EFFECT OF HONEY PROPORTION AND pH ON THE SENSORY QUALITY OF MEAD

by

Bunu Bhattarai

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2019

EFFECT OF HONEY PROPORTION AND pH ON THE SENSORY QUALITY OF MEAD

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

by

Bunu Bhattarai

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

May, 2019

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Effect of honey proportion and pH on the sensory quality of mead* presented by Bunu Bhattarai has been accepted as the partial fulfillment of the requirement for the B.Tech. Degree in Food Technology.

Dissertation Committee

1. Head of the department _____

(Prof. Basanta Kumar Rai)

2. External Examiner _____

(Asst. Prof. Birendra Kr. Yadav)

3. Supervisor _____

(Prof. Basanta Kumar Rai)

4. Internal Examiner _____

(Prof. Dr. Dhan B. Karki)

May, 2019

Acknowledgements

First of all, I would like to express my deep sense of gratitude to my respected supervisor Prof. Basanta Kr. Rai, Dept. of Food Technology, CCT, Hattisar, Dharan for his encouragement and constructive recommendations on doing this research.

I wish to express my deep sense of gratitude to Prof. Dr. Dhan Bahadur Karki (Campus Chief, CCT) for providing necessary facilities during the work.

I would like to acknowledge all the library and laboratory staffs of Central Campus of Technology, my seniors Mr. Samip Khadka, Mr. Ramesh Koirala and Ms. Shraddha Khanal. Mr. Sanjog Kharel for constant support and my juniors Ms. Sangita Sah, Ms. Sangita Rijal, Ms. Simran Shrestha, Ms. Nisha Marahatha, Ms. Barsha Baniya, Ms. Sunita karki, Mr. Aadarsha Poudel, Mr. Utsav Pokhrel for helping with great effort.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of day.

.

Date of submission: May, 2019 Bunu Bhattarai

Abstract

Honey brought from Chitwan was used for the preparation of mead. Honey was analyzed for TSS, Acidity, reducing sugar, and pH. Meads were prepared from twelve musts using different proportions of honey (100%, 75%, 50% and 25% contribution for a must of 25ºBx) and pH (3, 3.5 and 4), the final TSS and pH being maintaining using sugar and citric acid, respectively. Fermentation was carried out at 28º±2ºC using wine yeast (*Saccharomyces cerevisiae*, Lalvin EC 1118) for 21 days. The effects of honey content and pH on sensory attributes (appearance, odor, in-mouth sensation, finish and overall acceptance) of the meads were evaluated using 9- point hedonic scale rating test ranging from faulty (1) to exceptional (9) to identify the best product. The raw data were analyzed by one-way and two-way ANOVA using Genstat Discovery Edition 12, 2014 at 5% level of significance.

The minimum concentration of honey and pH in must evaluated by sensory evaluation of prepared mead and was found to be 25% honey and 3.5, respectively. This sample was also comparable with sample having honey content 50% and pH 3.5 in terms of in-mouth sensation and overall acceptability, and also sample having 100% honey content and pH 4.0 in terms of appearance, odor and overall acceptance. Sensory analysis showed significant difference among all the products with respect to appearance, odor, in-mouth sensation, finish and overall acceptance of product. Variation in honey content and pH of must significantly ($p<0.05$) affected mead quality. From sensory evaluation, mead prepared from 25% honey and pH 3.5 was found to be superior and contained 15.34 % (v/v) alcohol content, 9.2ºBx TSS, 3.40 pH, 0.43% (as % citric acid) acidity, 0.012% (as acetic acid) volatile acidity, 367 ppm ester, 317 ppm aldehyde, 68 ppm methanol content. Alcohol content, volatile acidity and other parameters of mead were within the range of a good quality wine.

Table of Contents

List of tables

List of figures

List of abbreviations

Abbreviation	Full form
ADY	Active dry yeast
ANOVA	Analysis of vaiance
ATP	Adenosine triphosphate
CFU	Colony forming units
EMP	Embden–Meyerhof Parna glycolytic pathway
FOS	Fructooligosacharides
KMS	Potassium metabisulfite
LSD	Least significance difference
MLF	Malolactic fermentation
NADH	Nicotinamide adenine dinucleotide
OIV	International organization for vine and wine
PPOs	Polyphenol oxidases
TSS	Total soluble solid

Part I

Introduction

1.1 General introduction

The term ‘wine’ is applied to the product made by alcoholic fermentation of grapes or grape juice, with an aging process. Fermented products derived from berries, fruits and honey are also called wines and the resultant wine is normally named after the substrate used. Wine is one of the God’s choicest gift to man and history is almost a romance. The oldest testament is the Bible gives evidence of wine existing but there is a definite evidence of its use in China and Egypt in 2000 and 3000 B.C. respectively (Andrew, 1980).

For the preparation of wine, either for flavor or for enrichment of wine with chief chemical constituents, different types of raw materials have been used (Gubhaju, 2006). Wine can be made from any fruit, which contains sufficient fermentable carbohydrate. The grape (*Vitis venifera* or, less commonly, *V. rotundifolia*) is a soul in wine making and have the great commercial importance, although wine is made on limited commercial scale from fruits such as strawberry, gooseberry and peach. Cider, produced by fermentation of apple juice, is not legally a wine, but shares a similar technology and, together with less common Perry (Varnam and Sutherland, 1994) . Different herb incorporated wines are also on practices throughout the world. For e.g., Ginger wine, is an alcoholic beverage made from a fermented blend of ground ginger (*Zingiber officinale*) and raisins fermenting by the yeast, *Saccharomyces cerevisiae*. It is a popular beverage in Europe (Rai, 2009b). The Japanese sake is also the form of wine derived from the fermentation of cereals as rice (Steinkraus, 1987).

Honey is produced by bees and some related insects, sweet in taste and really viscous (E. Crane, 2013). Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation.

Bees store honey in wax structure called honeycombs (Crane *et al.*, 1984) . Honey is suitable for long-term storage because of its chemical properties and composition and is easily assimilated even after long preservation. Honey and objects immersed in honey, have

been preserved for centuries. The key to preservation is limiting access to humidity. In its cured state, honey has a sufficiently high sugar content to inhibit fermentation. If exposed to moist air, its hydrophilic properties pull moisture into the honey, eventually diluting it to the point that fermentation can begin.

Long shelf life of honey is attributed to an enzyme found in the stomach of bees. The bees mix glucose oxidase with expelled nectar they previously consumed, which then creates two byproducts: gluconic acid and hydrogen peroxide, partially responsible for honey's acidity and ability to suppress bacterial growth.

Honey is composed mainly from carbohydrates, lesser amounts of water and a great number of minor components.

Mead (honey wine) is almost certainly the oldest alcoholic beverage known to man and likely discovered before the wheel was invented. As long as there have been bees and honey there has been mead. Mead occurs naturally when honey is mixed with water and yeast. A chance occurrence of mead was likely produced during the Stone Age when honey became wet from rain and wild yeast in the air settled into the mixture.

Mead's popularity is currently on the rise throughout the world. The history of mead has roots in royalty, religion, sex, and violence throughout the ages of time and cultures of the world. There are tales of Norsemen toasting one another with mead drunk from skulls of their slain enemies. According to Nordic mythology, the Norse gods lived wild lives of reckless abandon often given to lechery. Legends revealed stories of various gods giving goddesses cups of mead, which caused the goddesses' resistance to advances by the gods to be reduced so the scheming gods could take full advantage of their physical delights.

Mead is the oldest alcoholic beverage made by the fermentation of honey with water by adding yeast to the honey–water must, it takes weeks or months for fermentation (Iglesias et al., 2014). In modern mead production, Now a days the yeast *Saccharomyces cerevisiae* is commonly used(Pereira *et al.*, 2015). Sometimes different types of herbs, fruits, grains, spice or hops also used to make mead (Fitch, 2002). Here spice is a seed, root, bark or other plant substances primarily used for flavoring coloring or preserving food. Spices are distinguished from herbs, which are the leaves, flowers, or stems of plants used for flavoring or as a garnish. Many spices have antimicrobial properties. Spice such as fennel, nutmeg, black

pepper, mace, cinnamon, cloves, saffron, ginger etc. And herbs such as mint, rosemary, basil, lavender etc. And here plain mead (Odinsson, 2010), pyment (Tayleur, 1973), cyser, melomel (Tayleur, 1973), hippocras (Odinsson, 2010) and mulled are short list of the most popular mead type. The defining characteristic of mead is that the majority of the beverage's fermentable sugar is derived from honey (Gayre, 1986). It may be still, carbonated, or naturally sparkling; dry, semi-sweet, or sweet.

1.2 Statement of problem

Wine culture in Nepal is comparatively a new practice. Production and consumption of wine in Nepal does not date back so long. Since the beginning, wineries of Nepal have been focusing on the very regular types of wines using common raw materials like grapes, locally available seasonal fruits and to some extent, some Himalayan herbs and berries are found to be used. Almost all wineries currently operating in Nepal, lack the adequate knowledge about mead and also not enough technical knowledge about beekeeping due to which produced honey couldn’t fulfilled the requirements to make mead.

Dadaghare is the one and only honey wine found in Nepal but production has decreased over the years because of inadequate raw material, knowledge. Also less economic feasibility, delays and "pouts" fermentations, lack of product uniformity, and production of yeast off-flavors. Many factors might be related with these problems, such as honey variety, temperature, medium composition (vitamin and nitrogen content), fermentative yeast, and pH. Lack of adequate raw materials mainly honey because of inadequate production might have played some role in confining the mead behind the curtain in context of Nepalese market.

1.3 Objectives

1.3.1 General objective

The general objective of this dissertation was to prepare, effect of honey proportion and pH on sensory quality of mead and carry out its sensory and chemical analysis.

1.3.2 Specific Objective

The specific objectives of the study are as follows:

1. To find out effect honey proportions and pH on the sensory quality of mead.
2. To carry out sensory and physicochemical analysis of mead.
3. To optimize honey content in the must.

1.4 Significance of the study

Firstly significance of this study is product diversification. It is the way of expanding the original market for honey. This strategy increases the production and sales of honey. And hence among fermented rather than distilled beverage of an adult nature, those who don't like beer or cider can find themselves torn between mead vs wine. The latter is much more common and hence mead will be the best option for the people due to its own distinctive appeal. And also mead made with fresh honey, in particular, contains high levels of lactic acid bacteria, which comes from the stomach of the bees. This imbues the honey with powerful infection-fighting properties, mead boosts the immune system against antibiotic-resistant pathogens.

It is an alcoholic brew full of antioxidants, especially mead made with dark honey. This makes it an ideal way to lower chronic inflammation and free radical activity when consumed in moderation (Socha *et al.*, 2015). As well this study can be a helpful in winery industries of Nepal for making complete new product with superior quality in terms of aroma, taste, mouth feel and appearance. The results generated from this research may be an initiation for further study to make a good quality of mead.

1.5 Limitations of the study

1. The fermentation was done in ambient condition because of the unavailability of temperature control instrument in laboratory.
2. The fermentation was carried out at same TSS, temperature and adjustment of pH by addition of same acid. Hence, optimization on TSS, temperature and acid used was not done.
3. Prepared mead was not aged properly due time and technical constraints.
4. Only one yeast type was used.

Part II

Literature review

2.1 Historical background of alcoholic beverage

It is believed that Egypt and Mesopotamia are the origin of alcoholic beverage. The history of an alcoholic beverage dates back to ancient times and different civilization had developed some types of alcoholic beverage at every part of the world. The people have been producing alcoholic beverage and it is the man's oldest activities. For the countries like Germany and France, wine making was an important economic activities (Varnam and Sutherland, 1994). The use of wheat, rye, millet, rice, oats, barley, potatoes or grapes in early fermentation processes paved the way to the technologies that are in existence currently (Jones, 1995).

Alcoholic fermentation was first identified by Gay Lussac in 1810, but at that time yeast was not recognized as causative organism. Schwan in 1835, demonstrated that yeast could produce alcohol and carbon dioxide when introduced in sugar-containing solution. He termed yeast *Zuckerpilz* meaning sugar-fungus, from which the name *Saccharomyces* originated. Despite this early application of microbiology, the ability of microorganisms to stimulate the biochemical changes was demonstrated several years later. *Saccharomyces* group possesses almost all the credit of producing alcoholic beverages.

The yeast cells growing under anaerobic conditions caused the conversion of glucose to alcohol and researchers also demonstrated that fermentation could be carried out using cell free yeast juice, which led to the discovery of the role of enzymes in fermentation. He called the enzyme "Zymase". Such work of pioneers finally revealed the truth that the alcoholic fermentation was in fact anaerobic, due to the presence of an enzyme complex known as Zymase, which is made available by the yeasts. Having realized the importance of yeasts in fermentation, people started culturing valuable yeasts and exploiting them for the production of various alcoholic beverages. Today, yeasts are utilized throughout the world for the production of alcoholic beverages in many different forms and tastes. The starting materials normally comprise either sugary materials, which need to be hydrolyzed to simple sugars before fermentation (Buglass *et al.*, 2011). Over the year a vast range of alcoholic beverage have evolved although, in most cases, it is possible to place these in one of three categories beer, wine or distilled spirit – according to ingredient and method of manufacture (Varnam and Sutherland, 1994).

In Nepal, the history of alcoholic beverage dates back to ancient times. These technologies were developed by ethnic groups while celebrating various festivals and settlement of marriage. The knowledge of home brewing has been passed on to generations but they are quite ignorant about the broad dimensions of microbial biochemistry or their complex mechanisms. In fact the exact nature of fermentation is still not fully known to them (Gubhaju, 2006).

2.2 History of wine making

The history of wine and winemaking is as old as civilization itself. Viticulture, or grape-growing, began in Georgia some 9000 years ago. From here it spread to Middle East via the Tigris and Euphrates rivers to Mesopotamia, and then on to Persia. Stories abound about how wine was first discovered, and one of the more delightful tells of a mythical Persian king called Jamsheed. At his court, grapes were kept in jars for eating out of season. One jar was discarded because the juice had lost its sweetness and the grapes were deemed to be poisonous. A damsel from the king's hareem was suffering from nervous headaches and tried to take her life with the so-called poison. She fell asleep, to awake later feeling revived and refreshed. She told everyone what she had done and of the miraculous cure, and thereupon 'a quantity of wine was made and Jamsheed and his court drank of the new beverage'. Someone, somewhere in Asia Minor, possibly in modern Anatolia or Georgia, put wild grapes in a container, which were pressed by their own weight. The resulting juice began to ferment and a new drink was discovered that was to give untold pleasure to an untold number of people. Also the great civilizations of ancient Greece, and Rome trace wine back into their pre-history, with similar legends about its discovery (Sandler and Pinder, 2003).

2.3 Major wine producing region and current situation of world

According to International Organization for Vine and Wine (OIV), major vineyard surface area in world are in Spain, China, France, Italy, and Turkey which cover about 50% of world vineyard, however the vineyard area of world is on reducing pattern, since 2000 A.D. mainly due to the reduction of European vineyards. Total grapes production in 2015 was 75.7 MT. Total wine production in 2015 A.D was 274 mhl. Italy occupies top position by producing 50 mhl of wine followed by France, Spain, Argentina, Australia, China, South Africa, Chile, Germany, Portugal etc. however wine consumption seems to decreasing in traditional wine making countries of southern Europe but progressive increase in other countries. USA

occupies top wine consuming country followed by France, Germany, China, U.K. etc. while Spain occupies top exporter followed by France, Chile Australia etc.(OIV, 2016). The top wine producing countries with quantity, vine surface area, production of grapes, wine consumption and wine export data are given in Appendix D.

2.4 Winery in Nepal

In Nepal, the history of commercial wine making is not very long (Bhandari, 1992). The practice of making some forms of traditional wines can be traced to times immemorial. There is drastic change in wine drinking culture in Nepal within few years (Khatiwada, 2015).

Wine consumption in Nepal has seen steady growth in the recent years, scope has increased and numerous Nepalese wine brands have been launched in the market. These wines are mostly made from various fruits like apple, orange, black grapes, wild Himalayan barberries /raspberries and nettles (*Sishno)* with mixture of honey, saffron, spices, tea and various other herbs. As these raw materials are less in quantity, it gets a bit challenging to maintain the same level of production year on year. To avoid such issue, some manufacture import dark grapes (vine grapes) from India and China (Acharya and Yang, 2015). More than 50 brands of wine are produced in the country. Brands like Hinwa, Dandaghare and Divine hold a major share of the market while recently launched Black Stone and Moon Dance are struggling to gain fans with in the short period of time the consumers of Nepali wines have grown significantly. Where no one used to take a glance at the Nepal made wine bottle five years ago, around 100,000 bottles of Nepal wines are on demand in the market (monthly) (Nepal, 2014). Following are some of the popular brands of wines made in Nepal.

I. Dadaghare

The wine manufactured in Pokhara, *Dadaghare* is considered to be the first Nepali wine. It is not only popular among the local customers but also foreigners. The wine available in four different flavors- *Aangan*, *Pidi*, *Majheri* and *Aati*, is manufactured using various fruits, herbal fruits and honey and is absolutely chemical free.

II. Hinwa

One of the most popular wines, Hinwa is manufactured by Makalu wine industries at Sankhuwasabha. Made from wild fruits like raspberry, Himalayan barberry and saffron, this wine first started manufacture in 1995.

III. **Nettlange**

Manufactured by *Sakaro* Beverages, *Nettlange* is one of the popular Nepali wines 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**

One of the fast selling brands available in the market, Divine wine was introduced in 2010. The wine manufactured by Shree *Mahakali* wine, it is made of grapes, spices, tea and various other fruits (Rijal, 2016).

2.5 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 are (i) table wines, (ii) sparkling wines, and (iii) fortified wines. A summary of the classification scheme is given in Table 2.1 and composition of some wines is given in Table 2.1.

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)	Table wines
	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)
	Appertizer wine	Dry; fortified; taken before meal	Sherry (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: There is considerable overlapping of wine types in the classification shown above. For example, a Red Table wine can at the same time is sweet, sparkling, fortified, or natural. Similarly, a fortified wine can be sweet, sparkling, red, or white (Rai,2009a).

Table 2.2 Chemical composition of some wines

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	20.-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	0.3-0.60	0.30-0.45
% Ash	0.25-0.35	0.35-0.55	0.20-0.30	0.2-0.4	0.25-0.45
% sugar	2.5-12.0	2.0-7.0	0.0-0.7	0.03-0.55	8.5-16

Source: Egan *et al.* (1981)

2.6 General cultural condition for alcoholic fermentation

Cultural condition refers to the environment of yeast i.e. fermentative media on which the propagation of yeast as well as final quality of wine is largely depended. Following are the few parameters, which determines cultural condition of the fermentative media.

2.6.1 pH

The pH of wine is crucial not only to its flavor but also to nearly every aspect of the wine. The pH could affect flavor, aroma, color, tartrate precipitation, carbon dioxide absorption, malolactic fermentation, stability, agility, and fermentation rate. Also, the pH can influence many chemical reactions that take place in wine. The optimum pH for wine production varies from types of fruits and type of wine that should be made, pH range of 2.8 to 4 cover most wines. It is usually suggested that honey must for mead production have a pH range of 3 to 4 and grape musts for table wine production have a pH range of 3.1 to 3.3, however, must values closer to 3.5 for whites and 3.6 for reds are not uncommon. Musts for the production of sparkling wine or wine for distillation can have a pH range of 2.8 to 3.0. A low pH increases the efficacy of many preservatives such as sulfur dioxide and sorbic acid. The pH of must/wine do not remain static during course of fermentation and maturation. The most common adjustment to must pH is to lower it through the addition of acids like malic, citric, and tartaric acid. Tartaric acid is the most recommended acid for must adjustments because,

it is a stronger acid than malic and citric acid and less susceptible to breakdown by microorganisms during the alcoholic and malolactic fermentations as well (Butzke, 2010).

The generally low pH values found in wines are an important contributor to the relatively high stability they have compared to other foods and beverages. Many wine maker keeps wine pH below 3.65. Wine is a highly buffer liquid. This means that the corresponding pH decrease for a given addition in titrable acid (added acidity) is not directly proportional. Further, the change in pH for a given titrable acidity increase /decrease is unique to each individual wine, since every wine is buffered slightly differently. However, as a general rule, the addition of 0.5-1 g/L acid as tartaric tends to drop the pH by about 0.1 units (Rotter, 2008).

2.6.2 Temperature

Temperature plays important role on fermentation. Above 38ºC the yeast will certainly be killed; at too low a temperature it will ferment only very slowly (Berry, 1996). The optimum temperature for the fermentation is dependent upon the types of wines produced. For white wine the temperature is 10-15ºC and that for the red wine is 20-30ºC which is also for mead. There is possibility of stuck fermentation if it is carried at higher temperature. On the other hand, low temperature may delay onset of fermentation. At high temperature, the loss of alcohol and aroma substance takes place. Also, a large amount of by product like glycerol, acetaldehyde may be formed. An imbalance of these constituents can be very detrimental to wine quality. It has been reported that at higher temperature the formation of higher alcohol decreases. The advantage of lower fermentation temperature are the fresher and fruitier character of wine, smaller losses of ethanol and less danger of producing volatile acidity.

2.6.3 Sugar concentration

The ‘must’ having very high sugar concentration imparts high osmotic pressure, which in turn has a negative effect on yeast cells, since both growth of yeast and fermentation activity are lowered. The tolerance of higher sugar concentration varies according to the yeast species. The must having very high sugar concentration imparts high osmotic pressure which in turn has negative effect on yeast cells, since both growth of yeast and fermentation activity are lowered. The optimum sugar concentration in terms of total soluble solid is 20-24 ºBx. The tolerance of higher sugar concentration varies according to the yeast species.

2.6.4 Wine yeast

Wines can be prepared using either natural yeast flora of the grapes (spontaneous fermentation) or pure cultures (culture yeasts). Many manufacturers still depend on spontaneous fermentation which can produce wine of unique quality in terms of bouquet because the end product is the result of interaction of diverse yeast types. Each yeast type will contribute unique flavor to the wine. But yeast profile is diverse, spontaneous fermentation may sometimes lead to failure and also most strain of yeast do not produce large amount of wine as well few strains produce undesirable organic compounds such as organic acids, H_2S, higher alcohols, etc., that may affect the flavor (Rai, 2009a). Nowadays the must is partially 'sterilized' by the use of Sulphur dioxide, a bisulphate or a metabisulphite which eliminates most microorganisms in the must leaving wine yeasts. Yeasts are then inoculated into the must. The yeast which is used is *Saccharomyces cerevisiae* var, e*llipsoideus* (synonyms: *S. cerevisiae, S. ellipsoideus, S. vini*.) Other yeasts which have been used for special wines are *S. fermentati*, *S. oyiformis* and *S. bayanus* (Okafor, 2007).

There are two reasons for using starters. One is to start the alcoholic fermentation quickly after the harvest. Indeed, in some cases, and preferably at the beginning of the winemaking the yeast population is too low (less than 10^4 CFU/ml). Multiplication up to 10^6 and more takes several days especially if the temperature is low. During this time, other microorganisms can develop, yeasts with oxidative metabolism and acetic acid bacteria that take advantage of the presence of oxygen to produce volatile acidity and many other defects. Thus, inoculation with starters at the concentration of 10^6 CFU/ml prevents the growth of such microorganisms. The second reason for the winemaker to use yeast starters is to improve the final phase of alcoholic fermentation. Indeed, grape musts are so rich in sugar and sometimes so poor in essential nutrients that yeast cannot survive long enough to ferment all sugars. Stuck fermentation is one of the major problems in winemaking. Hence the use of selected yeast starters allow a better control of the process as well influence on the sensorial and hygienic quality of wine (Lonvaud, 2002).

Good wine yeast is one which will impart a vinous or fruit like flavor, will ferment sugar to a low content producing 14-18% alcohol, and is characterized by remaining in suspension during fermentation and then agglomerating to yield a coarse granular sediment that settles quickly and is not easily disturbed in racking (Pederson, 1980). In general, good wine yeast should have the following properties:

a) High alcohol tolerance, i.e. the yeast should continue to ferment despite the increasing concentration of the alcohol, giving stronger, drier wines with up to 16% alcohol (v/v), or even up to 18% (v/v) where the yeast is fed by periodic additions of sugar in small amounts.
b) Good degree of agglutination i.e. the tendency of the yeast to flocculate into small lumps that give a cohesive sediment as fermentation ceases, so that racking is simple and the wine clears easily.
c) Steady, persistent fermentation capacity; this leads to wines of better quality than when the fermentation falls away after a tempestuous start.
d) Absence of unpleasant flavors generated by dead and dying cells (Austin, 1968).
e) Growth at the relatively high acidity i.e., low pH of grape juice or must for fermentation.
f) Osmotolerance i.e. yeast should able to tolerant high osmotic pressure created by high concentration of sugar on must composition.
g) SO_2 tolerance, i.e. for partial sterilization of must SO_2 in the form of sulfite is used yeast should not be affected by applied Sulfite (Okafor, 2007).

2.7 Alcohol

The word "alcohol" derives from Arabic *al- kuhul,* which denotes a fine powder of antimony used as an eye makeup. Alcohol originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage (Shakhashiri, 2009)**.**

There are many different kind of alcohol, but when the term is used loosely by winemakers, it is invariably applies to the potable alcohol called ethyl alcohol or ethanol, the common ingredients of alcoholic drinks of all type. Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide The ethanol produced by fermentation ranges in concentration from a few percent up to about 14 percent. Above about 14 percent, ethanol destroys the zymase enzyme and fermentation stops. Ethanol melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C. It mixes easily with water in any proportion, and where quantities are mixed there is a contraction in volume. It is clear, colorless, inflammable liquid. It is good solvent for essential oil, ester, tannins,

various organic acids and certain other organic compounds. It burns easily in air, so that oxidation is possible and then gives a blue smokeless flame, producing water and CO_2 (Shakhashiri, 2009).

2.7.1 Alcoholic fermentation

Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide in presence of nitrogen compound. Fruit juices have the highest sugar concentration among the many substrates used for the production of ethanol by fermentation. As a result, the level of ethanol is among the highest seen and the importance of substrate and ethanol inhibition This process, which is carried out by yeast and also by some bacteria can be summarized by this overall reaction:

$$\underset{\text{Hexose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} \underset{\text{Ethanol}}{2\,C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

However, alcoholic fermentation is fortunately a much more complex process. At the same time as this overall reaction proceeds, a lot of other biochemical, chemical and physicochemical processes take place, making it possible to turn the grape juice into wine. Besides ethanol, several other compounds are produced throughout alcoholic fermentation such as higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin and 2, 3-butanediol. Simultaneously, some compounds of grape juice are also transformed by yeast metabolism. Without the production of these other substances, wine would have little organoleptic interest (Zamora, 2009).

2.7.2 Biochemistry of alcohol fermentation by yeast

The major function of the yeast (*Saccharomyces cerevisiae*) in fermentation is, of course, the production of ethyl alcohol (ethanol, C_2H_5OH) from the sugars including sucrose, glucose, fructose, galactose, mannose, maltose and maltotriose but not other sugars like arabinose, rhamnose and xylose, which may also be present in small quantity in the must for alcoholic fermentation (Varnam and Sutherland, 1994).

In wine, *Saccharomyces* metabolize glucose and fructose to pyruvate via the glycolytic pathway. One molecule of glucose or fructose yields two molecules each of ethanol and carbon dioxide. The particular enzyme present in the yeast has the general name zymase, but, in fact, yeast contains several enzymes, including invertase, which is necessary to split

the sucrose into its component sugars (glucose and fructose). The mechanism of the metabolic pathway from glucose and fructose to ethyl alcohol has been well established; the conversion proceeds primarily via the Embden–Meyerhof glycolytic pathway oxidation to pyruvate, then to acetaldehyde and ethyl alcohol. For growth and reproduction, yeast cells require a steady supply of ATP (adenosine triphosphate) together with the reducing power of NADH (nicotinamide adenine dinucleotide). There are metabolic intermediates, which result in the noted formation of succinates, glycerol, acetoin, diacetyl, acetic and succinic acids. Notably, the production of alcohol during fermentation assists the physical extraction of numerous compounds (e.g. terpenes) from grape cells, which appear in the fermented wine (Clarke and Bakker, 2004).

The organism uses EMP pathway, generating 2 ATP per mole of glucose converted to ethanol, plus CO_2. Ethanol, which is the end product, is primary metabolite. In an industrial fermentation, the basic strategy is to maintain Crabtree effect during the fermentation. A truncated form of the metabolic pathway for ethanol synthesis is given in Fig. 2.1.

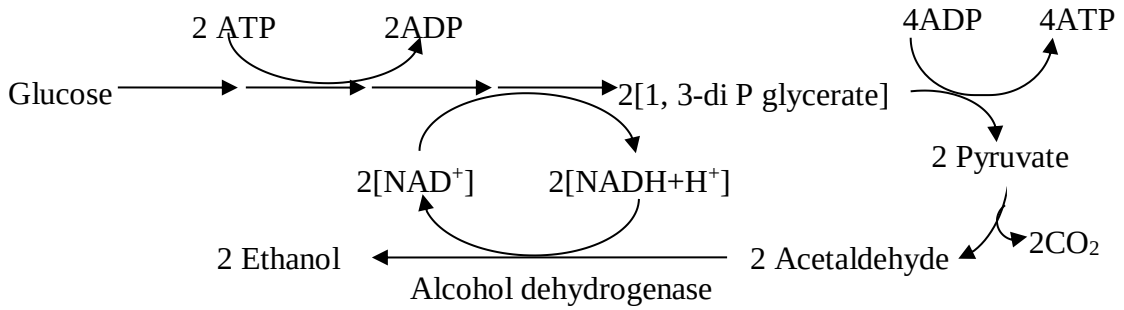


Fig. 2.1 Simplified pathway of alcohol synthesis by yeast

2.7.3 Malo-lactic fermentation

Malolactic fermentation (MLF) in wine is by definition the enzymatic conversion of malic acid to lactic acid, a secondary process which usually follows primary (alcoholic) fermentation of wine but may also occur concurrently. But, this reduction of malic acid to lactic acid is not a true fermentation (Costantini *et al.*, 2009). The MLF occurs as a result of metabolic activity by certain lactic acid bacteria and results in the conversion of malic acid

to lactic acid. The bacteria may also impact the flavor and aroma of the wine. Although spontaneous MLF may occur due to bacteria naturally present in musts and wines, specific starter cultures of bacteria are now commonly used as they allow more control over the process with more reliable results (Osborne, 2010). MLF is mainly performed by *Oenococcus oeni*, a species that can withstand the low pH (<3.5), high ethanol (>10 vol %) and high SO_2 levels (50 mg/L) found in wine. More resistant strains of *Lactobacillus*, *Leuconostoc* and *Pediococcus* can also grow in wine and contribute to MLF; especially if the wine pH exceeds 3.5. Wines with low levels of acidity should be protected from malo-lactic fermentation: wine quality decreases if the acid level falls too low 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 be easily prevented by early *racking*, cold storage, and maintaining 100 ppm or more of SO_2. On the other hand, if such a fermentation is desired it can be facilitated by leaving the wine on the *lees* (yeast sediments) for prolonged periods at higher temperatures. This storage causes lysis of yeast cells and releases amino acids and other nutrients needed for the growth of the 'contaminant' lactic acid bacteria. This fermentation is particularly useful if the titrable acidity of the wine is to be reduced malo-lactic fermentation has an important bearing in the quality of wine. It is a natural way of reducing acidity in wine Rai(2012) The biochemistry of fermentation is given in Fig. 2.2

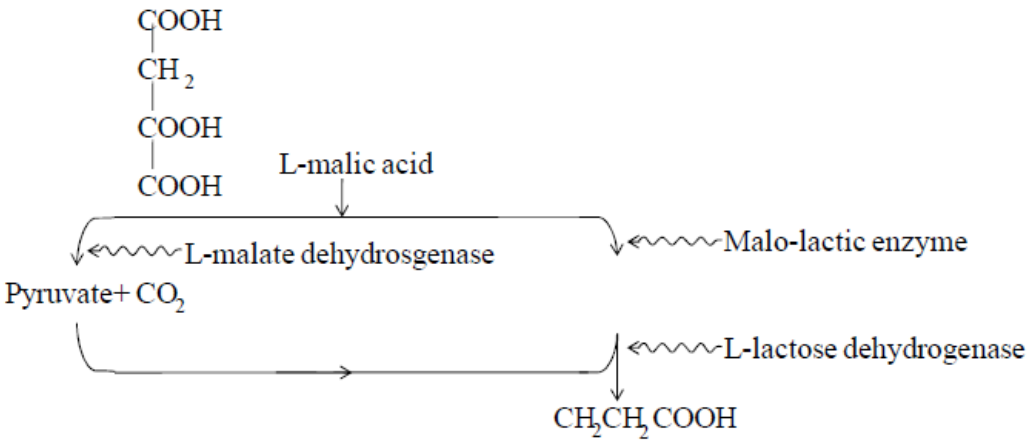
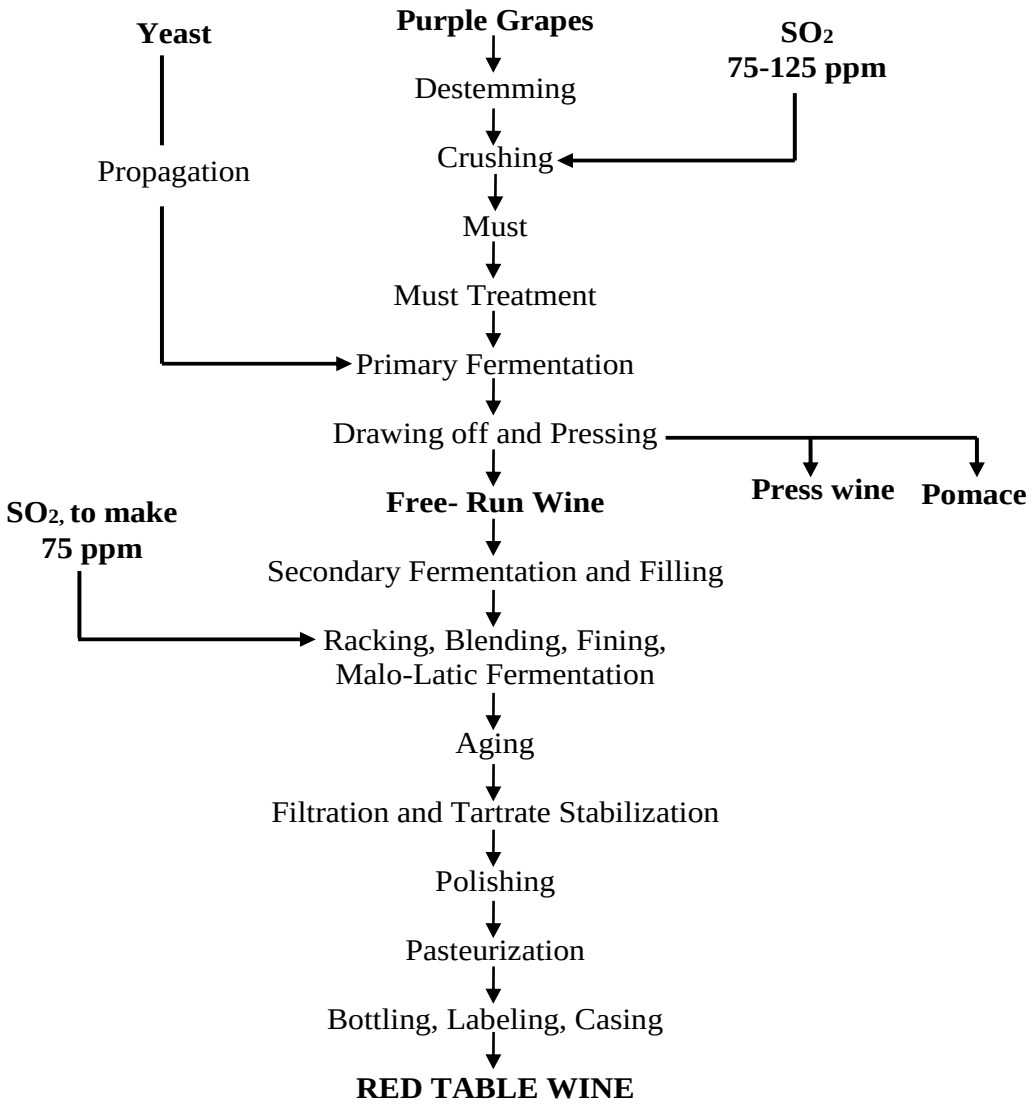


Fig. 2.2 The malolactic pathway

2.8 General method of wine preparation

Wild yeast and other microorganisms are present on the skin of the grapes and these pass into the juicy pulp (known as must) when the fruit is crushed. These are destroyed by adding sulphur dioxide (or KMS) in the required quantity. If the sugar content is low, sucrose is added to the desired strength and the pH is adjusted to 2.8 to 4 by the addition of tartaric acid. Next, the must is inoculated with a pure culture of actively growing yeast (*S. ellipsoideus*). The temperature and duration of fermentation depend upon whether dry or sweet wine is required. Fermentation usually lasts 4 to 10 days. When fermentation is complete, the clear wine is siphoned from the yeast sediment into barrels (racking) and the wine allowed to age. During this period, secondary fermentation takes place and wine also losses it's raw and harsh flavor and mellows down. During this period of maturation, clarification takes place in natural way. It can also be achieved by fining and filtration. Next, the wine is bottled and allowed to mature; the time of this maturation extends to a number of years depending upon the quality desired (Mmegwa, 1987). A simplified flow-sheet of wine reparation is given in Fig. 2.3.

`



Source: Rai (2012)

Fig. 2.3 Flow chart of red table wine preparation

2.8.1 Selection of raw material

Any suitable raw material is chosen to function as a substrate. Compared to cereals, fruit juices are more readily utilizable substrate by yeasts for the alcoholic fermentation. The latter is also a suitable media for the yeast to grow (Varnam and Sutherland, 1994). Following criteria should be fulfilled when selecting for proper raw material for fermentation.

- It should be readily available.
- It should be good source of carbon and nitrogen.
- It should have sufficient amount of fermentable sugar.

- It should not contain any toxic compound nor should impart any undesirable odor or taste.
- It should be clean sound and mature.

2.8.2 Crushing and blending

This step is carried out to extract the juice from the fruit. Selected ripe grapes are crushed to release the juice which is known as 'must', after the stalks which support the fruits have been removed. These stalks contain tannins which would give the wine a harsh taste if left in the must. The skin contains most of the materials which give wine its aroma and color. For the production of red wines the skins of purple grapes are included, to impart the color (Okafor, 2007).

In modern wine production, the grapes are harvested from vineyard and taken to the winery where these are passed through destemmer crusher machine. Three types of crusher are generally used: Roller type, disintegrator type, and garolla type the last one is more generally used (B. Rai, 2012). It has been suggested that the process should be very gentle. If the blending and crushing machine is constructed of mild steel or cast iron then iron causes “ferric cause-cloudiness” of wine due to iron; actually iron will react with the tannin of the juice to form ferric-tannin complex. Bronze equipment is also used but may cause dissolution of copper and tin from bronze equipment and it will affect the color. Usually, stainless steel is used for the crushing machine. Water may be added during blending/ crushing for smoothness of operation.

The grape juice meant for wine fermentation is called must. For consistent wine quality, the quality of must should also be consistent. If the must does not meet the requirement, grape juice concentrate, sugar, acid, etc., most be added for the adjustment. This manipulation to standardize the must is called amelioration (Rai, 2009a). Following methods can be used as per requirement:

I. Chaptalization: Chaptalization is another term used to imply addition of sugar only. Addition of sugar is supposed to produce substandard wine and is prohibited in some countries. In cooler climates, grapes often do not contain enough sugars to produce a balanced wine. This may be addressed by chaptalization, the addition of sucrose to the must or the juice in the early stages of fermentation. In some countries concentrated grape must is used instead of sugar.

II. Gallization**:** Gallization is a term used to imply addition of water and sugar prior to fermentation in order to increase alcohol content, total volume, and to decrease acidity.

III. Acidification: This may be necessary if the pH of the must is too high, that is, if the acidity is too low. The addition of tartaric acid, malic acid or citric acid or there mix called acid blend is the usual method employed.

IV. De-acidification: This may be necessary if the pH of the must is too low. It is not permitted in warmer regions of the European Union. There are a number of materials that may be used, including calcium carbonate ($CaCO_3$), potassium bicarbonate ($KHCO_3$), and potassium carbonate (K_2CO_3) (Grainger and Tattersall, 2005).

2.8.3 Sulfiting /preservatives

Sulfur dioxide (SO_2) has been used for thousands of years during winemaking as an antimicrobial and antioxidant agent. It is very effective in these roles, is readily available, and is relatively cheap and easy to use. Sulfur dioxide's main role is to prevent microbial infection of the juice and thereby prevent unwanted or spontaneous fermentations by yeasts other than that planned by the winemaker and infections by undesirable bacteria (e.g. *Acetobacter, lactobacillus*). There are three form of sulfites in wine. Molecular sulfur dioxide and bisulfite is the form that inhibits microbes. The sulfite ion (SO_3^{2-}) is mainly responsible for preventing oxidation (Ritchie, 2010). SO_2 is added before the fermentation process to prevent air from oxidizing the juice and converting the alcohol into vinegar. The air has bacteria principally *Acetobacter* i.e. it is alive in the presence of air of oxygen. These *Acetobacter* cannot convert alcohol into vinegar because SO_2 being hungry for oxygen, takes of the oxygen from the must to let the wine yeast which in anaerobic condition convert the fruit sugar into alcohol. SO_2 also forms a coating on the surface of juice to prevent the air entering the juice (Andrew, 1980).

Sulfur dioxide can react with compounds other than oxygen that may be found in musts (e.g., anthocyanin, acetaldehyde (acetaldehyde has undesirable organoleptic properties), to form `bound' SO_2, which is unable to prevent microbial spoilage or oxidation. Consequently, when we add sulfur dioxide to a juice or wine, not all will be available to protect the wine (depending on its distribution between the different forms), which complicates deciding how much to add. In practice, we have to make an estimate of how much will be in the bound form to ensure that there is sufficient molecular SO_2 (Ritchie, 2010). The most commonly

used source of SO_2 is potassium metabisulfite (KMS). In general, SO_2 is seldom used at a rate above 150 ppm. Moldy grapes may needed 200 ppm, though. Higher concentration of SO_2 make delay fermentation (sometimes as long as 2 months) (B. Rai, 2012).

2.8.4 Yeast

Wine yeasts are the member of genus of *Saccharomyces* and consequently of great individual importance (Austin, 1968). A good quality of wine yeast should have the following characters (Varnam and Sutherland, 1994).

i. Introduction of flocculation and reduction of H_2S production
ii. Reduced higher alcohol production
iii. Improved fermentation efficiency
iv. Reduced foaming.
v. Resistance to killer activity.

2.8.4.1 Yeast nutrition

Proper nutrient are must for the growth of yeast in cultural media. The cultural medium used must therefore contain all the essential elements for growth, in proportion similar to those occurring in yeast biomass. The elemental requirement (and the source) for yeast nutrition is given in Table 2.3.

2.7.4.2 Pitch development

Within the last 20 years or so, the use of active dry yeast (ADY) in winemaking has increased considerably. It has replaced the traditional practice of yeast starters in many wineries. In this formerly widespread method, a juice is strongly sulfited (10 g/hl) to eliminate spoilage yeasts and promote the growth of wine yeasts. It is then inoculated into newly filled fermenter at a concentration of 1– 3% after several days of spontaneous fermentation. Pitch of sufficient quantity is developed before preparation of must. The developing medium should have low sugar concentration so that the ‘Pasteur effect’ is maintained. Pitching is done when the culture of the pitch is at its optimum stage of growth. Vigorous agitation is done after pitching to help distribute the culture and also to help in their initial growth (Grainger and Tattersall, 2005)

Table 2.3 Elemental requirement and source for yeast nutrition

Element	Major source
Carbon	Sugar
Hydrogen	Water, organic compound
Oxygen	Water, dissolved oxygen, organic compound
Nitrogen	Inorganic source: NH_4Cl, $(NH_4)2SO_4$
Phosphorus	KH_2PO_4, Na_2HPO_4
Sulphur	Na_2SO_4, $Na_2S_2O_3$ and organic sulphur compound
Potassium	KH_2PO_4
Magnesium	$MgCl_2$
Sodium	NaCl
Calcium	$CaCl_2$
Iron	$FeCl_3$, $FeSO_4$

Source: Madigan *et al.* (2000)

2.8.5 Fermentation

Fermentation is the soul (heart) of wine making. All the desirable reactions take place during this step, so most of wine makers pay strict attention to this stage. Fermentation is the process of adding wine yeast (technically termed as *S. ellipsoidues*) to fresh juice to convert the natural sugar to ethyl alcohol. In this process, CO_2 is simultaneously released making fermentation violent at first and then slow. The yeast added is 1-3 % of the volume of the juice. Generally 14 days is required for complete alcoholic fermentation. Most of the fermentation takes place in three stages.

- An initial stage during which time the yeast cells are multiplying.
- A very vigorous stage accompanied by bubbling and marked rise in temperature.
- Quiet fermentation that can proceed for quite a long time at a lower and lower rate.

Fermentation time may range from 2-20 days depending upon numerous variables- types and condition of fruits, type of wine being made, climatic condition among others. Temperature is quite critical to the fermentation process (Douglas and Considine, 1982). The optimum temperature for fermentation of Red wine is higher than that of White wine. The optimum temperature is believed to be 21.1-27.4ºC (Johnson and Peterson, 1974). At temperature above 90ºF (32.2ºC), it is likely that wine flavor and bouquet will be injured. High temperature also encourages heat tolerant bacteria to produce acid, mannitol and off flavor (Douglas and Considine, 1982).

Johnson and Peterson (1974) reported that at the usual total sugar content of 19-24%, alcoholic fermentation proceeds rapidly and, with alcohol tolerant strains of yeast, to completion, producing about 10-12.5% alcohol (by volume). If the sugar content is greater than 24%, the high sugar content may inhibit fermentation and the rate of fermentation will be slower and may be incomplete. Under special condition of simulation, 16-18% alcohol can be reached. It is generally agreed that methanol is not produced by alcoholic fermentation, from glycine for example, but is primarily derived from hydrolysis of naturally occurring pectin. The amount of higher alcohols produced is less when ammonium phosphate is added prior to fermentation. At very low concentration the higher alcohols may play a desirable role in sensory quality (Amerine *et al.*, 1980).

Guymon *et al.* (1961) Showed that oxidative conditions during fermentation favor higher alcohol production. According to Gentillini and Cappelleri (1959), glycerol production is favored by low temperature, high tartaric content and by addition of SO_2. Most of the glycerol develops in the early stages of fermentation. Most enologists consider that glycerol is of considerable sensory importance because of its sweet taste and its oiliness. Acetaldehyde is a normal by-product of alcoholic fermentation. Kielhofer and Wurding (1960) showed that acetaldehyde retention is much greater when SO_2 is added before the fermentation. According to Kielhofer and Wurding (1960), the primary source of acetaldehyde is from enzymatic process, i.e., in the presence of yeast. Acetaldehyde reacts with ethyl alcohol to form acetal, a substance with a strong aldehyde like odor, found very little in wines (Amerine *et al.*, 1980).

The tartaric, malic and citric acids of the must are found in the resulting wines but in decreased amounts. They are important constituents of wine not only for their acid taste but also because they protect the wine from spoilage, maintain the color, and are themselves

sometimes attacked by microorganisms. Malic acid disappears during alcoholic fermentation to the extent of 10 to 30 %. Succinic acid is a product of alcoholic fermentation. Lactic acid has a slight odor and is a weak acid. It is a constant by-product of alcoholic fermentation, 0.04 to 0.75 g/L. Carbonic acid constitutes a very special case for both still and sparkling wines. It has no odor and very little taste. But it does have a feel and disengagement of the bubbles from the wine probably brings more oxygen away from the surface of wine (Amerine *et al.*, 1980).

The end of fermentation is signaled by a clearing of the liquid, by a vinous taste and aroma, and by a drop in temperature, and can be confirmed by checking degrees balling (sugar residual) (Douglas and Considine, 1982).

2.8.6 Racking

Racking is the process of transferring juice or wine from one vessel to another, leaving any sediment behind. One of the most important factors in producing clear, stable wine is racking, i.e. Siphoning (Grainger and Tattersall, 2005). After completion of fermentation, the wine must be separated from the dead cells because, it may lead to yeast autolysis and, at low redox potential, formation of H_2S which give off flavors and odors to wine. This dead yeast settle at the bottom of the fermentation vessel and the wine is carefully transferred (siphoned) to other vessel without disturbing the dead yeast leaving some wine at the bottom called lees. The advantages of racking are:

i. It helps removing CO_2.
ii. It raises O/R potential, which retards the formation of H_2S.
iii. It clarifies the wine(Andrew, 1980).

Normally, wine should be racked within a month of the end of fermentation. Racking process normally entails a sacrifice of 2-3% wine in lees (B. Rai, 2012).

2.8.7 Fining and filtration

Fining is a process of converting cloudy wine into clear wine. With the coarse sediment removed by racking or centrifuge, there remains other lighter matter suspended in the wine known as colloids. These are capable of passing through any filter. If not removed they will cause the wine to look ‘hazy’ and then form a deposit. The colloids are electrostatically charged and can be removed by adding another colloid with the opposite charge. Examples of such fining agents are egg whites, gelatin, isinglass (obtained from swim bladders of fish)

and bentonite. Quantities need to be carefully controlled otherwise the fining agent itself will form a deposit, or a further, opposite, electric charge may be created. Fining may also be used to remove excess tannin and so improve the taste of the wine. Phenolic compounds are absorbed by the substance PVPP (polyvinyl polypyrrolidone). This may be used at the fining stage to remove color from white wines and help prevent browning (Grainger and Tattersall, 2005).Typically, bentonite can be used at a rate of 1.5 g/L. However, it is essential that the fining agents be tested for dosage optimization before use because, over fining can cause a permanently cloudy wine (B. Rai, 2012) .

Filtration is the process used to remove solid particles, and may take place at various stages in wine making, for example must or lees filtration. However, one of its main uses is in the preparation for bottling. The processes of fining and filtration are not interchangeable. There are three principal categories of filtration, which may be used at different stages in the winemaking process.

Earth filtration

This filtration method is used for initial rough filtration and can remove large quantities of 'gummy' solids, which consist of dead yeast cells and other matter from the grapes. The filtration takes place in two stages. Firstly, a coarse grade earth called kieselguhr, which is commonly used as the filter medium, is deposited on a supporting screen within a filter tank. A mixture of water and kieselguhr may be used to develop the filter bed. This is known as precoating. Secondly, more earth is mixed with wine to form a slurry that is used continuously to replenish the filtration surface through which the wine passes. Wine is passed through the filter and the bed gradually increases in depth. Eventually it will clog and the kieselguhr will have to be completely replaced with fresh material (Grainger and Tattersall, 2005).

I. **Sheet filtration (plate and frame filter)**

A series of specially designed perforated steel plates are held in a frame. Sheets of filter medium (cloth or paper) are suspended between the plates, which are then squeezed together by screw or hydraulic methods. The filter sheets are available with various ranges of porosity filter aid such as hyflosupercel , diatomaceous earth, etc are used to facilitate the filtration process. Wine is pumped between pairs of plates to pass through the filter sheets into a cavity

in the plates and then to exit the system. Yeast cells and other matter are trapped in the fibres of the filter media (Grainger and Tattersall, 2005).

II. **Membrane filtration**

In recent years microfiltration has been increasingly applied as the final process before bottling. Microfiltration membranes are usually in a tubular configuration for use with wine. Pre-filtration is not required, but clarifying and stabilizing agents such as bentonite are still necessary to maintain a sufficiently high product flow. The capital cost of microfiltration system is relatively high, but this is offset by the operating efficiency, reliability and versatility. Maintenance and cleaning costs are also low (Varnam and Sutherland, 1994). The membrane operates as a molecular sieve which permits the passage of water, ethanol, flavor compounds, selected macromolecules and other dissolved species, but retains suspended material such as colloids and microbial cells. They also greatly reduce the number of bacteria. The process is not used for full-bodied red wines as it can reduce body and flavor (Grainger and Tattersall, 2005).

2.8.8 Stabilization of wine

Stabilization may be carried out to prevent tartrate crystals forming after the wine has been bottled. The tartrates are either potassium or calcium salts of tartaric acid and the crystal are also called *wine diamonds* and are totally harmless. They are sometimes found on the cork or as sediment in the bottle, and sometimes cause unwanted concern to consumers. To inhibit the precipitation of tartrate crystals in bottle, the wine is chilled to -4ºC, or colder in the case of liqueur (fortified) wines. After approximately 8 days the crystals will have formed, and the cleared wine can be bottled. Another method of removal is to reduce the temperature of the wine to approximately 0ºC and seed it with finely ground tartrates, followed by a vigorous stirring. The seeds then attract further crystals to them and the entire process of removal takes just 24 hour or so (Grainger and Tattersall, 2005).

2.8.9 Maturing and ageing of wine

This is one of the most interesting and one of the most important, yet one of the most complex processes of wine making. Newly fermented wine is cloudy, harsh in taste, yeasty in flavor and odor, and without the pleasing bouquet that develops later in its history (Rai, 2009a). Maturation in winemaking terms is the time period, and associated changes, that occur in a wine between alcoholic fermentation and bottling, while the wine is still in bulk

storage in the production facility. The period after bottling and before consumption in the life of a wine should be referred to as 'bottle ageing,' but for the purposes of discussion, it shall just be termed 'ageing' (Buglass *et al.*, 2011).

Immediately after fermentation, wines may taste rough and fairly unpleasant. A period of maturation is required. This period may be anything from 2 to 24 months, or longer, depending on the style of wine being made, and may include processes such as malolactic fermentation, oak coopering, racking, ageing in tanks or barrels, fining and filtration (Buglass *et al.*, 2011) . The choice of maturation vessel and the period of time depend upon the style of wine to be produced and quality and cost factors. There are many types of maturation vessels, including stainless steel vats and wooden barrels (Grainger and Tattersall, 2005).

Chemical processes during maturation and ageing include the oxidation of phenolics and other substances, formation of aldehydes and esters and hydrolysis of glycosides and other components. Physical effects include salt precipitation, loss of carbon dioxide, evaporation of volatile substance and dissolution of oak components. Effects may include loss of brightness, changes to the color of the wine and character of the bouquet, and rounding and softening of tannins (Buglass *et al.*, 2011). Aging of wines improves the flavor and bouquet due to oxidation and formation of esters. These esters of higher acids formed during aging give the ultimate pleasing bouquet to the well-aged wine (Clarke and Bakker, 2004).

2.8.10 Bottling

Following filtration and clarification the wine passes to storage tanks prior to bottling. The use of glass bottles is universal for high quality wine. Bottles are cleaned, dried with hot air and cool for this purpose. The cork is the traditional means of closing the bottle, and this is protected from dehydration and mold growth by a lead foil or, in recent years, a plastic outer cap. Wine is bottled under an inert atmosphere (CO_2 and / or nitrogen) to protect wine from oxidation. Additions may also be made before bottling to stabilize the wine against microbiological and chemical deterioration, SO_2 and sorbic acid are most commonly used (Varnam and Sutherland, 1994).

2.8.11 Pasteurization

Pasteurization is the process used to kill microorganisms present in the wine so that fermentation is stopped and increase the shelf life. Wine pasteurization usually occurs for

shorter periods or at lower temperatures than typical for products such as milk. This is possibly due to wine's low pH and ethanol content, both of which markedly depresses the thermal resistance of yeasts and bacteria. And approximately 3 min at 60ºC should be sufficient for a wine at 11% ethanol. Flash pasteurization at 80ºC usually requires only a few seconds as well hot bottling of wine at temperature 55-70ºC can also be done. Sulfur dioxide reduces still further the need for heating. High temperatures markedly increase the proportion of free SO_2 in wine. Although pasteurization kills most microbes, it does not inactivate the endospores of *Bacillus* species. On rare occasions, these bacteria may induce wine spoilage. The quality of some wine is reduced by pasteurization while that of other may be improved. Pasteurization inactivates the enzymes but injure the quality of the product Due to complexities of establishing the most appropriate time and temperature conditions for pasteurization, membrane filters have replaced pasteurization in most situations. Filters also result in few physical or chemical disruptions to the sensory characteristics of wine. Membrane filters with a pore size of 0.45 μm or less are standard (Jackson, 2014).

2.8.12 Finishing

The traditional method of finishing the wine was to turn the bottles on end, place them in racks at about 45º angle and turn them to the left and right daily to get the yeast deposit into the neck of the bottle and on the cork. The process is called riddling "reumage". The temperature of the whole bottle is then reduced to about 30ºF to 40ºF. the neck of the bottle containing the yeast deposit is then frozen (by placing in brine or other freezing solution) When the cork is removed the solid plug containing the yeast is ejected. This is called disgorging (Pederson, 1980).

2.8.13 Storage of wine

Storage of wine is an important consideration for wine that is being kept for long-term ageing. There are some factors that have the most direct impact on a wine's condition are temperature, light and humidity. The perfect storage temperature for wine, is supposed to be 52ºF (11ºC), anything between 40°F and 65ºF (5ºC and 18ºC) will in fact suffice for most styles of wines. All wines are affected negatively by the ultraviolet end of the light spectrum, hence, in the cellar, wines are stored in corrugated boxes or wooden crates to protect the wines from direct light. A certain humidity (between 60 and 70 percent) is essential to keep the cork moist and flexible, thereby avoiding oxidation. The position in which a wine bottle is stored is also extremely important. Most wines should be laid horizontal position so that

the wine keeps the cork moistened, and therefore fully swollen and airtight. Exceptions to this rule are sparkling wines and any wine that has been sealed with a screw top lid Wines should also be stored under vibration-free conditions, but this only becomes a significant factor over a long period for sparkling wines and mature wines with sediment (Stevenson, 2005).

2.8.14 Yield

The theoretical conversion of 180 g of sugar into 88 g of carbon dioxide and 92 g of ethanol means that yield of ethanol is 51.1% on a weight basis. This percentage may vary depending upon inoculum size, fermentation temperature and nutrient availability (Usansa, 2003). Under special condition of simulation 16-18 % alcohol can be reached, but normally in commercial operation, 13-15 % is the maximum (Johnson and Peterson, 1974).

2.9 Wine analysis

Throughout the history of wine making, analytical techniques have become increasingly important with the development of technology and increased governmental regulation. Analysis of wine is performed for a number of reasons such as quality control, spoilage reduction and process improvement, blending, export certification and global regulatory requirements (Fugelsang, 1996).

2.9.1 Physical and chemical analysis

All wines should be subjected to appropriate analyses during their production and storage to meet the requirements of regulatory agencies and to give the winemaker information to monitor the operations properly (Fugelsang, 1996).

Experimental wines often require additional analyses to obtain more complete information and study the specific effects of the experimental conditions. There is no sense in doing the experiments unless analytical methods are available to evaluate the results. Planning for these analyses and the labor and timing for them should precede initiation of the experiments. Some analyses can be done more or less at leisure on the finished wine, others must be done at specific moments or the experiment is spoiled. Sometimes interim samples can be quickly frozen and held for later analyses as a group. Other cases arise where this is not possible for experimental or logic reasons (Boulton, 1998).The components of wine and must can be broken into classes and are given in Table 2.4

Table 2.4 Component of wine.

Soluble solids:	sugar extract glucose and fructose
Acidity:	total volatile pH individual acids
Alcohols:	ethanol methanol fusel oils glycerol
Carbonyle compounds:	acetaldehyde HMF diacetyl
Esters:	ethyl acetate methyl anthranilate (labruscana)
Nitrogen compounds:	NH_3 amino acids Amines 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)

According to Amerine *et al.* (1980), the different parameters viz. alcohol by volume,(%), alcohol, glycerol, ash, total acids, volatile acids, reducing sugars, proteins, tannins and specific gravity of different wines were analyzed. According to Pearson (1981), analytical parameters of different wines were specific gravity, alcohol (g/100, % total solids, % free volatile acids (as acetic acid), % fixed acid (as acetic acid), % ash and % sugar.

Different dissertations related to wine held in Central Campus of Technology, Hattisaar, Dharan have mostly analyzed the parameters such as pH, TSS, alcohol content, acidity, reducing sugar, aldehydes, esters, specific gravity, total sugars, ash, methanol and higher alcohols (Raut, 2014).

2.9.2 Sensory evaluation

2.9.2.1 Development of sensory evaluation

Sensory tests of course have been conducted for as long as there have been human beings evaluating the goodness and badness of food, water, weapons, shelters, and everything else that can be used and consumed. The rise of trading inspired slightly more formal sensory testing. A buyer, hoping that a part would represent the whole, would test a small sample of a shipload. Sellers began to set their prices on the basis of an assessment of the quality of goods. With time, ritualistic schemes of grading wine, tea, coffee, butter, fish, and meat

developed, some of which survive to this day. Grading gave rise to the professional taster and consultant to the budding industries of foods, beverages, and cosmetics in the early 1900s. A literature grew up which used the term "organoleptic testing" to denote supposedly objective measurement of sensory attributes. In reality, tests were often subjective, tasters too few, and interpretations open to prejudice. Scientists have developed sensory testing, then, very recently as a formalized, structured, and codified methodology, and they continue to develop new methods and refine existing ones (Meilgaard *et al.*, 1999).

Sensory evaluation is an integrated, multidimensional measure with three important advantages: it identifies the presence of notable differences, identifies and quantifies important sensory characteristics in a fast way, and identifies specific problems that cannot be detected by other analytical procedures. The methods that have been developed serve economic interests. Sensory testing can establish the worth of a commodity or even its very acceptability. Sensory testing evaluates alternative courses in order to select the one that optimizes value for money. The principal uses of sensory techniques are in quality control, product development, and research. They find application not only in characterization and evaluation of foods and beverages, but also in other fields such as environmental odours, personal hygiene products, diagnosis of illnesses, testing of pure chemicals, etc. The primary function of sensory testing is to conduct valid and reliable tests, which provide data on which sound decisions can be made (Meilgaard *et al.*, 1999).

2.8.2.2 Sensory evaluation of wine and importance

Sensory evaluation has become a popular research tool in the food and beverage industries and is 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 they are perceived by the senses of sight, smell, taste, touch and hearing." Changes in product formulation may produce desirable or undesirable changes in the final product and must be assessed, analyzed, then interpreted in a meaningful way. Without the proper sensory evaluation techniques it is difficult to interpret sensory response and make logical and sound decision. Even the most sophisticated chemical analysis cannot now, and probably never will, define the subtle flavors that make one wine greater than another in the opinion of observant consumers. That is as it should be. As a consequence, it is almost always necessary to compare wines by sensory analysis in addition to chemical and physical methods. This is true of commercial wines, but often especially so with experimental wines (Savits, 2014).

Wine is an exceedingly complex beverage, containing an estimated several hundred volatile compounds. The compounds may arise from the grape itself, through the process of crushing and enzyme action, through fermentation, and over the period of maturation. In the greater sense, there are a collection of factors contributing to the vast number of wine types and styles: climate, grape variety, stage of ripeness at harvest, winemaking techniques, and storage methods. Thus, sensory evaluation methods have been applied to study characteristics in wine related to these factors. More than 1000 compounds have been identified in grapes and wine, with individual concentrations varying considerably. Our ability to perceive compounds is dependent not only upon their presence at or above a sensory threshold concentration, but also upon their interaction with other components. The sensory properties of a particular wine therefore, are dependent upon chemical and physical effects relating to the specific matrix or composition (Savits, 2014).

In spite of opinion to the contrary by wine writers and some wine makers, one person's opinion is hardly definitive on any wine's sensory character and quality. That is not to say that one tester may not be better than another in natural ability, concentrated effort, amount of experience, and/or comparative memory. In evaluation of the sensory qualities of one or more wines a panel of testers is necessary. This panel should be as sensitive and experienced as possible, but each individual is erratic, biased, or unobservant on some occasions, hence the need for panels and statistical evaluation of the testing results (Lesschaeve, 2007).

No technique is ideal for everyone. Probably the most essential property of a serious taster is the willingness, desire, and ability to focus his or her attention on the wine's characteristics. Peynaud (1987) advocates rinsing the mouth with wine before embarking on serious tasting. Where tasters are unfamiliar with the characteristics of the wines to be tasted, it can familiarize the senses to the basic attributes of the wines. However, the introductory sample must be chosen with care to avoid setting an inappropriate standard and distorting expectations. It is safer to encourage tasters to cleanse their palate between each sample. In contrast, olfactory adaptation may have an advantage. For example, it may "unmask" the presence of other aromatic compounds. Most wines are best sampled in clear, tulip-shaped wine bottle. The primary exception involves sparkling wines. These are normally judged in elongated, flute-shaped glasses. They facilitate observation of the wine's effervescence. All glasses in a tasting should be identical and filled to the same level (about one-quarter to one-third full). This permits each wine to be sampled under equivalent conditions. Between 30 and 50 ml is adequate for most tastings. Not only are small volumes economic, but they

facilitate holding the glass at a steep angle (for viewing color and clarity) and permit vigorous swirling (to enhance the release of aromatics) (Jackson, 2002).

2.10 Color of wine

The color of red wine is derived initially from anthocyanin pigments. The fermentation of grapes for wines has a marked effect upon the color of the product. The final color may be influenced by the SO_2 content and the alcohol content attained at the time of screening (Berg and Akiyoshi, 1962). Maximum color is attained between 3 and 6 % alcohol and the amount of color extracted increases with increasing SO_2 content up to 250 ppm. The color stability during the aging of wines was superior at the higher level of SO_2. Berg and Akiyoshi (1962) noted that non-fermented wines fortified with alcohol had much higher color retention during aging than those produced by fermentation Jackson (2009). Wine production practices including the level of SO_2 and alcohol content have an influence on the color equilibrium between anthocyanogens and anthocyanins. Often testers associate particular colors with certain wines. Young, dry, white wines generally ranges from nearly colorless to pale straw colored. A more obvious yellow tint may suggest long maceration or maturation in oak cooperage. Sweet white wine may vary from a pale straw to yellow- gold to brown. Ascorbic acid is an effective oxygen scavenger reacting with O_2 (which would otherwise react with phenolic to produce browning) around 1700 times more quickly than SO_2.(Somers and Evan, 1997).

2.11 Volatile components in wine

The volatile compounds, as the factors influencing taste and aroma of the final product are present in wine. In terms of volatile compounds, wine is one of the most complex beverages. More than 800 volatile compounds such as alcohols, esters, organic acids, aldehydes, ethers, ketones and terpenes, *etc*., have been identified in them, with a wide concentration range varying between hundreds of mg/L to the μg/L or ng/L levels, and their combinations form the character of wine and differentiates one wine from another (Jiang and Zhang, 2010). How many, and what types of volatile compounds are present depends on many factors such as the vineyard's geographical site, which is related to soil and climate characteristics, grape variety, yeast strain, and technical conditions during wine making (Usansa, 2003).

2.11.1 Alcohol

A range of alcohols is present in wine. The most important of these is ethanol. Although small quantities are produced in grape cells during carbonic maceration, the primary source of ethanol in wine is yeast fermentation. Ethanol is crucial to the stability, aging, and sensory properties of wine. The inhibitory action of ethanol, combined with the acidity of the wine, permits wine to remain stable for years in the absence of air. Ethanol has multiple effects on taste and mouth-feel. It adds directly to the perception of sweetness. It indirectly modifies the perception of acidity, making acidic wines appear less sour and more balanced. At high concentrations, alcohol produces a burning sensation, and may contribute to the feeling of weight (body), especially in dry wines. Ethanol can also increase the intensity of bitterness, decrease the astringency of tannins and influence the volatility of aromatic compounds. In addition to helping to dissolve pigment and tannin extraction from grapes, it is a solvent for many volatile compounds produced during fermentation, and formed during maturation in oak cooperage (Jackson, 2014).

Methanol occurs in wine, but only in trace amounts. Within its normal range (0.1–0.2 g/L), methanol has no sensory or health consequences. Of the over 160 esters found in wine, few are associated with methanol. Health concerns connected to methanol relate to its metabolism to formaldehyde and formic acid. Both are toxic to the central nervous system. One of the first targets of formaldehyde toxicity is the optic nerve, causing blindness. However, methanol never accumulates to toxic levels in wine, at least under legitimate winemaking procedures. The marginal amount of methanol that is found in wine comes almost exclusively from the demethylation of pectin. These methyl groups are released as methanol. Thus, methanol content is a partial function of the must pectin content. Unlike most fruits, grapes are low in pectin content. Thus, wine has the lowest methanol content of any fruit-based, fermented beverage. However, pectolytic enzymes, added to juice or wine as a clarification aid, can inadvertently increase the methanol content. Adding distilled spirits to a wine may also slightly increase the methanol content (Jackson, 2014).

Alcohols with more than two carbon atoms are commonly called higher or fusel alcohols. They commonly account for about 50% of the aromatic constituents of wine, excluding ethanol. The principal higher alcohols produced by yeast are the aliphatic alcohols n-propanol, isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-mthyl-1-butanol), and the aromatic alcohols hexanol and 2- phenethyl

alcohol. The higher alcohols content in wine should be 80-540 mg/L the concentration of higher alcohols below 300 mg/L strengthens the desirable aroma of wine, whereas these components are seen as a negative factor in creating the aroma when their level exceeds 400 mg/L (Usansa, 2003).

The higher alcohols are important as the immediate precursors of the more flavor active esters, so that the control of higher alcohol formation needs regulation to ensure that, in turn, ester production is controlled. The higher alcohols are produced by yeast as secondary metabolites of amino acid metabolism. The situation is actually complicated by the fact that yeast cells are capable of synthesizing their own higher alcohols from other pathways rather than from amino acids. Again, as for esters, yeast strain turns out to be the most important factor. Conditions which favor increased yeast growth, such as excessive aeration or oxygenation, promote higher alcohol formation, but this can be ameliorated by the application of a top pressure during fermentation (Baxter and Hughes, 2001).

2.11.2 Ester

There are a number of esters which contribute to the flavor of wines. Ester plays an important role in the formation of wine's sensory characteristics. They are formed from acids and alcohols during wine fermentation and fermentation process. There are a lot of different alcohols and acids in wines, so the number of possible ester is also very large. Ester in wine have two distinct origins; enzymatic esterification during the fermentation process and chemical esterification during long term aging (Usansa, 2003). Biosynthesis of esters mainly depends on fruit maturity, yeast species, must aeration, fermentation technology and temperature. Their amount in young wines varies over a wide range (from 25 to 300 mg/L). The majority of esters are formed at the beginning of fermentation, and during wine maturation their concentration changes only slightly. Among wine esters very important in terms of bouquet are isoamyl acetate (banana aroma), 2-phenylethyl acetate (rose aroma), and ethyl acetate (strong, sweet aroma) (Clarke and Bakker, 2004).

2.11.3 Aldehyde

Acetaldehyde is of special interest because of its role as the immediate precursor of ethanol. It has an unpleasant 'grassy' flavor and aroma. Acetaldehyde is formed during the early to mid-stages of fermentation and thereafter it declines to a low level. In some circumstances, it can accumulate during fermentation in concentrations above the flavor threshold of 10-20 ppm. The principal causes of high acetaldehyde concentrations in wine are the use of poor

quality pitching yeast, excessive must oxygenation, unduly high fermentation temperature and excessive pitching rates (Briggs *et al.*, 2004).

Generally, white and red wines have similar aldehyde contents. The aldehyde content is however, low and this may be explained by the fact that the sulphur dioxide added to wine reacts with aldehydes to form a-hydroxysulphonic acids, which reduce the free aldehyde content. Furthermore, aldehydes can be chemically bound to ethanol and higher alcohols as acetals. White and red wines produced in various countries contain I-propanol (11-125 mg/L), 2-methyl-1 propanol (15-174 mg/L), 2-methyl-1-butanol (12-311 mg/ L) and 3-methyl-1-butanol (isopentanol;49-180 mg/L). Aldehydes also play a role in color, by reacting with sulfites and preventing bleaching, and more importantly, by participating in the binding of anthocyanins to tannins and stabilizing color. Finally, aldehydes also play a role in texture, due to the above participation in tannin polymerization reactions (Frivik and Ebeler, 2003).

2.12 Nutritional aspects and health benefits of wine

The excessive abuse of distilled alcoholic beverages, combined with religious and political conservatism, created a backlash against all beverages containing alcohol. From a scientific standpoint, much more attention has been given by the researchers to the non-nutritional aspects of wine than to what substances, in addition to alcohol, it may contain of tangible value to the consumer. Now, research concentration has largely been directed to better understanding such aspects as flavor, bouquet, keeping qualities, better ways to utilize, chemistry and biochemistry etc. in processing (Douglas and Considine, 1982).

According to Louis Pasteur, wine is the “healthiest and most health-giving of drinks.” The use of wine as a medicine, or as a carrier for medications, has a long history. It goes back at least to the ancient Egyptians. Ancient Greek and Roman society used wine extensively in herbal infusions (Jackson, 2000).

According to Mmegwa (1987) beer and wine contain some nutrients present in the original malted barley and the fruit juice used in their proportion and naturally their energy value would be higher than that of distilled liquor; 100 ml of wine gives about 80 Kcal. Wine’s major nutritional value comes from the rapidly metabolized, caloric value of its ethanol content. Alcohol does not need to be digested, and can be absorbed directly through the intestinal wall. In rural viticultural areas, wine historically functioned as a major source

of metabolic energy for the adult population. Wine in those regions was a food (Jackson, 2000).

Wine contains small quantities of several vitamins, notably the B vitamins, such as B1 (thiamine), B2 (riboflavin), and B12 (cobalamin). Morgan et al (1939) reported that about 2/3rd of the thiamin and riboflavin in grape juice is lost during winemaking but that very little is lost during aging. They found that white wines contained more riboflavin as well as, the mineral contents of red wine generally exceed those of white wine, notably as regards potassium, sodium, phosphorus, magnesium, iron, strontium, manganese, zinc, copper, barium, and thus in terms of total ash. Red wines were slightly lower in calcium and Aluminium. As regards to vitamin content of wine, Lucia (1954) reported that when wines are taken along with a good and balanced diet, their content of thiamine, riboflavin pentothenate, niacin and vitamin B_6 contribute to total nutrition. Although wine contains soluble dietary fiber, especially red wines. It is insufficient to contribute significantly to the daily recommended fiber content in the human diet (Jackson, 2000).

Nowadays, it is becoming equally clear that moderate wine consumption (250– 300 ml/day) has undeniable health benefits. Multiple epidemiological studies suggest that daily, moderate, alcohol consumption and especially wine is associated with a reduction in all-cause mortality. This is expressed in a U-shaped curve, with increased mortality being associated with both excess alcohol intake and abstinence. This is particularly evident in the reduced incidence of cardiovascular disease in moderate alcohol consumers. In addition, it reduces the likelihood of non-insulin dependent diabetes, combats hypertension, and reduces the frequency of certain cancers and several other diseases. These epidemiological correlations are being supported by *in vivo* studies that provide molecular explanations for these associations. Wine also has several indirect benefits on food digestion. wine stimulates the production of gastric juices and foster a healthy appetite (Jackson, 2014).

2.13 Wine defects and spoilage

Like beer, wine has its defects from non-microbial causes and spoilage caused by microorganisms. Defect include those, due to metals or their salts, enzymes and agents employed in coloring the wine. Iron, for example, may produce a sediment known variously on grey, black, blue or ferric casse and in white wine, it may be responsible for a white precipitation of iron phosphate termed white casse. Tin and copper and their salts have been blamed for cloudiness. White wines may be turned brown and red wines may have their

color precipitated by peroxidase and oxidizing enzyme of certain molds. Gelatin used in clarifying wines, may cause cloudiness. The main role of microorganisms in winemaking is to convert grape sugars to alcohol, reduce wine acidity and contribute to aroma and flavor. They can also cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. Winemaking processes include multiple stages at which microbial spoilage is likely to occur and ends up with altering the quality and hygienic status of the wine. This may render the wine unacceptable, since the spoilage can include bitterness and off flavor, and cosmetic problems such as turbidity, viscosity, sediment and film formation. The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria (Mojsov *et al.*, 2006).

2.13.1 Wine defect caused by yeast

Yeasts play a central role in the spoilage of beverages, mainly those high acidity and reduced water activity. The spoilage caused in wine by yeasts is important because they cause re-fermentation, ester formation, hydrogen sulphide and volatile sulphur compounds, volatile acidity, the formation of volatile phenols, mousiness, film formation, deacidification and the formation of ethyl carbamate (Mojsov *et al.*, 2006).

The yeast *Schizosaccharomyces pombe* has been associated with wine spoilage when growing in bottled wine and forming a sediment at the bottom of the bottle. The yeast *Zygosaccharomyces bailii* is one of the major wine spoilage yeasts, re-fermenting juice or wine during storage. Yeasts *Hansenula anomala, Kloekera apiculata and Hanseniaspora uvarum* are associated with ester taint of faulty wines, which correlates with large amounts of acetic acid. These three species are associated with grape juice and result in spoilage at the early stages of alcoholic fermentation. The ester taint can be linked to the presence of ethyl acetate and methyl butyl acetate. Hydrogen sulphide is produced by yeasts during fermentation through the sulphate reduction pathway and has a flavor threshold of 50-80 mg/L and when exceeding this value will produce the rotten egg off flavor. One of the yeasts that can withstand the toxicity of ethanol levels and which has become the latest concern for most winemakers as a result of phenolic off flavors, is *Brettanomyces/Dekkera.* Wines typically associated with a “Bretty character” is commonly recognized by aromatic defects ranging from medicinal smells to farmyard like odors and even spicy clove like aromas (Mojsov *et al.*, 2006).

2.13.2 Wine defects caused by bacteria

Bacteria are part of the natural microbial ecosystem of wine and play an important role in winemaking by reducing wine acidity and contributing to aroma and flavor. They can cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. Lactic acid and acetic acid bacteria are the main families of bacteria found in grape must and wine. (Mojsov *et al.*, 2006).

In presence of air, the aerobic acetic acid bacteria, usually *Acetobacter aceti* of *Gluconobacter oxydane,* oxidize alcohol in wine to acetic acid, an undesirable process called 'acetification'. They also may oxidize glucose in the must to gluconic acid and may give a 'mousy' or 'sweet-sour' taste to the must. If the larger amounts of sugar in must or wine are fermented by the lactic acid bacteria, variable amounts of CO_2, ethanol, volatile acid and mannitol are formed depending on the particular species. Wine which have undergone changes in this manner are said to have a 'lactic acid flavor'(S. C. Prescott and Dunn, 2004).the growth of lactobacilli produces milky cloudiness, increase lactic and acetic acid and yield CO_2. It sometimes give 'mousy' or other disagreeable flavor and damages the color of the wine (Mojsov *et al.*, 2006).

2.13.3 Prevention of wine spoilage

Winemaking processes include multiple stages at which microbial spoilage is likely to occur. The first stage involves the fruit material to be processed and equipment to be used. One must attempt to reduce the numbers of microbes in the juice and on the equipment. This is achieved through processing the pulp by applying food hygiene practices and following the hazard analysis critical control point (HACCP) system. The second stage of microbial spoilage may occur during fermentation because at this stage, the fruit juice contains both the natural flora of the fruit and flora that may be harboured by the wine cellar and its equipment. Traditionally, sulphur dioxide has been used to control unwanted micro-organisms during winemaking, where it is usually added to bins of machine-harvested grapes and after malolactic fermentation. Sulphur dioxide acts as both an antimicrobial agent and an antioxidant in wine. Physical removal of microorganisms through filtration of juice or wine can also be used. However, filtration typically is mainly conducted prior to bottling and hence is not used to remove microorganisms during winemaking (Mojsov *et al.*, 2006).

However best way to avoid wine spoilage is not always clear-cut. As an initial barrier, the high ethanol concentrations (up to 16% v/v), high wine acidity (pH as low as 2.9) can

inhibit development of bacterial populations. Storage of wine at temperatures below 15˚C might assist with minimizing the ability of bacteria to proliferate in wine, but will also delay wine maturation. To prevent microbial spoilage of the finished wine, it is important to deactivate any residual microorganisms before or after bottling. This can be accomplished by pasteurization, addition of inhibitors such as SO_2 or by filtration. The delicate flavor of some wines is harmed by heating or by adding SO_2. For these wines, filtration is preferred method of removing microorganisms (Banwart, 2004).

2.14 Wine raw materials

Different fruits are taken as raw materials to prepare wine. Basically the term 'wine' is applied to the product made by alcoholic fermentation of grapes or grape juice, with an aging process. However, products of fermentation of other berries, fruits and honey are also called wines. Actually honey wine is called mead.These are designated by the substance from which they were made. For example, Perry (pear wine) is prepared from the juice of pears, Cider is prepared from the juice of apple, and Basi is prepared from banana juice (Jones, 1995). So honey can also be used to prepare wine as raw material.

2.15 Honey

2.15.1 Introduction

Honey is produced by bees and some related insects, sweet in taste and really viscous (Crane, 2013). Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation (Crane *et al.*, 1984).

Bees store honey in wax structure called honeycombs. Honey is suitable for long-term storage because of its chemical composition and properties and is easily assimilated even after long preservation. Honey, and objects immersed in honey, have been preserved for centuries (Prescott *et al.*, 1996) . To inhibit fermentation, honey has a sufficient high sugar content. If exposed to moist air, its hydrophilic properties pull moisture into the honey, eventually diluting it to the point that fermentation can begin (Root and Root, 2005).

Long shelf life of honey is attributed to an enzyme found in the stomach of bees. The bees mix glucose oxidase with expelled nectar they previously consumed, which then creates

two byproducts: gluconic acid and hydrogen peroxide, partially responsible for honey's acidity and ability to suppress bacterial growth (Geiling, 2013)

Depending on temperature, water content, type of floral used and the specific sugar it contains, the physical properties of honey vary. Fresh honey is a supersaturated liquid, containing more sugar than the water can typically dissolve at ambient temperatures (Tomasik, 2004).

At room temperature, honey is a super cooled liquid, in which the glucose will precipitate into solid granules. This forms a semisolid solution of precipitated glucose crystals in a solution of fructose and other ingredients. At the temperature of 20°C, density of honey typically ranges between 1.38 and 1.45 kg/L (Tomasik, 2004).

The melting point of crystallized honey is between 40 and 50 °C (104 and 122 °F), depending on its composition. Below this temperature, honey can be either in a metastable state, meaning that it will not crystallize until a seed crystal is added, or, more often, it is in a "labile" state, being saturated with enough sugars to crystallize spontaneously (Root and Root, 2005). The rate of crystallization is affected by many factors, but the primary factor is the ratio of the main sugars: fructose to glucose(Tomasik, 2004).

Honeys that are supersaturated with a very high percentage of glucose, such as brassica honey, crystallize almost immediately after harvesting, while honeys with a low percentage of glucose, such as chestnut or tupelo honey, do not crystallize (Tomasik, 2004).

Some types of honey may produce very large but few crystals, while others produce many small crystals. Crystallization is also affected by water content, because a high percentage of water inhibits crystallization, as does a high dextrin content(Tomasik, 2004).

Temperature also affects the rate of crystallization, with the fastest growth occurring between 13 and 17 °C (55 and 63 °F) (Tomasik, 2004).

2.15.2 History of beekeeping

Human started to collect honey since thousand years ago (Dams and Dams, 1977). Beekeeping in pottery vessels began about 9,000 years ago in North Africa (Salque *et al.*, 2016). Domestication of bees is shown in Egyptian art from around 4,500 years ago. (E. Crane, 1999). For the collection of honey normal hive and smoke were used and honey was

stored in a jar. After 18th century the European understanding of the colonies and biology of bees allowed the construction of the moveable comb hive so that honey could be harvested without destroying the entire colony.

After long time humans began to start to domesticate wild bees in artificial hives made from hollow logs, wooden boxes, pottery vessels, and woven straw baskets or "skeps". Traces of beeswax are found in pot sherds throughout the Middle East beginning about 7000 BCE (Salque *et al.*, 2016). Honeybees were kept in Egypt from antiquity. On the walls of the sun temple of Nyuserre Ini from the Fifth Dynasty, before 2422 BCE, workers are depicted blowing smoke into hives as they are removing honeycombs.

2.15.3 Honey in Nepal

Beekeeping and honey hunting is the tradition of Nepal dating back thousands of years and it is handed down from generation to generation. Simple native hive and smoke are used to collect honey from bee *Apis cerana* and it is still in a preliminary stage. *Apis cerana* colonies are generally kept in log and wall hives without any management except honey harvesting once or twice a year. Many farmers, who grow variety of crops, rear livestock and perform number of other activities to manage their livelihood for them beekeeping is a spare time activity. In some parts of Nepal beekeepers are able to earn sufficient income from sale of honey and beeswax particularly in *Diploknema butyracea (chiuri*) threshold areas. The Government of Nepal, together with national, bilateral agencies, has made various efforts to increase productivity of *Apis cerana* through the provision of supply inputs and embedded technical assistance and training.

The government took first initiative in 1968 to provide training on beekeeping through its department of cottage Industry and remote area development Beekeeping development section (BDS) Committee. To look after beekeeping and sericulture, Vocational Entomology Section was established in 1975 and in 1980 a separate unit; beekeeping development section was created to provide training and extension support services in beekeeping. SNV supported Beekeeping Training and the Extension Support Project (BETRESP) made good efforts to strengthen institutional capacity of Beekeeping Development Section. International Centre for Integrated Mountain Development (ICIMOD) has implemented various projects with the support of Austrian Government focusing on indigenous honeybee species with the objective to conserve and promote these honeybee species and improve the productivity of *Apis cerana*. In 1990, the Italian race of honeybee *Apis mellifera* ligustica

was introduced in the country. To promote this bee species for higher honey production, lots of efforts have been made by both government and non-government agencies. This species is performing well in Terai parts of the country however it did not do well in the hills and mountain districts of the country. Later on, *Apis mellifera carnica* and buckfast bees have also been introduced to the country but they did not perform well in Nepal. At present, there are about 20,000 colonies of *Apis mellifera* distributed from east to western part of Terai. The average honey yield is 20 kg per annum and total honey production from *Apis mellifera* colonies is estimated to be 500-750 MT (Joshi, 2008).

2.15.4 Honey bee species

There are only two types of honeybee species known in Nepal, first one is *Apis mellifera* and second one is *Apis cerana*. *A. cerana* produces honey two times a year, the first time is during the summer (March to May) and the second time during the winter (November to December) and lives at an altitude of 60 to 3500m(Thapa, 2001). *A. cerana* is very famous in Nepal because of the low cost of its beehive, the log hive. Farmers can build their own beehives. However, *A. cerana* is more resistant to cold and predators, most *Apis cerana* colonies are reduced because of the harsh weather and the low amount of flowers during winter. Nevertheless, compared to *A. mellifera, A. cerana* can survive in low temperatures (i.e. -0, 1°C) (Thapa, 2001) because their beehives (i.e. log hive) can protect themselves from the cold. *A. cerana* is resistant to the parasite, Varroa destructor. The acrid breed feeds on bees' larva. *A. melifera* is very sensitive to this parasite and causes an incapacity to fly, an abdominal malformation and appearance of cannibalism (Acarology, 2000). The principle is to dig a hole in a trunk (the size is about 50 cm in diameter and 65 cm in height), and then a cap is necessary to protect the top of the beehive from cold and predators (e.g. *Marte flavigula*).

2.15.5 Chemical composition of honey

Honey is composed mainly from carbohydrates, lesser amounts of water and a great number of minor components.

Chemical composition of honey (Blossom honey) is given in Table 2.5 (Bogdanov, 2016), values in g/100 g.

Table 2.5 Chemical composition of honey

	Average	Min-max
Water content	17.2	15-20
Fructose	38.2	30-45
Glucose	31.3	24-40
Sucrose	0.7	0.1-4.0
Other disaccharides	5.0	28
Melezitose	<0.1	
Erlose	0.8	0.56
Other oligosaccharides	3.6	0.5-1
Total sugar	79.7	
Minerals	0.2	0.1-0.5
Amino acids, proteins	0.3	0.2-0.4
Acids	0.5	0.2-0.8
pH	3.9	3.5-4.5

Source: Bogdanov (2016)

2.15.6 Health Benefits of honey

Over its history as a food, the main uses of honey are in cooking, baking, desserts, such as a spread on bread, as an addition to various beverages, such as tea, and as a sweetener in some commercial beverages. Honey barbecue and honey mustard are other common flavors used in sauces. But honey has many other uses. Surprisingly, many of the conditions that honey is used to treat are far more serious than the simple sore throat (Arawwawala and Hewageegana, 2017) and (Socha *et al.*, 2015).

2.15.6.1 Anti inflammation action

Honey reduces the activities of cyclooxygenase-1 and cyclooxygenase-2, thus showing anti-inflammatory effects and demonstrates immunomodulatory activities. Furthermore, ingestion of diluted natural honey showed reduction effect on concentrations of prostaglandins such as prostaglandin E2, prostaglandin F2α, and thromboxane B2 in plasma of normal individuals. Have proved that anti-inflammatory activity of honey was as effective as prednisolone, reference drug. Further, honey has an anti-inflammatory action free from adverse side effects such as suppression of immune response and tissue growth, formation of ulcers in stomach, etc.

2.15.6.2 Antioxidant activity

Honey has exhibited a strong antioxidant potential and its activity is strongly correlated with the content of total phenolic and the color of honey. It was found that dark honey has a higher total phenolic content, and consequently, a higher antioxidant capacity. Honey also inhibited oxidative stress which may be partly responsible for its neuroprotective activity. Hyperlipidemia and production of free radicals are risk factors for cardiovascular diseases. A wide range of phenolic compounds are present in honey which has promising effect in the treatment of cardiovascular diseases.

2.15.6.3 Antidiabetic properties

The use of honey in Type I and Type II diabetes was associated with significantly lower glycemic index than with glucose or sucrose in normal diabetes. Due to the low glycemic index of the honey, it helps to reduce the absorption of digested food. Honey compared with dextrose caused a significantly lower rise in plasma glucose levels in diabetic subjects. It also caused reduction of blood lipids, homocysteine levels and C-reactive protein levels in normal and hyperlipidemic subjects. In earlier observations, it was found that honey stimulates insulin secretion, decrease blood glucose levels, elevates hemoglobin concentration, and improves lipid profile.

2.15.6.4 Antimicrobial activity

Honey has been reported to have antibacterial activity against various bacterial species including *Bacillus anthracis, Corynebacterium diphtheriae, Haemophilus influenzae, Klebsiella pneumoniae, Listeria monocytogenes, Mycobacterium tuberculosis, Pasteurella*

multocida, Yersinia enterocolitica, Proteus species, Pseudomonas aeruginosa, Acinetobacter spp., *Salmonella diarrhea, Salmonella typhi, Serratia marcescens, Shigella dysentery, Staphylococcus aureus, Streptococcus faecalis, Streptococcus mutans, Strep. pneumoniae, Streptococcus pyogenes,* and *Vibrio cholerae.* An antifungal action has been reported for honey against *Aspergillus, Penicillium,* as well as all the common dermatophytes and *Candida albicans*. Honey has shown antiviral effect also. The topical application of honey on recurrent attacks of herpes lesions concluded that topical honey application was safe and effective in the management of the signs and symptoms of recurrent lesions from labial and genital herpes compared to acyclovir cream. In addition, honey has also been reported to have inhibitory effects on rubella virus activity.

2.15.6.5 Wound healing activity

Honey has cleansing action on wounds, stimulates tissue regeneration, reduces inflammation, and honey impregnated pads act as non-adhesive tissue dressing. Clinical trials have revealed that honey dressing showed better improvement (e.g., dressing in burns with amniotic membrane dressing; silver sulfadiazine dressing, and boiled potato peel dressing) and showed early healing with lesser degree of contracture and scarring.

Part III

Material and methods

3.1 Materials

3.1.1 Raw materials

3.1.1.1 Honey

Ten kilogram unprocessed honey was collected from farm of Bharatpur, Chitwan of bees *Apis cerana* and different physical and chemical analysis were carried out where moisture content of honey was 19.61%, ash content 0.5%, reducing sugar 75% and TSS and pH of honey were found to be 78ºBx and 4.2 respectively.

3.1.1.2 Table sugar

The table sugar was brought from local market of Dharan, Nepal.

3.1.1.3 Citric acid

The citric acid was added to adjust pH of must as well as for antioxidant property. It was provided from campus laboratory.

3.1.1.4 Yeast

Active wine yeast (*Saccharomyces cerevisiae*, Lalvin EC 1118).

3.1.3 Equipment

All equipments required for the experiment were obtained from laboratory of Central campus of Technology. List of equipments used for this work is shown in Table 3.2.

Table 3.2 List of equipment used

Physical apparatus	
Stainless steel vessels	Weighing arrangement
Hand refractometer (0-30 ºBx)	Heating arrangement
pH meter	Distillation set

Thermometer	Titration apparatus
Pycnometer	Other routine glassware

3.1.4 Chemicals

All the chemicals required for the experiment were obtained from laboratory of Central campus of Technology. List of chemicals used for this work is shown in Table 3.3.

Table 3.3 List of chemicals used

Chemicals	
Potassium metabisulfite (KMS)	Sodium hypochloride solution
Sodium hydroxide	Sulphuric acid
Buffer solution (4, 7 and 9.2)	Sodium thiosulphate solution
Folin-Denis reagent	Iodine solution
Tannic acid solution	Sodium bisulphite solution
Calcium hydroxide solution	Starch indicator
Sodium carbonate solution	Methylene blue indicator
Hydrochloric acid	Phenolphthalein
Fehling A solution	Fehling B solution
Carrez-I solution	Carrez-II solution

3.1.2 Other materials

All other required materials were obtained from local market of Dharan. List of other materials used for this work is shown in Table 3.1

Table 3.1 List of other materials used

Material	
Food grade silicon tube rubber pipe	Plastic jar
Muslin cloth	Wine bottle
Cotton	

3.2 Methodology

The total work was based on preparation of mead with varying pH and honey concentration and analysis of final mead.

3.2.1 Experimental procedure

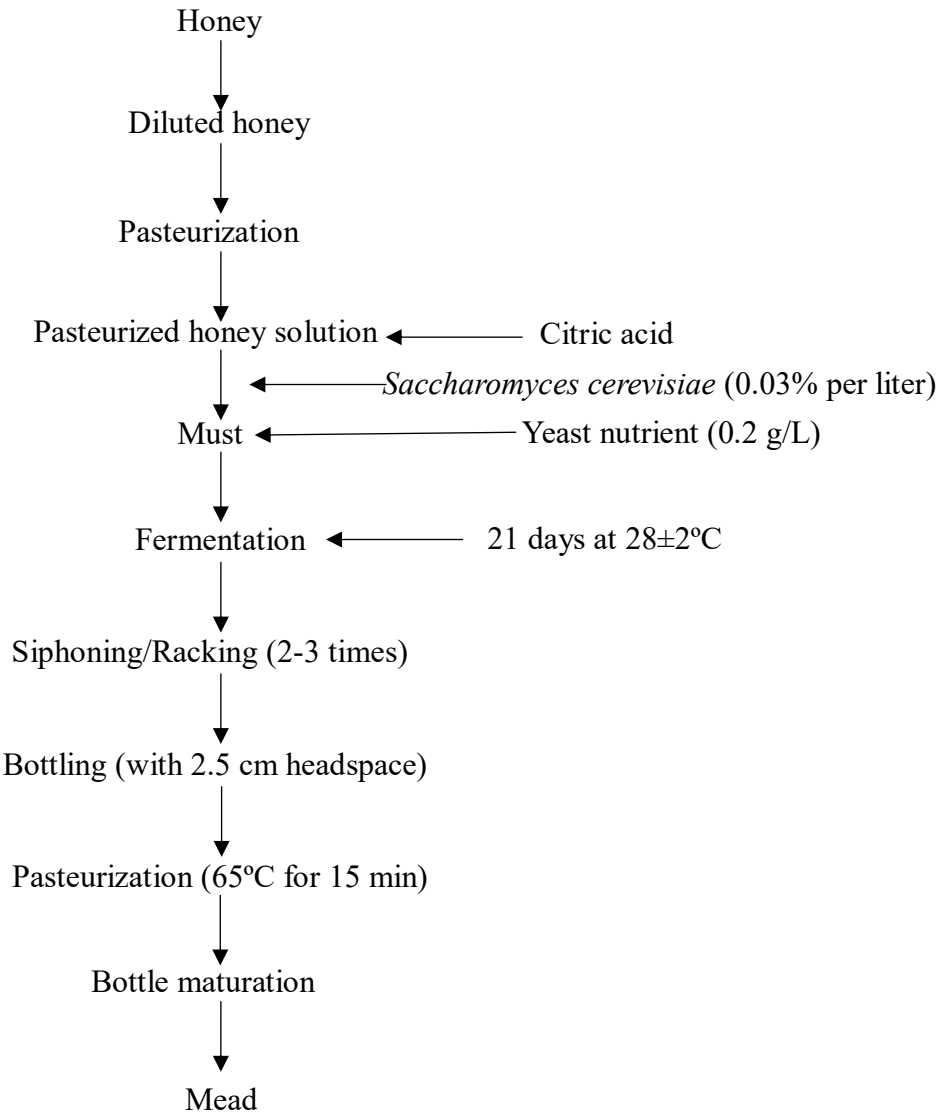


Fig. 3.1: Process of making mead

Source: (Gupta and Sharma, 2009)

3.2.1.1 Preparation of must composition

After analysis of honey different composition of must or sample were prepared. The honey and water was varied according to the honey content of 100%, 75% 50%, and 25%. The pH of must was varied as 3, 3.5 and 4 by addition of citric acid on must. The final TSS of 25˚BX was maintained by addition of table sugar as 25%, 50%, and 75%. The sample prepared for experiment is presented in below Table 3.4.

Table 3.4 Preparation of must

Samples	Honey concentration	Table sugar	pH
A	100%		3
			3.5
			4
B	75%	25%	3
			3.5
			4
C	50%	50%	3
			3.5
			4
D	25%	75%	3
			3.5
			4

3.2.1.2 Pitching

Wine yeast was used for pitching. It was activated with mildly heated honey and water solution and pitching was done at the rate of 0.3 g/L for all musts. The general flow sheet for procedure is given in Fig. 3.1.

3.2.1.3 Fermentation

Must after pitching were kept in plastic jars for fermentation. The exact process followed in this study is given in Fig. 3.1. It was necessary to create an anaerobic condition inside the jars during fermentation for improving the quality of product. The process of fermentation was followed by measuring the drop in degree brix. The fermentation was assumed to be completed after degree brix creased to drop below 10ºBx. It takes 15-18 days from pitching.

3.2.1.4 Racking, pasteurization and bottling

After fermentation the clear wine was drawn off from the sediment known as 'lees'. This Was done using a SO_2 treated food grade silicon tube rubber pipe into a sterile glass wine bottle and wine was pasteurized by heating bottle of wine by boiling water in order to maintain temperature of wine 63°C for 15 min and cooled to room temperature. The cooled wines were racked and filled into the pre-sterilized bottles and kept in room temperature until needed for further analysis (Gupta and Sharma, 2009).

3.2.2 Analytical procedure

Although different authors have described different methods and parameters to analyze honey, must and mead only those parameters and related methods, which were feasible in the laboratory, were determined in this study. The determination was conducted in triplicates.

For honey TSS, pH, mineral content, moisture content, acidity and reducing sugar were analyzed. The must were analyzed for TSS and acidity and for prepared mead sensory analysis based on following parameters appearance, odor, in mouth sensation, finish and overall acceptance was done to select best product. For the optimized (best product) was analyzed for chemical composition and properties like TSS, pH, total acidity, volatile acidity, specific gravity, alcohol content, ester, and aldehyde.

3.2.2.1 Determination of total soluble solid (TSS)

The TSS of the honey, must and mead were determined by using hand sugar refractometer.

3.2.2.2 Determination of pH

pH of honey, must and mead were determined by the digital pH meter of Labtronic ™ (Deluxe pH meter) of model LT-10 provided by Central Campus of Technology, Nepal and standardized with standard buffers at 25ºC.

3.2.2.3 Acidity determination

The total acidity was determined following the method of (K.C. and Rai, 2007). The volatile acidity of wine was determined following the method of (Jacobson, 2006).

3.2.2.4 Alcohol content

Alcohol by volume was determined by pycnometric method as per official method 935.21 described in AOAC (2005). 50 ml of each sample and 50 ml of distilled water was added to it; was distilled till 40 ml of distillate and volume maintained to 50 ml by distilled water. Then weight of dry picnometer, distillate in piconmeter and water in picnometer was taken; and room temperature was noted. The specific gravity of water was calculated, and alcohol % (v/v) was found by chart.

3.2.2.5 Ester content

200 ml of mead was taken for distillation and 50 ml of distillate was collected. Then it was neutralized with 0.1 N NaOH. Further 5 ml excess 0.1 N NaOH was added and reflux for 1 hour. Cool and back titrate the unspent alkali against 0.1N sulphuric acid carry out blank simultaneously taking 50 ml of distilled water. The difference in titer value in milliliter of standard sulphuric acid gives equivalent ester. The values were expressed in gram per 100 liter of ethyl alcohol as ethyl acetate. As per method of FSSAI (2012).

Ester express as ethyl acetate = $(V \times 0.0088 \times 100 \times 1000 \times 2) / V_1$

g/ 100 L of abs. alcohol

Where, V = difference of titer value of std. H_2SO_4 used for blank and sample in ml

V_1 = alcohol % by volume

3.2.2.6 Aldehyde content

Total aldehyde as g acetaldehyde/ 100 L alcohol was determined as per Kirk and Sawyer (1991) with slight modifications. Solution A, B, C and D was prepared as per followings:

A: Mix 15 g potassium metabisulphite with 70 ml conc. HCI and dilute to 1 L with distilled water

B: Dissolve 188 g $Na_2HPO_4.12H_2O$ + 21 g NaOH and 4.5 g EDTA in water and dilute to 1 L with distilled water.

C: Dilute 250 ml conc. HCI to 1:1 with distilled water.

D: Mix 100g boric acid with 170 g NaOH add water to dissolve and dilute to 1 L with distilled water.

In 1000 ml conical flask 300 ml boiled and cooled water and 10 ml of solution A were taken. To this mixture 40 ml of beer distillate was added, stopper was placed and the flask was swirled. It was then allowed to stand for 15 min. After this, 10 ml of solution B was added, mixed by swirling and allowed to stand for further 5 min. Then 10 ml of solution C and 10 ml fresh 0.2% starch solution was added, mixed by swirling and iodine was (can 0.1 M) added so that excess bisulphate was just destroyed and colour of the solution became faint blue. Finally 10 ml of solution D was added and the liberated bisulpite was titrated with 0.05 M iodine solution to the same faint blue end point. Total aldehyde as g acetaldehyde per 100 L alcohol was calculated using following expression:

$$\text{Total acetaldehyde (g acetaldehyde/ 100 L alcohol)} = \frac{\text{Titer} \times 2.2}{S}$$

Where, S = % alcohol by volume in the sample

3.2.3 Sensory evaluation

The prepared 12 mead samples by varying pH and honey content of must were subjected to sensory evaluation for consumer's acceptability. The samples were served in clean wine glass at silent environment around 1:00 pm and room temperature was 27ºC. Sensory attributes (appearance, odor, in mouth sensation, finish and overall quality) were evaluated using 7 points hedonic rating test ranging from faulty (1) to exceptional (7) as described by (Jackson, 2002) with the help of 8 semi- trained panelist whom were teachers and students of food technology (both Bachelor and Master level at CCT) and they were familiar with alcoholic beverages.

The sequence and method of wine sensory evaluation can be listed as following

I. **Appearance:** Firstly, view each sample at 30º to 45º against the bright white background. Then record separately the wine's clarity (absence of haze), color (shade or tint) and depth (intensity or amount of pigment), viscosity (resistance to flow) and effervescence (notably sparkling wines).
II. **Odor:** Firstly sniff each at mouth of glass before swirling and then, study and record the nature and intensity of fragrance. Now swirl the glass to promote release of the

aromatic constituents from wine, then smell the wine initially at the mouth and deeper into bowl. Now study and record the nature and intensity of fragrance.

III. **In-mouth sensations:** Take a small (6 to 10 ml) sample into mouth. Move wine into mouth to coat all surface of the tongue checks and palate. For various taste sensations (sweet ,acid, bitter) note where they perceived, when they first detected, how long they last, and how they changes in perception and intensity. Then, concentrate on the tactile (mouth feel) sensation of astringency, prickling, body temperature and heat. Record these perception and how they combine with each other.

IV. **Finish:** concentrate on the olfactory and gustatory sensations that linger in the mouth compare these sensations with those previously detected. Note their character and sensations.

V. **Overall quality:** After the sensory aspect have been studied individually, attention shift to the integration of their effects the wine's overall quality and finally, make and overall assessment of the pleasurableness, complexity, subtlety, elegance, power, balance and memorableness of wine (Jackson, 2002).

3.2.4 Statistical analysis

For all chemical analysis triplicates of the same sample were used for the determination of each constituent. Mean values with standard deviation were computed. Data were subjected to analysis of variance and read at 95% confidence level using statistical software GenStat Release 7.1 (Discovery Edition 3 developed by VSN International Limited).

Part IV

Results and discussion

Mead (honey wine) was prepared from honey obtained from Bharatpur, Chitwan as described in section 3. My study is not only about the preparation of mead, it shows the effect of honey proportion and pH on the sensory quality of mead. The results and discussion are as follows:

4.1 Chemical analysis of honey (*Apis cerana*).

Chemical analysis of honey was done at laboratory of central campus of technology Hattisar, Dharan. Parameters as TSS, acidity, pH, moisture content, reducing sugar were measured. And the values obtained from this analysis lies within the good quality honey. Chemical composition of honey obtained is given in Table 4.1.

Table 4.1 Chemical composition of honey

Parameter	Value
TSS (ºBx)	78 (0)
Acidity (% as citric acid)	0.5 (0.005)
pH	4.2 (0.029)
Moisture content (%)	19.61(0.003)
Reducing sugar (% as dextrose)	75 (0.040)

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

4.2 Effect of honey proportion and pH on the sensory quality of mead.

4.2.1 Appearance

Mean sensory score for appearance of twelve samples varying with honey concentration and pH are as follows in Table 4.2.

Table 4.2 Mean sensory score for appearance

Honey concentration (%)	pH	Mean score
25	3	6.50
25	3.5	7.75
25	4	8.38
50	3	7.38
50	3.5	7.75
50	4	6.50
75	3	7.13
75	3.5	7.25
75	4	6.38
100	3	6.63
100	3.5	6.63
100	4	6.13

The statistical analysis showed that there was a significant effect ($p<0.05$) of honey concentration and pH variation on appearance at 5% level of significance.

The mean sensory score was found to be highest for sample C which was of 4 pH and 25% honey content (Fig.4.1) which was significantly different from other samples. This indicates that panelists preferred mead with lowest honey concentration and high pH. The best samples with 25% honey and higher pH were of brilliant in appearance.

According to Jackson (2009) amount of pigment effects appearance of product. Honey content will effect on color intensity of mead, higher concentration of honey will have higher amount of pigments hence color intensity of mead with 25% honey content will have higher values which support above result.

Hence the appearance of mead with low honey concentration and higher pH gave the best sensory score. Which is in accordance to Jackson (2009).

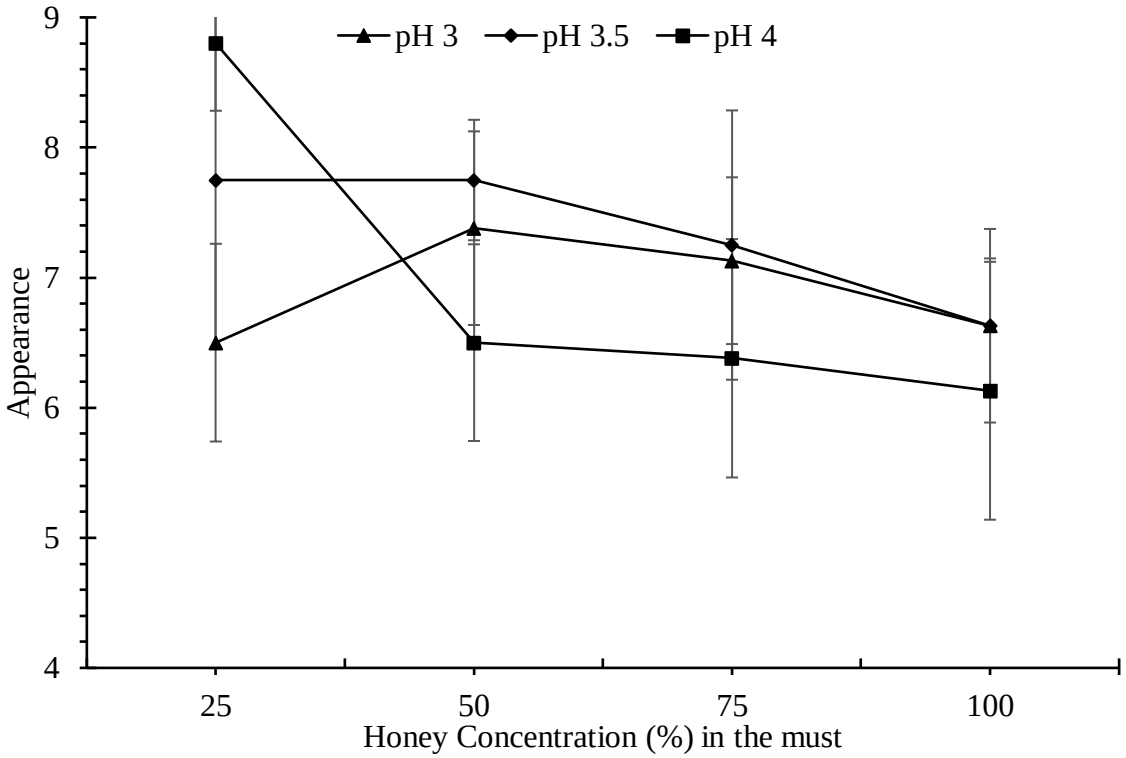


Fig. 4.1 Effect of honey proportion and pH on appearance of mead.

4.2.2 Odor

Mean sensory score for odor of twelve samples varying with honey concentration and pH are given in Table 4.3.

Table 4.3 Mean sensory score for odor

Honey concentration (%)	pH	Odor
25	3	7.00
25	3.5	7.00
25	4	7.13
50	3	6.50
50	3.5	7.63
50	4	6.63
75	3	6.63
75	3.5	6.63
75	4	7.38
100	3	8.00
100	3.5	6.68
100	4	7.25

The statistical analysis showed that there was significant effect ($p<0.05$) of honey percentage and pH variation on odor at 5% level of significance.

The mean sensory score was found to be highest for sample I which was of 3.5 pH and 50% honey concentration (Fig. 4.2) which was significantly different from other samples. This indicates that panelists preferred mead with lower honey concentration and average pH.

According to Butzke (2010) standard pH for must of white wine is 3.5. Since spoilage bacteria do not grow well below pH 3.6 preventing growth of acetic acid bacteria which caused formation of excessive volatile acid. If the volatile acidity is too high, the wine may smell vinegary, or of nail varnish remover which is undesirable.

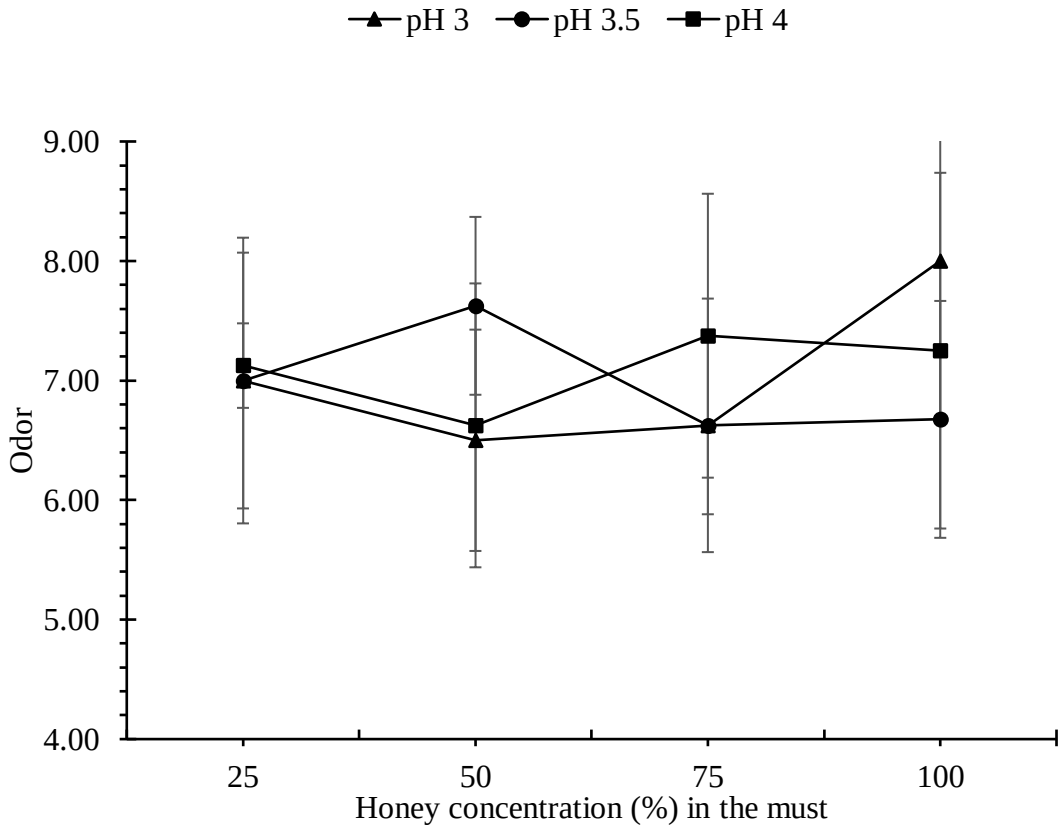


Fig. 4.2 Effect of honey proportion and pH on odor of mead.

4.2.3 In-mouth sensation

Mean sensory score for in mouth sensation of twelve samples varying with honey concentration and pH are given in Table 4.4.

Table 4.4 Mean sensory score for in-mouth sensation

Honey concentration (%)	pH	In mouth sensation
25	3	5.88
25	3.5	8.00
25	4	7.88
50	3	7.38
50	3.5	6.88
50	4	6.38
75	3	6.75
75	3.5	6.63
75	4	6.38
100	3	5.38
100	3.5	6.75
100	4	6.75

The statistical analysis showed that there was significant effect ($p<0.05$) of honey concentration and pH variation on in mouth sensation of product at 5% level of significance. The mean sensory score was found to be highest for sample F which was of 3.5 pH and 25% honey concentration (Fig. 4.3).

The mean score for samples with lowest honey concentration (25%) found to have higher values while that for samples with highest honey concentration (100%, and 75%) have lower values. This indicates that panelists preferred mead lowest honey concentration and average pH. 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.

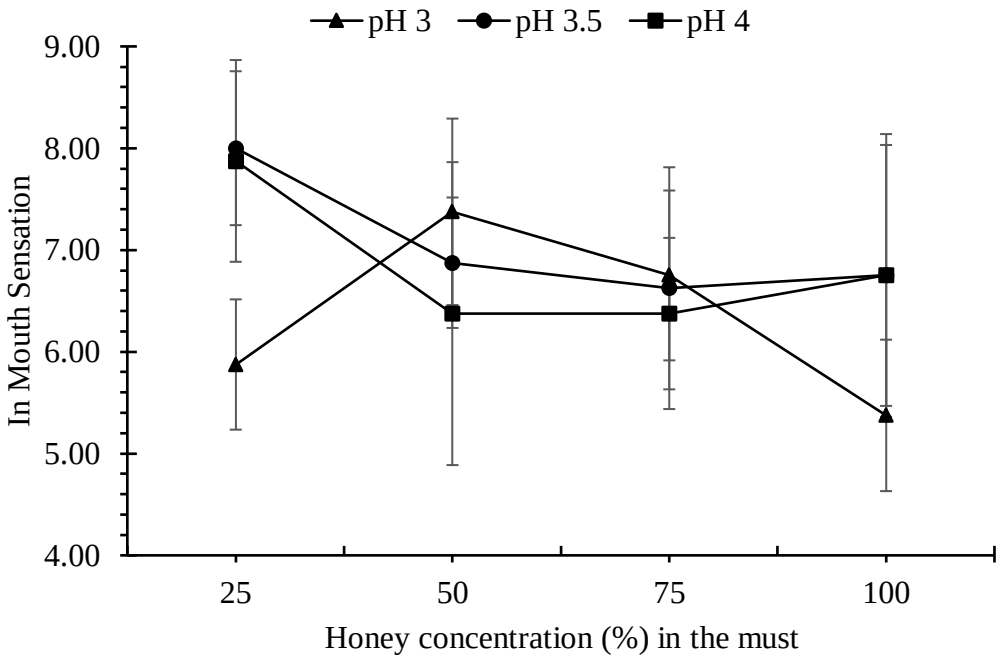


Fig. 4.3 Effect of honey proportions and pH on in mouth sensation of mead.

4.1.4 Finish

Mean sensory score for finish of twelve samples varying with honey concentration and pH are given in Table 4.5.

Table 4.5 Mean sensory score for finish

Honey concentration (%)	pH	Finish
25	3	6.13
25	3.5	7.88
25	4	7.50
50	3	6.75
50	3.5	6.75
50	4	7.00
75	3	6.25
75	3.5	6.00
75	4	5.75
100	3	5.25
100	3.5	6.13
100	4	6.38

The statistical analysis showed that there is significant effect ($p<0.05$) of honey percentage and pH variation on finish at 5% level of significance. The mean score for

samples with highest honey percentage (100%, 75%, 50%) were found to have lower values while that for sample F with lower honey concentration (25%) and average pH (3.5) have higher value (Fig. 4.4). This indicates that panelists preferred mead with lowest honey percentage and average pH. The mead sample which was proven to be best from the sensory analysis was found to have moderate lingering flavor in the mouth and pleasant aftertaste.

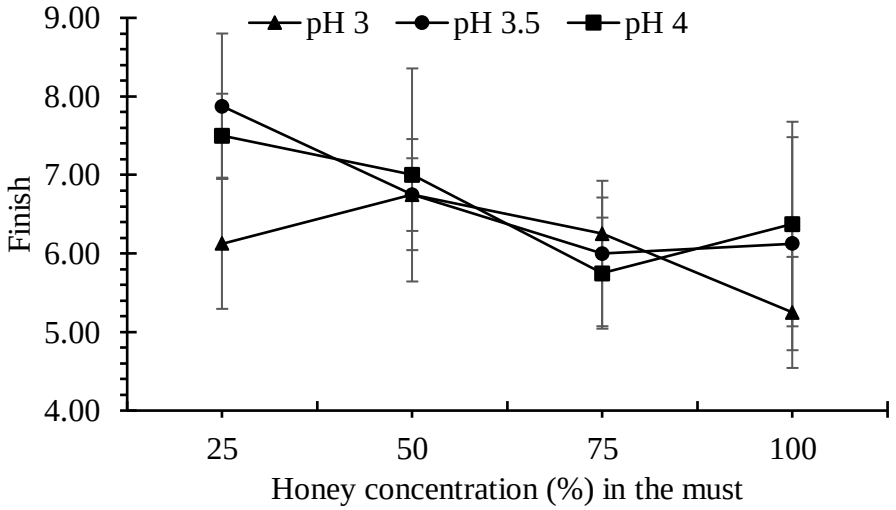


Fig. 4.4. Effect of honey proportions and pH on finish of mead.

4.2.5 Overall acceptance

Mean sensory score for finish of twelve samples varying with honey concentration and pH are given in Table 4.6.

Table 4.6 Mean sensory score for overall acceptance

Honey concentration (%)	pH	Overall acceptance
25	3	6.50
25	3.5	7.75
25	4	7.63
50	3	6.88
50	3.5	7.25
50	4	6.63
75	3	6.63
75	3.5	6.75
75	4	6.13
100	3	5.25
100	3.5	6.00
100	4	7.00

The statistical analysis showed that there is significant effect ($p<0.05$) of honey concentration and pH variation on overall acceptance at 5% level of significance. The mean sensory score was found to be highest for sample F which was of 25% honey content and 3.5 pH (Fig. 4.5). This indicates that panelists prefer mead with lowest honey concentration and average pH.

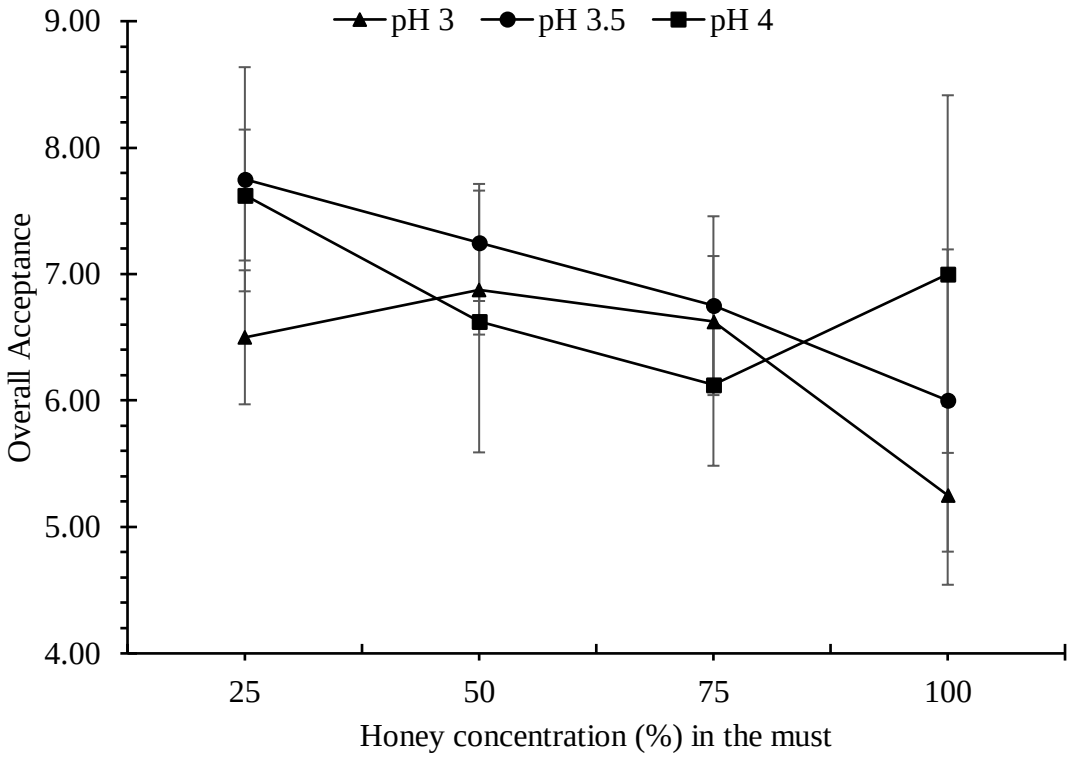


Fig. 4.5 Effect of honey proportion and pH on overall acceptance of mead.

In comparison with the mead made from other formulations of varied honey concentration and pH, mead made from formulation having 25% honey concentration and pH 3.5 keeping TSS at 25ºBx seems to be the best. Also analytical parameters of the best product (formulation of 25% honey content and pH 3.5) obtained from this study lies within the range of good quality wine.

4.4 Chemical composition

Chemical composition of best mead is given in Table 4.6. The TSS of mead was found to be 9.2ºBx, almost similar results were obtained as (Gupta and Sharma, 2009). pH of mead 3.4 and total acid 0.43% was found almost similar as compared to (Gupta and Sharma, 2009). Volatile acidity was found to be 0.012% as acetic acid which is within range. The legal limit for white wines is 0.12% and 0.14% for red wine. In case of mead there is not found enough legal limits or references.

Table 4.7 Chemical composition of optimized mead

S.N.	Parameter	Value (*)
1	TSS (°Bx)	9.2 (0.25)
2	pH	3.4 (0.051)
3	Acidity	
	a) Total acidity (% as citric acid)	0.43 (0.41)
	b) Fixed acidity (% as tartaric acid)	0.53 (0.025)
	c) Volatile acidity (% as acetic acid)	0.012 (0.005)
4	Specific gravity	0.9793 (0.003)
5	Alcohol (% v/v)	15.34 (0.32)
6	Ester (g/100 L abs. alcohol)	367.44 (10.40)
7	Total aldehyde (g/100 L abs. alcohol)	317 (12.05)
8	Methanol (g/100L)	68 (10.35)

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

The reducing sugar, 0.148% was higher than that of dry red wine, 0.134% and dry white wine, 0.146% reported by Amerine *et al.* (1980). The alcohol content in mead (15.34 v/v) was slightly higher than that of dry white wine (9.88% v/v) and dry red wine (10% v/v) reported by Amerine *et al.* (1980). For good quality wine, the aldehyde content should be within the range of 200-500 ppm (Rai, 2009a). The aldehyde content in the mead was found to be 317 ppm. The ester content in good quality wine should be within the range of 200-400 ppm (Rai, 2009a). The ester content of the mead was found to be 367 ppm. Methanol content in the mead was found to be 68 ppm which is highly lower than that of regular type of wine such as fruit wine, this results should reflect the raw material composition. Methanol is toxic to humans, therefore its maximum concentration in the final distillate is fixed at 1000 g/hL P.A. by EU regulation for grape marc spirits and fruit spirits, but not for honey spirits (EU, 2008). High methanol concentrations are often found in fruit spirits due to the enzymatic degradation of pectin during fermentation, so the low methanol content of honey spirits was expected.

Part V

Conclusions and recommendations

5.1 Conclusions

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

1. In comparison with the mead made from other formulations of varied honey concentration and pH, mead made from formulation having 25% honey concentration and pH 3.5 keeping TSS at 25ºBx seems to be the best.
2. Mead made from different honey concentration and different pH values differs significantly (at 5% level of significance) with respect to sensory properties.
3. Analytical parameters of the best product (formulation of 25% honey content and pH 3.5) obtained from this study lies within the range of good quality wine.
4. Mead can be produced at a cost within the means of common people. Consequently, mead holds a lot of promise from commercial point of view.

5.2 Recommendations

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

1. Mead can be prepared with varying TSS, temperature and acid used.
2. Study on the distillate of mead can be carried out.
3. Study on the quality of mead using different yeast can be carried out

Part VI

Summary

In this study, honey was taken from Chitwan, which is one of the districts for commercial cultivation of beekeeping in Nepal. Other essential materials (citric acid, sugar, yeast food and wine yeast) and other chemical and apparatus were obtained from local market of Dharan and campus laboratory. Physicochemical analysis of honey showed 78°Bx TSS, 4.2 pH and 0.5 (% citric acid) acidity. Fermentation was carried out in 12 different mashes of 25°Brix where the contribution of honey was varied (100%, 75%, 50% and 25%) to achieve the final TSS with sugar. pH was also varied (3.0, 3.5, and 4.0) keeping TSS constant at 25 °Bx and fermentation carried out for 21 days.

The twelve different meads were subjected to sensory analysis (9-point hedonic rating) and data obtained were analyzed by two-way ANOVA at 5% level of significance to study the difference among the mead types. There was significant difference ($p<0.05$) in case of appearance, odor, in-mouth sensation, finish and overall acceptability of all types. From the sensory evaluation, mead made from 25% honey and 4 pH, 50% honey and 3.5 pH got the highest score among other formulations. But for the further analysis sample having 25% honey concentration and pH 4 was taken which may be the best for commercial point of view.

Mead made from 25% honey content and 3.5 pH had 15.34 % (v/v) alcohol content, 9.2 °Bx TSS, 3.40 pH, 0.43% (as % citric acid) acidity, 0.012% (as acetic acid) volatile acidity, 367 ppm ester, 317 ppm aldehyde, 68 ppm methanol. Alcohol content, volatile acidity and other parameters of wine made from this research is within the range of a good quality wine.

References

Acarology, E. a. A. (2000). Personal communication [dnnlakly@hotmail.com]. 5 April 2019.

Acharya, T. D. and Yang, I. T. (2015). Vineyard suitability analysis of Nepal. *Int. J. Environ. Sci*. **6**, 13-18.

Amerine, M. A., Berg, H. W., and and Cruess, W. V. (1980). "The Technology of Wine Making". The AVI publishing Co. Westport, Connecticut.

Andrew, S. (1980). "The Food Beverage Service". Tata Mc graw Hill publishing co. Ltd. New Delhi.

AOAC. (2005). "Official Methods of Analysis " (18th ed.). Association of Official Analytical Chemists. USA.

Arawwawala, D. A. M. L. and Hewageegana, G. S. P. H. (2017). Health benefits and traditional uses of honey. *J. Apitherapy*. **2** (1).

Austin, C. (1968). "The Science of Wine Making". University of London Press Ltd.

Banwart, G. J. (2004). "Basic Food Microbiology" (2 ed.). CBS Publisher and Distributors. New Delhi

Baxter, E. D. and Hughes, P. S. (2001). "Beer Quality, Safety and Nutritional Aspects". Royal Society of Chemistry. Cambridge, UK.

Berg, H. W. and Akiyoshi, M. (1962). Wine fermentation. *In:* "Commercial Fruit Processing". (H. B. Woodruff, Ed.). pp. 1-531. Westport, Connecticut. The AVI Pub. Co. Inc.

Berry, C. J. J. (1996). "First Step on Winemaking" (3 ed.). The Amateur Winemaker.". North Croye, UK.

Bhandari, M. (1992). Quality evalution of beer produced in Nepal. B.Tech. Dissertation. Tribhuvan Univ., Nepal.

Boulton, R. B. (1998). "Principles and Practices of Winemaking". An Aspen Publication. Maryland, US.

Briggs, D. E., Boulton, C. A., Brookes, P. A. and Stevens, R. (2004). "Brewing Science and Practice". CRC Press,LLC. USA.

Buglass, A. J., Gooklee, C. and Mckay, M. (2011). Fermented beverages: Beers, ciders, wines and related drinks. *In:* "Handbook of Alcoholic Beverages Technical, Analytical and Nutritional Aspects" (Vol. 1). (A. J. Buglass, Ed.). pp. 63-455. United Kingdom. A John Wiley and Sons, Ltd.

Butzke, C. E. (2010). "Juice and Must Preparation in Winemaking" (1 ed.). Woodhead Publishing Limited. Cambridge, UK.

Clarke, R. J. and Bakker, J. (2004). "Wine Flavour Chemistry" (1st ed.). Blackwell Publishing Ltd. Oxford, UK.

Costantini, A., Garcıa-Moruno, E. and Moreno-Arribas, M. V. (2009). Biochemical transformations produced by malolactic fermentation. *In:* "Wine Chemistry and Biochemistry". (M. V. Moreno-Arribas and M. C. Polo, Eds.). pp. 28-49. New York, USA. Springer Science+Business Media.

Crane, Walker and Day. (1984). "Directory Of Important World Honey Sources". International Bee Research Association London UK. United Kingdom.

Crane, E. (1999). "The world History of Beekeeping and Honey Hunting". New York : Routledge, 1999. United state of America.

Crane, E. (2013). Honey from Honeybees and Other Insects. *Ethology Ecology & Evolution*. (3), 100-105.

Dams, L. and Dams, M. (1977). Spanish rock art depicting honey gathering during the Mesolithic. Nature. **268** (5617), 228-230.

Douglas, M. and Considine, P. E. (1982). "Food and Food Production Encyclopedia". Van Nostrand, Reinhold Company, Inc. New York, N.Y. 10020.

Egan, H., Kirk, R. S. and Sawyer, R. (1981). "Pearson's Chemical Analysis of Foods." (8th ed.). Longman group Ltd. Harlow,UK.

EU. (2008). "Regulation (Ec) No 110/2008 Of The European Parliament and of The Council On The Definition, Description, Presentation, Labelling and The Protection of Geographical Indications of Spirit Drinks and Repealing Council Regulation (Eec) No 1576/89". Retrieved from http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008R0110. [Accessed July 2017].

Frivik, S. and Ebeler, S. (2003). Influence of sulfur dioxide on the formation of aldehydes in white wine. *In: Am. J. Enol.* **54**, 31-38.

FSSAI. (2012). "FSSAI Manual of Methods of Analysis of Foods ". New Delhi. Food Safety and Standard Authority of India Ministry of Health and Family Welfare Government of India. pp. 1-30.

Fugelsang, K. C. (1996). "Wine microbiology". The Chapman and Hall Enology. USA.

Geiling, N. (2013). The science behind honey's eternal shelf life. Retrieved from https://www.smithsonianmag.com/science-nature/the-science-behind-honeys-eternal-shelf-life-1218690/. [Accessed 25 Jan 2019].

Grainger, K. and Tattersall, H. (2005). "Wine Production Vine to Bottle." (1 ed.). Blackwell Publishing Ltd. Oxford, UK.

Gubhaju, M. (2006). Preparation and quality evaluation of wine prepared from Rhododendron flower. B.Tech (Food) Dissertation. Tribhuvan Univ., Nepal.

Gupta, J. K. and Sharma, R. (2009). Production technology and quality characteristics of mead and fruit-honey wines. Natural Product Radiance. **8(4)**, 345-355.

Guymon, J. F., Ingraham, J. L. a. and Crowell, E. A. (1961). "The Technology of Wine Making" (2nd ed.). The AVI Pub. Co. Ltd.

Jackson, R. S. (2000). "Wine Science Principles, Practices and Perception" (2 ed.). Academic press. San Diago Califonia, USA.

Jackson, R. S. (2002). "Wine Tasting a Professional Handbook" (1 ed.). Elsevier Academic Press. San Diego, California, USA.

Jackson, R. S. (2014). "Wine Science Principles and Applications" (4 ed.). Elsevier Academic press. Burlington, USA.

Jacobson, J. L. (2006). "Introduction to Wine Laboratory Practices and Procedures". Springer Science+Business Media, Inc. United States of America.

Jiang, B. and Zhang, Z. (2010). Volatile compounds of young wines from cabernet sauvignon,cabernet gernischet and chardonnay varieties grown in the loess plateau region of china. *Molecules*. **15**, 9185-9196.

Johnson, A. H. and Peterson, M. S. (1974). "Encyclopedia of Food Technology". The AVI Pub. Co. INC. Westport, Connecticut USA.

Jones, K. C. (1995). Adoptation of fermentative organisms to alcoholic environments. *In:* "Alcoholic Beverages". (G. G. Birch and M. G. Lindly, Eds.). p. 171. London. Elsevier Appliedn Sc. Publication.

Joshi, R. S. (2008). Personal communication [+977-1-5555289].

K.C., J. B. and Rai, B. (2007). "Basic Food Analysis Handbook" (1 ed.). Mrs. Maya KC. Anamnagar, Kathmandu-32, Nepal.

Khatiwada, B. (2015). Study on the effect of *amla (Emblica officinalis)* juice addition on the sensory and chemical quality in the preparation of white wine. B.Tech. Dissertation. Tribhuvan Univ., Nepal.

Lesschaeve, I. (2007). Sensory evaluation of wine and commercial realities: Review of current practices and perspectives. *Am. J. Enol.* **58**, 252-257.

Lonvaud, F. A. (2002). Starter for wine making. *In:* "Applied Microbiology" (Vol. 2). (A. Durieux and J. P. Simon, Eds.). Institute Meurice, Brussels, Belgium. Kluwer Academic Publishers.

Madigan, M. E., Martinko, J. M. and Parker, J. (2000). "Brock Biology of Microorganisms" (9th ed.). Prenticae Hall. USA.

Meilgaard, M., Civille, G. V. and Carr, B. T. (1999). "Sensory Evaluation Techniques" (3rd ed.). Crc press. Boca Raton New York.

Mmegwa, S. V. A. (1987). Factors controlling organoleptic properties in palm wine fermentation. Ph.D Thesis. Univ. Nigeria, Nigeria.

Mojsov, K., Petreska, M. and Ziberoski, J. (2006). Risks of microbial spoilage of wine: A review. *J. Hygienic Engg. Design*. 266-270.

Nepal, B. (2014). Wines Made in Nepal. Retrieved from http://bossnepal.com/wines-nepal/. [Accessed 5 March, 2017].

Odinsson, E. (2010). "Northern Lore: A Field Guide to the Northern Mind-Body-Spirit" (1st ed.).United state of America.

OIV. (2016). "World Vitiviniculture Situation". Paris. International Organisation of Vine and Wine. pp. 1-16. Retrieved from http://www.oiv.int. [Accessed 24 march 2017].

Osborne, J. P. (2010). Malolactic fermentation in wine. *In:* "Winemaking Problems Solved." (1 ed.). (C. E. Butzke, Ed.). pp. 78-97. Cambridge, UK. Woodhead Publishing Limited.

Pearson, D. (1981). "The Chemical Analysis of Foods". Churchill Livingstone. Ravelson Terrace, Edindurg.

Pederson, C. S. (1980). "Microbiology of Food Fermentation". The AVI publishing Co. Inc. Westport, Connecticut USA.

Pereira, A. P., Ferreira, A. M. and Estevinho, L. M. (2015). Improvement of mead fermentation by honey-must supplementation. *J. The institute of Brewin*. **121** (3), 405-410

Prescott, Harley and Klein. (1996). "Microbiology " (15th ed.).United Kingdom.

Prescott, S. C. and Dunn, C. G. (2004). "Industrial microbiology" (3rd ed.). Tata McGraw-Hill Book Co. New York.

Rai, B. K. (2012). "Essentials of Industrial Microbiology" (4th ed.). Vol. 423. Lulu. Nepal.

Rai, B. K. (2009a). "Industrial Microbiology" (1 ed.).Dharan, Nepal.

Rai, P. L. (2009b). Preparation and quality evaluation of ginger wine. B.Tech. (Food) Disseration. Tribhuvan Univ., Nepal.

Raut, B. (2014). Effect of addition of tea extract on fermentation and quality of red wine. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal.

Rijal, P. (2016). Nepals wine industry predicts boom times. *The Kathmandu post*. Retrieved from http://kathmandupost.ekantipur.com/news/2016-05-28/nepals-wine-industry-predicts-boom-times.html. [Accessed 5 march, 2017].

Ritchie, G. S. (2010). Wine clarification, stabilization and preservation. *In:* "Winemaking Problems Solved". (C. E. Butzke, Ed.). pp. 119- 128. Cambridge, UK. Woodhead Publishing Limited,.

Rotter, B. (2008). The influence of pH. Retrieved from www.brsquared.org/wine. [Accessed 24 March,2017].

Salque, R. M., Regert, M., Evershed, P. R., Outram, K. A., Decavallas, O., Dunne, J., Gerbault, P. and Mileto, S. (2016). Widespread exploitation of the honeybee by early Neolithic farmers. Nature. **534**.

Sandler, M. and Pinder, R. (2003). "Wine a Scientific Exploration" (1 ed.). Taylor & Francis group. New York, USA.

Savits, J. R. (2014). Descriptive sensory analysis of wines produced from Iowa-grown LA Crescent grapes. Master of Science Thesis. Iowa State Univ., USA.

Shakhashiri, B. Z. (2009). Ethanol chemistry & physiology of the world's second-most popular drug. www.scifun.org. Retrieved from http://www.scifun.org/CHEMWEEK/PDF/Ethanol.pdf. (Last update Feb 5, 2009). [Accessed 15, June, 2017].

Socha, R., Pajak, T. and (2015). Phenolic profile and antioxidant activity of polish meads. *J. Food Properties* **18** (12).

Somers, T. C. and Evan, M. E. (1997). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2 “chemical age”. *J. Sci. Food Agri.* **28**, 279-287.

Steinkraus, K. H. (1987). Fermented foods: Traditional and current practice. *In:* "Microbial Technology in the Developing World". (E. J. Dasilva, Y. R. Dommergues, E. J. A. Nyns and C. Ratledge, Eds.). UK. Oxford Science Publication.

Stevenson, T. (2005). The Sotheby’s Wine Encyclopedia (4th ed.). Dorling Kindersley Limited. London, UK.

Tayleur, W. H. T. (1973). "The Penguin Book of Home Brewing and Wine-making (Penguin handbook)" (1st ed.). Penguin Books. United state of America.

Thapa, R. (2001). Himalayan honeybees and beekeeping in Nepal. *Apimondia*.

Tomasik, P. (2004). "Chemical and functional properties of food saccharides". CRC press. New York Washington, D.C.

Usansa, U. (2003). Effect of alcoholic fermentation temperatures on red wine flavor. Master in Biotechnology. Thesis. Suranaree Univ. of Technol., Thailand.

Zamora, F. (2009). Biochemistry of alcoholic fermentation. *In:* "Wine Chemistry and Biochemistry". (M. V. Moreno-Arribas and M. C. Polo, Eds.). pp. 3-22. New York, USA. Springer Science+Business Media.

Appendices

Appendix A

Specimen card of sensory evaluation by 7 point hedonic rating test

Sensory evaluation of mead

Name of panelist: ……. Date: ……

Name of Product: mead

Please evaluate the product by your sense organ to show your perception by checking at the point that best describes your feelings about the product and also write to any of the defect as described below. An honest expression of personal feeling will help me.

Parameters	Samples											
	A	B	C	D	E	F	G	H	I	J	K	L
Appearance												
Odor												
Inmouth sensation												
Finish (after taste and lingering)												
Overall acceptance												

Quality description:

1: Faulty 2: poor 3: Below average 4: Average 5: Above average

6: very good 7: Exceptional

Comments:

……

……

_____

Signature

Source: Johnson (2002)

Appendix-B

ANOVA result for sensory analysis of mead

Table B. 1 Mean sensory scores for different attributes

Sample code	Quality attributes				
	Appearance	Odor	In mouth sensation	Finish	Overall acceptance
A	6.125^{a}	7.250^{ab}	6.750^{bcd}	6.375^{bc}	7.000^{cde}
B	6.375^{a}	7.375^{ab}	6.375^{bc}	5.750^{ab}	6.125^{b}
C	8.375^{e}	7.125^{ab}	7.875^{e}	7.500^{de}	7.625^{de}
D	7.375^{d}	6.500^{a}	7.375^{de}	6.750^{cd}	6.875^{bcd}
E	6.500^{ab}	7.000^{ab}	5.875^{ab}	6.125^{bc}	6.500^{bc}
F	7.750^{de}	7.000^{ab}	8.000^{e}	7.875^{e}	7.750^{e}
G	6.500^{ab}	6.625^{a}	6.375^{bc}	6.750^{cd}	6.625^{bc}
H	7.125^{bcd}	6.625^{a}	6.750^{bcd}	6.250^{bc}	6.625^{bc}
I	7.750^{de}	7.625^{b}	6.875^{cd}	6.750^{cd}	7.250^{cde}
J	7.250^{cd}	6.625^{a}	6.625^{bcd}	6.000^{abc}	6.750^{bc}
K	6.625^{abc}	6.625^{a}	5.375^{a}	5.250^{a}	$5 .250^{a}$
L	6.625^{abc}	6.875^{ab}	6.750^{bcd}	6.125^{bc}	6.500^{bc}
LSD	0.6725	0.9608	0.9030	0.8260	0.7665

The values are the mean of 8 panelist score. The values having same superscript in column.did.not.vary.significantly.at.5%.level.of.significance.

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

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	11	41.2812	3.7528	8.22	<.001
Panelist	7	20.4896	2.9271	6.41	<.001
Residual	77	35.1354	0.4563		
Total	95	96.9062			

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD of appearance at 0.05 level of significance is 0.6725.

Table B.3 Two way ANOVA (No blocking) for odor.

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	11	11.1250	1.0114	1.09	<.001
Panelist	7	22.7917	3.2560	3.50	0.003
Residual	77	71.7083	0.9313		
Total	95	105.6250			

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD of odor at 0.05 level of significance is 0.9608.

Table B.4 Two way ANOVA (no blocking) for in-mouth sensation

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	11	49.5000	4.5000	5.47	<.001
Panelist	7	21.1667	3.0238	3.68	0.002
Residual	77	63.3333	0.8225		
Total	95	134.0000			

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD of in-mouth sensation at 0.05 level of significance is 0.9030.

Table B.5 Two way ANOVA (no blocking) for finish.

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	11	46.3333	4.2121	6.12	<.001
Panelist	7	14.5000	2.0714	3.01	0.008
Residual	77	53.0000	0.6883		
Total	95	113.8333			

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD of finish at 0.05 level of significance is 0.8260.

Table B.6 Two way ANOVA (no blocking) for overall acceptance

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	Variance ratio
Sample	11	39.1146	3.5559	6.00	<.001
Panelist	7	9.7396	1.3914	2.35	0.032
Residual	77	45.6354	0.5927		
Total	95	94.4896			

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD of overall acceptance at 0.05 level of significance is 0.7665.

Appendix C

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

Component	(g per 100 ml)				
	Dry White	Dry Red	Sweet White	Sweet Red	Sparkling
Alcohol by volume, (%)	2.45	12.61	18.38	19.30	13.22
Alcohol	9.88		10	14.58	10.48
Glycerol	0.7019	0.6355	0.3025	0.5089	0.4177
Ash	0.196	0.247	0.203	0.311	0.153
Total acids	0.586	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: Amerine *et al.* (1980)

Appendix D

Table D.1 According to OVI major wine producing countries of the world-2015.

Counties	Wine production (mhl)	Wine export (mhl)	Wine consumption (mhl)	Total grapes (MT)	Area of vine (kha)
Italy	50.0	20.0	21	8.2	682
France	47.4	14.0	27	6.3	786
Spain	37.3	22.9	10	6.0	1021
USA	22.1	4.2	31	7.0	419
Argentina	13.4	2.7	10	2.4	225
Australia	11.9	7.4	5	1.7	149
China	11.5	-	16	12.6	830
South Africa	11.2	4.2	4	2.0	130
chile	10.1	8.8	2.1	3.1	211
Germany	8.8	3.6	20	1.2	103
Portugal	7.0	2.8	5	-	217
Others	43.3	13.4	87.9	25.7	2738
Total	274	104	239	75.7	7511

Source: OIV (2016)

Color Plates

Beekeeping

Processed honey

Different sample of wine for sensory analysis

Panelist performing sensory analysis