รูปแบบการหมักและการปรับแต่งสูตรของไวน์ส้มจากน้ำส้มเขียวหวานสายน้ำผึ้ง

นางสาว วิจักขณา นวรัตน์ธารา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

FERMENTATION PROFILE AND FORMULATION OF ORANGE WINE FROM SAI NAM PHUENG TANGERINE JUICE

Miss Vijuckana Navarattara

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Food Technology

Department of Food Technology

Faculty of Science

Chulalongkorn University

Academic Year 2008

Thesis Title	FERMENTATION PROFILE AND FORMULATION OF ORANGE WINE FROM SAI NAM PHUENG TANGERINE JUICE
By	Miss Vijuckana Navarattara
Field of Study	Food Technology
Advisor	Assistant Professor Cheunjit Prakitchaiwattana, PhD.
Co-Advisor	Assistant Professor Varapha Kongpensook, PhD.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

S. Hannongbua ..... Dean of the Faculty of Science

(Professor Supot Hannongbua, PhD.)

THESIS COMMITTEE

Romanee Sanguandeekul ..... Chairman

(Assistant Professor Romanee Sanguandeekul, PhD.)

cheunjit Prakitchaiwattana ..... Advisor

(Assistant Professor Cheunjit Prakitchaiwattana, PhD.)

Varapha Kongpensook ..... Co-Advisor

(Assistant Professor Varapha Kongpensook, PhD.)

..... Examiner

(Kanitha Tananuwong, PhD.)

..... External Examiner

(Viyada Kunathigan, PhD.)

วิจักขณา นวรัตน์ธารา : รูปแบบการหมักและการปรับแต่งสูตรของไวน์ส้มจากน้ำส้มเขียวหวานสายน้ำผึ้ง. (FERMENTATION PROFILE AND FORMULATION OF ORANGE WINE FROM SAI NAM PHUENG TANGERINE JUICE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร. ชื่นจิต ประกิตชัยวัฒนา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.ดร. วรภา คงเป็นสุข, 122 หน้า.

งานวิจัยนี้ศึกษารูปแบบการหมักของไวน์ส้มจากน้ำส้มเขียวหวานสายน้ำผึ้ง (*Citrus reticulata*) และประเมินการยอมรับไวน์ส้มที่ปรับแต่งสูตร โดยศึกษารูปแบบการหมักของน้ำส้ม 3 ความเข้มข้น (30%, 50% และ 100%) ที่มีน้ำตาลรีดิวซ์และไดแอมโมเนียมฟอสเฟสเท่ากับ 25% และ 0.05% ตามลำดับ และหมักโดยใช้กล้าเชื้อเดี่ยวและกล้าเชื้อผสมของ *Saccharomyces cerevisiae* และ *Saccharomyces bayanus* ที่อุณหภูมิ 30 °C เพื่อหาสภาวะที่เหมาะสมในการผลิตไวน์ส้มพื้นฐาน โดยติดตามจำนวนประชากรยีสต์ ปริมาณน้ำตาลรีดิวซ์ และ ปริมาณแอลกอฮอล์ทุกวันตลอดระยะการหมักจนกระทั่งได้แอลกอฮอล์ 12% จากการศึกษารูปแบบการหมัก พบว่าการหมักของกล้าเชื้อเดี่ยว *S. bayanus* ในน้ำหมักที่เตรียมจากน้ำส้ม 100% มีอัตราการหมักเร็วที่สุด โดยใช้น้ำตาล 3.70% ต่อวัน และผลิตแอลกอฮอล์ 2.61% ต่อวัน จึงส่งผลให้ได้ปริมาณแอลกอฮอล์ 12% ภายใน 5 วัน น้ำส้มหมัก หลังทำให้ใสแล้วได้เป็นไวน์ส้มที่มีปริมาณน้ำตาลรีดิวซ์ 3.55% แอลกอฮอล์ 12% และกรดที่ไตเตรตได้ 0.32% แต่เนื่องจากไวน์ส้มที่ได้จากสภาวะการหมักนี้ยังมีน้ำตาลเหลืออยู่ในปริมาณมาก ซึ่งเป็นอุปสรรคต่อการปรับแต่งสูตรไวน์ ดังนั้นจึงศึกษารูปแบบการหมักของ *S. bayanus* ในน้ำหมักที่เตรียมจากน้ำส้ม 100% ที่มีน้ำตาลรีดิวซ์ประมาณ 16% 19% 22% และ 25% ผลการทดลองพบว่าน้ำหมักที่มีน้ำตาล 22% ได้รับคัดเลือกให้เป็นสภาวะสำหรับทำไวน์ส้มพื้นฐาน เนื่องจากยีสต์แสดงรูปแบบการหมักที่เหมาะสม และไวน์ที่ได้มีกลิ่นรสเป็นที่ยอมรับ ไวน์พื้นฐานที่ได้หลังทำให้ใสแล้ว มีปริมาณน้ำตาลรีดิวซ์ 1.93% แอลกอฮอล์ 11% และกรดที่ไตเตรตได้ 0.38% เมื่อนำไวน์พื้นฐานมาปรับแต่งสูตรโดยปรับปริมาณน้ำตาล (2%, 4% และ 6%) และค่าความเป็นกรด (0.5%, 0.7% และ 0.9%) ประเมินการยอมรับไวน์ทั้ง 9 สูตร ด้วยการวางแผนการทดลองแบบบล็อกไม่สมบูรณ์ (Balance Incomplete Block design) โดยใช้ผู้ประเมิน 108 คน (เพศหญิง 54 คน และ เพศชาย 54 คน) ผู้ประเมินแต่ละคนทดสอบ 4 ตัวอย่าง พบว่าการยอมรับตัวอย่างไวน์ส้มของผู้ประเมินระหว่างกลุ่มเพศหญิงและเพศชายมีความแตกต่างกันอย่างมีนัยสำคัญ ($p \leq 0.05$) ดังนั้นข้อมูลที่ได้จึงแยกวิเคราะห์ในแต่ละเพศ ในกลุ่มเพศหญิงพบว่าปริมาณน้ำตาล ค่าความเป็นกรด และอันตรกริยาของทั้งสองค่ามีอิทธิพลต่อการยอมรับในผลิตภัณฑ์อย่างมีนัยสำคัญ ($p \leq 0.05$) สูตรที่มีน้ำตาล 6% ได้คะแนนความชอบสูงกว่าสูตรที่มีน้ำตาลต่ำกว่า ในทุกๆ ระดับของค่าความเป็นกรด สูตรที่มีน้ำตาล 6% และมีค่าความเป็นกรด 0.5% ได้รับการยอมรับสูงสุดโดยมีคะแนนความชอบเฉลี่ย 7.13±1.33 ในกลุ่มเพศชายพบว่ามีเพียงค่าความเป็นกรดเท่านั้นที่มีผลต่อคะแนนความชอบอย่างมีนัยสำคัญ ($p \leq 0.05$) ไวน์ส้มที่มีน้ำตาล 6% และมีค่าความเป็นกรด 0.9% ได้รับคะแนนความชอบสูงสุด (5.75±1.73) ผลการทดลองเหล่านี้แสดงให้เห็นว่าเพศหญิงมีความชอบในผลิตภัณฑ์สูงกว่าเพศชาย ดังนั้นผลิตภัณฑ์นี้น่าจะเหมาะกับเพศหญิง ในขณะที่เพศชายไม่สามารถประเมินได้ว่าชอบหรือไม่ชอบในผลิตภัณฑ์เนื่องจากคะแนนความชอบใน 9-point hedonic scale ต่ำกว่า 6 คะแนน ส่วนประสบการณ์ในการดื่มไวน์ผลไม้ของผู้ประเมิน พบว่าไม่มีผลต่อการยอมรับในผลิตภัณฑ์อย่างมีนัยสำคัญ($p>0.05$) ไวน์ส้มสูตรที่ได้รับการยอมรับมีอายุการเก็บนานกว่า 2 เดือน เมื่อเก็บที่อุณหภูมิตู้เย็นและอุณหภูมิห้อง

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต Vijchana N.

สาขาวิชา เทคโนโลยีทางอาหาร ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก

ปีการศึกษา 2551 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม

4873601023 : MAJOR FOOD TECHNOLOGY

KEYWORDS : FERMENTATION PROFILE / FORMULATION OF ORANGE WINE

VIJUCKANA NAVARATTARA : FERMENTATION PROFILE AND FORMULATION OF ORANGE WINE FROM SAI NAM PHUENG TANGERINE JUICE. ADVISOR: ASST. PROF. CHEUNJIT PRAKITCHAIWATTANA, Ph.D., CO-ADVISOR: ASST. PROF. VARAPHA KONGPENSOOK, Ph.D., 122 pp.

This research investigated fermentation profiles of Sai Nam Phueng tangerine (*Citrus reticulata*) wine and evaluated the acceptability of tangerine wine formulation. The fermentation profiles of 3 juice concentrations (30%, 50%, and 100%) containing approximately 25% reducing sugar and 0.05% diammonium phosphate fermented under 30°C by single and mixed cultures (*Saccharomyces cerevisiae* and *Saccharomyces bayanus*) were investigated to find a proper condition for basic tangerine wine production. The yeast population, reducing sugar and alcohol content were monitored everyday during the fermentation until alcohol reached 12%. Based on fermentation profile evaluation, the fermentation of single culture of *S. bayanus* using 100% juice as a substrate could perform the fastest fermentation rate. It utilized 3.70% sugar/day and generated 2.61% alcohol/day, consequently alcohol reached 12% within 5 days. After the clarification, the fermented juice contained 3.55% reducing sugar, 12% alcohol, and 0.32% titratable acidity. However, sugar amount in the tangerine wine from this condition still remained in large amount. This was obstacle for wine formulation. Therefore the fermentation profile of *S. bayanus* in 100% must containing lower reducing sugar approximately 16%, 19%, and 22% were investigated. The must containing 22% sugar was selected to produce a basic wine due to its appropriate fermentation profile and acceptable flavor. The basic wine made from this condition after clarification contained 1.93% sugar, 11% alcohol, and 0.38% titratable acidity. The selected basic wine was then formulated by adjusting sugar content (2%, 4%, and 6%) and acidity (0.5%, 0.7%, and 0.9%). The acceptability of the 9 tangerine wine formulations were evaluated a Balanced Incomplete Block design with 108 assessors (54 females and 54 males). Each assessors evaluated 4 samples. The acceptability of the tangerine wine samples evaluated by female and male groups was significantly different ($p \leq 0.05$) therefore the data were analyzed separately for each gender. For the female group, sugar content, acidity and their interaction significantly ($p \leq 0.05$) influenced on the product acceptability. The formulas with 6% sugar had higher liking scores than the lower levels in every level of %acidity. The formula with 6%sugar and 0.5%acidity was the most accepted with the mean liking score 7.13±1.33. For the male group, only the %acidity had significantly ($p \leq 0.05$) effected the liking score. The tangerine wine containing 6%sugar and 0.9%acidity had the highest liking score (5.75±1.73). These results showed the higher preference in female over male group, therefore this product could suit for female while male perception was neither like nor dislike the product because its score was lower than six points in the hedonic scale. The experience in fruit wine drinking was not significantly ($p>0.05$) effect the acceptability of the product. Shelf life of the most accepted tangerine wine formula storaged under refrigerator and room temperature was longer than 2 months.

Department : Food Technology Student's Signature Vijuckana N.

Field of Study : Food Technology Advisor's Signature

Academic Year : 2008 Co-Advisor's Signature Varapha Kongpensook

ACKNOWLEDGEMENTS

First I would like to express my deepest appreciation and gratitude to Asst. Prof. Dr. Cheunjit Prakitchaiwattana and co-advisor Asst. Prof. Dr. Varapha Kongpensook for their unfailing availability when ever problems arose during all the time of study which make this work possible. Their wise guidance in the way of learning will always be grateful.

My appreciation is also passed to Asst. Prof. Dr. Romanee Sanguandeekul, Dr. Kanitha Tananuwong, and Dr. Viyada Kunathigan serving as the thesis committee.

I would like to acknowledge Food microbiology and Food chemistry laboratory units, Department of Food technology, Faculty of Science for providing all instruments for my research.

I am thankful to Khun Torkul Kumthakhrua for his kindly and support of raw material from Tipco company.

Special thank is also offered to Khun Jessada Laicharoenkiat for his support of all facilities in bottling process throughout the research.

My sincere gratitude is also given to Khun Worapoj Junnongsuang for his valuable cooperation at New Story Restaurant.

Finally, the greatest gratitude is expressed to my family and my friends for their encouragement, endless love, careness and support throughout my degree.

CONTENT

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

Tangerine (*Citrus reticulata*) is a citrus fruit widely grown in Thailand. Several popular cultivars of the tangerine such as Si Thong, Bang Mod, Shogun and Sai Nam Phueng are generally consumed in Thailand. Tangerine gives an unique sweet and sour taste of orange. Tangerine juice could be used as a raw material for wine making since it contains sugars, proteins, lipids, organic acids, vitamins and minerals that could provide sufficient nutrients for yeast fermentation. In addition, its color is unique which could produce as orange color wine. Therefore, making wine from tangerine juice could be a good alternative for value added to tangerine juice product.

For wine making, several factors such as must composition, fermentation condition including yeast strains are important in wine fermentation. Wine quality and value are determined by wine flavor and aroma which generated during fermentation by species and strains of yeasts. *Saccharomyces cerevisiae* and other species, in particular *Saccharomyces bayanus* have been studied and introduced to the yeast starter market allowing the wine maker to select a proper yeast strains for their wine production. Yeast species and strains should be selected based on their fermentative properties to give a unique wine flavor. Using of multistarter culture, which are now widely being studied, could be an alternative to improve the wine fermentation. These specific art and technology have been generally used to improve wine quality. There are many researches reported that fermentation profile of different yeast strains and culture types in each fruit juice were significantly different. These profiles influenced on wine flavor and quality (Romano *et al.,* 2003; Clemente-Jimenez *et al.,* 2004; Ciani *et al.,* 2006).

Since a few systematic researches on tangerine wine making development have been reported, the fundamental information of the tangerine wine making process should be investigated. Therefore, it is necessary to know, (i) proper yeasts used for tangerine juice fermentation, (ii) color stability and formation of chemical substances generated during tangerine juice fermentation. and (iii) appropriate tangerine wine formulation Thus, to add new knowledge in the field, this study aimed to evaluate; a proper yeast species and culture type for tangerine wine fermentation, a basic tangerine wine making condition and acceptable tangerine wine formulation.

CHAPTER II

LITERATURE REVIEW

1. Tangerine (*Citrus reticulata*)

Tangerine is a type of loose skinned orange belonging to the species *Citrus reticulata*. Tangerine is subclassed in the major varieties of orange belong to genus Citrus. It has small to medium size with small seeds and it is easily peeled rind and eaten as fresh fruit. Tangerine has deeper orange color than other types of citrus fruit and its flavor is very unique and richer than other types of citrus fruit (Kimball, 1999). The color of tangerine represents the yellow, orange and red that responded by carotenoid pigment, locating in the chloroplasts (Samson, 1986).

1.1. Cultivation

FAO production yearbooks demonstrate that tangerine is produced and consumed in tropical region whereas Japan supplies approximately half of the world production. The cultivation area of tangerine can be distinguished into 3 areas which are subtropical (between 30° and 40° latitude), semitropical (between 20° and 28° latitude) and tropical area (within 20° of the equator) according to the regional climate (Samson, 1986).

In Thailand, the tangerine cultivation can be done in both of highland and lowland areas where has no inundation. The cultivated period is around 8-10 months before harvesting. After harvesting, the tangerine is generally subjected to cleaned, graded, sized and packed before launched to the market. The postharvest technology is applied in order to prolong their shelflife.

Tangerine is treated by cold room storage, dark room storage and wax coating (Department of Agricultural, 2007).

1.2. Nutrition value of tangerine juice

Tangerine fruit is mainly used for dessert. Its flavor is also used in beverage, confectionery, cookies and bakery. The peel and juice of tangerine is commercially important. However, the juice is the main tangerine product since it can be easily processed to prolong shelflife. The nutrition of commercial UHT tangerine juice is shown in table 2.1. The juice is an important source of vitamin C, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_9 (folate), vitamin A, potassium and magnesium. It also contains carbohydrate, protein and amino acids. Its color naturally comes from carotenoid pigment which is an important antioxidant substance. Moreover, the flavonoid in tangerine is also a beneficial antioxidant to health (Samson, 1986 and USDA, 2008).

Table 2.1 The nutrition value of Tipco UHT tangerine *(Citrus reticulata)* juice

Nutrition value	Value per 100 grams	Units
Proximates		
Protein	<0.5	g
Total lipid (fat)	0	g
Carbohydrate	10.5	g
Total dietary fiber	1	g
Total Sugars	8.5	g
Minerals		
Calcium, Ca	16	mg
Iron, Fe	0.3	mg
Sodium, Na	15	mg
Lipids		
Total saturated fatty acids	0	g
Cholesterol	0	mg
Vitamins		
Vitamin C, total ascorbic acid	48	mg
Thiamin (Vitamin B_1)	0.06	mg
Riboflavin (Vitamin B_2)	0	mg
Vitamin A, IU	333	IU

Source: Tipco Nutrition Box Label.

1.3 Commercially processed tangerine juice

Several processing techniques used for tangerine juice production are generally applied in the industry. There are pasteurization, evaporation, freezing technique, sterilization and also ultra high temperature (UHT) processes. These variety of processes differently affect tangerine juice quality, such as color, flavor and in particular nutritional value.

a) Pasteurization in tangerine juice production is generally performed by using plate pasteurization system to provide heat to juice at 95 °C for 30 second then rapidly cool down to 4°C before transferring to packaging process. Pasteurized juice product is stored under refrigerated temperature (4°C) throughout storage time. The product has minimal loss of nutrition and flavor profile compared to fresh juice (Nisperos-Carriedo and Shaw, 1990; Gil-Izquierdo *et al.,* 2003).

b) Evaporation in the industrial is equipped with the double effect plate concentrators, two evaporators and a thermocompressor pump. Fresh crushed juice is pasteurized before loaded into the evaporation process. The evaporation process consists of two steps. The first step is evaporation of the juice at 78 °C to reach 20 °Brix. The second step is allowing the juice to reach 60 °Brix at 64 °C and cooling to 4 °C. The product is kept under refrigerated temperature (4°C) throughout storage time (Gil-Izquierdo *et al.,* 2003).

c) Freezing is the technique used in industry when tangerine exceeds market demand. This system is equipped with the tunnel, compressor, evaporator and evaporative condenser. The juice is pasteurized before transferring to freezing tunnel which process at

-40 °C ± 5 °C for 24 – 48 hours depended upon the load of product. The frozen juice is thawed by performing the second pasteurization before packing in aseptic packaging (Gil-Izquierdo *et al.,* 2003).

d) Sterilization in beverage is generally processed under high temperature –short time (HTST). This condition is used to minimize the nutrient degradation and off-flavor formation in the product. Juice is generally packed in closed container before loading into the thermal process by steaming at 104 °C for 3 min in retort equipment. The sterilized juice can be stored at room temperature for approximately 1 year. However, severe condition in the thermal process could cause the loss of vitamin and flavor in the juice (Toledo, 1986).

e) Ultra high temperature (UHT) process of juice is generally equipped with the plate heat exchanger to provide heat to juice 115°C for 3 sec then the juice is filled to packaging. The packaging is treated with hydrogen peroxide (H_2O_2) prior to fill, under aseptic zone. The product can be stored in room temperature for approximately 1 year. The loss of vitamin and flavor of juice is lower than the juice processed by HTST (Toledo, 1986; Yang and Tang, 2002).

Beside these processed tangerine juice products, there are several products made from tangerine which is found in the market such as jelly, pudding, cooler and wine. Since this research aimed to study wine made from

processed tangerine juice, wine science and relevant researches are therefore reviewed in the next section.

2. Wine

Wine is an alcoholic beverage produced through the partial or total fermentation of grapes juice. Wine is generally made from grapes since it contains all important ingredients for wine, including pulp, juice, and seeds that possess the acids, sugars, tannin, minerals, and vitamins that are found in wine. The other fruits such as apples, cherries, elder-berries, and palm can use to make wine normally called fruit wine. Classification of wine depends on the color, sweetness, alcohol content, presence of carbon dioxide, the variety of grape including viticultural practice and the region where the grapes are grown. However, wine is generally classified into 3 categories based on the taxation system which are still table wine, sparkling wine and fortified wine (Jackson, 2000).

a) Still table wine contains alcohol content ranged between 9% to 14% by volume. Wine could be characterized by their color which are red, white, and pink or rosé which made from different production method. Other than the color, the sweetness of wine also uses to distinguish the type of wine. The amount of residual reducing sugar divides wine into 3 types which are dry (<1% sugar), semi-sweet (1-3% sugar) and sweet wine (>3% sugar) (Lea and Piggott, 1995).

b) Sparkling wine contains alcohol content ranged between 9% to 14% by volume and carbon dioxide content approximately 3.9g L^{-1}. Sparkling wine is also divided into 3 types which are dry, semi-sweet and sweet wine as described above.

c) Fortified wine is table wines that elevate the alcohol content with by adding brandy or the other spirits. The wine contains alcohol content ranged between 17% to 22% by volume. This wine can be classified into 2 types which are wine added flavor and wine without added flavor.

The wine of all 3 categories have different properties in term of the color, sweetness (sugar content), alcohol content and carbon dioxide content. These wine characteristics are the result of wine making processes.

2.1 Wine making process

Wine making process includes the must preparation, fermentation and clarification process. Red, white and rosé wine are made by different process as shown in figure 2.1. The wine making, commences from the stemming step after the grape is harvested. Stemming process is the removal of the stems, leaves and stalk in order to prevent the contamination of phenolic and lipid from the vine to wine. The next step is crushing performed in order to rupture the cell of seed, skin and pulp of grape. The crushed parts will easily release juice, enzyme and flavors to grape must at the beginning stage of maceration. In the maceration step, the grape skin, seed and pulp are soaked or macerated under specific condition, depended upon wine types.

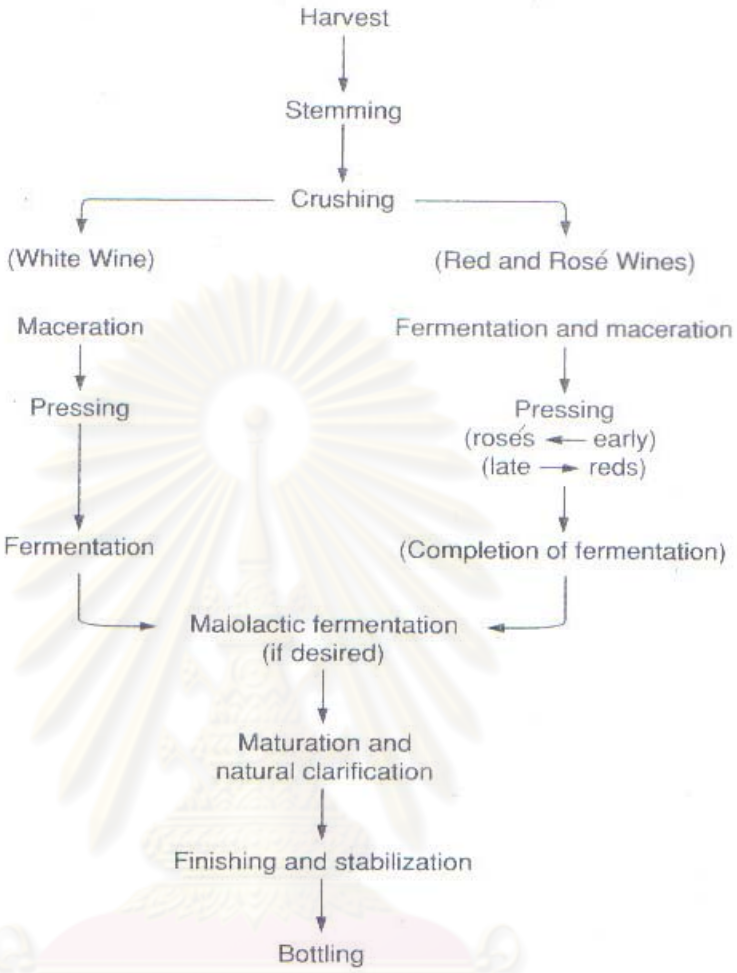


Figure 2.1 The flow diagram of wine making (Jackson, 2000).

For white wine, the grape skin is generally separated from the must by filtrated before the fermentation process. In red wine production, the whole crushed grape is initially fermented in order to allow alcohol generated to extract pigment, tannin and flavonoids from the grape skins. For red wine making, the skins are left in the tank for approximately 5 days to 3 weeks. For rose wine, the grape skins were left in the fermentor for a short time before separated out (< 24 hours). The fermentations are normally performed under warm temperature (24°C -27°C) for red wine making and under 10°C -15°C for white wine making (Jackson,

2000). Furthermore, the red wine making requires malolactic fermentation (MLF) and aging in barrel to improve flavor.

Clarification is done by removal of suspended material and minimization of substances associated to wine defect. The addition of bentonite is generally used in the clarification process to settle the sediment before racking (Jackson, 2000). After racking, the juice is subjected to filtration process. The filtration is normally processed through the several filter media which are diatomaceous earth, cellulose sheets, synthetic polymer membrane and inorganic and organic membranes (Ribéreau-Gayon *et al.,* 2006). For bottling, KMS is added after the wine is bottled to limit the oxidation and spoilage of microorganism.

In the following section, the details of important wine making process are described.

2.1.1 Must preparation

After the destemming, crushing and pressing processes, the concentration of important nutrients and pH of must are adjusted. Commonly, sugar content in term of total soluble solid in juice is measured with hydrometer as degree Brix (°Brix). The sugar content ranged in 15% to 25% (w/v) is suitable for the wine making since it is sufficient for yeast growth and fermentation (Ribéreau-Gayon *et al.,* 2006). Ammonium salt is generally used as nitrogen source for wine fermentation. The recommended nitrogen concentration in must is approximately 500mg L^{-1}. The pH of juice or must is necessarily adjusted before the fermentation. High pH must is adjusted by direct acidification. Tartaric acid is typically used for the must acidification. Deacidification of must is required for adjusting low pH must that is done by blending with low acid juice (Jackson, 2000).

2.1.2 Fermentation

The wine fermentation is divided to alcoholic and malolactic fermentations (primary and secondary fermentations) that are responded by different microorganisms as shown in figure 2.2.

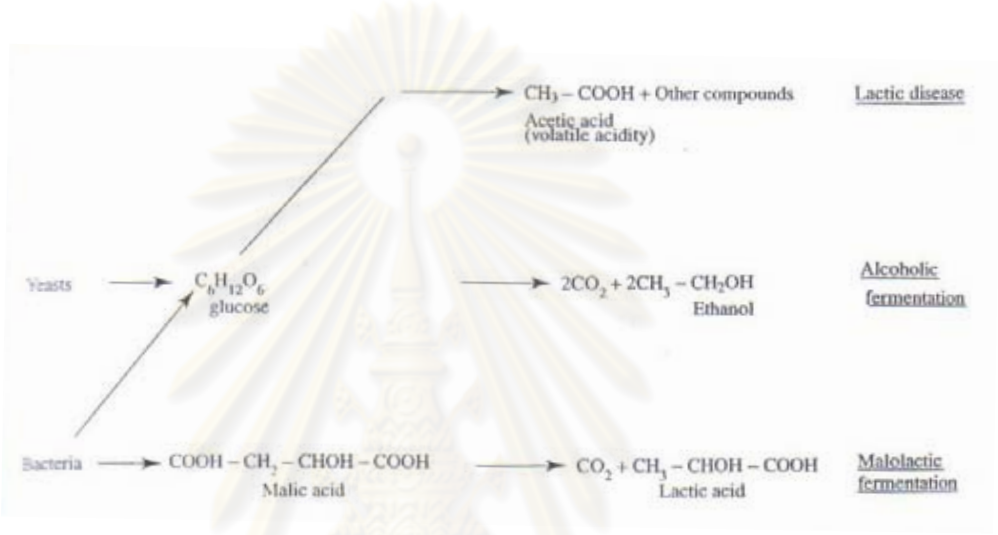


Figure 2.2 The microbial phenomena during primary and secondary fermentation (Ribéreau-Gayon *et al.,* 2006).

In term of alcoholic fermentation, it is processed by yeast to convert sugar in the system to be ethyl alcohol and carbon dioxide. Malolactic fermentation (MLF) is an another important process required in red wine making. MLF is processed by lactic acid bacteria to convert malic acid (a dicarboxylic acid) into lactic acid (a monocarboxylic acid). Beside, the main fermentation products during both fermentation processes, volatile compounds are also generated which could enrich the aromatic quality in wine (Henick-Kling, 1995; Moreno-Arribas and Polo, 2005; Pozo-Bayon *et al.,* 2005).

2.1.2.1 Alcoholic fermentation

Alcoholic fermentation is biochemical process which sugar in particular glucose is converted to ethanol and carbon dioxide by yeast under anaerobic condition. The chemical process of fermentation is shown as the chemical equation below:

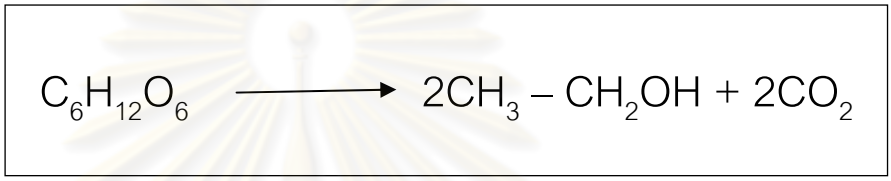


Figure 2.3 The biochemical reaction of alcohol formation (Delfini and Formica, 2001).

From this equation, there is one glucose molecule is converted to two ethanol molecules and two carbon dioxide molecules including ATP, via metabolism pathway called glycolysis as shown in figure 2.4. This metabolism process is performed under anaerobic condition by yeast, which utilizes glucose and fructose as a substrate to produce ethanol in wine.

The alcoholic fermentation contributes ethanol, and many metabolites into must, which known as the secondary metabolites. Secondary metabolites are directly produced via the Glycero-pyruvic fermentation (figure 2.5). The products are glycerol, pyruvic acid and acetaldehyde. Pyruvic acid from this pathway also oxidized to acetyl-Co-A before further enters to TCA cycle. Pyruvate is also decarboxylated to acetaldehyde, which is finally reduced to ethanol (Delfini and Formica, 2001).

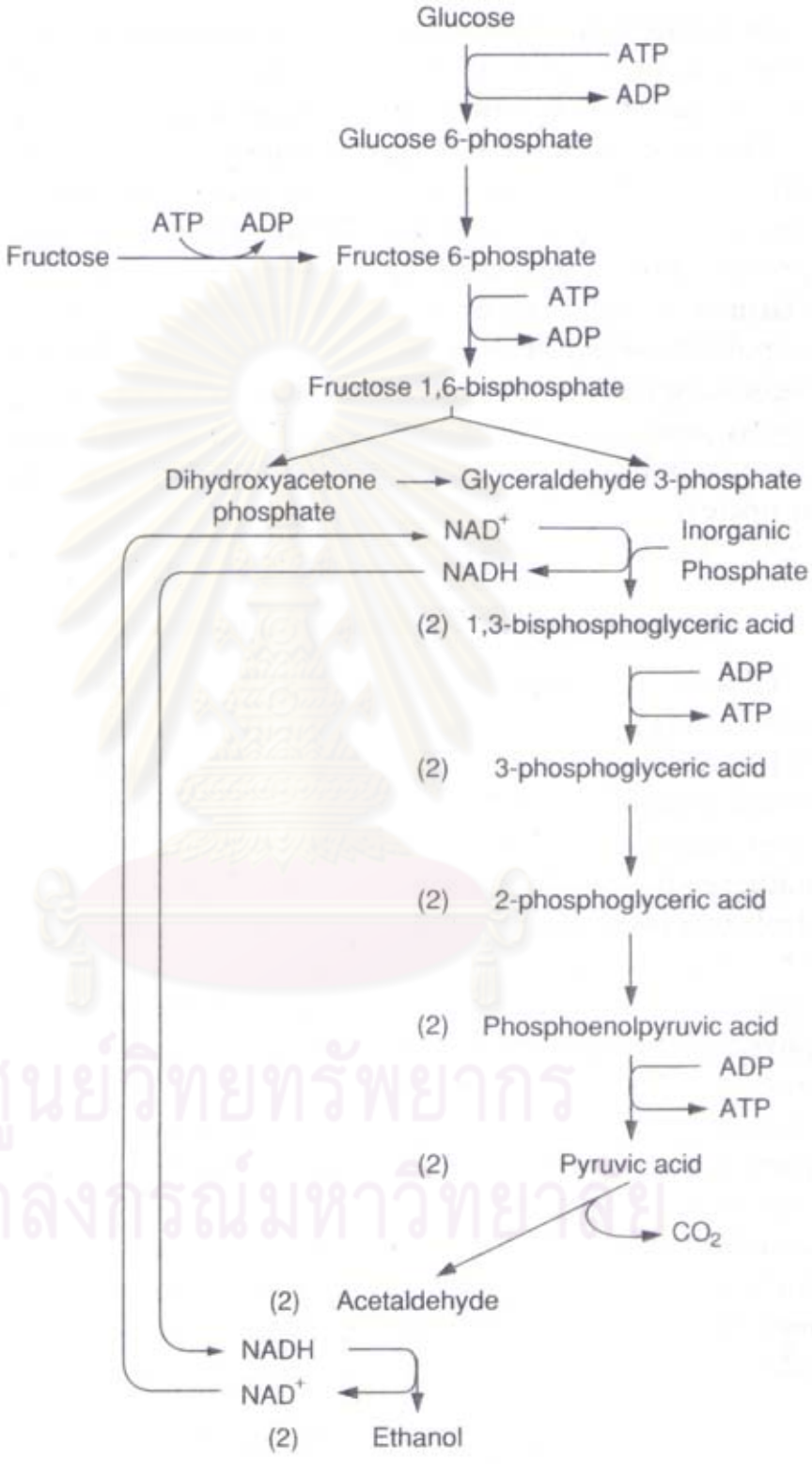


Figure 2.4 Glycolytic pathway utilized by yeast metabolism (Jackson, 2000).

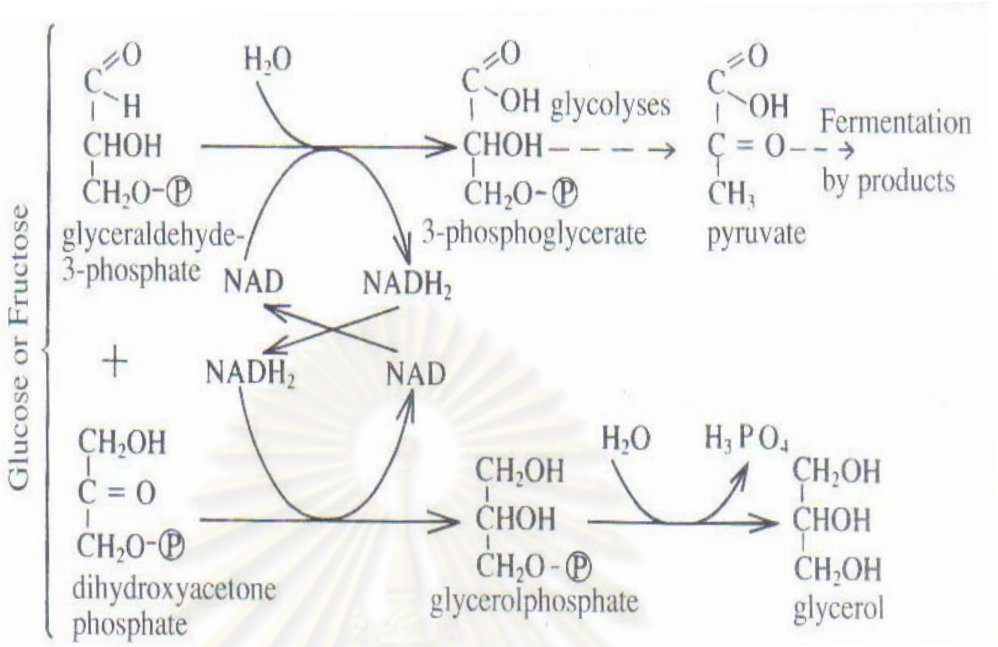


Figure 2.5 The Glycero-pyruvic fermentation (Delfini and Formica, 2001).

At the early stage of the alcoholic fermentation, the yeast needs the metabolic intermediates for cell growth and survival that is synthesized from TCA cycle with the presence of oxygen (figure 2.6). Then the yeast shifts to the glycolysis which the ethanol is produced when the oxygen is exhausted.

The TCA cycle produces several acids and intermediates in wine such as succinic acid, oxaloacetic acid, malic acid and fumaric acid. Other than TCA cycle, the enzymatic decarboxylation of pyruvic acid with the presence of vitamin B_1 also produces the ethanol. The evidence of chemical oxidation allows acetic acid formation including other acid developed (D, L- lactic acid and citramalic acid) by yeast during alcoholic fermentation (Ribéreau-Gayon *et al.,* 2006).

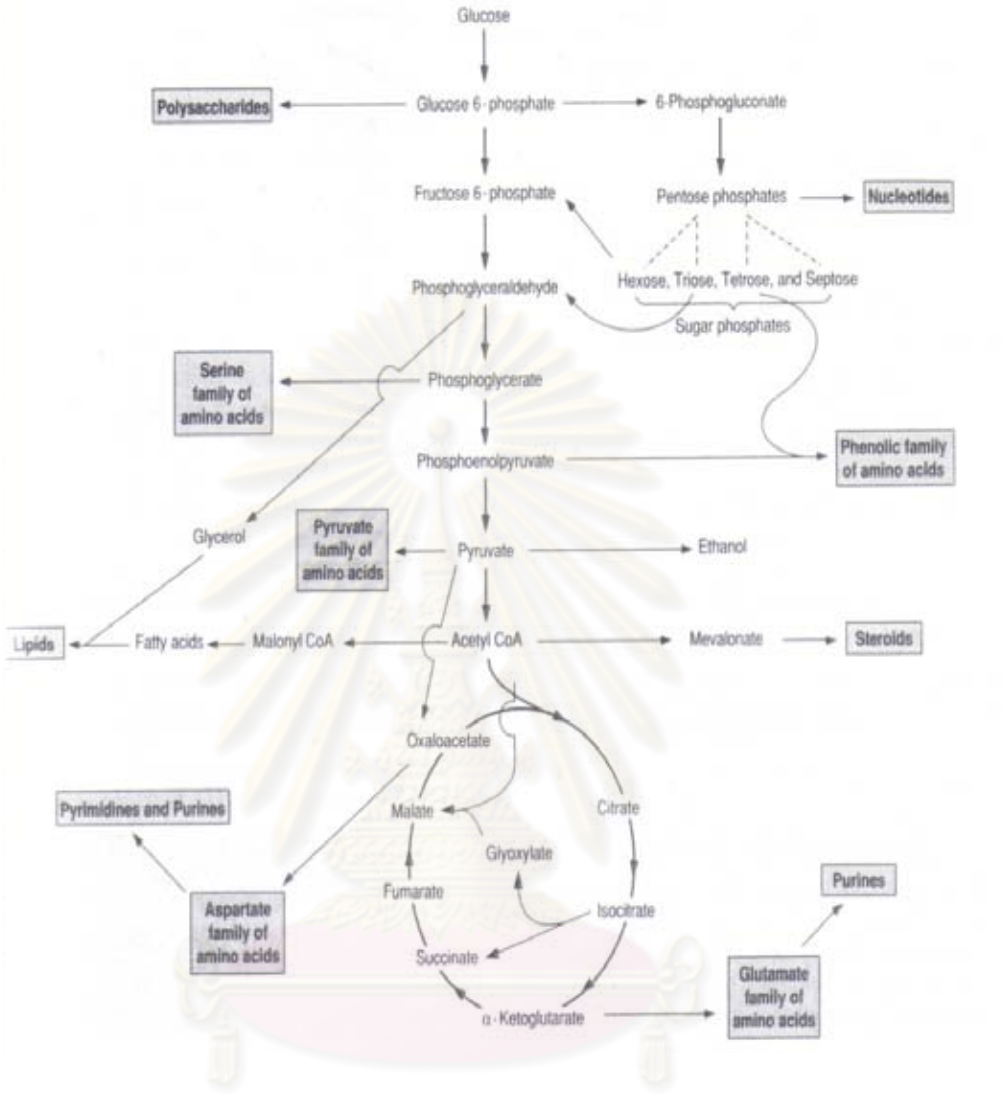


Figure 2.6 TCA cycle in alcoholic fermentation (Jackson, 2000).

Beside many organic acids formed during the alcohol fermentation, the volatile compounds is also generated in must which is induced by thiamine pyrophosphate (TPP) presence in the system as shown in figure 2.7.

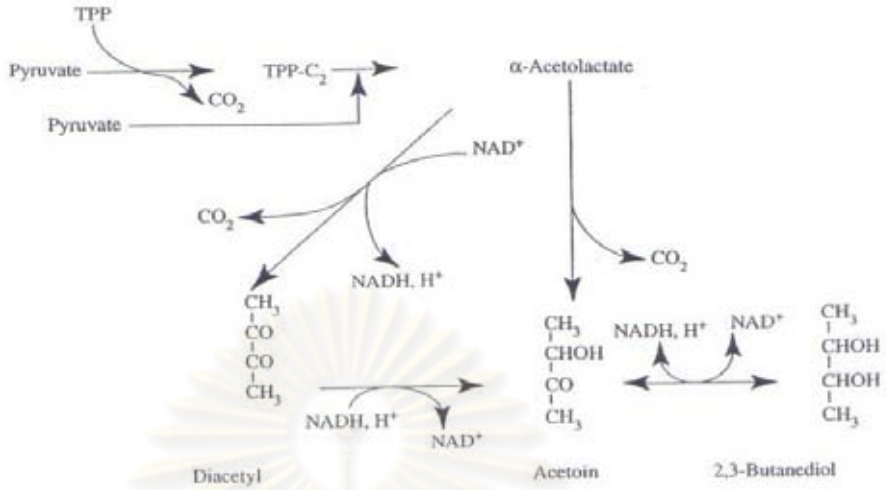


Figure 2.7 Diacetyl, acetoin and 2, 3-butanediol formation by yeast in anaerobiosis (Ribéreau-Gayon *et al.,* 2006).

Yeast is normally utilized pyruvate to form diacetyl, acetoin and 2, 3-butanediol under the anaerobic condition. The condensation of pyruvate and acetaldehyde bound with TPP allows the α–acetolactic acid formation. Then the oxidative decarboxylation of α–acetolactic acid form diacetyl. For acetoin formation, it comes from both of reduction of α–acetolactic acid and diacetyl. Acetoin is also reduced to 2, 3- butanediol (Ribéreau-Gayon *et al.,* 2006).

Moreover, the yeast also utilizes dissolved nitrogen and amino acids in must as the supplement which promote its growth and fermentation speed including higher alcohol formation via Ehrlich pathway as shown in figure 2.8. Higher alcohols are noted as the aromatic compounds in wine and also play an important role to wine quality in term of wine flavor (Fleet, 2003).

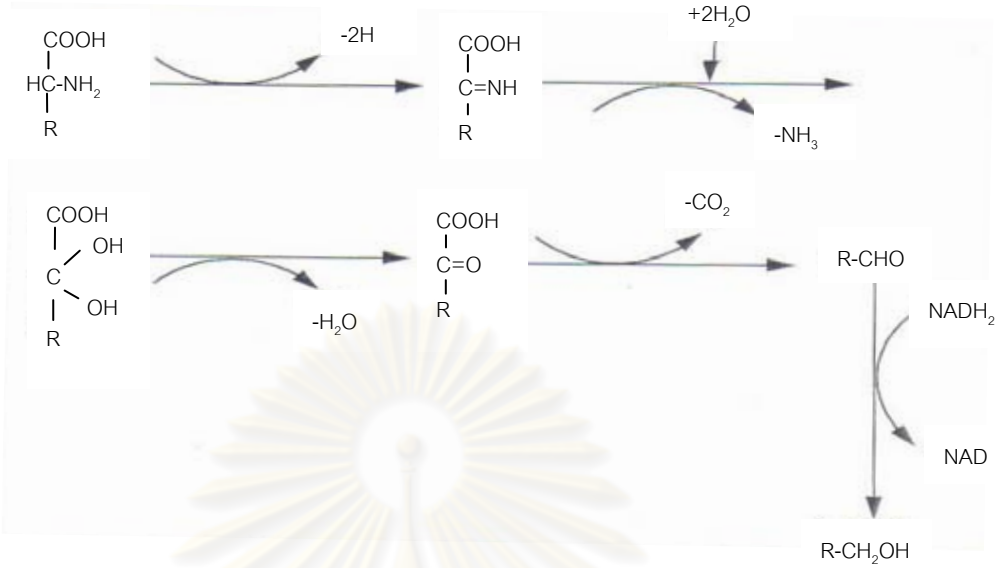


Figure 2.8 The higher alcohol formation during Ehrlich pathway (Delfini and Formica, 2001).

From the figure 2.8, the higher alcohols are formed by generic carbon radical which represents as R. The protein in must is an considerable nitrogen source that support yeast growth and fermentation. Amino acids can be utilized directly by yeasts and also can be deaminated and decarboxylated to ammonia and the higher alcohol via Ehrlich partway (Delfini and Formica, 2001).

In the alcoholic fermentation as mentioned, ethanol is the considerable primary product in the process. Other secondary products are also formed by many metabolisms such as organic acids, amino acids, glycerol, fatty acids, sterols and other volatiles. These compounds play important in fermentation process and contribute in wine characteristic. In red wine making process, the wine characteristic improvement was generally further conducted by the other processes that are the malolactic fermentation and aging in order to develop their flavor and reduce the acid taste in wine (Jackson, 2000).

2.1.2.2 Malolactic fermentation (MLF)

Malolactic fermentation is biochemical reaction of enzyme in wine performed by lactic acid bacteria. MLF is responded by lactic acid bacteria such as *Leuconostoc oenos, Leuconostoc eoni, Lactobacillus* and *Pediococcus*. The main purpose of this fermentation process is to reduce the acidity strength by the degradation of malolactic enzyme. This enzyme converts malic acid to lactic acid and carbon dioxide as shown in figure 2.9.

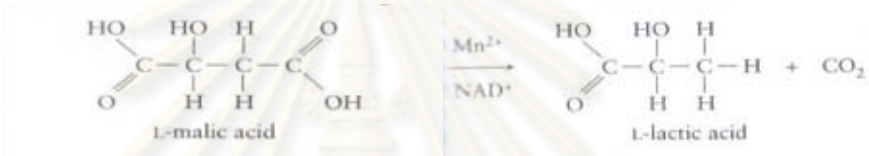


Figure 2.9 Lactate formation during malolactic fermentation (Delfini and Formica, 2001).

The beneficial aspect of the MLF is decreasing of hydrogen ion concentrations which is sufficient to significantly decrease of acid taste. MLF is required for red wine due to balance in the mouth-feel in wine. It also provides the benefit as stop yeast multiplication and extraction of excessive compounds (acetic acid, lactic acid, acetoin and diacetyl). Therefore, MLF is a considerate process for red wine making prior to aging or bottling (Delfini and Formica, 2001).

2.1.3 Post fermentation

After fermentation, acidity, sweetness and color of wine is generally adjusted prior to clarification. The acidity level significantly associated to the taste of wine. The total acidity is commonly determined as the titratable acidity (Ribéreau-Gayon *et al.,* 2006). An acceptable acidity in wine is ranged between

5.5-8.5 g L^{-1}. For pH adjustment, white wine is adjusted in range 3.1 to 3.4 whereas the pH range 3.3 to 3.6 is adjusted for red wine. Deacidification of wine is required if the pH of the wine is low, the wine is generally treated with calcium carbonate. This deacidification method is the precipitation of hydrogen ions of tartaric acid and malic acid forming with the cations of calcium carbonate. Sweetness in wine is related to the sugar content. Commonly, sugar content is served as reducing sugar remaining in wine. The wine is typically dry after the fermentation. Thus, the base wine is usually sweetened before bottling. The sweetness adjustment could be done by termed “sweet reserve”, such as adding sugar in form of syrup, and adding of partially fermented grape juice and unfermented juice. Both of wine and sweet reserve materials are necessarily decontaminated by filtration or pasteurization before bottling under aseptic condition (Jackson, 2000).

Wine color is normally improved by blending and clarifying processes. Blending wine is the wine made from mixing of variety wine from many regions. This process could also improve the flavor of wine. Clarifying is applied to wine in order to improve the physical property of wine. This method is typically stabilize wine against haziness including elimination of off-odor, excessive bitterness and astringent phenolics. In addition, clarifying is used to remove suspended particle and improve the flavor development.

For clarification, wine is treated by several techniques such as of adding fining agent (albumin, bentonite, casein, gelatin, kieselsol, isinglass and polyvinylpolypyrrolidone (PVPP)), racking, centrifugation and filtration (membrane filters and depth filters) (Jackson, 2000).

2.2 Factor associated to wine fermentation

Factors associated to wine fermentation include must condition, yeast strain as starter culture and fermentation condition (Jackson, 2000).

2.2.1 **Must condition** (Jackson, 2000)

The major carbon source for yeast fermentation is glucose and fructose. The sugar concentration in must ranged between 20 and 25% is a preferable condition for yeast fermentation. The sugar concentration above 25%-30% is generally fermented by *Saccharomyces cerevisiae* since it can tolerate high sugar concentration. The ethanol production rate relates to the sugar content in must. Therefore, an appropriate sugar content in must is also important to fermentation efficiency of yeasts which generates ethanol along with secondary metabolite determining wine quality.

For nitrogen source, sufficient nitrogen in must can promote the fermentation kinetic and complete the fermentation. The minimum level of sufficient nitrogen source is approximately 150 mg L^{-1} whereas the optimum level is suggested at 400-500 mg L^{-1}. Nitrogen is required as the significant growth factor of yeast. Nitrogen content in must is also influence on yeast fermentation to generate aroma compound impacting on wine flavor.

Beside the sugar and nitrogen sources, vitamin is considered as the co-factor for the yeast metabolisms. Yeast supplement includes vitamin B_1, vitamin B_2, biotin, folic acid and

niacin. Vitamin B_1 and biotin are the supplement associating to the decarboxylate reaction whereas vitamin B_2 and niacin are the supplement associating to dehydrogenation reaction. For folic acid, it is important in transamination and ergosterol synthesis of the yeast. The addition of vitamin in must is occasionally recommended to solve the stuck fermentation.

The bioavailability related to mineral inform of ion is the major inorganic elements for yeast metabolism requirement such as potassium, magnesium, calcium, zinc, iron and sodium. These minerals enhance the fermentation efficiency by providing the stability of yeast cell and preventing cell death in alcoholic fermentation. Many of them stimulate the sugar uptake in yeast cell and regulate the yeast metabolism.

2.2.2 Yeast strain

The fermentation of must is a complex process of different yeast genera and species. Wine characteristics are made from ambient yeasts, which naturally present in vineyards and on the grape berry, and isolated yeast culture for use in winemaking. The use of different strains of yeasts is a major contributor to the diversity of wine even among the same grape variety (Romano *et al.,* 2003).

2.2.2.1 Natural fermentation

The large variety of bacteria, molds and yeasts were found on grape skin and involve in alcoholic fermentation. Common genus of wild yeasts originated from grapes which are

Candida, Hanseniaspora, Metschnikowia, Pichia and *Kluyveromyces* that found as the predominant in winemaking. Wild yeasts produce unique flavor in wine. These species are indigenous, non- *Saccharomyces* species, which play important role in wine fermentation and wine characteristics. However, these microflora produce ethanol approximately 6% alcohol and then die off due to toxicity of alcohol. Consequently, the microbial population has been significantly decreased. Then, the *Saccharomyces* species dominate and complete the alcoholic fermentation which alcohol reaches approximately 12-14% (Fleet *et al.,* 2002).

2.2.2.2 Starter culture

Since the winemaking of the wild microflora (*Candida, Hanseniaspora, Metschnikowia, Pichia* and *Kluyveromyces*) perform low ethanol production in alcoholic fermentation and the quality of wine characteristics is also unpredictable. Therefore, the starter culture technology is introduced to the wine industry. The fermentation of the starter culture offers an advantage on rapid, efficient process and provides a consistent wine quality.

The common starter cultured yeasts used in wine making is *Saccharomyces cerevisiae*. Several hundred different yeast strains of this yeast species are used for wine fermentation to generate the flavor characteristics of wine. Therefore, the *Saccharomyces* strains are developed and widely used as starter cultures in commercial winemaking (Fleet *et al.,* 2002; Romano *et al.,* 2003; Prakitchaiwattana *et al.,* 2004).

Furthermore, the starter culture technology is improved by several techniques. The technique includes using of single, mixed and hybrid of culture for wine fermentation (Fleet *et al.,* 2002; Fleet, 2003; Serra *et al.,* 2005; Ciani *et al.,* 2006).

2.2.2.2.1 Single culture

a. *Saccharomyces cerevisiae*

The *Saccharomyces cerevisiae* is the main yeast species used for wine making. This species is widely used as the commercial starter culture due to its high alcohol tolerant and high sugar resistant (approximately 300g L^{-1}). The optimum temperature of this yeast in fermentation is approximately 15 °C to 30 °C. This yeast also generates many secondary metabolites which contribute in wine quality in term of flavor (Jackson, 2000; Romano *et al.,* 2003).

b. *Saccharomyces bayanus*

Apart from *Saccharomyces cerevisiae*. *Saccharomyces bayanus* is another species which is used in the commercial wine production. This yeast is considered as cryotolerant strain in wine making since its minimal fermentation temperature is approximately 5°C - 15°C. *Saccharomyces bayanus* produces more glycerol and less acetic acid relative to *Saccharomyces cerevisiae.* This yeast is reported as the specific strain for some fruit wine fermentation, for instance longan wine (Chomsri *et al.,* 2003; Serra *et al.,* 2005).

2.2.2.2.2 Mixed culture

Originally, wine is the product of the complex interaction between fungi, yeast and bacteria. Thus, the mixed culture is an alternative starter culture in wine making process. There are the wine characteristic responded by the interaction of yeast-yeast, yeast-fungi and yeast-bacteria. Mixed culture interaction could enhance and inhibit the growth of some species that also influence on wine flavor. Yeast-yeast interaction impacts on wine flavor in term of conduction of the alcoholic fermentation, catalyze the flavor component transformation, influence of the malolactic and spoilage bacteria. Yeast-fungi interaction impacts on wine flavor in term of production of the botrytized flavor in wine, mycotoxin, and the other metabolites. Yeast-bacteria interaction impacts on wine flavor in term of inducing the spoilage during storaged, causing of the stuck fermentation and also conduction of malolactic fermentation. The interaction of different species and strains is considered as the factor effect on wine quality since different multistarter influence on the flavor profiles in wine (Mateo *et al.,* 2001; Fleet, 2003; Ciani *et al.,* 2006). The controlled mixed culture of *Saccharomyces cerevisiae* and non- *Saccharomyces* were studied and reported that they can improve the analytical and aromatic profile of wine due to the positive metabolic interactions as shown in table 2.2.

Table 2.2 Utilization of controlled mixed culture in wine making processes

mixed cultures	aim	process
Saccharomyces cerevisiae *Torulaspora delbrueckii*	reduction of acetic acid production	Sequential cultures
Saccharomyces cerevisiae *Schizosaccharomyces pombe*	malic acid degradation	Sequential cultures Immobilized cells (batch process) Immobilized cells (continuous process)
Saccharomyces cerevisiae *Candida stellata*	enhancement of glycerol content	Immobilized cells (pre-treatment or Sequential cultures)
Saccharomyces cerevisiae *Hanseniaspora uvarum*	stimulation of natural fermentation (complex aroma)	Mixed or Sequential cultures
Saccharomyces cerevisiae *Kluyveromyces thermotolerans*	reduction of acetic acid production and enhancement of titratable acidity of wine	Sequential cultures

Source: Ciani *et al.* (2002)

2.2.3 Fermentation condition

In the wine making process, the composition of must is important for fermentation efficiency. Several factors such as the oxygen dissolved, carbon dioxide presence, pH and temperature are significantly influence on yeast fermentation.

For oxygen and aeration, the wine fermentation kinetic is influenced by the presence of oxygen (approximately 10mg O_2 L^{-1}). Aeration is limited for the fermentation process (approximately 40mg O_2 L^{-1}) due to the benefit of wine maturation. The oxygen dissolved enhances yeast cell viability and stimulate yeast cell division that might slow the alcoholic fermentation. Aeration also provide the wine quality in term of color and aroma since it is demonstrated that the aeration increase the acetaldehyde formation and anthocyanin-tannin polymers formation which dominate the flavoring color stability (Jackson, 2000).

Normally, during the fermentation, the large amount of carbon dioxide is generated in fermentor due to glucose utilization of yeast. Some research indicated that the carbon dioxide might affect loss of various aroma volatiles up to 25% such as acetate ester, monoterpenes and higher alcohols (Jackson, 2000).

pH in must normally play, a insignificant role in the fermentation rate and aromatic compound production of yeast. The yeast could grow in pH ranged 3-4. This low pH condition can inhibit competitive organisms in the fermentation system. The pH also affects to the by-product presence in wine such as leading to hydrolysis of ethyl and acetate ester.

Temperature is an another key factor playing role in the fermentation. It is directly associated to yeast metabolism and fermentation speed. The fermentation temperature approximately 10 °C-15 °C is recommended for wine flavor improvement whereas the warm fermentation conducted under higher 20°C is performed in order to speed up the fermentation rate (Jackson, 2000).

2.3 Fruit wine and development

Wine can be made from the other type of fruits that is usually called “fruit wine”. The fruit wines making process is relatively similar to grape wine. However, the specific art or technology used to improve quality of each type of the fruit wine is still at the initial stage of the development. Although not many systematic researches about fruit wine fermentation are reported, there are some studies about palm wine, mango wine, kiwi wine and orange wine fermentation and their properties published in scientific journals and proceeding books. These studies are reviewed in this part (Soufleros *et al.,* 2001; Selli *et al.,* 2003; Ezeronye, 2004; Reddy and Reddy, 2005).

2.3.1 Strain selection for fruit wine

There are many reports demonstrated that the interaction of yeast strains and species specific involve in the characteristic and flavor of wine. Many studies investigated the strain development related to their influences on the aromatic profile for use in winemaking process (Romano *et al.,* 2003). Ezeronye (2004) studied fermentation profiles of 11 *Saccharomyces cerevisiae* strains in tropical fruits which were pinop, mogo, cashew, and papaw. The results demonstrated that the fermentation profiles of different yeast strains in each fruit juice were

significantly different and volatile compounds generated were not identical. Thammarat (1978) reported that during palm wine fermentation, *Kloeckera apiculata* was the main predominant in the early stage of fermentation then *Saccharomyces chevalieri* dominated throughout the fermentation

Chomsri and research group (2003) compared the fermentation rate of *Saccharomyces bayanus* and *Saccharomyces cerevisiae* in longan wine. The result demonstrated that *Saccharomyces bayanus* could perform a rapid fermentation over the yeast *S. cerevisiae*.

From the research group of Deeraksa (2005), they used yeast isolates obtained from fermented mangosteen paste to make mangosteen wine compared to *Saccharomyces bayanus.* The results demonstrated that the natural isolates provided significantly higher acceptance of sensory test than the pure culture of *S. bayanus* in mangosteen wine.

2.3.2 Fruit wine formulation

In some wine making process, the yeast fermentation is not enough to provide the desirable characteristic of wine. For red wine, the malolactic fermentation is conducted to reduce the acid taste while the formulation by blending methodology can be applied to fruit wine production. Soufleros *et al.,* (2001) tried to develop new kiwi wine making process by selecting the yeast efficient to ferment kiwi juice. The basic wines obtained were fortified with sugars, CO_2 and alcohol to find an acceptable kiwi wine formulation for the consumer. The accepted kiwi

wine from this study contained 10% alcohol, 4.5% sugar and less than 1% acidity.

2.3.3 Orange/tangerine wine

For orange wine, Selli *et al.* (2003) reported about orange wine composition that alcohol, total sugar and total acidity contents in orange wine were relatively similar to grape wine. Total acidity of orange wine commonly expressed as citric acids whereas grape wine expressed as tartaric acid. The volatile compounds contributed to orange wine could be terpenes, alcohols, esters, volatile phenols, acids, aldehyde, and others. In year 1993, Jutajumpol and Panumastrakul (1993) provided useful information about tangerine wine fermentation from 3 strains of *Saccharomyces cerevisiae*. They reported that the strains of wine yeasts could be a key for tangerine juice fermentation. Their results also demonstrated that the strain generating large amount of aldehyde would give more desirable flavor characteristic of tangerine wine. The most accepted tangerine wine character in this study was the wine containing 10.5% alcohol, 10% reducing sugar, 0.188mg citric acid $100ml^{-1}$ of total acidity, and 3.8mg $100ml^{-1}$ of aldehyde.

The reports about orange wine properties of previous studies are summarized in table 2.3.

Although there are some reports of orange wine characteristics could be referred the influence of yeast strain on orange wine fermentation, color and the formulation of orange wine has not been reported.

Table 2.3 The properties and chemical composition of orange wine

components in orange wine	active compounds	references
total acidity	citric acid (ranged 0.02%-1.17%)	Jutajumpol and Panumastrakul, 1993; Selli *et al.*, 2003 and Selli, 2007
volatile acidity	acetic acid (ranged 0.03%-0.05%)	Selli *et al.*, 2003 and Selli, 2007
carbonyl compound	aldehyde	Jutajumpol and Panumastrakul, 1993 and Selli *et al.*, 2003
higher alcohol	2-phenylethanol and isoamyl alcohol	Selli *et al.*, 2003 and Selli, 2007
volatile ester	ethyl hexanoate and isoamyl acetate ethyl butanoate	Selli *et al.*, 2003 Selli, 2007
terpene	linalool, terpinene-4-ol and limonene	Selli *et al.*, 2003 and Selli, 2007
volatile phenol	4-vinyl guaiacol, 4-vinyl phenol and tyrosol	Selli *et al.*, 2003 and Selli, 2007
fatty acid	hexanoic and octanoic acids	Selli *et al.*, 2003 and Selli, 2007
lactone	γ-Butyrolactone	Selli *et al.*, 2003 and Selli, 2007
isoprenoid	norisoprenoid	Selli *et al.*, 2003

2.4 Wine flavor and color

The flavor of wine plays an important role on indicating the wine quality. Flavors are highly complex formation of organic molecules such as esters and terpenes that contain in grape juice and wine. Wine aroma comes from the interaction of the grape components and is also produced during winemaking processes, which are fermentation and aging. The phenolic compounds are the most important components of wines. They are directly related to its color, astringency, bitterness and oxidative level, and also act as the antioxidants in wine (Jackson, 2000). The compounds associate in wine flavor and their flavor notes as shown in table 2.4.

During storage and aging, red wine color changes from bright red to reddish-brown due to the attribution of the more stable polymeric pigments formation. The pigments are proceeded from anthocyanins and other phenolic compounds reactions (flavan-3-ol monomers and polymers). The reactions respond for the formation of these changes including acetaldehyde-mediated condensation, co-pigmentation and self-association. The most important key factor affecting to these compound contents in wine are their concentration in grape, the winemaking technology used, and their transformation during wine aging process (Jackson, 2000). Clarification techniques also affect to the wine quality. Fining process can eliminate some phenolic compounds of colloidal, implicated the oxidation and the excess astringency of wine. Clarifying also contribute the organoleptic on the characteristics improvement into wine. Fining agents such as PVPP, gelatin, egg albumin and casein can also reduce phenolic levels and alter the color in wines (Jackson, 2000; Maury *et al.,* 2001). To drink wine, serving wine at room temperature can increases the vaporization of aroma compounds, making the wine more aromatic.

Table 2.4 Chemical compounds associate to wine flavor

Chemical compounds	Sources	Flavor notes
Aldehydes and isoamyl acetate	Grapes	Grassy
Benzaldehyde	Grapes : Pinot noir	Bitter almonds
Ethyl esters, ethyl acetate and acetaldehyde	Yeasts	Fruity
2-phenylethanol and isoamyl alcohol	Yeasts	Floral
Acetates	Yeasts	Sweet
Octanoic	Yeasts	Mousy
Ionene	Aging	Rose-like
Acetoin	*H. uvarum*	Buttery
2,3-butanediol	*S. cerevisiae* and *Z. fermentati*	Bouquet and nutty
Ethyl acetate	*C. stellata* and *S. ludwigii*	Sour, off-odor

Source: Romano *et al.* (2003)

However, some phenolic can provide an undesirable flavor in wine such as banana flavor note derived from isoamyl acetate which are the product of yeast metabolism representing the spoilage aroma in wine (Romano *et al.,* 2003).

2.5 Wine regulation

Thai industrial standard institute provide a definition for fruit wine. The fruit wine is the fermented wine made from fruit without distillation process. The alcohol content is not over than 15%. The quality of color, clarity and flavor are acceptable with no presence of contamination and bubble from the second fermentation. The limit by regulation of considered contaminants is shown in table 2.5.

Table 2.5 Limit of contaminant in fruit wine

Contaminants	Limitation ($mg\ L^{-1}$)
Methyl alcohol	≤ 420
Sorbic acid	≤ 200
Benzoic acid	≤ 250
Sulfur dioxide	≤ 300
Copper	≤ 5
Iron	≤ 15
Lead	≤ 0.2
Arsenic	≤ 0.1
Ferrocyanide	0

Source: Thai Industrial Standard Institute. (2003)

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental materials and equipments

3.1.1 Materials

a) 100% UHT Tangerine juice (Tipco, Thailand)

b) Citric acid, food grade (Bangkok Chemical Industrial Co. Ltd., Thailand)

c) Sugar (Mitrphol, Thailand)

3.1.2 Equipments

a) pH meter (Cyberscan 1000, USA)

b) Colony counter (Gallenkamp, Germany)

c) Vortex mixer (Lab-line Instrument Inc., USA)

d) Laminar flow hood (BVT 123 Issco, USA)

e) Microwave (KOR-63-D7 Daewoo, Korea)

f) Incubator (Memmert, Germany)

g) Spectrophotometer (V530 Jasco, USA)

h) Autoclave (SS320 Tomy, USA)

i) Hot-air oven (Binder, Germany)

j) 1000 µl micropipette (LMS, Japan)

k) 2-Decimal place balance (Satorious BP 3100S, Germany)

l) Magnetic stirrer (Framo ®, Germany)

m) Muffle furnance (Furnance Carbolote, S336RB Parsons Lane, England)

n) Kjeldahl distillation unit (Kjeldahl and Vapodest, K424 Büchi, Switzerland)

o) Chromameter (CR-300 series, Minolta, Japan)

p) Refractometer (Atago, Japan)

q) Vinometer (Alla, France)

3.1.3 Chemicals

a) Sodium hydroxide, analytical grade (Merck, Germany)

b) Ethyl alcohol, analytical grade (Ajax Finechem, USA)

c) Sulfuric acid, analytical grade (J.T. Baker, USA)

d) Boric acid, analytical grade (Fisher scientific, UK)

e) Selenium reagent mixture, analytical grade (Merck, Germany)

f) Methyl red, analytical grade (Merck, Germany)

g) Bromocresol green, analytical grade (Merck, Germany)

h) Methylene blue, analytical grade (Riedel-de Haën, Germany)

a) Diatomaceous earth, analytical grade (Sigma-Aldrich, USA)

i) Bentonite, analytical grade (Riedel-de Haën, Germany)

j) Hydrochloric acid, analytical grade (Merck, Germany)

k) Potassium metabisulphite, analytical grade (Merck, Germany)

l) Copper II sulphate pentahydrate, analytical grade (Merck, Germany)

m) Potassium sodium tartrate tetrahydrate, analytical grade (Merck, Germany)

n) Phenolphthalein, analytical grade (Merck, Germany)

3.1.4 Microorganism cultural media

a) Potato dextrose agar (PDA) (Difco, France)

b) Peptone from casein (Merck, Germany)

c) de Man, Rogosa and Sharpe agar (MRS) (Difco, France)

3.1.5 Yeast culture

a) *Saccharomyces cerevisiae,* baker's yeast (Angel®, China)

b) *Saccharomyces bayanus* EC1118, wine yeast (Lavin®, Australia)

3.2 Experimental procedures

3.2.1 Determination of physical and chemical properties of tangerine juice.

3.2.1.1 Physical properties determination

The color of tangerine juice was determined in CIELAB system by Chromameter CR-300 connected with CT-310 and also investigated by using Munsell's book system (Appendix A). This experiment was conducted in 2 replication.

3.2.1.2 Chemical properties determination

The chemical composition of tangerine juice was investigated as the following methods. This experiment was conducted in 2 replication.

- The nitrogen content of tangerine juice was determined by following the Kjeldahl method (A.O.A.C., 1995) (Appendix A).
- The reducing sugar content of tangerine juice was investigated by Lane-Eynon method (A.O.A.C., 1995) (Appendix A).
- The titratable acidity of tangerine juice was determined by titration with 0.1N NaOH (A.O.A.C., 1995) (Appendix A).
- Ash content in tangerine juice was investigated by incineration in muffle furnance oven at 550 °C (A.O.A.C., 1995) (Appendix A).
- Total soluble solid and pH value were measured by refractometer and pH meter, respectively.

3.2.2 Determination of fermentation profiles of tangerine juice fermentation

The tangerine wine was made by following the process as shown in flow chart in figure 3.1.

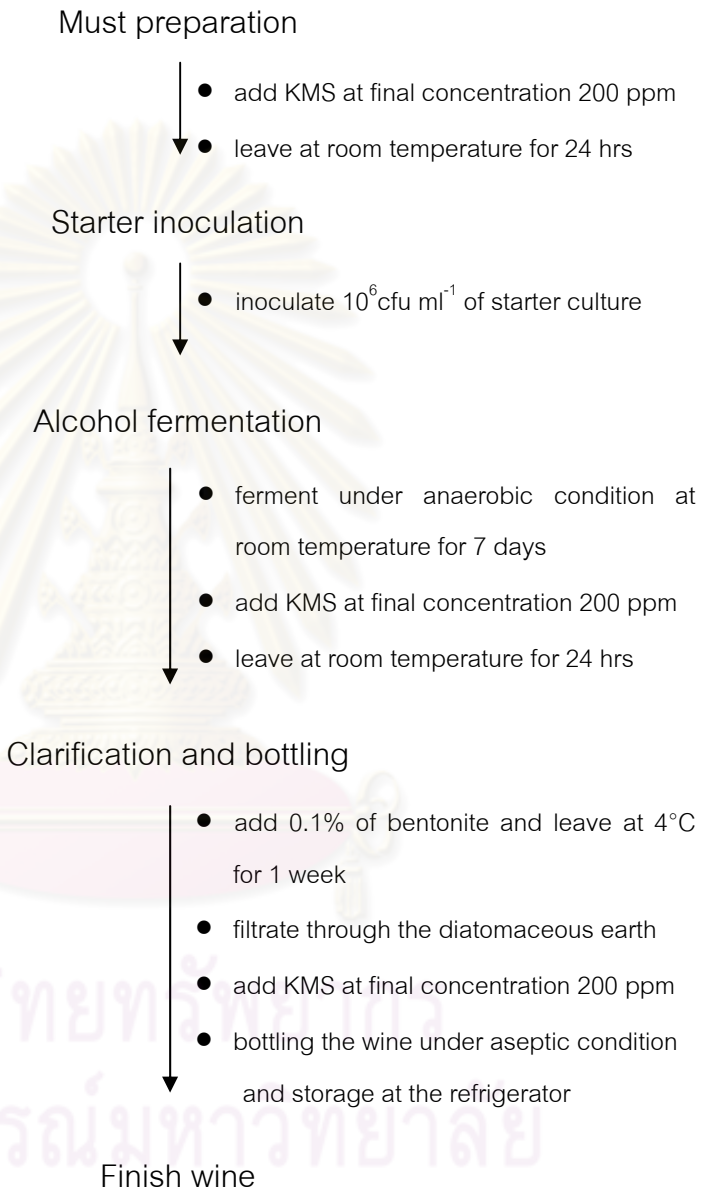


Figure 3.1 Tangerine wine making process

Each process shown in the flow chart was conducted as the following sections.

3.2.2.1 Must preparation and must condition

The 100% tangerine juice was diluted with water 30%, 50%, and 100% (v/v) concentrations. Carbon and Nitrogen sources in each concentration were adjusted to 25% (w/v) and 0.05% (w/v) by using sucrose and diammonium phosphate, respectively. pH of the juice was adjusted to 3.5 using 0.1N citric acid. Then, juice was decontaminated by adding potassium metabisulfite (KMS) giving final concentration 200 ppm and used as a must for fermentation (Jackson, 2000).

3.2.2.2 Starter culture preparation and the culture type used for fermentation

Yeast species and culture types used for tangerine juice fermentation in this study are listed in table 3.1. Starter culture was prepared by inoculated 1 loop of 48 hours yeast colony into 100 ml of tangerine juice (30%). Inoculated juice was orbitally shaked 200 rpm at room temperature for approximately 19 hours or until cell population number reached 8 log cfu ml^{-1}. The experiment was conducted in a 3x3 factorial design as shown in table 3.1.

Table 3.1 The tangerine wine fermentation conditions

must concentration	yeast species and culture types		
	single culture		mixed culture (1:1 ratio)
30% juice	*S. cerevisiae*	*S. bayanus*	*S. cerevisiae* + *S. bayanus*
50% juice	*S. cerevisiae*	*S. bayanus*	*S. cerevisiae* + *S. bayanus*
100% juice	*S. cerevisiae*	*S. bayanus*	*S. cerevisiae* + *S. bayanus*

3.2.2.3 Fermentation profile determination

Tangerine must was fermented using single and mixed starter culture as the conditions shown in table 3.1. The starter culture was inoculated into the must at initial population 10^6 cfu.ml^{-1} and fermented under anaerobic condition at 30 °C until alcohol content reached 12%. The fermentation profiles of 9 batches (table 3.1) were monitored everyday; by determination for alcohol and sugar content, yeast population, titratable acidity and color, using these following methods. This experiment was conducted in 2 replication.

- The alcohol content of fermented tangerine juice was observed by vinometer (Appendix A).
- The reducing sugar content of fermented tangerine juice was investigated by Lane-Eynon method (A.O.A.C., 1995) (Appendix A).
- The titratable acidity of fermented tangerine juice was investigated by titration with 0.1N NaOH (A.O.A.C., 1995) (Appendix A).
- The yeast population was investigated by spreading fermented tangerine juice onto PDA plate and incubated at 30 °C for 2-3 days and then counted the colony (Yeast & Mold count, A.O.A.C., 1995) (Appendix B).
- The color of fermented tangerine juice was determined in CIELAB system by Chromameter CR-300 connected with CT-310 and also investigated by Munsell's book system (Appendix A).

3.2.2.4 Wine clarification and bottling

The fermented juices containing 12% alcohol were added with potassium metabisulfite (final concentration 200 ppm) for decontamination. Then, 0.1% (w/v) of bentonite was added as the fining agent and let the

particle to settle down under the refrigerated temperature for 7 days. The clear liquid was racked into a new container. The racked fermented juices were further filtrated through diatomaceous earth by the vacuum pump. The 350 ml of clarified fermented juice was filled into the amber glass bottle (400ml) under aseptic area. Potassium metabisulphite (KMS) was added into the clarified fermented juice to a final concentration of 200 ppm, and then the bottle was closed with easy-open cap. The bottled wine was storaged in the refrigerator for a few day before subjecting to the preliminary sensory test. All experiments were conducted in 2 replication.

3.2.3 Basic wine selection

The finished wine of 9 batches after bottling were preliminary evaluated for off-flavor. Non-off flavor fermented juices were then subjected to methanol content determination using GC method (A.O.A.C., 2005), conducted by National Food Institute, a commercial facility. The experiment was conducted in 2 replication. The basic tangerine wine making condition and quality were selected based on the criteria of; a proper fermentation profile performing of the batch, residual sugar content, acceptable flavor and the methanol content limitation ($\leq$420mg L^{-1}). The selected basic wine was subjected to the formulation in the further study.

3.2.4 Evaluation of an acceptability for the formulated tangerine wine

To determine the most accepted formula of tangerine wine based on levels of sweetness and sourness, the basic wine selected from previous part (section 3.2.3) was formulated by adjusting reducing sugar content and acidity. The reducing sugar was adjusted into 3 levels; 2%, 4% and 6% (w/v) by sucrose syrup (Appendix D). this range represents as semi-sweet wine (1-3%

reducing sugar) and sweet wine (>3% reducing sugar) (Lea and Piggott, 1995). Total titratable acidity of wine was adjusted into 3 levels; 0.50%, 0.70%, and 0.90% (w/v) by adding citric acid (Appendix D). Jackson, 2000 reported that the accepted range of acidity in wine was 0.55% to 0.85% (w/v). The 350 ml of tangerine wine was filled into the amber glass bottle (400ml) under aseptic area. Potassium metabisulphite (KMS) was added into the clarified fermented juice to a final concentration of 200 ppm, and then the bottle was closed with easy-open cap. The bottled wine was storaged under the refrigerator for a few day before subjecting to the acceptance test.

Therefore, the 9 formulas of formulated tangerine wine were prepared as a 3x3 factorial experiment. The acceptance test was conducted in a Balanced Incomplete Block design by 108 assessors (54 females and 54 males), age over 20 years old. Each assessor evaluated only 4 out of 9 samples (Appendix D). Thus, each sample was evaluated by 24 assessors.

The acceptability of the 9 formulated wine samples were evaluated using a 9-points hedonic scale for the “overall liking”, the “liking of color”, the “liking of clarity”, the “liking of aroma” and the “liking of flavor” (Appendix E). The 5-point Just About Right scale was used to determine the perceived sweetness, sourness, astringency, bitterness and degree of alcohol (Appendix E). The evaluation was carried out in Department of Food Technology, Chulalongkorn University and “New Story” restaurant, Pattanakarn, Bangkok. All 9 wine samples were kept in the refrigerator (4°C). For each serving, 30 ml, was served chill in transparent, colorless glass containers covered with wrapping film and codified with a 3 digit random number.

The data was collected and analyzed. Analysis of Variance (ANOVA) was used to determine effect of factors and interaction (Appendix F). Comparison of means was conducted using Duncan’s New Multiple range test at 95% confident level.

3.2.5 Evaluation of shelflife of formulated tangerine wine

The shelflife of selected tangerine wine formula from section 3.2.4 was investigated. The 50ml of tangerine wine was kept in the amber glass bottle under aseptic area. Potassium metabisulphite (KMS) was added into the clarified fermented juice to a final concentration of 200 ppm, and then the bottle was closed with easy-open cap. The physical, chemical and microbiological properties of the wine were determined every week for 2 months as these following methods. This experiment was conducted in 2 replication.

3.2.5.1 Physical properties determination

- The color of tangerine wine was determined in CIELAB system by Chromameter CR-300 connected with CT-310 and also investigated by Munsell's book system (Appendix A).

3.2.5.2 Chemical properties determination

The chemical composition of tangerine wine was investigated as the following methods.

- The reducing sugar content of tangerine wine was investigated by Lane-Eynon method (A.O.A.C., 1995) (Appendix A).
- The titratable acidity of tangerine wine was investigated by titrated with 0.1N NaOH (A.O.A.C., 1995) (Appendix A).
- Total soluble solid and pH value were measured by Refractometer and pH meter, respectively.
- The alcohol content of tangerine wine was observed by vinometer (Appendix A).

3.2.5.3 Microbiological properties determination

The microorganism contamination of tangerine wine was investigated as the following methods.

- The yeast population was investigated by spreading 0.1 ml of tangerine wine onto PDA plate and incubated at 30 °C for 2-3 days and then counted the colony (Yeast & Mold count, A.O.A.C., 1995) (Appendix B).
- The lactic acid bacteria (LAB) population was investigated by spreading 0.1 ml of tangerine wine onto MRS plate and incubated at 30 °C for 2-3 days and then counted the colony (lactic acid bacteria (LAB) count, A.O.A.C., 1995) (Appendix B).

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Investigation of tangerine juices properties and formulation of must

The physical and chemical properties of 100% UHT Sai Num Phueng tangerine juices are displayed in table 4.1. L*, a* and b* values of juice color determined by Chromameter were 30.60, +8.95 and +49.32, respectively. Color of the juice was also observed by using Munsell's system. Hue was 7.5 and Yellow-Red shade was at 7/16. The tangerine juice contained 13.93% reducing sugar which total soluble solid was 14 degree Brix. The titratable acidity calculated as citric acid which was the main acid in tangerine juice, was 0.51%. pH of the juice was 3.41, Nitrogen and ash content were 0.02% and 0.27%, respectively.

Table 4.1 The physical and chemical properties of tangerine juices

physical and chemical properties	experimental result
color (CIELAB system)	L*= 30.60±0.05, a*= 8.95±0.05, b*= 49.32±0.09
color (Munsell's system)	Hue 7.5, YR at 7/16
nitrogen(%w/v)	0.02±0.00
reducing sugar(%w/v)	13.93±0.18
titratable acidity(%w/v)	0.51±0.00
ash(%w/v)	0.27±0.03
pH value	3.41±0.01
TSS (°Brix)	14.00±0.00

As the result of the juice composition, the content of nitrogen and carbon source would be adjusted in order to use as a must for yeast fermentation. After justification, the must contained 0.05% nitrogen and 25% reducing sugar. The pH value of the juice was also adjusted to 3.5 to follow the basic condition of must for wine fermentation referred from Jackson (2000).

4.2 Investigation of fermentation profile and selection of the basic wine

Based on physical and chemical properties of tangerine juice as investigated in section 4.1, 9 batches of tangerine musts (table 3.1) were prepared and adjusted the carbon and nitrogen source contents including the pH value. Adjusted musts of the 9 batches contained 0.05% nitrogen, 25% reducing sugar and pH of the must was 3.5.

The influences of must concentration and yeast species on the fermentation profile of tangerine juices were investigated. The experiment was conducted in a 3x3 factorial design as shown in table 3.1. The fermentation profiles were monitored everyday by determination of alcohol production rate, sugar consumption rate, yeast population number and titratable acidity changes, and color of fermented juice. The results are shown and discussed in the following section.

4.2.1 The fermentation profile determination

The alcoholic fermentation of tangerine juice in this study was conducted using two *Saccharomyces* species. The fermentation profiles of these yeasts in tangerine juice under different conditions are shown and discussed in the following figures 4.1 to 4.6.

4.2.1.1 Influence of must concentration on yeast fermentation profile

The influence of must concentration on the fermentation profiles of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* and the mixed culture were investigated in term of alcohol production rate, sugar consumption rate and the changes of yeast population number. The results are shown in figure 4.1, 4.2 and 4.3.

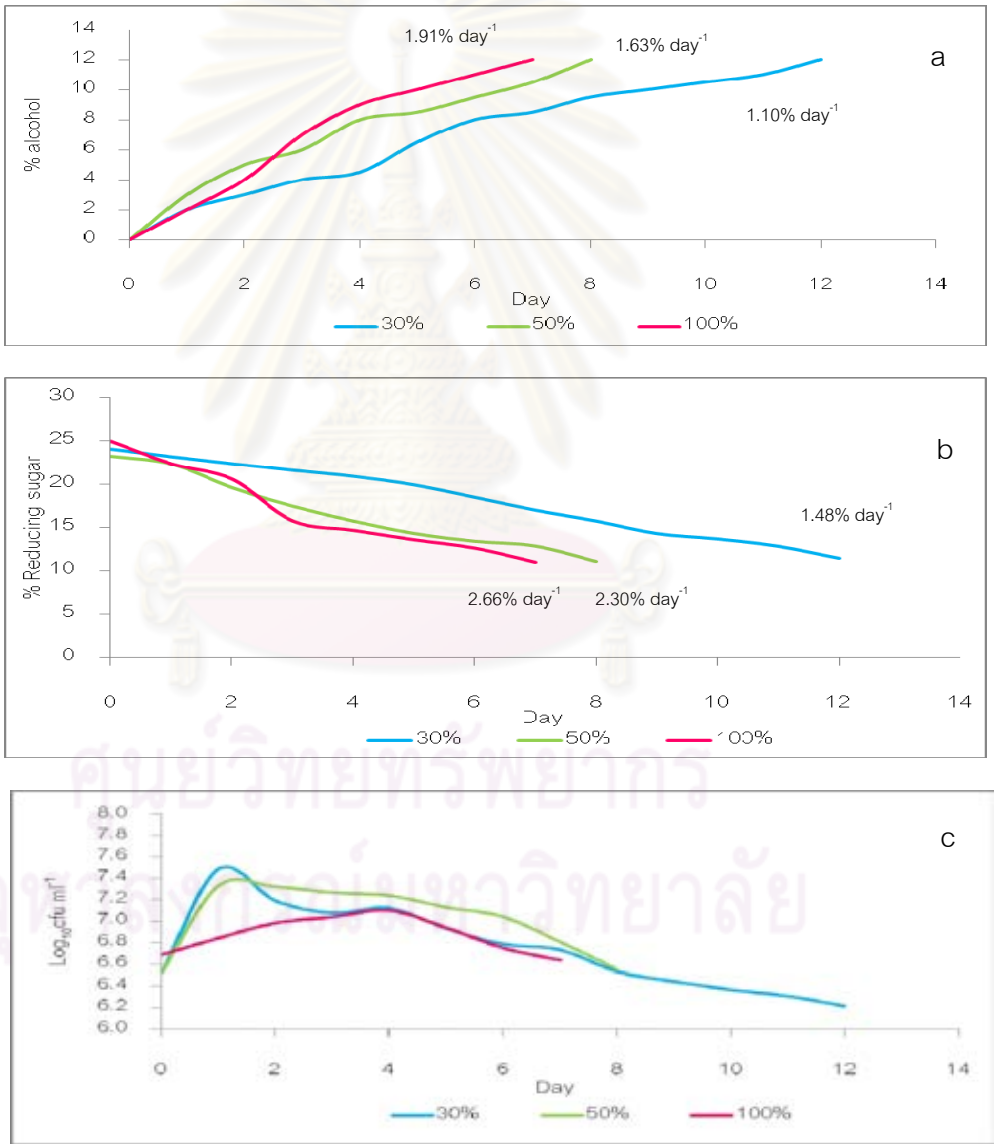


Figure 4.1 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of 30%, 50%, and 100% musts by *S. cerevisiae*

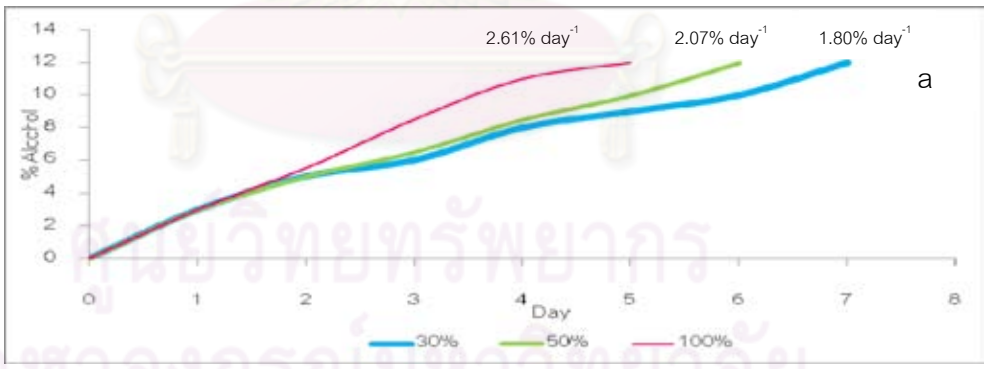
Figure 4.1a, 4.1b and 4.1c show the changes of alcohol, sugar contents and yeast population number during the fermentation of *S. cerevisiae* in 30%, 50%, and 100% musts. The sugar content in 100% must was constantly decreased at rate 2.66% day^{-1} during alcoholic fermentation of this yeast. Alcohol production was also constantly increased at rate 1.91% day^{-1} allowing alcohol to reach 12% within 7 days. The sugar consumption rate of the yeast *S. cerevisiae* in 50% must was 2.30% day^{-1} and the alcohol production rate was 1.63% day^{-1}. Therefore, alcohol reached 12% within 8 days. Whereas in 30% must, the yeast consumed sugar 1.48% day^{-1} and produced alcohol at rate 1.10% day^{-1} resulting in lately reached 12% alcohol for 12 days.

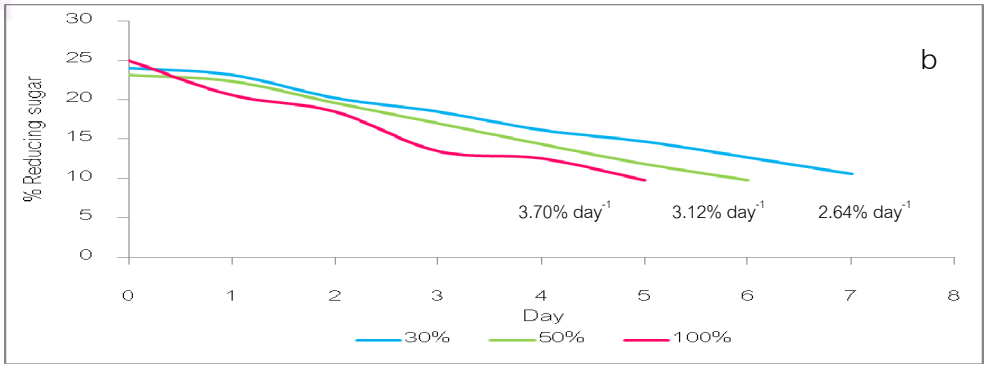
The number of yeast populations during the fermentation of 30% and 50% must increased from approximately 6 log cfu ml^{-1} to 7.3 and 7.5 log cfu ml^{-1} within 1 day then gradually decreased throughout the fermentation. Whereas the yeast population number in 100% must increased from initial around 6 log cfu ml^{-1} to 7 log cfu ml^{-1} within 2 days then slightly decreased to 6 log cfu ml^{-1} through the last day of fermentation.

The increase of yeast population number in 100% must concentration during the fermentation was slower than the other must concentrations while it could perform more rapid alcohol production rate. Since at the early stage of fermentation, in 100% must, yeast could shift to anaerobic pathway faster than the other batches. It might be explained that in the 100% must, large amount of nutrients were dissolved, which obstructed to the dissolving of oxygen. Therefore, the lack of oxygen would retard the growth and division of yeast cell and drive the cell to early perform alcoholic fermentation (Ribéreau-Gayon *et al.,* 2006). Consequently, the alcohol production rate of the yeast in this condition was higher than others. During the fermentation process, yeast population number would be generally dropped due to nutrient limitation and

toxicity of alcohol produced as present in these tangerine juice fermentation (Fleet, 1999).

The fermentation profiles of *S. bayanus* shown in figure 4.2 were similar to the fermentation profile of *S. cerevisiae* in the previous study. The alcohol production (fig. 4.2a) and sugar consumption rates (fig. 4.2b) of *S. bayanus* in 100% must concentration were also higher than others. The sugar consumption rate was 3.70% day^{-1} and alcohol production rate was 2.61% day^{-1} allowing alcohol to reach 12% within 5 days. The alcohol production and sugar consumption rates of *S. bayanus* in 50% must were lower than 100% must, which the sugar was consumed at rate 3.12% day^{-1} and the alcohol was produced at rate 2.07% day^{-1}. However, it was still higher than 30% must, which the sugar consumption rate was 2.64% day^{-1} and alcohol production rate was approximately 1.80% day^{-1}. The alcohol content in the batches of 50% and 30% reached 12% within 6 days and 7 days, respectively.





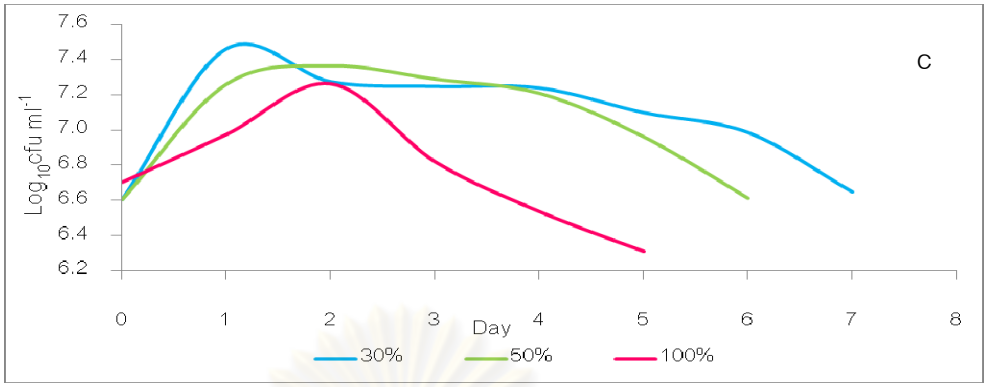


Figure 4.2 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of 30%, 50%, and 100% musts by *S. bayanus*

The changes of *S. bayanus* population (fig. 4.2c) was also similar to *S. cerevisiae* (fig. 4.1c), which demonstrated that although at the early stage of fermentation, the growth of *S. bayanus* in 100% must was slower than others, the yeast in this batch could still consume sugar and produce alcohol faster than 50% and 30% must. The pattern of yeast population change in each must concentration similar to the *S. cerevisiae* were influenced by limit of oxygen content in the must as just those described in the last section (Ribéreau-Gayon *et al.,* 2006).

The figure 4.3 shows the fermentation profiles of mixed culture in 30%, 50%, and 100% must. The results showed that the alcohol production (fig. 4.3a) and sugar consumption (fig. 4.3b) rates of mixed culture in 50% must were higher than 100% and 30% must. The sugar consumption rate of yeast in 50% must was 3.24% day^{-1} and alcohol production rate was 2.07% day^{-1} leading the alcohol to reach 12% within 6 days. The sugar consumption rate in 100% must was 2.81% day^{-1} and the alcohol production rate was 1.90% day^{-1} resulting in reached 12% alcohol within 7 days. While in 30% must, the sugar consumption rate was 2.38% day^{-1} and the alcohol production rate was just 1.67% day^{-1}. Therefore, the alcohol in this batch reached 12% within 8 days.

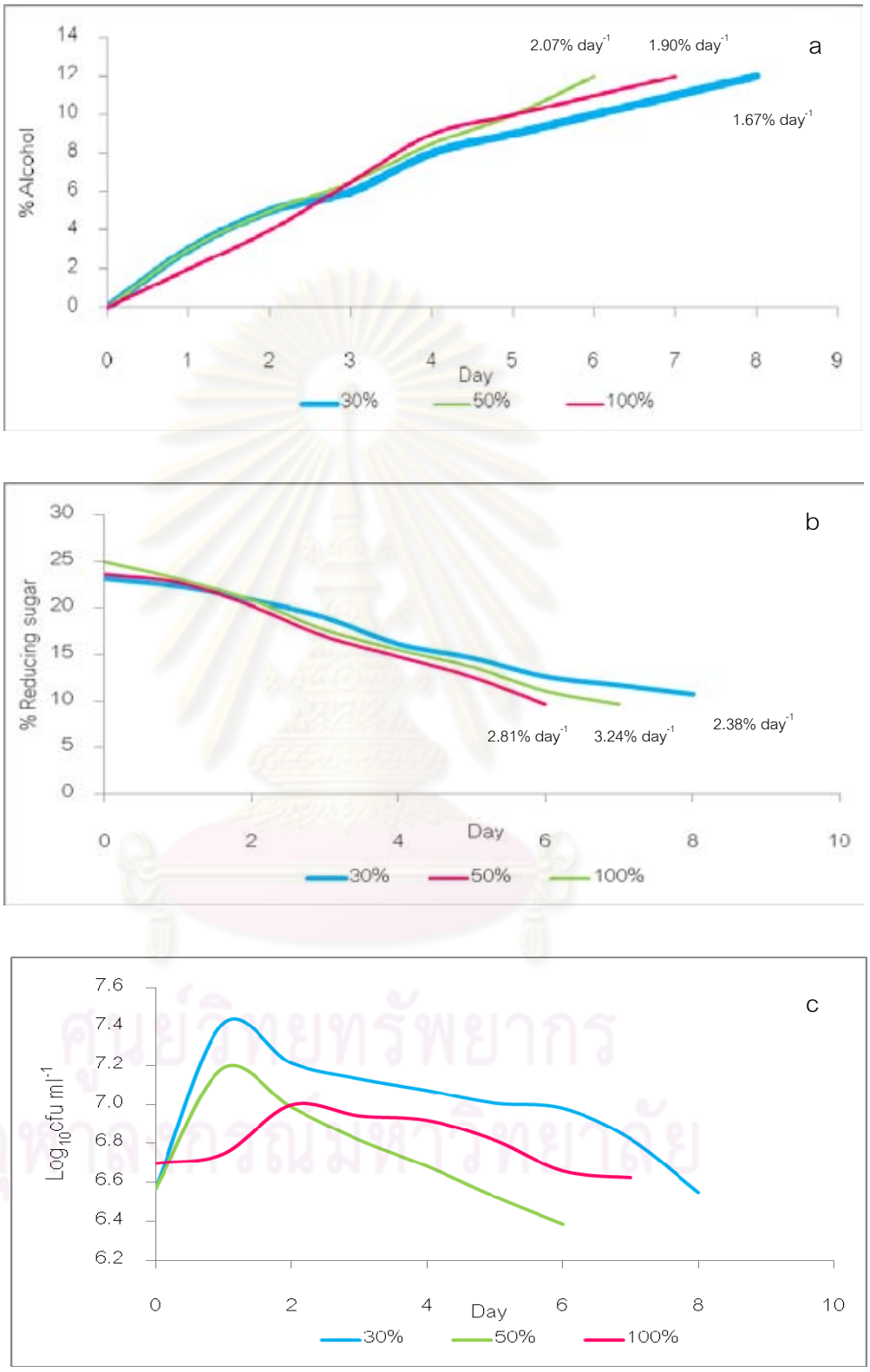


Figure 4.3 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of 30%, 50%, and 100% musts by mixed culture

From figure 4.3c, the change of yeast population of mixed cultures batches was also similar to both single cultures. The increasing of yeast population during the initial stage of the fermentation was still depended upon the concentration of the must.

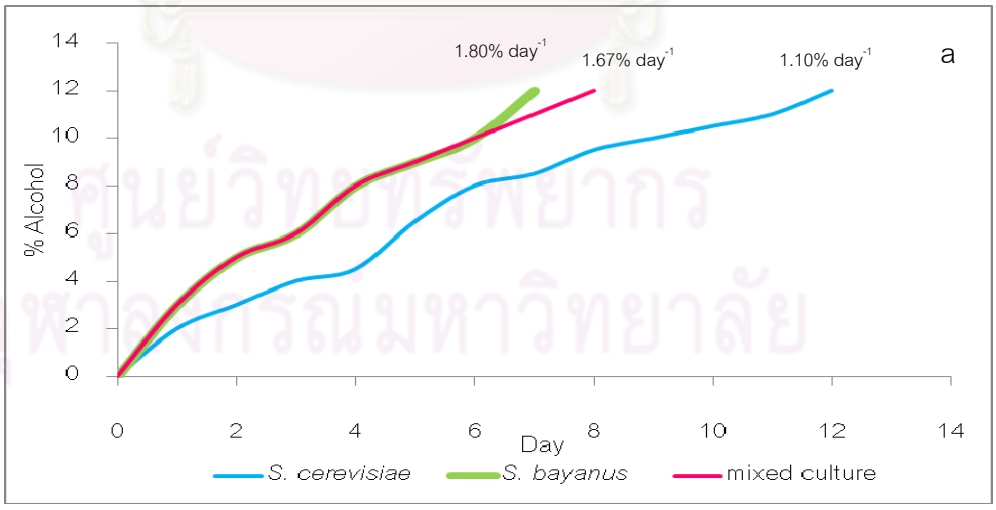
As the result of fermentation of yeasts in different must concentrations, it indicated that the must concentration influenced yeast fermentation profiles. Although all concentrations contained similar concentration of carbon and nitrogen sources, the growth factor and co-factor such as amino acids, vitamins, and minerals, available in each must were different. These factors significantly associate to the fermentation mechanism of the yeasts (Jackson, 2000). Tangerine juice contains the essential amino acids such as tryptophan, lysine, methionine, glutamic acid, glycine, histidine, and others which important to the yeast fermentation. These essential amino acids also significantly contribute the aroma composition in wine (Hernandes-Orte *et al.,* 2005). Regarding to minerals in tangerine juice, in particular calcium, magnesium, and zinc, they are necessary for yeast metabolism since the availability of the magnesium (Mg^{2+}) ion can make membrane stability and permeability of yeast cell to resist the heat shock and ethanol toxicity (Delfini and Formica, 2001). Beside the significance of amino acids and minerals described above, several vitamins in tangerine juice such as thiamin (vitamin B_1), riboflavin (vitamin B_2), and niacin can also promote an efficiency of yeast fermentation (USDA, 2008). Therefore, using of 100% tangerine juice as must, the co-factor and growth factor were not diluted and still sufficiently existed in the juice to promote the fermentation mechanism of yeast as described above. Furthermore, metabolize vitamins could also fulfill the biosynthesis of isoleucin and valine. Consequently, it can reinitiate the stuck of fermentation and increase the volatile acidity in wine (Eglington *et al.,* 1993). Adequate thiamins also reduce the carbonyl compounds synthesis which affect fermentation limits in the production of

pyruvate, acetaldehyde and acetic acid (Jackson, 2000). Therefore the availability of these nutrients as the growth factor and co-factor in the 100% tangerine juice could support the yeast to perform an efficient fermentation.

As the result of this study, it indicated that the different must concentration influenced an efficiency of the yeast fermentation. The basic condition of the must such as nitrogen and carbon source contents, and pH adjusted for yeast fermentation was not enough for use as the main criteria for the must preparation. The concentration of the juice used as the must should be also considered.

4.2.1.2 Influence of culture type on fermentation profile

Influence of culture types on the tangerine juice fermentation was studied. The 3 types of culture fermentation profile in 30%, 50% and 100% musts are shown in figure 4.4, 4.5 and 4.6.



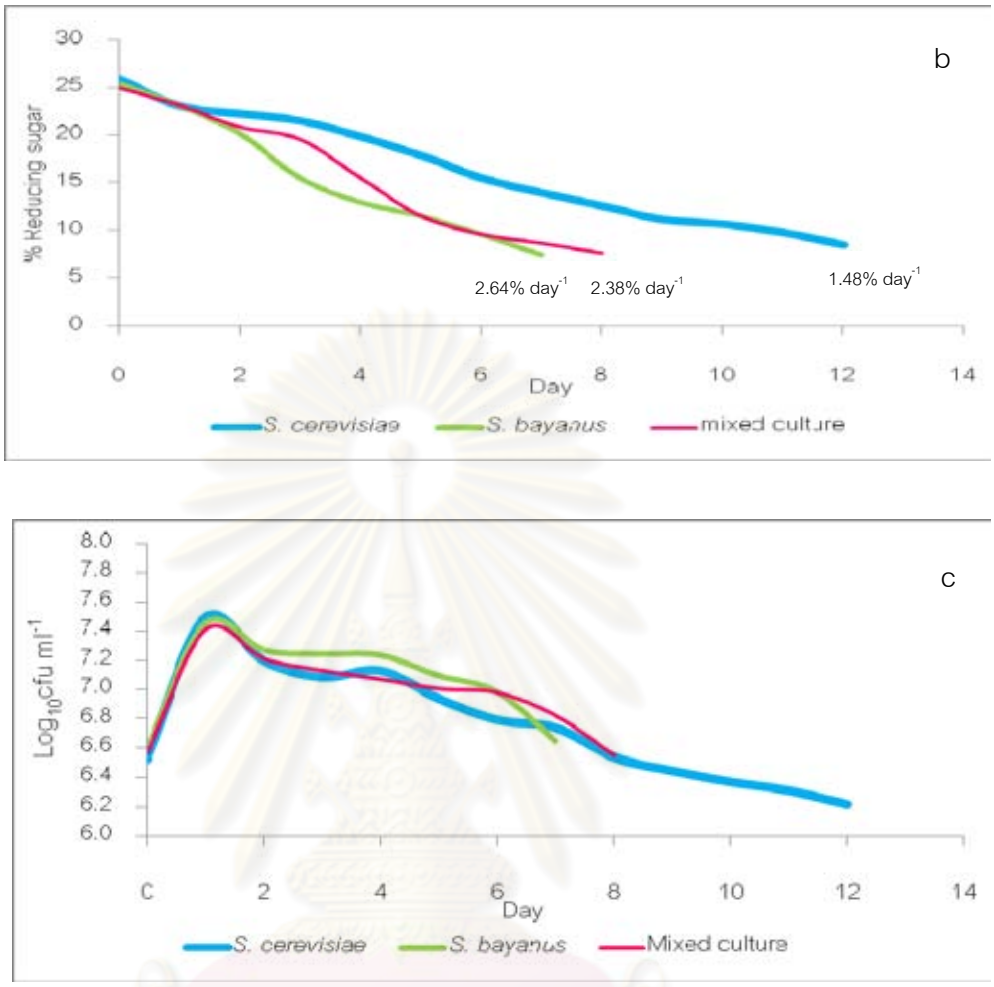


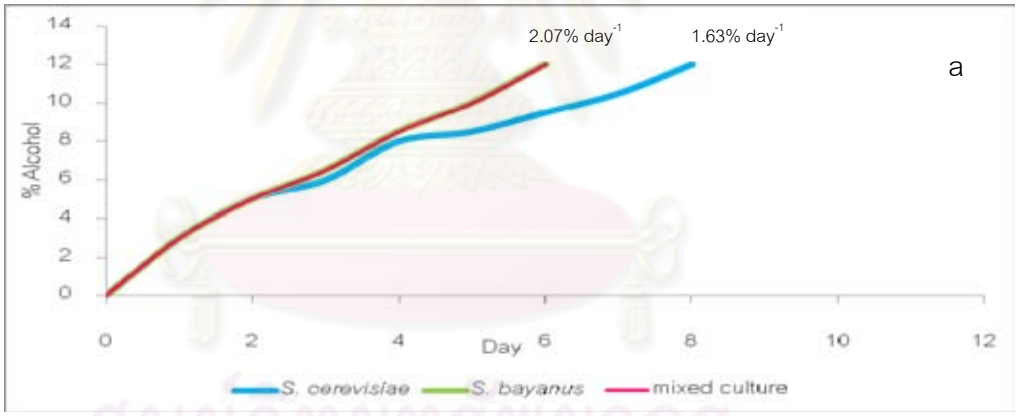
Figure 4.4 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of *S. cerevisiae*, *S. bayanus* and mixed culture in 30% must

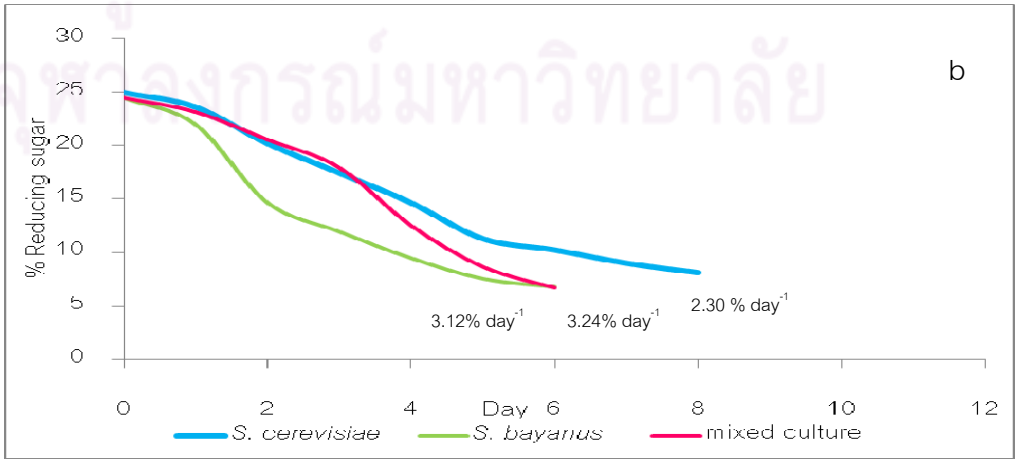
From figure 4.4a and 4.4b, the alcohol production rate of three types of culture in 30% must were different. The sugar in batch of *S. bayanus* decreased at rate 2.64% day^{-1} and the alcohol content rapidly increased at rate 1.80% day^{-1}.Consequently, the fermentation of this batch completed 12% alcohol within 7 days which was faster than the batches of *S. cerevisiae* and mixed culture. The sugar were decreased by mixed culture and *S. cerevisiae* at rate 2.38% day^{-1} and 1.48% day^{-1}, respectively and the alcohol were produced

at the rate 1.67% day^{-1} and 1.10% day^{-1} allowing alcohol to reach 12% within 8 days and 12 days, respectively.

From figure 4.4c, the pattern of yeast population changes of three culture types in 30% must were similar. The number of yeast population of all batches increased from initial 6 log cfu ml^{-1} to approximately 7 log cfu ml^{-1} within day 1 then gradually decreased throughout the fermentation.

Figure 4.5a, 4.5b and 4.5c show the fermentation profiles of three types of culture in 50% must. The sugar were consumed by *S. bayanus* and mixed culture at rate 3.24% day^{-1} and 3.12% day^{-1}, respectively, whereas alcohol content was rapidly produced at similar rate 2.07% day^{-1}.





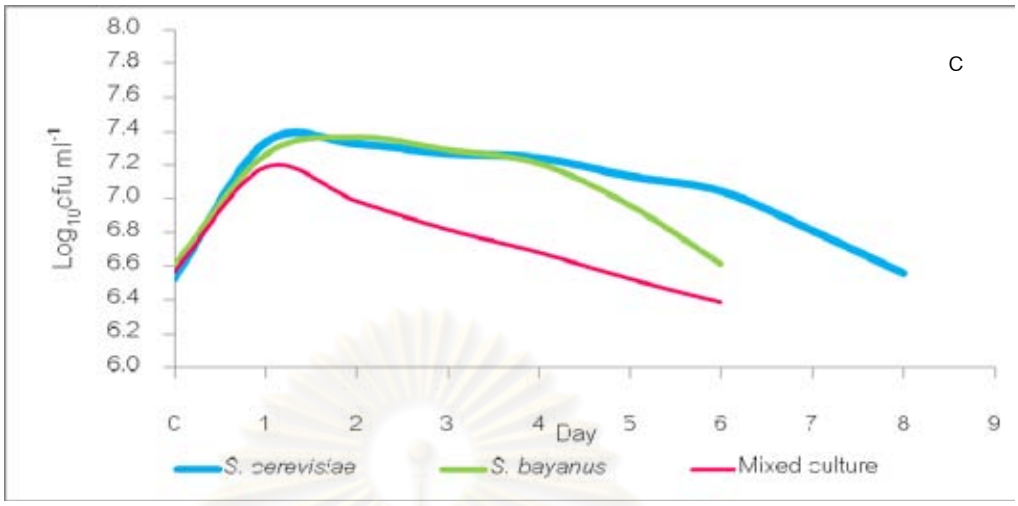
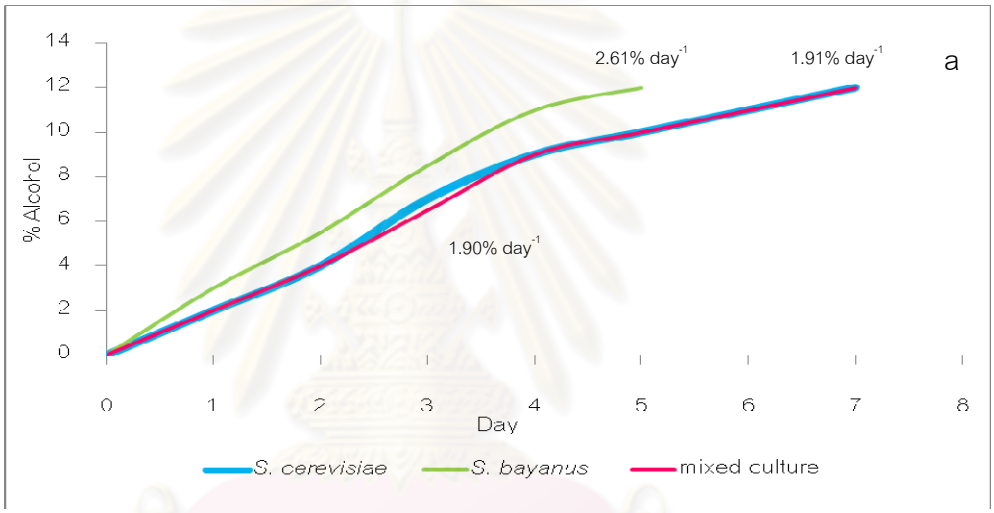


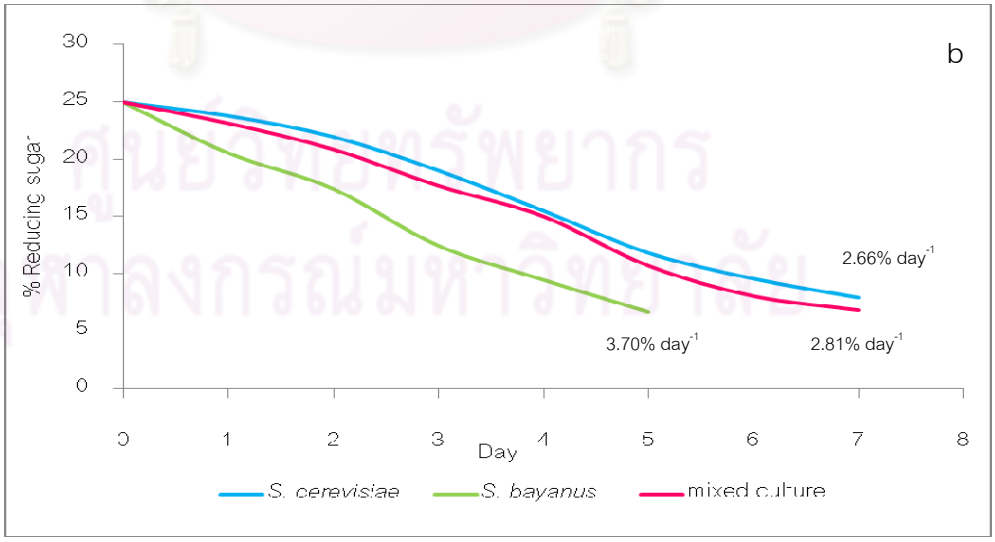
Figure 4.5 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of *S. cerevisiae*, *S. bayanus* and mixed culture in 50% must

Since the alcohol production rates of both batches were similar, alcohol reached 12% within the same day (day 6). While the sugar in *S. cerevisiae* batch was decreased at rate 2.30% day^{-1} and alcohol was produced at rate 1.63% day^{-1}. The alcohol reached 12% within 8 days which was much slower than the batches of *S. bayanus* and mixed culture.

The increasing of *S. cerevisiae* population number in 50% must during the initial stage of fermentation was similar to *S. bayanus* and mixed culture (fig. 4.5c). However, after day 1 of the fermentation, the decreasing of yeast population number of mixed culture was more rapid than both single culture batches. The population number of *S. cerevisiae* was larger than the other culture types throughout the fermentation time whereas this yeast could produce alcohol at the slowest rate since it could better utilize the sugar to support its growth (Delfini and Formica, 2001). Consequently, *S. cerevisiae* could begin the slower alcoholic fermentation slower than other culture types resulting in performing the lowest alcohol production rate.

From result shown in figure 4.6a and 4.6b, in 100% must, *S. bayanus* performed the fastest fermentation rate. It consumed 3.70% sugar and rapidly generated alcohol at rate 2.61% day^{-1}. Consequently, the fermentation completed to 12% alcohol within just 5 days. Whereas the alcohol production rate of mixed culture and *S. cerevisiae* batches were much slower (1.90% day^{-1}) which the sugar were consumed at rate 2.81% day^{-1} and 2.66% day^{-1}, respectively. Both batches similarly reached alcohol 12% within 7 days.





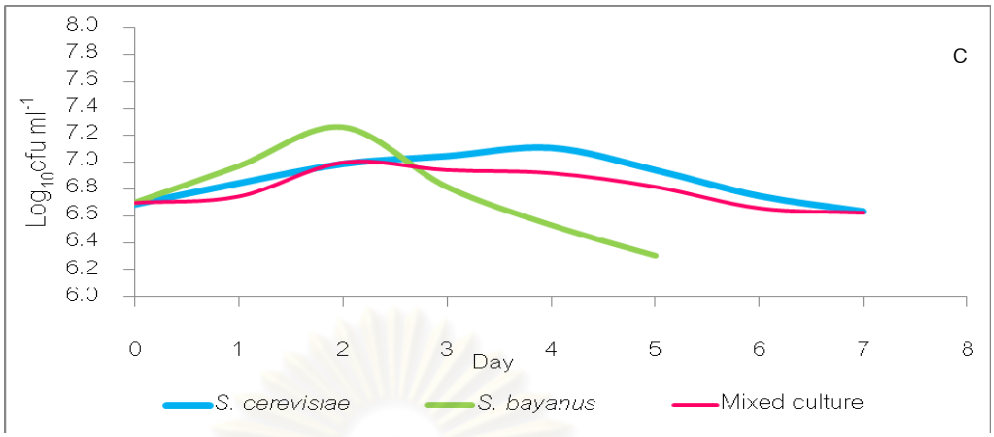


Figure 4.6 The changes of alcohol (a), sugar contents (b) and yeast population number (c) during fermentation of *S. cerevisiae*, *S. bayanus* and mixed culture in 100% must

Although the increasing of population number of *S. bayanus* in 100% must at the initial stage of the fermentation was significantly larger than *S. cerevisiae* and mixed culture, it still performed the highest alcohol production rate (fig. 4.6a and 4.6c). This was inconsistent with the previous investigation. However, it could be explained that *S. bayanus* might prefer some vitamins and citric acid mainly available in tangerine juice which could support the yeast to grow along with driving alcoholic fermentation. On the other hand, citric acid which is main acid available in many types of fruit including tangerine juice, could also retard *S. cerevisiae* during the fermentation. Therefore, it is possible that this yeast could be also a species specific for 100% tangerine juice fermentation since it could perform an efficient fermentation which indicated by performing efficient sugar consumption and rapid alcohol generation.

From these results studies, the data obtained were also summarized and shown in table 4.2 to illustrate the fermentation efficiency of each fermentation condition.

Data as shown in table 4.2 indicated that yeast species and culture types performed different fermentation profiles in different must concentrations. *S. bayanus* performed the fastest fermentation rate in every must. In 100% must *S. bayanus* showed the highest alcohol production rate and sugar consumption rate which were 2.61±0.00 % day^{-1} and 3.70±0.13 % day^{-1}, respectively. Therefore, the proper condition based on the fermentation rate for tangerine juice fermentation could be the condition of using 100% tangerine juice as must and fermented by *S. bayanus*. However, *S. bayanus* could convert 1.52 g of sugar to 1% alcohol in 1 L of wine which was relatively too high when compared to grape wine fermentation. Generally, in the grape wine fermentation 1.70 g of sugar was used to convert to 1% alcohol in 1 L of wine (Jackson, 2000). This indicated that *S. bayanus* might utilize sugar during fermentation to mainly produce alcohol. Therefore desirable secondary metabolite such as volatile compound determining flavor quality might be insufficiently produced.

Therefore, apart from an efficient fermentation of the yeasts, the other characteristic of tangerine wine associated with wine quality such as acid generated and color stability during fermentation including flavor acceptability by consumer should be further investigated.

Table 4.2 Summerized data of yeast fermentation profiles of each condition

must concentration	alcohol production rate (%alcohol day^{-1})			sugar consumption rate (%sugar day^{-1})			%sugar conversion^{NS} (%sugar %alcohol $^{-1}$)		
	A	B	C	A	B	C	A	B	C
30%	1.10^{f}±0.04	1.80^{d}±0.00	1.67^{e}±0.00	1.48^{e}±0.06	2.64^{c}±0.04	2.38^{d}±0.08	1.46 ±0.08	1.50 ±0.01	1.44±0.07
50%	1.63^{e}±0.06	2.07^{b}±0.06	2.07^{b}±0.06	2.30^{d}±0.16	3.12^{b}±0.08	3.24^{b}±0.04	1.41±0.07	1.48 ±0.00	1.49 ±0.02
100%	1.91^{c}±0.00	2.61^{a}±0.00	1.90^{c}±0.00	2.66^{c}±0.00	3.70^{a}±0.13	2.81^{c}±0.06	1.42±0.04	1.52 ±0.05	1.51±0.07

$^{a,b,c,....}$ value with significantly difference in each rate are indicated by different letters ($p \leq 0.05$)

^{NS} value with non significantly difference in each rate are indicated ($p > 0.05$)

A, B and C: culture types; *S. cerevisiae, S. bayanus* and mixed culture, respectively

4.2.2 The significant character of fermented juice influenced from fermentation condition

4.2.2.1 The change of titratable acidity

The changes of titratable acidity in fermented tangerine juice were determined. The titratable acidity calculated as citric acid, which was main acid in tangerine juice, in 9 tangerine musts and fermented juices was shown in figure 4.7.

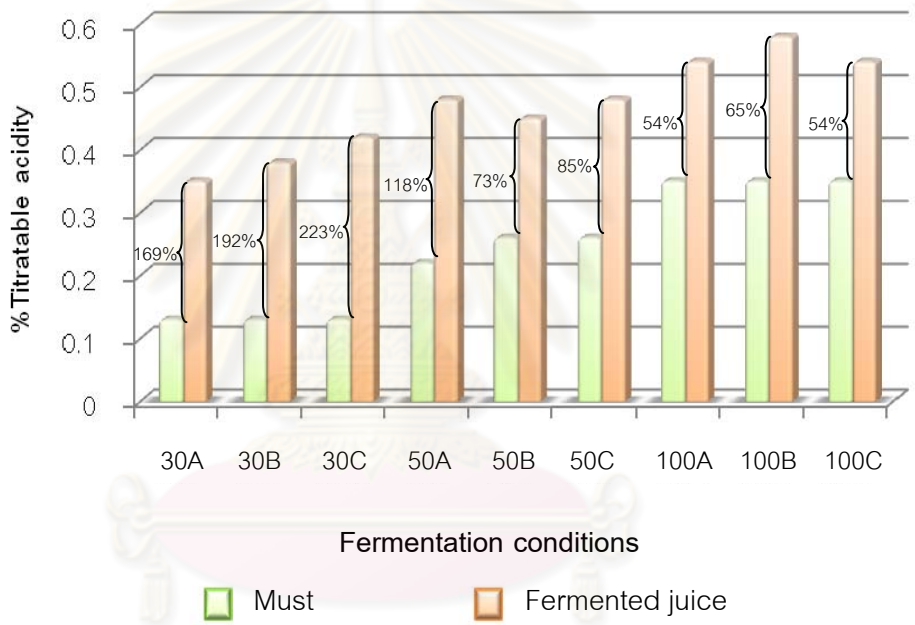


Figure 4.7 The titratable acidity as citric acid in all fermentation conditions.
A, B and C: culture types; *S. cerevisiae, S. bayanus* and mixed culture, respectively.
30, 50 and 100: must concentrations; 30% juice, 50% juice and 100% juice, respectively.

In figure 4.7, the titratable acidity in fermented juices from all conditions were significantly increased. The titratable acidity of the lowest must concentration (30% juice) was increased approximately 169%, 192% and 223% when fermented by *S. cerevisiae*, *S. bayanus* and mixed culture,

respectively. Whereas the increasing of titratable acidity of higher must concentration batches were totally lower. In 50% must fermented by *S. bayanus*, mixed culture and *S. cerevisiae,* the titratable acidity were increased approximately 73%, 85% and 118% and in 100% must fermented by these yeasts were increased approximately 54%, 54% and 65%, respectively. This showed that the citric acid would be decreasingly generated if the fermentation rate increased. These results also indicated that citric acid would be excessively generated if the fermentation was processed under improper condition, such as in lower must concentration which was co-factor and growth factor was not sufficient for supporting yeast to perform rapid fermentation. This was consistent to the report of Ribéreau-Gayon *et al.* (2006) demonstrating that citric acid was a general secondary metabolite of the yeast generated during alcoholic fermentation. If the condition was not suitable for alcohol production the yeast would generate excess secondary metabolite instead. Therefore, the fermentation batches using 100% must as substrate could be a proper condition for yeast fermentation in term of acid generation.

However, citric acid was not only main organic acid generated during fermentation. Other acids such as phosphoric acid and organic acids included malic acid, succinic acid, acetic acid, fumaric acid, glutamic acid, tartaric acid, and carboxylic acid could be significantly form during alcoholic fermentation (Ribéreau-Gayon *et al*., 2006). In addition, it has been reported that during orange wine fermentation, five acids were generated, which were hexanoic, octanoic, dodecanoic, 9-octadecenoic, and hexadecanoic acids. Hexanoic acid and 4-hexanoic acid were found as the volatile fatty acids in blood orange wine making (Selli *et al.,* 2003 and Selli*,* 2007). Therefore, to investigate the significant acid generated in fermented tangerine juice, the advance analytical method such as high performance liquid chromatography (HPLC) should be used to characterize the acid profile of the fermented tangerine juice.

4.2.2.2 Color of fermented juice

The color of must and fermented tangerine juice shown in table 4.3. The a* and b* value of all fermented juices were significantly different from the must color. L* value of all batches were significantly increased whereas a* value and b* value were significantly decreased. This might be the breakdown of carotenoid pigment during fermentation reaction. Consequently, leading to the loss of the pigment absorption which allowed L* value to increase. The carotenoids could be degraded by the enzymatic cleavage called CCD (carotenoid cleavage dioxygenase) to form volatile compounds named norisoprenoids as the aromatic compound in wine (Oliviera *et al.,* 2006; Ferreira *et al.,* 2008).

In addition, another pathway of carotenoid breakdown in tangerine juice, which was not an effect of the fermentation, could be also caused from carotenoid oxidation. This could decompose beta-carotene in tangerine juice due to an oxidation reaction stimulated by metal ion and in particular sulfite ion derived from potassium metabisulphite (KMS) which was generally added for decontamination of the must (Fennema, 1996).

Fermented juices of all batches in this study were then subjected to clarification to be finish wine following the method described in section 3.2.2.4. The characteristics of tangerine wine are listed in table 4.4.

From the table 4.4, the characteristic of all 12% alcohol finish tangerine wine contained different sugar content, acidity and color. These were influenced from the fermentation conditions. The sugar content and total acidity of all fermented tangerine juices were range in 3.40%-5.80% and 0.19%-0.32%, respectively.

Table 4.3 The color of must and fermented tangerine juices

fermentation condition	L* value		a* value		b* value	
	must	fermented juice	must	fermented juice	must	fermented juice
30% must						
• *S. cerevisiae*	69.56[b] ±0.11	70.66[a] ±0.15	2.92[a] ±0.08	1.64[b] ±0.06	37.43[a] ±0.05	30.10[b]±0.07
• *S. bayanus*	66.49[b] ±0.05	68.92[a] ±0.05	3.31[a] ±0.02	1.89[b] ±0.01	37.64[a] ±0.04	29.37[b]±0.06
• Mixed culture	68.13[b] ±0.17	70.05[a] ±0.05	3.13[a] ±0.02	2.13[b] ±0.10	37.29[a] ±0.14	32.19[b]±0.06
50% must						
• *S. cerevisiae*	57.62[b] ±0.10	60.44[a] ±0.06	5.80[a] ±0.06	2.04[b] ±0.05	46.62[a] ±0.04	44.75[b]±0.10
• *S. bayanus*	57.45[b] ±0.13	65.44[a] ±0.33	5.80[a] ±0.03	2.80[b] ±0.17	46.61[a] ±0.12	43.10[b]±0.07
• Mixed culture	58.36[b] ±0.77	68.14[a] ±0.47	6.16[a] ±0.08	2.88[b] ±0.02	46.86[a] ±0.09	40.65[b]±0.28
100% must						
• *S. cerevisiae*	31.55[b] ±0.05	36.62[a] ±0.20	7.19[a] ±0.09	3.57[b] ±0.16	48.34[a] ±0.06	46.12[b]±0.10
• *S. bayanus*	33.51[b] ±0.04	38.79[a] ±0.23	7.46[a] ±0.23	3.75[b] ±0.18	49.66[a] ±0.06	48.64[b]±0.45
• Mixed culture	31.19[b] ±0.18	35.34[a] ±0.25	7.60[a] ±0.21	3.60[b] ±0.06	50.25[a] ±0.02	48.30[b]±0.09

[a,b] value with significantly difference in each fermentation conditions (comparing between must and fermented juice) are indicated by different letters. ($p \leq 0.05$)

Table 4.4 The characteristics of finish tangerine wine

fermentation condition		finish tangerine wine characteristic					
					Color		
juice concentration	culture types	alcohol	% reducing sugar	% titratable acidity^{NS}	L* value	a* value	b* value
30% must	*S. cerevisiae*	12%	5.78±0.16^{a}	0.19±0.05	82.62±0.13^{a}	-3.57±0.10^{e}	36.12±0.06^{c}
	S. bayanus		4.64±0.19^{c}	0.19±0.07	82.79±0.05^{a}	-3.75±0.02^{d}	38.64±0.09^{c}
	Mixed culture		4.28±0.24^{d}	0.19±0.11	82.34±0.11^{a}	-3.60±0.17^{e}	38.30±0.12^{c}
50% must	*S. cerevisiae*	12%	4.52±0.07^{cd}	0.26±0.09	67.44±0.24^{b}	0.04 ±0.02^{c}	48.75±0.05^{ab}
	S. bayanus		3.87±0.12^{e}	0.26±0.00	67.44±0.06^{b}	0.80 ±0.02^{c}	48.10±0.19^{b}
	Mixed culture		3.54±0.16^{e}	0.26±0.13	67.14±0.17^{b}	0.88 ±0.06^{c}	48.65±0.18^{b}
100% must	*S. cerevisiae*	12%	5.12±0.18^{b}	0.32±0.07	30.66±0.19^{d}	8.64 ±0.06^{b}	49.10±0.04^{a}
	S. bayanus		3.55±0.15^{e}	0.32±0.05	30.92±0.46^{cd}	8.89 ±0.12^{a}	49.37±0.16^{a}
	Mixed culture		3.47±0.22^{e}	0.32±0.00	30.05±0.11^{c}	9.13 ±0.08^{a}	49.19±0.17^{a}

$^{a,b,c,...}$ value with significantly difference in each column are indicated by different letters. ($p \leq 0.05$)

^{NS} value with non significantly difference in each rate are indicated ($p>0.05$)

4.2.3 Selection of the basic wine making condition

Although the fermentation efficiency was an important factor for wine making, to select a proper fermentation condition, flavor and odor generated were also considered as one of the key criteria for making a basic wine in further study. Therefore, flavor and odor of all tangerine wine batches were preliminarily tested by five wine researchers. The main criteria for basic wine selection are listed in table 4.5.

Table 4.5 Main criteria considered for basic wine selection

fermentation condition		fermentation efficiency		preliminary sensory evaluation		
juice concentration	culture types	sugar conversion[NS]	fermentation time (day)	color	flavor test	methanol test
30% must	*S. cerevisiae*	1.46±0.08	12	accept	reject	N/A
	S. bayanus	1.50±0.01	7	accept	accept	<50mg L^{-1}
	Mixed culture	1.44±0.07	8	accept	reject	N/A
50% must	*S. cerevisiae*	1.41±0.07	8	accept	reject	N/A
	S. bayanus	1.48±0.00	6	accept	accept	<50mg L^{-1}
	Mixed culture	1.49±0.02	6	accept	accept	<50mg L^{-1}
100% must	*S. cerevisiae*	1.42±0.04	7	accept	accept	<50mg L^{-1}
	S. bayanus	1.52±0.05	5	accept	accept	<50mg L^{-1}
	Mixed culture	1.51±0.07	7	accept	reject	N/A

[NS] value with non significantly difference in each rate are indicated ($p>0.05$)

N/A: Not applicable

From table 4.5, five wines from nine fermented conditions were accepted from 5 researchers based on their color and flavor. These five wines were then determined the methanol content. The methanol content in all 5 wines was less than 50 mg L^{-1} which standardized by the regulation of alcoholic beverage (Thai

Industrial Standards Institute, 2003). According to table 4.5, based on the fermentation profile, *S. bayanus* in 100% must concentration performed the highest fermentation rate (2.61% day^{-1}) and could convert 1.52 g sugar to 1% alcohol, therefore it could produce alcohol 12% within 5 days. Citric acid was not excessively generated during fermentation in relative to the other batches (figure 4.10). The acceptation of color and flavor were also positive. Methanol content was also less than 50 mg L^{-1} which was below a lower limit of wine regulation.

Therefore, the 100% juice fermented by *S. bayanus* was selected as the condition for basic wine making in the further study, based on criteria of performing efficient fermentation. In addition, the wine obtained was also accepted in term of flavor and color and methanol generated in wine also lower than 50 mg L^{-1}.

However, the %sugar conversion of this condition was relatively high when compared to the %sugar conversion in grape wine (1.7%) as mentioned in section 4.2.1.2. Therefore, too large amount of sugar (3.55%, table 4.4) remained in this finish tangerine wine when compared to the grape wine. Normally, the residual sugar in grape wine was extremely dry (<1%). This large amount of sugar remaining could be an obstacle to tangerine wine formulation in the further study. Hence, the evaluation of the initial sugar for basic wine making was investigated in the following section to produce basic wine containing small amount of sugar.

4.2.4 Evaluation of the initial sugar in must for basic wine making by *S. bayanus*

This part aimed to evaluate a proper initial sugar content in 100% must used for making wine to obtain the finish wine containing small amount of residual sugar. The experiment in this part was conducted by varying the initial sugar in must for four levels (16%, 19%, 22%, and 25% sugar) which were the optimum sugar concentration recommend for wine making (Ribéreau-Gayon *et al.,* 2006). The

fermentation profiles of all four fermentation conditions were monitored everyday. The fermentation profile of four conditions were investigated by determination of alcohol production and sugar consumption rate, the yeast population number. Titratable acidity, and the color of fermented juice, %residual sugar, alcohol content, acidity and flavor acceptation were also investigated. The results are shown in table 4.6.

Table 4.6, reported the four fermentation conditions of *S. bayanus* that conducted different fermentation efficiency. The *S. bayanus* in each fermentation condition could generate different alcohol content which was an influence of osmolality level of must depending upon the sugar content added. The fermentation of 16% initial sugar rapidly completed the fermentation process within 3 days whereas the other conditions completed the fermentation process within 5 days. In 16% initial sugar batch, the yeast performed 1.77% of sugar conversion and maximally generated 8% alcohol, allowing sugar remained in finish wine 1.16%. The flavor of this wine was not accepted. Although the other batches complete the fermentation within a similar time (5 days), the alcohol produced and sugar remained in wine were different. In 19%, 22% and 25% initial sugar batches, the yeast converted sugar 1.64,1.75 and 1.57 g to 1% alcohol in 1 L and maximally generated 10%, 11% and 12% alcohol, allowing sugar to remained in finish wine 1.41%, 1.93% and 3.86%, respectively. The flavor of 19% initial sugar batch was not accepted whereas the other two batches were accepted. Acidity in wine of all batches were not significantly different ($p>0.05$). The 22% and 25% initial sugar batches were similarly generated 0.38% acidity, while the 16% and 19% initial sugar that batches similarly generated 0.32% acidity, respectively.

Table 4.6 Fermentation efficiency and wine characteristic from must contained different initial sugar concentrations fermented by *S. bayanus*

%initial sugar	Fermentation efficiency			maximum obtained alcohol content	%reducing sugar	% titratable acidity	accepted flavor
	sugar conversion	fermentation time (day)	alcohol producion rate (% day^{-1})				
16%	1.77±0.03[a]	3	2.67±0.00[a]	8%	1.16±0.03[d]	0.32±0.05[b]	Reject
19%	1.64±0.06[b]	5	2.00±0.00[d]	10%	1.41±0.05[c]	0.32±0.13[b]	Reject
22%	1.75±0.07[a]	5	2.20±0.00[c]	11%	1.93±0.11[b]	0.38±0.07[a]	Accept
25%	1.57±0.05[c]	5	2.40±0.00[b]	12%	3.86±0.41[a]	0.38±0.11[a]	Accept

[a,b,c,....] value with significantly difference in each rate are indicated by different letters ($p \leq 0.05$)

The fermentation profile of different initial sugar batches were not similar since the sugar concentrations could influence sugar utilization of yeast cell during the fermentation. Normally, yeast could process the fermentation in must containing 15-25% sugar concentrations. However, for the wine fermentation, the most suitable sugar concentration was in range 20-22% (Jackson, 2000). Since under the proper condition, yeast could process the alcoholic fermentation along with volatile compound generation. Therefore, if the sugar concentration is not appropriate, the alcohol production will not be processed properly. Therefore, based on sugar concentration, the yeast could not complete the fermentation if the sugar concentration lower than 20%. Consequently, it could also generate excess undesirable metabolites giving off-flavor in wine as presented in the 16% and 19% of initial sugar batches.

In conclusion, the 100% juice containing 22% sugar fermented by *S. bayanus* was selected as the condition for basic wine making based on criteria of performing the efficient fermentation and the flavor of wine was accepted. Importantly, an amount of sugar remaining in the wine was also in the range which could be formulated to be semi-sweet (1%-3%) and sweet wine (>3%). The basic tangerine wine characteristic after clarification was the wine contained 1.93% sugar, 11% alcohol, and 0.38% titratable acidity as shown in table 4.6.

4.3 Evaluation of an acceptability of formulated tangerine wine

The means of overall liking scores of the 9 formulated wines were shown in table 4.7. The result of all assessors showed that the formulas containing higher reducing sugar content were more accepted than the formulas with lower level. The mean scores of the 6% reducing sugar formulas were higher than the 4% and 2% formulas, respectively. The statistical analysis showed that there were significant effects of the sugar and the sugar and acidity interaction ($p \leq 0.05$) on the "overall liking" score, and the effect of acidity was not significant ($p > 0.05$). However, the acceptance of female and male was significantly different ($p \leq 0.05$).The female assessors accepted the tangerine wine more than male assessors did. Therefore, the data were analyzed separately by gender.

The result showed that females preferred sweet wine (4% and 6% reducing sugar) to the semi-sweet wine (2% reducing sugar), with low acidity. From ANOVA, there were significant effects ($p \leq 0.05$) of the reducing sugar, the acidity and the interaction. Thus, the formulas no. 7 and 8, 6% reducing sugar with 0.5% or 0.7% acidity were liked the most. The formulas no. 2 and 3, 2% reducing sugar with 0.7% or 0.9% acidity were liked the least.

Table 4.7 The means of overall liking scores of the 9 formulas

sample			female (54 assessors)	male (54 assessors)	all assessors (108 assessors)
formulated wine No.	%reducing sugar	%acidity			
1	2%	0.50%	5.13±1.78^{de}	4.13±1.85^{c}	4.63±1.86^{c}
2		0.70%	4.63±1.50^{e}	5.00±1.82^{abc}	4.81±1.66^{c}
3		0.90%	4.63±1.74^{e}	5.00±1.41^{abc}	4.81±1.58^{c}
4	4%	0.50%	6.13±1.26^{bc}	5.13±1.85^{ab}	5.63±1.65^{ab}
5		0.70%	5.63±1.95^{cd}	4.63±1.66^{bc}	5.13±1.86^{bc}
6		0.90%	6.13±1.85^{bc}	5.25±1.73^{ab}	5.69±1.82^{ab}
7	6%	0.50%	7.13±1.33^{a}	5.00±1.50^{abc}	6.06±1.77^{a}
8		0.70%	6.63±1.10^{ab}	5.13±1.45^{ab}	5.88±1.48^{a}
9		0.90%	6.38±1.50^{b}	5.75±1.73^{a}	6.06±1.63^{a}

$^{a,b,c,....}$ value with significantly difference in each column are indicated by different letters. ($p \leq 0.05$)

For males, the acidity was only one factor that significantly effected on the "overall liking" score ($p \leq 0.05$). For each level of % reducing sugar, the formulas with 0.9% acidity had the highest liking score. The formula with 2% reducing sugar and 0.5% acidity had the lowest liking score and the formula with 6% reducing sugar and 0.9% acidity had the highest liking score. However, none of the 9 formulas had the liking score over than 6. This meant all formulas of tangerine wine were not well accepted by males.

Table 4.8 shows the mean scores of "liking of color", "liking of clarity", "liking of aroma" and "liking of flavor" of the 9 formulas evaluated by female assessors. From ANOVA, there were interaction between reducing sugar and acidity ($p \leq 0.05$) effected the liking scores of these 4 properties. The means of "liking of color" and "liking of

clarity" were higher than 6 (like slightly) for all 9 formulas. These showed that the female assessors accepted the appearance of the tangerine wine.

Table 4.8 The means of liking scores of the 9 formulas from the female assessors

sample			mean liking scores (9-points hedonic scale)			
formulated wine No.	%reducing sugar	%acidity	Color	clarity	aroma	Flavor
1	2%	0.50%	7.13 ± 1.15^{ab}	7.25 ± 1.39^{a}	5.50 ± 1.32^{d}	4.75 ± 1.73^{d}
2		0.70%	6.88 ± 1.26^{bc}	6.75 ± 1.39^{cd}	6.13 ± 1.26^{abc}	5.00 ± 1.41^{cd}
3		0.90%	6.50 ± 1.10^{cd}	6.63 ± 1.21^{d}	6.25 ± 1.07^{ab}	4.50 ± 1.50^{d}
4	4%	0.50%	6.38 ± 1.81^{d}	6.88 ± 1.45^{bcd}	5.75 ± 1.29^{bcd}	5.50 ± 1.41^{c}
5		0.70%	6.63 ± 1.41^{cd}	6.75 ± 1.29^{cd}	6.00 ± 1.41^{bcd}	5.54 ± 1.89^{c}
6		0.90%	7.33 ± 1.06^{a}	7.13 ± 1.23^{ab}	6.13 ± 1.15^{abc}	6.25 ± 1.65^{b}
7	6%	0.50%	7.25 ± 1.07^{ab}	7.00 ± 1.32^{abc}	6.63 ± 1.31^{a}	7.00 ± 1.56^{a}
8		0.70%	6.63 ± 1.21^{cd}	6.75 ± 1.39^{cd}	6.25 ± 1.48^{ab}	6.38 ± 1.41^{b}
9		0.90%	6.63 ± 1.21^{cd}	6.63 ± 1.31^{d}	5.63 ± 1.21^{cd}	6.25 ± 1.29^{b}

$^{a,b,c,....}$ value with significantly difference in each column are indicated by different letters. ($p\leq0.05$)

For aroma, the result showed different trends depending on the reducing sugar containing in wine. For the formulas containing 2% and 4% reducing sugar, the samples with higher %acidity had higher liking scores, but the formulas with 6% reducing sugar gave the contrast result.

The liking score of flavor had a similar trend to the "overall liking". From ANOVA, there was the significant effect of reducing sugar ($p\leq0.05$). The higher levels of %reducing sugar represent the higher liking scores for flavor. The formula no. 7 (6% reducing sugar and 0.5% acidity) had the highest score for "liking of flavor". Because of the interaction between reducing sugar and acidity, the trends of liking scores for flavor

at each level of reducing sugar were difference depending on %acidity. The flavor was a key attribute driving the acceptability of the tangerine wine.

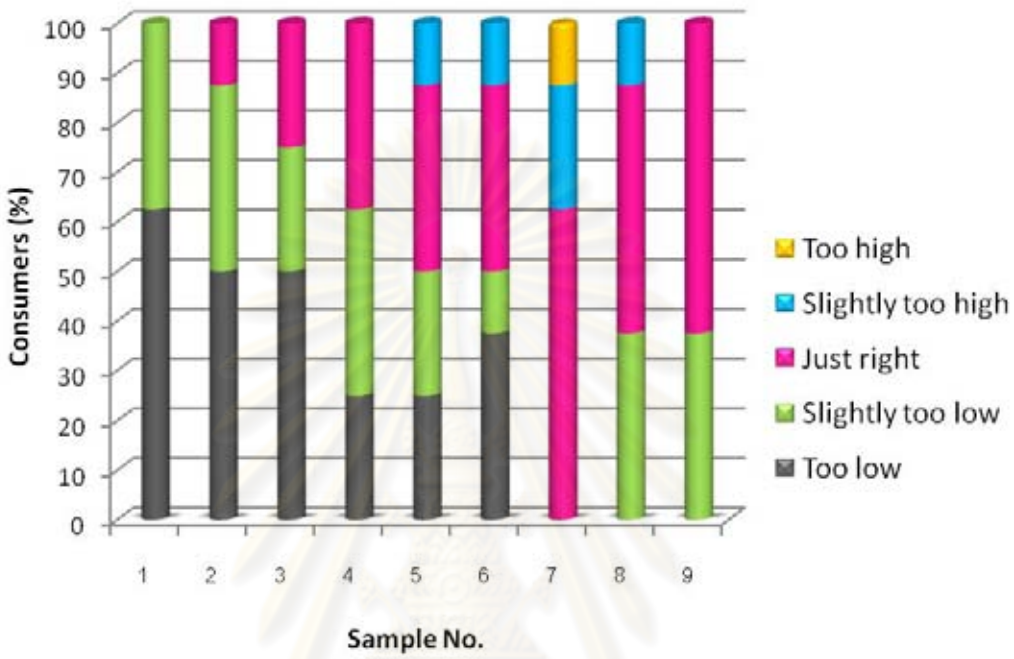


Figure 4.8 The percentage of consumers who selected each categories of the sweetness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 females).

From figure 4.8, among 3 levels of reducing sugar, the formulas containing 6% reducing sugar had the highest percentage of female assessors selected the “just about right” for sweetness. The sample no. 7 had 62.5% of female assessors accepted the sweetness, 37.5% thought this formula was too sweet. In contrast, the sample no. 9 also had 62.5% of female assessors accepted the sweetness, but 37.5% thought the sweetness was too low. This showed that %acidity also effected the perception of sweetness.

For the 3 formulas containing 2% reducing sugar, most of assessors thought that the sweetness was too low. These showed that the sweetness played the key role on the perceived sweetness and may be a key drive of the acceptance in flavor of wine.

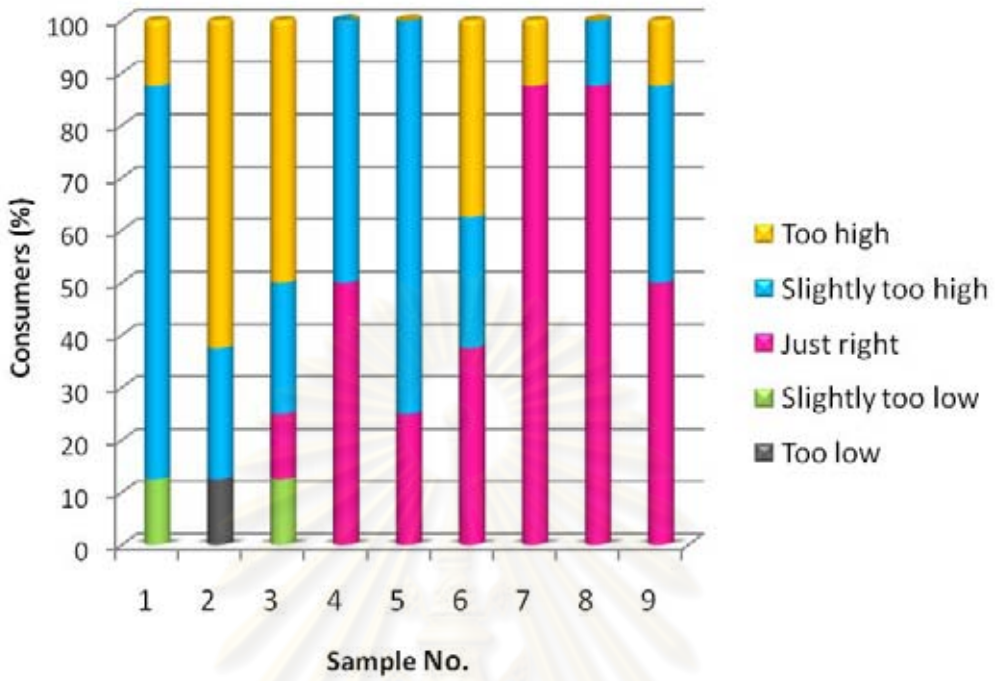


Figure 4.9 The percentage of consumers who selected each categories of the sourness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 females).

From figure 4.9, among 3 levels of reducing sugar, the formulas containing 6% reducing sugar had the highest of female assessors selected the “just about right” in sourness. The sample no.7 and 8 had 87.5% of female assessors accepted the sourness, with 12.5% thought this formula was too sour. The sample no. 9 also had 50% of female assessors accepted the sourness, but 50% thought the sourness was too high. For the 3 formulas containing 2% reducing sugar, most of assessors thought that sourness was too high. These showed that %reducing sugar played the important role on the sourness, while increasing in %acidity did not effect on the perceived sourness that much.

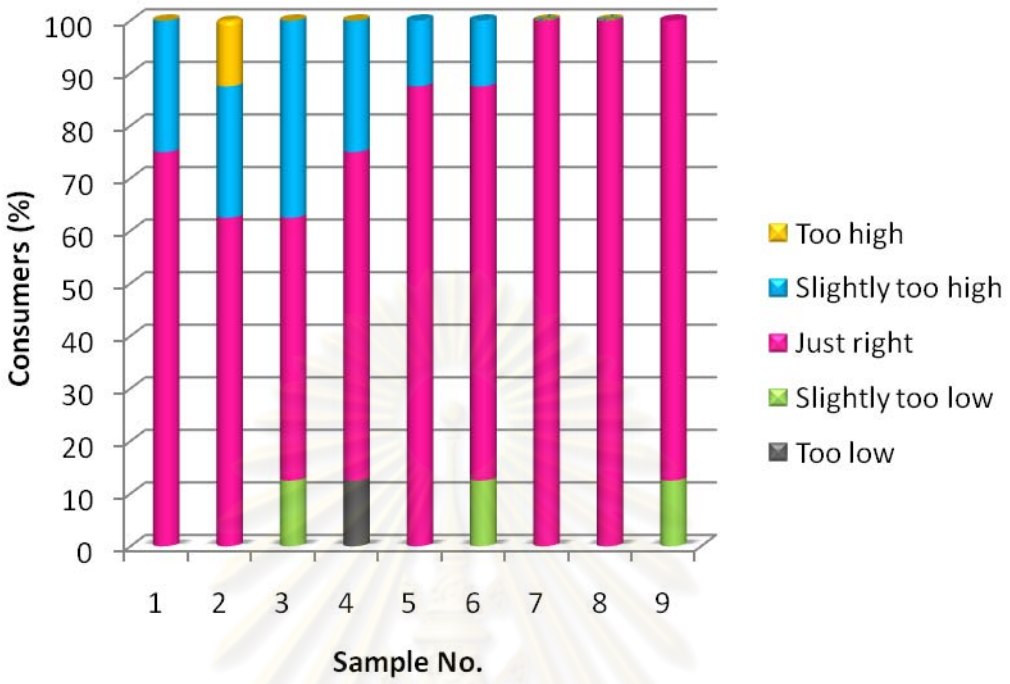


Figure 4.10 The percentage of consumers who selected each categories of the astringency evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 females).

From figure 4.10, most formulas had high percentage of “just about right”, more than 60% of female assessors accepted the astringency except the sample no. 3 which had 50% for “just about right”. While the 3 formulas with 6% reducing sugar (wine no. 7, 8 and 9) had over 90% in this category. The lower level of reducing sugar, the percentages of the “too high” category got higher.

The 3 formulas with 0.9% acidity (wine no. 3, 6 and 9) had 12.5% selected in “slightly too low”. These showed that the sweetness of tangerine wine samples play more important role on the perception of astringency. From the result, the % reducing sugar could play the important role on the astringency.

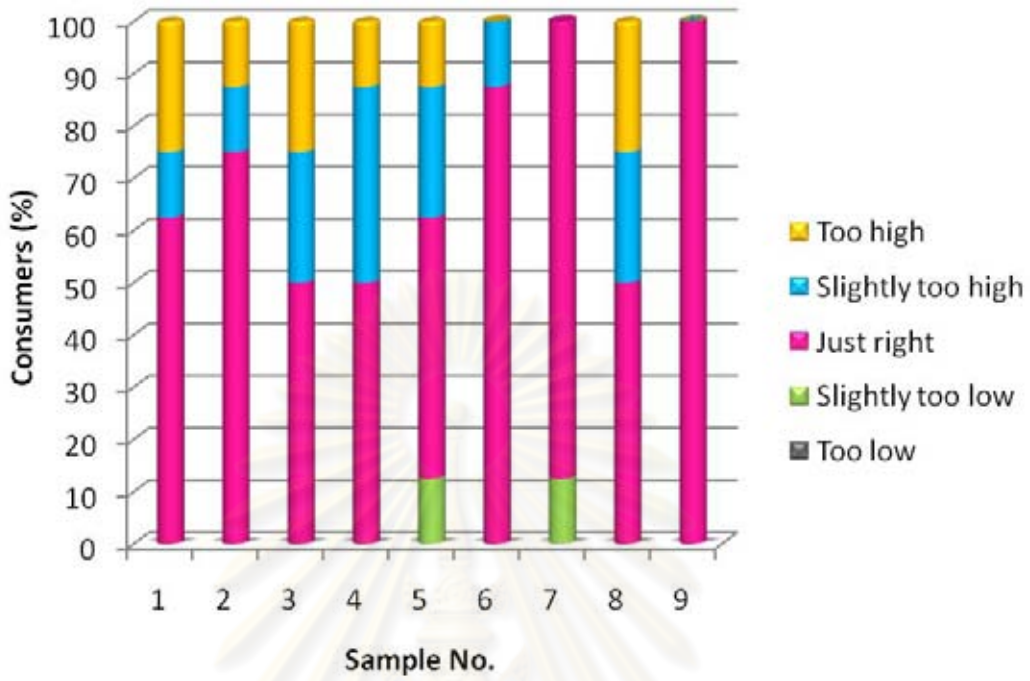


Figure 4.11 The percentage of consumers who selected each categories of the bitterness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 females).

From figure 4.11, the samples with 6% reducing sugar had high percentage in “just about right” for bitterness (>87.5%), except the wine sample no.8. For the formulas containing low level of reducing sugar, especially in sample no. 1-5, showed the greater percentage of assessors in “too high” and “slightly too high” categories for bitterness. These showed that sweetness and sourness had effect on the perception of bitterness in tangerine wine.

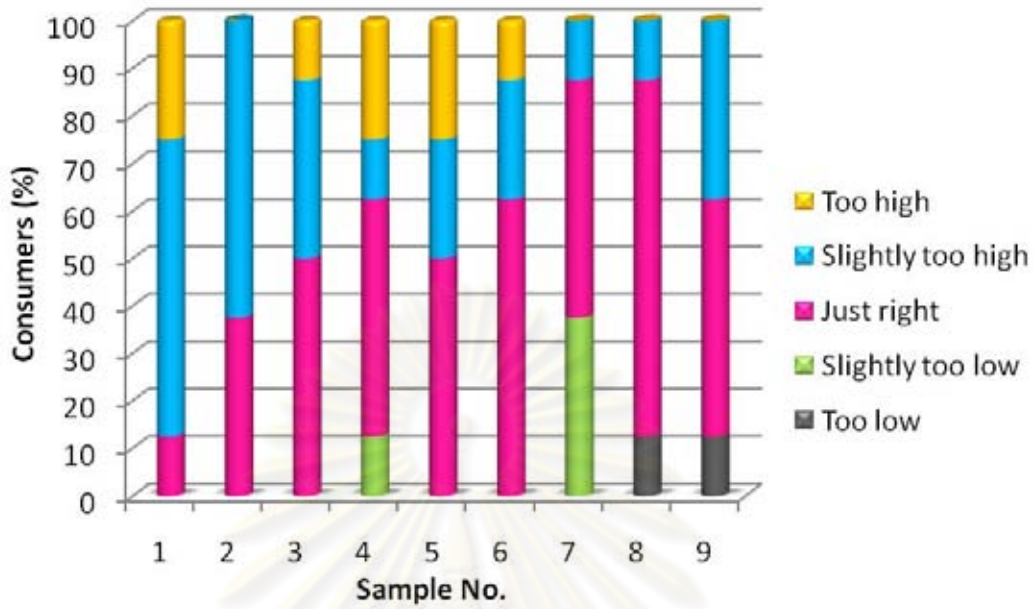


Figure 4.12 The percentage of consumers who selected each categories of the degree of alcohol evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 females).

From figure 4.12, the samples containing 4% and 6% reducing sugar (wine no.3-9), tended to have higher percentage of assessors in “just about right” for degree of alcohol than the samples with 2% reducing sugar (wine no.1 and 2) which more assessors perceived the degree of alcohol as “too high” or “slightly too high”. These showed that the sweetness influenced on the perception of the degree of alcohol in tangerine wine samples.

From the results of perception on sweetness, sourness, astringency, bitterness and degree of alcohol using Just About Right, showed that the level of reducing sugar had higher influenced than acidity. However, both factors played role in acceptability of tangerine wine. The sweeter wine (formulas with 6% reducing sugar) was well accepted by female assessors.

Table 4.9 shows the mean scores of “liking of color”, “liking of clarity”, “liking of aroma” and “liking of flavor” of the 9 formulas evaluated by male assessors. The means of liking scores for these 4 properties were rarely higher than 6 (like slightly) for all 9 formulas. These showed that the male assessors were not accepted the tangerine wine that well, and supported the “overall liking” scores.

Table 4.9 The means of liking scores of the 9 formulas from the male assessors

sample			mean liking scores (9-points hedonic scale)			
formulated wine No.	%reducing sugar	%acidity	color[NS]	clarity	aroma	flavor
1	2%	0.50%	6.00±1.59	5.88±1.70 [abc]	5.00±1.89 [bc]	4.25±1.73 [c]
2		0.70%	5.50±1.50	6.25±1.29 [a]	4.63±2.20 [c]	5.13±1.85 [b]
3		0.90%	5.04±2.12	4.88±1.85 [e]	5.25±1.80 [abc]	5.13±1.36 [b]
4	4%	0.50%	5.63±1.58	5.63±1.66 [bcd]	5.88±1.78 [a]	5.00±1.89 [bc]
5		0.70%	5.13±1.36	5.38±1.50 [cde]	4.75±1.65 [c]	4.88±1.85 [bc]
6		0.90%	5.88±1.36	5.75±1.29 [abc]	5.75±1.29 [a]	5.63±1.66 [ab]
7	6%	0.50%	5.88±1.54	6.13±1.26 [ab]	5.00±1.67 [bc]	5.00±1.82 [bc]
8		0.70%	5.75±1.65	5.75±1.65 [abc]	5.63±1.21 [ab]	5.25±1.57 [b]
9		0.90%	5.38±1.66	5.13±1.92 [de]	5.75±1.29 [a]	6.25±1.29 [a]

[a,b,c,....] value with significantly difference in each column are indicated by different letters. ($p \leq 0.05$)

[NS] value with non significantly difference in each column are indicated ($p > 0.05$)

From ANOVA, %reducing sugar, acidity and their interaction did not significantly effect the “liking of color” ($p > 0.05$). The interaction between reducing sugar and acidity were significantly effect on the “liking of clarity” ($p \leq 0.05$). However, the mean scores of “liking of color” and clarity of the 9 formulas were around 5 to 6, which were “neither like nor dislike” or “like slightly”. These meant the appearance of the tangerine wine was not quite appealing to males.

For the “liking of aroma”, the reducing sugar containing in wine had significantly impact the acceptability. The formulas with 6% reducing sugar had slightly higher in the liking scores.

The scores for “liking of flavor” showed the similar trend to the “overall liking” scores. From ANOVA, there was the significant effect of acidity ($p \leq 0.05$). The higher levels of %acidity had the higher scores for “liking of flavor”. The formula no. 9 (6% reducing sugar and 0.7% acidity) had the highest score which is the only one that was greater than 6. Therefore, %acidity played more important role on “liking of flavor” which was a drive the acceptability of wine.

The figure 4.13 to 4.17 showed the results from Just About Right scale for sweetness, sourness, astringency, bitterness and degree of alcohol. The formulas no.9 (6% reducing sugar with 0.9% acidity), which were most acceptable by male assessors, had the highest percentage in “just about right”category for sweetness, sourness and astringency (75%, 62.5% and 87.5%, respectively). However, 75% of assessors percepted that the bitterness, and 62.5% percepted the degree of alcohol of this formula as “slightly too low” and “too low”. Apart from product image, a lady wine product, tangerine wine was not accepted by male assessors might be cause by another error. Since the “overall liking” attribute of tangerine wine was evaluated, its score could tend to influence the other attribute scores due to the halo effect (Meilgaard *et al.,* 2007).

Thus, from the Just About Right result (sweetness, sourness, astringency, bitterness and degree of alcohol), showed that the level of acidity had more influence on the acceptability of tangerine wine. Comparing to females, males like wine with more intense in flavor, which must had higher degree of alcohol and bitterness.

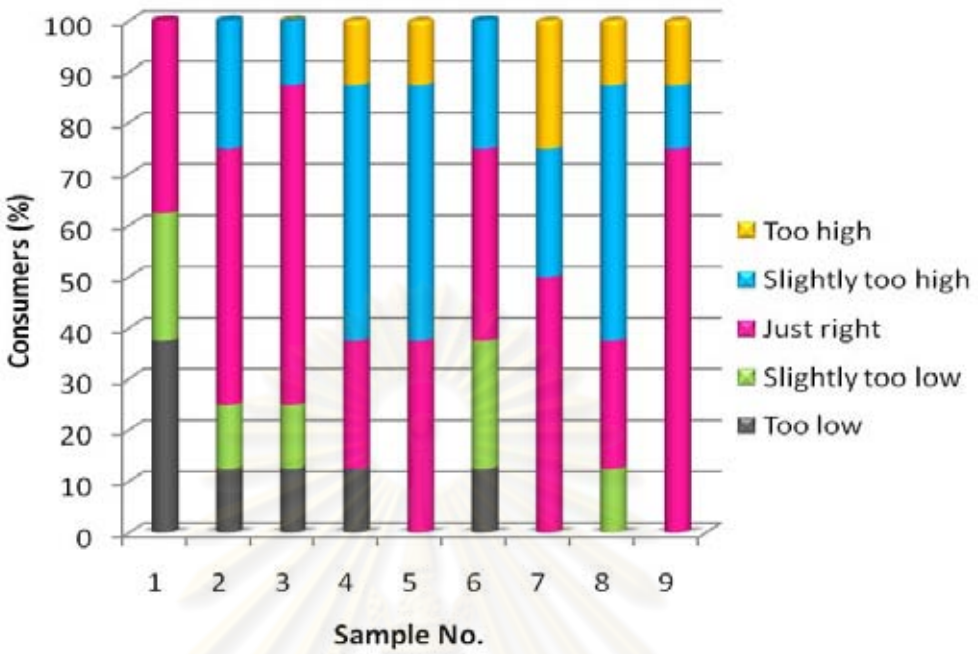


Figure 4.13 The percentage of consumers who selected each categories of the sweetness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 males).

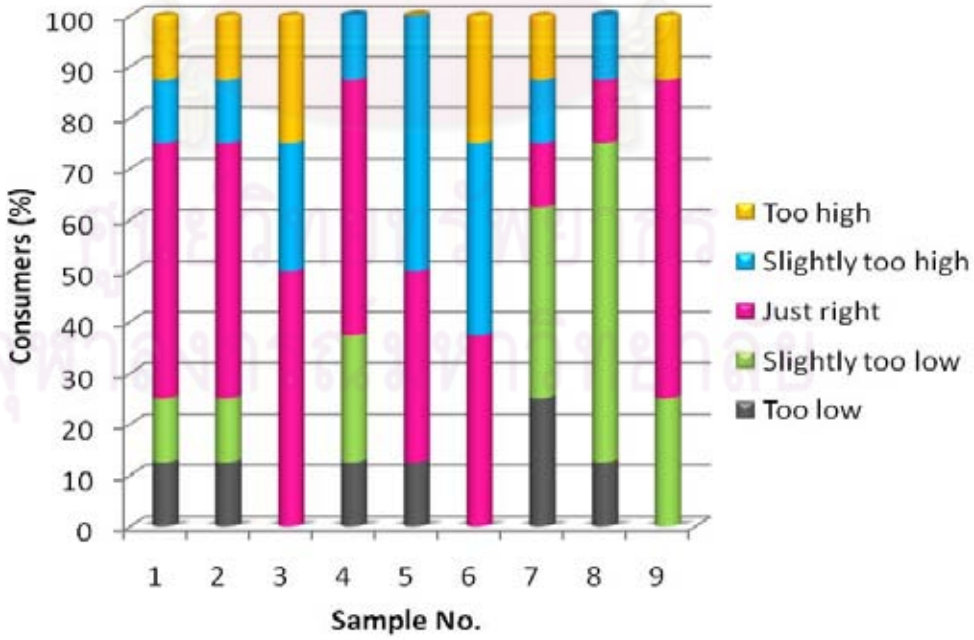


Figure 4.14 The percentage of consumers who selected each categories of the sourness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 males).

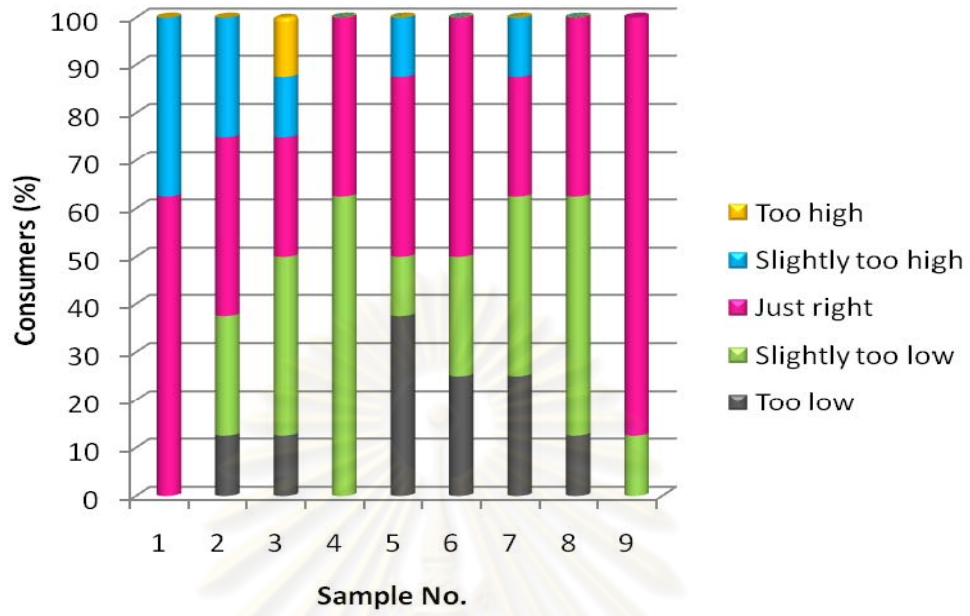


Figure 4.15 The percentage of consumers who selected each categories of the astringency evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 males).

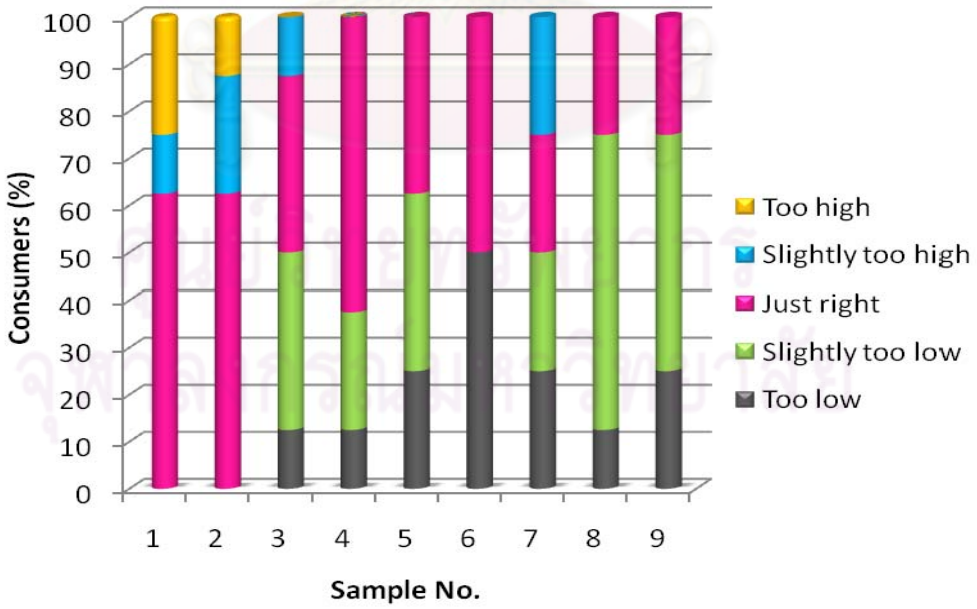


Figure 4.16 The percentage of consumers who selected each categories of the bitterness evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 males).

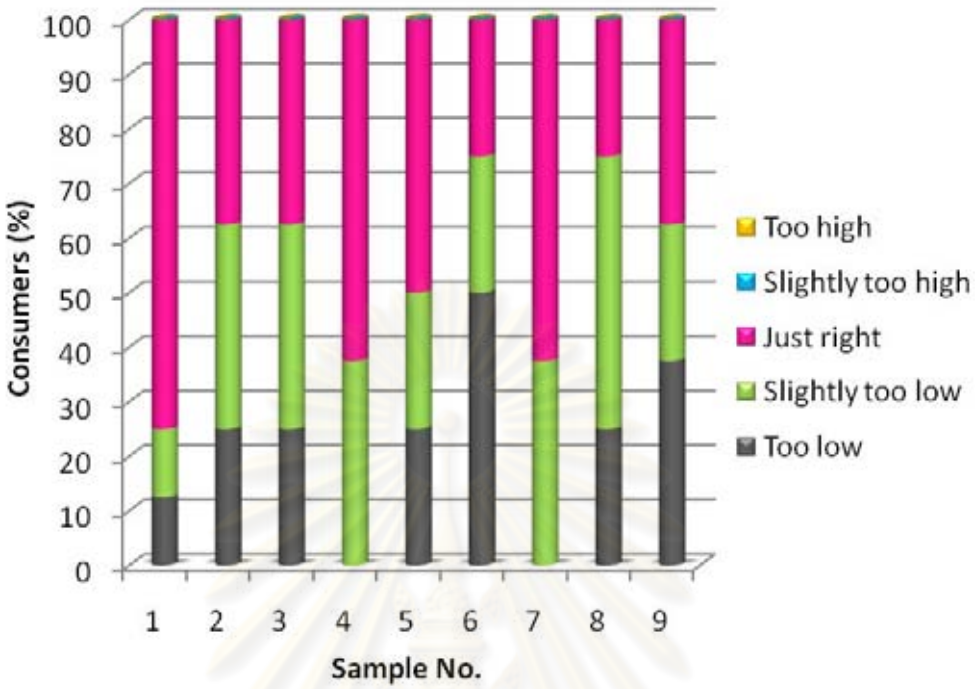


Figure 4.17 The percentage of consumers who selected each categories of the degree of alcohol evaluated by Just About Right (JAR) scale of all tangerine wine samples (24 males).

From the result, the acidity was significantly effect on the “overall liking” score of tangerine wine samples in male ($p \leq 0.05$). The acidity and the interaction of sugar and acidity also was significant effect on the “liking of clarity” ($p \leq 0.05$). The “liking of aroma” was significant influenced by the sugar content ($p \leq 0.05$) whereas the “liking of color” score of tangerine wine was not significantly different ($p > 0.05$). In conclusion, the result showed that the formulated tangerine wine no. 9, which is the sweet wine, contained 6% sugar and 0.9% acidity, respectively, was not accepted with the highest overall liking score by male assessors.

Table 4.10 and 4.11 showed the influence of the experience in fruit wine drinking on the “overall liking” score of the 9 formulas by female and male assessors, respectively. The “overall liking” score of formulated tangerine wine were ranged in 4.44 – 7.33. For each formula, the result showed that the “overall liking” scores between the experience and no experience groups were not significantly different ($p>0.05$) for both female and male assessors. It could conclude that the experience in fruit wine drinking was not influence on the “overall liking” score in female assessors.

Table 4.10 The effect of the experience in fruit wine drinking to the mean score in female.

sample			experience group		no experience group	
no.	%reducing sugar	%acidity	mean±SD	N	mean±SD	N
1	2%	0.50%	5.00±1.73	7	5.18±1.85	17
2		0.70%	4.71±1.38	7	4.59±1.58	17
3		0.90%	5.17±1.47	6	4.44±1.82	18
4	4%	0.50%	6.33±0.52	6	6.06±1.43	18
5		0.70%	5.86±1.86	7	5.53±2.03	17
6		0.90%	6.67±0.52	6	5.94±2.10	18
7	6%	0.50%	7.33±1.21	6	7.06±1.39	18
8		0.70%	6.75±0.96	4	6.60±1.14	20
9		0.90%	6.29±1.38	7	6.41±1.58	17

No significantly different between the 2 groups in each formula

Table 4.11 The effect of the experience in fruit wine drinking to the mean score in male.

sample			experience group		no experience group	
no.	%reducing sugar	%acidity	mean±SD	N	mean±SD	N
1	2%	0.50%	4.00±1.85	8	4.19±1.91	16
2		0.70%	4.88±1.89	8	5.06±1.84	16
3		0.90%	4.88±1.13	8	5.06±1.57	16
4	4%	0.50%	5.00±1.77	8	5.19±1.94	16
5		0.70%	4.50±1.51	8	4.69±1.78	16
6		0.90%	5.13±1.46	8	5.31±1.89	16
7	6%	0.50%	4.88±1.36	8	5.06±1.61	16
8		0.70%	5.00±1.07	8	5.19±1.64	16
9		0.90%	5.63±1.60	8	5.81±1.83	16

No significantly different between the 2 groups in each formula

According to this study, the result showed that the acceptability of 2 formulated tangerine wines were tangerine wine with 11% alcohol, contained 6% of reducing sugar with 0.5% and 0.9% of acidity, respectively, by all assessors. For females, the result reported that 5 accepted tangerine wines were tangerine wine with 11% alcohol, contained 4% of reducing sugar with 0.5% and 0.9% of acidity and 6% of reducing sugar with 0.5%, 0.7% and 0.9% of acidity, respectively. In contrast in males, although the highest overall liking score of formulated tangerine wine was tangerine wine with 11% alcohol contained 6% of reducing sugar with 0.9% of acidity, this formulated wine was not accepted. From the result in this study compared to the study of wine making from kiwi fruit, the characteristics of the accepted tangerine wine was nearly similar to

kiwi wine, contained 10% alcohol, 4.5% of sugar and less than 1% of acidity, in term of alcohol content, sugar content and acidity (Soufleros *et al.,* 2001).

4.3.3 Shelf life of formulated tangerine wine

From sensory evaluation study, the tangerine wine formula no.7 was accepted with the highest score. This formula was selected for shelflife evaluation. The shelflife of tangerine wine was evaluated by determination of physical, chemical, microbiological and off-odor properties during storage under refrigerator temperature (4°C) and room temperature (30°C) for 2 months. These properties were monitored every week. The results are shown in table 4.12 and table 4.13, respectively.

Data in table 4.12 reported properties of tangerine wine during storaged under 4°C. The color of wine was determined using CIELAB and Munsell's system. The color determined by Munsell's system was not changed during storage whereas the color in CIELAB was not significantly changed ($p>0.05$). From the result in CIELAB, L* value was not significantly ($p>0.05$) increased whereas a* value and b* value were not significantly ($p>0.05$) decreased. The increasing of L* value might be the breakdown of carotenoid pigment during storage, lead to the lost of pigment absorption which allowed L* value to increase (Oliviera *et al.,* 2006; Ferreira *et al.,* 2008). Beside the color, the precipitation is normally observed in tangerine wine. The white precipitation is also reported as significant detect normally found in wine during storage, which is caused by ferric formation from the spoilage yeast and yeast autolysis (Jackson, 2000). However, under storage condition at temperature 4°C, the precipitation of tangerine wine was not found throughout 2 months.

Table4.12 Properties of tangerine wine storaged under refrigerator temperature (4°C)

storage time (weeks)	physical properties			chemical properties		microbiological properties		off-odor
	color		precip-itation					
	CIELAB[NS]	Munsell's system		%reducing sugar[NS]	% acidity[NS]	yeast mold count (cfu ml^{-1})	LAB count (cfu ml^{-1})	
0	L*= 67.96 a*= -1.54 b*= 51.53	2.5YR 8/14	ND	5.92±0.19	0.51±0.00	<100	<100	ND
1	L*= 68.18 a*= -1.56 b*= 51.33	2.5YR 8/14	ND	5.92±0.04	0.48±0.05	<100	<100	ND
2	L*= 68.22 a*= -1.56 b*= 51.08	2.5YR 8/14	ND	5.98±0.12	0.54±0.05	<100	<100	ND
3	L*= 68.52 a*= -1.57 b*= 50.96	2.5YR 8/14	ND	5.92±0.12	0.51±0.00	<100	<100	ND
4	L*= 68.61 a*= -1.57 b*= 50.96	2.5YR 8/14	ND	5.95±0.31	0.54±0.05	<100	<100	ND
5	L*= 68.67 a*= -1.58 b*= 50.93	2.5YR 8/14	ND	5.98±0.28	0.48±0.05	<100	<100	ND
6	L*= 68.78 a*= -1.59 b*= 50.93	2.5YR 8/14	ND	5.90±0.23	0.48±0.05	<100	<100	ND
7	L*= 68.81 a*= -1.59 b*= 50.91	2.5YR 8/14	ND	5.97±0.04	0.54±0.05	<100	<100	ND
8	L*= 69.14 a*= -1.61 b*= 50.89	2.5YR 8/14	ND	6.00±0.08	0.51±0.00	<100	<100	ND

[NS] value with non significantly difference in each column are indicated (p>0.05)

LAB: Lactic acid bacteria

ND: Not detectable

During wine storage, the sugar content and acidity should be observed since decreasing of sugar and increasing of acidity might be occurred due to yeast contamination. They utilize sugar and acid for their growth (Ribéreau-Gayon *et al.,* 2006). Furthermore the utilization of sugar and generation of acid are also originated by the lactic acid bacteria (LAB) that produced mousy odor (Jackson, 2000). Therefore, the changes of sugar and acidity were monitored and it was found that sugar and acidity were not changed. Jackson (2000) reported that spoilage yeast and bacteria approximately 10^5 cfu ml^{-1} could cause off odor and lead to wine spoilage. From the microbiological property determination, yeast and lactic acid bacteria were not observed throughout storage time in this study. In addition, the off-odor was not also detected. Under this condition, the changes of physical, chemical, and microbiological properties were not presence and off-flavor was not also observed. It could be concluded that, based on these properties the shelflife of tangerine wine storaged under refrigerator temperature (4°C) was longer than 2 months.

Data shown in table 4.13 reported the properties of tangerine wine during storage under room temperature (approximately 30°C) for 2 months. The results were relatively similar to the result of tangerine wine during storage under 4°C which was all properties were not changed during storage. Therefore, it could conclude that the shelflife of tangerine wine storaged under room temperature (30°C) was also longer than 2 months.

In conclusion based on the chemical, physical, microbial properties and off-odor, tangerine wine formula no.7 could be storage under both refrigerator and room temperature longer than 2 months.

Table4.13 Properties of tangerine wine storage under room temperature (30°C)

storage time (weeks)	physical properties			chemical properties		biological properties		Off-odor
	color		precip-itation					
	CIELAB[NS]	Munsell's system		%reducing sugar[NS]	% acidity[NS]	yeast mold count (cfu ml^{-1})	LAB count (cfu ml^{-1})	
0	L*= 67.96 a*= -1.54 b*= 51.53	2.5YR 8/14	ND	5.95±0.16	0.54±0.05	<100	<100	ND
1	L*= 68.36 a*= -1.54 b*= 51.17	2.5YR 8/14	ND	5.92±0.12	0.51±0.00	<100	<100	ND
2	L*= 68.54 a*= -1.57 b*= 50.98	2.5YR 8/14	ND	5.92±0.19	0.51±0.09	<100	<100	ND
3	L*= 68.58 a*= -1.59 b*= 50.72	2.5YR 8/14	ND	5.95±0.24	0.51±0.00	<100	<100	ND
4	L*= 68.76 a*= -1.59 b*= 50.70	2.5YR 8/14	ND	5.95±0.08	0.51±0.09	<100	<100	ND
5	L*= 68.89 a*= -1.59 b*= 50.69	2.5YR 8/14	ND	5.98±0.28	0.48±0.05	<100	<100	ND
6	L*= 68.91 a*= -1.61 b*= 50.66	2.5YR 8/14	ND	5.92±0.19	0.51±0.00	<100	<100	ND
7	L*= 68.96 a*= -1.61 b*= 50.66	2.5YR 8/14	ND	6.06±0.16	0.54±0.05	<100	<100	ND
8	L*= 69.24 a*= -1.63 b*= 50.64	2.5YR 8/14	ND	5.98±0.28	0.51±0.00	<100	<100	ND

[NS] value with non significantly difference in each rate are indicated (p>0.05)

LAB: Lactic acid bacteria

ND: Not detectable

CHAPTER V

CONCLUSION

1. The proper basic tangerine wine making condition was using 100% juice concentration contained approximately 22% reducing sugar as must, and fermented by single culture of yeast *Saccharomyces bayanus* under 30°C. The fermentation profile of this condition demonstrated that the yeast could convert 1.75 g of sugar to 1 g of alcohol. The sugar consumption rate was 3.71% day^{-1} and generated the alcohol at rate 2.57% day^{-1} allowing alcohol to reach 11% within 5 days. The basic tangerine wine characteristic after clarification contained 1.93% sugar, 11% alcohol, and 0.38% titratable acidity.

2. The acceptance of formulated tangerine wines in both of female and male assessors was different. From the sensory evaluation of formulated tangerine wines, it was found that the sweetness, sourness and their interaction significantly ($p \leq 0.05$) influenced on the product acceptability in female assessors whereas the sweetness was the key factor that played role the liking scores. In female assessors, 5 out of 9 formulated wine were significantly ($p \leq 0.05$) accepted. The most accepted formula was the wine contained 6%sugar and 0.5%acidity (7.13±1.33). Although the sourness was the key factor that played role the liking scores in male assessors, all formulated tangerine wines were significantly ($p \leq 0.05$) unaccepted.

3. Based on the physical, chemical, microbiological and flavor properties. The accepted tangerine wine formula could be storaged under refrigerator and room temperature longer than 2 months.

RECOMMENDATION

This study could be an alternative model for development of other fruit wine making, in order to value adding fruit juice products.

REFERENCES

A.O.A.C. 1995. Official Methods of Analysis. 16th ed. Washington D.C.: Association of Official Analytical Chemists.

Ciani, M., Fatichenti, F., and Mannazzu I. 2002. Biodiversity and Biotechnology of Wine Yeasts. Yeasts in winemaking biotechnology, pp. 111-123. India: Research Signpost.

Ciani, M., Beco, L., and Comitini, F. 2006. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. International Journal of Food Microbiology. 108: 239-245.

Clemente-Jimenez, J.M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Heras-Vázquez, F.J.L., and Rodríguez-Vico, F. 2004. Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. Journal of Food Microbiology. 21: 149-155.

Chomsri, N., Chanrittisen, T., Srisamatthakarn, P., Schmitz, C., and Jaokondee, W. 2003. Effects of composition in longan must on the growth rates, cell biomass, and fermentation of wine yeasts. Proceedings of The 1st International Symposium and Workshop on Insight into The World of Indigenous Fermented Foods for Technology Development and Food Safety (IWIFF), pp. 340-348. The 1st International symposium and workshop. Thailand.

Deeraksa, S., Warasawas, P., Daengprok, W., and Chanrittisen, T. 2005. A comparative study of mangosteen wine quality using natural microbial and pure yeast culture. Advancing Food Technology: Bringing Thailand into The World's Kitchen, pp.12-13. The 7th Agro-industrial conference. Thailand.

Delfini, C., and Formica, J.V. 2001. Wine Microbiology: Science and Technology. USA: Marcel and Dekker, Inc.

Department of Agricultural, Thailand. 2007. Good Agricultural Practice (GAP) for Tangerine [Online]. Available from: http://210.246.186.28/plant/gap/gap_Tangerine.html [2009, January 10].

Eglington, J.M., Buckingham, L., and Henschke, P.A. 1993. Increased volatile acidity of white wines by chemical vitamin mixtures is grape juice dependent. Australian Wine Industry Technical Conference, pp. 197-198. The 8th Australian Wine Industry Technical Conference. Australia.

Ezeronye, O.U. 2004. Nutrient utilization profile of *Saccharomyces cerevisiae* from palm wine in tropical fruit fermentation. Antonie Van Leeuwenhoek. 86: 235-240.

Fennema, O.R. 1996. Food Chemistry, 3rd ed. USA: Marcel and Dekker, Inc.

Ferreira, A.C.S., Monteiro, J., Oliveira, C., and de Pinho, P.G. 2008. Study of major aromatic compounds in port wines from carotenoid degradation. Food Chemistry. 110: 83-87.

Fleet, G.H. 1999. Microorganisms in food ecosystems. International Journal of Food Microbiology. 50: 101-117.

Fleet, G.H., Prakitchaiwattana, C., Beh, A.L., and Heard, G. 2002. Biodiversity and Biotechnology of Wine Yeasts. The yeasts ecology of wine grapes, pp. 1-17. India: Research Signpost.

Fleet, G.H. 2003. Yeast interactions and wine flavour. International Journal of Food Microbiology. 86: 11-22.

Gil-Izquierdo, A., Gil, M.I., Tomas-Barberan, F.A., and Ferreres, F. 2003. Influence of industrial processing on orange juice flavanone solubility and transformation to chalcones under gastrointestinal condition. Journal of Agricultural and Food Chemistry. 51: 3024–3028.

Henick-Kling, T. 1995. Control of malo-lactic fermentation in wine: Energetics, flavour modification and methods of starter culture preparation. Journal of Applied Bacteriology. (Symposium Supplement) 79: 29S-37S.

Hernandez-Orte, P., Ibarz, M.J., Cacho, J., and Ferreira, V. 2005. Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine. Food Chemistry. 89: 163-174.

Jackson, R.S. 2000. Wine Science: Principles, Practice, Perception, 2nd ed. USA: Academic Press.

Jutajumpol, A. and Panumastrakul, W. 1993. Orange Wine. Senior Project. Department of Food Technology, Chulalongkorn University. (In Thai)

Kimball, D.A. 1999. Citrus Processing: a Complete Guide, 2nd ed. USA: Aspen Publishers, Inc.

Lea, A., and Piggott, J. 1995. Fermented Beverage Production, 1st ed. UK: Blackie Academic & Professional.

Mateo, J.J., Jimenez, M., Pastor, A., and Huerta T. 2001. Yeast starter cultures affecting wine fermentation and volatiles. Food Research International. 34: 307-314.

Maury, C., Sarni-Manchado, P., Lefebvre, S., Cheynier, V., and Moutounet, M. 2001. Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. American Journal of Enology and Viticulture. 52: 140-145.

Meilgaard, M.C., Civille, G.V., and Carr, B.T. 2007. Sensory Evaluation Techniques, 4th ed. USA: CRC Press.

Moreno-Arribas, M.V., and Polo, M.C. 2005. Winemaking biochemistry and microbiology: current knowledge and future trends. Critical Reviews in Food Science and Nutrition. 45: 265–286.

Nisperos-Carriedo, M., and Shaw, P. 1990. Comparison of volatile flavor components in fresh and processed orange juices. Journal of Agricultural and Food Chemistry 38: 1048–1052.

Oliveira, C., Barbosa, A., Ferreira, A.C.S., Guerra, J., and de Pinho, P.G. 2006. Carotenoid profile in grapes related to aromatic compounds in wines from Douro region. Journal of Food Science. 71: S1-S7.

Pozo-Bayon, M.A., G-Alegría, E., Polo, M.C., Tenorio, C., Martín-Álvarez, P.J., Calvo de la Banda, M.T., Ruiz-Larrea, F., and Moreno-Arribas, M.V. 2005. Wine volatile and amino acid composition after malolactic fermentation: effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. Journal of Agricultural and Food Chemistry. 53: 8729–8735.

Prakitchaiwattana, C.J., Fleet, G.H., and Heard, G.M. 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. Federation of European Microbiological Societies Yeast Research. 4: 865-877.

Reddy, L.V.A. and Reddy, O.V.S. 2005. Production and characterization of wine from mango fruit (*Mangifera indica* L). World Journal of Microbiology and Biotechnology. 21: 1345-1350.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., and Lonvaud A. 2006. Handbook of Enology: The Microbiology of Wine and Vinifications, vol. 1, England: John Wiley & Sons.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., and Lonvaud A. 2006. Handbook of Enology: The Chemistry of Wine: Stabilization and Treatments. vol. 2, England: John Wiley & Sons.

Romano, P., Fiore, C., Paraggio, M., Caruso, M., and Capece, A. 2003. Function of yeast species and strains in wine flavor. International Journal of Food Microbiology. 86: 169-180.

Samson, J.A. 1986. Tropical Fruits. 2nd ed. UK: Blackwell Publishing.

Selli, S., Cabaroglu, T., and Canbas, A. 2003. Flavour components of orange wine made from a Turkish cv. Kozan. International Journal of Food Science and Technology. 38: 587-593.

Selli, S. 2007. Volatile constituents of orange wine obtained from Moro oranges (*Citrus sinensis* [L.] Osbeck). Journal of Food Quality. 30: 330-341.

Serra, A., Strehaiano, P., and Taillandier P. 2005. Influence of temperature and pH on *Saccharomyces bayanus* var. *uvarum* growth; impact of a wine yeast interspecific hybridization on these parameters. International Journal of Food Microbiology. 104: 257-265.

Soufleros, E.H., Pissa, I., Petridis, D., Lygerakis, M., Mermelas, K., Boukouvalas, G., and Tsimitakis, E. 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimisation of its composition. Food Chemistry. 75: 487-500.

Thai Industrial Standard Institute. 2003. Thai Community Product Standard (TCPS) [Online]. Available from: http://library.tisi.go.th/E/fulltext_e/Cps_e/P1_e.html [2008, December 17].

Thammarat, P. 1978. Investigation the Efficiency of Yeast Culture in Palm Wine and Strains Selection for Alcoholic Fermentation. Thesis. Department of Microbiology, Kasetsart University. (In Thai)

Toledo, T. 1986. Postprocessing changes in aseptically packed beverages. Journal of Agricultural and Food Chemistry. 34 (3): 405-408.

USDA. 2008. USDA National Nutrient Database for Standard Reference: Tangerine juice, pack, canned. Release 21. [online]. Available from: http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl [2008, October 19].

Yang, X.H., and Tang, J. 2002. Advances in Bioprocessing Engineering. 1st ed. USA: World Scientific Publishing, Inc.

APPENDICES

APPENDIX A

Determine the physical and chemical properties

A1: Determination of juice color

Instruments

1. Minolta CR-300 Chromameter
2. Minolta CT-310

Methods

1. Switch on the equipment then press all data clear
2. Press calibrate and select mode D65
3. Set channel-00 with distilled water
4. Place sample container, press measure mode and record data

A2: Protein determination (A.O.A.C.,1995)

Instruments

1. Distillation unit (Kjedahl and Vapodest, K424 Büchi, Switzerland)
2. Kjeldahl flask
3. Conical flask
4. Burette

Chemicals

1. Sulfuric acid (concentrated)
2. 0.1 N sulfuric acid
3. 50% (w/v) sodium hydroxide
4. 4% (w/v) boric acid
5. Selenium reagent mixture
6. Methyl red-methylene blue indicator
7. 0.1 N hydrochloric acid

Methods

1. Accurately weigh out 0.7-2.2 g of sample on a low ash paper and transfer to a digestion flask.

2. Add 5 g of selenium reagent mixture.

3. Add 30 mL of concentrated H_2SO_4

4.Place the rack and tubes in the digestion apparatus. Connect the exhaust manifold onto the tubes and turn on the water pump. Set the thermostat to 400°C and turn on and leave for 45 min and digest until the solution become clear.

5. After the 45 min, lift the rack out of the digestion block and place on the stand to cool. Leave the water pump and manifold connected. Then, remove the manifold when the tubes are cooled.

6. Place the tube to the distillation apparatus and add 80 ml of distilled water and 120 ml of 50% w/v sodium hydroxide.

7. Place a conical flask containing 50 ml of 4% w/v boric acid and 4 drops of indicator (0.1% methylene blue + 0.2% methyl red).

8. Run the distillation process.

9. Remove the flask from the apparatus and titrate the ammonia in the flask to the original purplish color with 0.1N HCl.

Calculation

Protein (%) = (mL of titer × N of HCl × 14 × 6.25)/(Weight of sample (g) × 10)

Nitrogen content (%) = %Protein content /6.25

A3: Ash determination (A.O.A.C., 1995)

Instruments

1. Muffle furnace (Furnace Carbolote, S336RB Parsons Lane, Hope England)
2. Hot plate
3. Crucible
4. Fume hood
5. Desiccator

Methods

1. Weigh a crucible, which was previously dried in a muffle furnace at 500 °C and cooled in a desiccator for 2 hours.
2. Weigh accurately 3-5 g of sample into a crucible.
3. Place sample on a hot plate in the fume hood.
4. Transfer the crucible to a muffle furnace heated to 550 °C.
5. Leave the crucible in the muffle furnace for 4 hr. until the ash is white or grayish-white.
6. After incineration, place in a desiccator for 2 hour and reweigh.

Calculation

$$\text{Ash (\%)} = ((A-B) \times 100)/C$$

Where, A = weight of crucible + weight of sample after incineration (g)

B = weight of crucible (g)

C = weight of sample before incineration (g)

A4: Titratable acidity (TA) determination (A.O.A.C., 1995)

Instruments

1. Burette
2. Pipette
3. Conical flask

Chemicals

1. 0.1 M sodium hydroxide solution (NaOH)
2. 0.1% phenolphthalein

Methods

1. Fill 100ml of sample into dry beaker
2. Pipette 10 ml of this filtered into conical flask
3. Dilute to 80 ml with water
4. Add 3 drops of phenolphthalein
5. Titrate with 0.1M sodium hydroxide solution until obtain pink end point

Calculation

$TA = 10 \times T$

$\%\text{Citric acid} = T \times 192 / 3 \times 1000$

Where TA is titratable acidity

T is quantity (ml) of 0.1M sodium hydroxide solution in titration

A5: Total sugar content determination by Lane-Eynon method (A.O.A.C., 1995)

Instruments

1. Burette
2. Volumetric flask
3. Glass wool

Chemicals

1. Fehling's solution A and B

1.1. Dissolve 69.28 g of copper II sulphate pentahydrate ($CuSO_4.5H_2O$) and 1ml of 1M sulphuric acid in 1 litre of distilled water

1.2. Dissolve 346 g potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$) and 100g sodium hydroxide (NaOH) in distilled water then make volume up to 1 litre. Filtrate through glass wool after standing.

1.3. Mix Fehling's solution A and B by adding equal volumes into dry glass container, gentle swirl. Store the solution in the dark place.

2. 1% Methylene blue solution indicator

Methods

1. Weight 4-5 g of sample into beaker and add 100ml of water
2. Filtrate through glass wool into 250ml volumetric flask, then make up volume of sugar solution
3. Add 100 ml of the solution into conical flask
4. Add 10 ml of diluted HCl and boil for 5 minutes, then leave cool
5. Neutralize with 10% NaOH
6. Make volume to 250ml in volumetric flask
7. Fill sugar solution to burette
8. Pipette 10ml of Fehling's solution into conical flask
9. Add 4 drops of Methylene blue and boil
10. Titrate with sugar solution until reach end point

Calculation

%Total sugars = factor×250×2.5/ T×W×10

Where T is sugar solution (ml)

W is weight of used sample (g)

Invert sugar factor = 5.09 mg

A6: Alcohol content determination

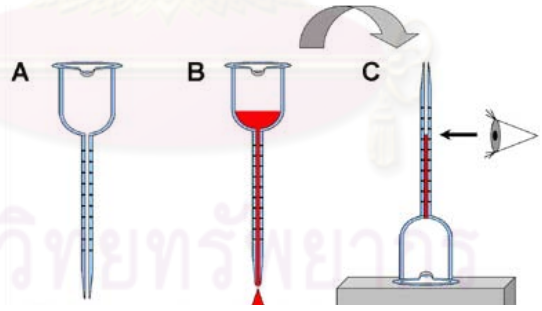
Instruments

1. Vinometer

Figure A1 vinometer instrument

Methods

1. Add 1 ml of sample into vinometer and wait until sample reach the end of vinometer (B)
2. Upside down the vinometer and wait until sample is still (C)
3. Observe the alcohol scale (C)



APPENDIX B

Determination of the microbiology properties

B1. Yeast and Mold count (A.O.A.C., 1995)

Instruments

1. Incubator (Memmert, Germany)
2. Petri dish
3. 1000 µl micropipette
4. Colony counter
5. Vortex mixer
6. Spreader

Chemicals

1. Ethyl alcohol
2. 0.1% peptone solution
3. Potato dextrose agar

Methods

1. Take 1 ml of sample into 9 ml 0.1% peptone solution
2. Make serial dilution of the sample
3. Spread 0.1 ml of the sample dilution onto PDA plate
4. Incubate at 30 °C for 2-3 days
5. Count the colony

B2. Lactic acid bacteria (LAB) count (A.O.A.C., 1995)

Instruments

1. Incubator (Memmert, Germany)
2. Petri dish
3. 1000 µl micropipette
4. Colony counter
5. Vortex mixer
6. Spreader

Chemicals

1. Ethyl alcohol
2. 0.1% peptone solution
3. de Man, Rogosa and Sharpe agar

Methods

1. Take 1 ml of sample into 0.1% peptone solution
2. Make serial dilution of the sample
3. Spread 0.1 ml of the sample dilution onto MRS plate
4. Incubate at 30 °C for 2-3 days
5. Count the colony

APPENDIX C

The standard curve of yeast population

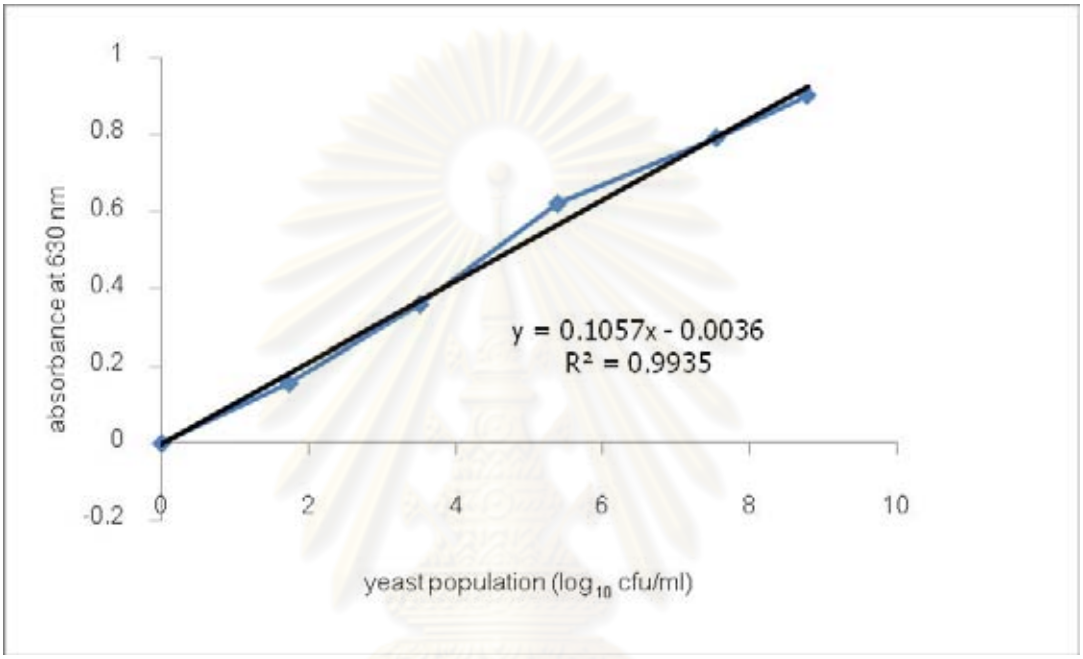


Figure C1 The relationship of yeast population (log10 cfu/ml) and their absorbance values (λ_{max} = 630 nm)

Calculation

The linear equation: $y = 0.1057x - 0.0036$

Where y = the absorbance at 630 nm

X = the yeast population

APPENDIX D

D1: Must preparation

Table D1 The carbon and nitrogen sources adjustment for 1L of must

must concentration	sugar added (g)	diammonium phosphate added (g)
30% juice	208.21	0.44
50% juice	180.35	0.40
100% juice	110.70	0.30

D2: Formulated tangerine wine preparation

Methods

1. Prepare 50 ml of syrup by mix sugar, citric acid and water together for each formula
2. Add syrup to 950 ml of basic wine mix well

Table D2 The preparation of 50 ml syrup for 1L of formulated tangerine wine

Wine no.	1	2	3	4	5	6	7	8	9
sugar added (g)	1.66			21.66			41.66		
citric acid added (g)	1.39	3.39	5.39	1.39	3.39	5.39	1.39	3.39	5.39

D3: The sample arrangement of sensory evaluation

Table D3 Balance Incomplete Block table for sensory evaluation using 54 females and males

assessor	sample No.			
1	1	4	6	7
2	2	6	8	9
3	1	3	8	9
4	1	2	3	4
5	1	5	7	8
6	4	5	6	9
7	2	3	6	7
8	2	4	5	8
9	3	5	7	9
10	1	2	5	7
11	2	3	5	6
12	3	4	7	9
13	1	2	4	9
14	1	5	6	9
15	1	3	6	8
16	4	6	7	8
17	3	4	5	8
18	2	7	8	9
19	1	4	6	7
20	2	6	8	9
21	1	3	8	9
22	1	2	3	4
23	1	5	7	8
24	4	5	6	9
25	2	3	6	7
26	2	4	5	8
27	3	5	7	9
28	1	2	5	7

TableD3 Balance Incomplete Block table for sensory evaluation using 54 females and males (continue)

assessor	sample No.			
29	2	3	5	6
30	3	4	7	9
31	1	2	4	9
32	1	5	6	9
33	1	3	6	8
34	4	6	7	8
35	3	4	5	8
36	2	7	8	9
37	1	4	6	7
38	2	6	8	9
39	1	3	8	9
40	1	2	3	4
41	1	5	7	8
42	4	5	6	9
43	2	3	6	7
44	2	4	5	8
45	3	5	7	9
46	1	2	5	7
47	2	3	5	6
48	3	4	7	9
49	1	2	4	9
50	1	5	6	9
51	1	3	6	8
52	4	6	7	8
53	3	4	5	8
54	2	7	8	9

APPENDIX E

Sensory evaluation questionaire

แบบสอบถาม

ชุดที่.....

เรื่อง การทดสอบการยอมรับของผู้บริโภคในการบริโภคไวน์ส้มจากน้ำส้มสายน้ำผึ้ง

เรียน ท่านผู้ตอบแบบสอบถาม

คำชี้แจง แบบสอบถามชุดนี้เป็นการทดสอบการยอมรับของผู้บริโภค ในการบริโภคไวน์ส้มจากน้ำส้มสายน้ำผึ้ง เพื่อประกอบวิทยานิพนธ์ของ นางสาววิจักขณา นวรัตน์ธารา นิสิตปริญญาโท ภาควิชาเทคโนโลยีทางอาหาร คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

คำอธิบาย ไวน์ส้มในการวิจัยซึ่งทำมาจากน้ำส้มสายน้ำผึ้ง ได้ปรับปรุงกลิ่นรสเพื่อประเมินหาสูตรของไวน์ส้มที่ได้รับการยอมรับจากผู้บริโภค ทั้งนี้เพื่อเพิ่มคุณค่าของผลิตภัณฑ์ในอนาคต จึงใคร่ขอความกรุณาจากท่านตอบแบบสอบถามให้สมบูรณ์ ข้อมูลทั้งหมดที่ท่านตอบจะเป็นประโยชน์อย่างยิ่งสำหรับงานวิจัยนี้ และจะไม่มีผลกระทบใดๆ ต่อท่านทั้งสิ้น ขอขอบพระคุณอย่างสูงที่ให้ความกรุณาในการตอบแบบสอบถามค่ะ

ขอบพระคุณค่ะ

นางสาววิจักขณา นวรัตน์ธารา

ผู้วิจัย

คำแนะนำ : กรุณาใส่เครื่องหมายถูก (**√**) ลงในช่อง ☐ หน้าคำตอบที่ต้องการเลือก

ส่วนที่ 1 : ข้อมูลส่วนบุคคล

1. เพศ

☐ ชาย ☐ หญิง

2. อายุ

☐ ต่ำกว่า 20 ปี ☐ 20-30 ปี

☐ 31-40 ปี ☐ 41-50 ปี

☐ 51 ปี ขึ้นไป

3. อาชีพ

☐ นักเรียน/ นักศึกษา ☐ รับราชการ/รัฐวิสาหกิจ

☐ ค้าขาย/ธุรกิจส่วนตัว ☐ พนักงานบริษัทเอกชน

☐ อื่นๆ (โปรดระบุ).....

4. ระดับการศึกษา

☐ 4.1 กำลังศึกษา

☐ มัธยมศึกษา ☐ อาชีวศึกษา/อนุปริญญา

☐ อาชีวศึกษา/อนุปริญญา ☐ ปริญญาตรี

☐ ปริญญาโท ☐ ปริญญาเอก

☐ 4.2 สำเร็จการศึกษาแล้ว และระดับการศึกษาขั้นสูงสุด

☐ ต่ำกว่ามัธยมศึกษา ☐ มัธยมศึกษา

☐ อาชีวศึกษา/อนุปริญญา ☐ ปริญญาตรี

☐ ปริญญาโท ☐ ปริญญาเอก

5. รายได้ส่วนตัว (บาท/เดือน)

☐ ยังไม่มีรายได้ ☐ ต่ำกว่า 5,000 บาท

☐ 5,001- 9,000 บาท ☐ 9,001-15,000 บาท

☐ 15,001- 30,000 บาท ☐ 30,001 -50,000 บาท

☐ มากกว่า 50,000 บาท

6. ท่านบริโภคเครื่องดื่มแอลกอฮอลล์บ่อยมากแค่ไหน

☐ ไม่ดื่มเลย ☐ 1-2 ครั้ง/สัปดาห์

☐ 3-5 ครั้ง/สัปดาห์ ☐ 1-2 ครั้ง/เดือน

☐ น้อยกว่า 1 ครั้ง/เดือน

7. ท่านเคยบริโภคเครื่องดื่มแอลกอฮอลล์ประเภทไวน์ผลไม้หรือไม่ และเป็นชนิดใด (ตอบได้มากกว่า 1 ข้อ)

☐ เคยดื่ม ☐ ไม่เคยดื่ม

☐ ไวน์ลิ้นจี่ ☐ ไวน์สับปะรด

☐ ไวน์สละ ☐ ไวน์มังคุด

☐ อื่นๆ โปรดระบุ.....

ส่วนที่ 2: **การทดสอบการยอมรับทางด้านประสาทสัมผัสของไวน์น้ำส้มสายน้ำผึ้ง**

คำแนะนำ : กรุณาประเมินลักษณะทางประสาทสัมผัสต่างๆของตัวอย่าง โดยใส่เครื่องหมายถูก (**√**) ลงในช่อง

รหัสตัวอย่างทดสอบ .....

กรุณาตอบคำถามข้อ1-3 **<u>ก่อน</u>**ดื่มตัวอย่าง

1. เมื่อพิจารณาตัวอย่างแล้ว คุณมีความชอบด้านสีของผลิตภัณฑ์ในระดับใด

☐	☐	☐	☐	☐	☐	☐	☐	☐
ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	เฉยๆ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

2. เมื่อพิจารณาตัวอย่างแล้ว คุณมีความชอบต่อลักษณะปรากฏที่ไม่ใช่สี (ความใส) ของผลิตภัณฑ์ในระดับใด

☐	☐	☐	☐	☐	☐	☐	☐	☐
ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	เฉยๆ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

3. เมื่อดมกลิ่นของตัวอย่างแล้ว คุณมีความชอบในกลิ่นของผลิตภัณฑ์ (aroma)ในระดับใด

☐	☐	☐	☐	☐	☐	☐	☐	☐
ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	เฉยๆ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

กรุณาตอบคำถามข้อ 4-10 **<u>หลัง</u>**ดื่มตัวอย่าง

4. เมื่อดื่มตัวอย่างแล้ว คุณมีความชอบโดยรวมต่อผลิตภัณฑ์นี้ในระดับใด

☐	☐	☐	☐	☐	☐	☐	☐	☐
ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	เฉยๆ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

5. เมื่อดื่มตัวอย่างแล้ว คุณมีความชอบในกลิ่นรสโดยรวม (flavor) ของผลิตภัณฑ์ ในระดับใด

☐	☐	☐	☐	☐	☐	☐	☐	☐
ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	เฉยๆ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

6. เมื่อดื่มตัวอย่างแล้ว คุณเห็นว่ารสหวาน (sweetness) ของผลิตภัณฑ์ นี้เป็นอย่างไร

☐	☐	☐	☐	☐
น้อยไป		พอดี		มากไป

7. เมื่อดื่มตัวอย่างแล้ว คุณเห็นว่ารสเปรี้ยว (sourness) ของผลิตภัณฑ์ นี้เป็นอย่างไร

☐	☐	☐	☐	☐
น้อยไป		พอดี		มากไป

8. เมื่อดื่มตัวอย่างแล้ว คุณเห็นว่ารสฝาด (astringency) ของผลิตภัณฑ์ นี้เป็นอย่างไร

☐	☐	☐	☐	☐
น้อยไป		พอดี		มากไป

9. เมื่อดื่มตัวอย่างแล้ว คุณเห็นว่ารสขม (bitterness) ของผลิตภัณฑ์ นี้เป็นอย่างไร

☐	☐	☐	☐	☐
น้อยไป		พอดี		มากไป

10. เมื่อดื่มตัวอย่างแล้ว คุณเห็นว่าความแรงของแอลกอฮอลล์ ของผลิตภัณฑ์ นี้เป็นอย่างไร

☐	☐	☐	☐	☐
น้อยไป		พอดี		มากไป

ข้อเสนอแนะ

.....

.....

.....

ขอขอบพระคุณค่ะ

APPENDIX F

The statistic analysis

Table F1 The statistic analysis of alcohol production rate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.670(a)	8	.334	256.719	.000
Intercept	62.347	1	62.347	47959.402	.000
must	1.180	2	.590	453.850	.000
culture	1.144	2	.572	440.017	.000
must * culture	.346	4	.086	66.504	.000
Error	.012	9	.001		
Total	65.029	18			
Corrected Total	2.682	17			

Table F2 The statistic analysis of sugar consumption rate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.076(a)	8	.635	56.016	.000
Intercept	75.195	1	75.195	6638.078	.000
must	2.175	2	1.087	96.002	.000
culture	2.572	2	1.286	113.521	.000
must * culture	.329	4	.082	7.270	.007
Error	.102	9	.011		
Total	80.373	18			
Corrected Total	5.178	17			

Table F3 The statistic analysis of sugar conversion

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.027[a]	8	.003	1.214	.387
Intercept	38.837	1	38.837	13981.472	.000
must	.002	2	.001	.344	.718
culture	.017	2	.009	3.104	.094
must * culture	.008	4	.002	.704	.609
Error	.025	9	.003		
Total	38.889	18			
Corrected Total	.052	17			

Table F4 The statistic analysis of overall liking of 108 assessors

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	792.479(a)	115	6.891	3.861	.000
Intercept	12642.521	1	12642.521	7083.398	.000
panel	667.563	107	6.239	3.496	.000
acidity	.343	2	.171	.096	.909
sugar	67.898	2	33.949	19.021	.000
acidity * sugar	30.009	4	7.502	4.203	.002
Error	564.000	316	1.785		
Total	13999.000	432			
Corrected Total	1356.479	431			

Table F5 The statistic analysis of overall liking of females

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	473.653(a)	61	7.765	6.218	.000
Intercept	7315.042	1	7315.042	5857.950	.000
panel	324.319	53	6.119	4.900	.000
acidity	15.130	2	7.565	6.058	.003
sugar	100.074	2	50.037	40.070	.000
acidity * sugar	18.741	4	4.685	3.752	.006
Error	192.306	154	1.249		
Total	7981.000	216			
Corrected Total	665.958	215			

Table F6 The statistic analysis liking of color of females

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	273.875(a)	61	4.490	8.032	.000
Intercept	10045.042	1	10045.042	17970.220	.000
panel	249.792	53	4.713	8.431	.000
acidity	.796	2	.398	.712	.492
sugar	.796	2	.398	.712	.492
acidity * sugar	15.074	4	3.769	6.742	.000
Error	86.083	154	.559		
Total	10405.000	216			
Corrected Total	359.958	215			

Table F7 The statistic analysis liking of clarity of females

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	332.278(a)	61	5.447	18.414	.000
Intercept	10168.167	1	10168.167	34373.363	.000
panel	322.944	53	6.093	20.598	.000
acidity	1.167	2	.583	1.972	.143
sugar	2.074	2	1.037	3.506	.032
acidity * sugar	6.704	4	1.676	5.665	.000
Error	45.556	154	.296		
Total	10546.000	216			
Corrected Total	377.833	215			

Table F8 The statistic analysis liking of aroma of females

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	248.278(a)	61	4.070	5.332	.000
Intercept	7848.167	1	7848.167	10281.247	.000
panel	224.444	53	4.235	5.548	.000
acidity	1.056	2	.528	.691	.502
sugar	4.667	2	2.333	3.057	.050
acidity * sugar	13.222	4	3.306	4.330	.002
Error	117.556	154	.763		
Total	8214.000	216			
Corrected Total	365.833	215			

Table F9 The statistic analysis liking of flavor of females

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	475.426(a)	61	7.794	7.637	.000
Intercept	6981.407	1	6981.407	6840.743	.000
panel	339.917	53	6.414	6.284	.000
acidity	4.570	2	2.285	2.239	.110
sugar	81.422	2	40.711	39.891	.000
acidity * sugar	29.342	4	7.335	7.188	.000
Error	157.167	154	1.021		
Total	7614.000	216			
Corrected Total	632.593	215			

Table F10 The statistic analysis of overall liking of males

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	314.056(a)	61	5.148	2.609	.000
Intercept	5400.000	1	5400.000	2736.026	.000
panel	276.556	53	5.218	2.644	.000
acidity	12.056	2	6.028	3.054	.050
sugar	2.722	2	1.361	.690	.503
acidity * sugar	17.278	4	4.319	2.189	.073
Error	303.944	154	1.974		
Total	6018.000	216			
Corrected Total	618.000	215			

Table F11 The statistic analysis liking of color of males

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	404.611(a)	61	6.633	6.624	.000
Intercept	6711.185	1	6711.185	6702.320	.000
acidity	4.965	2	2.483	2.479	.087
sugar	1.385	2	.692	.691	.502
acidity * sugar	6.947	4	1.737	1.734	.145
panel	382.380	53	7.215	7.205	.000
Error	154.204	154	1.001		
Total	7270.000	216			
Corrected Total	558.815	215			

Table F12 The statistic analysis liking of clarity of males

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	436.778(a)	61	7.160	8.961	.000
Intercept	6868.167	1	6868.167	8595.286	.000
acidity	10.019	2	5.009	6.269	.002
sugar	.889	2	.444	.556	.575
acidity * sugar	16.037	4	4.009	5.017	.001
panel	398.194	53	7.513	9.402	.000
Error	123.056	154	.799		
Total	7428.000	216			
Corrected Total	559.833	215			

Table F13 The statistic analysis liking of aroma of males

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	462.264(a)	61	7.578	7.369	.000
Intercept	6048.375	1	6048.375	5881.809	.000
acidity	2.074	2	1.037	1.008	.367
sugar	14.389	2	7.194	6.996	.001
acidity * sugar	8.926	4	2.231	2.170	.075
panel	419.514	53	7.915	7.697	.000
Error	158.361	154	1.028		
Total	6669.000	216			
Corrected Total	620.625	215			

Table F14 The statistic analysis liking of flavor of males

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	364.111(a)	61	5.969	3.308	.000
Intercept	5766.000	1	5766.000	3195.392	.000
acidity	27.630	2	13.815	7.656	.001
sugar	5.167	2	2.583	1.432	.242
acidity * sugar	5.815	4	1.454	.806	.523
panel	307.111	53	5.795	3.211	.000
Error	277.889	154	1.804		
Total	6408.000	216			
Corrected Total	642.000	215			

VITA

Ms. Vijuckana Navarattara was born on 10 September, 1981, in Bangkok, Thailand. She studied at Assumption University, Faculty of Biotechnology, majoring in Agro-Industry, and received Bachelor of Science in 2003. In 2005, she enrolled in the master degree program in Food Science and Technology (English program) at Department of Food Technology, Faculty of Science, Chulalongkorn University. She completed her master degree program in the 2008 academic year. Her present address is 79/76 Sukhumwit road, Bangna, Bangkok, 10250.