# EFFECTS OF JUICE CONTENT AND pH OF MUST ON THE QUALITY OF YACON (*SMALLANTHUS SOCNCHIFOLIUS*) WINE


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# Effects of Juice Content and pH of Must on the Quality of Yacon (*Smallanthus Sonchifolius*) Wine

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

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## Approval Letter

**This *dissertation* entitled *Effects of Juice Content and pH of Must on the Quality of Yacon (Smallanthus Sonchifolius) Wine* presented by Lila Mani Pokhrel has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology.**

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# Abstract

Yacon brought from Phaksib, Dhankuta was used for the preparation of yacon wine. The fruit was sorted, washed, disinfected peeled for juice extraction. Yacon juice was analyzed for TSS, acidity, reducing sugar, and pH. Yacon wines were prepared from nine musts using different proportion of juice content (100%, 50% and 25%) and pH (3, 3.5 and 4) maintaining constant TSS (25ºBx) with addition of sugar and pH of must were maintained with the help of citric acid. Fermentation was carried out at ambient temperature ranging 23-27ºC using baker yeast and aged for 3 months. Effect of juice content and pH on sensory attributes of the wines were evaluated using seven point Hedonic scale rating test to identify the best product. The data were analyzed by one-way and two-way ANOVA using Genstat (Genstat Discovery Edition 12, 2009) at 5% significance level.

Sensory analysis showed that there was significant difference among all the products with respect to appearance, odor, in-mouth sensation, finish and overall acceptance. Variation in juice content and pH of must significantly ($p<0.05$) affected wine quality. From sensory evaluation wine prepared from 100% juice and pH of 3.5 was found to be superior and had 12.50 % (v/v) alcohol content, 9.2ºBx TSS, 3.40 pH, 0.376% (as % lactic acid) total acidity, 0.012% (as acetic acid) volatile acidity, 367 ppm ester and 317 ppm aldehyde. Alcohol content, volatile acidity and other parameters of wine were within the range of a good quality wine. The cost of wine prepared using 100% juice and pH of 3.5 was NRs.121 per 750 ml) and hence this wine had great potential for commercialization.

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## List of abbreviations

| Abbreviaion | Full form |
|---|---|
| ADY | Active dry yeast |
| ANOVA | Analysis of vaiance |
| ATP | Adenosine triphosphate |
| CFU | Colony forming units |
| EMP | Embden–Meyerhof 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. However, products of fermentation of others 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).

Different types of raw materials have been used for the preparation of wine, either for flavor or for enrichment of wine with chief chemical constituents (Gubhaju, 2006). Wine can be made from any fruit, which contains sufficient fermentable carbohydrate. The grape (*vitis venifera* or, less commonly, *v. rotundifolia*) is of overwhelming commercial importance, although wine is also 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, 2009). Cereals like rice can also be used to make some forms of wine, for examples, the Japanese sake (Steinkraus, 1987).

Yacon (Smallanthus sonchifolius) is a perennial herbaceous plant of the family Asteraceae, native to the Andean regions of South America. This plant has a branching system that gives rise to aerial stems about 2 to 2.5 m high with large dark green leaves. Yacon yields starchy, fruit-like roots of different shapes and sizes that are usually consumed raw and taste sweet. Their crunchy texture very much resembles that of an apple. One plant is estimated to produce more than 10 kilos of roots (Hermann and Heller, 1997). The fact that the yacon plant adapts to different climatic regions, altitudes and soils explains its expansion outside the Andean region. Yacon is currently cultivated in Argentina, Bolívia, Brazil, the Czech Republic, Ecuador, Italy, Japan, Korea, New Zealand, Peru and the United States. Yacon root's water content usually exceeds 70% of the fresh weight while the major portion of the dry matter consists of fructooligosacharides (FOS). FOS content ranges from 6.4% to 70% of the dry matter (0.7% to 13.2% of the fresh weight) depending upon the specific crop and location. Fructooligosacharides (FOS) The high content of FOS in yacon roots is considered to offer health benefits, as it can reduce glycemic index, body weight and the risk of colon cancer (Valentova and Ulrichova, 2003).

The moisture content in yacon is relatively high, hence the shelf life of fresh yacon does not exceed 15-20 days at normal condition. Hence farmers of Andes region use simple technique to give added value to their crops by producing food and sweets from its roots, such as pickles, juice, syrup, tea and dehydrated snack (Castilo *et al.*, 2016).The production of yacon wine can also be a good product for better utilization and exploitation of yacon which is normally spoiled or provide minimum financial benefit in peak season of its production.

## 1.2 Statement of problem

Yacon is a plant for health-conscious people, considered as a functional food due to its components such as dietary fiber with prebiotic function. These components are fructooligosaccharides (FOS) which are stored in large amounts in yacón roots (underground storage organs). FOS are difficult to digest by enzymes in the human gastrointestinal tract, stimulating growth and activity of intestinal health promoting bacteria (Daniela *et al.*, 2015). Yacon is an underutilized and scientifically neglected root crop. In Nepal, yacon is produced but not yet utilized in market efficiently. Consumer demand for its root in market of Nepal is poor. This is due to the root's short shelf life of few days and lack of consumer familiarity. Resulting most fruits are spoiled or the farmer are obliged to sell them at nominal prices. In Andean region, farmer use simple techniques to give added value to their crops by producing foods and sweets from its roots, such as pickle, juice, syrup, tea, dehydrated snacks (Castillo et al., 2016). But in Nepal, as far as research on the fruits is concerned, very little work has been attempted. In addition, appropriate technologies for transforming fruits in to new products are almost non-existent (Shrestha, 2015).

In Nepal, wine are not made from grape only. These wine are mostly made from various fruits with mixture of honey, saffron, spices and herbs. As these raw materials are less in quantity, it gets a bit challenging to maintain the same level of production throughout the year. Hence the need for alternative material which are available locally needed to be used along with grapes for wine production (Subedi, 2015).

Hence, the process of fermenting fruit is old, but continues to draw attention by using unconventional raw material such as yacon tuber. The preparation of wine from yacon would come against the problem of perishability of the tubers, as well as the demand for new products in the consumer market.

## 1.3 Objectives

### 1.3.1 General objective

The general objective of this dissertation was to prepare, optimize must composition of yacon wine and carry out its sensory and chemical analysis.

### 1.3.2 Specific objective

The specific objectives of the study are as follows:

1. To prepare yacon juice and to carry out its analysis.
2. To optimize juice content in wine making.
3. To optimize pH of must in wine making.
4. To carry out sensory and physicochemical analysis of yacon wine.
5. To study on total cost of the product.

## 1.4 Significance of the study

This study can be a useful to find out the possible utilization of yacon by using it as a substitute source of juice needed for wine making so that spoilage resulting from its underutilization can be minimized. This work mainly focus on optimization of must composition in terms of juice content and pH hence, the study can provide information to the cultivars to produce yacon wine in domestic scale so that they can produce a value added product from the fruit. 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 yacon wine. In addition, fermentation is one of the food preservation methods, it provides distinctive new foods with characteristics taste, body, appearance and texture, and it provides more nutritious foods than their unfermented counterparts. Therefore, this work may suggest preserving by fermentation that is one of the good preservative methods to minimize its spoilage.

## 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 yacon wine 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

Alcoholic beverages are believed to have originated in Egypt and Mesopotamia around 6000 years ago. At every part of world different civilization had developed some types of alcoholic beverage. The production and consumption of alcoholic beverage is one of the man's oldest activities. Wine making was an important economic activity, in the traditional countries of the 'old world' such as France and Germany (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).

Despite this early application of microbiology, the ability of microorganisms to stimulate the biochemical changes was demonstrated several years later. 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. *Saccharomyces* group possesses almost all the credit of producing alcoholic beverages (Prescott and Dunn, 2004).

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'. And that is it in a nutshell. 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 covers 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. Although, production of grapes is in increasing pattern, since 2000 A.D. due to continual improvement of viticulture technique. 7.5 mha is the global area under vines in 2015 A.D. 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 (E).

## 2.4 Winery in Nepal

In Nepal, the history of commercial wine making is not very long (Bhandari, 1992). Although 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). The taste and high qualities of Nepali wines have been able to pull the consumers towards them. 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 2.2.

**Table 2.1** Classification of wine

| Basis of classification | Class/type | Description | Example |
|---|---|---|---|
| Color | Red wine | Contain the red coloring matter of skin, pulp and seeds | Burgundy |
| | White wine | Do not contain the red coloring matter of skin, pulp and seeds | Rhine wine |
| | Pink wine | Low concentration of red coloring matter is maintained | Rose |
| Relative sweetness | Sweet wine | Contain up to 7% sugar | Sherry (sweet) |
| | Dry wine | Contains less than 0.12% sugar | Sherry (dry) |
| Alcohol content | Natural | Contains 8.5 – 16 % alcohol by volume ( % abv) | 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, 2009).

**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 (g/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 (Prescott and Dunn, 2004). 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 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. 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 (Prescott and Dunn, 2004). In case of red wine temperature range of 20-30ºC is much more beneficial as fruity flavors and aromas don't get preserved and better color and tannin extraction took place. And for white wine ideal fermentation temperature is 7-16ºC, these lower temperatures help to preserve fruitiness and volatile aromatics, characteristics more in line with white wine.

In general temperature of primary fermentation should be 20ºC, temperature of secondary fermentation should be 15ºC and finish wine storage temperature should be 10ºC (Berry, 1996).

### 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 (Prescott and Dunn, 2004).

### 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, 2009). 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 *Saccaromyces 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 *Sacch. 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.

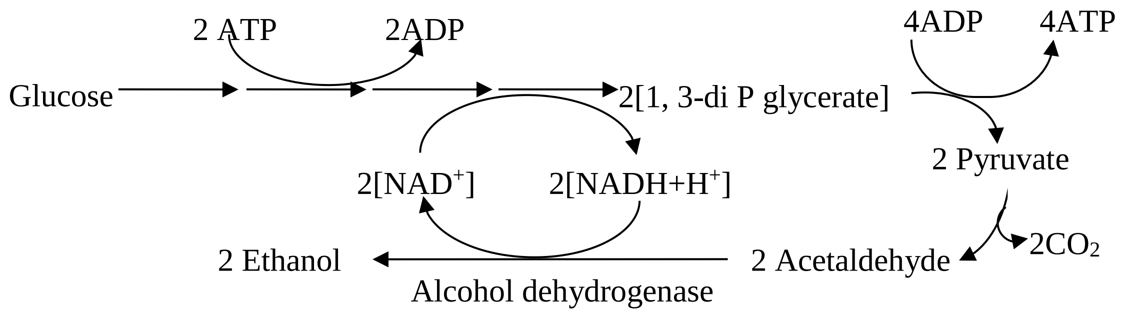


**Fig. 2.1** Simplified pathway of alcohol synthesis by yeast

### 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, 2009). The biochemistry of fermentation is given in Fig. 2.2

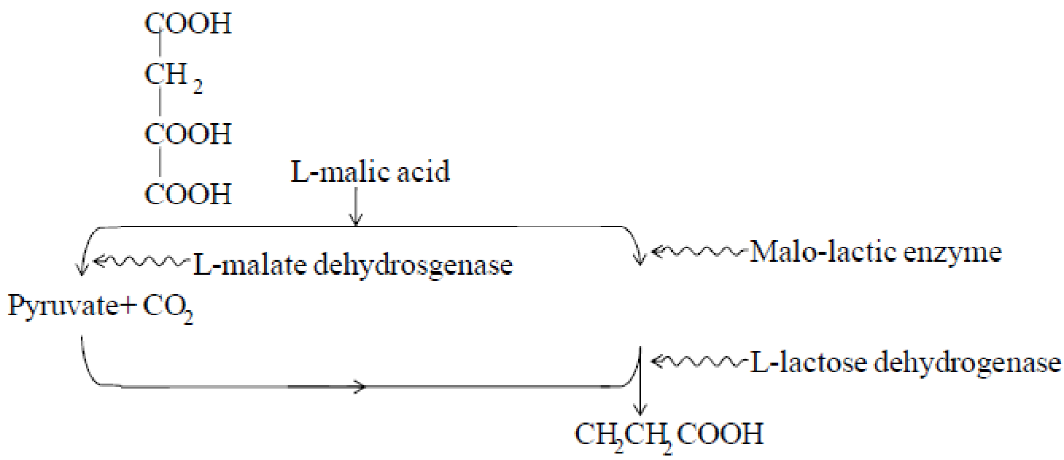


**Fig. 2.2** The malolactic pathway

## 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 preparation is given in Fig. 2.3.

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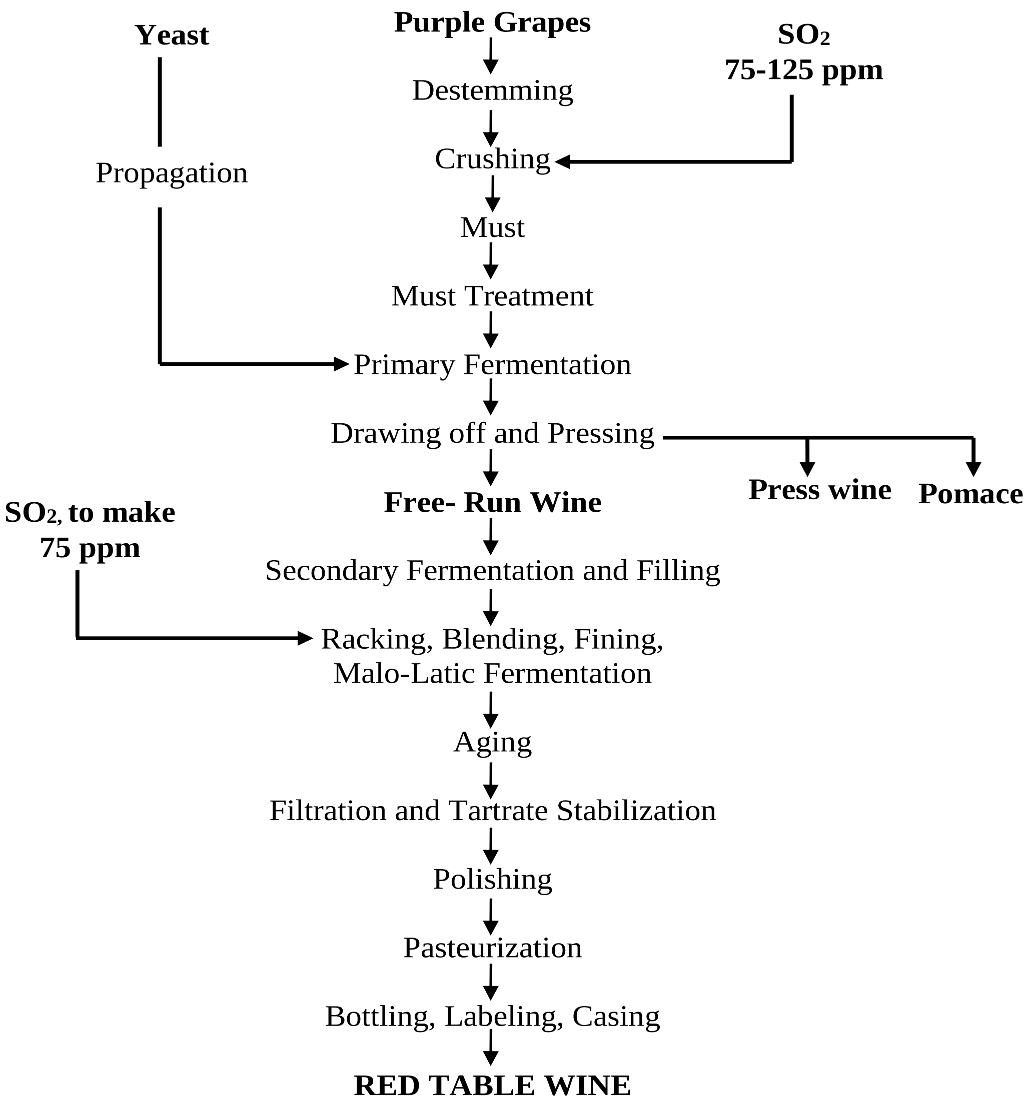



Source: Rai (2009)

**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 (Prescott and Dunn, 2004).

- 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 (Rai, 2009). 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 (Prescott and Dunn, 2004).

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, 2009). 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) (Rai, 2009).

### 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 %. Succininc 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 (Rai, 2009).

### 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 polypyrolidone). 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 (Rai, 2009).

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.

I. **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).

II. **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).

III. **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 hours 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, 2009). 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 %) 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 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).

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

I. **Appearance:** Firstly, view each sample at 30˚ to 45˚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 there 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).

## 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. 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/liter), 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'(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. 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). Yacon root juice has also been used for wine making by some authers (Shrestha, 2015) although literature regarding yacon wine is still scarce.

## 2.15 Yacon

### 2.15.1 Introduction

The yacon (*Smallanthus sonchifolius*) is a perennial plant traditionally grown in the Northern and Central Andes from Colombia to Northern Argentina for its crisp, sweet-tasting tuberous fruits. Their texture and flavor are very similar to jicama, mainly differing in that yacon has some slightly sweet, resinous and floral (similar to violet) undertones to its flavor, probably due to the presence of inulin, which produces the sweet taste of the fruits of elecampane, as well. Another name for yacon is Peruvian ground apple. The tuber is composed mostly of water and fructooligosaccharide. It has gradually received more attention due to its abundant content of fructooligosaccharides (FOS) and phenolic compounds (Quinteros, 2000).

Yacon is a perennial plant of the Andes; however, it is considered an annual in the cultivation system. It is traditionally grown for its root tubers and medicinal infusion from leaves rich in phenolic components with strong antioxidant effects. Yacon plants can grow to over 2 meters in height and produce small, inconspicuous yellow flowers at the end of the growing season. Yacon is not photoperiod sensitive, and can produce a commercial yield in the subtropics yacon is highly adaptable to various climates and altitudes. It can tolerate temperatures as low as 4ºC. The optimal development is reached within 18ºC and 25ºC. It needs near 200 days of no-frost climate before the tuberous roots are ready for harvest (Farnandez *et al.*, 2007)

Until the end of the 1980s, with the exception of Peru and Japan, the scientific community paid only vague attention to this plant. Each Yacon fruit typically weighs 100 – 2,000 g and mainly stores water (860 – 900 g/ kg) and carbohydrates (90 – 130 g/ kg). It also contains small amounts of fat, potassium, fructose, glucose, saccharose, vitamin A, some free amino acids such as L-tryptophan, and carotenoids such as β-carotene and neurosporene (Quinteros, 2000). The majority of the carbohydrates (600 to 700 g/kg) are inulin-type oligofructans and β-(2→1)-fructooligosaccharides. which are short fructose polymers with a polymerization degree of 3 – 10 fructans (Granato *et al.*, 2011). Fructooligosaccharides (FOS) which are stored in large amounts in yacón roots (underground storage organs). FOS are difficult to digest by enzymes in the human gastrointestinal tract, stimulating growth and activity of intestinal health promoting bacteria. Of the total carbohydrate content, 60 to 80% of the dry matters are FOS (Castilo *et al.*, 2016).

Fructans are considered part of functional fiber, which includes isolated non digestible carbohydrates that have beneficial physiological effects in humans. Fructans, especially inulin, can modulate the growth of bacteria in the intestines and boost the immunological system, offering beneficial health effects like enhance colon health and aid digestion (Capito, 2001).Yacon tubers have various antioxidant, anti-diabetic, antifungal and anticholesteremical properties (Granato *et al.*, 2011). Hence, it is a plant for health-conscious people, considered as a functional food due to its components such as dietary fiber with prebiotic function. Yacon is commonly consumed by diabetics because of its known positive effects on the digestive system (Zardini, 1991).

The Yacon tuber contains carotenoids that confer its yellow color (Quinteros, 2000). It also contains chlorogenic acid, ferulic acid, and caffeic acid which make the tubers susceptible to enzymatic browning reactions caused by polyphenol oxidases (PPOs). To inhibit these reactions, PPOs are inactivated by the heat or by the use of reducing agents, such as sulphites and organic acids (ascorbic, malic, citric acids) (Manrique *et al.*, 2005). Nowadays, Companies have also developed novel products such as yacon syrup and yacon tea. Both products are popular among diabetics and dieters. Beside this, Yacon juice treated with active carbon powder, Yacon vinegar, yacon wine, chocolate cake, and Yacon juice blended with peach or lemon juice, are some other products that have been developed (Granato *et al.*, 2011).

### 2.15.2 Historical background and current situation of yacon

Yacon *(Smallanthus sonchifolius*) is the name commonly given to the plant and its storage root. Yacon is native to the Andean region and is known to have been cultivated and consumed since pre-Inca times. Yacon belongs with 21 *Smallanthus* species to the family of *Asteraceae.* The origin of yacon and its relatives are the humid slopes in the Andean region of Latin America. In Peru seven *smallanthus* species are found, from which only yacon is domesticated species (Polreich, 2003). The first written record of yacon was in 1615 when Felipe Guaman Poma de Ayala included it in a list of 55 native crops cultivated by the Andeans. Attempts to establish yacon outside the Andes have been mixed. An early experiment with yacon in Italy was successful, but discontinued with the onset of World War II. A single variety from Ecuador was introduced to New Zealand in 1979. In the 1980s and 1990s, yacon was established in Japan, Brazil, South Korea, and the Czech Republic (Manrique *et al.*, 2005). It has since spread around the world and is grown on a small scale in many countries in Europe, North America and Asia. Yacon is believed to have entered Nepal from Japan since a decade ago, *Sudarshan Karki* named for first commercial cultivar and promotor of yacon in Nepal (Sthrestha, 2016).

Despite this, and unlike other Andean root crops such as potato and sweet potato, yacon remains relatively unexploited. Until now yacon has generally been cultivated only as a subsistence crop by Andean farmers, and more recently for sale in small provincial market towns. It is only in the last few years that the health benefits of yacon have become known and it has reached the market places of the big cities where efforts have begun to commercialize it and to experiment with processing techniques. Therefore, also be grown in many other regions of the world. It has been successfully cultivated in several different regions with varying climates including: Brazil, Czech Republic, China, Korea, Japan, New

Zealand, Russia, Taiwan and United States (Manrique *et al.*, 2005). Yacon is still not as widely known as other tubers, but it still generates demand around the world. Japan is the top consumer of the plant, closely followed by Brazil, and commercial cultivation is alive and well in those countries. Peru is the world's top producer, where 18 out of 20 regions of the country grow it on an agricultural scale, exporting to places like the U.S., New Zealand, and Korea at a free on board value of $633,000 in 2010 alone

### 2.15.3 Botanical characteristics and morphology of yacon plant

Yacon, a native of the Andes closely related to the sunflower, is a vigorous, herbaceous perennial plant (family Compositae or Asteraceae sunflower family). The plant produces large tuberous fruits similar to sweet potatoes in appearance, but they have a much sweeter taste and crunchy flesh. The plants are extremely hardy and are able to grow under hot or cold conditions. Yacon grows up to a height of two meters, has large opposite sagittate leaves with serrate margins, and multiple yellow-orange flowers 3 cm in size (Polreich, 2003).

The plant is distinguished by having two kinds of tuberous fruits, a central rhizome with "eyes" for producing new stems, and multiple edible tuberous fruits radiating from the rhizome. Generally, the root system is composed of 4- 20 fleshy tuberous storage roots that can reach a length of 25 cm by 10 cm diameter. The flesh color of storage roots varies considerably: white, cream, white with purple striations, purple, pink, and yellow. The tuberous root bark is brown, pink purplish, cream or ivory white and very thin (1–2 mm).

The edible tuberous fruits are crunchy like a crisp, sweet, and juicier than any pear. Stem is cylindrical or sub angular ramified in most clones, hollows at maturity, density pubescent and green to purplish colored bark. Like the sunflower, the yacon presents distributed big leaves of to even along very little ramified shafts. Lower leaves are broadly ovate and hastate or sub hastate  Cannale and auriculate at base; upper and lower surface are densely pubescent, the inflorescences are terminal, composed of one to five axes each with three capitula. The color of the flower varies between yellow to bright orange, ray flowers are two or three toothed (Polreich, 2003). Tuberous fruit crops, in which tuberous fruits are formed after cessation of stem growth, seem to have a similar mechanism of tuberous fruit formation to potato (Lachman *et al.*, 2003b). The scientific classification of yacon fruit is given in Table 2.5.Yacon morphological aspects are given in Fig. 2.4.

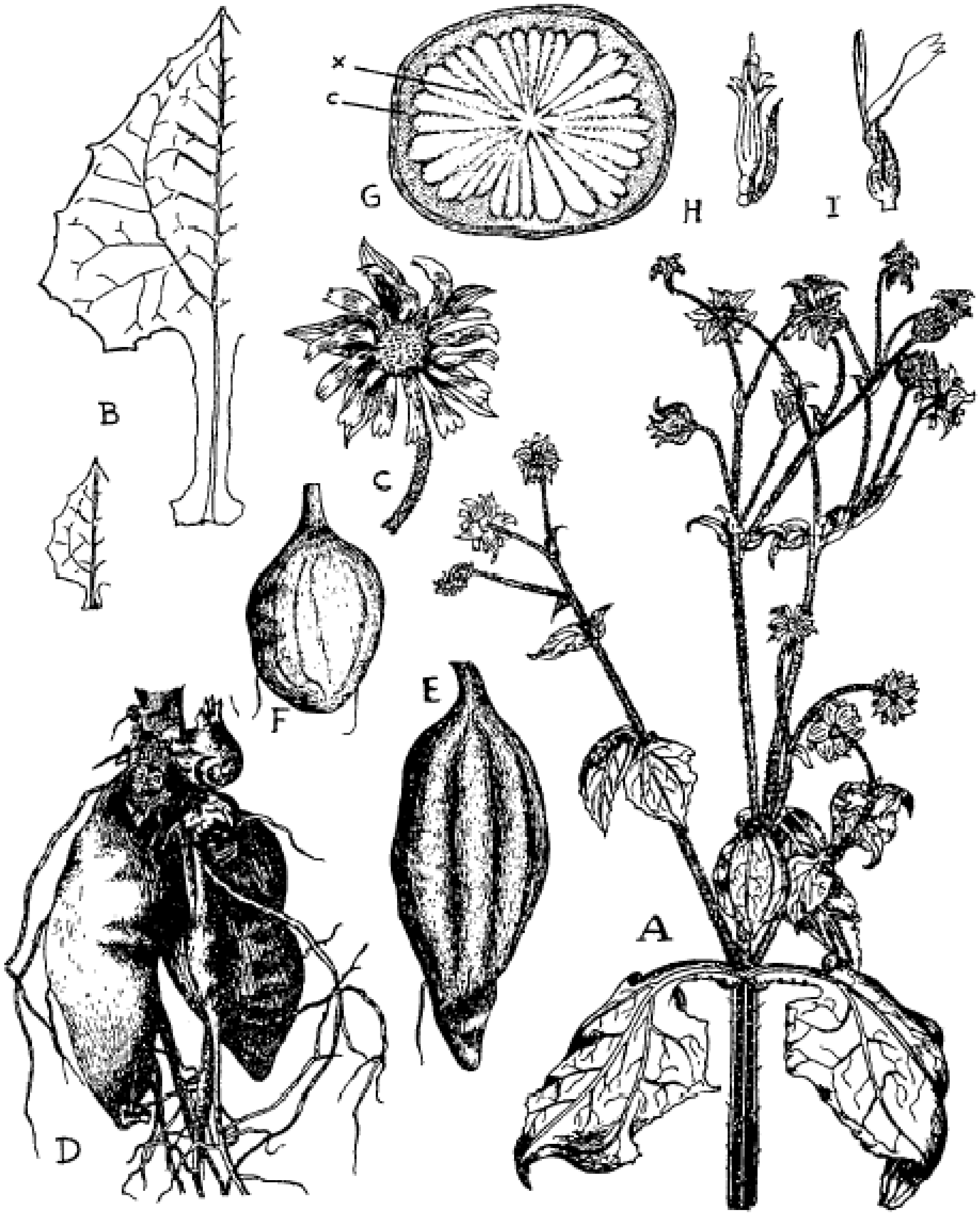



**Fig. 2.4** Yacon *(Smallanthus sonchifolius)* morphological aspects.

(A: flowering branches. B: leaves. C: flowerhead. D-F: tuberous roots. G: transverse section of the tuberous root (x, xylem; c, cortex tissues). H: staminate disk flower. I: pistilate ray flower.)

Source: Hermann and Heller (1997).

**Table 2.5** Scientific classification of yacon fruit

| Scientific classification | |
|---|---|
| Kingdom | Plantae |
| (unranked) | Angiosperms |
| (unranked) | Eudicots |
| (unranked) | Asterids |
| Order | Asterales |
| Family | Asteraceae |
| Genus | *Smallanthus* |
| Species | *S. sonchifolius* |

Source: Valentova and Ulrichova (2003)

#### 2.15.4 Cultivation condition for yacon

It is considered an annual in the cultivation system. It is traditionally grown for its root tuber and medicinal infusion from leaves rich in phenolic components with strong antioxidant effects. The yacon root system forms fleshy rhizomes as well as tuberous roots. These rhizomes are used for the propagation of the plant. On the surface of each rhizome there are many buds or points of growth. A mature rhizome can be broken into ten or twenty parts, each of which is traditionally used as seed and has between 3 and 5 growing points. Yacon is a member of the sunflower family and while it grows in the warm, temperate valleys of the Andes. Yacon is highly adaptable to various climates and altitudes (Zardini, 1991). Yacon is not photoperiod sensitive, and can produce a commercial yield in the subtropics, It can tolerate temperatures as low as 4ºC. The optimal development is reached within 18ºC and 25ºC and altitude from 1800 m. to 3200 m. Yacon is harvested from six to twelve months after sowing. Location and altitude most affect the length of the growing season (Manrique *et al.*, 2005).

The mature plants possess well-developed foliage, with a high transpiration capacity, so that they require a regular and important water supply. Yacon grows better in soils slightly retentive of humidity, with regular watering. Yacon develops well within a wide range of soil conditions; its development is favored by deep, well-tilled soils, rich or moderately rich in organic matter and well drained. It can tolerate a wide range of pH, from acid to slightly alkaline. In terms of the root tubers formation, yacon was described as a crop with negative reaction to photoperiod (Popenoe *et al.*, 1989). The yacon leaves has got di- sesquiterpenes with protective effects against insects are present. This property allows cultivation of yacon without pesticides, what is prerequisite good for organic farming of cultivation and utilization of yacon like dietetic food and raw material for production of bioproducts (Viehmannova *et al.*, 2007).

### 2.15.5 Chemical composition of yacon

A major portion of fruit biomass is composed of water that usually between 85 and 90% of the fresh weight of the storage roots is water. Due to high water content, the fruit energy value is low. The calculated yacon food energy 619–937 kJ/kg of fresh matter is very low and has similar properties like dietary fiber. In contrast to the majority of other edible roots yacon does not store its carbohydrates in the form of starch but rather as FOS, fructose glucose and sucrose. 70–80%of dry matter is composed of saccharides, mainly fructooligosaccharides. Proteins and lipids account for just 2.4 - 4.3% and 0.14 - 0.43% of dry weight respectively (Manrique *et al.*, 2005). Potassium accounts for an average 230 mg/100g of fresh weight. Other micronutrients occur in much lower concentrations and include calcium, phosphorous, magnesium, sodium and iron. The chemical composition of yacon fruit is given in Table 2.6.

**Table 2.6** Chemical composition of yacon tuber, stem and leaf

| Compound | Part of Yacon | | |
|---|---|---|---|
| | Stem | Leaf | Tuber |
| Water (%) | 86.7 | 83.20 | 93-70 |
| Protein (%) | 1.51 | 2.87 | 0.4- 2.0 |
| Saccharides (%) | 1.55 | 1.44 | 12.5 |
| Lipids (%) | 6.30 | 1.24 | 0.1- 0.3 |
| Ash (%) | 1.35 | 2.68 | 0.3-2.0 |
| Fibre (%) | 1.51 | 1.68 | 0.3 -1.7 |
| Calcium (mg/ 100 g) | 967 | 1805 | 23 |
| Phosphorus(mg/ 100 g) | 415 | 543 | 21 |
| Iron (mg / 100 g) | 7.29 | 10.82 | 0.3 |
| Copper (mg / 100 g) | ≤0.5 | ≤0.5 | 0.963 |
| Manganese (mg/ 100 g) | ≤0.5 | 3.063 | 0.541 |
| Zinc (mg / 100 g) | 2.93 | 6.20 | 0.674 |
| Retinol (mg / 100 g) | | | 10 |
| Thiamine (m g/ 100 g) | | | 0.01 |
| Ascorbate (mg / 100 g) | | | 13.10 |
| Carotene (mg / 100 g) | | | 0.02 |
| Riboflavin (mg / 100 g) | | | 0.11 |

Source: Valentova and Ulrichova (2003)

#### 2.14.5.1 Saccharides

Yacon tubers contain as storage compounds mainly fructans with low glucose content. Fructans are non-digestible carbohydrates derivatives of sucrose, formed by several units of fructose with a glucose residue. They can be produced by bacteria, algae, fungi and plants. In plants fructans are used as reserves of carbohydrates, found in different organs such as leaves, roots (including yacón), tubers, rhizomes and fruits. The term fructan includes both oligosaccharides and polysaccharides. Inulin-type fructans with degree of polymerization from 2 to 10 are known as fructooligosaccharides (FOS), whereas for those with a higher polymerization degree the term most often used is inulin. FOS are chemically composed of 1 molecule of glucose connected to between 2 and 10 fructose molecules They have a favourable influence on the human intestinal flora and can modify some hyperlipidemias. Humans have no enzyme capable of hydrolysing the $\beta(2\rightarrow1)$bond. $\beta(2\rightarrow1)$fructans of the inulin type are thus dietary fibre or the indigestible residues of plant origin in human diet.

Recently, oligofructans have been classified as prebiotics. These are not digested in the human gastrointestinal tract and they are transported to the colon where they are fermented by selected species of gut micro-flora, especially *Bifidobacterium* and *Lactobacillus*, both indicators of a balanced gut flora. Studies have demonstrated that prebiotic consumption modifies gut flora composition and its metabolic activities. Probably through this action they also modulate lipid metabolism, calcium absorption, childhood immune systems and gut function. The composition of saccharides in in yacon tuber is given in Table 2.7.

#### 2.14.5.2 Other important chemical components

In comparison with other roots and tubers yacon contains a high level of polyphenols, which account for approximately 200 mg/100 g of fresh weight. The most abundant polyphenols are chlorogenic acid and at least four soluble phenols derived from caffeic acid. Other compounds reported with antioxidant activity are trypthophan, quercetin, ferulic acid and galic acid. Despite the high levels of polyphenols in the root, much higher levels are found in the leaves and in the stem. Polyphenols are chemical components that have antioxidant properties. That is to say that they neutralize the oxidization caused by unstable molecules known as free radicals (Valentova and Ulrichova, 2003).

**Table 2.7** Contain of saccharides in yacon tuberous root

| Saccharide | Content mg/g dry matter |
|---|---|
| Fructose | 350 ± 42.0 |
| Glucose | 158.3 ± 28.6 |
| Sucrose | 74.5 ± 19.0 |
| $GF_2$ | 60.1 ± 12.6 |
| $GF_3$ | 47.4 ± 8.2 |
| $GF_4$ | 33.6 ± 9.3 |
| $GF_5$ | 20.6 ± 5.2 |
| $GF_6$ | 15.8 ± 4.0 |
| $GF_7$ | 12.7 ± 4.0 |
| $GF_8$ | 9.6 ± 7.2 |
| $GF_9$ | 6.6 ± 2.3 |
| Inulin | 13.5 ± 0.4 |

Source: Valentova and Ulrichova (2003)

### 2.15.6 Health benefits of yacon fruit

Yacon also known as Peruvian ground apple is a native of South America. It produces juicy, edible tubers, from which its name, translated as "watery fruit," is derived. People in the Andes have enjoyed yacon as a food source for more than a thousand years. Yacon offers certain health benefits.

#### 2.15.6.1 Blood sugar management

Sugar like molecules in yacon known as fructooligosaccharides, which are derived from a no digestible carbohydrate called inulin, provide about half the sweetness of sugar without raising blood sugar levels. In the study, obese volunteers with insulin resistance, which is the inappropriate response of cells to the presence of insulin, were given two daily doses of yacon syrup containing 0.29 g and 0.14 g of fructooligosaccharides per kilogram of body weight. At the end of the 120-day study, the participants had achieved significantly lower fasting insulin levels. However, fasting blood sugar levels were not affected. The study also showed that yacon syrup promoted weight loss and suppressed appetite.

#### 2.15.6.2 Cancer prevention

Potential anticancer benefits of yacon were demonstrated in a tissue culture study of human cervical cancer cells published in the October 2011 issue of the journal "Fitoterapia." Yacon compounds inhibited the growth and reproduction of cancer cells and promoted early cell death. In a tissue culture study published in the December 2010 issue of the journal "Chemistry and Biodiversity," a fungus that grows on the fruits and leaves of yacon demonstrated anticancer benefits against skin, colon, nerve and blood cancers.

#### 2.15.6.3 Liver health

A combination of yacon and silymarin, which is the active component in milk thistle, improved cholesterol and blood sugar levels in patients with metabolic syndrome in a study published in the March 2008 issue of "Food and Chemical Toxicology." Metabolic syndrome is a combination of conditions that increases risk for heart disease and diabetes. In the study, participants consumed 2.4 g of yacon and 0.8 g of silymarin a day for 90 days. Results showed the combination supplement improved cholesterol levels and prevented fat accumulation in the liver, leading researchers to conclude that yacon and silymarin may promote healthy heart and liver function

#### 2.15.6.4 Dietary uses

Fresh yacon has a slightly sweet taste and a crunchy texture similar to that of an apple, while its flavor is close to that of watermelon. South Americans eat the tubers, which can range from yellow to purple, as a fruit, with lemon juice and honey, or they add it to fruit salad. You can also stir-fry, roast or bake yacon as a vegetable. Use yacon leaves, also high in inulin, to wrap other foods as you would cabbage or grape leaves, or brew them to make an herbal tea. Yacon syrup and powdered yacon supplements are also available (Shresha, 2015).

### 2.15.7 Use of yacon and its potential in Nepal

Yacón can be eaten raw or cooked and have traditionally been used in fruit salads, jams, puddings, and juices. Their peeled skin, once dried, can also be used to make nutritious organic tea. Farmers in Brazil and Japan produce a number of processed yacon products, such as air-dried tuber slices unrefined yacon syrup that has a consistency of honey and can be marketed as a dietetic sweetener.or a juice without addition of sweeteners, synthetic colorants and preservatives, with only small additions of vitamin C. The yacon tuberous roots serve as a source of raw material for the production of sweet pastries, fermented vegetables and ethanol; they can be used as "chips" in dehydrated form. Another product is yacon juice treated with active carbon powder to obtain its clarification, decolorisation and deodorization, acetic acid fermentation of yacon juice with *Acetobacter pasteurianus* for production of improved yacon vinegar containing natural fructooligosaccharides. Yacon slices and stripes retain crunchiness during cooking and could be used in Asian stir-fried dishes (Manrique *et al.*, 2005).

One of the main yacon properties is medicinal. Antidiabetic medicinal properties were attributed mainly to yacon leaves. Dried yacon leaves were used to prepare a medicinal infusion or mixed with common tea leaves in Japan. The yacon tuberous roots as well as stems and leaves containing a high level of proteins could be used as a food for cattle and other domestic animals (Lachman *et al.*, 2003a).

Geographically, the climate and agricultural conditions of Nepal are quite similar to those€ of the Andes in South America. Unfortunately, due to their resemblance to a vegetable and the general lack of knowledge, yacóns are not as popular as initially imagined. However commercial production of yacon begen in Nepal and currently cultivated on Kavre, Ilam, Pachthar, Dhankuta like hilly region of Nepal. In Nepal works on yacon and its possible utilization has been started Shrestha (2015) conducted a study on preparation and quality analyses of yacon ready to serve (RTS) and wine. The commercial production of yacon syrup also started in Nepal. Perhaps in the future, innovative farmers will adopt newer crops from Latin America to Nepal as well and commercial farming and processing will take its place slowly (Karki, 2014).

# Part III

## Material and methods

### 3.1 Materials

### 3.1.1 Raw materials

#### 3.1.1.1 Yacon (ground apple)

The yacon fruit (35 kg) of good quality was brought from the farm of Phaksib, Dhankuta, Nepal.

#### 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

The baker yeast (*Saccharomyces cerevisiae*) marketed by Angel yeast Co. Ltd., Yichang Hubai, China was brought from market of Dharan because viable and active wine yeast was not present in Central Campus of Technology.

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

### 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 | Physical apparatus |
|---|---|
| Stainless steel vessels | Weighing arrangement |
| Hand refractometer (0-30 °Bx) | Screw press juice extractor |
| pH meter | Handheld potato peeler |
| Stainless steel vessels | Knives |
| Thermometer | Distillation set |
| Titration apparatus | Heating arrangement |
| Pycnometer | Other routine glaswares |

### 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 | 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 | Phenopthalein |
| Fehling A solution | Fehling B solution |
| Carrez-I solution | Carrez-II solution |

## 3.2 Methodology

The total work was based on preparation of yacon wine with varying pH and juice percentage and analysis of optimized wine.

### 3.2.1 Experimental procedure

#### 3.2.1.1 Selection of raw material

Yacon was brought from Phaksib Dhankuta where one of the very few commercial yacon farming have been initiated. Due to treacherous journey during the transportation of raw materials, many of the yacon tubers were damaged on arrival at work place, damaged tubers were segregated from the good ones.

#### 3.2.1.2 Washing and disinfecting the raw material

Washing was done using plenty of water, rubbing the roots against one another and with the use of a scrubbing brush which easily remove the soil that adheres to the surface of the root. After washing, the roots were submerged in a solution of sodium hypochloride and water with a concentration of 200 ppm. This reduces the microorganism content which is still present on the yacon surface. Sodium hypochloride is one of the most effective, economical and easy to use disinfectants available (Manrique *et al.*, 2005).

#### 3.2.1.3 Peeling of roots

The roots were peeled manually using handheld potato peeler and they were submerged in KMS solution of 100 ppm in order to control browning. The concentration of sugars found in the root increases from center to surface of root. For this reason care was taken not to remove an excessive amount of flesh when peeling since this is where the highest concentration of sugar is found. On the other hand, the skin contains the highest concentration of substances that are catalysts for the oxidation of the juice, must be completely removed (Butler and Rivera, 2004).

#### 3.2.1.4 Extraction of juice and oxidation control

Screw press juice extractor was used for the extraction of yacon juice. Thus obtained juice was susceptible to oxidation hence, immediate action had to be taken to prevent juice. According to Manrique *et al.* (2005) for this purpose juice was collected in the vessel containing calculated amount of antioxidant i.e. citric acid ( 0.15 g/kg of yacon weight) and preservative KMS (150 ppm $SO_2$ by weight of juice). In this way the juice came into immediate contact with antioxidants, which prevented yacon juice from oxidation and KMS controlled microbial loads too. For complete separation of juice from solid particle the pulp was recycled twice.

#### 3.2.1.5 Straining of juice

The juice obtained from the screw press juice extractor contains significant amount of suspended insoluble solid that should be eliminated. For this purpose juice was allowed to strain through double folded muslin cloth and analysis of TSS, pH, acidity, total sugar and reducing sugar content was carried out.

#### 3.2.1.6 Preparation of must composition

After analysis of yacon juice different composition of must or sample were prepared. The juice and water was varied according to the juice content of 100%, 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. The sample prepared for experiment is presented in below Table 3.4.

**Table 3.4** Preparation of must

| Parameter | Sample | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I |
| Juice (%) | 100 | 100 | 100 | 50 | 50 | 50 | 25 | 25 | 25 |
| pH | 3.0 | 3.5 | 4.0 | 3.0 | 3.5 | 4.0 | 3.0 | 3.5 | 4.0 |

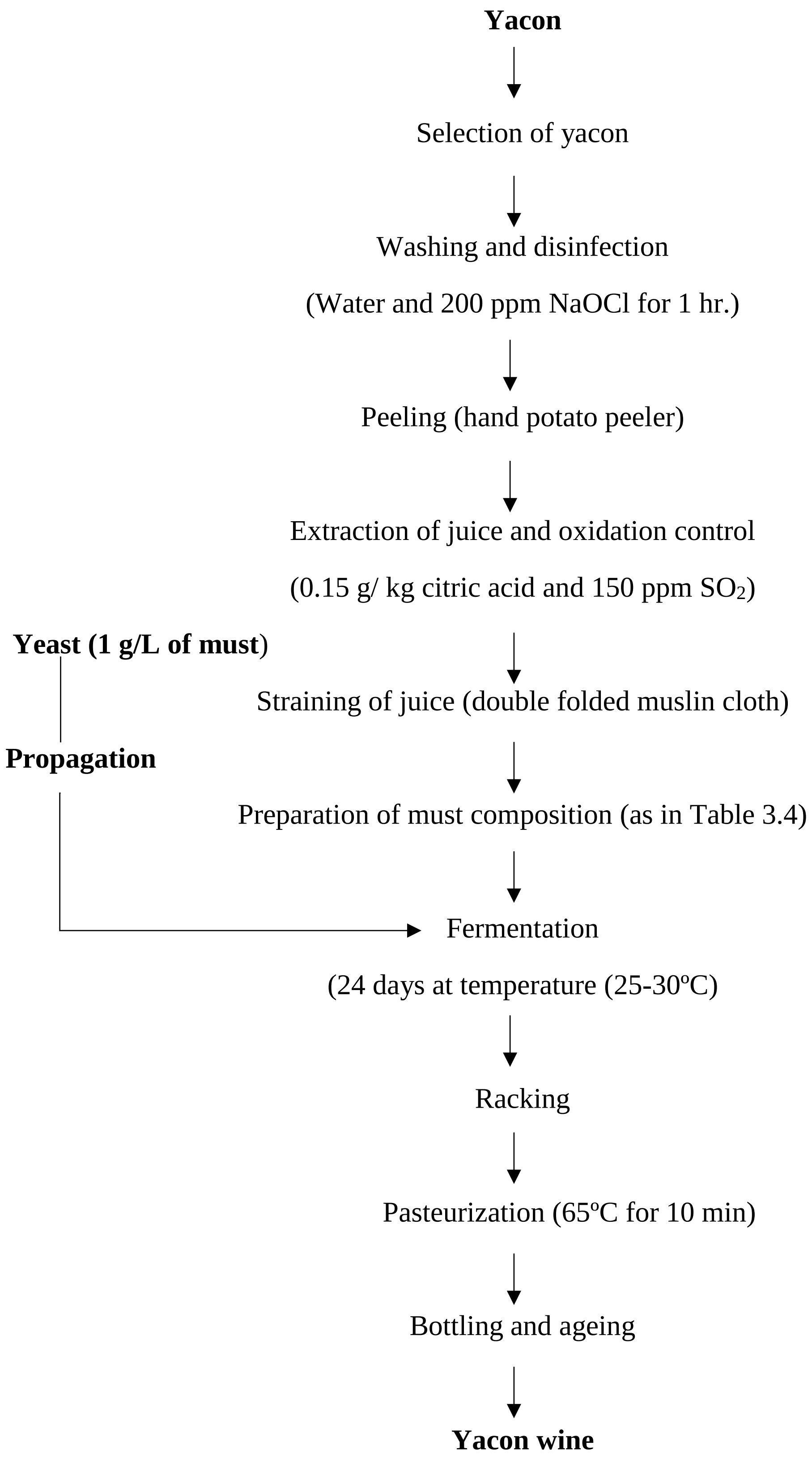



**Fig. 3.1** Flowsheet for preparation of yacon wine

Source: Manrique *et al.* (2005), (Jackson2014)

#### 3.2.1.7 Pitching

Baker yeast, was used for pitching. It was activated with mildly heated sugar and water solution and pitching was done at the rate of 1 g per liter for all musts. The general flow sheet for procedure is given in above Fig. 3.1.

#### 3.2.1.8 Fermentation

Must after pitching were kept in plastic jars for fermentation. After 5 days of pitching when vigorous evolution of $CO_2$ creased, the primary fermentation was completed. Then the necks of jars were closed tightly with cotton plugs for secondary 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 secondary 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 24 days from pitching.

#### 3.2.1.9 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 65°C for 10 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 (Jackson, 2014).

### 3.2.2 Analytical procedure

Although different authors have described different methods and parameters to analyze juice, must and wine only those parameters and related methods, which were feasible in the laboratory, were determined in this study. The determination was conducted in triplicates.
For juice TSS, pH, acidity and reducing sugar were analyzed. The must were analyzed for TSS and pH and for prepared wine sensory analysis based on following parameters appearance, odor, in mouth sensation, finish and overall acceptance was done to select best product. And for optimized (best product) was analyzed for chemical composition and

properties like TSS, pH, total acidity, volatile acidity, specific gravity, alcohol content, ester, aldehyde and tannin content were analyzed.

#### 3.2.2.1 Determination of total soluble solid (TSS)

The TSS of the juice, must and wine were determined by using hand sugar refractometer.

#### 3.2.2.2 Determination of pH

pH of juice, must and wine 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 25 ºC.

#### 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 Reducing sugar

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

#### 3.2.2.5 Specific gravity

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

#### 3.2.2.6 Alcohol content

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

#### 3.2.2.7 Tannin content

Tannin content of wine was determined by colorimetric determination as per K.C. and Rai (2007).and values were expressed in mg/ml.

#### 3.2.2.8 Ester content

The ester contents of wine sample were determined as per method described by FSSAI (2012). Briefly, 200 ml of wine 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.

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

#### 3.2.2.9 Aldehyde content

The aldehyde content of wine samples were determined as per FSSAI (2012). Briefly, 50 ml of distillate (from specific gravity determination) was taken in 250 ml iodine flask 10 ml of sodium bisulphite (0.05N) solution was taken then after flask was kept in dark place for 30 minute with occasional shaking. 25 ml of standard iodine solution (0.05N) was added and back titrated excess iodine against standard sodium thiosulphate solution (0.05N) using starch indicator (1%) to light green end point. Following same procedure blank sample was carried out using 5o ml distilled water. The difference in titer value in milliliter of sodium thiosulphate gives equivalent aldehyde content. The values were expressed in gram per 100 liter of absolute alcohol as acetaldehyde.

Aldehydes expressed acetaldehyde = $V \times 0.0011 \times 100 \times 1000 \times 2 / V_1$
g/100 L of abs. alcohol
V= difference in titer of blank and sample in ml of sodium thiosulphate solution
$V_1$ = alcohol percentage by volume.

### 3.2.3 Sensory evaluation

The prepared 9 wine samples by varying pH and juice 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 (such as 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 12 semi- trained panelist whom were teachers and students of food technology both Bachelor and Master level at CCT and they were familiar with alcoholic beverage.

### 3.2.4 Statistical analysis

The data were analyzed for two way and one way ANOVA, mean ANOVA ( No blocking at 5% level of significance ), LSD and interaction effects using Genstat (Genstat Discovery Edition 12, 2009) at 5% significance level were obtained to determine whether the sample were significantly different from each other and to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in Appendix A. The mean is compared using LSD method. Standard deviation and means were also analyzed from the same statistical tool.

# Part IV

## Results and discussion

The yacon was collected from Phaksib, Dhankuta and different physical and chemical analysis were carried out where moisture content of yacon was 90% and TSS and pH of yacon juice were found to be 9ºBx and 5.85 respectively. Different proportion of yacon juice and pH of juice were made for the preparation and optimization of yacon wine. In this experiment, must composition were prepared by using different juice concentration i.e.100, 50, 25 percent juice as well different pH were maintained by using citric acid the final pH was maintained 3, 3.5 and 4. TSS was maintained at 25ºBx by using table sugar. The fermentation was carried out at room temperature around 27ºC using baker yeast until the TSS reduces to 10ºBx it takes 24 days then pasteurized the product at 63ºC for 10 min. and allow to ageing for 2 months. The optimum product was determined by using sensory analysis and the chemical analysis was carried out for optimized product.

### 4.1 Chemical analysis of yacon fruit juice (*smallanthus sonchifolius*).

Chemical composition of yacon fruit juice is given in Table 4.1.

**Table 4.1** Chemical composition of yacon fruit juice

| Parameter | Value |
|---|---|
| TSS (ºBx) | 9 (0) |
| Acidity (% as lactic acid) | 0.0924 (0.005) |
| pH | 5.85 (0.029) |
| Juice yield ( % total fresh weight) | 50 |
| Reducing sugar ( % as dextrose) | 0.7037 (0.040) |

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

Above parameters for yacon fruit juice resemble to the data obtained from (Shrestha, 2015) in terms of TSS but slightly different in terms of acidity, pH and juice yield this is because in this experiment antioxidant ie. citric acid was used to prevent juice from oxidation hence acidity increases and pH decreases slightly and in case of juice yield percentage juice obtained was slightly reduces because heat treatment facilitate extraction of juice but in this experiment heat treatment was not done.

## 4.2 Formulation of must composition

The chemical composition of must was maintained according to design and is presented in Table 4.2.

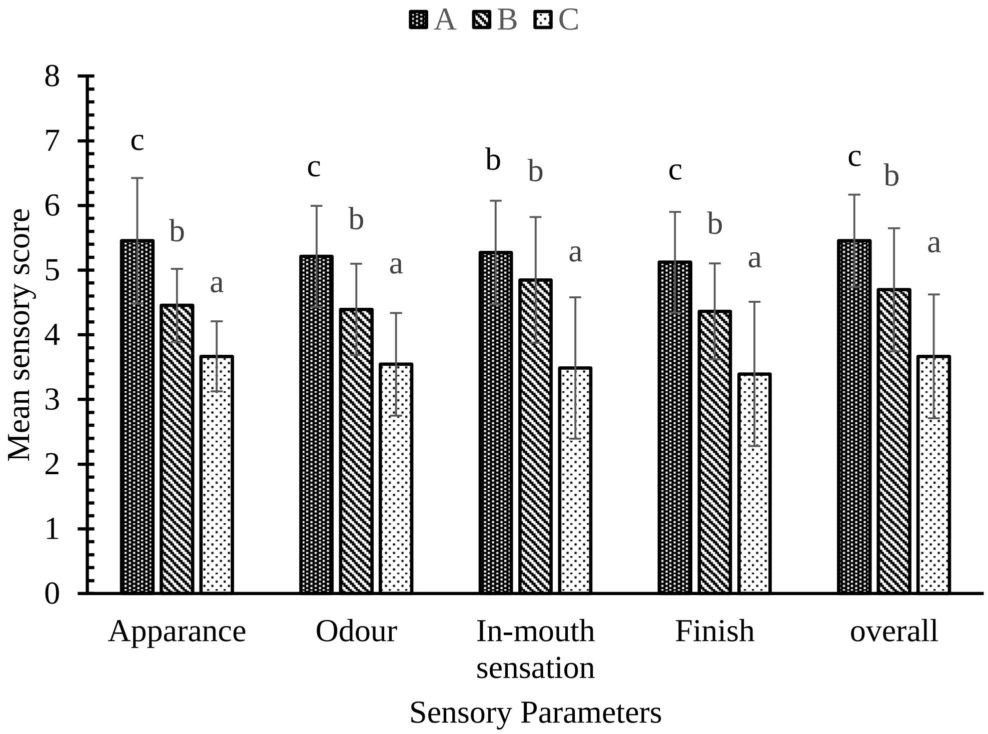
**Table 4.2** Chemical composition of must.

| Sample | Juice content (%) | pH | TSS |
|---|---|---|---|
| A | 100 | 3 | 25 |
| B | 100 | 3.5 | 25 |
| C | 100 | 4 | 25 |
| D | 50 | 3 | 25 |
| E | 50 | 3.5 | 25 |
| F | 50 | 4 | 25 |
| G | 25 | 3 | 25 |
| H | 25 | 3.5 | 25 |
| I | 25 | 4 | 25 |

## 4.3 Effect of juice content and pH of must on sensory quality of yacon wine

The sensory evaluation of all 9 sample were performed on the basis of 7 point hedonic rating test. The parameter for sensory analysis were appearance, odor, in-mouth sensation finish and overall acceptance of product. The obtained data from sensory analysis were analyzed using two-way ANOVA (Appendix B) at 5% level of significance to study the significance difference among formulation made and among panelists. However there is no significance difference in all panelist to judge the specified parameter of the product.

### 4.3.1 Effect of juice content of must on sensory quality of wine

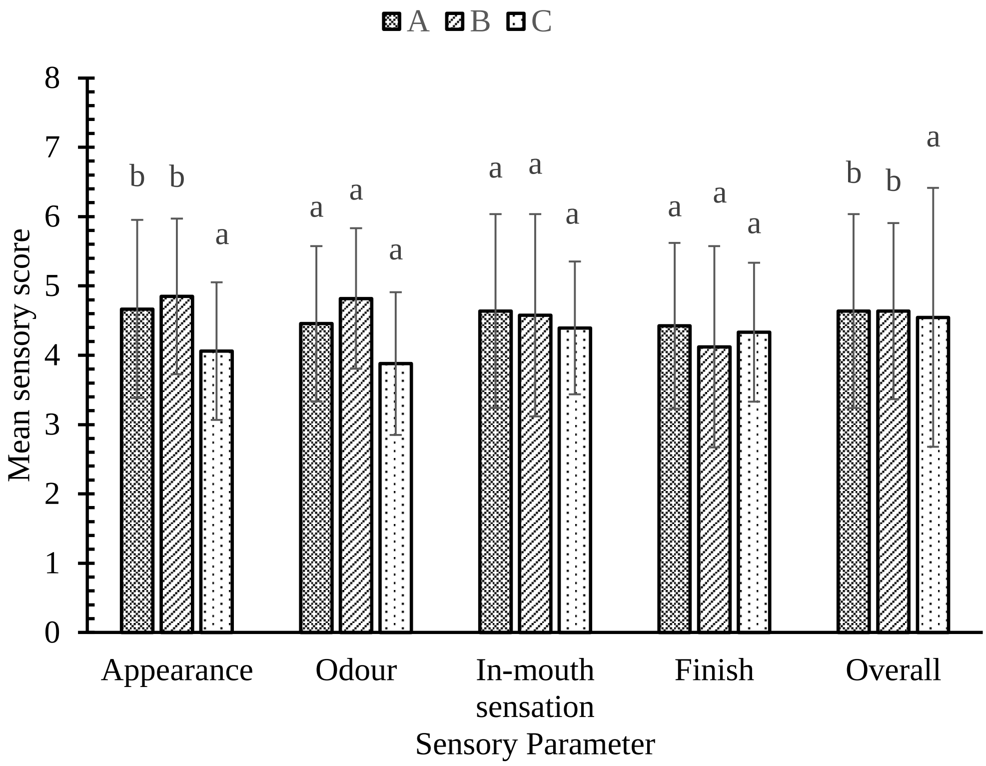



A = 100% juice content B = 50% juice content C = 25% juice content

**Fig. 4.1** Effect of juice content on sensory quality of wine

In the graph, "A" represents the group of samples containing hundred percent fruit juice, while "B" and "C" represents the samples containing fifty and twenty five percent fruit juices respectively. After the sensory analysis, samples containing hundred percent juice was found to be praised by the panelists. Sample having hundred percent juice was found to be superior to other samples having fifty and twenty five percent juice content, in almost all sensory attributes. While statistical analysis of data obtained from sensory pointed towards the superiority of sample completely made from juice only, it had no significant difference with sample containing fifty percent fruit juice in terms of in-mouth sensation.

### 4.3.2 Effect of pH of must on sensory quality of wine




A = pH 3.0 B = pH 3.5 C = pH 4

**Fig. 4.2** Effect of pH of must on sensory quality of wine

In the graph, "A" represents the group of samples containing pH 3.0, while "B" and "C" represents the samples containing pH 3.5 and 4.0 respectively. Statistical analysis of sensorial data showed that the pH ranging from 3 to 4 had no significant effects on the sensory attributes like odour, in mouth sensation and finish of the wine. Appearance of the wine was found to be a bit better in the samples having the pH 3 and 3.5 with no significant difference with each other than that of having pH 4. Also overall acceptability of wine was found to be better in case of samples with pH 3 and 3.5 with almost no differences than that of sample having pH 4.

### 4.3.3 Effect of juice content and pH of must on sensory quality of wine

Combine effect of juice content and pH on sensory parameters of wine were analyzed using 7 point hedonic rating test.

#### 4.3.3.1 Effect of juice content and pH of must on appearance of wine

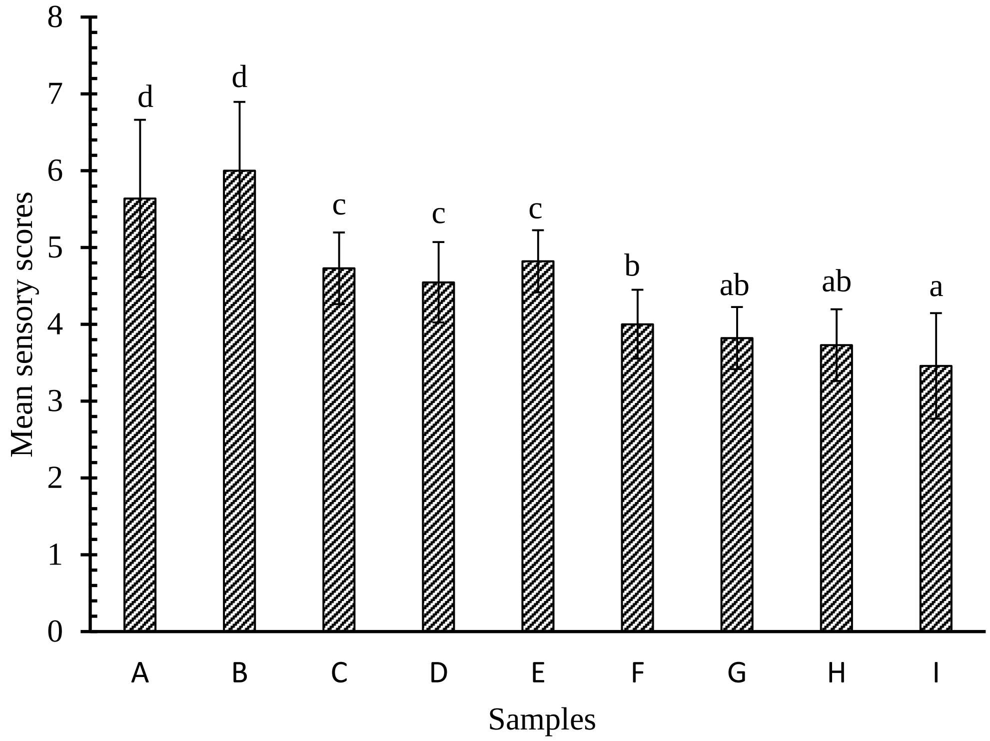
The mean sensory score ±standard deviation for appearance of nine samples A, B, C, D, E, F, G, H and I were found to be 5.63±0.53, 6.0±0.83, 4.72±0.30, 4.54±0.69, 4.81±0.47, 4.0±0.53, 3.81±0.70, 3.72±0.83 and 3.45±0.80 respectively. The statistical analysis showed that juice content and pH variation had significant effect on appearance of wine at 5% level of significance.

The maximum and minimum mean sensory score for appearance among nine samples were found to be 6.00 (sample B) which contained 100 % juice and of pH 3.5 and 3.45 (sample I) which contained 25 % juice and pH 4 respectively. Sample B was significantly different from other samples except sample A. The mean score for samples with highest juice content (100%) and average pH (3.5) was found to have higher values while that for samples with lower juice content (50%,and 25%) and with both lowest (3.0) and highest (4.0) pH values have lower sensory values. This indicates that panelists preferred yacon wine with highest juice conent and lower pH value. The best samples with 100% juice and 3.5 pH was of straw yellowish in appearance.

According to Jackson (2009) analysis of appearance includes clarity (absence of haze), color hue (shade or tint) and depth (intensity or amount of pigment), viscosity (resistance to flow) and effervescence (notably sparkling wines). According to Granato *et al.* (2011) the Yacon tuber contains carotenoids that confer its yellow color . It also contains chlorogenic acid, ferulic acid, and caffeic acid which make the tubers susceptible to enzymatic browning reactions caused by polyphenol oxidases (PPOs). PPOs catalyze the oxidation of *o*-phenolic substrates to *o*-quinones, which are subsequently polymerized to dark-colored pigments. The PPOs contained in yacon tubers lead to browning. To inhibit these reactions, PPOs are inactivated by the heat or by the use of reducing agents, such as sulfites and organic acids (ascorbic, malic, citric acids). According to Ioannou and Ghoul (2013) PPO is sensitive to pH variations while the optimum pH for PPO has been reported as ranging from acid to neutral, in most fruits and vegetables, optimum PPO activity is observed at pH 6.0 to 6.5, while little activity is detected below pH 4.5. It has also been reported that irreversible inactivation of PPO can be achieved below pH 3.0.

According to Jackson (2009) amount of pigment effects appearance of product. Juice content will not affect clarity, viscosity and effervescence of wine but juice content will effect on color intensity of wine higher percent juice will have higher amount of pigments hence color intensity of wine with 100 percentage juice content will have higher values which support above result.

Hence the appearance of wine with high juice content (100%) and low pH (3.5) gave the best sensory score which is in accordance to Jackson (2009), Granato *et al.* (2011) and Ioannou and Ghoul (2013).




A= 100% juice and pH 3 D = 50% juice and pH 3 G = 25% juice and pH 3

B= 100% juice and pH3.5 E = 50% juice and pH 3.5 H = 25% juice and pH 3.5

C = 100% juice and pH 4 F = 50% juice and pH 4 I = 25% juice and pH 4

**Fig. 4.3** Mean sensory scores for appearance of yacon wine samples

The wine samples were subjected for sensory evaluation and the results are shown in Fig. 4.3. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 11 panelists.

### 4.3.3.2 Effect of juice content and pH of must on odor of wine

The mean sensory score ±standard deviation for odor of nine samples A, B, C, D, E, F, G, H and I were found to be 4.81±0.60, 6.00±0.63, 4.81±0.40, 4.90±0.70, 4.27±0.46, 4.00±63, 3.63±0.50, 4.18±0.60 and 2.81±0.60 respectively. The statistical analysis showed that juice content and pH variation had significant effect on odor of wine at 5% level of significance. The maximum and minimum mean sensory score for appearance among nine samples were found to be 6.00 (sample B) which contained 100 % juice and of pH 3.5 and 3.45 (sample I) which contained 25 % juice and pH 4 respectively. The mean score for samples with highest juice content (100%) and average pH (3.5) were found to have higher values while that for samples with lower juice content (50%,and 25%) and both lowest (3.0) and highest (4.0) pH values have lower values. This indicates that panelists preferred yacon wine with highest juice content (100%) and average pH (3.5) value.

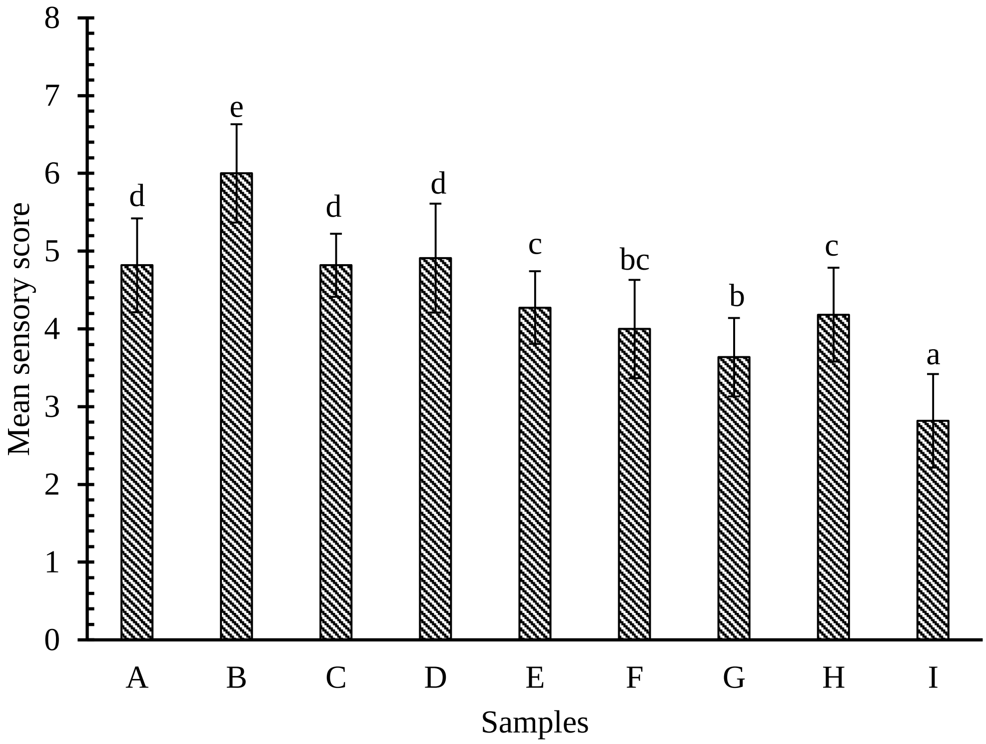



**Fig. 4.4** Mean sensory scores for odor of yacon wine

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 acidty is too high, the wine may smell vinegary, or of nail varnish remover which is undesirable. According to Jia (2016) optimum initial fermentation condition to have a pleasant smell for Marquette grape wine was of pH range from 3.4 to 3.6.

The wine samples were subjected for sensory evaluation and the results are shown in Fig. 4.4. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 11 panelists.

#### 4.3.3.3 Effect of juice content and pH of must on in mouth sensation of wine

The mean sensory score ±standard deviation for in mouth sensation of nine samples A, B, C, D, E, F, G, H and I were found to be 5.09±0.70, 5.81±0.75, 4.90±0.70, 5.36±0.50, 4.72±1.1, 4.45±1.03, 3.45±1.12, 3.18±1.07 and 3.81±1.07 respectively. The statistical analysis showed that juice content and pH variation had significant effect on in-mouth sensation of wine at 5% level of significance.

The maximum and minimum mean sensory score for in-mouth sensation among nine samples were found to be 5.81 (sample B) which contained 100 % juice and of pH 3.5 and 3.18 (sample H) which contained 25 % juice and pH 3.5 respectively. Sample B which was significantly different from C, E, F, G, H and I but not from A and D samples.

The mean score for samples with highest juice conent (100%) and average pH (3.5) were found to have higher values while that for samples with lower juice content (50%,and 25%) and both lowest (3.0) and highest (4.0) pH values have lower values. This indicates that panelists preferred yacon wine with highest juice content and average pH value. The wine sample which was proven to be best from the sensory analysis was found to have moderate sweetness, slight hint of sourness and acceptable amount of astringency.

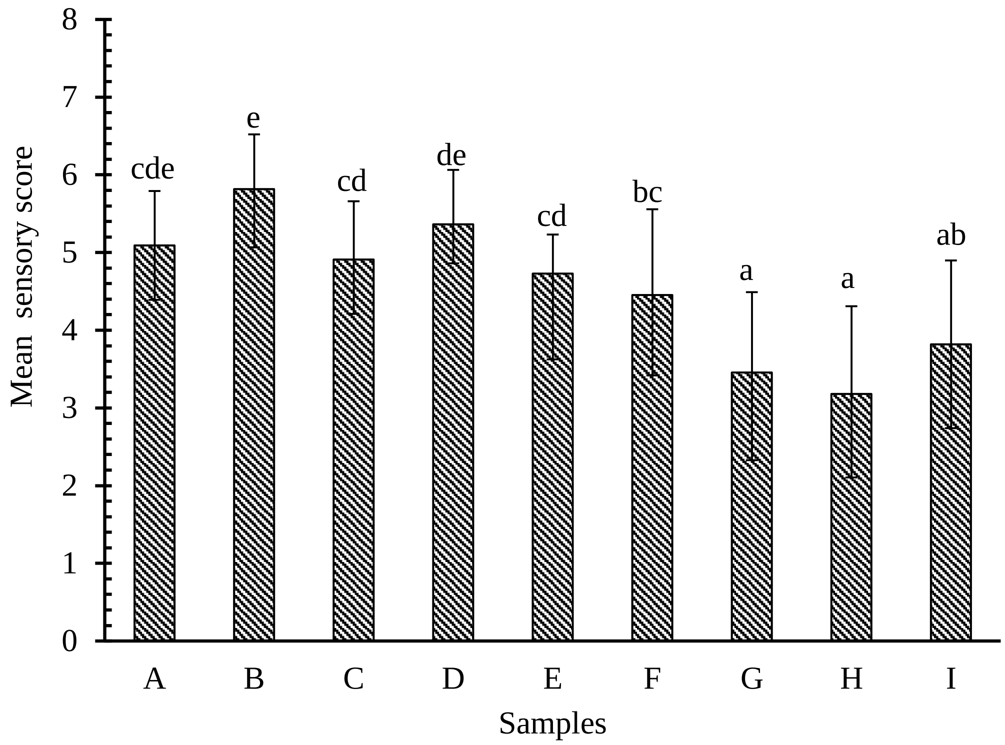



**Fig. 4.5** Mean sensory score for in-mouth sensation of yacon wine

According to Cheynier and Sarni-Manchado (2010) in mouth sensation includes taste and mouth-feel, odor and after smell .Major taste qualities in wine are sweetness, sourness and bitterness, contributed by sugars, organic acids and ethanol, respectively and are major sensory attributes of red wines while white wines are usually not astringent but can be perceived as sour or bitter. Sourness is due to organic acids and related to pH. Also astringency perception due to organic acids increases as the pH decreases but does not depend on the concentration or nature of the acid. Similar results were obtained by Baral (2011) in preparation of *junar* wine.

The wine samples were subjected for sensory evaluation and the results are shown in Fig. 4.5. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 11 panelists.

#### 4.3.3.4 Effect of juice content and pH of must on finish of wine

The mean sensory score ±standard deviation for finish of nine samples A, B, C, D, E, F, G, H and I were found to be 5.18±0.30, 5.54±0.93, 4.90±0.83, 4.72±0.64, 4.09±0.70, 4.27±0.78, 3.63±1.12, 2.72±1.0 and 3.81±0.98 respectively. The statistical analysis showed that juice content and pH variation had significant effect on finish of wine at 5% level of significance.

The maximum and minimum mean sensory score for finish among nine samples were found to be 5.54 (sample B) which contained 100 % juice and of pH 3.5 and 2.72 (sample H) which contained 25 % juice and pH 3.5 respectively. Sample B was significantly different from D, E, F, G, H and I but not from A and C samples.

The mean score for samples with highest juice content (100%) and average pH (3.5) was found to have higher values while that for samples with lower juice content (50%,and 25%) and both lowest (3.0) and highest (4.0) pH values have lower values. This indicates that panelists preferred yacon wine with highest juice content and average pH value. The wine sample which was proven to be best from the sensory analysis was found to have moderate lingering flavor in the mouth and pleasant aftertaste.

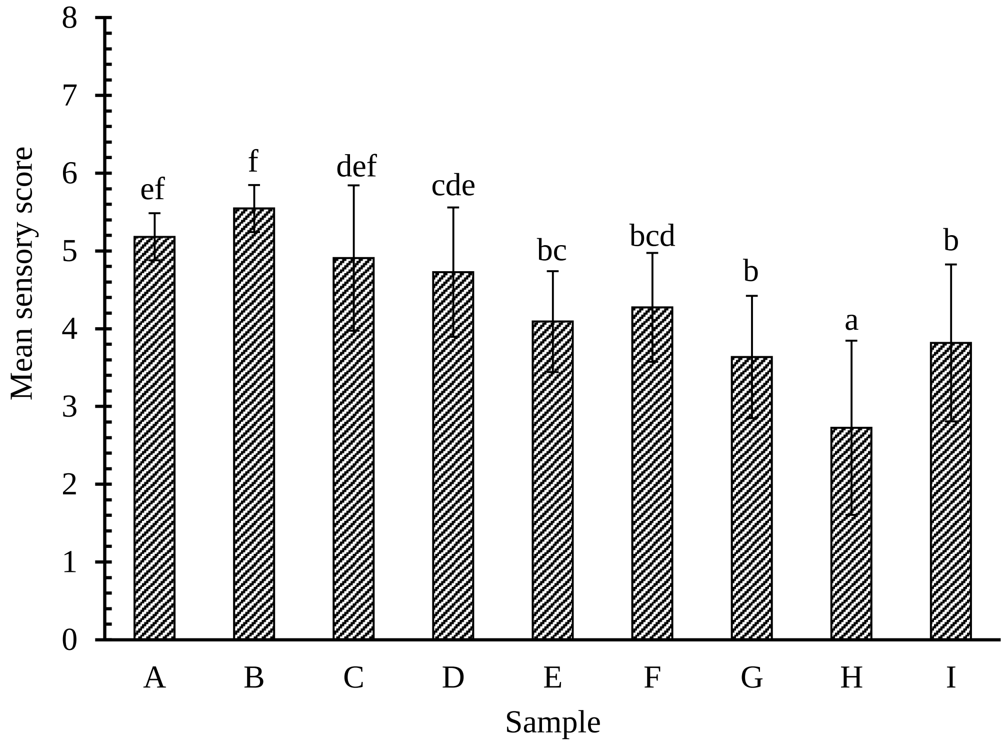



**Fig. 4.6** Mean sensory score for finish of yacon wine.

The wine samples were subjected for sensory evaluation and the results are shown in Fig. 4.6. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 11 panelists.

#### 4.3.3.5 Effect of juice content and pH of must on overall acceptance of wine

The mean sensory score ±standard deviation for overall acceptance of nine samples A, B, C, D, E, F, G, H and I were found to be 4.364±1.02, 6.27±0.89, 4.63±0.46, 4.27±0.52, 5.36±0.40, 3.90±0.44, 3.81±0.40, 3.81±0.46 and 3.45±0.68 respectively. The statistical analysis showed that juice content and pH variation had significant effect on overall acceptance of wine at 5% level of significance.

The maximum and minimum mean sensory score for overall acceptance among nine samples were found to be 6.27 (sample B) which contained 100 % juice and of pH 3.5 and 3.45 (sample I) which contained 25 % juice and pH 4 respectively. Sample B was significantly different from other samples. The mean score for samples with highest juice percentage (100%) and average pH (3.5) was found to have higher values while that for samples with lower juice percentage (50%, and 25%) and both lowest (3.0) and highest (4.0) pH values have lower values

According to Butzke (2010) the pH of wine is crucial to nearly every aspect of the wine. The pH could affect flavor, aroma, color, tartrate precipitation, carbon dioxide absorption, malolactic fermentation, stability and fermentation rate. Similar result was obtained by Sevda and Rodrigues (2011) in optimization of guava wine fermentation. And Baral (2011) in preparation of *junar* wine.

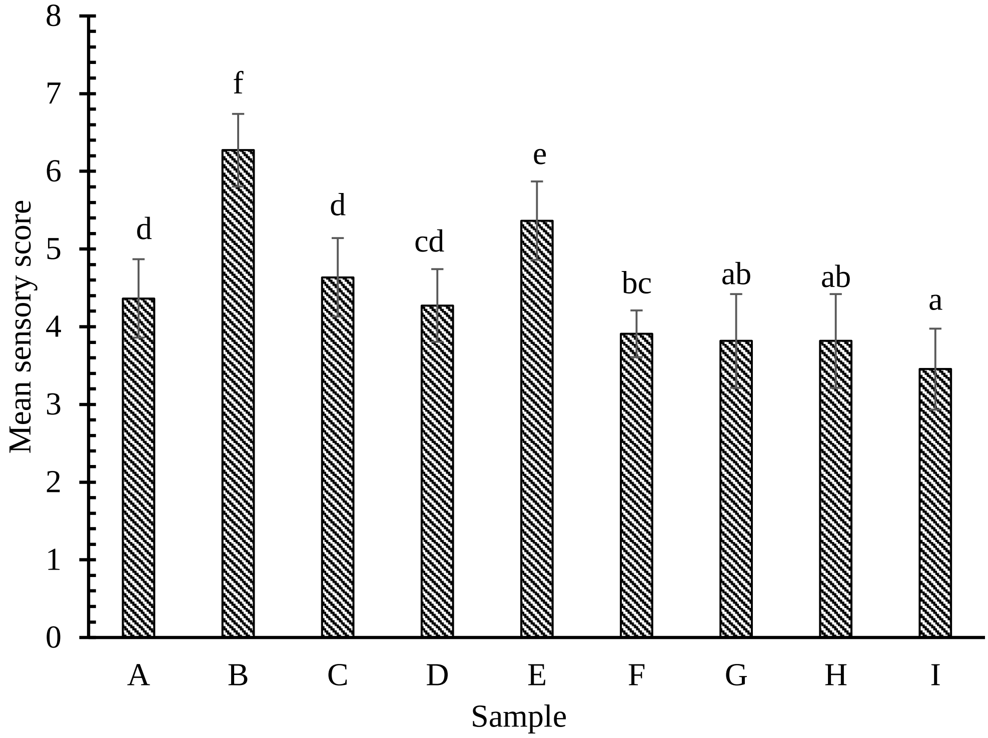



**Fig. 4.7** Mean sensory score for overall acceptance of yacon wine

The wine samples were subjected for sensory evaluation and the results are shown in Fig. 4.7. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 11 panelists.

## 4.4 Chemical composition of superior yacon wine.

Chemical composition of best yacon wine made from must containing 100% juice content, 3.5 pH and 25ºBx was analyzed and the results are shown in Table 4.3.

**Table 4.3** Chemical composition of the superior yacon wine

| S.N. | Parameter | Value (*) |
|---|---|---|
| 1 | TSS (ºBx) | 9.2 (0.25) |
| 2 | pH | 3.4 (0.051) |
| 3 | Acidity | |
| | a) Total acidity (% as lactic acid) | 0.376 (0.41) |
| | b) Fixed acidty (% as lactic acid) | 0.358 (0.025) |
| | c) Volatile acidity (% as acetic acid) | 0.012 (0.005) |
| 4 | Reducing sugar (% as dextrose) | 0.527 (0.029) |
| 5 | Specific gravity | 0.969 (0.003) |
| 6 | Alcohol (% v/v) | 12.50 (0.32) |
| 7 | Ester ( g/100 L abs. alcohol ) | 305.44 (10.40) |
| 8 | Total aldehyde ( g/100 L abs. alcohol ) | 264 (12.05) |
| 9 | Tannin (mg tannic acid/ L) | 17 (2.081) |

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

The TSS of wine was found to be (9.2ºBx), and the value was similar to those reported by Shrestha (2015) and Subedi (2015). pH of wine (3.4) found in this wine was low as compared to wine made from yacon by Shrestha (2015) and wine made from purple grapes by Raut (2014). The total acid of dry white wine and dry red wine were found to be 0.586 and 0.649 respectively as reported by Amerine *et al.* (1980).Total acidity of wine (0.376% as lactic acid) was found to be similar with Subedi (2015) But found to be low as compared to the wine produced by Raut (2014) and Baral (2011) Fixed acidity of wine (0.358 % as lactic acid) was found to be higher than wine made by Shrestha (2015). 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 for red wine (0.14%).

The reducing sugar, (0.527% ) was lower than that of sweet wine reported by Amerine *et al.* (1980). The alcohol content in the yacon wine (12.50 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 of wine, the aldehyde content should be within the range of (200-500 ppm) reported by Rai, (2009). The aldehyde content in the yacon wine was found to be (317 ppm.) The ester content in good quality wine should be within the range of (200-400 ppm) as reported by Rai, (2009). The ester content of the yacon wine was found to be (367 ppm). The tannin (0.017 g per 100 ml) was lower than that of dry white wine, (0.039 g per 100 ml) and dry red wine, (0.236 g per 100 ml) reported by Amerine *et al.* (1980).

## 4.5 Cost evaluation

The total cost associated with the best product was calculated and the cost per bottle (750 ml) of wine was NRs 121, excluding labor cost and tax. Mass production reduces this cost. The calculation is made in Appendix C.

# Part V

## Conclusions and recommendations

### 5.1 Conclusions

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

1. Yacon must containing 100% juice 25ºBx and 3.5 pH resulted best wine among all the treatment used.
2. Yacon wine made from different juice content and different pH values differs significantly (at 5% level of significance) with respect to sensory properties.
3. The physio-chemical properties of the best yacon were within the range of the reported commercial wines.
4. Yacon wine can be produced at a cost within the means of common people.
   Consequently, yacon wine 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. Yacon wine can be prepared with varying TSS, temperature and acid used.
2. Study on changes during ageing of yacon wine in terms of sensory and chemical properties can be carried out.
3. Study on the distillate of yacon wine can be carried out.
4. Study on the quality of yacon wine using different yeast can be carried out

# Part VI

## Summary

In this study, yacon was taken from Dhankuta, which is one of the district for commercial cultivation of yacon in Nepal. And other essential materials (citric acid, sugar and yeast) and other chemical and apparatus were obtained from local market of Dharan and campus laboratory. First, yacon was subjected to preliminary operation sorting, washing with plenty of water and scrubbing brush, disinfecting them by 200 ppm sodium hypochloride solution, peeling them with potato peeler and immediately submerged to 100 ppm KMS solution in order to protect from quick browning. After this yacon was subjected to screw press juice extractor for extraction of yacon juice which is susceptible to oxidation hence to preserve it calculated amount of antioxidant i.e. citric acid and preservative KMS was added. Physicochemical analysis of yacon juice showed 9 ºBx TSS, 5.85 pH and 0.0924 (% lactic acid) acidity. After that, fermentation was carried out in nine different mashes where juice content were varied to 100%, 50% and 25% and pH were varied to 3.0, 3.5, and 4.0 keeping TSS 25 ºBx without varying to other parameters for 24 days.

Nine different wines were subjected to sensory analysis and data obtained from this was analyzed by two-way ANOVA at 5% level of significance to study the difference among the all types. There was significant difference in case of appearance, odor, in-mouth sensation, finish and overall acceptability of all types. From the sensory evaluation, wine made from 100% juice and 3.5 pH obtained the highest score among other formulations. This sample is also comparable with sample having juice content 50% and pH 3.50 in terms of in mouth sensation, odor and overall acceptability. And sample having 100% juice content and pH 3.0 in terms of appearance and finish.

The cost of the best product was calculated and found to be Rs. 121 per bottle (750 ml) of wine made from 100% juice content and 3.5 pH contains 12.50 % (v/v) alcohol content, 9.2 ºBx TSS, 3.40 pH, 0.376% (as % lactic acid) total acidity, 0.358% (as lactic acid) fixed acidty, 0.012% (as acetic acid) volatile acidity, 367 ppm ester, 317 ppm aldehyde. Alcohol content, volatile acidity and other parameters of wine made from this research is within the range of a good quality wine.

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# Appendices

## Appendix A

**Specimen card of sensory evaluation by 7 point hedonic rating test**

**Sensory evaluation of *Yacon* wine**

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

Name of Product: Yacon wine

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 |
| Appearance | | | | | | | | | |
| Odor | | | | | | | | | |
| In mouth 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 yacon wine

**Table B. 1** Mean sensory scores for different attributes

| Sample code | Quality attributes | | | | |
|---|---|---|---|---|---|
| | Appearance | Odor | In mouth sensation | Finish | Overall acceptance |
| A | $5.636^{d}$ | $4.818^{d}$ | $5.091^{cde}$ | $5.182^{ef}$ | $5.364^{e}$ |
| B | $6^{d}$ | $6^{e}$ | $5.818^{e}$ | $5.545^{f}$ | $6.273^{f}$ |
| C | $4.727^{c}$ | $4.818^{d}$ | $4.909^{cd}$ | $4.909^{def}$ | $4.364^{d}$ |
| D | $4.545^{c}$ | $4.909^{d}$ | $5.364^{de}$ | $4.727^{cde}$ | $4.273^{cd}$ |
| E | $4.818^{c}$ | $4.273^{c}$ | $4.727^{cd}$ | $4.091^{bc}$ | $4.636^{d}$ |
| F | $4^{b}$ | $4^{c}$ | $4.455^{bc}$ | $4.273^{bcd}$ | $3.909^{bc}$ |
| G | $3.818^{ab}$ | $3.636^{b}$ | $3.455^{a}$ | $3.636^{b}$ | $3.818^{ab}$ |
| H | $3.727^{ab}$ | $4.182^{c}$ | $3.182^{a}$ | $2.727^{a}$ | $3.818^{ab}$ |
| I | $3.455^{a}$ | $2.818^{a}$ | $3.818^{ab}$ | $3.818^{b}$ | $3.455^{a}$ |
| LSD | 0.5316 | 0.4826 | 0.7646 | 0.7094 | 0.4258 |

The values are the mean of 11 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 | 8 | 67.0505 | 8.3813 | 21.36 | <.001 |
| Panelist | 10 | 4.2424 | 0.4242 | 1.08 | 0.387 |
| Residual | 80 | 31.3939 | 0.3924 | | |
| Total | 98 | 102.6869 | | | |

Since, F pr <0.05, there is significantly different between the sample so LSD testing is necessary. LSD at 0.05 = 0.5316

**Table B. 3** LSD for appearance

| Sample | Mean score | Mean difference | | | |
|---|---|---|---|---|---|
| A | 5.636 | A-B< LSD | B-D>LSD* | C-G>LSD* | E-G>LSD* |
| B | 6.0 | A-C>LSD* | B-E>LSD* | C-H>LSD* | E-H>LSD* |
| C | 4.727 | A-D>LSD* | B-F>LSD* | C-I>LSD* | E-I>LSD* |
| D | 4.545 | A-E>LSD* | B-G>LSD* | D-E<LSD | F-G<LSD |
| E | 4.818 | A-F>LSD* | B-H>LSD* | D-F>LSD* | F-H<LSD |
| F | 4.0 | A-G>LSD* | B-I>LSD* | D-G>LSD* | F-I>LSD* |
| G | 3.818 | A-H>LSD* | C-D<LSD | D-H>LSD* | G-H<LSD |
| H | 3.727 | A-I>LSD* | C-E<LSD | D-I>LSD* | G-I<LSD |
| I | 3.455 | B-C>LSD* | C-F>LSD* | E-F>LSD* | H-I<LSD |

*= Significantly different

**Table B.4** LSD for odor.

| Source of variation | Degree of freedom | Sum of squares | Mean squares | Variance ratio | Variance ratio |
|---|---|---|---|---|---|
| Sample | 8 | 71.2323 | 8.9040 | 27.53 | <.001 |
| Panelist | 10 | 4.3030 | 0.4303 | 1.33 | 0.229 |
| Residual | 80 | 25.8788 | 0.3235 | | |
| Total | 98 | 101.4141 | | | |

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 0.4826.

**Table B.5** LSD for odor.

| Sample | Mean score | Mean difference | | | |
|---|---|---|---|---|---|
| A | 4.818 | A-B> LSD | B-D>LSD* | C-G>LSD* | E-G>LSD* |
| B | 6.0 | A-C<LSD | B-E>LSD* | C-H>LSD* | E-H>LSD* |
| C | 4.818 | A-D>LSD* | B-F>LSD* | C-I>LSD* | E-I>LSD* |
| D | 4.273 | A-E<LSD | B-G>LSD* | D-E>LSD* | F-G<LSD |
| E | 4.909 | A-F>LSD* | B-H>LSD* | D-F<LSD | F-H<LSD |
| F | 4.0 | A-G>LSD* | B-I>LSD* | D-G>LSD* | F-I>LSD* |
| G | 3.636 | A-H>LSD* | C-D>LSD* | D-H<LSD | G-H>LSD* |
| H | 4.182 | A-I>LSD* | C-E<LSD | D-I>LSD* | G-I>LSD* |
| I | 2.818 | B-C>LSD* | C-F>LSD* | E-F>LSD* | H-I>LSD* |

*= significantly different

**Table B.6** 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 | 8 | 69.7172 | 8.7146 | 10.73 | <.001 |
| Panelist | 10 | 11.9596 | 1.1960 | 1.47 | 0.165 |
| Residual | 80 | 64.9495 | 0.8119 | | |
| Total | 98 | 101.4141 | | | |

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 0.7646.

**Table B.7** LSD for in-mouth sensation.

| Sample | Mean score | Mean difference | | | |
|---|---|---|---|---|---|
| A | 5.091 | A-B< LSD | B-D<LSD | C-G>LSD* | E-G>LSD* |
| B | 5.818 | A-C<LSD | B-E>LSD* | C-H>LSD* | E-H>LSD* |
| C | 4.909 | A-D<LSD | B-F>LSD* | C-I>LSD* | E-I>LSD* |
| D | 5.364 | A-E<LSD | B-G>LSD* | D-E<LSD | F-G>LSD* |
| E | 4.727 | A-F<LSD | B-H>LSD* | D-F>LSD* | F-H>LSD* |
| F | 4.455 | A-G>LSD* | B-I>LSD* | D-G>LSD* | F-I<LSD |
| G | 3.455 | A-H>LSD* | C-D<LSD | D-H>LSD* | G-H<LSD |
| H | 3.182 | A-I>LSD* | C-E<LSD | D-I>LSD* | G-I<LSD |
| I | 3.818 | B-C>LSD* | C-F<LSD | E-F<LSD | H-I<LSD |

*= significantly different

**Table B.8** Two way ANOVA (no blocking) for finish.

| Source of variation | Degree of freedom | Sum of squares | Mean squares | Variance ratio | Variance ratio |
|---|---|---|---|---|---|
| Sample | 8 | 99.7475 | 8.3434 | 11.94 | <.001 |
| Panelist | 10 | 12.9899 | 1.2990 | 1.86 | 0.064 |
| Residual | 80 | 55.9192 | 0.6990 | | |
| Total | 98 | 101.4141 | | | |

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 0.7094.

**Table B.9** LSD for finish.

| Sample | Mean score | Mean difference | | | |
|---|---|---|---|---|---|
| A | 5.182 | A-B< LSD | B-D>LSD* | C-G>LSD* | E-G<LSD |
| B | 5.545 | A-C<LSD | B-E>LSD* | C-H>LSD* | E-H>LSD* |
| C | 4.909 | A-D<LSD | B-F>LSD* | C-I>LSD* | E-I<LSD |
| D | 4.727 | A-E>LSD* | B-G>LSD* | D-E<LSD | F-G<LSD |
| E | 4.091 | A-F>LSD* | B-H>LSD* | D-F<LSD | F-H>LSD* |
| F | 4.273 | A-G>LSD* | B-I>LSD* | D-G>LSD* | F-I<LSD |
| G | 3.636 | A-H>LSD* | C-D<LSD | D-H>LSD* | G-H>LSD* |
| H | 2.727 | A-I>LSD* | C-E>LSD* | D-I>LSD* | G-I<LSD |
| I | 3.818 | B-C<LSD | C-F<LSD | E-F<LSD | H-I>LSD* |

***=** significantly different

**Table B.10** Two way ANOVA (no blocking) for overall acceptance

| Source of variation | Degree of freedom | Sum of squares | Mean squares | Variance ratio | Variance ratio |
|---|---|---|---|---|---|
| Sample | 8 | 69.4141 | 8.6768 | 34.46 | <.001 |
| Panelist | 10 | 2.7677 | 0.2768 | 1.10 | 0.373 |
| Residual | 80 | 20.1414 | 0.2518 | | |
| Total | 98 | 92.3232 | | | |

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 0.4258.

**Table B. 11** LSD for overall acceptance

| Sample | Mean score | Mean difference | | | |
|---|---|---|---|---|---|
| A | 4.636 | A-B>LSD* | B-D>LSD* | C-G>LSD* | E-G>LSD* |
| B | 6.273 | A-C<LSD | B-E>LSD* | C-H>LSD* | E-H>LSD* |
| C | 4.364 | A-D<LSD | B-F>LSD* | C-I>LSD* | E-I>LSD* |
| D | 4.273 | A-E>LSD* | B-G>LSD* | D-E>LSD* | F-G<LSD |
| E | 5.364 | A-F>LSD* | B-H>LSD* | D-F<LSD | F-H<LSD |
| F | 3.909 | A-G>LSD* | B-I>LSD* | D-G>LSD* | F-I> LSD* |
| G | 3.818 | A-H>LSD* | C-D<LSD | D-H>LSD* | G-H<LSD |
| H | 3.818 | A-I>LSD* | C-E>LSD* | D-I>LSD* | G-I<LSD |
| I | 3.455 | B-C>LSD* | C-F>LSD* | E-F>LSD* | H-I<LSD |

*= significantly different

# Appendix C

## Cost evaluation for yacon wine

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

| Particulars | Quantity | Rate (Rs) | Total (Rs) |
|---|---|---|---|
| Yacon | 1.5 kg | 50/kg | 75 |
| Sugar | 140 g | 90/kg | 13 |
| Wine bottle | 1 pcs | 20/piece | 20 |
| Wine yeast | 1 g | 2000/kg | 2 |
| Total cost | | | 110 |
| Final cost with 10% overhead | | | 121 |

The price of 750 ml wine cost Rs. 121. Thus, the price of 1 liter wine is Rs. 161.

## Appendix D

**Table D.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 E

**Table E.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)

## Photo gallery

**Plate 1** Yacon plant

**Plate 2** Yacon edible part (tuber))

**Plate 3** Different sample of wine for sensory analysis

**Plate 4** Panelist performing sensory analysis