Effect of Four Isolated Yeast Strains on Aged Sala Wine

By

Ms. Sasivimon Seemachaiboworn

ID: 471-8803

A Special project submitted to the Faculty of Biotechnology, Assumption University in part fulfillment of the repuirements for the degree of Bachelor of Science in Biotechnology

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Level of study	:	Bachelor of Science
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Effect of Four Isolated Yeast Strains on Aged sala wine

Abstract

In wine, the flavor and aroma of wine could be further modified by various yeasts and bacteria during aging period. The aim of this project was to study the effect of 4 isolated yeast strains on aged sala wine. The four isolated yeast strains (Yeast-No.1, Yeast-No.14, Yeast-No.15 and Yeast-No.30 recovered from Wanjaroen, 2006) were added into sala wine except the control and aged in glass bottle for 8 weeks. The aged sala wines were compared for both chemical and sensory effect. The chemical compositions of aged wine samples including total soluble solid (TSS), pH, total acid (%TA), volatile acid (%VA), alcohol content and reducing sugar were analyzed. The sensory characteristics of aged wine samples were analyzed using intensity test and hedonic scales by 5 partially trained panels. The attribute descriptors that identified by panel including 7 attributes as follow; sala, vinegar, Hale's blue boy, sun-dried banana, black pepper, solvent and dried-honey banana aroma. Both chemical and sensory analyses of five aged sala wines from 3 separate batches were evaluated by One-way ANOVA (SPSS). From statistical analysis, there were non-significant different in chemical compositions for all the wines. For sensory analysis, there were significant different in some aroma attributes, including sala, Hale's blue boy, black pepper, solvent and dried honey banana aroma between aged wine samples. From panelist's acceptability, all aged sala wine samples are acceptable by the panels with no significant different scoring. In conclusion, it's indicated the effect of 4 isolated yeast strains on aged wine had non-significant different in chemical compositions but had significant different in sensory characteristics.

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Introduction

Wine was famous beverages for long time, since it represents a high value added product from fruit and also vinegar as by-product of fruit manufacture (Karuwanna, 2005). The quality of wine is dependent on various factors. The enzyme in different yeast species is being an important factor to give the quality of wine. It is very important to use high quality yeast in all winemaking process. Yeast is the workhorse that converts the initial sweet syrupy must into great-tasting wine. Not all wines are made from grapes. In fact, some of the earliest wines were made from honey and berries. Over the years, grapes have become popular for commercial wineries but fruits are also made into delicious wines as well. During fermentation, yeast spores will reproduce exponentially until all of the fermentable sugars have been consumed. During this fermentation process, the sugars are converted into alcohol and carbon dioxide (Snop, 2007). However, there are many people confused about the role of aging wines. Some don't realize that wines can change in the bottle. Some think that wines will last forever .There is no food product is infinitely stable. Even aging wine under ideal storage condition, deterioration of quality can be occurred. During aging, re-fermentation with the residual sugar that present in the bottle of wine. It occurs when sweet wines are bottled in non-sterile conditions, allowing the presence of microorganisms. The strains of yeast, *Saccharomyces cerevisiae* are mostly used for fermentations of sugar into alcohols. At the start of 2004; press services reported that world wide wine industry had grown as large as the global cosmetics industry. However, in Thailand wine consumption accounts for only one percent of the total figure of alcoholic beverage consumptions. Regardless, the wine trade in Thailand remains lively, spirited and competitive with quite a few interesting trends developing. Those trends to keep an eye on during 2005 are the effect of free trade agreements (FTAs) with wine-production countries, the emergence of new wine distributors with high quality products and the likelihood of an increase in the wine excise tax following the national election (Karuwanna, 2005).

The differences between yeast strains used in wine-making may account for some of the variation seen in their fermentation properties and may also produce different sensory characteristics in the final wine product.

Objectives

- To compare chemical and sensory effect of 4 isolated yeast strains on aged sala wine
- Help to understand more about wine aging

Literature Review

Wine

Generally wine making follows a simple standard procedure which fruit juice is inoculated with a yeast starter culture. The fermentation usually takes around one week. During the week most of the sugar is converted to ethanol, yeast cells and carbon dioxide (Boulton *et. al.*, 1996). The dead yeast cells are allowed to settle. The relatively clear wine is then racked to remove the yeast cell before it is stabilized and filtered ready for bottling (Gawel, 2008).

Winemakers have long noted that using different strains of wine yeasts, even when used to ferment the same juice under identical conditions. It can yield very different wines in term of sensory characteristics (Dunn *et. al.*, 2005).

Sala or Salak

The Sala or Salak or snake fruit (*Salacca zalacca*) is a dioecious lepidocaryoid palm belonging to the Arecaceae family (Cape trib, 1999). The fruits are consumed fresh, candied or canned when fully ripe. Unripen fruits are used for pickles (Sauerborn, 2000). The Sala fruit is one of the many fruits available in Thailand. The Sala is a typical example for fruit species which are presently available only in local markets. Sala is the seasonal fruit which can harvest during May–July. The name "snake fruit" is a reminder of the fruit skin, which is very similar to reptile's skin in structure and color. The fruit derive from small, spiny palm trees, growing as understorey plants in the tropical rain forest of the lowlands. The female plants of this dioecious species develop fruits with the size of a fig, edible portion is sweet and aromatic (Lestari *et. al.*, 2002). Sala is cultivated in Thailand, throughout Malaysia and Indonesia. Sala palm is usually cultivated for its fruits. The flesh is exceptionally firm and crisp for a tropical fruit. It is quite sweet when fully ripe, but unripe fruit is sour and astringent due to the presence of tannic acid. It has a unique flavor, smoky with a tangy aftertaste. The unique taste is somewhat comparable to a combination of apple, pineapple and banana (Schuiling, 1992).

In Thailand, Sala wine had been made as OTOP products (One Tumbon One Product) which can add value to Sala fruit (Thaitambon, 2008). The high amount of dietary fibres (pectin, lignin, hemicellulose, and cellulose) and carbohydrate fractions (fructose, glucose, sucrose) reflected not only the high nutritional value, but also the sensory and textural quality compounds. The sugar and nutrient content of sala and generally low pH provides an ideal environment for yeast to grow and ferment (Lestari *et. al.*, 2003).

Fermentation

Wine fermentation has two distinct stages: Alcoholic fermentation or primary fermenatation and Malolactic fermentation as a part of secondary fermentation. Also sometimes described as aerobic and anaerobic fermentations (Kraus, 2005).

I. Alcoholic fermentation

Fruit juice or Sala must is converted into wine through the action of yeasts presented in the juice. It can be fermented spontaneously by inoculating yeast starter culture which turns sugar into alcohol. This alcoholic fermentation is also known as primary fermentation. The temperature for fermentation should be in between 15-18°C and take about 1-2 weeks (Karuwanna, 2005, Boulton *et. al.*, 1996). Some aromas and flavors were developed in this stage. After that, some were continues develop in malolactic fermentation.

II. Malolactic Fermentation

Malolactic fermentation (MLF) usually occurs shortly after the end of the primary fermentation. Malolactic fermentation of wine relies on lactic acid bacteria to convert malic acid into softer lactic acid and carbon dioxide. Their action can also significantly modify the wine's aroma, flavor and mouth feel. These changes may be either good or bad depending on which of the lactic acid bacteria dominates this step of fermentation (Karuwanna, 2005, Boulton *et. al.*, 1996). The bacteria also modify fruit flavor of wine and add some flavor compounds from their metabolism. The content of these organic compounds are organic acid esters, hydroxybenzene and terpene etc. Furthermore, the odor of wine is due to four ester (ethyl acetate, isoamyl acetate, ethyl hexanoate and octanoate) along with two alcohol, (isobutyl and isoamyl alcohol) and acetaldehyde (Boulton *et. al.*, 1996). However after malolactic fermentation has completed, the bacteria or yeast may still be presented in wine, where they can metabolize other compounds and caused wine faults (unpleasant characteristic of wine such as high level of acetic acid, estery off-flavors and etc.) (Karuwanna, 2005). After fermentation is complete and wine is racked several times to remove the largest solids ,the young wine is usually rough, raw and "green" and needs aging for a period of time.

Aging

Aging is the method of storing finished wine in proper container, to improve the quality of wine such as clarity, color, smell and taste. Usually wine is put in a wooden container made of oak wood. Alcohol may extract some chemical from wood for improve quality. A storing wine will have light oxidation by O_2 which slowly penetrate through small pores of wood. There are many chemical changes of wine from aging period. Therefore, aging period is important for the quality of wine (Karuwanna, 2005, Boulton *et. al.*, 1996).

Before bottling, wine usually aged for improvement, modification and to attract to consumer and command a higher price. Moreover, wine maturation and aging to some degree is inevitable. After wines are bottled, it take some time not reach customers immediately. If the wines are continuously available in shops and are often consumed months or years later, it obviously needs to consider changes that may result in wine. Every change is not an improvement. Loss of fruit aroma may be undesirable with some wines (Boulton *et. al.*, 1996, Wanjaroen, 2006).

Wine aging can be considered to be all the reactions and changes that occur after the first racking that lead to improvement at some stage rather than spoilage. Aging like all groups of reactions, takes more or less time depending upon temperature, yeast quality and other conditions (Boulton *et. al.*, 1996). In all wine making process, using high quality yeast is very important. The yeasts use for fermentation should be a selected one that has good agility to ferment wine, including the production of good aroma, flavor, and taste.

At the end of aging, the wine microbial community is stabilized but the total microbial population in the wine before bottling is often 10^3 to 10^4 viable and culturable cells per milliliter (Renouf *et. al.*, 2004).

During aging, where environmental conditions were more favorable, the main wine yeast *S. cerevisiae*, can be detected and will present at very low proportion in older wine. The presence of oxygen was essential for acetic acid bacteria which had never been detected in bottled wines. Therefore, non-*Saccharomyces* yeast and lactic acid bacteria (LAB) were the main species surviving in bottled wines. Among the LAB, *Oenococcus oeni* and *Pediococcus parvulus* were most often detected. Another yeast species occasionally detected in bottle is *Sygosaccharomyces bailii*. This species could be more problematic because of its ability to re-ferment sweet bottled wines (Renouf *et. al.*, 2004).

Re-fermentation

Re-fermentation might occur by the yeasts re-fermenting residual sugar that present in the bottled wine. Re-fermentation of the wine can cause turbidity, swampy, and slightly effervescent or spritzy. It can be prevented by bottling wines dry (with residual sugar levels < 1.0 g/L), sterile filtering wine prior to bottling, or adding preservative chemicals such as dimethyl dicarbonate (Champion, 2007).

The changes encountered in musts and wines during storage in barrel, tank or bottle due to microbial activity can be summarized as follows:

I. Changes due to yeasts:

i. Spoilage by flor or flowering yeast including *Candida, Pichia, Hanseniaspora* and also *Brettanomyces* sp. Flor is yeast that forms after fermentation, producing a film on the wine's surface and imparting a distinctive flavor. These yeasts may also increase volatile acid in the wine. Flor yeasts multiply on the surface of wines in contact with air and form a discontinuous film that clots when it reaches a certain thickness. This alteration by itself is not a serious fault, and if corrected in time, does not result in qualitatively unsatisfactory wine. But if instead the situation is not corrected, then the wine is predisposed to acetic spoilage, which obviously is more damaging.

ii. Unwanted secondary alcoholic fermentation (or re-fermentation). In very sweet wines, re-fermentation can cause the bottles to explode due to build up of CO_2 pressure. Re-fermentation depends on numerous factors, such as the presence of high alcohol producing yeast strains or very active re-fermenting yeasts, like the *Saccharomyces bayanus* and *Zygosaccharomyces bailii*.

iii. Hydrogen sulfide production (odor of rotten eggs, garlicky odors) and undesirable odors of dregs. Most yeasts are high sulfur dioxide producers including *Saccharomyces* spp. and lactic acid bacteria. It provides unpleasant odor of rotten eggs, odor of lees and hydrogen sulfide. During the malolactic fermentation, sulfur or garlicky odors can also occur due to the production of small quantities of H_2S, particularly if it occurs in bottled wine.

II. Changes due to bacterial activity such as acetic spoilage by acetic acid bacteria (*Gluconobacter* and *Acetobacter)*, unwanted malolactic fermentation, ferocious lactic fermentation or lactic acidity, glycerol degradation by *Lactobacillus*, oily ropy wine , ropiness due to *Pediococcus, Leuconostoc*, sweet sour or mannitic fermentation by *Leuconostoc*, Tartaric acid degradation by *Lactobacillu*s (Delfini *et. al.*, 2001). The above microbe can be produce different spoilage and change characteristics of wine.

Further information regarding contribution of different yeast strain to wine during aging will be discussed in the following section.

The yeast

I. Fermentation yeast

- ***Saccharomyces cerevisiae*** is a species of budding yeast used for wine fermentation. It is perhaps the most useful yeast since ancient times in baking and brewing. The spoilage reaction of *S. cerevisiae* can occur by re-fermentation of wines with residual sugar.

o **Pasteur red wine** yeast is also called French red, a strain of *S. cerevisiae*, as fermented yeast. It is a mixed population strain. In dry form, Pasteur Red is rapidly becoming the yeast of choice for producing full-bodied red wines. It is suit for red wines because it is tolerant to heat and sulfur dioxide and hardly ever causes stuck fermentation. It's optimum fermentation temperature range is 18-30 °C (64-86 °F) *(Quality Wine and Ale Supply, 2008, Keller, 2008).*

II. Aging yeast

- ***Zygosaccharomyces bailii*** is spoilage yeast that is tolerant of high sugar concentrations and is resistant to sorbate. It is commonly found throughout the winery environment and is often associated with grape juice concentrates that are used to adjust color and sugar in final wine blends. The yeast can cause turbidity and CO_2 gas in bottled wines (Boulton *et. al.*, 1996).

- ***Hansenula anomala*** has both fermentative and oxidative capabilities (growing as film yeast). *H. anomala* is capable of producing alcohol along with potentially large amounts of acetic acid and ethyl acetate and isoamyl acetate. Ester production, at much lower concentration, before and during the early stages of alcoholic fermentation may play a positive sensory role in wine complexity. If it exists in wine, part of the film yeast community where it utilizes ethanol, glycerol and wine acids in the production of acetic acid and acetaldehyde as well as esters. Acid utilization by *H. anomala* may be substantial, resulting in measurable decreased titritable acidity and upward pH shifts (Fugelsang, 1997).

- ***Hanseniaspora uvarum*** is wild apiculate yeast that is often present at high levels on incoming fruit. It is capable of producing volatile acids, including acetic acid and ethyl acetate in high concentrations before and during the early stages of fermentation. Population levels usually decline as alcohol concentration increases (Fugelsang, 1997, Boulton *et. al.*, 1996).

- ***Pichia*** is wild yeast that is often present at high levels on incoming fruit. *Pichia* can initiate fermentation, resulting in production of high levels of volatile acids, including acetic acid and ethyl acetate (Boulton *et. al.*, 1996). *Pichia* is also oxidative yeast growing as a chalky film in aging wine. It has also been observed during the initial phase of fermentation (Fugelsang, 1997).

• ***Brettanomyces bruxellensis,*** among the yeast, *B. bruxellensis* was predominant and could grow during bottle storage over a long period. As *Brettanomyces/ Dekkera* sp. can potentially spoil a wine, it is generally seen as wine spoilage yeast and these yeasts produce high concentrations of volatile acids, phenolic compounds, of which the ethyl phenols are the most prominent. which could produce 4-ethylphenol and 4-ethylguaiacol at a concentrations exceeding critical olfactory thresholds during the first months of storage (Renouf *et. al.*, 2004). These volatile phenols are largely responsible for off-flavors or taint associated with *Brettanomyces*. Its presence in wine is a cause of wine fault. The growth of *Brettanomyces* is best controlled by the addition of sulfur dioxide to which the yeast is particularly sensitive. On the other hand, when *Brettanomyces* grows in wine it produces several compounds that can alter the palate and bouquet. At low levels some winemakers agree that the presence of these compounds has a positive effect on wine, contributing to complexity. Many wines even rely on *Brettanomyces* to give their distinctive character but at high concentrations it is regarded as wine spoilage. The three most important known aroma active compound are 1) 4-ethyl phenol (4-ep), which has been variously described as having the aromas of Band-aid, antiseptic and horse stable 2) 4-ethyl guaiacol (4-eg) which has a rather pleasant aroma of smoked bacon, spice or cloves and 3) isovaleric acid which has an unpleasant smell of sweaty animal, cheese and rancidity. Other characters associated with Brett include wet dog, creosote, brunt beans, rotting vegetation, plastic and (but not exclusively caused by Brett) mouse cage aroma and vinegar (Boulton *et. al.*, 1996). Its presence in wine is an almost certain indicator of a Brett infection, and this is what most diagnostic labs test for to indicate the presence of Brett (Vuuren *et. al.*, 2003, Cocolin *et. al.*, 2003).

The differences between yeast strains used in wine-aging may account for some of the variation in fermentation properties and differing sensory characteristics in final wine product.

Wine quality and consumer acceptance

In order to determine the wine quality and consumer acceptance, the analysis can be separated into two parts; chemical composition and sensory characteristic.

Chemical composition of wine

I. Total soluble solid

The total soluble solid (TSS) in the wine represents not only the sugars but also includes many other soluble substances such as salts, acids and tannins. However, sugar is the compound in greatest quantity for all practical purposes. °Brix is a measure of sugar level (Delfini *et. al.*, 2001). Refractometer is usually measure °Brix of wine. It is fully required range of ambient operating temperature. Temperature is one of the single most important factors influencing accurate Refractometer readings and is one of the largest sources of error in measurement. It is well known that substantially all materials expand when heated (become less dense) and contract when cooled (become more dense). The speed of light in a liquid increases with temperature, and the refractive index, therefore, decreases (MISCO, 2004).

II. The Acidity of Wine

Acidity is acids in a wine (principally tartaric, malic, citric and lactic) provide liveliness, longevity and balance. Acids are very important structural components of wine. If a wine is too low in acid, it tastes flat and dull. If a wine is too high in acid, it tastes too tart and sour. The pH of must for table wine is between 2.9 - 4.2. But the winemaker usually prefer a pH range of 3.0–3.5, lower pH values are known to improve the stability (Karuwanna, 2005). Usually, the winemaker can easily manipulate the acidity of wine (Pandell, 1999). There are two kinds of acidity; total acidity and volatile acidity.

- **Total acidity**

In the U.S., the total acidity (TA) of a wine is measured assuming all the acid is tartaric. This allows one to determine a value for total acidity that is consistent. A high total acidity is 1.0%. Most people would find this level of acidity too tart and too sour for consumption. A low total acidity is 0.4%, results in flat tasting wine that is more susceptible to infection and spoilage by microorganisms (Pandell, 1999). Total Acidity should be 0.6-0.9% as tartaric acid (Karuwanna, 2005).

- **Volatile acidity**

The analysis of volatile acid can be used to detect the spoilage that may be developed during storage of aging new wine. High volatile acid is an indication of high acetic acid means that there is contamination of acetic acid bacteria in wines. It is a measurement of spoilage. Volatile acids in wine are formic acid, acetic acid, butyric acid, and etc. Both tartaric and malic acids are *nonvolatile* which means that they do not evaporate or boil off when the wine is heated. This is to be distinguished from volatile acidity (VA) in wine that commonly represents acetic acid (vinegar). Acetic acid does boil off when heated and high volatile acidity is undesirable in a wine. A %VA of 0.03-0.06% as acetic acid is produced during fermentation and is considered a normal level. The maximum permitted volatile acid in white and red wine in California and France is 0.11 - 0.12% as acetic acid (Pandell, 1999, Karuwanna, 2005).

III. Alcohol concentration

Alcohol concentration is recommended to analyze percent alcohol of wine by using Ebulliometer. The principle of Ebulliometer is to compare the boiling point of water and wine sample. Standard wine will contain 8–14 % alcohol by volume (Karuwanna, 2005).

IV. Reducing sugar

The reducing sugar content of wine in the range of 0.1-0.2% is classified as dry wines. The reducing sugar 2–5 % of wine content classified as semi dry wines and sweet wines which reducing sugars are higher than 5%. Reducing sugar was measured by using the rapid method called "Glucotest" (Pilone *et. al.*, 1966, Robinson, 2003).

Sensory characteristics of wine

The sensory characteristics of wine are critical in determining wines acceptability. Assessments of wines are often made by expert wine tasters, however the use of sensory analysis methods offers an alternative approach that is less reliant on the expertise. These include the use of scaling methods to evaluate changes in particular sensory characteristics as a result of changes in processing parameters, the use of sensory profiling to characterize a wine and determine changes that occur during storage, and the use of consumer testing to determine the key sensory characteristics of importance to a particular market segment (Eves, 1994).

The differences in sensory properties perceived including wine aroma and flavor between wines could be due to a variety of factors, including regional characteristics, fruit handling, vinification, yeast strain used, oak treatment or winemaking techniques.

Wine tasting is actually a complex proposition involving much more than simply sipping some fermented grape juice. There are many variable factors that affect an individual's perception of flavor in wine; chemical, physical, mechanical, physiological, and psychological variables. The chemical make-up of wine includes many trace elements that contribute to the combination of smells (Professional Friends of Wine, 2008).

Principle of wine tasting

Principle steps of 3-S are Sight Smell and Sip.

I. **Sight**: look for clarity, color, tears or legs of wine. Tears or legs are indicator of body or wine. If wine has good leg, it meant high alcohol, high body, and high glycerol. Except sweet wine, it usually has every high leg.

II. **Smell**: should detect both a good smell and bad smell (defects). A bad smell e.g. vinegar, sufury or sulphite, yeasty, moldy, too oaky, earth, oxidized, H_2S and etc.

III. **Sip**: have a small volume of wine in mouth, do not drink it. For detecting the balance, acidity, alcohol (hot, burn your throat or not), tannin or bitter, sweetness, salty, for more experience tasters, they should mention about Finish feeling and Aftertaste feeling (Karuwanna, 2005).

One important wine-tasting skill is the ability to recognize and provide accurate words to describe the odors and flavors that are encountered. Different terms are used to describe what being smelled depending on experiences, environment and social surrounding. Different part of the world have different attributes characterize the aroma of wine because of the smell of fruit and flower that they have known were different e.g. people who live in Europe surrounding by different types of fruit from Asian people, they will characterize white wines with peach, melon, grapefruit, banana, apricot, cherry and etc. But Asian people who live in tropical area are not familiar with these fruit aromas. They characterize the wine aroma by the tropical fruits that they have known such as grape, mango, banana, lychee and etc. Best descriptive terms of aroma should be created starting from general terms to more specific one e.g. fruity or chemical then follow by more specific aroma such as vanilla, battery and etc. The ability to produce detailed descriptions of the wine's aroma, depends on their ability to perceive the odor, to recognize what it is (Gawel, 2008). One of the most comprehensive and informative tools used in sensory analysis is descriptive sensory analysis. Descriptive Analysis techniques should be used when comparing wines to determine differences in sensory characteristics and quality of wine. Overall, descriptive analysis should be used when there are large differences between wine samples or changes in the winemaking process (Vinquiry, 2006).

Wine Aroma

A wine's aroma, or nose, is the smell of the wine. It can be floral, citrus, woodsy, buttery, oak, vanilla or any number of familiar scents depending on the grapes used, the winemaking process and the storage conditions. In older wines, the aroma is often referred to as the bouquet (Slinkard, 2005). A wine's aroma is an excellent indicator of its quality and unique characteristics.

There are currently more than 500 wine aroma descriptors known such as fruity, citrus, floral, sweet and honey/caramel aromas. These depend upon and are influenced by the sort of grapes or fruit must, the production process and the type of aging (Gesso, 2008).

If wine aroma is particularly complex, bouquet, should be assessed for each new wine after looking at its color. Swirling helps to volatilize particles in the wine and also allows the wine to develop and grow in complexity in the glass. (Wine-Tasting-Guide, 2008). The objective of the olfactory examination is to judge the intensity, complexity and quality of a wine's aroma, and subsequently to identify and describe the character of the specific odors of which it is composed. The sense of smell is the key to enjoying wine, because much of your sense of taste is really based on what you smell. So, when tasting wine, significant time should be taken to evaluate and enjoy the wine aroma.

I. **Intensity:** Intensity is made up of various odorous sensations coming together at once. It is a measure of quantity but not necessarily quality. In a fine wine, an intense bouquet is an attribute but in a poor-quality wine, a strong odor can be negative factor.

II. **Complexity:** Term for wine exhibiting depth of aromas, and variety of odorous sensations after being inhaled.

III. **Quality:** Represents the synthesis relating both to olfactory intensity and complexity, as well as finesse, elegance, frankness and tipicity. The taster's experience and knowledge of wines play an important role in the evaluation of the olfactory quality (Gesso, 2008).

This research aims to study the effect of four yeast strains previously isolated from aged wine by Wanjaroen (2006), in aged sala wine. The different yeast strains which used in aged sala wine may result in different sensory characteristics in the final wine product. Then the aged wines were evaluated for both chemical compositions and sensory characteristics to determine developing quality of aged wine. Therefore this research will help to understand more about wine aging and enable the development of the process for the production of high quality wine.

Method

PART 1: Recovered and selected the yeast from yeast stock

Material and Equipments

- Yeast stock from (Wanjaroen, 2006) -17 yeast strains

Procedure

1.1 Recovered yeast from yeast stock

Recovered 17 isolated yeast stains (Wanjaroen, 2006) on YM agar. Selected single colony and inoculated on YM slant for 24 hours. Prepared 20% glycerol 1 ml in eppendoft and transfer all yeast stains from YM slant into eppendoft to prepare stock culture then store in the freezer.

1.2 Selective and yeast culture preparation

The 24 hours old 17 isolated yeast stains were observed the characteristics under microscope by using wet mouth technique. Four isolated yeast strains were selected by the different biochemical characteristics including spore-forming and sugar utilization (studied from previous study). Each of them were stocked in YM slant and prepared for further step. Inoculated the selected yeast strains into YM broth mixed with 5% sterile alcohol for 2 nights before inoculate in aged wine, helped them adjust themselves with alcohol and to reduce lag phase during fermentation.

PART 2: Preparation of, base wine and aged with 4 different yeast strains on aged sala wine

Material and Equipments

- Sala *(Salacca zalacca)*
- Pineapple
- Pasteur Red wine yeast (*Saccharomyces cerevisiae*) (Red Star)
- Yeast for aging period
- Refractometer 0-32°Brix
- pH meter (HI 98127 HANNA instrument)

Procedure

2.1 Starter Culture preparation

The musts for starter culture were prepared from pineapple. The pineapple were peeled, cut and blended. The volume of must used for this experiment was about 800 ml (1:1 fruit: water). The total soluble solid (TSS) was 5 °Brix. Boil to sterilized and cool it down. Then add DAP (di-Ammonium Hydrogen Orthophosphate) 2 g/l (as nitrogen source). The must was inoculated with Pasteur Red wine yeast for 2-3 loops (from YM slant). The starter culture was left in a bottle covered with a plastic bag. The bottle of starter culture was leaved at room temperature (25– 32°C) for 24 - 48 hours to increase the amount of starter culture. Then the starter culture was transferred into based wine.

Figure 1: Pasteur Red (Yeast) on YM slant

2.2 Based wine preparation

The musts were prepared from Sala fruit (*S. zalacca*). The Sala were peeled, cut and seeded. The volume of must prepared for this experiment was 6 liters (1:3 fruit: water). Add 2 g/l DAP (nitrogen source), 1g/l citric acid (adjust pH to 3-4) and sugar until the TSS closed to 22 °Brix then add 200 ppm KMS (Leave it at least 10 hours or overnight before inoculation yeast starter). The musts were transferred to a fermentation room maintained at 25 - 32 °C and mixed with 10% yeast starter culture (Pasture red wine yeast, with pineapple) and let it primary fermented to dryness for 8-9 days , checked the TSS (°Brix) by Refractometer everyday until it reach 5-6 °Brix or stable. The dry wines were racked and stored in glass jar at 10 °C (secondary fermentation) for a week then add 200 ppm KMS. And filter by using diatomite earth and filter paper Whatman No.1 in order to develop clarity of based wine. Method modified from (Kundee, 1968, Karuwanna, 2005). Three batches of based wine were made separately with different batch of fruit.

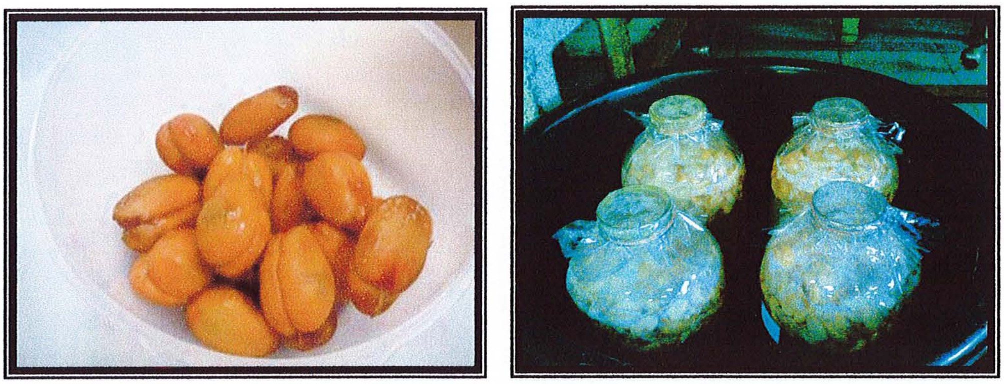

Figure 2: Sala fruit (left) and Sala must in glass jar in primary fermentation (right)

2.3 Aging

The based wines were performed on laboratory scale with 750 ml of wine in standard wine bottles. Each bottle was aged with addition of one of the 4 selected yeast strains and kept one bottle as a control. Then the wine samples were aged at 10°C for 2 months.

Table 1: Wine samples aged with different isolated yeast strains

Sample	Aging yeast	Tentative Yeast
Control	-	-
Sample No.1	Yeast No.1	*Saccharomycetaceae*
Sample No.14	Yeast No.14	*Saccharomycetaceae*
Sample No.15	Yeast No.15	Unidentified
Sample No.30	Yeast No.30	*Candidaceae (Brettanomyces sp.)*

After two months of aging, The KMS was added to all bottles to stop the reaction.

Figure 3: Five aged sala wine samples

PART 3: Analyze characteristics of aged wine

The effect of 4 isolated yeast strains were analyzed in both chemical and sensory characteristics.

3.1 Chemical analysis

Material and Equipments

- Refractometer
- pH meter (HI 98127 HANNA instrument)
- Ebulliometer (Dujardin Salleron)
- Glucotest strip (Diabur test 5000)

Procedure

All wine samples were subjected to chemical analysis including pH, TSS, total acidity, volatile acidity, alcohol content and reducing sugar. The pH of wine was measured by pH meter. The TSS (°Brix) was measured by Refractometer. Total acidity was measured by using a Titration apparatus. Then analyzed volatile acidity to detect the spoilage that may be developed during storage of aging new wine. The collected distillate samples is titrated with a sodium hydroxide (0.1 M NaOH_) solution to determine the volatile acidity. Alcohol concentration is analyzed percent alcohol of wine by using Ebulliometer. The principle of Ebulliometer is to compare the boiling point of water and wine sample. The reducing sugar content will be measured by using the rapid equipment called "Glucotest strip" (Karuwanna, 2005, Wanjaroen, 2006).

For TSS and pH were measured every week (once a week) during aging period to follow the stability of aged wine for all 8 weeks. While the %VA, %TA, %Alc and reducing sugar were analyzed for only one time after finish based wine. And they were analyzed every 4 weeks during aging period.

3.2 Sensory analysis

Material and Equipments

- Wine glasses
- Seven standard descriptors conducted from Descriptive analysis; Sala Aroma, Vinegar Aroma, Sun-dried Banana, Hale's Blue Boy, Black pepper, Solvent (Acetone) and Dried Honey Banana

Procedure

For Sensory analysis, the appearances were observed including color, clarity and aroma of wine. The aroma was analyzed by observing the intensity of 7 aroma attributes in wine. Then the sensory was evaluated; tasting the wine by panelists.

Descriptive analysis and training

The sensory panels were recruited from Assumption University. The panels consisted of five females who studies Bachelor's degree in Biotechnology faculty. All panelists performed the descriptive analysis to describe specific characters of wine aroma which it is composed. Three samples; sala wine, Roselle wine and sala juice

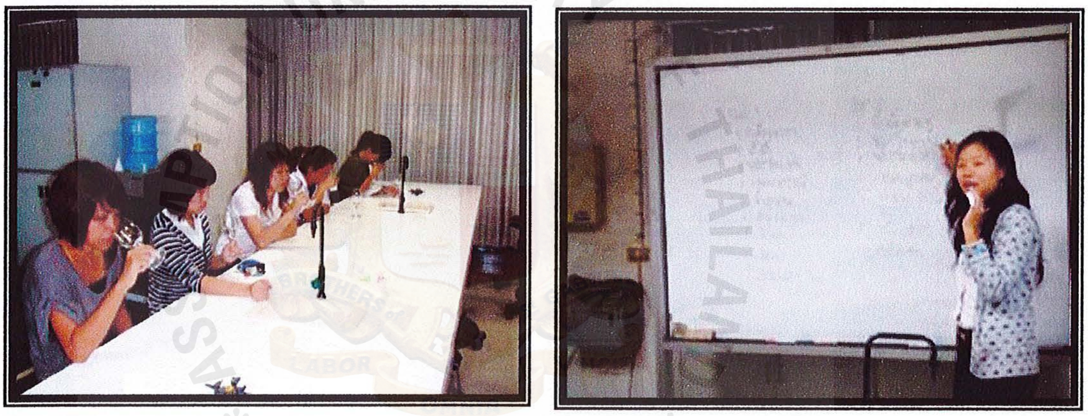

Figure 4: Process of descriptors generation

were provided to introduce different aroma attributes in wine. The panel was instructed to generate descriptors for the aroma of each sample. Then they were provided five aged sala wine samples (4 wine samples from four treatments and one as a control). They were asked to smell them and list as many attributes as possible for each sample and discuss term as a group to come up with a consensus. The panel leader facilitated the process of discussing terms and looked for overlap and redundancy among descriptors. After 4 repetition of descriptive analysis, the panelists had agreed on a list of seven descriptors that best characterize attributes for aged sala wine samples (Table 2).

Table 2: Standard descriptors with corresponding reference compositions

Descriptor	Reference compositions
Sala	10 ml Sala juice
Vinegar	2 drops in 10 ml Sala wine
Sun-dried Banana	1g in 10 ml Sala wine
Red Hale's blue boy	2 drops in 10 ml Sala wine
Black pepper	0.1g in 10 ml Sala wine
Solvent (Acetone)	2 drops in 10 ml Sala wine
Dried-Honey Banana	1g in 10 ml Sala wine

Figure 5: Examples of preparation for standard descriptors

All panels were trained with these standard descriptors including Sala aroma, Vinegar aroma, Sun-dried banana, Hale's blue boy, Black pepper, Solvent (acetone) and Dried honey banana for many times to let them familiar with these selected descriptors of wine. The training provided several times in order to reduce potential bias. In the session training, 5 aged wine samples were provided to them. Wine samples were consistently presented blind, in wine glasses (with 3-digit codes). In subsequent training session, reference standards were developed and evaluated for suitability of specific aroma in wine samples. For each aroma descriptor, "Intensity test" 15-cm line scales were developed, with the scale end indented 1 cm to avoid end-point effects. The left end of each scale was anchored with the phrase "Very

Low" at the 1-cm indent mark, and the right end with "Very High" at the corresponding 1-cm indent mark. The panel gained experience with rating the aroma intensities of both samples and control wine follow the standard descriptors (Pickering, 2004).

Sensory evaluation

The aged wine samples were assessed 2 weeks after bottling, and each batch was evaluated in separate sessions. Before each flight, panelists were instructed to refamiliarize themselves with each standard descriptor. The standard descriptors were also available during sensory taste for reference if required. All wines were presented as 10-mL samples in tasting glasses at room temperature (21-25°C) covered with plastic petri dishes. They were also presented as blind test. Each flight also had a glass of water for rinse the nose during tasting. There were 3 batches of 5 wine samples. The same batch were represented to the panel triplicate (with changed 3-digit codes) and assessed for aroma under the same assessment protocol. The aroma of each batch was assessed separately. Each batch was evaluated for aroma, with a minimum 3 hours break between batches.

Figure 6: Wine samples for sensory evaluation covered with plastic petri dish

The results from descriptive analysis were made as the standard descriptors for sensory tasting. In sensory evaluation session, the panels performed 1) the intensity test to evaluate the aroma intensity of 5 wine samples using a 15-cm line scales follow seven standard descriptors. The data were quantified by measuring the distance of the judge's mark from the origin. In addition, panelists were asked to list any additional descriptive terms they felt applicable. 2) The 9-point Hedonic scales was used to determine the acceptance of samples on 5 panelists to evaluate 3 appearances, including clarity, color and overall aroma for panelists' acceptability.

The evaluations were conducted individually in Sensory test Laboratory (room E81). The sensory characteristics of five aged wine samples were analyzed by intensity test and hedonic scales. Both chemical and sensory analyses of five aged sala wines from 3 separate batches were evaluated by One-way ANOVA (SPSS version 14.0). If the p of the treatment F-value was > 0.05, then the samples were not significantly different from others.

Figure 7: Training session for the panelists

Result and Discussion

PART 1: The characteristics of 4 isolated yeast strains

From 17 yeast strains previously isolated by Wanjaroen (2006), the selected 4 yeast strains which have different biochemical character or spore forming were used for varying wine samples during aging period in the experiment. The purpose of the research was to discover whether the wine could be characterized by chemical analysis, by the strain of yeast which was responsible for the aging period. Four selected yeast had different properties. Yeast No.1 capable of fermenting glucose, sucrose, maltose and lactose. Yeast No.14 and No.15 was similar to Yeast No.1 except cannot utilize lactose. Yeast No.30 cannot utilize all four sugar mentioned above but it's had unique growth characteristics (film formation). Both Yeast No.15 and Yeast No.30 were non-spore forming as observed within three weeks of previous study which is different from other yeast listed above (Wanjaroen, 2006). All selected yeasts were observed under light microscope and their characteristics were shown in Figure 8.

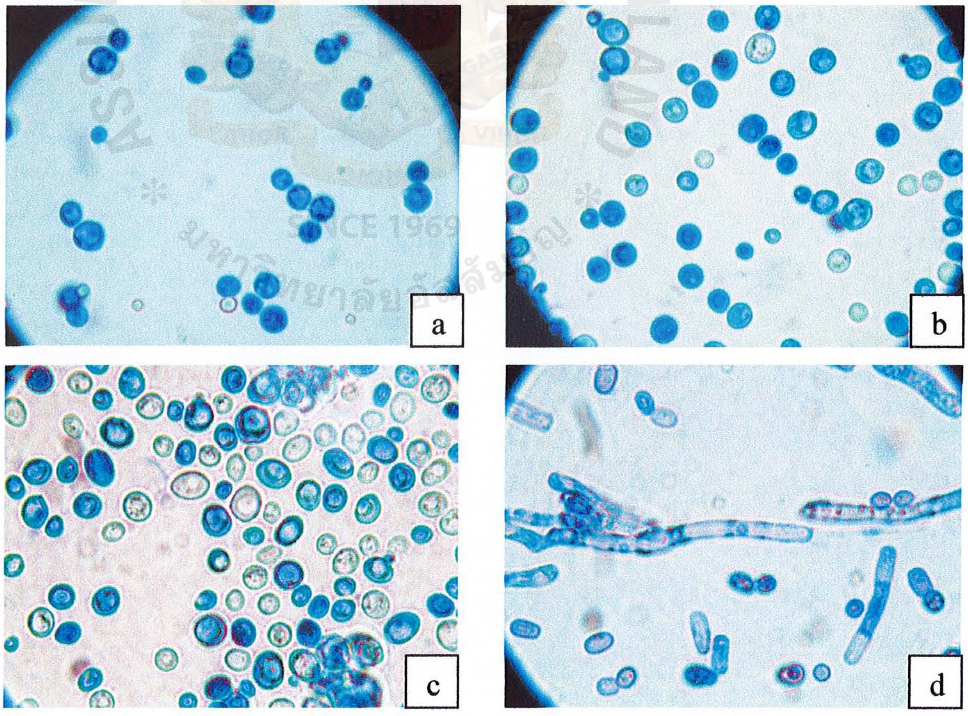

Figure 8: Wet mouth characteristic of selected 4 yeast strains under microscope (400x). (a)Yeast No.1: *Saccharomycetaceae* (b) Yeast No.14: *Saccharomycetaceae* (c) Yeast No.15: Unidentified (d) Yeast No.30: *Candidaceae (Brettanomyces sp.)*

PART 2: Chemical analysis

Three batches of based wines both before aging and after aging were analyzed for their chemical compositions, including TSS, pH, %Alc, %TA, %VA, and reducing sugar. Moreover, during aging period, the five wine samples were also analyzed for TSS and pH every week for 2 months to follow the stability of wine-aging.

There were 3 parts of chemical analysis; 2.1) Based wine 2.2) Aged wine samples during aging period (TSS and pH) 2.3) Finished aged wine samples.

2.1 Chemical analysis of based wine

In this experiment, three batches of based wines were prepared from Sala fruit. Each batch was made separately with different batch of fruit. Based wine were made following typical winemaking method and used Pasteur Red wine yeast, a strain of *Saccharomyces cerevisiae,* as fermented yeast. The TSS of based wine has been measured everyday until it reached 5 - 6 °Brix or stable. The chemical compositions of based wines (3 batches) were shown in Table 3.

Table 3: Chemical compositions of based wine (3 batches)

Wine Sample	°Brix	pH	%Reducing sugar (w/v)	%Alcohol (v/v)	%TA (g/100ml)	%VA (g/100ml)
Batch1	6.0	2.5	0.00	13.1	0.60	0.030
Batch2	7.5	2.6	0.10	12.9	0.60	**0.042**
Batch3	6.5	2.7	0.10	12.9	0.57	0.030

The TSS of Batch1 had taken only 8 days to reach 6 °Brix. The TSS of Batch2 and Batch3 had taken about 11 days to be stable but not reached 6 °Brix. The reducing sugar of Batch1, Batch2 and Batch3 were 0%, 0.1% and 0.1% which were classified as dry wines (Boulton *et. al.*, 1996). Subsequently, all chemical characteristics of 3 batches of based wine are similar to each other and were in the range of standard wine except pH and %VA. The pH of based wines in 3 batches are a little lower than standard range. It might occur because of adding too much of citric acid during fermentation. However, the low pH of three batches provided a stable condition that still suit with the optimum pH of yeast growth (pH 2.0 – 8.0) and can prevent bacterial growth during fermentation (Boulton *et. al.*, 1996). While the volatile acid of based wine in Batch 2 was very high. It showed that based wine of Batch2 has higher risk of contamination than the others. It might occurred because of the gas produced from yeast starter in the glass jar, uncovered the plastic bag and the open the system for 2 nights during primary fermentation. However, the volatile acidity of Batch2 was still not more than 0.11% which was still in the acceptable range and not represent as wine fault (Karuwanna, 2005).

2.2 Chemical analysis of aged wine samples during aging period

All wine samples were bottle-aged for two months. Each bottle was aged with addition of one of the 4 selected yeast strains and kept one bottle of based wine as a control. During aging period, each sample was inoculated with Yeast No.1, Yeast-No.14, Yeast No.15 and Yeast No.30 recovered from Wanjaroen, 2006. The TSS and pH of aged wine samples had been measured every weeks (once a week) during 8 weeks of aging period in order to follow the stability and ensured the usual of the processes.(Data not shown)

For all 8 weeks of aging period, The TSS and pH of all aged wine samples were present as usual. From the observation, the pH trend of aged wine samples during 8 weeks should be stable (not increase) but the increasing and larger variation of pH since forth week 4 occurred because of the pHs of first three weeks were analyzed by un-calibrated pH meter and cause incorrect value.

2.3: Chemical analysis of finished aged wine samples

After 2 months, the average chemical composition aged wine sample (control) was compared with average chemical composition of based wine before aging (Table 4).

Table 4: Comparing average chemical compositions of based wine (Table 3) and the wine after aging (Control)

Wine Sample	°Brix	pH	%Reducing sugar (w/v)	%Alcohol (v/v)	%TA (g/100ml)	%VA (g/100ml)
Based wine (Average)	6.67	2.6	0.07	13.0	0.59	0.03
Aged wine (Control)	**7.30**	2.7	**0.12**	12.9	**0.64**	0.03

From Table 4, all chemical compositions in aged wine were increasing from based wine except %Alc and %VA which were stable. The increasing of total soluble solid (°Brix) and reducing sugar after aged wine might occur because of the increasing of %TA. The increase of proton concentration of the solution makes faster fructo-oligosaccharides degradation (Matusek, 2008). The increasing of %TA in aged wine might come from acid production of some yeast during aging period. And it still need further study to prove this chemical effect on aged wine. From the data of each batch, the increasing of reducing sugar after aged wine had found in only Batch 2 which had increase from 0.1 to 0.25% reducing sugar (Table 9). Batch 2 might have higher acid production during fermentation that caused by oxidation to the air during the earlier period in primary fermentation as mentioned above in 2.1.

Table 5: Chemical compositions of aged wine samples average from 3 batches

Wine Sample	°Brix	pH	%Reducing sugar (w/v)	%Alcohol (v/v)	%TA (g/100ml)	%VA (g/100ml)
Control	7.30	2.70	0.12	12.90	0.64	0.027
Sample No.1	7.30	2.70	**0.28**	12.90	0.63	0.031
Sample No.14	7.47	2.70	**0.28**	12.83	0.62	0.031
Sample No.15	7.53	2.70	0.13	12.87	0.64	0.030
Sample No.30	7.50	2.70	0.07	12.77	0.62	**0.035**

After 2 months, the aged wines were analyzed for their final chemical compositions. The chemical compositions of five aged wine samples average from 3 batches has shown in Table 5.

From statistical analysis, there were non-significant different between all aged wine samples in chemical compositions. From the observation, there were some noticeable values in %reducing sugar and volatile acidity (Table 5). The %reducing sugar in Sample No.1 and No.14 had higher value than the other samples. They were aged by Yeast No.1 and Yeast No.14 that classified in *Saccharomycetaceae* (from previous study). Therefore Yeast No.1 and Yeast No.14 can produced higher % reducing sugar than the other types of yeast strains during aging period. The volatile acidity of all sample No.30 was 0.035%VA which was the highest volatile acidity compared to other samples. Sample No.30 aged with Yeast No.30 which classified as *Brettanomyces* sp (from previous study). It might able to produce high concentrations of volatile acids in wine sample No.30 (Vuuren *et. al.*, 2003).

Of all the wine samples, there were non-significant different between 5 aged wine samples in chemical compositions (Table 5). It's indicated that various yeast strains that added during aging period did not decrease or improve much the quality in chemical compositions of wines. Or the chemical composition might need longer aging time to aged wine. In order to see the improvement of wine besides chemical compositions, the wines were analyzed for sensory differences, especially in aroma to determine whether they could be characterized as to the yeast responsible for the aging.

Part: 3 Sensory analysis

The appearances of aged sala wine samples were analyzed by sensory analysis. They were evaluated by five partially trained panels using Descriptive analysis, intensity test and hedonic scales.

3.1 Descriptive analysis and training

The wines have been subjected to sensory evaluation by partially trained panels. The sensory panel was recruited from student who studied Bachelor's degree in Biotechnology at Assumption University – Hua Mak Campus.

Five female panelists were trained and performed the descriptive analysis to describe specific characters of wine aroma on aged wine. After 4 times of descriptive analysis, panelists had agreed on a list of seven descriptors that best characterize aroma attributes for aged sala wine samples, the list of standard descriptors were Sala aroma, Vinegar aroma, Sun-dried banana, Hale's blue boy, Black pepper, Solvent (acetone) and Dried honey banana (Table 2). These descriptors were set as standard descriptors for sensory analysis in intensity test.

By the way, with time limitation only a few training sessions were done. Therefore the panels in this experiment were not classified as trained panel but as partially trained panel instead. After the trained session, the SD was reduced form 4 to around 1.2.

3.2 Intensity test

The seven descriptors from descriptive analysis were set as standard descriptors in intensity test. In each flight, the panels re-familiarize with each standard descriptors and evaluate the aroma intensity of each sample follow the standard descriptors, using 15-cm line scales to analyzed. The results of all aroma intensities were summarized in Table 13.

From statistical analysis, there were significant different in all aroma attributes except vinegar and sun-dried banana aroma (Table 13).

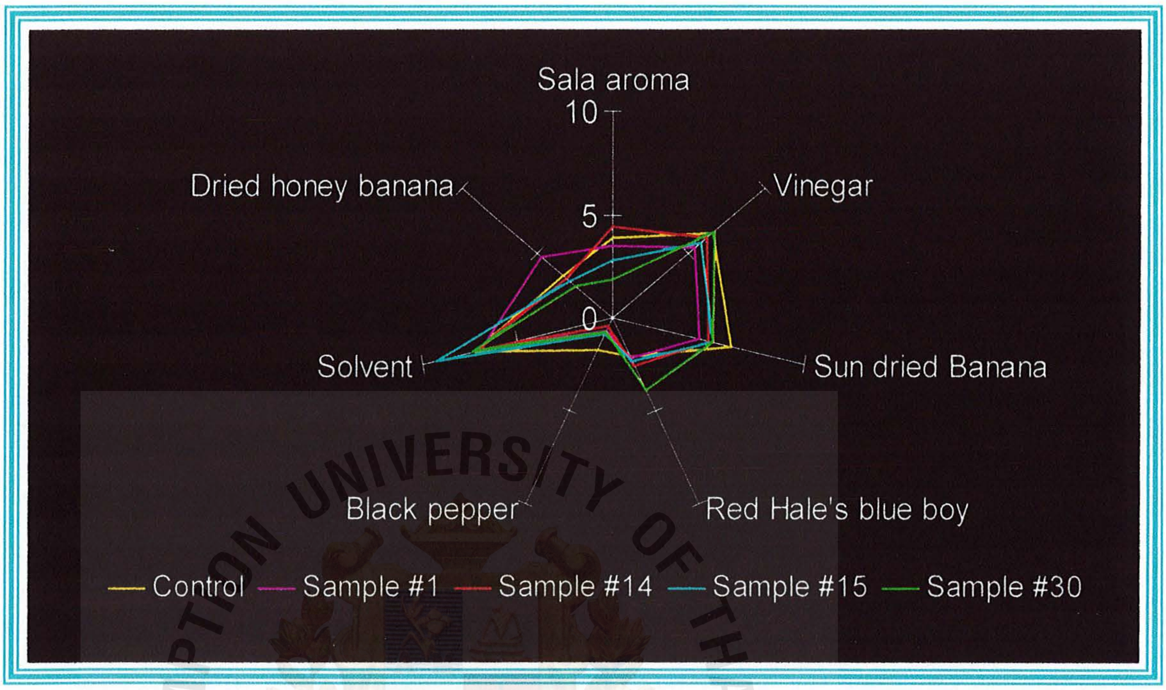

Figure 9: Spider-web chart of seven aroma attributes of 5 aged wine samples

Changes of aroma attributes on aged wine by the 4 isolated yeast strains showed significant variation (Figure 9). Comparing all aged wine samples, from the least; sample No.14 had significantly more sala intensity from sample No.15 and 30. While sample No.1 had significantly more dried honey banana aroma than the others. Sample No.15 had significantly more solvent and less sala intensity. Sample No.30 had significantly more Red Hale's blue boy and less sala intensity than the others. And all aged wine samples had significantly less black pepper intensity from control. Regardless of statistical analyzed, it is noticeable vinegar intensity of sample No.30 was highest. It may come from some specific production of yeast No.30 (classified as *Brettanomyces* sp.) that need further study and it had non-significant different in vinegar intensity.

3.3 Hedonic scale

The panelist's acceptability on the appearances of aged wine including color, clarity and overall aroma were using hedonic scale to analyze. (Table 14)

For panelist' acceptability, there were non-significant different in clarity, color and overall aroma acceptability of 5 aged wine samples. And the mean scores showed that all aged wine samples are acceptable.

From the experiment, the differences in sensory characteristics of wines between these aged wine samples could be due to different yeast strains used during aging period. Because of yeasts have the ability to ferment a large amount of sugar to perform the fermentation then leads to dramatic increase in volatile and non-volatile that largely characterize the wine. Although the various yeast strains during aging period had small effect on chemical compositions, many consumers seem especially attracted more to aromas. There are some aromas that are liked by some consumers but disliked by others, for most aromas, there are at least several compounds acting together that give rise to the flavors. Therefore the sensory taste of wine should be more focus than chemical composition of wine in order to develop the wine industry, since the consumers would have different preferences of wine aroma.

Meeting these quality requirements in the future will require a better understanding of the biology of human perception, and the relationship between composition and the production of wine to an evolving market preference and specification.

For further study, the differences in sensory characteristics also due to a variety of factors, including, fruit handling, vinification or winemaking techniques.

Conclusion

There were four isolated yeast strains (Yeast No.1, Yeast No.14, Yeast No.15 and Yeast No.30 recovered from Wanjaroen, 2006) were compared in both chemical and sensory effect on aged wines. From statistical analysis, the effect of 4 isolated yeast strains on aged sala wine had non-significant different in chemical compositions. For sensory analysis, there is significant different in all aroma attributes follow the standard descriptors except vinegar and sun-dried banana aroma. From panelist's acceptability, all aged wine samples are acceptable with no significant different scoring. It's indicated that the effect of 4 isolated yeast strains on aging wine had non-significantly differences in chemical compositions but had significantly different in sensory characteristics.

Suggestions

1. Microbial load in wine should be checked before and after aging to ensure the role of yeast in the change of wine.
2. Different properties of based wine will affect aged wine. It is difficult to control quality of based wine even it made under identical process therefore preparation of more batches of based wine may need to help reduce error.
3. Longer aging period may allow larger (obvious) differences of result in both chemical and sensory characteristics.
4. In this experiment, each bottle of samples were used for evaluation several times. It caused the space and oxidation in wine bottles. It may cause a change in sensory attributes of wine and cause error in sensory analysis. Therefore, the higher amount of sample should be prepared and separated into small portion for each time sensory analysis to prevent oxidation effect.
5. Apart from limitation time, the panelists need more train to get more experience and be able to differentiate aroma attributes of wine. And repeating many times of sensory evaluation will give more precision result and reduce high SD.

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Appendix A

Method and Formula

YM agar

Yeast extract	3	g/liter
Malt extract	3	g/liter
Peptone	5	g/liter
Glucose	10	g/liter
Agar	15	g/liter

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

Wet mount technique

Use Methylene Blue to stain the yeast cell observed under the microscope at 400-1000x (Wanjaroen, 2006).

Sugar addition (Karuwanna, 2005)

The amount of sugar to be added (kg) $= \dfrac{^{\circ}\text{Brix original} - {^{\circ}\text{Brix original}}}{100 - {^{\circ}\text{Brix wanted}}} \times \text{volume (l)}$

Chemical Analysis of composition in Wines (Karuwanna, 2005)

1. **Total Acidity or TA**

Standard

Dry table wine	0.6-0.9%	(as tartaric acid)
Sweet and Dessert wine	0.4-0.65%	(as tartaric acid)

Acids found in musts and wines

Tartaric, Malic, Lactic, Citric, Acetic acid and etc. The quantity of acids is usually expressed as percent or g/100ml of Tartaric acid.

Wine should be low in CO_2 in wines, so we have to degas wines before analysis total acidity.

To find percent total acidity, it should use clear must.

Total Acidity (TA) = (g/100ml) as Tartaric acid

$$\% \text{ TA} = \frac{V \times N \times 75 \times 100}{1000 \times v}$$

$$= V \times 0.075$$

2.pH

It should be measured by pH meter.

Standard pH of wines and musts

Ph of must for table wine 3.1 -3.6

3. Volatile Acidity (VA)

Volatile Acids in wine are formic acid, acetic acid ,butyric acid, and etc.

Standard Volatile acid

0.03% as acetic acid, usually present percent volatile acid as percent acetic acid.

Maximum permitted volatile acid

In white and red wine in France is 0.11-0.12% as acetic acid.

$$\% \text{ VA} = \frac{V \times N \times 60 \times 100}{1000 \times v}$$

$$= V \times 0.06$$

4. Percent Alcohol

Standard for table wine is 8-14 % alcohol by volume

<u>Standard wine</u>

%TA	Dry wine		0.6 - 0.9
%TA	Sweet wine		0.4 - 0.65
%VA	Dry wine	usually present	0.03
%VA	Sweet wine	Maximum	0.11

(Karuwanna, 2005)

Appendix B

Observation Data

Chemical analyses of aged wine samples

Table 6: Average TSS (°Brix) of 3 batches of wine samples (**Figure 10**)

Week	Control	Sample No.1	Sample No.14	Sample No.15	Sample No.30
0	6.67	6.67	6.67	6.67	6.67
1	6.80	6.47	6.77	6.53	6.77
2	6.87	6.93	6.83	6.73	6.93
3	6.80	6.83	6.83	6.90	6.83
4	7.43	7.43	7.53	7.40	7.50
5	7.13	7.13	7.17	7.13	7.17
6	7.13	7.10	7.13	7.20	7.13
7	7.13	6.73	6.73	6.57	6.83
8	7.30	7.30	7.47	7.53	7.50

Table 7: Average pH of 3 batches of aged wine samples (**Figure 11**)

Week	Control	Sample No.1	Sample No.14	Sample No.15	Sample No.30
0	2.2	2.2	2.2	2.2	2.2
1	2.3	2.4	2.3	2.4	2.3
2	2.4	2.4	2.4	2.4	2.4
3	2.3	2.3	2.4	2.4	2.3
4	**2.7**	**2.7**	**2.7**	**2.7**	**2.6**
5	**2.7**	**2.7**	**2.7**	**2.7**	**2.7**
6	**2.7**	**2.7**	**2.7**	**2.7**	**2.7**
7	**2.7**	**2.7**	**2.7**	**2.7**	**2.7**
8	**2.7**	**2.7**	**2.7**	**2.7**	**2.7**

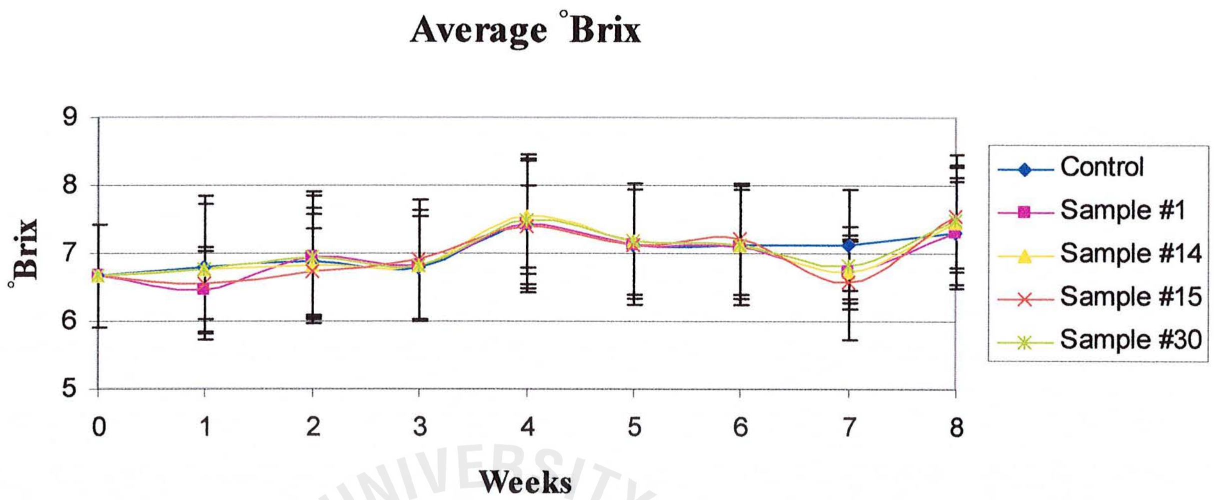

Figure 10: The average total soluble solid of 3 batches during 8 weeks

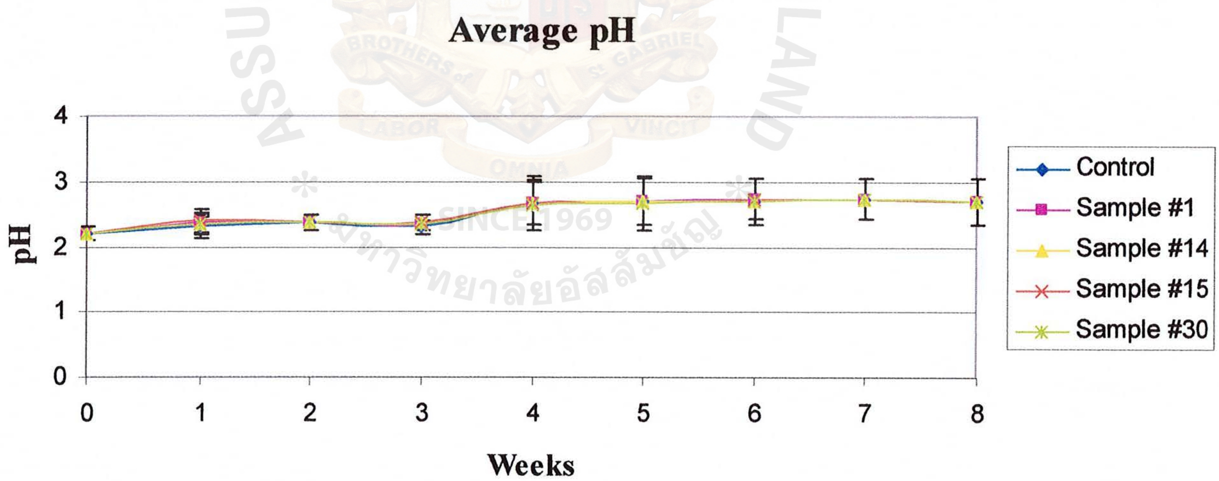

Figure 11: The average pH of 3 batches during 8 weeks

Chemical compositions of aged wine samples

Table 8: Properties of aged wine samples every 4 weeks during aging period (Batch1)

Week	Sample	°Brix	pH	reducing sugar	%Alc	%TA	%VA
0	**Based wine**	**6.0**	**2.1**	**0.00**	**13.1**	**0.6000**	**0.0300**
4	Control	6.7	2.3	0.00	-	0.5887	0.0240
	Sample No.1	6.7	2.2	0.10	-	0.6187	0.0900
	Sample No.14	6.9	2.2	0.10	-	0.6337	0.0300
	Sample No.15	7.0	2.2	0.00	-	0.6285	0.0330
	Sample No.30	6.9	2.2	0.00	-	0.6450	0.0330
8	Control	6.6	2.3	0.00	13.1	0.6712	0.0270
	Sample No. 1	6.6	2.3	0.10	13.1	0.6187	0.0300
	Sample No.14	6.8	2.3	0.10	13.1	0.6225	0.0300
	Sample No.15	7.0	2.3	0.05	13.3	0.6712	0.0330
	Sample No.30	7.0	2.3	0.00	13.1	0.6375	0.0330

Table 9: Properties of aged wine samples every 4 weeks during aging period (Batch2)

Week	Sample	°Brix	pH	reducing sugar	%Alc	%TA	%VA
0	**Based wine**	**7.5**	**2.2**	**0.10**	**12.9**	**0.6000**	**0.0420**
4	Control	8.5	2.8	0.25	-	0.6150	0.0330
	Sample No.1	8.6	2.8	0.50	-	0.6075	0.0300
	Sample No.14	8.5	2.8	0.50	-	0.6150	0.0360
	Sample No.15	8.1	2.8	0.25	-	0.6150	0.0300
	Sample No.30	8.6	2.8	0.25	-	0.6150	0.0330
8	Control	8.1	2.8	0.25	13.1	0.6338	0.030
	Sample No. 1	8.2	2.8	0.50	13.0	0.6488	0.036
	Sample No.14	8.6	2.8	0.50	13.0	0.6375	0.036
	Sample No.15	8.4	2.8	0.25	13.1	0.6300	0.030
	Sample No.30	8.4	2.8	0.10	13.1	0.6263	0.042

Table 10: Properties of aged wine samples every 4 weeks during aging period (Batch3)

Week	Sample	°Brix	pH	reducing sugar	%Alc	%TA	%VA
0	**Based wine**	**6.5**	**2.3**	**0.10**	**12.9**	**0.5700**	**0.0300**
4	Control	7.1	2.9	0.10	-	0.5625	0.0270
	Sample No.1	7.0	3.0	0.10	-	0.5475	0.0270
	Sample No.14	7.2	3.0	0.10	-	0.5550	0.0240
	Sample No.15	7.1	3.0	0.10	-	0.5475	0.0300
	Sample No.30	7.0	2.9	0.10	-	0.6038	0.0300
8	Control	7.2	3.0	0.10	12.5	0.6113	0.024
	Sample No. 1	7.1	3.0	0.25	12.6	0.6075	0.027
	Sample No.14	7.0	3.0	0.25	12.4	0.6038	0.027
	Sample No.15	7.2	3.0	0.10	12.2	0.6075	0.027
	Sample No.30	7.1	3.0	0.10	12.1	0.6038	0.030

Table 11: Average of 6 characters of aged wine (3 batches)

Week	Sample	°Brix	pH	reducing sugar	%Alc	%TA	%VA
0	**Based wine**	**6.7**	**2.2**	**0.07**	**13.0**	**0.5900**	**0.0340**
4	Control	7.4	2.7	0.12	-	0.5887	0.0280
	Sample No.1	7.4	2.7	0.23	-	0.5912	0.0490
	Sample No.14	7.5	2.7	0.23	-	0.6012	0.0300
	Sample No.15	7.4	2.7	0.12	-	0.5970	0.0310
	Sample No.30	7.5	2.6	0.12	-	0.6213	0.0320
8	Control	7.3	2.7	0.12	12.9	0.6388	0.0270
	Sample No. 1	7.3	2.7	0.28	12.9	0.6250	0.0310
	Sample No.14	7.5	2.7	0.28	12.8	0.6213	0.0310
	Sample No.15	7.5	2.7	0.13	12.9	0.6362	0.0300
	Sample No.30	7.5	2.7	0.07	12.8	0.6225	0.0350

Appendix C

Statistical Analysis

Chemical compositions

Table 12: One Way Anova of chemical compositions of aged wine samples

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Brix	Between Groups	.151	4	.038	.055	.993
	Within Groups	6.793	10	.679		
	Total	6.944	14			
pH	Between Groups	.000	4	.000	.000	1.000
	Within Groups	.133	10	.013		
	Total	.133	14			
%Reducing Sugar	Between Groups	.121	4	.030	1.354	.316
	Within Groups	.223	10	.022		
	Total	.344	14			
%Alcohol	Between Groups	.037	4	.009	.046	.995
	Within Groups	2.020	10	.202		
	Total	2.057	14			
%TA	Between Groups	.001	4	.000	.332	.850
	Within Groups	.006	10	.001		
	Total	.007	14			
%VA	Between Groups	.000	4	.000	1.242	.354
	Within Groups	.000	10	.000		
	Total	.000	14			

Note : $^{n.s.}$ **means no significant different**

* **means significant different**

Sensory characteristics

Table 13: Aroma Intensities of 7 aroma descriptors in aged sala wines

Wine Sample	Sala aroma	Vinegar	Sun dried banana	Red Hale's blue boy	Black pepper	Solvent (acetone)	Dried honey banana
Control	3.86[bc]	6.56	6.20	2.07[a]	1.68[b]	7.18[a]	3.26[ab]
Sample No.1	3.48[bc]	5.39	4.59	2.16[a]	0.70[a]	6.91[a]	4.69[b]
Sample No.14	4.40[c]	6.22	5.00	2.55[a]	0.50[a]	7.04[a]	3.02[a]
Sample No.15	2.76[ab]	5.76	5.24	2.29[a]	0.88[a]	9.30[b]	2.80[a]
Sample No.30	1.86[a]	6.67	5.27	3.90[b]	0.76[a]	7.36[a]	2.39[a]

Remark: Each aroma was analyzed separate from each other. Different in a, b and c of the same vertical column means significant different at $\alpha = 0.05$ level.

Table 14: Mean scores of wine's appearances using 9-poing Hedonic scales

Wine Sample	Clarity	Color	Overall aroma
Control	7.43	7.10	6.60
Sample No.1	7.50	7.40	6.13
Sample No.14	7.60	7.53	6.10
Sample No.15	7.50	7.57	5.97
Sample No.30	7.47	7.40	6.17

Table 15: One Way Anova of sensory characteristics of aged wine samples

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Sala Aroma	Between Groups	117.580	4	29.395	3.379	.011*
	Within Groups	1261.269	145	8.698		
	Total	1378.849	149			
Vinegar Aroma	Between Groups	34.735	4	8.684	.719	.581
	Within Groups	1752.305	145	12.085		
	Total	1787.040	149			
Sun-Dried Banana	Between Groups	42.257	4	10.564	.923	.453
	Within Groups	1659.985	145	11.448		
	Total	1702.242	149			
Hale's Blue Boy (Sala Syrup)	Between Groups	67.931	4	16.983	3.030	.020*
	Within Groups	812.702	145	5.605		
	Total	880.633	149			
Black Pepper	Between Groups	24.826	4	6.207	3.832	.005*
	Within Groups	234.871	145	1.620		
	Total	259.698	149			
Solvent (Acetone)	Between Groups	116.706	4	29.176	2.482	.046*
	Within Groups	1704.440	145	11.755		
	Total	1821.145	149			
Dried-Honey	Between Groups	92.012	4	23.003	2.486	.046*
	Within Groups	1341.870	145	9.254		
	Total	1433.883	149			
Clarity	Between Groups	.467	4	.117	.086	.987
	Within Groups	197.033	145	1.359		
	Total	197.500	149			
Color	Between Groups	4.067	4	1.017	.563	.690
	Within Groups	261.933	145	1.806		
	Total	266.000	149			
Overall Aroma	Between Groups	6.893	4	1.723	.484	.748
	Within Groups	516.500	145	3.562		
	Total	523.393	149			

Note : $^{n.s.}$ **means no significant different**

* **means significant different**

Homogeneous Subsets

Remark: Only homogeneous subsets which had significant different in attributes will be shown below.

Table 16: Mean scores of Sala aroma in five aged wine samples

Sala Aroma

	TRT	N	Subset for alpha = .05		
			1	2	3
Duncan[a]	sample#30	30	1.8633		
	sample#15	30	2.7600	2.7600	
	sample#1	30		3.4800	3.4800
	control	30		3.8633	3.8633
	sample#14	30			4.4033
	Sig.		.241	.175	.257

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Table 17: Mean scores of Hale's blue boy aroma in five aged wine samples

Hale's Blue Boy (Sala Syrup)

	TRT	N	Subset for alpha = .05	
			1	2
Duncan[a]	control	30	2.0733	
	sample#1	30	2.1567	
	sample#15	30	2.2867	
	sample#14	30	2.5500	
	sample#30	30		3.9000
	Sig.		.485	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Table 18: Mean scores of black pepper aroma in five aged wine samples

Black Pepper

	TRT	N	Subset for alpha = .05	
			1	2
Duncan[a]	sample#14	30	.5000	
	sample#1	30	.7000	
	sample#30	30	.7633	
	sample#15	30	.8767	
	control	30		1.6800
	Sig.		.303	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Table 19: Mean scores of solvent aroma in five aged wine samples

Solvent (Acetone)

	TRT	N	Subset for alpha = .05	
			1	2
Duncan[a]	sample#1	30	6.9133	
	sample#14	30	7.0400	
	control	30	7.1767	
	sample#30	30	7.3633	
	sample#15	30		9.2967
	Sig.		.650	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Table 20: Mean scores of dried-honey banana aroma in five aged wine samples

Dried-Honey

	TRT	N	Subset for alpha = .05	
			1	2
Duncan[a]	sample#30	30	2.3933	
	sample#15	30	2.8033	
	sample#14	30	3.0233	
	control	30	3.2633	3.2633
	sample#1	30		4.6933
	Sig.		.320	.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Appendix D

Forms Used in Sensory Evaluation of Aged Wine

HEDONIC TEST

Name: ______________________________
Product: ______________________________
Date: ______________________________

Instruction

1. Please smell fresh water before starting. You may smell it again at anytime during the test you need to.
2. Please smell the samples in the order presented, from left to right.
3. evaluate hedonic scale in each attribute of sample by using the following number

1 = dislike extremely
2 = dislike very much
3 = dislike moderately
4 = dislike slightly
5 = neither like nor dislike

6 = like slightly
7 = like moderately
8 = like very much
9 = like extremely

Attribute	______	______	______	______	______
Clarity					
Color					
Overall Aroma					

Note : ______________________________

____

____

INTENSITY TEST

Name :____
Product :____________________________**Date :**__________________

Instruction : Evaluate the sample for appearance by placing a mark on each line below

Sala Aroma

Low **High**

Vinegar Aroma

Low **High**

Sun-Dried Banana Aroma

Low **High**

Hale's blue boy Red Aroma

Low **High**

Black pepper Aroma

Low **High**

Solvent (Acetone) Aroma

Low **High**

Dried Honey Banana Aroma

Low **High**