The Study of Purple Rice Wine Production Using Different kinds of Starter Cultures And Cooking Conditions

By

Ms. YEN TRAN HAI

MASTER'S RESEARCH PROJECT

Submitted in partial satisfaction of the requirement for

Master degree of Food Biotechnology

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Faculty: Food Biotechnology

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ABSTRACT

Purple brown rice is one of the new species in Thailand. The species had been genetically bred to improve in nutrition and color in order to promote consumer's preference and health enhancement. Up to present, purple brown rice wine has not been attracting too many attentions. Furthermore, there are only a few research works mentioned the role of using pure culture and commercial local starter culture in rice wine making. Therefore, the quality of the purple brown rice wine was studied by using different kind of starter cultures to make the wine. Four local starter cultures from different collection were chosen to isolate pure culture strains in this research. The traditional isolation method was applied. However, each sample had different profiles of microorganisms. Especially, the local starter culture number 1 consisted of mold (*Actinomucor* spp., *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp.), yeast (*Saccharomyces* spp., *Candida* spp. and *Schizosaccharomyces* spp.) and LAB (*Lactobacillus casei*). Only yeast was isolated in the local starter number 4. On the other hand, the rice wine produced by both cooking and steaming rice process was tested and compared. The result showed that the steamed rice produced the higher amount of alcohol content than cooked rice. In addition, the color of the steamed purple brown rice wine was also better than the cooked rice wine. The quality of purple brown rice wine was checked by the chemical analysis and sensory evaluation. The wine produced from local starter culture number 1 and number 2 gave the best result. Furthermore, the alcohol content of the wine added with pure culture from local starter culture number 1 (9% v/v) is higher than the wine produced by adding local

starter culture number 1 (8.9 % v/v) and the mixture of local starter culture number 1 and dry active wine yeast (8.6% v/v).

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LIST OF ABBREVIATIONS

LAB: Lac tic acid bacteria

LS1: The purple brown rice wine produced by local starter culture number 1

LS2: The purple brown rice wine produced by local starter culture number 2

LS3: The purple brown rice wine produced by local starter culture number 3

LS4: The purple brown rice wine produced by local starter culture number 4

LSDY1: The purple brown rice wine produced by combination of dry active wine yeast and local starter culture number 1

LSDY2: The purple brown rice wine produced by combination of dry active wine yeast and local starter culture number 2

LSDY3: The purple brown rice wine produced by combination of dry active wine yeast and local starter culture number 3

PS1: The purple brown rice wine produced by pure strains of local starter culture number 1

PS2: The purple brown rice wine produced by pure strains of local starter culture number 2

PS3: The purple brown rice wine produced by pure strains of local starter culture number 3

CHAPTER I

INTRODUCTION

Rice is one of the most important grains for human consumption. Besides, rice is used for wine and vinegar production in the Orient. For instance, the purple glutinous rice wine has been produced for a long time because of its sherry-like taste, flavour and attractive brown-red color. The rice production is increasing dramatically around the world every year. Thailand is the top rice-exporting country in recent years. The genera of Thai rice are very diverse according to the customers' demand. At present, the purple brown rice is gaining more and more popular due to its color and nutritional content. Nevertheless, the purple brown rice wine production has not been focused up till now.

The most important ingredient used in the process of wine making is the starch-based rice wine fermentation starter cultures. However, a few research characterized about 100 types of microorganisms (moulds, yeast and lactic acid bacteria) which are important strains in solid state starter cultures. Each producer may have a different way of starter culture production, depending on the available ingredients and local custom preferences. In most cases, the quality of local starter cultures is not stable which lead to affecting on the rice wine quality. Until now, only yeast diversity are identified in Thai loog-pang (Thai starter culture) by Limtong, Sintara, Suwanarit and Lotong (2002) . Although, *Saccharomyces cerevisiae* is the most important yeast in the wine production, Limtong, Sintara, Suwanarit and Lotong (2002) indicated that it produced the low ethyl alcohol which is only 4.68% (w/v). The mold identification has not been

seriously studied yet in Thailand. Actually, the rice wine was produced by two step fermentation which is solid state fermentation in which the mold will produce amylase enzyme to change the starch to sugar and later on, yeast will use sugar to produce alcohol in the liquid state fermentation.

Objective

The objective of this study is to find the most appropriate cultures and processes to produce the purple brown rice wine with the acceptable quality by the taste panels. Local starter cultures which are available commercially from different sources were used for isolating and identifying for the beneficial organisms for wine making process. Traditional method was used for the isolation and identification of these cultures.

CHAPTER II

LITERATURE REVIEW

2.1 Purple brown rice

2.1.1 History

Chang (2003) and Rosell and Marco (2008) described rice always associates with human civilization. The earliest ancestor of rice is a grass which came from various humid regions of the southern landmass now called the Gondwana supercontinent more than 130 million years ago. After the Gondwana was separated to many landmasses, the genetic diversity of rice increased quickly. About 22 wild species was found and two kinds of rice are cultivated such as *Oryza glaberrima* and *Oryza sativa*. The origination of *O. glaberrima* is in West Africa and another is in South and Southeast Asia. Rice is the predominant staple food in many developing countries. An illustration of this is more than 100 countries of the world across a south-to-north span from 40°S to 53°N latitude are growing the rice. Global rough rice (paddy) production reached 534 million metric tons in 1994, of which 482 million metric tons were harvested in Asia. Until now, Chang and Luh (in Chang 2003, p. 4) stated that demand of rice and wheat production will be increased dramatically in order to support about 4 billion people by year of 2020.

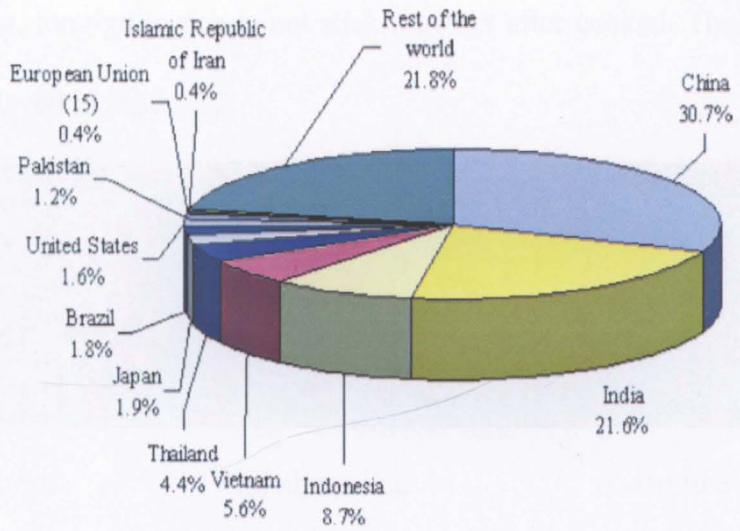


Fig 2.1: Distribution of the world paddy rice production (average 1999-2003) (Source: UNCTAD Secretariat from the Food and Agriculture Organization of the United Nations (FAO) data)

2.1.2 Rice classification

According to Rosell and Marco (2008) and the Pechsiam group's website (2000), rice can be classified by length, color, region of origin, quality, and texture.

2.1.2.1 Length

There are three sizes of rice grain including long (longer than 6.6 mm), medium (between 5.5 to 6.6mm), or short (shorter than 5.5mm). Different grain size would have various characteristic. For example, short grain rice tended to be very sticky after cooking. However, some of the starchiness was reduced when the steaming method was applied to cook the rice. The short grain rice is usually used for sushi, risottos, stir-fry recipes, and desserts. Another word, short-grain rice is stickier than medium-grain rice after cooking. The medium grain rice was clumped together when cooled. But it holds plenty of moisture when it was cooked, and it remains a bit firmer than short-

grain. In contrast, long-grain rice is not stick together after cooked. The different size of rice was displayed in figure 2.2.

Short rice Medium rice Long rice

Fig 2.2: Various grain size of rice

2.1.2.2 Color

Two different colors were used for rice classification such as brown or white. The complete milling process was applied for white rice production. Consequence, the bran layer and some nutritional components were lost. However, some of Western countries tried to enrich the number of lost nutrient in white rice such as iron, niacin, thiamin, and riboflavin. In addition, they also added the color to white rice in order to get the green rice. The distinctive color is known as chlorophyll. On the other hand, all unprocessed rice is a shade of brown, varying from dark yellow to red to deep brown or black, depending on the color of the bran and how much of the bran remains after processing. The figure 2.3 below showed the various color of rice.

Fig 2.3: Different color of rice

2.1.2.3 Region

Rice is also classified by the area or countries where the rice was cultivated. In fact, the popular market of rice production is Asia. The Asian eat rice as their staple food.

- United States: Texmati, Carolina®, Kasmati
- Japan: Mochi, Sushi
- Spain: Bahia, Bomba, Valencia
- Italy: Arborio, Roma, Carnaroli, Vialone Nano
- India: Basmati, Indian Red
- Thailand: Jasmine, Thai Black, Thai Red
- Iran: Sadri, Dom Siah
- Indonesia: Indonesian Red, Indonesian Black, Fragrant
- China: Bamboo, Chinese Black

2.1.2.4 Quality

According to the quantity of broken grains, "Top Quality," "Standard," "Household" and "Broken" are four categories of rice. In term of the top quality of rice, the percentage of broken grains is less than 5 percent of the total while 15 percent broken grains is a ratio of standard rice. On the other hand, household rice had two groups such as a maximum of 25 percent broken grains in the batch and a maximum of 40 percent broken grains in the batch. The final one is broken rice which had more than 40 percent of the grains were broken.

2.1.2.5 Texture

The rice genetic diversity is abundant, so the cooked rice could be classified from very sticky and soft to very firm and fluffy. The difference is defined by the amylose/amylopectin ratio. The sticky rice (glutinous rice) is very popular in Asia while the normal rice is usually consumed in United State. Two different textures of cooked rice were displayed in figure 2.4.

Cooked sticky rice High amylose cooked rice

Fig 2.4: Various rice textures

(Source:http://www.visit-chiang-mai-online.com/thai-sticky-rice.html, http://www.virginmedia.com/homefamily/fooddrink/food-poisoners.php?ssid=2)

2.1.3 Rice nutritients

Table 2.1 below shows the different nutrient contents of various rice grains. The milling process is the main factor which affected to the color of rice grains. There are four main nutrients content was mentioned in this case such as protein, iron, zinc and fibre.

Table 2.1: Nutrient contents of rice varieties

Type of rice	Protein (g/100g)	Iron (mg/100g)	Zinc (mg/100g)	Fibre (g/100g)
White - polished[a]	6.8	1.2	0.5	0.6
Brown[a]	7.9	2.2	0.5	2.8
Red[b]	7.0	5.5	3.3	2.0
Purple[b]	8.3	3.9	2.2	1.4
Black[a]	8.5	3.5	-	4.9

Sources: [a] = Association of Southeast Asian Nations (ASEAN) food composition table; [b] = Chinese food composition table.

(Source: International year of rice 2004)

2.1.4 Definition of purple brown rice

Nowadays, traditional plant breeding techniques and genetic engineering are the popular methods which improved the nutritional content of common rice varieties (International year of rice 2004). Especially, purple brown rice is developed using conventional cross breeding between two most famous rice that are Thai Jasmine and Kao Hom Nin (L H Rice International Company's website 2009). Kao Hom Nin is one of the high iron rice in Thailand. The whole grain of purple brown rice is dark purple, enriched with natural nutrients, and a distinct aroma. In fact, the milling process is not happened in the purple brown rice so the outer bran layer is still remained that in rich of nutritional value like protein, vitamin B and mineral. The color of rice is one of the most important parameter to identify the rice quality when they have so many different colors from black to red and brown. L H Rice International Company's website (2009) showed the purple pigment in rice grains is anthocyanin which contained several folds more antioxidant activities than the similar pigments of red grape, prune and blueberry. However, the rice without the husk had the bitter taste because the bran layer turned to

be rancid quickly. Therefore, the un-milled rice is usually used for fermentation process. The structure of rice and the cooked purple brown rice was showed in figure 2.5.

Fig 2.5: The schematic shows the most distinctive structures of a rice seed.

(Source: http://www.goldenrice.org/Content3-Why/why3_FAQ.html)

2.2 Starter cultures

2.2.1 Local starter cultures

2.2.1.1 Local starter cultures (Amylolytic fermentation starters) roles

Steinkraus's study (cited in Wang 1991, p.197) and Hutkins (2006) indicated that microbial starter cultures were added to the food directly in order to get useful effects of the fermented products at the end. Ellis et al.'s study (cited in Wang 1991, p.197) showed that there were different names of rice wine making starter cultures belong to the literature such as Indonesian *ragi-tape*, Malaysian *ragi-tapai*, Thai *loogpang*, Philippine *bubod levadura*, Chinese *ch'u* and Indian *bakhar*. Those

local starter cultures consist of mixed cultures of starch-degrading molds and fermenting yeasts. Molds produce functional enzymes such as α-amylase, amyloglucosidase which hydrolyzed starches to dextrin, maltotriose, maltose and glucose. Especially, glucoamylase (glucan 1,4- α-glucosidase) is the most important enzyme in rice wine fermentation because they could transform starches to glucose directly. In addition, acid and alkaline proteases are also produced to hydrolyze proteins to peptides and amino acids which contributed to the flavor content of finished products. Moreover, the role of yeast is alcohol production in this case. Nevertheless, some yeast species could mobilize starch to assimilate carbon sources and supply low alcohol content.

2.2.1.2 Local starter cultures production

Local starter culture making is a solid-state fermentation in which the ingredients after putting all together was left for air dry. The characteristics of milled raw grains define the selection and enrichment of natural microbes in the starter cultures. The size and texture of amylolytic substrates also affects to the growth of native-appearing microbes. So, the general process of local starter cultures production was described by Saono et al. (Steinkraus 1996) and Aidoo, Nout and Sarkar (2006). At the beginning of the starter culture production, the rice flour might be mixed with various herbs and additives such as dry powdered ginger, pepper, chili, garlic, etc. Then, the water or sugar cane juice was added to mixture in order to get the predicted moisture content. After that, dry powdered starters from previous batches were added for mixture inoculation. The cake was shaped into small balls or flattened tablets. These cakes were put on a bamboo tray and incubated for several days at ambient

temperature so that the desired microorganisms could be grown on flattened cake of rice flour. Finally, the starter cakes were either air or sun-dried to preserve the essential microorganism for several months at room temperature in the tropical area. Figure 2.6 showed the different shape of local starter culture which was produced by various sources.

Fig 2.6: Amylolytic fermentation starter cultures: Men (left) and Ragi (right).

(Source: Aidoo, Nout and Sarkar, 2006)

2.2.1.3 Current issues

Because of the unstable quality of local starter cultures, some researches tried to isolate and identify the name of those mold and yeast. For instance, Saono et al. (Aidoo, Nout and Sarkar 2006, p. 33) stated that *Amylomyces rouxii, Rhizopus* spp., *Mucor* spp. *and Aspergillus* spp. are amylolytic molds which usually found in Indonesian ragi, Chinese *ch'u* and *tane-koji* preparation. Moreover, Shrestha (Aidoo, Nout and Sarkar 2006, p. 34) showed that the genera *Mucor* and *Rhizopus* were discovered in Indian *bakhar* that were useful for the production of rice wines in India and Nepal. Another word, *Hansenula* spp., *Saccharomycopsis fibuligera, Candida* spp. *and Saccharomyces cerevisiae* were common yeasts in many starter tablets. Not only

traditional technique but also modern method was applied for mycelia fungi isolation. PCR-mediated DGGE was used for investigating the diversity of fungi and bacteria associated with traditional Vietnamese alcohol fermentation starter cultures. According to Thanh, Mai and Tuan (2008), a lot of species of yeast, molds and bacteria (lactic acid bacteria) were described in that study. Nevertheless, the appearance of some opportunistic contaminants in the traditional starter was suggested for further researches. In term of Thai traditional fermentation starter cultures, the yeast was isolated by using traditional method, *S. fibuligera* showed the strong amylolytic activity and flavor contribution in rice wine production. In contrast, *S. cerevisiae* was not the main ethyl alcohol producer in loog-pang. Until now, some researches were successful to select the molds and yeast which had the high amylolytic activities. An illustration of that was defined granulated starters containing *A. rouxii* and *S. cerevisiae* make high-quality Vietnamese rice wine (Dung et al. (Aidoo, Nout and Sarkar 2006, p. 34)). Moreover, Thitisararak, Plumcharoen and Rungsardthong (2003) isolated two fungi (*Aspergillus* sp. and *Rhizopus* sp.) and three yeast strains from a commercial look-pang lao, Thai starter cakes. Those microorganisms were used for lab starter cultures. Then, they indicated that the rice wine made from local starter cultures together with the pure commercial dry culture was better than the rice wine added with local starter cultures along.

2.2.2 Dry active wine yeast

Fleet and Heard (Krieger-Weber 2009) showed *Saccharomyces cerevisiae* is dominant in spontaneous alcoholic fermentations. According to Dittrich and Grossmann (Krieger-Weber 2009), when the alcohol content of spontaneous alcoholic

fermentations was around 4% v/v, non-*Saccharomyces* yeast was died. In contrast, *S. cerevisiae* could be existed and finished the fermentation. On the other hand, not only the dominant strains but also the *Saccharomyces* genus decided the successful fermentation. Therefore, more than 200 different yeast strains were available in the world market nowadays. However, *S. cerevisiae* and *S. bayanus* are the most popular commercial wine yeasts. In term of nutritional requirements of selected yeast strains, Julien et al. and Sablayrolles et al. (Krieger-Weber 2009, p.490) demonstrated that different selected yeast strains had various nitrogen and oxygen demanding. The advantage of the combined additions oxygen and nitrogen is prevention of stuck alcoholic fermentations. In addition, temperature is one of the important factors which affected to yeast growth strongly. Krieger-Weber (2009, p.490) in reporting Henick-Kling's study suggested that the optimum temperature for alcoholic fermentation was between 20 and 30°C and most selected yeast strains would tolerate up to 14% v/v. The role of selected yeast strains in wine making just ferment sugar into ethanol for a long time. On the other hand, both sensory properties and overall wine quality are becoming the most important choices of selected yeast strains these days. Moreover, the population of the indigenous yeasts already in the juice, choice of yeast strain and its adaptation to specific wine environment are also main factors affected to the finished products. Because of the short shelf-life of liquid starter cultures, Degre's study (cited in Krieger-Weber 2009) indicated that the active dry yeasts (ADY) was used and produced by the United States in the seventies and eighties. After that, they are more and more popular in wine industry. Monk (Krieger-Weber 2009, p.493) indicated that active dry yeast starter cultures production should be supplied an adequate nutritional

and oxygen demanding. Those nutrients were protein, ergosterol, unsaturated fatty acids, and reserve materials. Moreover, Degre (Krieger-Weber 2009, p.493) showed that the resistance of yeast to drying and subsequent rehydration is affected by the trehalose content in the cell. As a result, yeast producers tried to stimulate formation of trehalose during production so that the resistance of yeast cells to the stresses of dehydration and rehydration could be increased. Then, the yeast was dried to conserve it during transport and storage. On the other hand, residual moisture of commercial dry yeast normally contains less than 6%-8%. Therefore, active dry yeast must be rehydrated for revitalization. However, Henick-Kling (cited in Krieger-Weber 2009) indicated that rehydration of active dry yeast has a little bit problems when it was not done properly, consequence: it could leak large amounts of cellular components and loose viability and vitality.

2.3 Rice wine

2.3.1 Classification of traditional alcohol beverages in Southeast Asia Countries

In term of wine making process, alcoholic beverages were classified to three broad types such as brewing alcohol beverages (wine, beer, sake, etc), distilled spirit (whiskey, brandy, vodka, tequila, etc), and mixed of brewing and distilled spirit (liquor, vermouth, etc). On the other hand, according to fermentation processes, there were two kinds of brewing alcohol beverages like single fermentation process (wine) and combination fermentation process (beer and sake). Wine is the raw material fermentation directly while beer and sake must be spent two periods. First, starches are

converted to sugar. Second, yeast would transfer sugar to alcohol. (Lisdiyantti and Kozaki, 2003)

2.3.2 Rice wine origins

Lisdiyantti and Kozaki (2003) recorded that the rice wine making was originated from Yunggui area of China. Until now, the distilled spirit production is much more popular than the traditional alcohol beverages in that place. Then, the rice wine process spread to Southeast Asia. However, the development of rice wine was limited by religion in Indonesia and Malaysia. The rice wine consumption is still in local custom because of the household rice wine making. Nowadays, Nout and Aidoo (cited in Aidoo Nout and Sarkar 2006) Japan, China, Korea, Thailand, the Philippines and Vietnam are famous countries which making commercial rice wine.

2.3.3 Characteristics of traditional rice wine

Rice chaff addition led to difference between the continental and the islands of Asia in traditional rice wine making. Therefore, there are four way of making rice wine in accordance to application of rice chaff (figure 2.7)

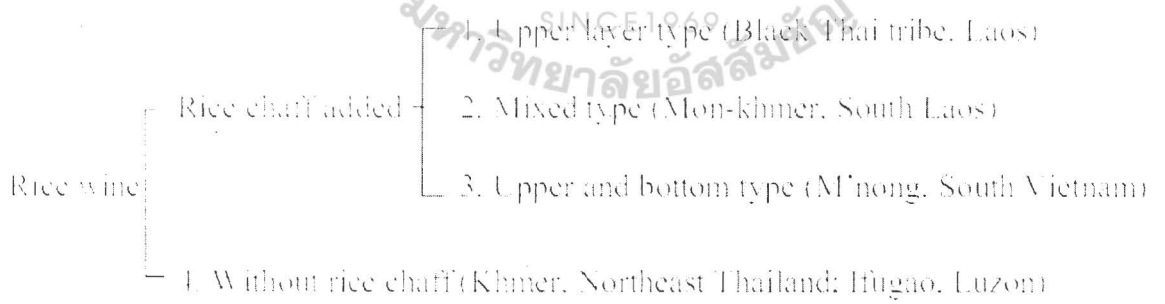


Fig 2.7: Classification of rice chaff application.

Lisdiyantti and Kozaki (2003) described some characteristics of traditional rice wine. First, the red (white) rice or waxy (non-waxy) rice was raw materials for making

rice wine. Second, the amylolytic starters were used for this process. Third, rice chaff was applied or not during fermentation. Fourth, the fermentation process of rice wine was conducted in one or two jars. Finally, the traditional drinking could be used fine bamboo straw without filtration depending on the different places. The rice wine process was displayed in figure 2.8 from the beginning to the end of starch transfer.

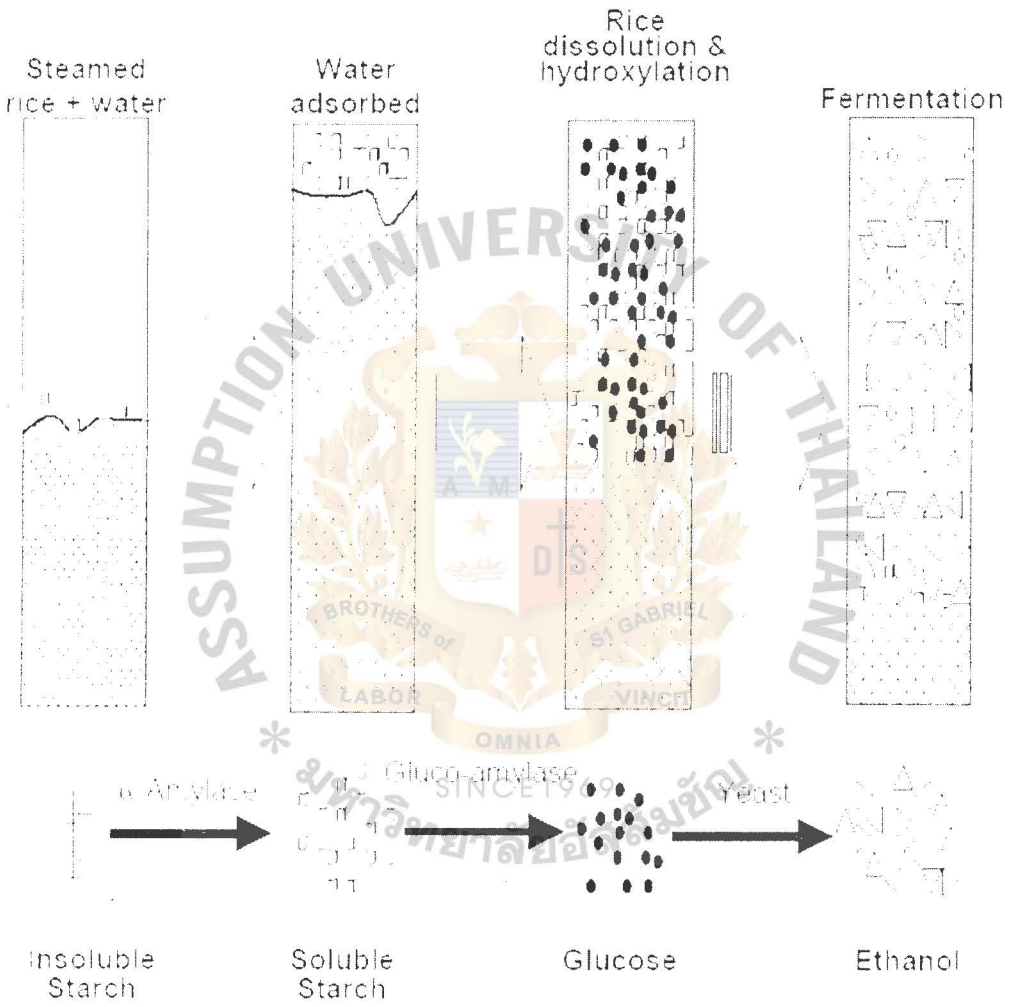


Fig 2.8: A model of dissolution of steamed rice, hydroxylation of starch, and ethanol fermentation

(Source: Yoshida Technology Development of Sake Fermentation in Japan)

CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection

Four dry commercial traditional rice wine starter cultures were obtained from leading producers in Thailand. Those local starter culture samples were stored at 4^0C prior to use for isolating the pure strains and identifying the name of selected microorganisms.

Dry active wine yeast used in this study was *Saccharomyces bayanus* which is a product of Danstar Ferment AG company (Denmark). They were storage in refrigerator until used.

3.2. Isolating and identifying the pure culture of local starter cultures

3.2.1 Isolation of microorganisms

1g of each selected local starter culture was homogenized with 99ml sterile saline (NaCl 0.85 g/100ml) in a Stomacher Lab-blender for 1 minute at high speed, and the serial dilutions (10^{-3}, 10^{-4} and 10^{-5}) were made in the same diluents. Each dilution was spread onto a plate containing sterile agar. The single colonies that appeared after incubation were transferred to slants of sterile agar. All plates and slants were incubated at 37^0C for 24-48 hours.

Mold was selected on the PDA agar (Himedia laboratories Pvt. Ltd).

Yeast malt extract (YM) agar was used for selection of yeasts. It contained malt extract (3 g/l), glucose (10 g/l), Yeast extract (3 g/l), agar (15 g/l), peptone (5 g/l)

For lactic acid bacteria: LAB was determined on MRS agar (Himedia laboratories Pvt. Ltd). Especially, the LAB was incubated in anaerobic condition.

3.2.2 Identification of mould, yeast and LAB

Isolated mold and yeasts were identified based on morphological examination and cultural properties according to established taxonomic keys and descriptions. (Webster 1970 and The University of Adelaide's website)

LAB species were determined depending on Bergey's Manual of Determinative Bacteriology

3.3 Compare the difference result of adding pure strains of local starter cultures to local starter culture and dry active wine yeast in purple brown rice wine making

After isolating the pure culture of local starter cultures, 3%-5% of local starters were used as the control sample in purple brown rice wine production.

3.3.1. Cultures preparation

Yeast and molds continued to be grown on slants of Yeast Malt Extract Agar (YM agar) and PDA agar at 37^0C for 2 days. Grown cultures were maintained in refrigerator.

3.3.2 Preparation of inoculums

Inoculums suspensions were made by adding sterile physiological salt solution (0.85% w/v NaCl) onto each slant. The biomass was gently scraped off the

agar by means of an inoculation loop. Then, 1 ml of the suspension from prepared one slant was used for mold, yeast and LAB inoculation.

3.3.3 Inoculation process

The traditional fermentation process was chosen in this study. Purple brown rice (50 g) was steamed or cooked at 100^0C. The steamed/cooked rice was cooled to 35- 40^0C and inoculated with pure cultures (mould, yeast and LAB) or 2 g of local starter cultures at room temperature. Solid-state fermentation period was 5 days. Then, adding 70ml of sterile water to the mold mass for submerged alcoholic fermentation and maturation for 10 days at 35^0C in the same flask, closed with an air lock.

For fermentation test of dry active wine yeast addition, purple brown rice (50 g) was steamed /cooked at 100^0C. The steamed/cooked rice was cooled to 35–40^0C and mixed well with local starters (2g) at room temperature. After 5 days of solid-fermentation, 1 g of dry active wine yeast and 70 ml of water was added to the mold mass for liquid state fermentation. Then, maturation for 10 days at 35^0C in the same flask, closed with an air lock. Finally, clear liquid was harvested after filtration for further analysis.

3.3.4 Chemical analysis

The chemical analysis was done by the method mentioned by Richard, Ellen & Theresa (Ovartvoraporn, 2002). The pH was measured by pH meter, Denver Instrument, Model 15. Total alcohol content was identified by Ebulliometer. In addition, the total soluble solid (^{0}Brix) was measured by refractometer. The amount of total acidity was analyzed by titration. Each sample was tested in duplicate.

3.3.5 Sensory evaluation

Purple brown rice wine sensory test was set up by my advisor. The number of experience panelists invited to test these purple brown rice wine products was ten. The amount of the rice wine in each sample was 50 ml for each examiner. The temperature of the rice wine was controlled at 20^0C. There were four descriptive factors for this evaluate such as: clarity and color, taste and flavor, aroma/bouquet and over impression.

3.4 Statistic analysis

The intensity score analysis of panelists in sensory evaluation was analyzed by using SPSS 16.0 for window program. Randomized Complete Block Design was set up for this research.

CHAPTER IV

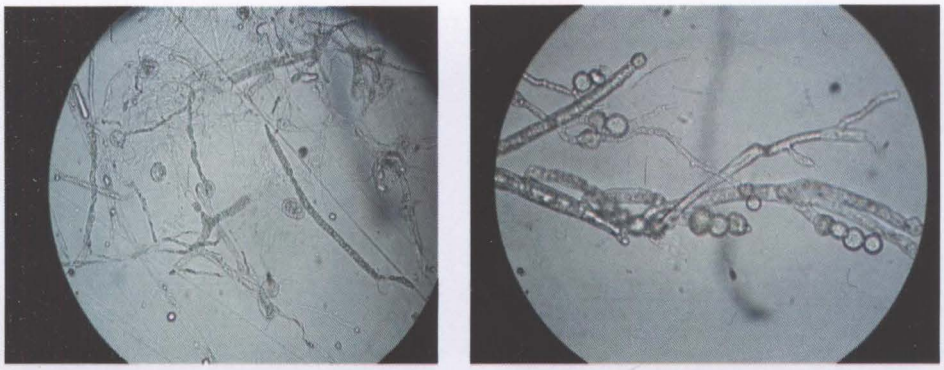
RESULTS AND DISCUSSION

4.1 Isolating and identifying the pure culture of local starters

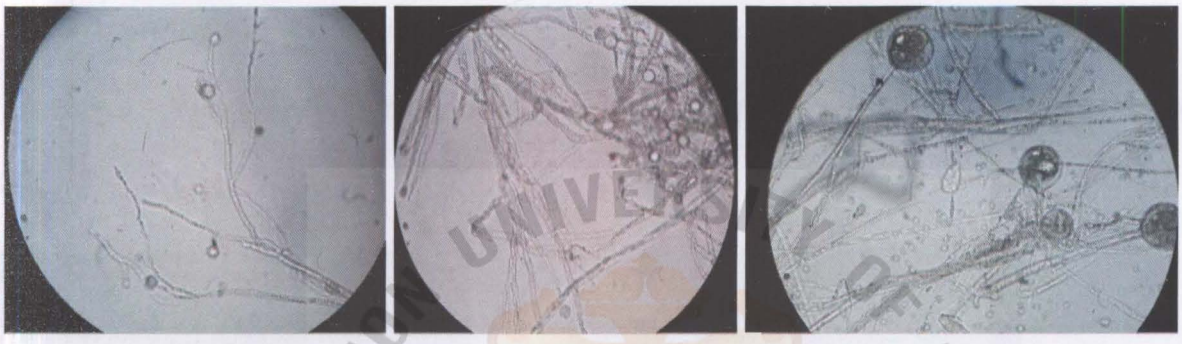
In this study, four different kinds of commercial starter cultures were used for isolation and identification of microorganisms. According to the microscopic morphology of single colony, four species of mold was identified such as *Actinomucor* spp., *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp.. The yeast cultures like *Saccharomyces* spp., *Candida* spp. and *Schizosaccharomyces* spp. were also isolated from the local starter cultures. Because of the different local producers, each commercial local starter culture consists of various yeast, mold and LAB. Illustrations of the organisms isolated from local starter culture number 1, 2, 3 and 4 were shown in figure 4.1, 4.2, 4.3 and 4.4 respectively. In table 4.1, organisms isolated from different local starter cultures sample were shown.

Table 4.1: Isolated microorganisms in local commercial starter cultures

Local starters	Microorganism		
	Mould	Yeast	LAB
1	*Actinomucor* spp., *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp.	*Saccharomyces* spp., *Candida* spp. and *Schizosaccharomyces* spp.	*Lactobacillus casei*
2	*Mucor* spp.	*Saccharomyces* spp., *Candida* spp.	Not found
3	*Actinomucor* spp.	*Saccharomyces* spp., *Candida* spp.	Not found
4	Not found	*Saccharomyces* spp., *Candida* spp.	Not found

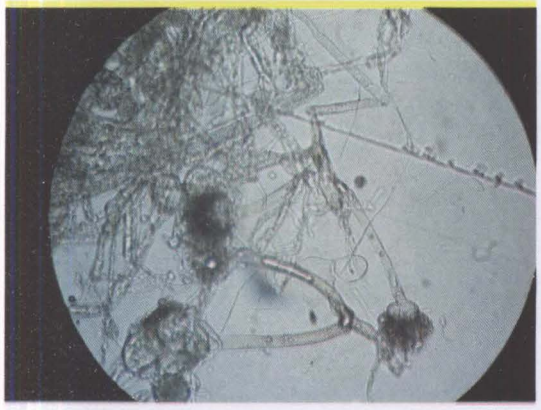


Actinomucor spp. (40x)

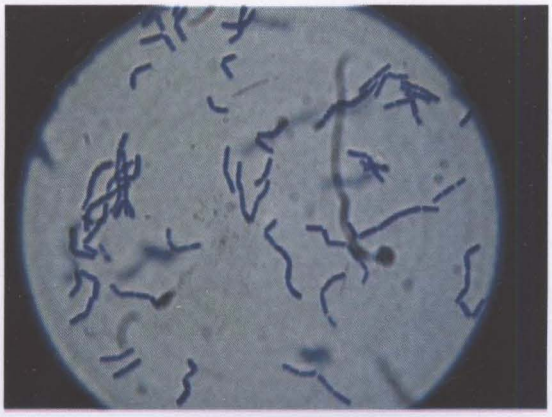


Mucor spp. (40x)

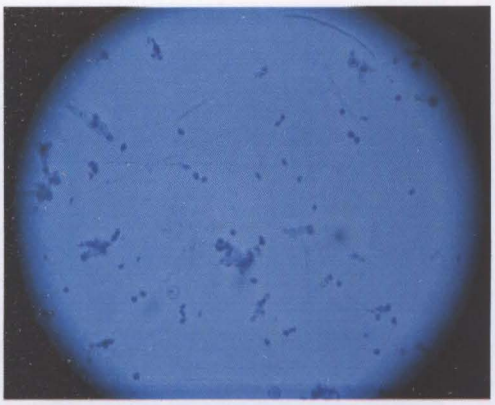
Rhizopus spp. (40x)



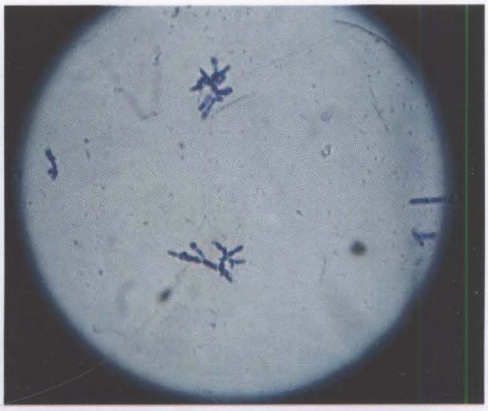
Aspergilus spp. (40x)



Schizosaccharomyces spp. (100x)

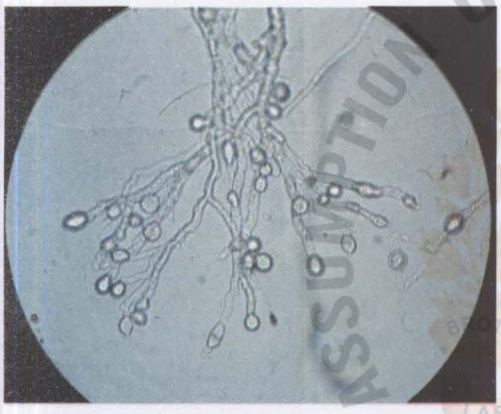


Saccharomyces spp. (40x)

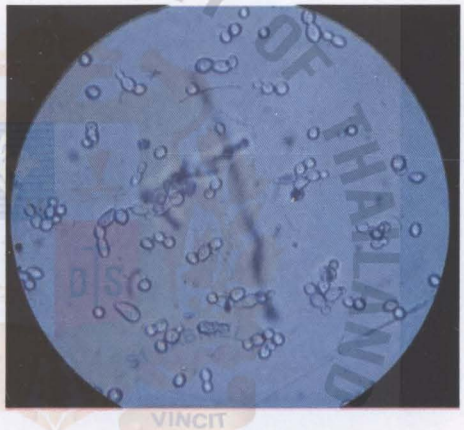


Candida spp. (100x)

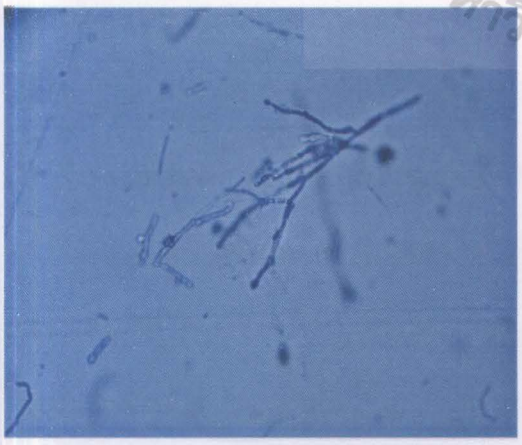
Figure 4.1: Yeast and mold morphology of sample 1 under microscopic (40x, 100x)



Mucor spp. (40x)

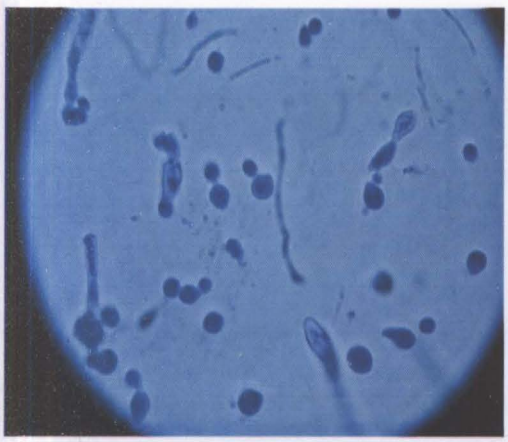


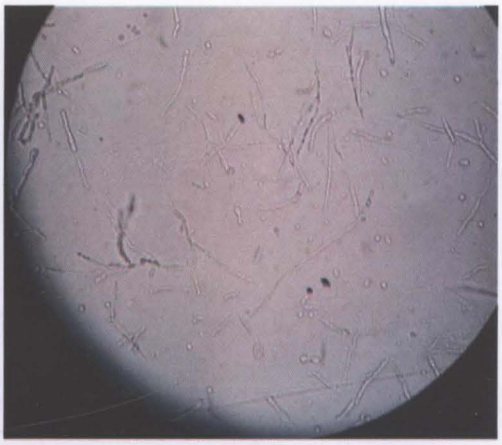
Saccharomyces spp. (100x)



Candida spp. (40x)

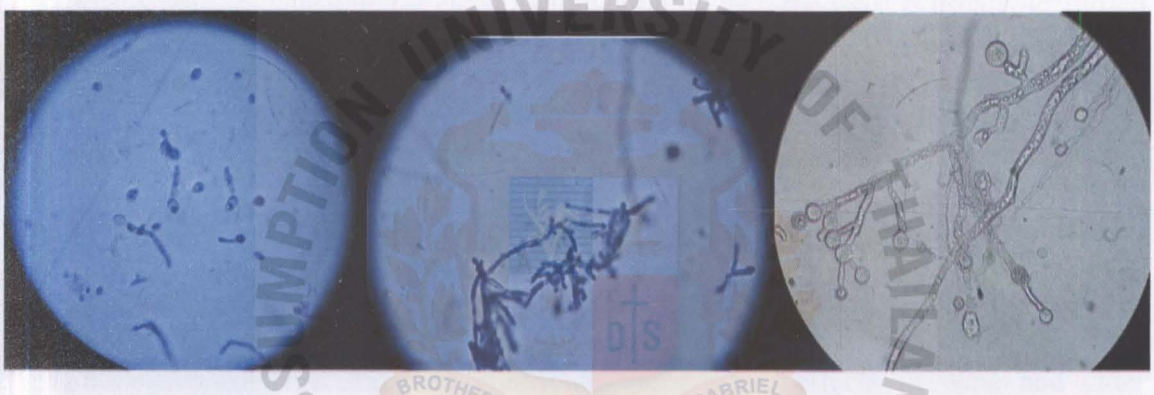
Figure 4.2: Yeast and mold morphology of sample 2 under microscopic (40x, 100x)





Saccharomyces spp. (100x) *Candida* spp. (40x)

Figure 4.3: Yeast morphology of sample 4 under microscopic (40x, 100x)



Saccharomyces spp. (100x) *Candida* spp. (40x) *Actinomucor* spp. (40x)

Figure 4.4: Yeast and mold morphology of sample 3 under microscopic (40x, 100x)

Wang (1991) indicated that *Mucor* and *Rhizopus* are common molds which found in the commercial starter cultures although the production is always unsanitary. In addition, they are the main enzyme producers for the production of rice wines in India and Nepal. On the other hand, Thitisararak et al (2003) had successfully isolated *Aspergillus* sp. and *Rhizopus* sp. from a commercial look-pang lao (Thai starters). *Aspergillus* sp. is the most important mold in the Japanese Sake production. They grow very well in the highly aerobic condition and on carbon-rich substrates. *Aspergillus*

species usually exist in the starchy food such as bread and potato. Especially, *Actinomucor* spp. was found in this study. *Actinomucor* spp., *Mucor* spp., and *Rhizopus* spp. are genera of the Zygomycetes. These molds break down starch to simpler sugar so that the yeast can use it to produce other products.

Regarding to the yeast identification, *Saccharomyces* spp. is the predominant yeast in the isolation of this study. *Saccharomyces* species are the facultative anaerobes. The nutrient requirement is very simple such as a reduced carbon source, minerals, nitrogen and vitamins. The genera of *Saccharomyces* can use fermentable carbohydrates as substrate for alcohol production. In fact, the yeast is usually identified in the local starters such as *Saccharomyces* spp. and *Candida* spp. (Aidoo, Nout and Sarkar 2005). For instance, Limtong, et al (2002) described those yeast were existed in the Thai loog-pang. However, not only *Saccharomyces* spp. and *Candida* spp. but also *Schizosaccharomyces* spp. was identified in this study. Fleet (2006) showed that *Schizosaccharomyces* spp. can contribute the positive effects in wine and cider production. In fact, *Schizosaccharomyces* is fission yeast which has one specific property in the fermentation such as alcoholic tolerance (about 5%-7% alcohol by volume). Overall, selected microorganisms contribute not only alcoholic beverage fermentation but also the quality of final products.

4.2 Inoculums

The cooking and steaming process was applied to the purple brown rice to find out which cooking process can be used to produce the better rice wine. Ovartvoraporn (2002, p.6) reported that from Jackson's study, found that the quality of wine is defined by chemical property and physical property. Color, clarity, tear and viscosity are

measured for identifying the physical characteristic. As a result, table 4.2 and 4.3 showed the chemical analysis of the products made from the purple brown rice in this study.

Table 4.2: Chemical analysis of cooked purple brown rice wine production

Name of rice wine	pH	Total soluble solid (^{0}Brix) after solid fermentation	Total soluble solid (^{0}Brix) after alcoholic fermentation	Total acidity (%)	Alcohol content (% v/v)
LS1	3.84±0.01	7	5	0.54±0.01	7.25
LS2	3.91±0.01	6.2	5	0.56±0.03	7.61
LS3	4.18	11	5.1	0.47±0.01	7.8
LSDY1	3.78±0.04	7.1	4	0.56±0.02	6
LSDY2	3.62±0.01	7	5	0.66±0.03	6.7
LSDY3	4.12±0.01	7	6.5	0.58±0.02	6.45

Table 4.3: Chemical analysis of steamed purple brown rice wine production

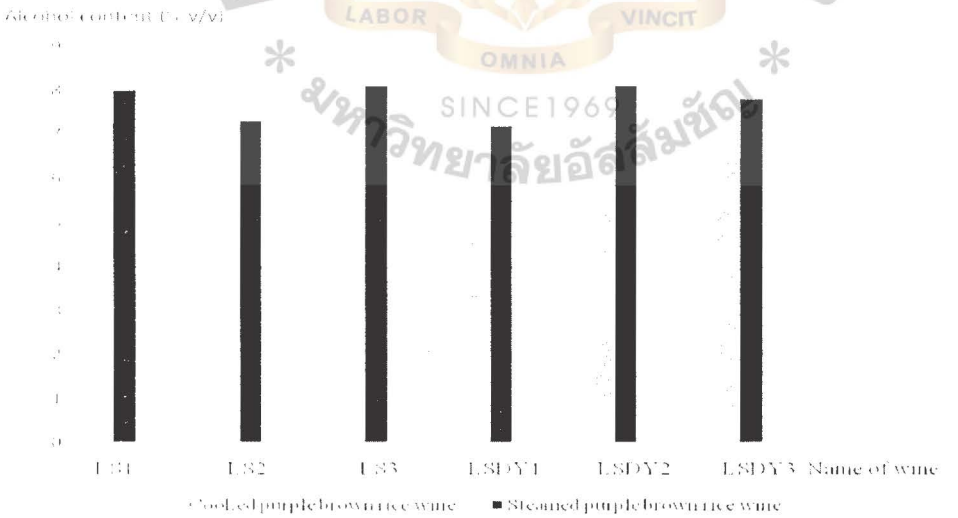
Name of rice wine	pH	Total soluble solid (^{0}Brix) after solid fermentation	Total soluble solid (^{0}Brix) after alcoholic fermentation	Total acidity (%)	Alcohol content (%v/v)
LS1	4.31±0.01	10.5	4.9	0.38	8
LS2	3.76±0.01	14	5.2	0.65±0.01	7.3
LS3	3.97	14	5.1	0.53±0.01	8.1
LSDY1	3.74±0.01	13.5	5.5	0.68±0.01	7.2
LSDY2	3.89±0.02	10.5	5.9	0.68	8.1
LSDY3	3.97±0.01	14	5.9	0.63±0.02	7.8

The result displays that rice wine making from the steaming process has the better quality than wine produced from the cooking process. Malakar and Banerjee

(Luh 1991, p.35) indicated that one-third of the mineral and half of the water-soluble vitamins were lost after cooking method. The vitamin was identified such as thiamin, riboflavin and niacin. Moreover, it is not easy to control the water at the beginning of the cooking process. The excess water also affects the amount of vitamin lost. In contrast, the steaming process is the impression technology which helps to improve the quality characteristics of rice. An illustration of that is up to 80% of vitamins and minerals contained in the rice shell moved into the grain of rice, and the grains become less brittle. Besides, the steamed grain rice is not stick together. Therefore, the taste, crispness and color are still remained in the rice grain.

Figure 4.5 displayed the alcohol content of steamed purple brown rice wine is much higher than the cooked rice wine. Therefore, the steamed purple brown rice wine process was chosen for the further study.

Figure 4.5: The alcohol content (%) by volume of cooked/steamed purple brown rice wine production



4.3 Comparing the difference of various starter cultures used in purple brown rice wine production

4.3.1 Chemical analysis

To compare the role of each starter culture in purple brown rice wine fermentation, the chemical properties was measured in this experiment. In term of pH value, the purple brown rice wine produced by the pure culture of local starter cultures had the pH lower than the others. In general, the pH value should not more than 3-4. Especially, the taste of wine would be affected significantly if the pH of the wine is above 3.7. All samples in this study had the pH around 3.5. This is good for protecting the wine from pathogenic microorganism. Although the pH value of the purple brown rice wine produced by the local starter culture number 3 (LS3) or the mixture of local starter culture number 1 and dry active wine yeast (LSDY1) was a little bit above 4, the wine is still considered acceptable. Besides, low pH also has another advantage which is less oxidation.

In addition, the total soluble solid (^{0}Brix) was measured after solid-state fermentation and liquid-state fermentation. The result in the table 4.4 displayed that the fermentable sugar utilization of yeast is the main reason to explain why the ^{0}Brix was decreased at the end of fermentation process. The alcohol content is directly related to the decrease of ^{0}Brix value when the fermentation was going on. The sample PS2 and PS3 have the lowest alcohol content (figure 4.6).

Table 4.4: Chemical analysis of steamed purple brown rice wine production after using each starter cultures

Name of rice wine	pH	Total soluble solid (^{0}Brix) after solid fermentation	Total soluble solid (^{0}Brix) after alcoholic fermentation	Total acidity (%)	Alcohol content (% v/v)
LS1	3.96 ± 0.02	9.5	5.5	0.54 ± 0.03	8.9
LS2	4.01 ± 0.01	8	5	0.57 ± 0.01	9.5
LS3	4.09 ± 0.01	10	4	0.55 ± 0.02	11
LSDY1	4.06 ± 0.01	8	5	0.54	8.6
LSDY2	3.89 ± 0.01	8	6	0.62 ± 0.01	7.75
LSDY3	4.02 ± 0.01	9.5	5	0.62 ± 0.08	8.25
PS1	3.79 ± 0.01	7	5	0.44 ± 0.01	9
PS2	3.50 ± 0.01	10	15	0.80 ± 0.01	1.6
PS3	3.31 ± 0.01	2	2.5	0.72 ± 0.01	2.6

The purple brown rice wine produced by the pure strains isolated from the local starter cultures are not working well and producing very low ethanol content which is 1.6% v/v and 2.6% v/v. The low alcohol content may also due to the sensitivity of isolated culture to the alcohol in the wine, or the alcohol was transformed to acetic acid. These samples also have the lowest pH measured.

The purple brown rice wine from pure culture of local starter number 1 (PS1) had satisfactory alcohol content (9% v/v). The different alcohol content after using various pure cultures of local starter culture is the result of the microorganisms' activities. Especially, the isolated culture from local starter culture number 1 contained mold, yeast and LAB (table 4.1). These species are the dominated species in the rice

wine industry for a long time. For instance, *Aspergillus* spp. and *Rhizopus* spp. are the common molds which used for sake production. *Saccharomyces* spp. is the strong fermenting yeasts (Dung, et al. 2006). Moreover, the characteristic of *Schizosaccharomyces* spp. is alcohol tolerant which is very important in fermentation process. *Lactobacillus casei* was also selected as inoculum to ferment the purple brown rice wine PS1. Krieger-Weber (2009) indicated that the role of LAB is bacterial degradation of malic acid to lactic acid and CO_2 (malolactic fermentation). Furthermore, Bamforth's study (2005) showed that lactic acid bacteria produce antimicrobial substances known as bacteriocins (acids and hydrogen peroxide). So, it is good for wine fermentation because they inhibited the spoilage microorganisms. The purple brown rice wine production is specific two-state fermentation which consists of mold and yeast activity. The mold transfers starch to fermentable sugar not only in solid-state fermentation but also in liquid-state fermentation. In addition, some filamentous fungi use the glucose for mycelium production such as *Rhizopus* spp. Dung (2006, p.339) in reporting Underrkofler's study, emphasized the glucogenic enzyme system is more prefer than the successful of saccharification activity of α-amylase.

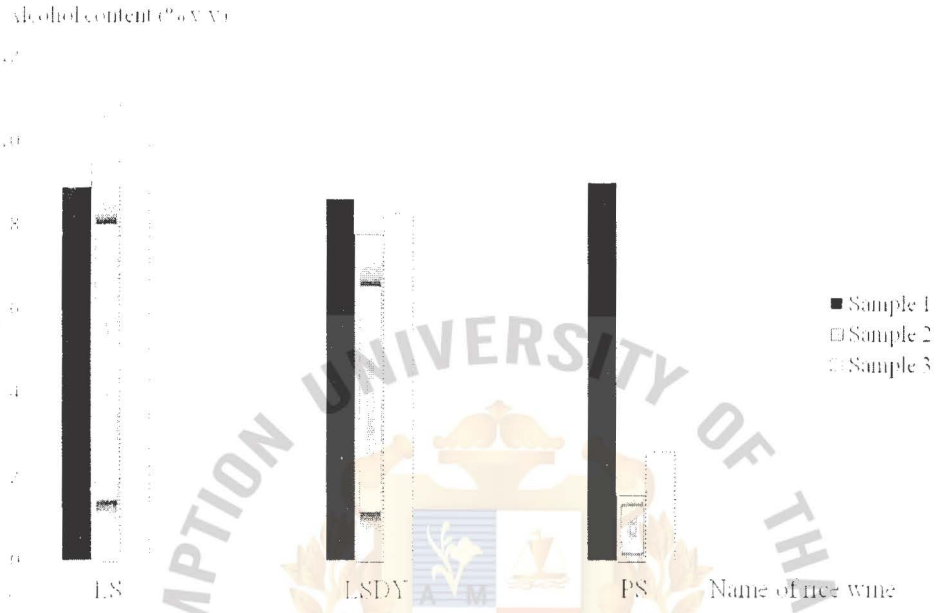
When the mixture of local starter culture and dry active wine yeast was used for purple brown rice wine making in sample LSDY1, the volume of ethanol produced was 8.6% v/v. Furthermore, the wine from pure culture of local starter number 1 had the highest alcohol content when comparing with LSDY1 and the control sample (LS1). However, the alcohol content from the wine used the local starter culture number 2 and 3 is higher than LSDY2 and LSDY3. Therefore, there are many important factors

affected to the fermentation. The role of yeast is identified significantly in the alcoholic fermentation. The nutrition requirements of yeast consist of a carbon source, nitrogen source, metal and vitamin. In term of the carbohydrate utilization, the yeast can take up and metabolize monosaccharides, disaccharide maltose and trisaccharide maltotriose by using the special enzyme such as invertase, α-galactosidase and glucoamylase. The product of yeast activity is alcohol and carbon dioxide. The dry active wine yeast (*Saccharomyces bayanus*) was used together with local starter culture in all samples LSDY. Local starter cultures are the mixture of mold, yeast and LAB. The successful fermentation is depended on many important factors such as indigenous yeasts of local starter culture, choice of dry active wine yeast strain and its adaptation to specific wine environment. Schütz and Gafner (1995) displayed that the high sugar uptake of yeast seems to be correlated to the structure of hexokinase encoded sequences. Nevertheless, the *Saccharomyces bayanus* did not consume fructose higher than *Saccharomyces cerevisiae*. The lower fructose uptake capacity of *S. bayanus* led to an excess of residual fructose at the end of alcoholic fermentation. As a result, alcoholic fermentation activity was decreased dramatically. The dry active wine yeast (*S. bayanus*) might compete with the microorganism in the local starter cultures during liquid-state fermentation; consequence, the alcohol content was affected in this case.

For the purple rice wine produced from local starter cultures, the highest alcohol content (11% v/v) was obtained from sample LS3. Actually, the commercial local starter cultures production is at the household scale. The number and kind of microorganism are different from sample to sample. The quality is also very different, depending on the local producers. Moreover, the biological reaction between mold,

yeast and LAB is still not known very well until now. Due to the limitation of time, only the mold (yeast) function was identified in this study.

Figure 4.6: Alcohol content (%) by volume of wine produced by each starter culture



4.3.2 Sensory evaluation

Ten panelists were invited to evaluate eight purple brown rice wine samples which focused on color and clarity, flavor, taste, bouquet and overall impression.

A description of wine tasting procedure was invented; clarity is the term judges use to describe the absence of suspended materials in wine. Table 4.6 indicated that the purple brown rice wine LS1 and LS2 were nearly clear and clean in term of color and clarity attribute. Therefore, suspended bacteria and yeast cells did not cause a hazy-white appearance. According to table 4.5 and figure 4.7, color is affected by pH value. Especially, the wine with lower pH gave much purple color while the wine with high pH gave a lighter color (shading toward more blue color). The color of LS1 and LS2 is

much darker than the others. In addition, overall impression (general quality) of LS1 and LS2 was received the highest score after statistic adjustment. That result from the tasting also showed that some wines might not look very good but taste great. Other wines might score well in all other factors but may not taste well.

Besides, the smooth, even and pleasant contributed to the flavor and taste of purple brown rice wine goes to samples LS1, LS2 and LS3. All purple brown rice wine produced from local starter culture also got the highest score in the bouquet attribute. The bouquet is the complex smell which describes the odors produced by the winemaking process. Rice wine bouquet is generated by fermentation process, ingredient used in the local starter culture, controlled wine oxidation, bottle aging, etc. The table 4.5 indicated that sample LS1, LS2 and LS3 produced from local starter cultures and dry active wine yeast have the higher the alcohol content. Sample LS1, LS2 and LS3 also received higher evaluated score than LSDY1, LSDY2 and LSDY3 in sensory test.

Overall, LS1 and LS2 received the best score in all attribute. Sensory evaluation of the purple brown rice wine was affected by many factors such as low pH, high acidity and high alcohol content. From the chemical analysis, all wine produced by local starter culture number 1 and 2 had lower acidity and higher alcohol content than others samples (LSDY1, LSDY2 and LSDY3). As the consequences these wine the best score in sensory test.

The result was showed in table 4.5. Sample LS1 and LS2 received the best result in all attributes due to the highest score and no significant difference ($p \geq 0.05$) was identified. Most of the panelist indicated that they appreciated sample LS3 due to its

aroma; flavor and taste attribute than the overall impression, color and clarity attribute. All purple brown rice wine produced by the combination between the local starter cultures and dry active wine yeast were not received the best result in sensory tests.

Figure 4.7: All purple brown rice wine produced by different starters

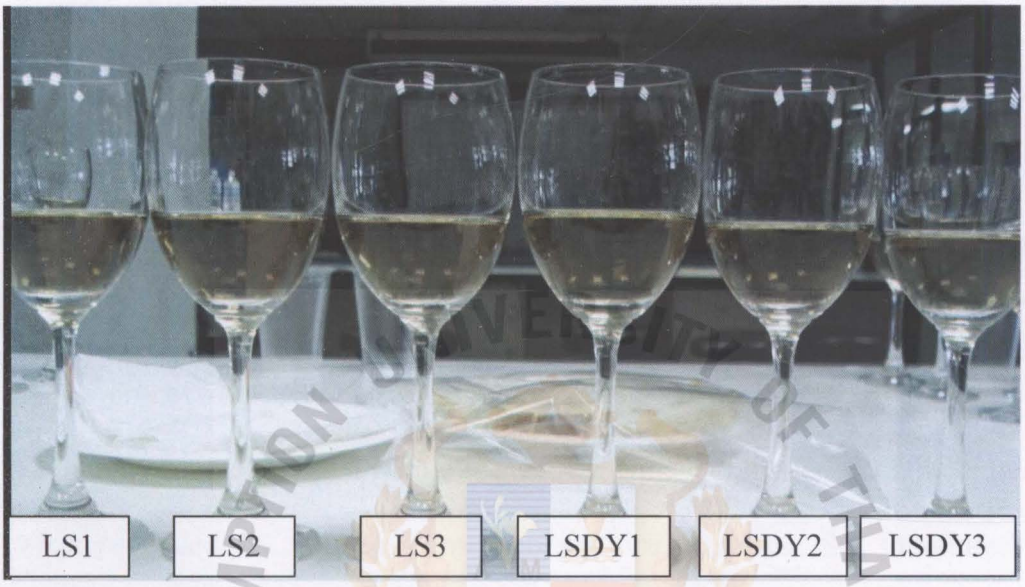


Table 4.5: Intensity score out of sensory test of purple brown rice wine produced by different starters

Sample	Color and Clarity	Aroma	Flavour and Taste	Overall impression
LS1	3.6 ± 0.84^{a}	2.9 ± 0.57^{a}	2.5 ± 0.85^{a}	2.5 ± 0.71^{a}
LS2	3.4 ± 0.70^{ab}	2.7 ± 0.82^{ab}	2.3 ± 0.82^{ab}	2.5 ± 0.85^{a}
LS3	2.6 ± 0.70^{c}	2.4 ± 0.52^{abc}	2.2 ± 1.03^{abc}	1.9 ± 0.74^{b}
LSDY1	2.9 ± 0.74^{bc}	1.5 ± 1.08^{d}	1.6 ± 0.70^{cd}	1.5 ± 0.71^{b}
LSDY2	3.0 ± 0.94^{bc}	1.8 ± 0.92^{cd}	1.4 ± 0.52^{d}	1.5 ± 0.71^{b}
LSDY3	2.8 ± 1.03^{c}	2.1 ± 1.1^{bcd}	1.7 ± 0.67^{bcd}	1.9 ± 0.88^{b}

*Remark: same alphabet in the same column means not significant difference ($p \geq 0.05$)

CHAPTER V

CONCLUSION

It can be conclude that some pure strains of local starter cultures were isolated in this study. Microbial identification was done by the traditional method. Because of the local starter cultures used for this experiment were collected from different sources; various microorganisms were obtained. Due to the time constrain, yeast and LAB were isolated from the local starter culture number 1 while only yeast was isolated from the local starter culture number 4.

The process of steaming and cooking purple brown rice was compared. From chemical analysis, the steamed purple brown rice wine had higher alcohol content than the wine produced by cooked purple brown rice. Besides, the literatures many researches reported that the nutrients of steamed rice were lost less than the cooked rice. The color of steamed purple brown rice was also better than the cooked purple brown rice. Therefore, the steaming process of purple brown rice was chosen to further study. Result from this study showed that the purple brown rice wine produced by local starter cultures gave the highest alcohol content. Sample LS3 produced the high alcohol content (11% v/v). When the local starter cultures were combined to dry active wine yeast in purple brown rice wine production, the result was not showing any significant improvement than the control sample. For the sensory evaluation, the purple brown rice wines of sample LS1 and LS2 obtained the highest score from the judges. Sample LS3 obtained high scores in aroma, flavor and taste attribute but the color and clarity is not good.

From the results of this study, I conclude that pH value, acidity and volume of alcohol are the most important factors which affected the quality of purple brown rice wine in sensory test.

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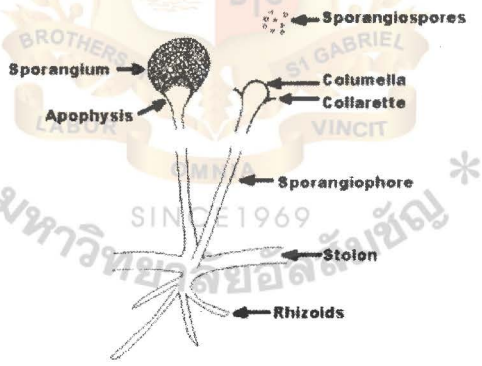
<http://www.doctorfungus.org/thefungi/aspergillus_spp.htm>

APPENDIX

I. Description and morphology of mold and yeast

1. Rhizopus spp. (The University of Adelaide's website, 2010)

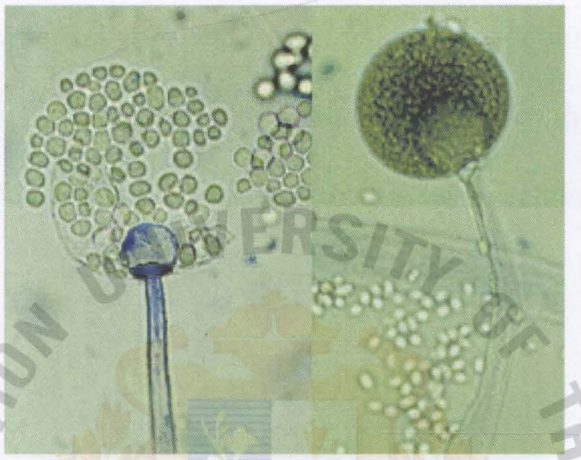
The genus *Rhizopus* is characterized by the presence of stolons and pigmented rhizoids, the formation of sporangiophores singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multi-spored, generally globose sporangia. After spore release the apophyses and columella often collapse to form an umbrella-like structure. Sporangiospores are globose to ovoid, one-celled, hyaline to brown and striate in many species. Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.



2. Mucor spp. (The University of Adelaide's website, 2010)

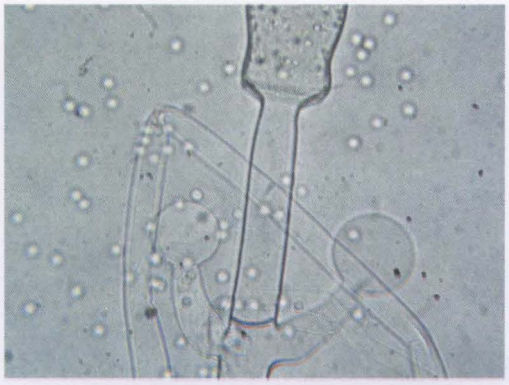
The genus *Mucor* can be differentiated from *Absidia, Rhizomucor* and *Rhizopus* by the absence of stolons and rhizoids. Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia. Sporangiophores are erect, simple or branched, forming large (60-300 μm in diameter), terminal, globose to spherical, multispored sporangia, without apophyses and with

well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal. Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled or finely ornamented. Chlamydospores and zygospores may also be present.



3. **Actinomucor spp.** (Benjamin and Hesseltine, 1957)

Actinomucor is one of several monotypic genera of the family Mucoraceae. It was closely related to Mucor, but differed in having branched stolons which gave rise to rhizoids and sporangiophores. There is no apophysis present in Actinomucor.



4. Aspergilus spp.

Hyphae are septate and hyaline. The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. Vesicle is the typical formation for the genus *Aspergillus*. The morphology and color of the conidiophore vary from one species to another. Covering the surface of the vesicle entirely ("radiate" head) or partially only at the upper surface ("columnar" head) are the flask-shaped phialides which are either uniseriate and attached to the vesicle directly or are biseriate and attached to the vesicle via a supporting cell, metula. Over the phialides are the round conidia (2-5 μm in diameter) forming radial chains.

II. Raw data for SPSS calculation

Treatment	Replication	Color and Clarity	Aroma	Flavor and Taste	Overall impression
1	1	2	3	3	3
1	2	4	3	3	3
1	3	4	3	3	3
1	4	4	3	2	2
1	5	2	2	1	1
1	6	4	2	2	2
1	7	4	3	2	3
1	8	4	3	2	2
1	9	4	3	4	3
1	10	4	4	3	3
2	1	2	2	3	3
2	2	3	3	3	3
2	3	4	4	3	3
2	4	4	2	2	3
2	5	3	2	1	1
2	6	4	2	2	2
2	7	4	3	2	3
2	8	3	3	1	1
2	9	3	2	3	3
2	10	4	4	3	3
3	1	2	3	3	3
3	2	2	2	2	2
3	3	2	2	1	1
3	4	3	3	2	2
3	5	3	2	1	1
3	6	2	3	4	3
3	7	3	2	3	2
3	8	4	3	1	1
3	9	3	2	2	2
3	10	2	2	3	2
4	1	2	2	2	2
4	2	2	0	1	1
4	3	3	1	1	1
4	4	3	2	2	1
4	5	2	1	1	1
4	6	4	1	3	2
4	7	4	4	2	3
4	8	3	1	1	1
4	9	3	2	2	2

4	10	3	1	1	1
5	1	1	2	1	1
5	2	3	1	1	1
5	3	3	1	1	1
5	4	3	2	2	2
5	5	2	1	1	1
5	6	4	1	1	1
5	7	4	4	2	3
5	8	4	2	1	1
5	9	3	2	2	2
5	10	3	2	2	2
6	1	1	2	2	2
6	2	2	0	1	1
6	3	3	2	1	2
6	4	2	2	1	2
6	5	3	2	1	1
6	6	4	3	2	2
6	7	4	4	3	4
6	8	4	2	2	2
6	9	3	3	2	2
6	10	2	1	2	1

III. SPSS analysis

Tests of Between-Subjects Effects

Dependent Variable: Color

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.833[a]	14	2.131	6.386	.000
Intercept	558.150	1	558.150	1672.592	.000
Treatment	7.150	5	1.430	4.285	.003
Replication	22.683	9	2.520	7.553	.000
Error	15.017	45	.334		
Total	603.000	60			
Corrected Total	44.850	59			

a. R Squared = .665 (Adjusted R Squared = .561)

Color

	Treatment	N	Subset		
			1	2	3
Duncan[a]	LS4	10	2.6000		
	LSDY4	10	2.8000		
	LSDY1	10	2.9000	2.9000	
	LSDY2	10	3.0000	3.0000	
	LS2	10		3.4000	3.4000
	LS1	10			3.6000
	Sig.		.165	.073	.443

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .334.
a. Uses Harmonic Mean Sample Size = 10.000.

Tests of Between-Subjects Effects

Dependent Variable: Aroma

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.400[a]	14	1.957	3.222	.001
Intercept	299.267	1	299.267	492.695	.000
treatment	14.333	5	2.867	4.720	.002
replication	13.067	9	1.452	2.390	.026
Error	27.333	45	.607		
Total	354.000	60			
Corrected Total	54.733	59			

a. R Squared = .501 (Adjusted R Squared = .345)

Aroma

	Treatment	N	Subset 1	2	3	4
Duncan[a]	LSDY1	10	1.5000			
	LSDY2	10	1.8000	1.8000		
	LSDY4	10	2.1000	2.1000	2.1000	
	LS4	10		2.4000	2.4000	2.4000
	LS2	10			2.7000	2.7000
	LS1	10				2.9000
	Sig.		.110	.110	.110	.183

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .607.
a. Uses Harmonic Mean Sample Size = 10.000.

Tests of Between-Subjects Effects

Dependent Variable: Flavor

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.433[a]	14	1.674	3.879	.000
Intercept	228.150	1	228.150	528.760	.000
treatment	9.750	5	1.950	4.519	.002
replicatio n	13.683	9	1.520	3.524	.002
Error	19.417	45	.431		
Total	271.000	60			
Corrected Total	42.850	59			

a. R Squared = .547 (Adjusted R Squared = .406)

Flavor

	Treatment	N	Subset 1	2	3	4
Duncan[a]	LSDY2	10	1.4000			
	LSDY1	10	1.6000	1.6000		
	LSDY4	10	1.7000	1.7000	1.7000	
	LS4	10		2.2000	2.2000	2.2000
	LS2	10			2.3000	2.3000
	LS1	10				2.5000
	Sig.		.342	.059	.059	.342

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .431.

a. Uses Harmonic Mean Sample Size = 10.000.

Tests of Between-Subjects Effects

Dependent Variable: Overall impression

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.400[a]	14	1.886	5.463	.000
Intercept	232.067	1	232.067	672.296	.000
treatment	10.133	5	2.027	5.871	.000
replication	16.267	9	1.807	5.236	.000
Error	15.533	45	.345		
Total	274.000	60			
Corrected Total	41.933	59			

a. R Squared = .630 (Adjusted R Squared = .514)

Overall impression

	Treatment	N	Subset 1	Subset 2
Duncan[a]	LSDY2	10	1.5000	
	LSDY1	10	1.5000	
	LS4	10	1.9000	
	LSDY4	10	1.9000	
	LS1	10		2.5000
	LS2	10		2.5000
	Sig.		.172	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .345.

a. Uses Harmonic Mean Sample Size = 10.000.

Wine Judging Score Sheet

Date:

Panelist:

Sample: Purple brown rice wine

Remark: Please rinse your mouth before starting and rinse your mouth with bread and water before you change another sample.

Description	Sample					
	1	2	3	4	5	6
COLOR and CLARITY (4 Points) Clear, clean = 4 Nearly correct, attractive = 3 Slightly off = 2 Off, brown = 1						
BOUQUET (4 Points) Varietal, complex, flowery = 4 Pronounced, developed = 3 Clean, pleasant, scented, delicate = 2 Simple, underdeveloped = 1 Defective, off = 0						
FLAVOR and TASTE (4 Points) Varietal, complex = 4 Smooth, even, pleasant = 3 Agreeable, clean, simple = 2 Off, chemical, off = 1						
OVERALL IMPRESSION (4 Point) Noble, elegant, grand, distinguished = 4 Charming, fine, graceful = 3 Fine, sound = 2 No exceptional features = 1						

Comment: