PREPARATION AND QUALITY EVALUATION OF YACON (*Smallanthus Sonchifolius*) - BEETROOT (*Beta Vulgaris)* WINE

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Preparation and Quality Evaluation of Yacon (S*mallanthus sonchifolius*) - Beetroot (*Beta vulgaris*) Wine

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Approval Letter

This *dissertation* entitled *Preparation and Quality Evaluation of Yacon (Smallanthus sonchifolius) - Beetroot (Beta vulgaris) Wine* presented by Kumar Prasad Subedi has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology.

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Abstract

Yacon (*Smallanthus sonchifolius*) and beetroot (*Beta vulgaris*) was mixed in the ratio 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and the wine was prepared using wine yeast (*S. cerevisiae,* SC 22) at the rate 0.3gm/L of the must. Yacon wine without the addition of beetroot juice in the must was taken as a control sample. The pH and Total Soluble Solid of the must were maintained at 3.8 and 25ºBx respectively and fermentation was carried out for 24 days at 25° ± 2°C. The prepared wine was analyzed for pH, TSS, alcohol content, acidities, total aldehyde, total esters, methanol content, tannin, total sugar, reducing sugar, and antioxidant properties. Sensory attributes (color, flavor, clarity, mouthfeel, and overall acceptability) of the wines were evaluated using nine-point hedonic scale rating test. The assessed data were analyzed by ANOVA using Genstat Discovery$_{®}$ Edition 12, 2014 at 5% significance level.

The optimum concentration of yacon and beetroot juices on must evaluated by sensory evaluation of prepared wine was found to be superior in 80% yacon juice and 20% beetroot juice. Sensory analysis showed that there was significant difference among all the products with respect to color, clarity, flavor, mouth feel and overall acceptability of product. Variation in juice content of beetroot and yacon of must significantly (p<0.05) affected the wine quality. From sensory evaluation wine prepared from 20% beetroot juice and 80% yacon juice was found to be superior and contained 8.6 % (v/v) alcohol content, 7.8 ºBx TSS, 4.02 pH, 0.47% acidity (as % citric acid), 0.031% volatile acidity (as acetic acid) , 280.56 ppm ester, 270.37 ppm aldehyde and 86 % DPPH inhibition. The cost of superior product wine prepared in this study is within the means of common people (NRs.137 per 750 ml) hence this wine has great potential for commercialization.

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List of abbreviations

Abbreviation	Full form
ADY	Active dry yeast
ANOVA	Analysis of variance
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 (*vitisvenifera* 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 branchingsystem 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 kg of roots (Hermann and Heller,1997). Yacon is currently cultivated in Argentina, Bolivia, 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 fructo-oligosaccharides (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. Fructo-oligosaccharides (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.

The beetroot is the taproot portion of the beet plant, usually known in North America as the beet, also table beet, garden beet, red beet, or golden beet. It is one of several of the cultivated varieties of *Beta vulgaris* grown for their edible taproots and their leaves (called beet greens) (Boswell, 1967). These varieties have been classified as *B.vulgaris* subsp. *vulgaris* Conditiva group. Beetroot is a good tonic food for health. *Betavulgaris var. rubra* revealed significant tumor inhibitory effects in skin and lung cancer (Kapadia *et al.*, 1996). These fin dings suggest that beetroot ingestion can be a useful means to prevent development and progression of cancer. But extracts of beetroot also showed some antimicrobial activity on *Staphylococcus aureus* and on *Escherichia coli* and also antiviral effect was observed (Rauha*et al.*, 2000). Garden beet juice is a popular health food betanins, obtained from the roots, are used industrially as red food colorants e.g. to improve the color of tomato paste, sauces, desserts, jams and jellies, ice cream, sweets and cereals. Red beet also makes a rich, red, Burgundy style wine (Hughes and Mitchell, 2006).

1.2 Statement of the problem

Yacon is an under-utilized fruit crop but it has great potential to become a profitable product in small scale farming using organic cultivation. In Nepal, yacon is being commercially produced in various parts of the country but still they aren’t being efficiently utilized in the market. Yacon itself is an abundant source of fructo-oligosaccharide (FOS). FOS is a type of sugar that has a lower calorie value than other sugar types and its intake favors the growth of health-promoting bacteria while reducing pathogenic bacteria populations. So far, research work on the production of yacon wine has been carried out by using yacon juice only and works regarding the possibility of preparing wines from yacon pulp and yacon syrup are scarce. Moreover, studies related to the preparation and quality analysis of herbal wines in

Nepalese context has not been carried out so far. Information on the antioxidant activity of these wines is also scanty. With this view, the present study was undertaken to investigate the possibility of preparing beetroot-based yacon wine. 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. The work mainly focuses on optimization of must composition in terms of juice recipe in must. Grape is expensive and habit of consuming wine is increasing. Beetroot has positive medicinal aspects and catchy color. So, new product modification and commercialization of low-cost red wine can be done. Hence, the study can provide information to the cultivars to produce yacon-beetroot 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 completely new product with superior quality in terms of color, 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 grape-beetroot 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.

1.3 Objectives

1.3.1 General objective

The general objective of this dissertation was to prepare and evaluate yacon-beetroot wine using different proportion of yacon and beetroot juice.

1.3.2 Specific Objective

The specific objectives of the study are as follows:

- To prepare wines using different ratio of yacon juice and beetroot juice (100:0, 95:5, 90:10, 85:15, 80:20, 75;25)
- To select the best wine based on sensory evaluation.
- To carry out physicochemical analysis of best yacon-beetroot wine.

1.4 Significance of the study

This study can be a useful to find out the possible utilization of yacon and beetroot by using it as a substitute source of juice needed for wine making so that spoilage resulting from its underutilization can be minimized. The work mainly focuses on optimization of must composition in terms of juice recipe in must. Grape is expensive and habit of consuming wine is increasing. Yacon is cheaper than grapes and beetroot has positive medicinal aspects and catchy color. So, new product modification and commercialization of low-cost red wine can be done. Hence, the study can provide information to the cultivars to produce yacon-beetroot 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 completely new product with superior quality in terms of color, 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-beetroot 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. Only fresh wines were used for physicochemical and sensory analysis.

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. mainly 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 274mhl. Italy occupies top position by producing 50mhl 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 andYang, 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.

- **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.

- **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.

- **Nettlange**

  Manufactured by *Sakaro* Beverages, *Nettlange* is one of the popular Nepali wines in the local market made from nettles (*Sishno*) and oranges.

- **Grapple**

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

- **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	Contains the red coloring matter of skin, pulp and seeds	Burgundy
	White wine	Does 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	Contains upto 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 wine
	Fortified	Contains 17-21 % abv	Sherry
Effervescence	Still	Does not contain CO_2	Chianti
	Sparkling	Contains CO_2 (natural or added)	Champagne
Wine Advisory Board, USA	Dessert wine	Contains sugar, taken after meal	Sherry (Sweet)
	Appetizer wine	Dry, fortified. Taken before meal	Sherry (dry)
	Sparkling wine	Contains 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 types 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 malo-lactic 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 makers keep 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 is 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 activities 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 yeasts does 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 *Saccaromyce scerevisiae* var, e*llipsoideus* (synonyms: *Sacch. cerevisiae, Sacch. ellipsoideus, Sacch, vini*). Other yeasts which have been used for special wines are *Sacch. fermentati*, *Sacch pyriformis* 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 allows 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:

- 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.
- 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.

- Steady, persistent fermentation capacity; this leads to wines of better quality than when the fermentation falls away after a tempestuous start.
- Absence of unpleasant flavors generated by dead and dying cells (Austin, 1968).
- Growth at the relatively high acidity i.e., low pH of grape juice or must for fermentation.
- Osmotolerance i.e. yeast should able to tolerant high osmotic pressure created by high concentration of sugar on must composition.
- 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 kinds of alcohol, but when the term is used loosely by winemakers, it 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 juiceshave 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.

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 no other sugars like arabinose, rhamnoseand 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 toethanol, 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 below.

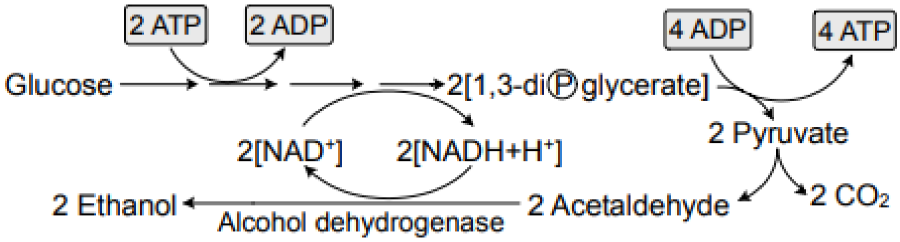


Fig. 2.1 Simplified pathway of alcohol synthesis by yeast

2.7.3 Malo-lactic fermentation

Malo- lactic 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 mainlyperformed 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 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.

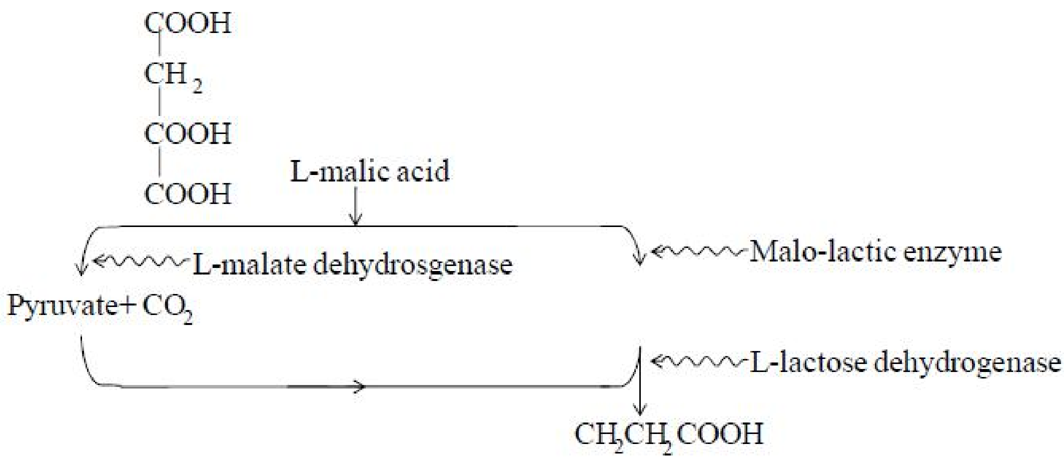


Fig. 2.2 The malolactic pathway

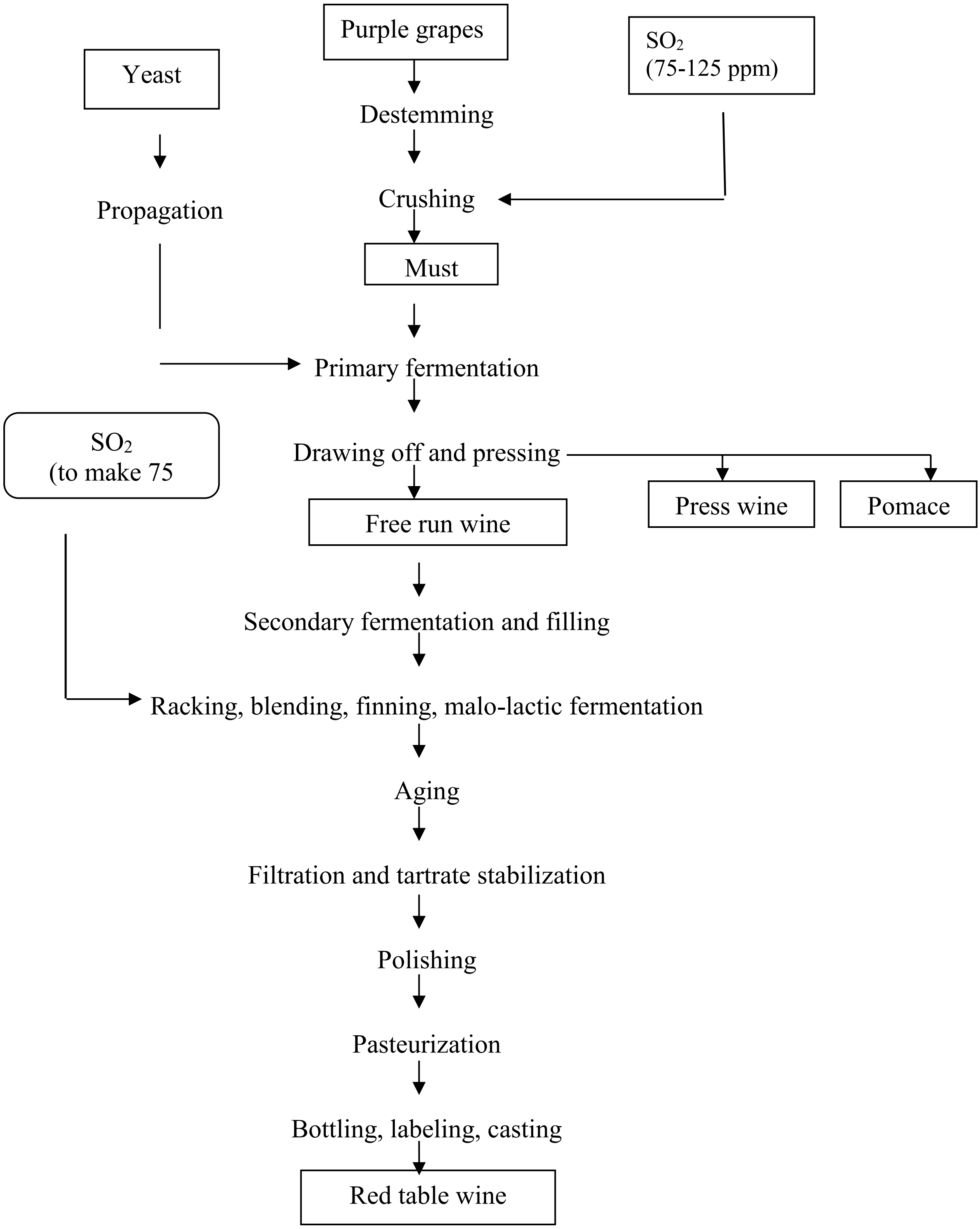
2.8 General method of wine preparation

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 reparation is given in Fig. 2.3.

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



Source: Rai (2009)

Fig. 2.3 Flow chart of red table wine preparation

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 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:

- **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.
- **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.

- **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 their mix called acid blend is the usual method employed.
- **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 wine making 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_2being 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 meta-bisulfite (KMS). In general, SO_2 is seldom used at a rate above 150 ppm. Moldy grapes may need 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 is 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.

2.8.4.2 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 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:

- It helps removing CO_2.
- It raises O/R potential, which retards the formation of H_2S.
- 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 polypyridines). 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 winemaking, for example must or lees filtration. However, one of its main uses is in the preparation for bottling. The processes of fining and filtration are not interchangeable. There are three principal categories of filtration, which may be used at different stages in the winemaking process.

- **Earth filtration**: This filtration method is used for initial rough filtration and can remove large quantities of "gummy" solids, which consist of dead yeast cells and other matter from the grapes. The filtration takes place in two stages. Firstly, a coarse grade earth called kieselguhr, which is commonly used as the filter medium, is deposited on a supporting screen within a filter tank. A mixture of water and kieselguhr may be used to develop the filter bed. This is known as precoating. Secondly, more earth is mixed with wine to form 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).

- **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 fibers of the filter media (Grainger and Tattersall, 2005).

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 bouquetto 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 depress 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 55-70 ºC 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 "remuage". The temperature of the whole bottle is then reduced to about -1.1ºC to 4.4ºC. The neck of the bottle containing the yeast deposit is then frozen (by placing in brine or other freezing solution) When the cork is removed the solid plug containing the yeast is ejected. This is called disgorging (Pederson, 1980).

2.8.13 Storage of wine

Storage of wine is an important consideration for wine that is being kept for long-term ageing. There are some factors that have the most direct impact on a wine's condition are temperature, light and humidity. The perfect storage temperature for wine, is supposed to be 52ºF (11ºC), anything between 40°F and 65ºF (5ºC and 18ºC) will in fact suffice for most styles of wines. All wines are affected negatively by the ultraviolet end of the light spectrum, hence, in the cellar; wines are stored in corrugated boxes or wooden crates to protect the wines from direct light. A certain humidity (between 60 and 70 percent) is essential to keep the cork moist and flexible, thereby avoiding oxidation. The position in which a wine bottle is stored is also extremely important. Most wines should be laid horizontal position so that the wine keeps the cork moistened, and therefore fully swollen and airtight. Exceptions to this rule are sparkling wines and any wine that has been sealed with a screw top lid Wines should also be stored under vibration-free conditions, but this only becomes a significant factor over a long period for sparkling wines and mature wines with sediment (Stevenson, 2005).

2.8.14 Yield

The theoretical conversion of 180 g of sugar into 88 g of carbon dioxide and 92 g of ethanol means that yield of ethanol is 51.1% on a weight basis. This percentage may vary depending upon inoculums 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 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.

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).

Table 2.4 Components of wine.

Parameters	Components
Soluble solids	sugar extract glucose and fructose
Acidity	total volatile pH individual acids
Alcohols	ethanol methanol fuel oils glycerol
Carbonyl 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)

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 andAkiyoshi (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).

2.9.2 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 usesof 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).

2.9.2.2 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

- **Appearance:** Firstly, view each sample 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).

- **Odor:** Firstly, sniff each at mouth of glass before swirling and then, study and record thenature 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.
- **In-mouth sensations:** Take a small (6 to 10 ml) sample into mouth. Move wine intomouth 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 change in perception and intensity. Then, concentrate on the tactile (mouth feel) sensation of astringency, prickling, body temperature and heat. Record this perception and how they combine with each other.
- **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.
- **Overall quality:** After the sensory aspect have been studied individually, attention shiftto the integration of their effects the wine's overall quality and finally, make and overall assessment of the complexity, subtlety, elegance, power and balance of wine (Jackson,2002).

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, have been identified in them, with a wide concentration range varying between hundreds of mg/L to the µg/L or ng/L levels, and their combinations form the character of wine and differentiates one wine from another (Jiang and Zhang, 2010). How many, and what types of volatile compounds are present depends on many factors such as the vineyard's geographical site, which is related to soil and climate characteristics, grape variety, yeast strain, and technical conditions during wine making (Usansa, 2003).

2.11.1 Alcohol

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

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

Alcohols with more than two carbon atoms are commonly called higher or fusel alcohols. They commonly account for about 50% of the aromatic constituents of wine, excluding ethanol. The principal higher alcohols produced by yeast are the aliphatic alcohols n-propanol, isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-mthyl-1-butanol), and the aromatic alcohols hexanol and 2-phenethyl alcohol. The higher alcohols content in wine should be 80-540 mg/L the concentration of higher alcohols below 300 mg/L strengthens the desirable aroma of wine, whereas these components are seen as a negative factor in creating the aroma when their level exceeds 400 mg/L (Usansa, 2003).

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

2.11.2 Ester

There are a number of esters which contribute to the flavor of wines. Ester plays an important role in the formation of wine's sensory characteristics. They are formed from acids and alcohols during wine fermentation and fermentation process. There are a lot of different alcohols and acids in wines, so the number of possible esters 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 viticulture 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), B_2 (riboflavin), and B_{12} (cobalamine). 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 Aluminum. 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 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). So yacon can also be used to prepare wine as raw material.

2.13.1 Yacon (Smallanthus sonchifolius)

2.13.1.1 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 fructo oligosaccharide. It has gradually received more attention due to its abundant content of fructo oligosaccharides (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 andneurosporene(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 anti-cholesteremical 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).

2.13.1.2 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 (Shrestha, 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.

2.13.1.3 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 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.

2.13.1.4 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 withstrong 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).

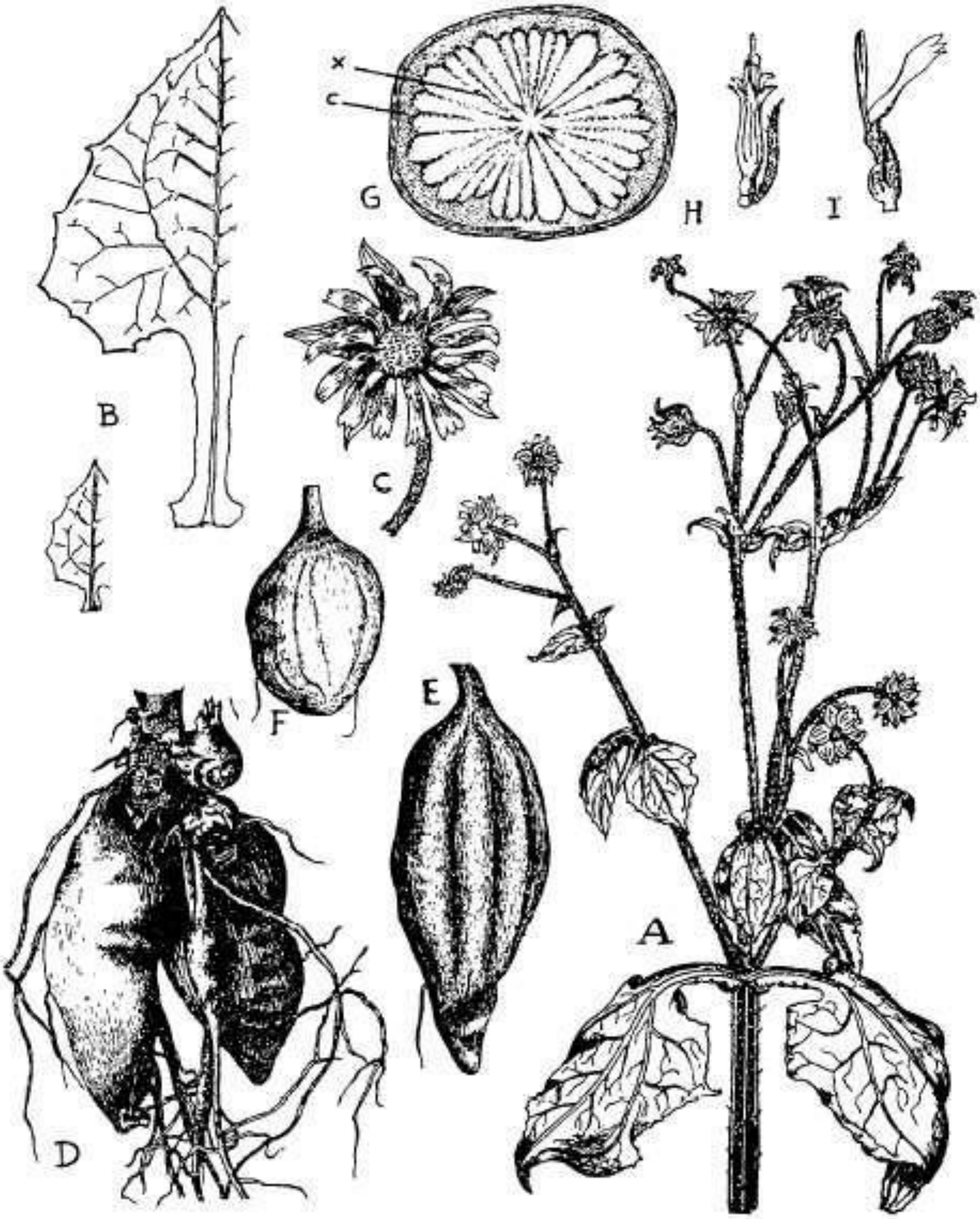


Fig. 2.4 Yacon *(Smallanthus sonchifolius)* morphological aspects.

(A: floweringbranches. 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)	Asteroids
Order	Asterales
Family	Asteraceae
Genus	*Smallanthus*
Species	*S. sonchifolius*

Source: Valentova and Ulrichova (2003)

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 bio-products (Viehmannova *et al.*, 2007).

2.13.1.5 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 fructo-oligosaccahrides. 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.

2.13.1.6 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 and10 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 β (2→1) bond. B (2→1)fructans of the inulin type are thus dietary fibre or the indigestible residues of plant origin in human diet.

Table 2.6 Chemical composition of yacon tuber, stem and leaf

Components	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 (mg/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)

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.

2.13.1.7 Other important chemical components

Table 2.7 Contents of saccharides in yacon tuberous root

Saccharides	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)

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 gallic 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).

2.13.1.8 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.

2.13.1.8.1 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.

2.13.1.8.2 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.

2.13.1.8.3 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 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

2.13.1.9 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 (Shrestha, 2015).

2.13.1.10 Use of yacon and its potential in Nepal

Yacon 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, decolorization 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 began 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).

2.13.2 Beetroot

2.13.2.1 History

Beets are native to the Mediterranean. Although the leaves have been eaten since before written history, the beetroot was generally used medicinally and did not become a popular food until French recognized their potential in the 1800's. Beet powder is used as a coloring agent for many foods. Some frozen pizzas use beet powder to color the tomato sauce. The most common garden beet is a deep ruby red in color, but yellow, white, and even candy-striped are available in specialty markets. Outside the United States, beets are generally referred to as beetroot. It is estimated that about two-thirds of commercial beet crops end up canned. They state the earliest written mention of the beet comes from 8th century Mesopotamia (Maria and Daniel, 2000). The Greek Peripatetic Theophrastus later describes the beet as similar to the radish, while Aristotle also mentions the plant (Hill and Langer, 1991). Zohary and Hopf also argue that it is very probable that beetroot cultivars were also grown at the time, and some Roman recipes support this. Later English and German sources show that beetroots were commonly cultivated in Medieval Europe.

2.13.2.2 Origin of beetroot

The ancient Babylonians were the first to use it for various applications. Early Greeks and Romans used the root for its medicinal properties and the leaves as vegetables. Moving ahead with time, beetroot held an important place in medicine. In England, beetroot juice or broth was recommended as an easily digested food for the aged, weak, or infirm. Even in mythology, Aphrodite is said to have eaten beets to retain her beauty. In folk magic, if a woman and man eat from the same beet, they will fall in love. In Africa, beets are used as an antidote to cyanide poisoning (Kumar, 2015).

2.13.2.3 Cultivation and Collection

Beet or Beetroot or table Beet is biennial plant that is cultivated for its thick flashy roots in early spring. Beet seeds are planted one half inch deep at three to five- inch intervals and the rows are eight inches apart. Seed germination takes from five to ten days. Roots are formed during the first season. Leafy stem having tall branch and clusters of minute green flowers arises in the second season. Stem will not produce the flowers until their roots mature and live one month at cold temperature. The flowers develop into brown corky fruits, commonly called seed balls. According to the Federal Germination Standard in commercially grown seeds there are approximately 2,800 seeds per ounce and germination rate is 75%. Beets are harvested during the summer, when the leaves are dehydrated. They can be harvested when the roots are 2.5- 3 inches in diameter. Beets can be stored for long periods of time if they are very cool and very damp (Yadav, 2016).

2.13.2.4 Nutritional Benefits

Beetroots mainly consist of water (87%), carbohydrates (8%) and fiber (2-3%).Raw or cooked beetroots contain about 8-10% carbohydrates. Simple sugars, such as glucose and fructose, make up 70% of the carbs in raw beetroots, and 80% in cooked beetroots. Beetroots are also a source of fructans, short-chain carbs which are classified as FODMAPs. Some people cannot digest these FODMAPs, causing unpleasant digestive symptoms. Beetroots have a glycemic index score of 61, which is considered to be in the medium range. Beetroots are high in fiber, providing about 2-3 g in each 100 g serving. Dietary fibers important as part of a healthy diet, and has been linked to reduced risk of various diseases. Beetroots are good sources of vitamins and minerals, such as folate, manganese, potassium iron and vitamin C. Plant compounds are natural plant substances, some of which have beneficial effects in humans. Beetroots are high in several beneficial plant compounds, especially betanin (beetrootred), vulgaxanthin and inorganic nitrates (Anon., 2015a).

Inorganic nitrates include nitrates, nitrites and nitric oxide. Beetroots, and beetroot juice, are exceptionally high in nitrates. There has been some debate about these substances in the past. Some believe that they are harmful and cause cancer, while others think the risk is overstated. Most dietary nitrate (80-95%) comes from fruits and vegetables. On the other hand, dietary nitrite comes from food additives, processed cured meats, baked goods and cereals. Dietary nitrates, such as those coming from beetroots, can get converted into a biological messenger molecule called nitric oxide. Nitric oxide travels through the artery walls, sending signals to the tiny muscle cells around the arteries and telling them to relax. When these tiny muscle cells relax, our blood vessels dilate and blood pressure goes down (Anon., 2015a).

2.13.2.5 Uses of beetroot

Young leaves of the garden beet are sometimes used for eating. The midribs of Swiss chard are eaten boiled while the whole leaf blades are eaten as spinach beet. In some parts of Africa, the whole leaf blades are usually prepared with the major as one dish (Grubben and Denton, 2004).

The leaves and stems of young plants are steamed briefly and eaten as a vegetable, older leaves and stems are stir-fried. The usually deep-red roots of garden beet are eaten boiled either as a cooked vegetable or cold as a salad after cooking and adding oil and vinegar. A large proportion of the commercial production is processed into boiled and sterilized beets or into pickles. In Eastern Europe beet soup, such as cold soup, is a popular dish. Yellow-colored garden beets are grown on a very small scale for home consumption. Beetroot can be peeled, steamed, and then eaten warm with butter as a delicacy; cooked, pickled, and then eaten cold as a condiment; or peeled, shredded raw, and then eaten as a salad. Pickled beets are a traditional food of the American South (Kumar, 2015). The Nutritional value of fresh beetroots per 100 g are given in Table 2.8.

Table 2.8 Nutritional value of fresh beetroots per 100 g

Constituents	Amount	Constituents	Amount
Carbohydrates	9.96 g	Vitamin B6	0.067 mg
Sugars	7.96 g	Folate (Vit. B9)	80 µg
Dietary fiber	2.0 g	Vitamin C	3.6 mg
Fat	0.18 g	Calcium	16 mg
Protein	1.68 g	Iron	0.79 mg
Vitamin A equiv.	2 µg	Magnesium	23 mg
Thiamine (Vit. B_1)	0.031 mg	Phosphorus	38 mg
Riboflavin(Vit.B_2)	0.027 mg	Potassium	305 mg
Niacin (Vit. B_3)	0.331 mg	Zinc	0.35 mg
Pantothenic acid (B_5)	0.145 mg	Sodium	77 mg

Source: (Anon., 2015 b)

Beetroot pigment is used commercially as a food dye. It changes color when heated so can only be used in ice-cream, sweets and other confectionary, but it is both cheap and has no known allergic side-effects. Beetroot itself, of course, is a common salad ingredient when cooked, vinegar is added to the water to lower the pH. Beetroot juice is very potent, and it's recommended that you drink the raw juice diluted at least 4 times with other milder juices such as carrot, cucumber. Plus it tastes better, a beautiful rich ruby red color it is known to help purify the blood. There are nine other species in the *Beta* genus and all also have the common name beet, although *Beta vulgaris* is the most well-known and commercially important and is known as the common beet (NRCS, 2006). Beets, with large leaves, are also grown as ornamental plants. Ecologically, they provide food for many animals, including the larvae of a number of pest species. Beets are delightful for their color and flavor as well as for their beet nutrition. Their juice is wonderful mixed with carrot juice and can also be used as a dye. In some countries the beet juice, betanin, is processed commercially for coloration in various products (Kumar, 2015).

2.13.2.6 Health benefits

Beets have long been known for its amazing health benefits for almost every part of the body.

- **Anemia:** The high content of iron in beets regenerates and reactivates the red blood cells and supplies fresh oxygen to the body. The copper content in beets helps make the iron more available to the body.
- **Blood pressure:** All its healing and medicinal values effectively normalize blood pressure, lowering high blood pressure or elevating low blood pressure.
- **Cancer:** Betaine, an amino acid in beetroots, has significant anti-cancer properties.
- **Constipation:** Drinking beets juice regularly will help relieve chronic constipation.
- **Detoxification:** The chlorine from the juice detoxifies (which remove toxic substance).
- **Kidney ailments:** Coupled with carrot juice, the excellent cleansing virtues are exceptional for curing ailments (Grubben and Denton, 2004).
- **Liver toxicity or bile:** The cleansing virtues in beets juice is very healing for liver toxicity or bile ailments, like jaundice, hepatitis, food poisoning, diarrhea or vomiting.
- **Skin disorders:** The water in which beetroots and tops have been boiled is an excellent application for boils, skin inflammation and out breaks of pimples and pustules.
- **Tonic effects:** Beetroot is a nutritious and suitable tonic for the entire digestive tract.
- **Increases sex drive:** It contains high amounts of boron, which is directly related to the production of human sex hormones (Anonymous, 2008).

- **Lowers cholesterol:** Beetroot contains soluble fiber, which has also been shown to have cholesterol lowering capabilities (Kumar, 2015)

Part III

Material and methods

3.1 Materials

3.1.1 Raw materials

3.1.1.1 Yacon (ground apple)

The yacon fruit of good quality was brought from Hile, Dhankuta, Nepal.

3.1.1.2 Beetroot

Beetroot was purchased from local market of Dharan.

3.1.1.3 Table sugar

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

3.1.1.4 Citric acid

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

3.1.1.5 Yeast

Wine yeast was brought from wine industry of Dhangadi. Wine yeast, *Saccharomyces cerevisiae* (SC 22) manufactured in Canada by Lallemand Inc. was used for wine preparation

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 equipment required for the experiment were obtained from laboratory of Central Campus of Technology. List of equipment 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 ºBx)	Screw press juice extractor
pH meter	Handheld potato peeler
Stainless steel vessels	Knives
Thermometer	Distillation set
Titration apparatus	Heating arrangement
Pycnometer	Other routine glassware

3.1.4 Chemicals

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

Table 3.3 List of chemicals used

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

3.2 Methodology

The total work was based on preparation of yacon wine with varying the percentage of beetroot juice.

3.2.1 Experimental procedure

3.2.1.1 Collection of raw material

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

Yacon and beet 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

Five wine samples were prepared using different concentration of yacon and beetroot juice. During the musts preparation beetroot juice was blended with yacon juice at the concentration of 0%, 5%, 10%, 15%, 20% and 25% and pH 3.5 was maintained using critic acid. 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

	Samples					
Parameters	A	B	C	D	E	F
Beet root juice (%)	0	5	10	15	20	25
Yacon Juice (%)	100	95	90	85	80	75

3.2.1.7 Pitching

Wine yeast *Saccharomyces cerevisiae* (SC 22) was used for pitching. It was activated by mildly heated about 35-40°C with sugar and water solution and pitching was done at the rate of 0.3 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 was kept in plastic jars for fermentation. After 10 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.

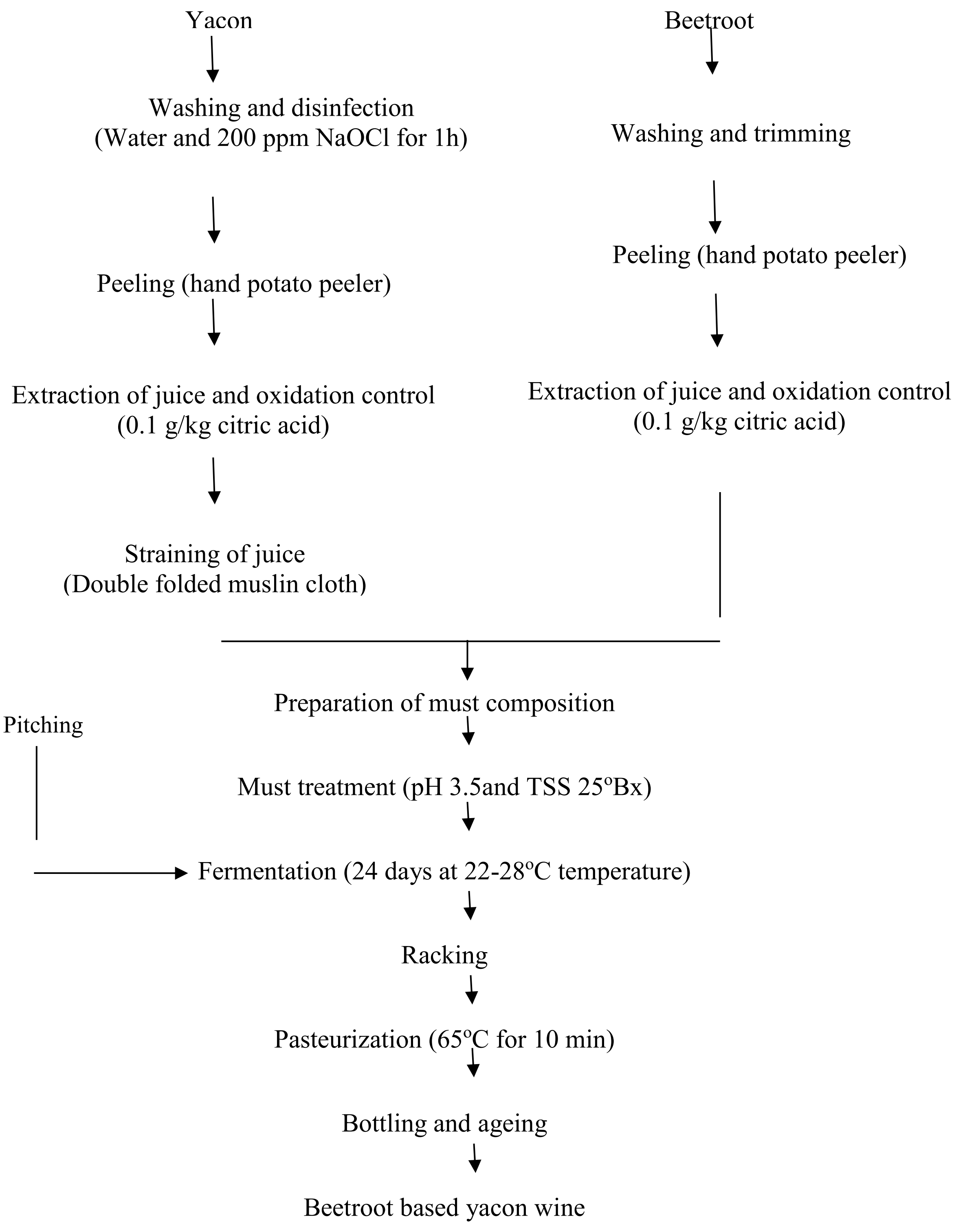


Fig. 3.1 Flowsheet for preparation of beetroot based yacon wine

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 bulk pasteurized by heating of wine in vessel with continuous stirring until temperature of wine reached at 65°C after which the heat was turned off and left for 10 min followed by immediate cooling. 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. Chemical composition and properties like TSS, pH, total acidity, volatile acidity, specific gravity, alcohol content, ester, aldehyde and tannin content were analyzed for optimized (best product).

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 Methanol content

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

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

3.2.2.5 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).

3.2.2.6 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.

3.2.2.7 Ester content

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 ethylacetate. As per method of FSSAI (2012).

Ester express as ethyl acetate= $(V\times 0.0088 \times 100 \times 1000 \times 2) / V_1)$ g/100 L of abs. alcohol

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

V_1 = alcohol % by volume

3.2.2.8 Aldehyde content

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 minutes 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 50 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. As per method of FSSAI (2012).

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.

3.2.2.9 Determination of antioxidant activity

The antioxidant activity of wine was determined by DPPH method as per (Sing *et al.*, 2008). Briefly, wine sample was filtered through Whatman No. 41 filter paper. One ml of the filtered wine was diluted to 10 ml with distilled water. One ml of the diluted wine was taken in a test tube and 4 ml of 0.004% methanolic solution of DPPH was added. Then the test tube was incubated at room temperature (28°C) for 30 min in the dark and absorbance was measured at 517 nm using a UV-vis spectrophotometer. Similarly, blank was also run using methanol instead of the sample. The DPPH scavenging activity was calculated as follows:

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

3.2.3 Sensory evaluation

The prepared 6 wine samples by varying beetroot juice content of must were subjected to sensory evaluation. The samples were served in clean wine glass at silent environment around 1:00 pm and room temperature was 25±2ºC. Sensory attributes (such as clarity, color, mouth feel, flavor, and overall acceptability) were evaluated using 9 points hedonic rating test with the help of 10 semi- trained panelist that included 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 was analyzed for one way and two way ANOVA, mean ANOVA (No blocking), 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 Hile, 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 9.5˚Bx and 5.34 respectively. Beetroot was bought from local market of Dharan and physical and chemical analysis was carried out where TSS and pH of beetroot juice were found to be 6 and 5.4 respectively. Different proportion of yacon juice and beetroot juice were blended for the preparation and optimization of yacon-beetroot wine. In this experiment, yacon-beetroot wines were prepared from six musts using different proportion of beetroot juice content (0%, 5%, 10%, 15%, 20% and 25%) and pH 3.5 maintaining constant with the help of citric acid and TSS was maintained at 25ºBx with addition of sugar. The fermentation was carried out at room temperature around 25±2˚C using wine yeast then pasteurized the product at 65ºC for 10 min. The best product was determined by using sensory analysis and the chemical analysis was carried out for control (0%beetroot juice) and best product.

4.1 Chemical analysis of yacon juice (*Smallanthus sonchifolius*).

Chemical compositions of yacon and beetroot juice are given in Table 4.1 and 4.2 respectively.

Table 4.1 Chemical composition of yacon juice

Parameter	Value*
TSS (ºBx)	9.5 (0)
Acidity (% as citric acid)	0.13 (0.012)
pH	5.34 (0.023)
Juice yield (% total fresh weight)	50
Reducing sugar (% as dextrose)	0.713 (0.034)

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

Above parameters for yacon juice resemble to the data obtained from Pokhrel (2017) and Shrestha (2015) in terms of TSS but slightly different in terms of acidity, pH and juice yield this is because in this experiment antioxidant i.e. citric acid was used to prevent juice from oxidation hence acidity increased and pH decreased slightly and in case of juice yield percentage juice obtained was slightly reduced because heat treatment facilitates extraction of juice but in this experiment heat treatment was not done.

Table 4.2 Physiochemical analysis of Beetroot juice

Parameter	Value
TSS (ºBx)	6 (2.5)
Acidity (% as citric acid)	0.6 (0.135)
pH	5.4 (0.4)
Juice yield (% total fresh weight)	45
Reducing sugar (% as dextrose)	1.7 (0.3)

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

Above parameters for beetroot juice resemble to the data obtained from Rai (2018).

4.2 Formulation of must composition

The chemical composition of must was maintained according to design and is presented in Table 4.3

4.3 Effect of juice percentage on sensory parameters of wine

The sensory analysis was carried out in the room of Central Campus of Technology. The panelists were trained in sensory of wine they were experience professor and lecturers of Central Campus of Technology, and some semi trained panelist but who were familiar with alcoholic beverage. The sensory of evaluation of all 6 samples were performed on the basis of 9 point hedonic rating test.

Table 4.3 Preparation of must

	Samples					
Parameter	A	B	C	D	E	F
Beet root juice (%)	0	5	10	15	20	25
Yacon juice (%)	100	95	90	85	80	75

The parameter for sensory analysis were color, clarity, mouth feel, flavor and over all acceptability of product. The obtained data from sensory analysis was analyzed using two-way ANOVA (Appendix B) at 5% level of significance to study the significance difference among formulation made and among panelists. However, there is no significance difference in all panelists to judge the specified parameter of the product. In sensory analysis, the significant difference in products based on specified parameters was in the sense of liked or disliked of the product as judged by the panelists but not in sense of physicochemical characteristics of the products.

4.3.1 Effect of beetroot juice percentage of must on color of product

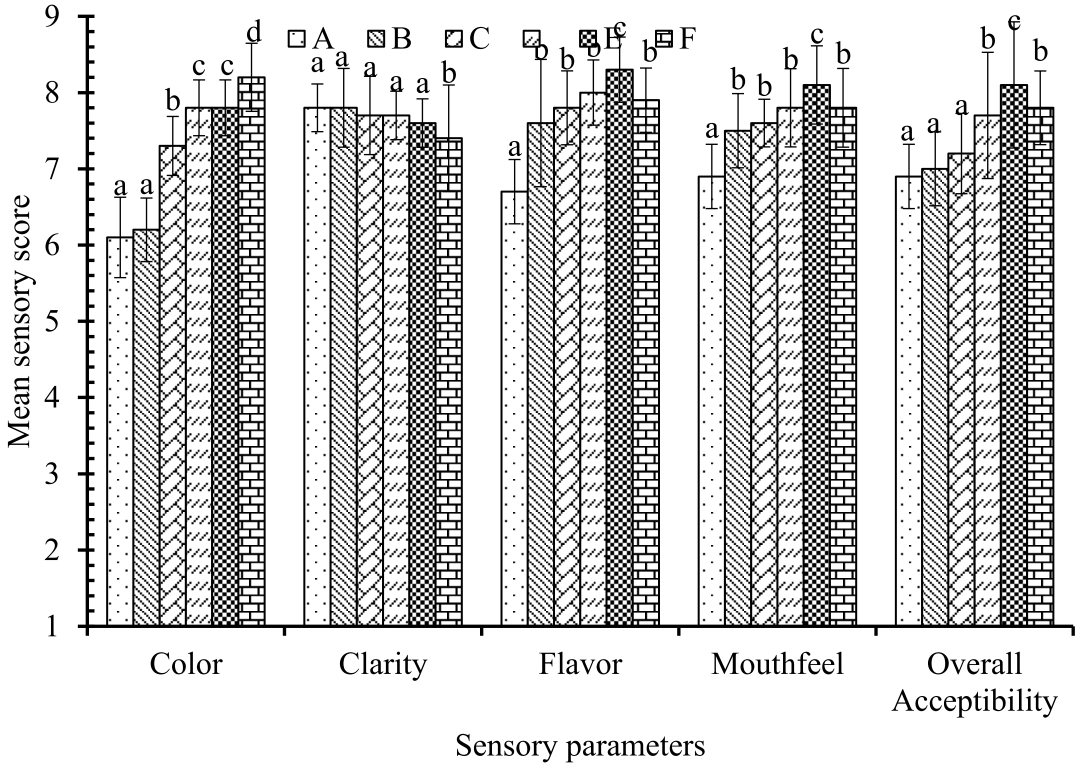


Fig. 4.1 Effects of yacon and beetroot juice proportions on sensory quality yacon-beetroot of wines.

A refers yacon juice: beetroot juice 100:0 (control)

B refers yacon juice: beetroot juice 95:5

C refers yacon juice: beetroot juice 90:10

D refers yacon juice: beetroot juice 85:15

E refers yacon juice: beetroot juice 80:20

F refers yacon juice: beetroot juice 75:25

4.3.2 Effect of beetroot juice percentage of must on color of product

The mean sensory score for color of six samples A, B, C, D, E and F were be 6.1, 6.2, 7.3, 7.8, 7.8, and 8.2 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of beetroot juice percentage variation on color at 5% level of significance.

The maximum and minimum mean sensory score for appearance among five samples were found to be 8.2 (sample F) and 6.1 (sample A) respectively. Statistically the mean sensory score was found to be highest for sample F which was of 25% beetroot juice which was significantly higher from all other sample. The mean score for samples with highest beetroot juice percentage (25%) was found to have higher values while that for samples with lower juice percentage (0%, 20%, 15%, 10%, 5%) have lower values. This indicates that panelists preferred wine with highest beetroot juice percentage in terms of color. The best sample with 25% beetroot juice was of red-violet color.

According to Jackson (2009) analysis of color 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 Singh and Hathan (2012) beetroot is composed of different pigments all belonging to the class betalaine; main colouring principle consists of betacyanins (red) of which betanine accounts for 75-95%; minor amounts of betaxanthine (yellow) and degradation products of betalaines (light brown) may be present.

According to Jackson (2009) amount of pigment effects appearance of product. Juice content will effect on color intensity of wine, higher percent juice will have higher amount of pigments hence color intensity of wine with 25% beetroot juice will have higher values which support above result.

Hence the appearance of wine with high juice percentage gave the best sensory score which is in accordance to Jackson (2009), Singh and Hathan (2012).

4.3.3 Effect of beetroot juice percentage of must on clarity of product

The mean sensory score for color of six samples A, B, C, D, E and F were be 7.8, 7.8,7.7, 7.7, 7.6 and 7.4 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of beetroot juice percentage variation on clarity at 5% level of significance.

The maximum and minimum mean sensory score for clarity among six samples were found to be 7.8 (sample A) and 7.4 (sample F) respectively. The mean sensory score was found to be highest for sample a (control) which was significantly similar to all other sample except sample F. From the graph we can observe the score for clarity is gradually decreasing. Therefore, based on clarity preference, beetroot juice could be mixed up to 20% of the total must for preparing yacon-beetroot wine.

4.3.4 Effect of beetroot juice percentage of must on flavor of product

The mean sensory score for flavor of six samples A, B, C, D, E and F were 6.7, 7.6, 7.8, 8, 8.3, and 7.9 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of beetroot juice percentage variation on clarity at 5% level of significance.

The maximum and minimum mean sensory score for flavor among six samples were found to be 8.3 (sample D) and 6.7 (sample A) respectively. The mean sensory score was found to be highest for sample D which was of 20% beetroot juice which was significantly different from all other sample. This graph shows that on increasing the beetroot juice percentage flavor also increased but on increasing above 20% flavor was again scored less thus 20% beetroot juice gave optimum flavor to the wine. Increased beetroot percentage contributed muddy off flavor to the wine.

4.3.5 Effect of beetroot juice percentage of must in mouth feel of product

The mean sensory score for color of six samples A, B, C, D, E and F were be 6.9, 7.5, 7.6, 7.8., 8.1, and 7.8 respectively. The statistical analysis showed that there is significant effect ($p<0.05$) of beetroot juice percentage variation on color at 5% level of significance.

The maximum and minimum mean sensory score for mouth feel among six samples were found to be 8.1 (sample D) and 6.9 (sample A) respectively. The mean sensory score was found to be highest for sample D which was of 20% beetroot juice which was significantly different from all other sample. The mean score for samples with beetroot juice percentage (20%) was found to have higher values while that for samples with lower juice percentage (0%, 5%, 10%, 15%) have lower values. This indicates that panelists preferred wine with highest beetroot juice percentage in terms of mouth feel.

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. 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.

4.3.6 Effect of beetroot juice of must on overall acceptability of product

The mean sensory score for overall acceptability of six samples A, B, C, D, E, and F were found to be 6.9, 7, 7.2, 7.7, 8.1 and 7.8 respectively. The statistical analysis showed that there is significant effect (p<0.05) of juice percentage on overall acceptance at 5% level of significance.

The maximum and minimum mean sensory score for overall acceptance among five samples were found to be 8.1 (sample D) and 6.9 (sample A) respectively. The mean sensory score was found to be highest for sample D which was of 80% yacon content and 20% beetroot juice which was significantly different from other samples.

The juice content could affect flavor, aroma, color, tartarate 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.

Wine containing 25% beetroot juice was superior in terms of color, 20% beetroot juice was superior in terms of flavor, mouth feel, overall acceptability and up to 20% beetroot juice was superior in terms of clarity. Most of the of the sensory attributes were found superior for wine prepared by using 80% yacon and 20% beetroot juice, hence this proportion was selected for further study.

4.4 Chemical composition

Chemical composition of best formulated yacon-beetroot wine i.e. 80 % yacon juice and 20 % beetroot juice in must and yacon wine (control) i.e. 100% yacon juice in must is given in Table 4.4.

Table 4.4 Chemical composition of control and best formulated wine.

Parameter	Control sample*	Best formulated*
TSS (ºBx)	7.3[a]	7.8[b]
pH	3.8[a] (0.07)	3.74[a] (0.04)
Acidity		
a) Total acidity (% as citric acid)	0.47[b] (0.12)	0.38[a] (0.14)
b) Fixed acidty (% as tartaric acid)	0.44[b] (0.25)	0.37[a] (0.22)
c) Volatile acidity (% as acetic acid)	0.03[b] (0.05)	0.019[a] (0.01)
Reducing sugar (% as dextrose)	0.448[a] (0.03)	0.621[b] (0.08)
Methanol (% v/v)	0.0011[a] (0.03)	0.0013[a] (0.23)
Alcohol (% v/v)	9.8[b] (0.32)	8.6[a] (0.65)
Ester (g/100 L abs. alcohol)	320.44[b] (10.40)	280.56[a] (6.88)
Total aldehyde (g/100 L abs. alcohol)	252.54[a] (8.05)	270.37[b] (7.65)
Tannin (mg tannic acid/ L)	17[a] (1.081)	24[b] (2.03)
Antioxidant activity (%)	55[a] (0.34)	86[b] (0.23)

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

The TSS of yacon wine was found to be 7.3ºBx while that of best formulated wine was 7.8, almost similar results were obtained by Shrestha (2015) and Subedi (2015). pH of yacon wine was found to be 3.8 which was similar to the result of Pokhrel (2017) while that of best formulated wine was 3.74. The total acid of dry white wine and dry red wine were found to be 0.586 and 0.649 respectively reported by Amerine*et al.* (1980).Total acidity of yacon wine was 0.47% as citric acid and of best formulated wine was 0.38% as citric acid which 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 yacon wine and best formulated wine were 0.44% and 0.37% as tartaric acid respectively which was found to be higher than wine made by Shrestha (2015). Volatile acidity was found to be 0.03 % and 0.01 as acetic acid for yacon wine and best formulated wine respectively which is within range. The legal limit for white wines is 0.12% and 0.14% for red wine.

The reducing sugar of yacon wine and best formulated 0.448% and 0.621 % was also slightly higher than that of dry white wine, 0.134% and dry white wine, 0.146 % reported byAmerine *et al.* (1980). The alcohol content in the yacon wine (9.8 % v/v) and best formulated (8.6 % v/v) was similar to that of dry white (9.88 % v/v) wine and dry red wine (10 % v/v) reported by Amerine *et al.* (1980).The methanol content in the yacon wine and best formulated wine was similar and also reported by Karki (2019). For good quality of wine, the aldehyde content should be within the range of 200-500 ppm (Rai, 2009). The aldehyde content in the yacon wine and best formulated wine was found to be 303.23 ppm and 324.64 ppm. The ester content in good quality wine should be within the range of 200-400 ppm (Rai, 2009). The ester content of the yacon wine and best formulated wine were 385.02 ppm and 337.10 ppm respectively. The tannin of yacon juice wine and best formulated wine were 0.01 g per 100 ml and 0.02 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). The antioxidant activities of yacon juice wine and best formulated wine were 55% and 86% respectively.

4.5 Cost evaluation

The total cost associated with the best product was calculated and the cost per bottle (750ml) of wine was NRs 137, 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 juice and beetroot juice was prepared and chemical analysis of yacon wine as well as best wine and sensory analysis of all yacon-beetroot wine was carried out.
2. In comparison with the wine made of all formulations, wine made from formulation having 80% yacon and 20% beetroot juice seems to be the best.
3. Yacon-beetroot wine (i.e. best formulated) has better antioxidant property then yacon wine based on above sample result.
4. Yacon-beetroot wine can be produced at a cost with the use of underutilized roots that resembles a good quality red wine.

5.2 Recommendations

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

1. Yacon beetroot wine can be prepared with varying TSS, temperature and acid used.
2. Study on changes during ageing of yacon wine interms of sensory and chemical properties can be carried out.
3. Detail study on physiochemical changes during fermentation and its ageing can be carried out.
4. Study on the quality of beetroot based yacon wine using different yeast can be carried out
5. Yacon and beetroot can be the alternative source for making red wine with low cost.

Consequently, yacon-beetroot wine holds a lot of promise from commercial point of view.

Part VI

Summary

In this study, yacon was taken from Dhankuta, which is one of the districts for commercial cultivation of yacon in Nepal. And other essential materials beetroot was bought from local market of Dharan and other materials (citric acid, sugar and yeast) and other chemical and apparatus were obtained from local market of Dharan and campus laboratory. First, yacon and beetroot were 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 yacon to 100 ppm KMS solution in order to protect from quick browning. After this yacon and beetroot were subjected to screw press juice extractor for extraction of yacon juice and beetroot juice. Yacon juice 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 9.5ºBx TSS, 5.34 pH and 0.13 acidity (% as a citric acid). Similarly, physiochemical analysis of beetroot juice showed 6ºBx TSS, 5.4 pH and 0.6 acidity (% as a citric acid). After that, fermentation was carried out in six different mashes where beetroot juice content was varied to 0%, 5%, 10%, 15%, 20% and 25% and pH as 3.5 keeping TSS 25 ºBx without varying to other parameters for 24 days.

Six different wines were subjected to sensory analysis and data obtained from this was analyzed by one-way ANOVA at 5% level of significance to study the difference among the all types. There was significant difference in case of color, clarity, flavour, and overall acceptability of all types. From the sensory evaluation, wine made from 80% yacon juice and 20% beetroot juice obtained the highest score among other formulations.

The cost of the best product was calculated and found to be Rs. 137 per bottle (750 ml) of wine made from 80% yacon juice and 20% beetroot juice content contains 8.6 % (v/v) alcohol content, 7.8 ºBx TSS, 3.74 pH, 0.38% acidity (% as a citric acid), 0.019% (as acetic acid), volatile acidity, 337.10 ppm ester and 324.64 ppm aldehyde. Alcohol content, volatile acidity and other parameters of wine made from this research are within the range of a good quality wine. The antioxidant activities were 86% in the best formulated which shows that the best formulated wine is superior in term of health conscious also.

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Appendices

Appendix A

Specimen card of sensory evaluation by 9 point hedonic rating test

Sensory evaluation of *yacon-beetroot* wine

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

Name of Product: Yacon-beetroot 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
Color						
Clarity						
Flavor						
Mouth feel						
O. A.						

Give points as follows:

Like extremely **9**.

Like very much **8**.

Like moderately **7**.

Like slightly **6**–

Neither like nor dislike **5**–

Dislike slightly **4**-

Dislike moderately **3**.

Dislike very much **2**.

Dislike extremely **1**.

Comments………………………………………………………………………………………

Signature

Appendix B

ANOVA result for sensory analysis of yacon-beetroot wine

Table B1 Two way ANOVA (no blocking) for color in the optimization of yacon- Beet root wine.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	5	31.1000	7.7750	14.51	<.001
Panelist	9	8.3438	1.1920	2.23	0.062
Residual	45	15.0000	0.5357		
Total	59	54.4437			

Table B2 Two way ANOVA (no blocking) for clarity in the optimization of yacon-Beet root wine.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	5	18.9	4.725	18.9	<.001
Panelist	9	8.3438	1.192	4.77	0.001
Residual	45	7	0.25		
Total	59	34.2438			

Table B3 Two way ANOVA (no blocking) for flavor in the optimization of yacon-beetroot wine.

Source of variation	d. f.	s.s.	m.s.	v.r.	F. pr.
Samples	5	26.475	6.6188	15.81	<.001
Panelist	9	6.9938	0.9991	2.39	0.048
Residual	45	11.725	0.4187		
Total	59	45.1937			

Table B4 Two way ANOVA (no blocking) for mouthfeel in the optimization of yacon-beetroot wine.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	6	32.8500	8.2125	32.62	<.001
Panelist	9	13.0750	1.8679	7.42	<.001

Residual	54	7.0500	0.2518		
Total	69	52.9750			

Table B5 Two way ANOVA (no blocking) for overall acceptability in the optimization of yacon-beetroot wine.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	5	16.4667	8.2333	13.64	<.001
Panelist	9	5.3333	0.5926	0.98	0.487
Residual	45	10.8667	0.6037		
Total	59	32.6667			

Appendix C

Cost evaluation for beetroot- yacon wine

Table C1 Cost evaluation (for every 750ml bottle)

Particulars	Quantity	Rate (Rs)	Total (Rs)
Yacon	1.2 kg	50/kg	60
Beetroot	0.3 kg	80/kg	24
Sugar	140 g	90/kg	13
Wine bottle	1 pcs	20/piece	20
Wine yeast	1 g	2000/kg	2
Total cost			119
Final cost with 15% overhead			137

The price of 750 ml wine cost Rs. 137. Thus, the price of1 liter wine is Rs. 183.

Appendix D

Table D1 Average chemical analysis of prize-winning high quality wines.

Components	**Dry white**	**Dry red**	**g per 100 ml**		
			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 E1 According to OVI major wine producing countries of the world-2015.

Countries	production (Million hecto-liter)	export (million hecto-liter)	consumption (million hector-liter)	Total grapes (metric ton)	Area of vine (kilo- hector)
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)