Sodium hydroxide (99% Purity, Alfa chemicals)
- Buffer solution (4 and 7)
- Folin-ciocalteau's reagent (A. R. grade, Fisher Scientific, India)
- Tannic acid solution (Fizer Merk)
- Calcium hydroxide solution (Fisher Scientific)
- DPPH assay (95% Sisco Research Laboratories, Pvt. Ltd, India)
- Sodium bisulfite solution (Fizer Merk)
- Methylene blue indicator (MERCK)
- Fehling A solution (LOBA CHEMIE PVT. LTD)
- Fehling B solution (LOBA CHEMIE PVT. LTD)
- Sodium bicarbonate (>99%, Qualigens, India)
- Sodium hypochlorite solution (Fisher Scientific)
- Hydrochloric acid (Echo Chem)
- Carrez-I solution (BICCA)
- Carrez-II solution (BICCA)
- Sulfuric acid (Echo Chem)
- Sodium thiosulphate solution (Fisher Scientific)
- Iodine solution (Chemigens)
- Starch indicator (Fisher Scientific)
- Phenolphthalein indicator (British drug houses Ltd)
- Methanol (Methanex Corporation)

A.2 Equipment used:

- Stainless steel vessels
- Hand refractometer (0-30°Bx)
- pH meter
- Thermometer
- Titration apparatus
- Pycnometer
- Screw press juice extractor
- Weighing arrangement
- Knives
- Measuring cylinder
- Distillation set
- Heating arrangement
- Other routine glassware's

A.3 Other materials

- Food grade silicon tube rubber pipe
- Muslin cloth
- Cotton
- Plastic bottle
- Wine bottle
- Cone

Appendix B

Specimen card of sensory evaluation by a 9-point hedonic rating test Sensory evaluation of *plum-white grape* juice wine

Name of panelist: ………………………………. Date: ………………

Name of Product: Plum-white grape wine

Please assess the product using your sense organs to demonstrate your perspective by checking at the place that best represents your feelings about the product and writing to any of the defects listed below. An open sharing of personal feelings will be beneficial to me.

Parameters	Sample						
	A	B	C	D	E	F	G
Color							
Flavor							
Smell							
Taste							
Clarity							
Overall acceptance							

Quality description:

1: Dislike extremely 2: Dislike very much 3: Dislike moderately

4: Dislike slightly 5: Neither like nor dislike 6: Like slightly

7: Like moderately 8: Like very much 9: Like extremely

Any comments: ………………………………………………………………………………

Signature

B.1 ANOVA result for sensory analysis of plum-white grape wine

Table B.1 Mean sensory scores for different attributes

Sample code	Sensory parameters					
	Color	Flavor	Smell	Taste	Clarity	Overall
A	5.700^{a}	6.900^{c}	6.700^{cd}	7.000^{cd}	7.700^{c}	7.200^{c}
B	6.700^{b}	6.700^{bc}	7.100^{cd}	6.700^{bcd}	7.600^{c}	7.150^{c}
C	7.500^{c}	7.200^{c}	7.400^{d}	7.400^{d}	7.500^{c}	7.300^{c}
D	6.800^{bc}	6.500^{bc}	6.400^{bc}	6.400^{abc}	6.300^{b}	6.600^{bc}
E	6.400^{ab}	5.900^{ab}	5.700^{ab}	5.800^{ab}	5.500^{a}	5.950^{ab}
F	5.900^{a}	5.500^{a}	5.500^{a}	5.500^{a}	5.300^{a}	5.450^{a}
G	5.700^{a}	5.200^{a}	5.800^{ab}	5.800^{ab}	5.250^{a}	5.600^{a}
LSD	0.5213	0.6353	0.4915	0.6115	0.4501	0.4685

The values are the means of 10 panelist score. The values having the same superscript in column did not vary significantly at 5% level of significance.

Table B.2 Two-way ANOVA (No blocking) for color

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	26.8857	4.4810	13.25	<.001
Panelist	9	5.4429	0.6048	1.79	0.092
Residual	54	18.2571	0.3381		
Total	69	50.5857			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD for color at 0.05 level of significance = 0.5213

Table B. 3 LSD for color

Sample	Mean score	Mean difference		
A	5.700	A-B>LSD*	B-D<LSD	C-G>LSD*
B	6.700	A-C>LSD*	B-E<LSD	D-E<LSD
C	7.500	A-D>LSD*	B-F>LSD*	D-F>LSD*
D	6.800	A-E>LSD*	B-G>LSD*	D-G>LSD*
E	6.400	A-F<LSD	C-D>LSD*	E-F<LSD
F	5.900	A-G<LSD	C-E>LSD*	E-G>LSD*
G	5.700	B-C>LSD*	C-F>LSD*	F-G<LSD

*= Significantly different

Table B.4 Two-way ANOVA (No blocking) for flavor

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	33.7429	5.6238	11.20	<.001
Panelist	9	2.9857	0.3317	0.66	0.740
Residual	54	27.1143	0.5021		
Total	69	63.8429			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD of flavor at 0.05 level of significance = 0.6353

Table B. 5 LSD for flavor

Sample	Mean score		Mean difference	
A	6.900	A-B<LSD	B-D<LSD	C-G>LSD*
B	6.700	A-C<LSD	B-E>LSD*	D-E<LSD
C	7.200	A-D<LSD	B-F>LSD*	D-F>LSD*
D	6.500	A-E>LSD*	B-G>LSD*	D-G>LSD*
E	5.900	A-F>LSD*	C-D>LSD*	E-F<LSD
F	5.500	A-G>LSD*	C-E>LSD*	E-G>LSD*
G	5.200	B-C<LSD	C-F>LSD*	F-G<LSD

*= Significantly different

Table B.6 Two-way ANOVA (No blocking) for smell

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	32.3429	5.3905	17.94	<.001
Panelist	9	1.7714	0.1968	0.65	0.745
Residual	54	16.2286	0.3005		
Total	69	50.3429			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD of smell at 0.05 level of significance = 0.4915

Table B.7 LSD for smell

Sample	Mean score		Mean difference	
A	6.700	A-B<LSD	B-D>LSD*	C-G>LSD*
B	7.100	A-C>LSD*	B-E>LSD*	D-E>LSD*
C	7.400	A-D<LSD	B-F>LSD*	D-F>LSD*
D	6.400	A-E>LSD*	B-G>LSD*	D-G>LSD*
E	5.700	A-F<LSD*	C-D>LSD*	E-F<LSD
F	5.500	A-G>LSD*	C-E>LSD*	E-G<LSD
G	5.800	B-C<LSD	C-F>LSD*	F-G<LSD

*= Significantly different

Table B.8 Two-way ANOVA (No blocking) for taste

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	29.7429	4.9571	10.66	<.001
Panelist	9	7.4857	0.8317	1.79	0.092
Residual	54	25.1143	0.4651		
Total	69	62.3429			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD of taste at 0.05 level of significance = 0.6115

Table B.9 LSD for taste

Sample	Mean score		Mean difference	
A	7.000	A-B<LSD	B-D<LSD	C-G>LSD*
B	6.700	A-C<LSD	B-E>LSD*	D-E<LSD
C	7.400	A-D<LSD	B-F>LSD*	D-F>LSD*
D	6.400	A-E>LSD*	B-G>LSD*	D-G<LSD
E	5.800	A-F<LSD*	C-D>LSD*	E-F<LSD
F	5.500	A-G>LSD*	C-E>LSD*	E-G<LSD
G	5.800	B-C>LSD*	C-F>LSD*	F-G<LSD

*= Significantly different

Table B.10 Two-way ANOVA (No blocking) for clarity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	76.7500	12.7917	50.76	<.001
Panelist	9	1.7179	0.1909	0.76	0.655
Residual	54	13.6071	0.2520		
Total	69	92.0750			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD of clarity at 0.05 level of significance = 0.4501

Table B.11 LSD for clarity

Sample	Mean score		Mean difference	
A	7.700	A-B<LSD	B-D>LSD*	C-G>LSD*
B	7.600	A-C<LSD	B-E>LSD*	D-E>LSD*
C	7.500	A-D>LSD*	B-F>LSD*	D-F>LSD*
D	6.300	A-E>LSD*	B-G>LSD*	D-G>LSD*
E	5.500	A-F<LSD*	C-D>LSD*	E-F<LSD
F	5.300	A-G>LSD*	C-E>LSD*	E-G<LSD
G	5.250	B-C<LSD	C-F>LSD*	F-G<LSD

*= Significantly different

Table B.12 Two-way ANOVA (No blocking) for overall acceptability

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	6	37.6857	6.2810	23.01	<.001
Panelist	9	5.2321	0.5813	2.13	0.043
Residual	54	14.7429	0.2730		
Total	69	57.6607			

Since, F pr <0.05, there is significantly different between the samples so LSD testing is necessary. LSD of clarity at 0.05 level of significance = 0.4685

Table B.13 LSD for overall acceptability

Sample	Mean score		Mean difference	
A	7.200	A-B<LSD	B-D>LSD*	C-G>LSD*
B	7.150	A-C<LSD	B-E>LSD*	D-E>LSD*
C	7.300	A-D>LSD*	B-F>LSD*	D-F>LSD*
D	6.600	A-E>LSD*	B-G>LSD*	D-G>LSD*
E	5.950	A-F<LSD*	C-D>LSD*	E-F<LSD
F	5.450	A-G>LSD*	C-E>LSD*	E-G<LSD
G	5.650	B-C<LSD	C-F>LSD*	F-G<LSD

*= Significantly different

Appendix C

Cost evaluation for plum-white grape wine

Table C.1 Cost evaluation (for every 750 ml bottle)

Particulars	Quantity	Rate (Rs)	Total (Rs)
Plum	670 g	50/kg	34
Grape	1.335 kg	200/kg	267
Sugar	150 g	95/kg	15
Wine bottle	1 pcs	15/piece	15
Wine yeast	1 g	2000/kg	2
Total cost			333
Final cost with 10% overhead			366

The price of 750 ml wine cost Rs. 366. Thus, the price of 1 liter wine is Rs. 488.

Appendix D

Table D.1 Independent samples t-test for composition of control sample and best formulated sample (C)

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TSS	Equal variances assumed	16.000	.016	-1.000	4	.374	-.03333	.03333	-.12588	.05921
	Equal variances not assumed			-1.000	2.000	.423	-.03333	.03333	-.17676	.11009
TA	Equal variances assumed	.400	.561	2.500	4	.067	.01667	.00667	-.00184	.03518
	Equal variances not assumed			2.500	3.200	.082	.01667	.00667	-.00382	.03715
VA	Equal variances assumed	.400	.561	-2.000	4	.116	-.01333	.00667	-.03184	.00518
	Equal variances not assumed			-2.000	3.200	.134	-.01333	.00667	-.03382	.00715
Reducing	Equal variances assumed	.352	.585	30.301	4	.000	.09367	.00309	.08508	.10225
	Equal variances not assumed			30.301	3.404	.000	.09367	.00309	.08446	.10288
pH	Equal variances assumed	5.000	.089	-2.940	4	.042	-.03667	.01247	-.07130	-.00204
	Equal variances not assumed			-2.940	2.306	.083	-.03667	.01247	-.08405	.01072

Phenolic	Equal variances assumed	1.288	.320	19.449	4	.000	.09098	.00468	.07799	.10397
	Equal variances not assumed			19.449	2.645	.001	.09098	.00468	.07490	.10706
Alcohol	Equal variances assumed	16.000	.016	23.000	4	.000	.76667	.03333	.67412	.85921
	Equal variances not assumed			23.000	2.000	.002	.76667	.03333	.62324	.91009
Ester	Equal variances assumed	.069	.806	-7.356	4	.002	-14.08000	1.91396	-19.39400	-8.76600
	Equal variances not assumed			-7.356	3.882	.002	-14.08000	1.91396	-19.45830	-8.70170
Aldehyde	Equal variances assumed	.787	.425	-2.000	4	.116	-.04667	.02333	-.11145	.01812
	Equal variances not assumed			-2.000	3.174	.134	-.04667	.02333	-.11868	.02534
Tanin	Equal variances assumed	.055	.825	-1.102	4	.332	-2.00000	1.81484	-7.03880	3.03880
	Equal variances not assumed			-1.102	3.893	.334	-2.00000	1.81484	-7.09395	3.09395
Methanol	Equal variances assumed	.032	.866	-2.949	4	.042	-.00067	.00023	-.00129	-.00004
	Equal variances not assumed			-2.949	3.883	.044	-.00067	.00023	-.00130	-.00003
Antioxidant	Equal variances assumed	5.393	.081	-4.386	4	.012	-4.66667	1.06399	-7.62078	-1.71255

	Equal variances not assumed			-4.386	2.241	.039	-4.66667	1.06399	-8.80362	-.52971
FA	Equal variances assumed	.400	.561	3.500	4	.025	.02333	.00667	.00482	.04184
	Equal variances not assumed			3.500	3.200	.036	.02333	.00667	.00285	.04382

Appendix E

Table E.1 Average chemical analysis of prize-winning high-quality wines.

Component	(g per100 ml)				
	Dry White	Dry Red	Sweet White	Sweet Red	Sparkling
Alcohol by volume (%)	3.35	12.16	18.83	19.03	13.22
Alcohol	9.89	10	10	14.85	10.84
Glycerol	0.7091	0.6353	0.3025	0.5098	0.4178
Ash	0.196	0.247	0.203	0.311	0.135
Total acids	0.568	0.649	0.412	0.502	0.658
Volatile acids	0.101	0.128	0.092	0.122	0.082
Reducing sugars	0.134	0.146	11.30	10.20	3.409
Protein	0.162	0.150	0.162	0.232	0.214
Tannins	0.039	0.236	0.036	0.096	0.035
Specific gravity	0.9917	0.9947	1.0298	1.0276	1.0045

Source: L. Pokhrel (2018)

Appendix F

Table F.1 The major wine producing countries of the world-2022.

Countries	Wine production	Wine expert	Wine consumption	Total grapes	Area of vineyard
	(mhl)	(mhl)	(mhl)	(mt)	(kha)
Italy	49.468	18.225	26.232	8.2	682
France	48.421	14.417	30.823	6.3	786
Spain	35.906	15.803	12.190	6.0	1021
USA	21.805	3.384	27.027	7.0	419
Argentina	13.708	2.379	10.865	2.4	225
China	11.084	-	13.635	12.6	830
Australia	11.179	5.871	4.745	1.7	149
South Africa	9.369	4.406	3.790	2.0	130
Chile	8.715	5.761	2.5	3.1	211
Germany	9.123	3.123	19.912	1.2	103
Portugal	6.697	2.695	4.866	-	217
Others	43.3	13.4	87.9	25.7	2738
Total	268.775	89.464	244.485	75.7	7511

Source: Ohana-Levi and Netzer (2023)

Appendix G

Photo gallery

Plate 1: Sensory analysis of wine sample

Plate 2: Sensory analysis of wine sample

Plate 4: Propagation of yeast

Plate 5: In bottle pasteurization

Plate 6: Total reducing sugar determination.