Effect of Three Isolated Aging Yeast Strains on Sensory and Chemical Characteristics of Roselle Wine

By
Ms. Tshering Lhamo
ID.5418063

A special project submitted to the Faculty of Biotechnology,
Assumption University in part fulfillment of the requirements for the degree of
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Abstract

The aim of this project was to observe differences in the sensory and chemical properties of roselle wine after aging with 3 different aging yeast strains. Yeast no.9, Yeast no.15 and Yeast no.16 were recovered from Ms. Wanjaroen (2006) who isolated 17 yeast strains from Assumption University winery. The yeast strains were added to roselle base wine and aged for 12 weeks. Chemical compositions of the different wine such as total soluble solids (°Brix), pH, reducing sugar (μg/mL), % alcohol, % total acid, % volatile and color were measured every 3 weeks during aging. Descriptive sensory analysis was done to measure the sensory attributes of the wine. 6 panelists selected from Assumption Biotechnology Faculty were trained. 5 sessions of training were done which led to compilation of 12 sensory attributes. The definition and understanding of the terms were made uniformly across all the panelists by the use of reference standards. The panelists used a 15-point intensity scale and analyzed the wine samples. Duplication was done. The chemical and sensory data were analyzed using SAS program. There were significant differences in the effect on the chemical compositions of the different wine ($p<0.05$). In replication 1 there were significant differences in reducing sugar (2650-3075μg/mL), alcohol content (9.4-10.5%), total acidity (0.83-1.01%) and volatile acidity (0.12-0.26%). Replication 2 showed significant differences in reducing sugar (700-1130μg/mL), total acidity (0.98-1.02%) and volatile acidity (0.25-0.34%). However there were very less sensory characteristics that were significantly from each other. In replication 1 sample with yeast 16 was seen to be significantly different from the control in clarity and sample with yeast 15 was in bitter taste ($p\geq0.05$).

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Finally, I am grateful for every experience that I have gained through this project. Some of which were very memorable while others were pretty challenging. Either ways, I am have become much matured and well informed in this respective field of study.

Ms. Tshering Lhamo
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Introduction

Wine is a product of grape fermentation by yeast (*Saccharomyces cerevisae*). The history of wine dates back to as early as 3000 B.C. Grape wine production is known to be one of the most ancient beverages made by our ancestors in early civilizations. The Neolithic, Egyptian, Greek and Roman civilization all show the importance of wine in their culture. The earliest record of wine making was written in the old testament of the Bible. The ability of wine to create a sensation of euphoria is one of the reasons that made it a very popular beverage. Ancient Egyptians offered wine when a pharaoh died which was a sign of offering for afterlife. During the Greek and the Roman times, wine was domesticated, cultivated and used as an integral part of their cultures.

The knowledge of viniculture spread throughout northern Africa, Middle East and Europe through trade links and colonization by civilizations (Rivard, 2009). It is assumed that the quality of wine during those ancient times is not as good as it is today. However, there were ratings done on the quality of wine and best wines were kept for the most important people and occasions (Vernon, 1965). Currently the best quality wine can cost up to $38000. Understanding the mechanism of grape wine production, people have now begun making wine from fruits and flowers.

Wine fermentation depends on several factors such the composition of the juice that is used for fermentation, temperature and the type of yeast used. Yeast is one of the most important components in wine production. Traditionally, a starter is used to introduce yeast into the grapes. Nowadays active dry yeast (ADY) is used. Yeast utilizes the sugar in the grapes/fruits to produce ethanol with the release of carbon dioxide. The Ethanol that is produced is consumed as wine. Various strains of yeast are known to produce different types and grades of wine. Genetics of the yeast determines whether the wine is fermented faster, alcohol is produced in larger amount, the aroma of the wine, etc. Today there are around 30 different types of dry active yeast strains in the market (Ribéreau-Gayon P, 2007). The strain of the yeast determines whether the yeast is tolerant to the ethanol produced, temperature used, and their dominance over other microorganisms during fermentation. A yeast strain with low tolerance will eventually produce low quality wine.

Yeasts are not only important in fermenting the wine but also in the aging process. Wine aging is also a crucial step to make the wine possess more sensory characteristics that consumers like. Usually aging is done in oak barrels however bottle aging is also practiced after barrel aging. A complex and favorable aroma develops during the aging process due to volatile substances that gets released. In the barrels the oak wood releases it's aroma. Microorganisms such as lactic acid bacteria and yeasts contribute to the development of good sensory characteristics in wine during aging. Bacteria and yeasts produce some important volatile compounds such as vinylphenol and vinylguaiacol (Victoria Moreno-Arribas M, 2009).

Aim

To observe differences in the sensory and chemical properties of roselle wine after aging with 3 different aging yeast strains.

Objectives

1. To perform chemical and sensory analysis on the aged roselle wine samples.
2. To analyze the differences between the aged roselle wine samples.
3. To study the effects of the 3 aging yeasts on chemical and sensory properties of roselle wine.

Literature review

The methods of wine production may have improved and better facilities might be available today but the basic steps can be coined into 6 crucial steps; destemming, crushing, pressing, fermentation, racking and bottling (Curran, 2006).

1. Destemming

By using either hand or mechanical means, the ripe grapes are picked. The grapes are then destemmed. Destemming is also done while the grapes get crushed. This step is one of the important steps depending on the type of wine produced. The stems of the grape vines contain tannins that influence the color and flavor of the wine (Curran, 2006).

2. Crushing

The destemmed grapes are then crushed. There are many ways to crush the grapes. In the olden times a method called threading was used to press the juice out of grapes. This method simply involved putting grapes in a big container and pressing the grapes by our feet (Shaw, 2000). However nowadays there are many advanced methods to carry out the same procedure in more efficiency. Machines with screws at the bottom that are covered by rubbers are used nowadays for the purpose of crushing (Winemaker's Academy, 2014). "Must" is the product that is gotten from crushing step. There are different ways to treat the must depending on the type of wine to be produced. In the production of white wine the solids (skin, seeds, stem) are removed before fermentation. Red wine is produced by fermenting the whole must (Curran, 2006).

3. Pressing

Pressing simply means pressing the grapes to give juice. In case of the white wine, when the solids are removed they are pressed to give the remaining juice. Pressing happens after fermentation in case of red wine. There are many ways to press the grapes; basket press, pneumatic press, cage press. Pressing is an important step in wine making because if it were not done one would be drinking wines with chunks of grapes in it.

4. Fermentation by yeast

Yeasts are the microorganisms that are responsible for the production of wine mainly because of the enzymes that is housed by them. There are 5 enzymes that are mainly responsible for the conversion of grapes into wine (Sanchez, 2008). Enzyme hexokinase converts hexose sugars into two triose sugars. Oxidoreductase or aldolase converts the triose sugars into glyceric acid and glycerine. Then enzyme enolase converts the glyrceric acid to pyruvic acid. Carboxylase converts the pyruvic acid to acetaldehyde and carbon dioxide. Finally zymase converts some of the acetaldehyde to ethanol.

The most important group of yeast that is important in wine making is the *Saccharomyces* (*Saccharomyces cerevisiae* and *Saccharomyces bayanus*). Normally

there are many species of yeasts that come with the grape fruits themselves. Out of the many species of yeast, *Candida, Hanseniaspora* and *Kloeckera* are the dominating yeasts during the initial stage of fermentation. However, the ethanol produced inhibits the growth of these yeasts and only the alcohol tolerant strains of *S.cerevisiae* survive in the later stages of fermentation (Lisbeth Meunier-Goddik, 2004).

There are about 10^3-10^6 yeast cells on the surface of grapes. These yeast cells produce varieties of metabolites. It is however debatable whether these metabolites are desirable. Some argue that these metabolites are important in forming the bouquet of wine characters while others consider them otherwise. Yeast of *Candida* spp. such as *C.vini, C. krusei, C.valida*, etc. grow as surface film. They are considered spoilage yeasts because they give a 'damp basement' smell, which is considered undesirable in wine. *Pichia membanefaciens*, one of the *Candida* spp., produces surface film along with high amounts of acetic acid, acetaldehyde and acetate esters. They have higher tolerance to ethanol and SO_2. *Hansenula anomala* is another type of spoilage yeast that produces ethyl acetate and acetic acid. Yeast such as *Saccharomycodes lugwigii* is problematic because it forms sediments and is highly resistant to SO_2. On the other hand species like *Zygosaccharomyces bailii do* not create undesirable metabolites but can be problematic in the production of sweet wines, as they tend to referment them (Panchal, 2001).

One of the most problematic yeast species is called *Brettanomyces.* It is sensitive to SO_2 but can tolerate high amount of ethanol. It is very difficult to get rid of once it infests the wine barrels. Wineries take multiple measures to get rid of this microbe. Ozone sterilization and treating barrels with alternate hot and cold temperatures are few such measures (Victoria Moreno-Arribas M, 2009). This yeast is known to produce caproic, isobutyric, isovaleric acid and ethyl acetate. These components are however found to give the wine a peculiar fruity smell (Panchal, 2001). *Brettanomyces* and *Dekkera* synthesize ethyl phenols such as 4-ethylphenol and 4-ethylguaiacol from hydroxycinnamic acids. With increasing aging time, these ethyl phenols increase in the wine content (Victoria Moreno-Arribas M, 2009). These ethyl phenols are found to give phenolic, animal and stable odors to the aging wine (Pascal Chatonnet, 1992).

The winemaking industry nowadays destroys the initial flora by adding sulphur dioxide and only adding the desired strains of *S.cerevisiae* and *S.bayanus.* There are many strains of *S.cerevisiae* that are used today for better production of wine. There are several reasons why yeast strains are improved and developed constantly. The genes of the strains are developed to increase the efficiency of fermentation process, to increase the efficiency of wine processing, improve the biological control of wine spoilage, improvement of wine wholesomeness and for improvement of wine flavor and sensory qualities. For example *S.cerevisiae* strains like 71B increases the fermentation aroma of the dry white wines (Pascal Ribéreau-Gayon, 2006).

The best way to produce a high quality wine is to select the best strain of yeast. Experiments that were done previously have shown the clear influence of yeast strains on mango wine. Volatile compounds produced during yeast fermentation are also noticed to influence the aroma and flavor of the wine. Different levels of compounds (such as organic acids and esters) are also results of various strains of yeast (Hui Y.H, 2012). To select the best strain of yeast, one can perform chemical and sensory tests

on the wines. The yeast used to produce the wine that has the highest scoring in the sensory tests and has higher ethanol amount, organic acids and esters would be considered as a good strain.

Since fermentation is meant to carry out by the selected active dry yeast (ADY) sulphur dioxide (SO_2) is added to the must to get rid of the existing yeast cells and other microorganisms. Thus SO_2 acts as a preservative in terms of inhibiting the growth of spoilage microorganisms and also helps prevent oxidation and browning reaction (Cooke, 1988).

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Enzymes)}}{\text{Zymase}} \longrightarrow \underset{\text{(Ethanol)}}{2\,C_2H_5OH} + \underset{\text{(Carbon dioxide)}}{2\,CO_2}$$

The fermentation happens as per the above equation is called alcoholic fermentation. This is the process when the yeast cells utilize the sugar, glucose and fructose in the must and produce ethyl alcohol and carbon dioxide.

During the initial period (12-24 hours) after inoculation of yeast into the must, the yeast cells multiply in number and grow in size. This is called budding off. This is the lag and log phase of the yeast cell growth. There is an exponential growth in the number of yeast cells. The yeast cells use the nutrients in the must for their growth and multiplication (Jackson, 2000). Even though the calculated/estimated production of ethanol is 51.1%, the actual production is lower because the yeast cells utilize the nutrients in the must for their growth.

The yeast cells are high enough in number to commence the actual alcoholic fermentation. The yeast cells now ferment the sugar and produce ethanol. The yeast cells have reached their stationery phase of growth because the production of alcohol, the limiting supply of sugar and the population density limits the growth of the yeast cells. A lot of sugar gets fermented in this stage. The skins of the grapes rise up in the surface forming "cap". There is frothing in this stage, as there is production of carbon dioxide gas. As the cap forms like a compact layer on the surface and the production of ethanol is exothermic reaction, there is a lot of accumulation of heat. This heat if left unattended will kill the yeast cells leading to incomplete fermentation. To avoid this wine producers use various methods to reduce control the temperature. Some use special fermenters with cooling jackets but the most inexpensive method used by many is called punching. Using a wooden stick the cap is punched down into the fermenting must easily releases the accumulated heat (Cooke, 1988).

The third stage of alcoholic fermentation takes places after pressing step. This is when the unfermented grape fruits are exposed to the remaining yeast cells. This is a slow fermentation step. By now most of the yeast cells are killed. Alcoholic fermentation is not only significantly important because of production of ethanol but also due to the production of many aromatic and flavor compounds that give wine it's characteristic features. The main volatile compounds that constitute to the aroma of the wine are alcohol itself. There are other alcohols that are produced along with ethanol such as 2-methyl-1-butanol, tyrosol and 2-phenyl ethanol. At lower concentration these alcohols give pleasant complexity to the wine aroma however at higher concentration the quality of the wine can be lowered. Volatile acids are produced which can have either

consequence. Volatile organic acids are used during fermentation in ester formation. Acids such as Octanoic and hexanoic acids contribute to the fruity and fresh notes of the wine. However higher concentration of acids such as acetic acid could lead to wine smelling like vinegar (E. Evranuz, 2012).

Aldehydes and ketones produced also contribute to the aromatic characteristics of wine. Acetaldehydes gives a nutty note to the wine but when in high concentration gives rise to off-flavors. Diacetyl that is produced is known to give wines a buttery flavor. Production of Hydrogen sulfide is considered to be a negative feature because it gives the wine the smell similar to rotten eggs (E. Evranuz, 2012).

5. Racking

This is the step, which basically clarifies the wine. The liquid wine is separated from the solid parts called the "lees". Racking can be done by either centrifugation or filtration methods. This step is necessary because the sediment of lees leads to wine spoilage.

6. Bottling

Bottles are sterilized and fluxed with nitrogen to ensure that there is no dust and oxygen. This process is called 'sparging'. Wine shouldn't be agitated and the bottling procedure should be done as quickly as possible to limit the contact of oxygen with wine. However some parts of wine do come in contact with oxygen, which leads to dull flavor. This is called 'bottle shock'. This doesn't create a significant change in the flavor and aroma of the wine (Frank, 2008).

After the alcoholic fermentation there are two processes that the wine can either go through. It can either go through malolactic fermentation or aging or both.

Malolactic fermentation

This is different from alcoholic fermentation. Lactic acid bacteria particularly those species of genus *Leuconostoc* carries out this fermentation. The malic acid that is naturally present in the grapes and must will be converted to lactic acid by the bacteria. The malic acid is higher in acidity than lactic acid so malolactic fermentation reduces the acidity of the wine. This step is important because this ensures stability of wines before bottling. If wine has not undergone malolactic fermentation, the wine might ferment in the bottles leading to bursting out of the corks and eventually leading to spoilage. Thus for this reason wine producers inoculate culture of lactic acid bacteria into the wine before bottling (Cooke, 1988). Organoleptic properties of the wines are changed in favorable ways during malolactic fermentation. The lactic acid has a powerful flavor that replaces the unpleasant malic acid flavor. Diacetyl and esters are formed which leads to fruity, buttery, floral, vanilla, honey, etc. characteristics (E. Evranuz, 2012).

Aging

Dr. Murli Dharmadhikar from Iowa State University defines wine aging as "a group of reactions that tend to improve the taste and flavor of a wine over time" (Dharmadhikari, 2015). Aging is traditionally done after racking process in oak barrels. Bottle aging is done after barrel aging. Usually red wines are mostly aged in barrels however some white wines are also aged in the similar ways (Victoria Moreno-Arribas M, 2009). There is a general misconception that aging wine will make wine better in taste and sensory attribute. However it is known that only 1% of the wine verities can be aged. Rest of the varieties has to be consumed within a year. Red wines contain more tannin in them and can be aged better than white wines without spoiling (Zraly, 2010).

In traditional aging, oak barrels play a significant role in development of the favorable sensory characteristics of wine. Volatiles from the oak barrels get diffused into the wine. Compounds such as guaiacol, furfural and 5-methylfurfural get diffused into the wine. The type, age, geographical origin, drying treatment performed and usage of the oak affect the volatile compounds released by it into the wine. Oak lactones increase in the wines that are aged in barrels that have been used twice but however when the same barrel is used for third time, this compound decreases in wine (Victoria Moreno-Arribas M, 2009).

After the oak compounds have been extracted in the wine, microbial actions take place that alters the composition further. The microbes convert furanic and phenolic aldehydes to their alcohols and then into ethyl esters. Furfural alcohol is a great indicator for microbial activity as furfural in wine are very sensitive and thus gets reduced pretty easily. Eugenol and guaiacol stay stable throughout after they have been extracted from oak barrels. Lactones that have been extracted also go through very little change. Compounds such as Vinylphenol and vinylguaiacol are mainly due to yeast metabolisms. Vinylguaiacol gives off a spicy aroma. This compound gets converted to alcohol adducts and wine pigments in red wines, thus reduces in wines that are aged for a long time (Victoria Moreno-Arribas M, 2009).

Nowadays, traditional barrel aging is not practiced so widely. Bottle aging and bulk aging in carboys are practiced currently. So changes in aging is mainly due to spontaneous chemical reactions and microbial activity. The wine will consist a variety of fungi, yeast and bacteria carried forward from the raw materials and fermentation process. It was found out in sherry wines that the flor yeasts that are involved in aging are species of s.*cerevisaie* such as *beticus*, *cheresiensis*, *montuliensis* and *rouxii*. They have different metabolisms than the typical alcohol fermenting yeasts y (E. Evranuz, 2012).

Wine aged with yeast lees show a lot of changes happening in the wine because of yeast especially in the case of sparkling wine. During aging, refermentation occurs where the yeast cells utilize the remaining sugar and produces ethanol. Yeast also uses up the amino acids to grow this leads to decrease in the amino acid content of the wine (Andrew G.H. Lea, 2003). The yeast cells also use alcohol as carbon and energy source that leads to decrease in alcohol level. Through this metabolism there are many compounds formed such as acetaldehyde, acetic acid, butanediol, diacetyl and acetoin (E. Evranuz, 2012). These are volatiles and contribute to the aroma of the wine.

The membranous content of the cell walls of the yeasts become degraded and plasmolyzed with time. The polysaccharide content of the wine increases during this time. Lipid content of the wine is changed. Triglycerides that are released from yeast broken down into smaller molecules that contributes to the aroma of the wine (Andrew G.H. Lea, 2003). When the amount of sugar becomes low, the yeast gives out the absorbed amino acids back into the wine.

Yeast cells die off but they remain intact and enzymes inside are still active. These are intracellular protease enzymes autolyze the yeast cells releasing proteins, protein fragments and amino acids into the wine. These amino acids are precursors of flavor and aroma compounds. Amino acids are sweet and the compounds that are produced such as higher alcohol, polyamines and amino acid esters contribute to the flavor and aroma of the wine. The peptides bind to the volatile compounds thus decreasing their effect on the aroma of the wine (Andrew G.H. Lea, 2003).

Due to the activity of the yeast on the wine researches are done to find out more about yeast activity in wine aging. The changes happening in the wine depends on the strain of yeast. Researches are into finding the high quality strain of yeast to age wine better. 17 yeast strains were isolated from ABAC winery by Ms. Wanjaroen (2006), among which were believed to be aging wine yeasts. The yeasts extracted were then identified to be in the family of *Saccharomycetaceae* and *Candidaceae*. There were some that could not be identified (Nitayakarnsakun, 2008). Following that research Ms. Sasivimon used 4 of the yeast strains to age sala fruit wine. She found out that there is no significant difference in the chemical composition of the wines aged with the 4 yeast strains. However, it was found out that there is a significant difference in the sensory characteristics of the different wines (Seemachaiboworn, 2008).

There are also many spontaneous chemical reactions that occur during aging that changes the sensory characteristics of the wine. The most noticeable sensory changes in wine that one can perceive are in the color, taste, mouth feel and aroma. The golden color of white wines tends to become darker and eventually brown due to oxidation of the phenolic compounds. Anthocyanin is responsible for the bright red color of young red wines. In presence of oxygen anthocyanin goes through condensation reaction that leads to forming stable polymeric pigments. With time, more polymeric pigments develop changing the color of the wine from red to orange to brick red. This polymerization of pigments is also observed at a slower rate in aerobic conditions (Dharmadhikari, 2015).

Wine before aging has fruity and floral aroma. This is due to the esters and higher alcohols formed during fermentation. During aging these esters get hydrolyzed and new esters get synthesized such as isoamyl acetate and diethyl succinate. This leads to the loss of the fresh and fruity aroma. Terpene is one of the compounds that are contained in some wine varieties. During aging monoterpene alcohols change to monoterpene oxides; in Riesling the monoterpene linalool that has a floral aroma is seen to change to its oxides such as alphaterpineol. The wine then develops a pine like odor (Dharmadhikari, 2015).

Flavonoid phenols are responsible for bitterness and astringency of wine. During aging flavonoid phenols get polymerized. The wine becomes less bitter and more astringent because monomeric flavonoids contribute to the bitter taste. With further

polymerization, the flavonoids phenols precipitate and reduce the astringency. This leads to a smoother and softer taste. With aging the acidity of the wine is also seen to reduce. Since acidity enhances the astringency, lower acidity gives wine more mellow taste (Dharmadhikari, 2015).

Chemical composition of commercial grape wine

Total soluble solids content means it is the concentration of soluble solids in the wine. It is a good way to approximate the amount of fermentable sugar contained in the fruit. An estimation of 90-95% of soluble solids being sugar makes it easier for winemaker to estimate the resulting alcohol percent. Though other soluble substances also contribute to total soluble solids such as pectin, tannins, pigments, acids and salts. In theory 180g of fermentable sugar (glucose) can result in 92g of ethanol, that is 51.1% ethanol. Many wine makers use a conversion range from 0.54-0.62 depending on the region where the grapes are grown as grapes grown on higher altitude has more sugar content. One can use a hydrometer or a refractometer to measure this attribute. The standard TTS in California wine type Angelica, white port, muscatel and port wines is >5.5°Brix while dessert wines contain >3.5°Brix (Panda, 2011). Total soluble solids value is used not only to calculate the final percent of alcohol but also to see a progressive fermentation indicator.

The main alcohol wines contain is ethanol. Wines can be classified into different types according to their ethanol content. Table wines are mainly seen to have 7-14% ABV. Fortified wines have more than 14% ABV (Schaechter, 2009). Red wines have 13% ABV while white wines have 11.5% ABV. Champagne has 12% ABV (The Australian Government; Department of Health, 2010)

Reducing sugar is the sugars that are fermentable by yeasts. They have an aldehyde or alpha-hydroxy ketone that can be oxidized. The main reducing sugars in wine are mainly glucose and fructose. Pentoses that cannot be fermented are also included in the reducing sugar content of a wine. However it is very negligible (Mansfield, 2011). According to the reducing sugar content of a wine, it can be classified into sweet or dry wines. Sweet wines have 25-125 g/L, semi dry wines have 5-25 g/L and dry wines have <5 g/L of reducing sugar (International Organization of Vine and Wine, 2009).

Acidity in wine plays an important role. It determines its sensory qualities and the wine's susceptibility of getting contaminated by unwanted microbes. Too high acid content makes wine too tart and sour for consumers and too low acid content makes wine flat. Wine with low acid is susceptible to microbial invasion and eventually spoilage. Generally most table wines have an acid content of 0.6-0.9% tartaric acid and white wines have higher than red wines. Volatile acid content of wine is another important factor that tells whether a wine is contaminated or not. Acetic acid or vinegar is a predictor of wine spoilage because spoilage microbes produce acetic acid. High content of VA signifies that the wine is spoilt and undesirable for consumption. An acceptable content of VA in wine is 0.03-0.06%. Since Acidity and pH are somehow related. Table wines in general are seen to have a pH of 3.3 – 3.7. (Pandell, 1999).

Fruit Wines

Fruit wines are often used in the olden times as that of grape wines. They were popular where grapes were not available. History has shown that the fruit wines were a part of Indian and Chinese ancient cultures. Fruit wines especially apple fruit wine were extremely popular in medieval America. Fruit wines are found to be difficult in grouping them into a category of wine. Since they are found to be sweet, they are categorized into sweet wine or dessert wine group. Global Wine market is worth 22.6 billion liters as of now. However, fruit wines constitute only 2% of that market. Thus the global demand for fruit wines is highly unmet. A definite demand cannot be designated but cases have shown that there is a high demand on fruit wine. A fruit winery opened in Australia in 2003 is seen to produce 250,000 bottles annually and also exports to the UK, the USA, Japan and South Africa. Many wineries such as in Australia and Canada are keeping up with the high demand. There is a lot of potential in the market for a good fruit wine business (Rivard, 2009).

Fruit wines have unique advantages over grape wines in terms of shorter maturation period. There are several ranges of fruits available especially in the tropical countries. Fruits can be frozen without losing their intrinsic identities and thus can be used for wine production. Fruits can also be less costly compared to that of grapes (Rivard, 2009). In Europe fruit wines are divided into two types according to their alcohol fortification. A fruit wine without addition of alcohol is said to have alcohol strength of 8-14% $_{ABV}$. On the other hand fruit wine with added alcohol has an alcohol content of 12-15% $_{ABV}$. In Europe fruits such as apples, cherries, currants, plums, strawberries and wild berries/fruits were used to make fruit wines. However fruit wine production from sub-tropical and tropical fruits such as apricots, banana, carambola, kiwi, mango, orange, muskmelon, plantain and persimmon are used to make fruit wines (D. Arthey, 1996).

Fruit wines are not usually aged. The main reason is that fruits do not contain the required acidity, alcohol content, tannin and phenolic compounds like grapes (WineMaker, 2006). In cases of apple wines, additive sugars are used during the fermentation process and this makes it difficult for it to age well (Thomas, 2012). Fruit wines are usually ready to consume after 6 months of aging. Depending on the fruit, some wines can be aged to 12 months. However it is advised to consume fruit wines within 3-5 years (Midwest Homebrewing and Winemaking Supplies, 2015).

Roselle

Roselle is a shrub that is native to India and south East Asia. It grows approximately 2 meters in height. This plant belongs to the genus *Hibiscus.* The scientific name of this plant is *Hibiscus Sabdariffa* L. However this plant is also called by many names such as Rosella, Indian sorrel, Jamaican sorrel, Florida cranberry, Oseille rouge and Flor de Jamaica (Roberts, 2000). Roselle is a bushy herbaceous plant that grows in regions that have rainfall of about 1500-2000 mm/year and grows till an altitude of 600m from sea level. This plant adapts very well in terms of soil type. Since it grows in countries like India, Malaysia, Saudi Arabia, Thailand,

Figure 1 Roselle Calyces

Indonesia and other tropical countries, it tolerates warm and humid conditions. Before the flowers are matured and collected, it takes the plant 3-4 months to reach there (Amin Ismail, 2008).

Roselle is an annual plant. It is grown along the tropical regions for various reasons. The most important part utilized for commercial purposes of this plant is the Calyces. They are removed from the plant by removing the flower petals from the capsule that contains seeds (Amin Ismail, 2008). The red pigments extracted from the calyces of the plant are used for making products such as jelly, jam, beverages and food colorant (Bajaj, 1993). They are also used for making wine, syrup, gelatin, cakes and pudding, and dried roselle calyces are used for making tea, marmalade, ice cream, pies, butter, tarts and sherbets (Amin Ismail, 2008).

Roselle calyces were previously used to make wine in one of the researches. The extract contained 4.21% protein, 0.69% titratable acid and 21°Brix total soluble solids. The final wine after fermentation had a pH of 3.43, 0.75% titrable acid and 10.8%(w/v) alcohol. Through this research it was concluded that there were no significant difference in sensory properties between commercial red wine and roselle wine (Offonry, 2009).

The main component of roselle calyces that makes it a good food colorant is because of the anthocyanin content in it. Anthocyanin gives roselle its characteristic orange-red color. This makes it a very good potential anti-oxidant too. The major pigment contained in this plant is daphniphylline along with other flavonoids such as gosypetine and sabdaretine (Amin Ismail, 2008). The Dried calyces are found to contain organic acids such as citric, malic and ascorbic acid, sugars and anthocyanin (Grubben, 2004).

The seeds of roselle are used lesser than the calyces. Some people used it in making soaps and consuming in soups after grounding into powder. The seeds contain very high protein, dietary fiber and minerals (Amin Ismail, 2008). Another research found that the oil from roselle seeds contain linoleic, palmitic, oleic, stearic fatty acids (Grubben, 2004)

Roselle has many medicinal properties too. One research screened this plant for it's medicinal importance and it was found that it contains alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols and tannins are present in petals. It was found out that the phenol content was very high while flavonoid content was lower (Obouayeba A Pacome, 2014). Its very high vitamin C, amino acid, iron and potassium content make it a good plant to consume for health. When people have cough, cold and sore throat, consuming Roselle helps. It has good diuretic, anti-spasmodic and antibacterial properties. It helps in stimulating digestive system and improves immune system (Roberts, 2000).

Descriptive Sensory Analysis

This is a technique used for sensory scientists to obtain complete sensory descriptions of products. The sensory qualities to be described include aroma, flavor, texture and sound of the food. Descriptive analysis is used in new product development, quality control, to define product attributes for consumers, to tract sensory changes over time,

for shelf life study and to measure sensory attributes to compare with instrumental measurements (Walker, 2004).

There are various steps to implementing this technique:

1. Selection and screening of panel members

Descriptive analysis needs panelists. The number of panelist depends on the type of descriptive analysis one does. They will be trained and be given orientation before the final testing of the products. However panelists should be screened in regards to their ability to discriminate products, allergies, dentures, physiology, and motivation and product usage. For the entire analysis to go smoothly it is recommended to do individual interviews to know their motivation and personality. Dietary questionnaires can be designed to find out the eating habits of the possible panelists (J.M. Murray, 2001).

2. Training phase

The selected panelists are exposed to wide range of products in a specific product category. Then the panelists led by an experienced panel leader generate terms to describe the products presented. Panelists should have the same concept of the terms developed. Consensus is developed of the definition of the terms generated (Walker, 2004). Reference standards are used and scorecards are developed.

3. Assess panelist's performance

The panelists are assessed for their accuracy and precision in their responses. Few samples from the real samples are used for this step. If the panelists perform well, they proceed to evaluation of the samples step. However if they perform poorly, the training step is done again.

4. Product Evaluation

The trained panelists in duplicates or triplicates analyze the final products. The factors for the test controls are kept constant for each session. And data are analyzed according to the appropriate statistical program.

There are few methodologies carried out in descriptive sensory analysis:

1. Flavor profile method

This is the first method to be developed in 1940s. This is a consensus technique; vocabulary development and attribute ratings are from group discussion. This technique uses 4-6 panelists. They are trained 2-3 weeks. Numbers and symbols are used for the scale. However, regarding this method the number of panelists is too less. Data are difficult to interpret because of the inclusion of symbols (Walker, 2004).

2. Texture profile method

Texture can be classified into mechanical, geometrical and other characteristics. This method tries to bridge gap between expert and consumer texture terminology. A panel of 10 panelists is trained for 6-7 months. They use standardized terminologies. There are specific protocols and procedures for panelists to follow during the training and testing. The description is recorded from first bite through complete mastication. Results are consistent and accurate. However this method is time-consuming (Walker, 2004).

3. Sensory Spectrum

This is a technique that has been built upon the idea of texture profile method. This method evaluates the whole "spectrum" of the product attributes. Panelists generate their own terms and could borrow language from other developers. Training is done for several months. Panel leader teaches the panelists to rate. Reference points are used and intensity scales are absolute. Panelists use the scale in the same manner and this is a good method for quality assurance. However, this method is time consuming and sometimes the reference products are not always available (Walker, 2004).

4. Quantitative Descriptive Analysis

This method analyzes all the sensory attributes of a product. 10-12 panelists are chosen from frequent users of the product. They are trained for 3-4 weeks. Everyday language is used to generate terms. Reference standards are selected. Panel leader acts as a facilitator. This method has many advantages such as there are no influence from the panel leader, less time consuming and statistics can be used to analyze results. However this method could be expensive (Walker, 2004).

5. Free – choice profiling

This method is developed to assist the demands of marketing and product development teams. It is assumed here that the panelists do not differ in their perceptions but differ in how they describe the products. Consumers of the products are used to generate product sensory profile. And then panelists quantify product attributes and come up with their own score sheet. This method requires minimal panel training and non-technical language. However it can be time consuming when individual ballots are handled and interpretation can be difficult.

Methodology

Part 1: Preparation of starter culture and base wine

1.1 Starter Culture

5% starter was prepared for 15L of base wine. A dried roselle: water ratio of 200g: 5L was used. 48g of dried roselle were weighed. 1.2L of water was put into a pot. Then it was brought to boil. The weighed dried roselle was put in the boiling water and let to boil for 10 minutes. While the roselle was boiling, sugar was constantly added in little amounts till the final brix of the must was 15°Brix. This was measured using the refractometer (0-32°Brix). The pH was checked using the pH meter (HI 98127 HANNA instrument) to see if it was between 2.5-3.5. $CaCO_3$ was added to bring the pH up. After the must was stopped from boiling, 2.4g of DAP was added into the mixture. The final pH and °Brix are recorded. This was poured into a sterilized container and let to cool. Pasteur red is inoculated after it was cooled. Then the must is left to ferment. Every day the °Brix of must was measured using refractometer and it was observed whether the fermentation was gone right. This starter culture was used when the °Brix became constant.

1.2 Base wine

570g of dried roselle wine were weighed. 14,250 L of water was put to boil. The dried roselle was put into the boiling water. While the roselle was boiling, sugar was constantly added in little amounts till the final brix of the must was 20-23°Brix. The pH of the mixture was checked to see if it were between 2.5-3.5. $CaCO_3$ was added to bring the pH up. After the must was stopped from boiling, 28.5g of DAP was added into the mixture. The final pH and °Brix are recorded. This was poured into a sterilized vat and let to cool. 1.2 L of the starter culture was poured into the vat. Everyday the pH and °Brix of the fermenting wine was recorded and observed using the refractometer. Punching was done with a sterilized wooden stick to extract the color of the roselle and also to prevent the fermenting vat from bursting due to accumulation of carbon dioxide. Fermentation was allowed to carry on till the °Brix was stabilized. Two batches of base wine were prepared as replicates.

1.3 Aging

3 bottles each with 200mL fermented roselle wine aging starter were prepared. 3 strains of aging yeast were inoculated in each bottle. For this project yeast 9, yeast 15 and yeast 16 strains were chosen. The strains were let to grow for a week. Each batch of base wine was divided into five aliquots. One was reserved for panel training. One was kept as control and no aging yeasts were inoculated. Other 3 aliquots were inoculated with the 200mL of above-mentioned 3 aging yeasts respectively. The wines were allowed to age for 12 weeks at 10°C.

Part 2: Chemical Analysis of the wine

Chemical analyses were done before the wine samples are aged and every 3 weeks during the aging period till the end of aging (12 weeks). pH of the wine samples was measured using the pH meter. The total soluble solid content was measured using

refractometer. Total acidity and volatile acidity are measured and calculated using titration method. Wine samples are titrated using 0.1M NaOH. Ebulliometer was used to measure the percentage alcohol. The Ebulliometer compares the boiling point of water and wine samples. Reducing sugar is measured using a 3,5-dinitrosalicylic acid (DNS) assay (Alexander V. Gusakov, 2011). A standard curve using glucose solution was constructed. Using the spectrophotometer at 575nm, the absorbance values of the samples were measured. And plotting the absorbance values on the standard curve, reducing sugar contents were derived. Colors of the samples were measured using the colorimeter. L*, a* and b* were measured and recorded.

Part 3: Sensory Analysis

1. Selection and training of panelists

6 panelists were chosen from ABAC fourth year class. 20mL of base wine was given to the panelists. They were told to generate all the sensory attributes that they could perceive. A general discussion was done to coin down the characteristics to 10. A vote was taken on the sensory attributes mentioned by each panelist. The commonly perceived characteristics were put on the list.

After the list of ten characteristics was drawn, training was done among the panelists. Reference standards were decided on. Using the reference standards the understanding of the attributes were equalized and made uniform among the panelists.

Table 32 Reference standards for sensory attributes

Attribute	Reference standard
Purplish red	[redacted]
Over ripe grape aroma	20 mL of fermented grape juice
Ethanol aroma	20 mL of 70% ethanol
Woody/oaky flavor	Roselle wine aged in oak wood chips
Body/ Mouth feel	20 mL milk and 20 mL drinking water
Astringent	20 mL of green tea

2. Assessment of panelists and final sensory analysis

Panelists after 5 sessions of training, one mock sensory test was done. 3 samples from the 8 samples were used for this test. The panelists that were doing poorly were trained again. Final analysis was done after that. 8 samples were presented from the two batches of wine samples. 2 duplications were done for each sample. In total 16 data were collected. Intensity scores on a 15-point hedonic scale were used for both the mock and final sensory tests.

Part 3: Statistical Analysis

Using SAS program, the chemical data and sensory data were analyzed. Randomized block design was used to analyze both the data types. Multiple comparison tests were done. LSD was used to analyze the data between the aged samples. Dunnett test was done to analyze the difference from the base wine after the wine samples have been aged. Dunnett test was also done in sensory data to analyze the difference from control. All the tests were analyzed at 5% level of significance.

Results and Discussions

The two batches were to be treated as replications. However in this project, through statistical analysis it was found that there were significant differences between the two batches. That's why the batches were analyzed separately. This is common in wine industry. However to keep the quality of the raw materials consistent, one can measure the raw material characteristics such as the pH, TTS, %reducing sugar, %TA, %VA and color. The batches of raw materials that resembled the characteristics of the measured batch should be used for this project. This step wasn't taken in this project. Another factor that led to the two batches being different could be because of the aging time of the two batches. There was a gap of 2 weeks between the two wine batches but the sensory testing was done at the same time. To control this, one can do separate sensory testing for the different batches.

Chemical Attributes

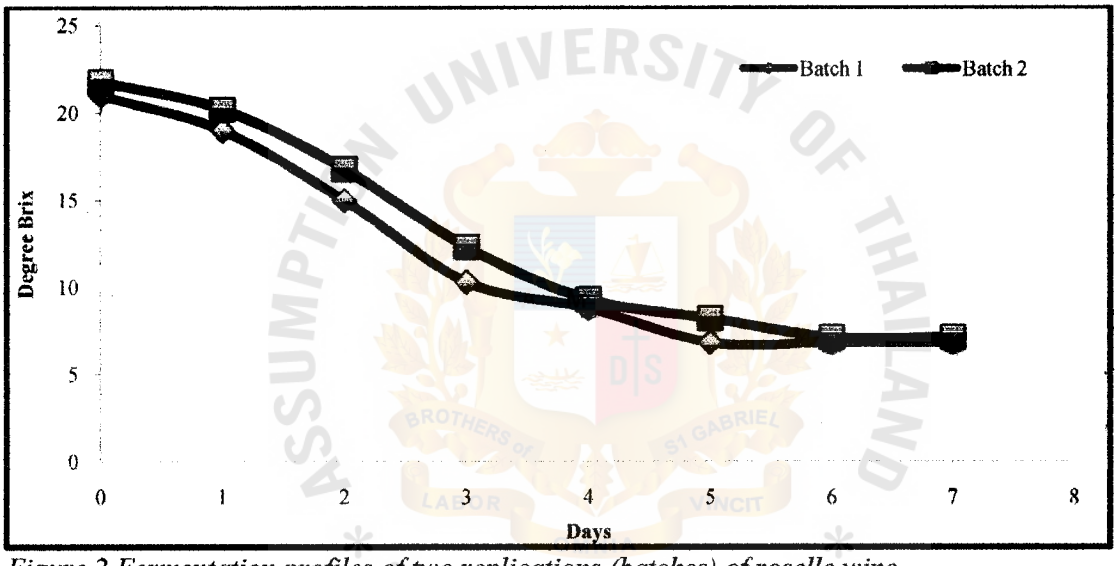

Figure 2 Fermentation profiles of two replications (batches) of roselle wine

Table 33 Chemical characteristics of base wine of two replications of roselle wine

Wine Sample	°Brix	pH	Reducing sugar (µg/mL)	%Alcohol	%TA	%VA	Color
Rep 1	6.8	3.59	1180	11.15	0.96	0.31	L* =8.84 a*=23.03 b*=6.68
Rep 2	7.4	3.58	774	12.85	1.11	0.41	L*=17.10 a*=44.18 b*=26.27

Fermentation profile showed that both the replications took about 7 days to stabilize the total soluble solids content (°Brix). From the figure 1, replication 2 started off with a higher total soluble content than replication 1. Through the two profiles shown in the graph replication 2 had better fermentation, which explains the higher alcohol content as shown in table 1.

The two replications of base wine had characteristics that were in the acceptable range for a standard commercial wine except for total acid and volatile acid contents. The total acid and volatile acid content of the two replications of base wine were higher than the standard level of 0.6-0.9% tartaric acid and 0.11- 0.12% acetic acid used for table grape wine (Pandell, 1999). This could have been because of the already present high amount of acid in the roselle calyces. The roselle extracts are found to have high amount of organic acids. Organic acids such as hibiscus acid (13-24%), citric acid (12-20%), malic acid (2-9%) and tartaric acid (8%) are found in roselle extract (Inês Da-Costa-Rocha, 2014).

The base wines can be classified whether they are sweet wine or dry wine by looking at their reducing sugar level. According to International Organization of Vine and Wine (2009) standard, both the replicates fall under the category of dry wine (<5 g/L).

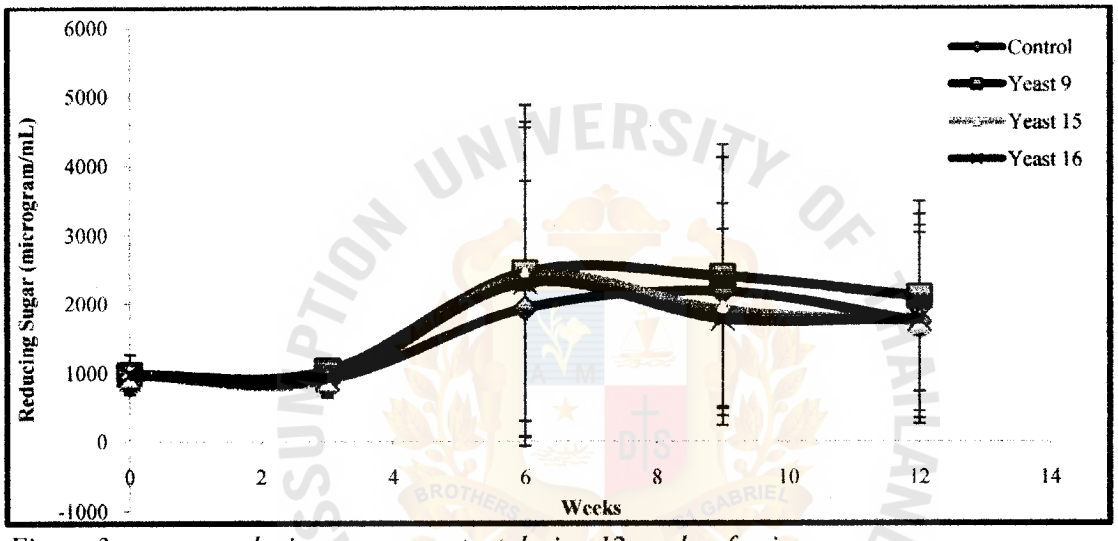

Figure 3 average reducing sugars content during 12 weeks of aging

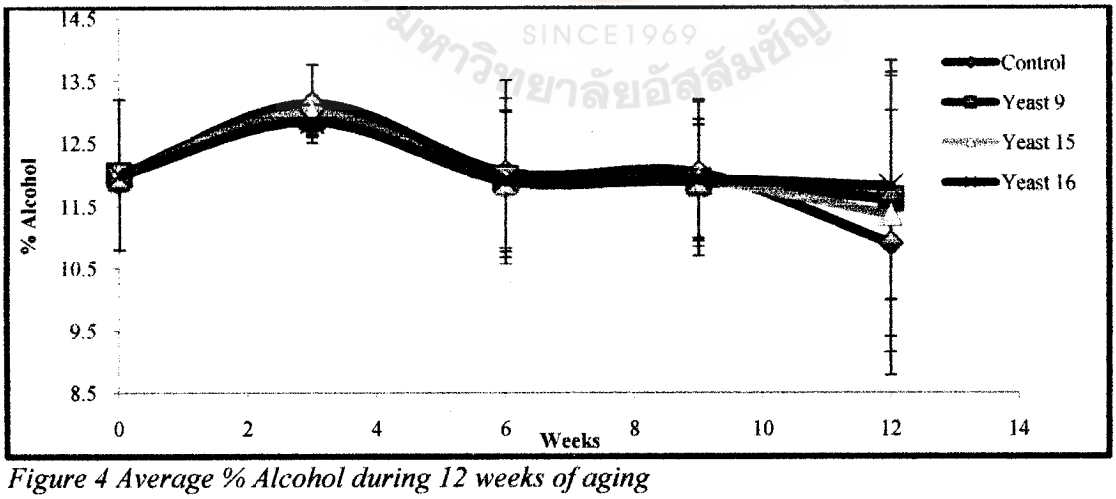

Figure 4 Average % Alcohol during 12 weeks of aging

Theoretically, reducing sugar gets utilized by microorganisms and converted to alcohol. There is a negative correlation between these two attributes. This can be generally seen in the above two graphs. At week12, yeast 16 is seen have lower reducing sugar and highest % alcohol. Control is seen to have the least % alcohol that

suggests that the aging yeasts have utilized reducing sugar available in the wine and converted them to alcohol.

There is a high increase in the % alcohol even though there isn't much increase in the reducing sugar. This suggests that the phenomenon of refermentation could have occurred where the fermentation yeasts might have been carried forward and utilizes the remaining sugar in the wine. The amount of reducing sugar is seen to risen in other samples except the control. This could be because of other oligosaccharides and monosaccharides that are there in roselle that *S.cerevisaie* cannot utilize but the aging yeasts can. Roselle is found to contain arabinose, galactose, glucose, rhamnose and smaller amounts of galacturonic acid, glucuronic acid, manose and xylose. Studies have been undergoing to develop strains of *S.cerevisaie* to ferment arabinose, xylose, galacturonic acid and rhamnose (Maris AJ, 2006) but a commercial Pasteur Red strain of *S.cerevisaie* is unlikely to be able to ferment the above sugars.

The aging yeasts could also have been able to utilize some of those sugars and converted them to alcohol. Or it could be that the aging yeasts themselves have produced exopolysaccharides during aging that could have been broken down to alcohol. Some species of yeasts were previously observed to produce exopolysaccharides. *Candida boidinii, Cryptococcus laurentii, Hansenula capsulata, Lipomyces starkeyii, Rhinocladellia elatior*, and *Rhodotorula glutinis* were found to produce exopolysaccharide that composed of glucose and mannose in ethanol media (G.R. Petersen, 1990).

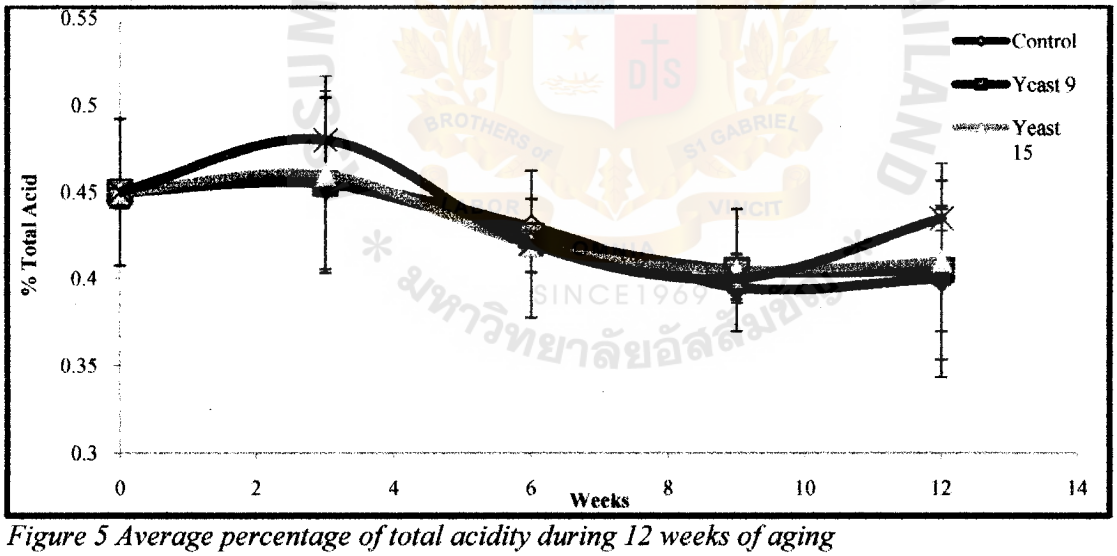

Figure 5 Average percentage of total acidity during 12 weeks of aging

The acidity of the samples was seen to decrease gradually during the aging period. This is because of the acid precipitates and esters are also formed (Dharmadhikari, 2015). Esterification occurs when acid reacts with alcohol. So when the alcohol level increases in the wine, the esterification rate increases thus decreasing the acidity of wine. The hydrogen ions in the wine act as catalyst in esterification reaction ethanol and the acids. Wine is said to balance and reach the equilibrium state where there are equal parts of alcohol, esters, acids and water (Hesseling, 2014). This could explain the fluctuation of the levels. At the end of aging, the acidity of the wine samples seem to increase especially in sample with yeast 16, this could be because of the wine trying to balance its chemical attributes as mentioned before.

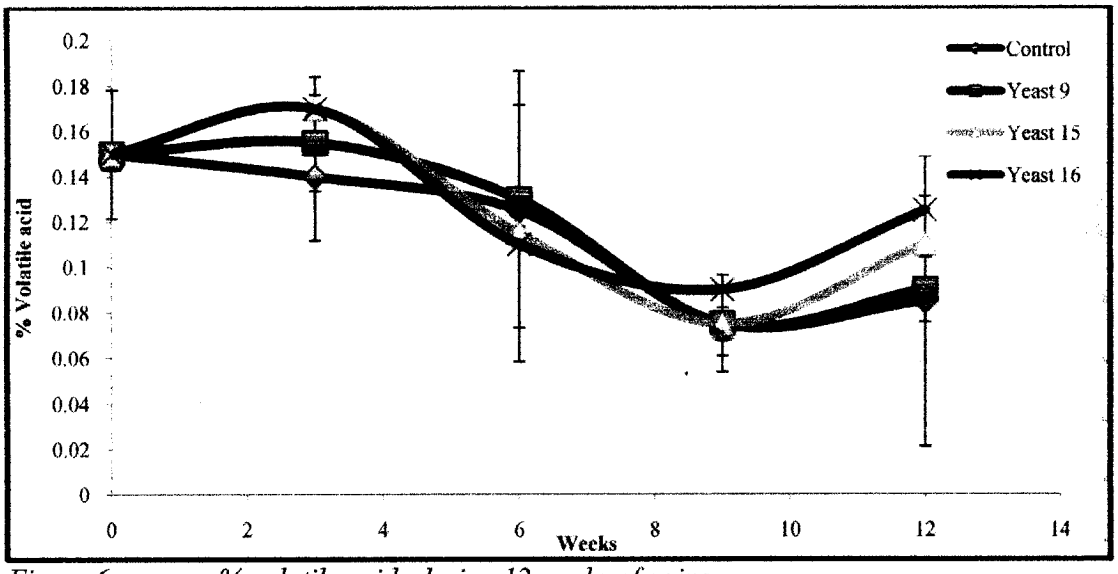

Figure 6 average % volatile acids during 12 weeks of aging

The % volatile acid content follows the same trend as that of % total acid; it fluctuates during the course of aging period. From figure 5 one can see that during the earlier period of aging, the samples that have been inoculated with aging yeasts seem to have produced higher volatile acid content. This must be due to the aging yeasts producing volatile acids. In beer aging, yeasts are found to produce volatile acids such as acetic acid, caprylic acid, capric and lauric acid (Hill, 2015). Many yeast species in wine aging are seen to produce volatile acid such as acetic acid. One research even saw drastic increase in volatile acid content of white wine (0.31g/L to 0.75g/L) in the presence of yeast specie *Brettanomyces.*

Table 34 Chemical characteristics of aged wine samples and base wine from replication 1

Sample	Reducing sugar (µg/mL)	%Alcohol	%TA	%VA
Base wine (0 week)	1180 ± 70.7	11.2 ± 0.2	0.96 ± 0.03	0.31± 0.04
Control (week 12)	2650 ± 70.7*	9.4 ± 0.1*	0.83 ± 0.00*	0.12 ± 0.03*
Yeast 9 (week 12)	3075 ±106.1*	10.1 ± 0.1*	0.87 ± 0.00*	0.18 ± 0.02^{ns}
Yeast 15 (week 12)	2725 ± 35.4*	9.8 ± 0.1*	0.85 ± 0.03*	0.19 ± 0.01^{ns}
Yeast 16 (week 12)	2850 ± 70.7*	10.5 ± 0.2^{ns}	1.01 ± 0.01^{ns}	0.26 ± 0.03^{ns}

Note: Dunnett Test was done to compare samples with base wine
* Means significantly different (p=0.05)

Table 35 Chemical characteristics of aged wine samples and base wine from replication 2

Sample	Reducing sugar (µg/mL)	%Alcohol	%TA	%VA
Base wine (0 week)	774 ± 19.8	12.9 ± 0.2	1.11 ± 0.01	0.41 ± 0.03
Control (week 12)	820 ± 28.3^{ns}	12.4 ± 1.3^{ns}	1.01 ± 0.01*	0.30 ± 0.01*
Yeast 9 (week 12)	1130 ± 42.4*	13.2 ± 0.5^{ns}	1.00 ± 0.01*	0.25 ± 0.01*
Yeast 15 (week 12)	750 ± 98.9^{ns}	12.9 ± 0.1^{ns}	1.02 ± 0.01*	0.34 ± 0.01*
Yeast 16 (week 12)	700 ± 28.3^{ns}	13.1 ± 0.0^{ns}	0.98 ± 0.01*	0.32± 0.01*

Note: Dunnett Test was done to compare samples with base wine
* Means significantly different (p=0.05)

As expected the chemical characteristics of the aged wine are different from the base wine; though some were significant while others not. Replication 1 showed significant

differences in terms of reducing sugar in all samples and in replication 2 only the sample with yeast 9 did. In both the replications, sample with yeast 9 had the highest reducing sugar. However the % alcohol in both the replications display samples with yeast 9 and yeast 16 to have the highest alcohol content. The theory of having lower reducing sugar and higher alcohol content can be seen in the sample with 16 especially in replication 2. However sample with yeast 9 having high alcohol content and reducing sugar is peculiar. This could be because yeast 9 produces exopolysaccharides that is more than the other 2 yeast strains. Total acidity and volatile acidity seemed to decrease in both the replications over the course of aging.

When the aged samples were compared, there were significant differences (tables 4&5). Samples with yeast 9 and 16 had the highest level of reducing sugar and alcohol while control displayed the opposite. Yeast 9 displayed to have the lowest % volatile acid compared to the other two yeast strains, this could be because yeast 9 has low volatile acid such as acetic acid producing characteristics.

Table 36 Comparison of chemical characteristics of aged wine samples from replication 1

Sample	Reducing sugar (µg/mL)	%Alcohol	%TA	%VA
Control	2650 ± 70.7^{B}	9.4 ± 0.1^{C}	0.83 ± 0.00^{B}	0.12 ± 0.03^{B}
Yeast9	3075 ± 106.1^{A}	10.1 ± 0.1^{AB}	0.87 ± 0.00^{B}	0.18 ± 0.02^{AB}
Yeast15	2725 ± 35.4^{B}	9.8 ± 0.1^{BC}	0.85 ± 0.03^{B}	0.19 ± 0.01^{AB}
Yeast16	2850 ± 70.7^{AB}	10.5 ± 0.2^{A}	1.01 ± 0.01^{A}	0.26 ± 0.03^{A}

Note: RCBD and LSD was done to compare samples after aging (p=0.05)

Table 37 Comparison of chemical characteristics of aged wine samples from replication 2

Sample	Reducing sugar (µg/mL)	%Alcohol	%TA	%VA
Control	820 ± 28.3^{B}	12.4 ± 1.3^{A}	1.01 ± 0.01^{AB}	0.30 ± 0.01^{A}
Yeast9	1130 ± 42.4^{A}	13.2 ± 0.5^{A}	1.00 ± 0.01^{AB}	0.25 ± 0.01^{B}
Yeast15	750 ± 98.9^{B}	12.9 ± 0.1^{A}	1.02 ± 0.01^{A}	0.34 ± 0.01^{A}
Yeast16	700 ± 28.3^{B}	13.1 ± 0.0^{A}	0.98 ± 0.01^{B}	0.32 ± 0.01^{A}

Note: RCBD and LSD was done to compare samples after aging (p=0.05)

Sensory Attributes

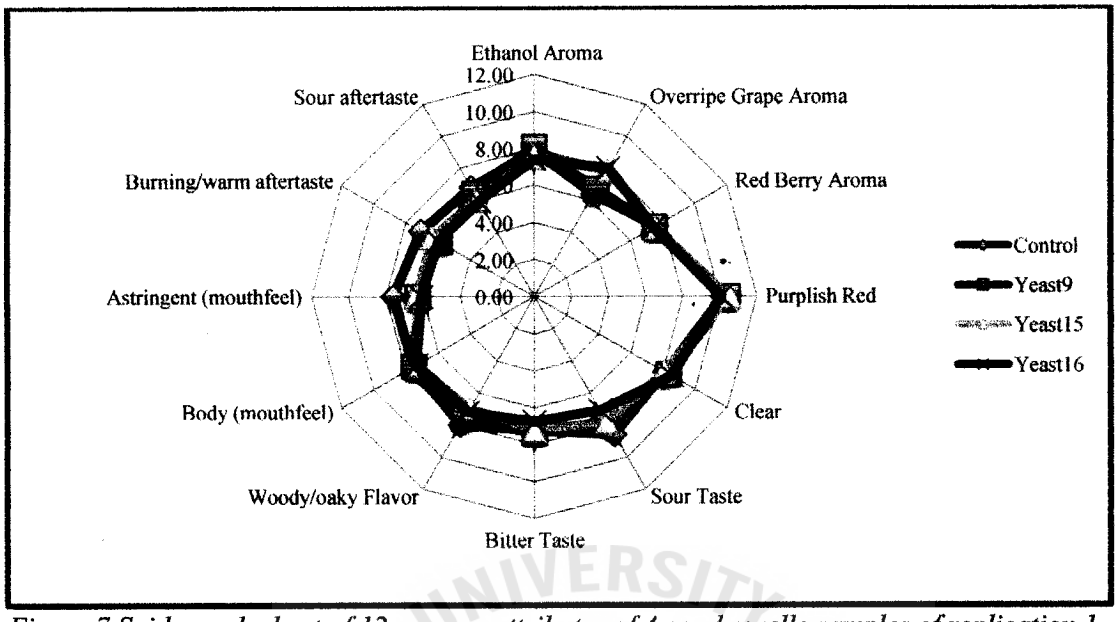

Figure 7 Spider-web chart of 12 sensory attributes of 4 aged roselle samples of replication 1

In replication 1 all the characteristics had no significant differences between the samples except for burning/warm aftertaste. Samples with yeast 9 and yeast 16 were seen to be significantly different from the control ($p<0.05$).

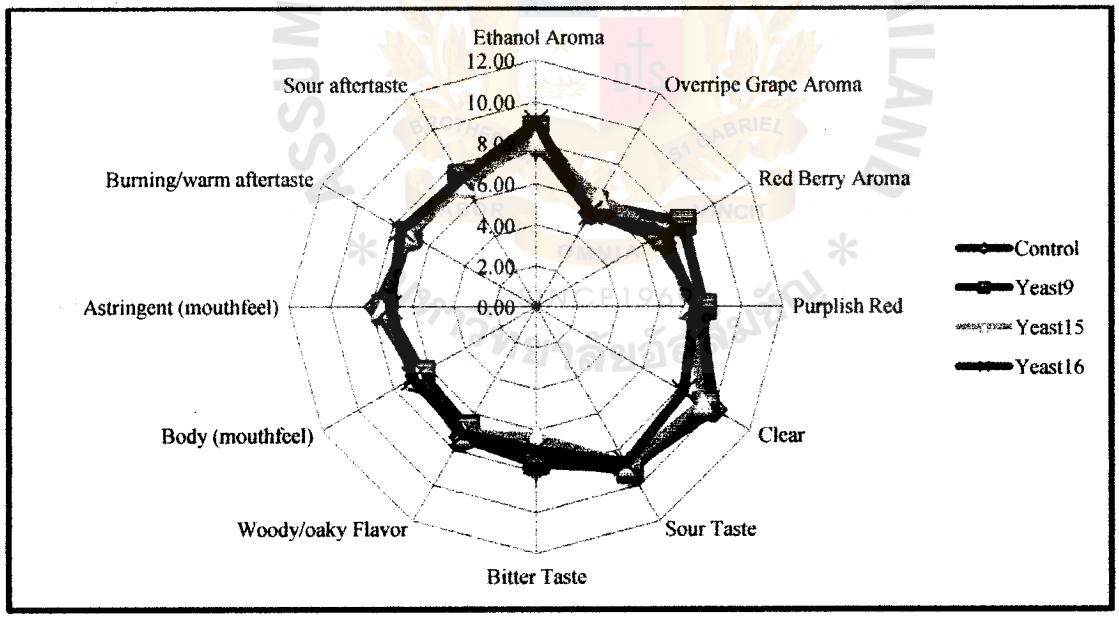

Figure 8 Spider-web chart of 12 sensory attributes of four aged roselle wine of replication 2

There are no significant differences in the characteristics in replication 2 either except for clear and bitter taste. Sample with yeast 16 was seen to be significantly different from the control in clarity and sample with yeast 15 was in bitter taste ($p<0.05$).

Overall, there were minimal significant differences in the sensory characteristics of the samples. There could be many reasons as to why the results. By looking at the chemical characteristics there definitely are changes happening in the wine samples. However it could have been that the panelists could not detect the changes. The

threshold to sense the characteristics might have been too low in the samples despite the chemical chemicals.

Another reason definitely could be due to the panelists. The training session might have been too short and the panelists were chosen from graduating class of Assumption who necessarily are not regular wine drinkers. If this project were to be repeated, the selection of the panelists should be done very carefully. There should be screening questionnaire that could screen the regular consumers from those who aren't. A panel of regular consumers of wine would be able to devise characteristics of the samples in more diverse ways. And since they are already used to the taste of wine, they would be able to detect changes more accurately. The training session of the panelists should be longer. However if the panel is composed of regular consumers, the training time may be short.

Through the previous works of Ms. Sasivimon, yeast 9 was seen to have more solvent in it (2008). And through unofficial testing of the wine samples among regular drinkers it was noted that sample with yeast 9 had different mouth feel compared to other samples. The sample was observed to have higher body compared to others. However this claim cannot be scientifically and statistically proven in this project. Further studies and research has to be done to be able to prove this.

Conclusions

Through statistical analysis it was found that there were some significant differences in the chemical characteristics of the wine samples that have been aged with yeast 9, yeast 15 and yeast 16. In replication 1 there were significant differences in reducing sugar (2650-3075μg/mL), alcohol content (9.4-10.5%), total acidity (0.83-1.01%) and volatile acidity (0.12-0.26%). Replication 2 showed significant differences in reducing sugar (700-1130μg/mL), total acidity (0.98-1.02%) and volatile acidity (0.25-0.34%). However there were very less sensory characteristics that were significantly from each other. In replication 1 sample with yeast 16 was seen to be significantly different from the control in clarity and sample with yeast 15 was in bitter taste ($p \geq 0.05$).

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Appendix

Method and Formula

YM agar

Glucose	10 g/liter
Peptone	5 g/liter
Yeast extract	3g/liter
Malt extract	3g/liter

Autoclave at 121°C for 15 minutes (Wanjaroen, 2006)

3,5 –dinitrosalicylic acid (DNS) reagent

Dinitrosalicylic acid	10 g/liter
Phenol	2 g/liter
Sodium sulfite	0.5 g/liter
Sodium hydroxide	10 g/liter

40% Potassium tartrate solution

Potassium sodium tartrate	40 g
Distilled water	100 mL

Chemical Analysis

1. Total acidity

%Total Acid = g/100mL as Malic acid

$$\% \text{ TA} = \frac{V \times N \times 134}{100}$$

Where V is the volume of NaOH and N is the normality of NaOH. 134 g/mol is the molecular weight of malic acid.

2. Volatile acidity

%Volatile acid = g/100mL as acetic acid

$$\% \text{ VA} = \frac{V \times N \times 60}{100}$$

60 g/mol is the molecular weight of acetic acid.

3. Reducing sugar

Sugar stock solution = 0.1 g/mL

Table 38 Preparation of solutions for standard reducing sugar curve

Conc. (mg/mL)	Sugar solution (µL)	Water (µL)	Total volume (mL)
000	0000	5000	5
200	1000	4000	5
400	2000	3000	5
600	3000	2000	5
800	4000	1000	5
1000	5000	0000	5

Procedure

3mL of DNS reagent was added to 3mL of the supernatant in a lightly capped test tube. This was then heated at 90°C for 5-15 minutes until red brown color was developed. 1 mL of 40% potassium sodium tartrate solution was added to stabilize the color change. This was then cooled to room temperature. Using the spectrophotometer, absorbance was measured at 575nm. Standard curve was constructed. The samples were then plotted in the graph to find the amount of reducing sugar.

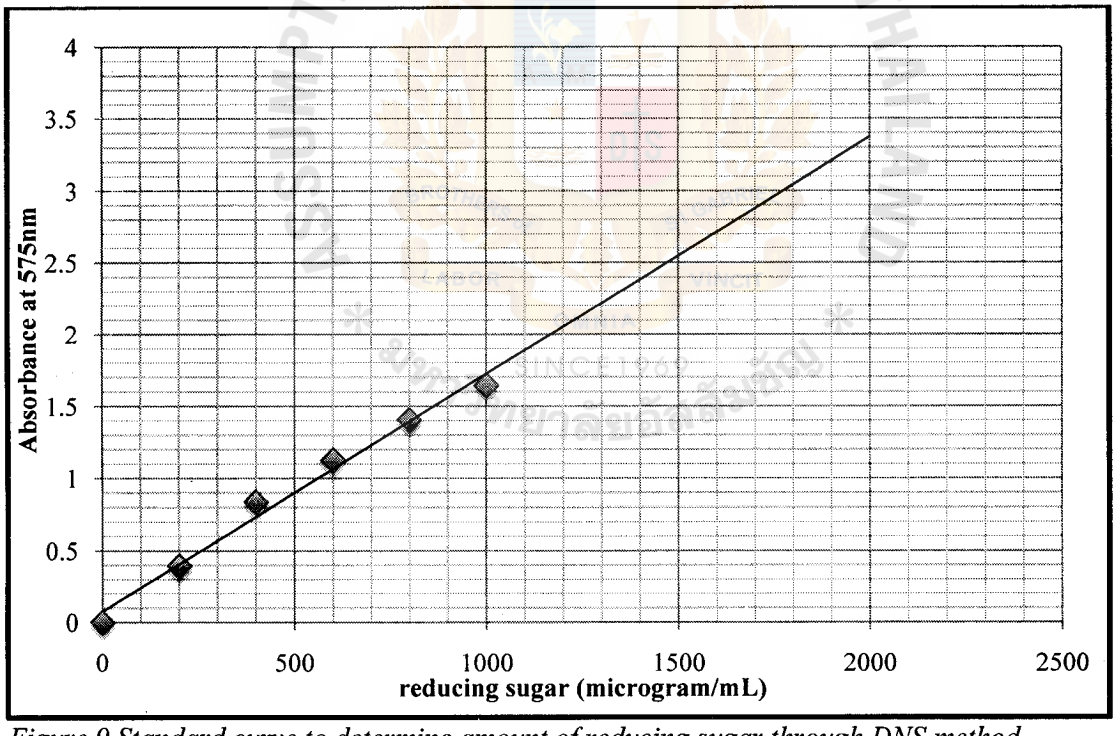

Figure 9 Standard curve to determine amount of reducing sugar through DNS method

Chemical Data

Table 39 Chemical data for replication 1

Week	Sample	TTS	pH	Reducing sugar ($^{\mu g}/_{ml}$)	%Alc.	%TA	%VA	L*a*b*
0	Base wine	6.8	3.59	1180	11.15	0.96	0.31	L* =8.84 a*=23.03 b*=6.68
3	Control	6.6	3.58	1100	12.70	0.96	0.29	L* =9.94 a*=30.15 b*=10.83
	Yeast 9	6.0	3.59	1090	12.70	0.95	0.31	L* = 9.16 a*=34.88 b*= 13.49
	Yeast 15	6.4	3.62	1060	12.80	0.96	0.33	L* = 8.74 a*=34.73 b*= 13.15
	Yeast 16	6.6	3.59	1100	12.70	1.04	0.35	L* = 9.93 a*=34.31 b*=13.22
6	Control	7.0	3.74	3240	11.00	0.98	0.30	L* = 7.06 a*=31.53 b*=10.37
	Yeast 9	6.9	3.78	4000	11.05	0.99	0.37	L* =7.29 a*=30.40 b*= 9.57
	Yeast 15	6.8	3.76	4150	11.10	0.95	0.25	L* = 7.32 a* =29.45 b*=9.29
	Yeast 16	6.9	3.69	3900	11.15	1.02	0.33	L* = 5.39 a* =21.58 b*=4.73
9	Control	6.2	3.77	3550	11.20	0.92	0.17	L*= 8.33 a*= 31.76 b*= 11.66
	Yeast 9	6.8	3.79	3750	11.25	0.99	0.22	L* = 8.76 a* = 32.58 b*= 12.20
	Yeast 15	6.7	3.77	3000	11.23	0.94	0.18	L* = 9.11 a* = 33.66 b*= 13.43
	Yeast 16	6.6	3.77	2700	11.06	0.94	0.18	L* = 11.65 a* =37.97 b*=17.84
12	Control	6.6	3.71	2650	9.40	0.83	0.11	L* = 8.29 a* = 29.38 b*= 10.94
	Yeast 9	6.4	3.85	3075	10.05	0.87	0.18	L* = 8.02 a* = 31.38 b*= 11.07
	Yeast 15	6.4	3.74	2725	9.80	0.85	0.18	L* = 5.87 a* = 22.19 b*= 5.92
	Yeast 16	6.6	3.77	2850	10.53	1.01	0.25	L* = 9.15 a* = 32.25 b*= 12.71

Table 40 Chemical data for replication 2

Week	Sample	TTS	pH	Reducing sugar ($^{\mu g}/_{ml}$)	%Alc.	%TA	%VA	L*a*b*
0	Base wine	7.4	3.58	774	12.85	0.48	0.17	L* =17.10 a*=44.18 b*=26.27
3	Control	7.2	3.42	720	13.58	0.49	0.16	L*=14.35 a*=41.44 b*=22.70
	Yeast 9	7.6	3.43	980	13.17	0.49	0.17	L*=14.44 a*=42.10 b*=22.88
	Yeast 15	7.4	3.41	740	13.19	0.50	0.20	L* =14.48 a*=42.11 b*=22.96
	Yeast 16	7.4	3.42	760	13.00	0.50	0.18	L*=13.86 a*=40.79 b*=21.68
6	Control	7.6	3.45	620	13.08	0.43	0.12	L* =14.27 a*=40.43 b*=22.53
	Yeast 9	7.4	3.45	930	12.85	0.41	0.09	L* = 13.94 a*= 40.12 b*= 21.90
	Yeast 15	7.2	3.42	660	12.70	0.42	0.12	L* = 14.42 a*= 40.53 b*= 22.20
	Yeast 16	7.4	3.45	720	12.68	0.39	0.07	L* = 13.87 a*=39.92 b*=21.46
9	Control	7.0	3.58	800	12.85	0.39	0.08	L* =14.10 a*= 39.45 b*= 21.72
	Yeast 9	7.0	3.59	1060	12.53	0.38	0.06	L* = 13.56 a*= 38.66 b*= 20.11
	Yeast 15	7.2	3.57	820	12.60	0.4	0.08	L* = 13.93 a*= 39.83 b*= 21.33
	Yeast 16	7.0	3.49	860	12.80	0.39	0.10	L* = 16.08 a*= 41.92 b*= 24.78
12	Control	7.4	3.58	820	12.40	0.44	0.13	L* =13.50 a*=37.41 b*=19.78
	Yeast 9	7.4	3.65	1130	13.18	0.43	0.10	L* = 13.58 a*= 37.26 b*= 19.49
	Yeast 15	7.2	3.62	750	12.93	0.45	0.15	L* = 13.65 a*=38.29 b*= 19.63
	Yeast 16	6.8	3.58	700	13.10	0.43	0.17	L* = 13.67 a*=38.24 b*=19.98

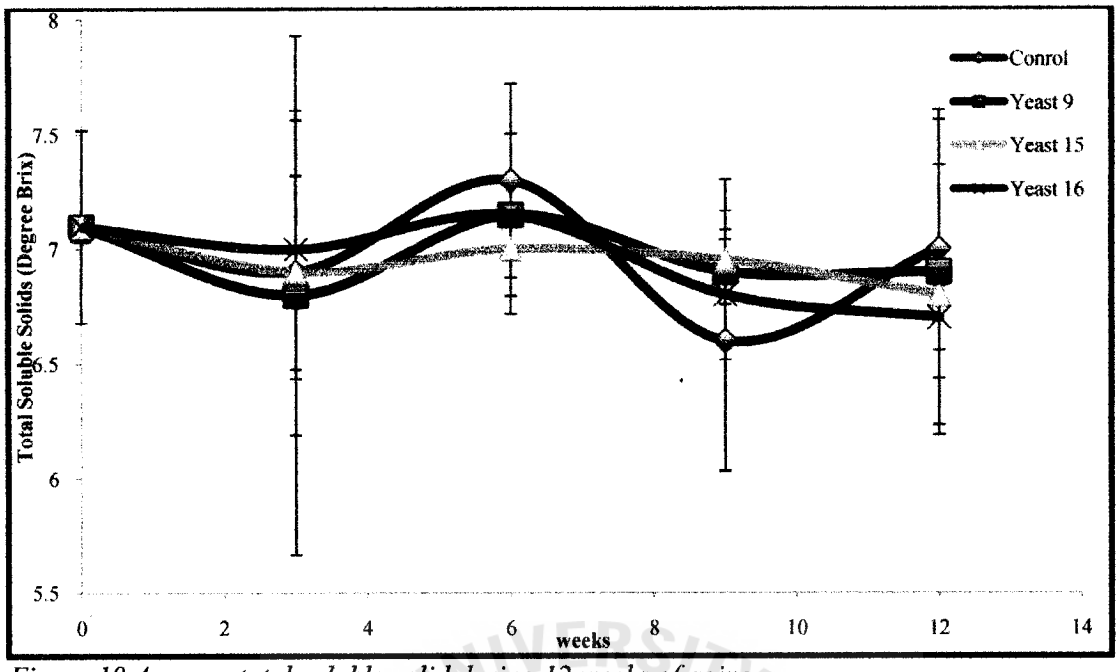

Figure 10 Average total soluble solid during 12 weeks of aging

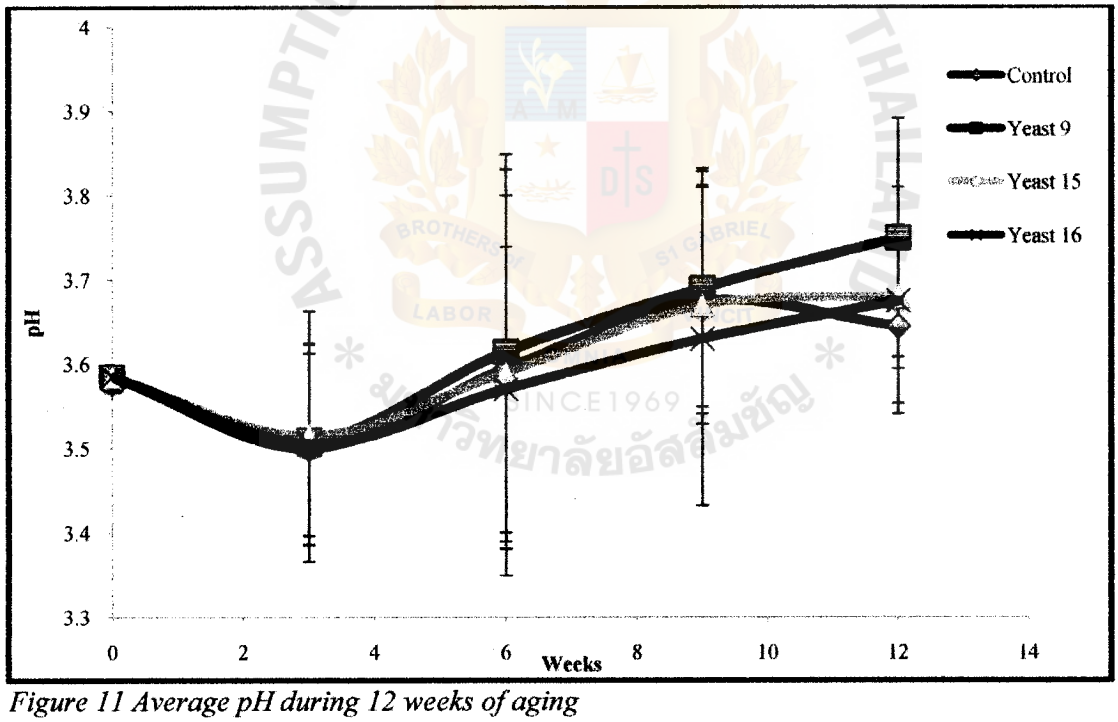

Figure 11 Average pH during 12 weeks of aging

Statistical analysis

Table 41 ANOVA output comparing aged roselle wine to base wine of replication 1

Attributes		df	Sum of Squares	Mean Square	F	Sig.
Reducing sugar	Treatment	4	4537140.000	1134285.000	193.07	<.0001*
	Rep	1	4000.000	4000.000	0.68	0.4557
Alcohol	Treatment	4	3.65900000	0.91475000	31.82	0.0027*
	Rep	1	0.00625000	0.00625000	0.22	0.6653
Total Acid	Treatment	4	0.04694000	0.01173500	22.79	0.0052*
	Rep	1	0.00004000	0.00004000	0.08	0.7943
Volatile Acid	Treatment	4	0.04514000	0.01128500	7.12	0.0418*
	Rep	1	0.00121000	0.00121000	0.76	0.4316

Table 42 Dunnett test comparing reducing sugar of aged roselle wine to base wine of replication 1

Comparisons significant at the 0.05 level are indicated by ***.

Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast9 - Basewine	1895.00	1601.32	2188.68	***
Yeast16 - Basewine	1670.00	1376.32	1963.68	***
Yeast15 - Basewine	1545.00	1251.32	1838.68	***
Control - Basewine	1470.00	1176.32	1763.68	***

Table 43Dunnett test comparing % alcohol of aged roselle wine to base wine of replication 1

Comparisons significant at the 0.05 level are indicated by ***.

Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast16 - Basewine	-0.6250	-1.2747	0.0247	
Yeast9 - Basewine	-1.1000	-1.7497	-0.4503	***
Yeast15 - Basewine	-1.3500	-1.9997	-0.7003	***
Control - Basewine	-1.7500	-2.3997	-1.1003	***

Table 44 Dunnett test comparing % TA of aged roselle wine to base wine of replication 1

Comparisons significant at the 0.05 level are indicated by ***.

Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast16 - Basewine	0.04000	-0.04695	0.12695	
Yeast9 - Basewine	-0.09500	-0.18195	-0.00805	***
Yeast15 - Basewine	-0.11500	-0.20195	-0.02805	***
Control - Basewine	-0.13500	-0.22195	-0.04805	***

Table 45 Dunnett test comparing %VA of aged roselle wine to base wine of replication 1

Comparisons significant at the 0.05 level are indicated by ***.

Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast16 - Basewine	-0.05500	-0.20754	0.09754	
Yeast15 - Basewine	-0.12500	-0.27754	0.02754	
Yeast9 - Basewine	-0.13000	-0.28254	0.02254	
Control - Basewine	-0.19500	-0.34754	-0.04246	***

Table 46 ANOVA output comparing chemical data among aged roselle wine for replication 1

Attributes		df	Sum of Squares	Mean Square	F	Sig.
Reducing sugar	Treatment	3	207500.0000	69166.6667	9.76	0.0467*
	Rep	1	1250.0000	1250.0000	0.18	0.7027
Alcohol	Treatment	3	1.33093750	0.44364583	17.53	0.0209*
	Rep	1	0.00031250	0.00031250	0.01	0.9185
Total Acid	Treatment	3	0.03763750	0.01254583	51.03	0.0045*
	Rep	1	0.00011250	0.00011250	0.46	0.5472
Volatile Acid	Treatment	3	0.01963750	0.00654583	9.19	0.0506
	Rep	1	0.00361250	0.00361250	5.07	0.1098

Note: * means significantly different

Table 47 LSD test of reducing sugar of aged roselle wine for replication1

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	3075.00	2	Yeast9
AB	2850.00	2	Yeast16
B	2725.00	2	Yeast15
B	2650.00	2	Control

Table 48 LSD test for % alcohol of aged roselle wine samples for replication 1

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	10.5250	2	Yeast16
AB	10.0500	2	Yeast9
BC	9.8000	2	Yeast15
C	9.4000	2	Control

Table 49 LSD test of % Total Acid of aged roselle wine for replication 1

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	1.00500	2	Yeast16
B	0.87000	2	Yeast9
B	0.85000	2	Yeast15
B	0.83000	2	Control

Table 50 LSD test of % volatile acid of aged roselle wine for replication 1

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	0.25500	2	Yeast16
AB	0.18500	2	Yeast15
AB	0.18000	2	Yeast9
B	0.11500	2	Control

Table 51 ANOVA output comparing aged roselle wine to base wine of replication 2

Attributes		df	Sum of Squares	Mean Square	F	Sig.
Reducing sugar	Treatment	4	232841.6000	58210.4000	19.65	0.0068*
	Rep	1	1742.4000	1742.4000	0.59	0.4859
Alcohol	Treatment	4	0.73650000	0.18412500	0.45	0.7700
	Rep	1	0.25600000	0.25600000	0.63	0.4726
Total Acid	Treatment	4	0.02106000	0.00526500	30.09	0.0030*
	Rep	1	0.00000000	0.00000000	0.00	1.0000
Volatile Acid	Treatment	4	0.02906000	0.00726500	25.49	0.0042*
	Rep	1	0.00016000	0.00016000	0.56	0.4954

Table 52 Dunnett test comparing reducing sugar of aged roselle wine to base wine of replication 2

Comparisons significant at the 0.05 level are indicated by ***.				
Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast9 - Basewine	356.00	147.46	564.54	***
Control - Basewine	46.00	-162.54	254.54	
Yeast15 - Basewine	-24.00	-232.54	184.54	
Yeast16 - Basewine	-74.00	-282.54	134.54	

Table 53Dunnett test comparing % alcohol of aged roselle wine to base wine of replication 2

Comparisons significant at the 0.05 level are indicated by ***.			
Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
Yeast9 - Basewine	0.3250	-2.1220	2.7720
Yeast16 - Basewine	0.2500	-2.1970	2.6970
Yeast15 - Basewine	0.0750	-2.3720	2.5220
Control - Basewine	-0.4500	-2.8970	1.9970

Table 54 Dunnett test comparing % TA of aged roselle wine to base wine of replication 2

Comparisons significant at the 0.05 level are indicated by ***.				
Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast15 - Basewine	-0.09000	-0.14069	-0.03931	***
Control - Basewine	-0.10500	-0.15569	-0.05431	***
Yeast9 - Basewine	-0.11500	-0.16569	-0.06431	***
Yeast16 - Basewine	-0.13000	-0.18069	-0.07931	***

Table 55 Dunnett test comparing %VA of aged roselle wine to base wine of replication 2

Comparisons significant at the 0.05 level are indicated by ***.				
Treatment Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Yeast15 - Basewine	-0.07000	-0.13468	-0.00532	***
Yeast16 - Basewine	-0.09500	-0.15968	-0.03032	***
Control - Basewine	-0.11000	-0.17468	-0.04532	***
Yeast9 - Basewine	-0.16500	-0.22968	-0.10032	***

Table 56 ANOVA output comparing chemical data among aged roselle wine for replication 2

Attributes		df	Sum of Squares	Mean Square	F	Sig.
Reducing sugar	Treatment	3	223600.0000	74533.3333	22.36	0.0148*
	Rep	1	3200.0000	3200.0000	0.96	0.3994
Alcohol	Treatment	3	0.73250000	0.24416667	0.45	0.7361
	Rep	1	0.21125000	0.21125000	0.39	0.5773
Total Acid	Treatment	3	0.00170000	0.00056667	3.78	0.1519
	Rep	1	0.00005000	0.00005000	0.33	0.6042
Volatile Acid	Treatment	3	0.00970000	0.00323333	19.40	0.0182*
	Rep	1	0.00000000	0.00000000	0.00	1.0000

Note: * means significantly different

Table 57 LSD test of reducing sugar of aged roselle wine for replication 2

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	1130.00	2	Yeast9
B	820.00	2	Control
B	750.00	2	Yeast15
B	700.00	2	Yeast16

Table 58 LSD test for % alcohol of aged roselle wine samples for replication 2

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	13.1750	2	Yeast9
A	13.1000	2	Yeast16
A	12.9250	2	Yeast15
A	12.4000	2	Control

Table 59 LSD test of % Total Acid of aged roselle wine for replication 1

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	1.02000	2	Yeast15
AB	1.00500	2	Control
B	0.99500	2	Yeast9
B	0.98000	2	Yeast16

Table 60 LSD test of % volatile acid of aged roselle wine for replication 2

Means with the same letter are not significantly different			
t group	Mean	N	Treatment
A	0.34000	2	Yeast15
A	0.31500	2	Yeast16
A	0.30000	2	Control
B	0.24500	2	Yeast9

Table 61 ANOVA output comparing sensory attributes of aged roselle wine for replication 1

		df	Sum of Squares	Mean Square	F	Sig.
Ethanol Aroma	Treatment	3	5.29166667	1.76388889	0.60	0.6239
	Panelist	5	16.35416667	3.27083333	1.12	0.3934
	Rep	1	1.33333333	1.33333333	0.45	0.5103
Overripe Grape Aroma	Treatment	3	8.3906250	2.7968750	2.49	0.1003
	Panelist	5	172.1510417	34.4302083	30.61	<. 0001*
	Rep	1	15.7552083	15.7552083	14.01	0.0020*
Red Berry Aroma	Treatment	3	2.14062500	0.71354167	0.22	0.8827
	Panelist	5	45.35937500	9.07187500	2.77	0.0578
	Rep	1	2.29687500	2.29687500	0.70	0.4158
Purplish Red	Treatment	3	1.93229167	0.64409722	0.17	0.9159
	Panelist	5	28.67187500	5.73437500	1.50	0.2479
	Rep	1	0.63020833	0.63020833	0.16	0.6904
Clarity/clear	Treatment	3	0.5208333	0.1736111	0.07	0.9738
	Panelist	5	226.2916667	45.2583333	18.88	<. 0001*
	Rep	1	4.6875000	4.6875000	1.96	0.1823
Sour Taste	Treatment	3	25.06250000	8.35416667	1.43	0.2723
	Panelist	5	69.25000000	13.85000000	2.38	0.0889
	Rep	1	0.52083333	0.52083333	0.09	0.7691
Bitter Taste	Treatment	3	14.0416667	4.6805556	1.25	0.3279
	Panelist	5	144.8125000	28.9625000	7.71	0.0009*
	Rep	1	0.0000000	0.0000000	0.00	1.0000
Woody/Oaky Flavor	Treatment	3	5.1250000	1.7083333	0.69	0.5711
	Panelist	5	132.9166667	26.5833333	10.76	0.0002*
	Rep	1	4.0833333	4.0833333	1.65	0.2180
Body	Treatment	3	1.26562500	0.42187500	0.24	0.8685
	Panelist	5	32.71354167	6.54270833	3.69	0.0224*
	Rep	1	0.25520833	0.25520833	0.14	0.7097
Astringent Mouth feel	Treatment	3	13.93229167	4.64409722	2.85	0.0724
	Panelist	5	62.65104167	12.53020833	7.70	0.0009*
	Rep	1	0.88020833	0.88020833	0.54	0.4734
Burning Mouth feel	Treatment	3	20.89062500	6.96354167	3.39	0.0460
	Panelist	5	87.15104167	17.43020833	8.48	0.0006*
	Rep	1	0.00520833	0.00520833	0.00	0.9605
Sour Aftertaste	Treatment	3	8.85416667	2.95138889	0.86	0.4840
	Panelist	5	71.16666667	14.23333333	4.14	0.0146*
	Rep	1	1.68750000	1.68750000	0.49	0.4943

Note: * means significantly different

Table 62 ANOVA output comparing sensory attributes of aged roselle wine for replication 2

		df	Sum of Squares	Mean Square	F	Sig.
Ethanol Aroma	Treatment	3	4.05729167	1.35243056	0.40	0.7532
	Panelist	5	49.42187500	9.88437500	2.94	0.0479*
	Rep	1	0.00520833	0.00520833	0.00	0.9691
Overripe Grape Aroma	Treatment	3	2.1822917	0.7274306	0.39	0.7605
	Panelist	5	138.4010417	27.6802083	14.92	<. 0001*
	Rep	1	13.5468750	13.5468750	7.30	0.0164*
Red Berry Aroma	Treatment	3	8.16666667	2.72222222	0.93	0.4513
	Panelist	5	25.22916667	5.04583333	1.72	0.1907
	Rep	1	1.68750000	1.68750000	0.58	0.4599
Purplish Red	Treatment	3	4.6406250	1.5468750	0.39	0.7612
	Panelist	5	108.0885417	21.6177083	5.46	0.0046*
	Rep	1	0.6302083	0.6302083	0.16	0.6954
Clarity/clear	Treatment	3	27.5625000	9.1875000	2.76	0.0788
	Panelist	5	139.3541667	27.8708333	8.36	0.0006*
	Rep	1	0.0833333	0.0833333	0.02	0.8765
Sour Taste	Treatment	3	2.3489583	0.7829861	0.20	0.8972
	Panelist	5	162.7760417	32.5552083	8.17	0.0007*
	Rep	1	0.8802083	0.8802083	0.22	0.6452
Bitter Taste	Treatment	3	24.68229167	8.22743056	8.49	0.0016*
	Panelist	5	68.15104167	13.63020833	14.06	<. 0001*
	Rep	1	4.38020833	4.38020833	4.52	0.0505
Woody/Oaky Flavor	Treatment	3	1.55729167	0.51909722	0.34	0.7933
	Panelist	5	22.77604167	4.55520833	3.03	0.0438*
	Rep	1	1.17187500	1.17187500	0.78	0.3915
Body	Treatment	3	4.22916667	1.40972222	1.03	0.4059
	Panelist	5	38.91666667	7.78333333	5.71	0.0038*
	Rep	1	3.52083333	3.52083333	2.58	0.1290
Astringent Mouth feel	Treatment	3	2.8072917	0.9357639	0.24	0.8669
	Panelist	5	158.3593750	31.6718750	8.13	0.0007*
	Rep	1	0.4218750	0.4218750	0.11	0.7466
Burning Mouth feel	Treatment	3	0.3541667	0.1180556	0.09	0.9662
	Panelist	5	195.3750000	39.0750000	28.71	<. 0001
	Rep	1	0.5208333	0.5208333	0.38	0.5455
Sour Aftertaste	Treatment	3	3.0572917	1.0190972	0.24	0.8664
	Panelist	5	114.4635417	22.8927083	5.41	0.0048*
	Rep	1	1.8802083	1.8802083	0.44	0.5150

Note: * means significantly different