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Tangerine wine fermentation and acceptability of formulated wine

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Abstract - This research studied on fermentation of wine from Sai Nam Phueng tangerine (*Citrus x aurantium L.*) juice and evaluated the acceptability of tangerine wine formulations. The experiment with100% juice chaptalized (25% reducing sugar) fermented under 30°C by single and mixed cultures (*Saccharomyces cerevisiae* and *Saccharomyces bayanus*). The fermentation profiles ~~of~~ were investigated until alcohol reached 12%. Based on fermentation profile, single-*S. bayanus* performed the fastest fermentation rate. Next, wine fermentation using *S. bayanus* was studied by varying reducing sugar of the must (16-25% reducing sugar). Results showed basic wine made from 22% reducing sugar must (contained 1.93% sugar, 11% alcohol, and 0.38% titratable acidity) was selected for further formulation. The basic wine was 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 was evaluated by fruit wine consumers (54 females and 54 males). Different acceptability of formulated wines was found between female and male assessors. In female assessors, 5 out of 9 wine formulars were significantly accepted ($p \leq 0.05$) with the liking score higher than 6 (form 9-point hedonic scale), as both sugar content and acidity were driving factors affecting the liking of flavor and overall liking. The most accepted formula was the wine contained 6%sugar and 0.5%acidity (7.13±1.33). Although the acidity was the key factor that played role the liking scores in male assessors, all formulated tangerine wines were significantly ($p \leq 0.05$) unaccepted (the liking score lower than 6).

Keywords: Fruit wine, formulation, Sai Nam Phueng, tangerine juice

1. Introduction

Tangerine (*Citrus reticulata*) is a citrus fruit widely grown in Thailand. Several popular cultivars of the tangerine such as Bang Mod, Shogun and Sai Nam Phueng *(Citrus x aurantium L.)* are generally consumed in Thailand. Tangerine gives sweet and sour taste and unique citrus flavor. 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 (Kimball, 1999; Chanprasartsuk & Prakitchaiwattana, 2015). 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 *S. bayanus* have been studied and introduced to the yeast starter market allowing the wine maker to select 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 multi-starter culture could be an alternative to improve the wine fermentation. There are many studies 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; Chanprasartsuk and Prakitchaiwattana, 2015).

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 *S. 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. 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. 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. 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.

2. Materials and methods

2.1 Fermentation profile determination

2.1.1. Study on effect of starter culture

The tangerine juice (100% UHT Tangerine juice Tipco, Thailand), which had physical and chemical properties as shown in Table 1, was prepared as the must for wine fermentation. Carbon and nitrogen sources were adjusted to 25% (w/v) and 0.05% (w/v) by 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, 2020).

Table 1. The physical and chemical properties of tangerine juice

Tangerine juice properties	Value ± S.D.
Color (CIELAB system)	
L*	30.60 ± 0.05
a*	8.95 ± 0.05
b*	49.32 ± 0.09
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

Two yeast cultures; *S. cerevisiae*, baker's yeast (Angel®, China) *and S. bayanus* EC1118, wine yeast (Lavin®, Australia), were used. 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.

The tangerine must was fermented using each single culture or the mixed starter culture. The starter culture was inoculated into the must at initial population 10^6 CFU/mL and fermented under anaerobic condition at 30 °C until alcohol content reached 12%. The fermentation profiles of fermented juice were monitored daily; by determination for alcohol and sugar content, yeast population, titratable acidity and color, using these following methods. The alcohol content of fermented tangerine juice was observed by vinometer. The reducing sugar content of fermented tangerine juice was investigated by Lane-Eynon method (A.O.A.C., 1995). The titratable acidity of fermented tangerine juice was investigated by titration with 0.1N NaOH (A.O.A.C., 1995). 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). The color of fermented tangerine juice was determined in CIELAB system by Chromameter CR-300 connected with CT-310. This experiment was conducted in 2 replications. The starter culture which performs the most efficient fermented profile was selected for the next step.

2.1.2. Study on effect of initial sugar in tangerine must

This part aimed to evaluate a proper initial sugar content in tangerine must fermented with the starter culture selected from 2.1.1 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 4 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 daily. The fermentation profile of 4 conditions were investigated as in 2.1.1. The primary sensory quality was also investigated by researchers. A basic wine fermented from tangerine juice was selected for the next part.

2.2 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 selected basic wine obtained from previous part was formulated by adjusting reducing sugar content and acidity. The reducing sugar of the formulated wine was adjusted into 3 levels; 2, 4 and 6%(w/v). This range represents as semi-sweet wine (1-3% reducing sugar) and sweet wine (>3% reducing sugar) (Lea & Piggott, 2003). Total titratable acidity of wine was adjusted into 3 levels; 0.50, 0.70, and 0.90% (w/v). Jackson (2020) reported that the accepted range of acidity in wine was 0.55 to 0.85%(w/v). For preparation of the 9 wine formulations, the 950 ml. of selected basic wine (from 2.1) was added with 50 ml. sugar mixed with citric acid solution. The 350 ml of tangerine wine was filled into the amber glass bottle (400 ml) 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 stored in the refrigerator (4°C) for a few days before subjecting to the acceptance test.

The sensory acceptance test of the 9 formulas of formulated tangerine wine, prepared as a 3x3 factorial experiment, was conducted in a Balanced Incomplete Block (BIB) design by 108 panelists. Each assessor evaluated only 4 out of 9 samples. Thus, each sample was evaluated by 24 panelists. The acceptance test was conducted at a restaurant in Pattanakarn district, Bangkok. All volunteer panelists (54 females and 54 males), age over 20 years old, were recruited by interception after their dinner at the restaurant. They were screened as alcohol beverage and fruit wine consumers.

For each sample serving, 30 ml of formulated wine was served chill in clear transparent glass containers covered with wrapping film and coded with a 3-digit random number. Each assessor evaluated 4 samples (out of 9 formulars) in the random serving order. 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". Water was used as palate cleanser between test samples.

2.3 Statistical analysis

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

3. Results and discussions

3.1 Fermentation profile of single and mix culture yeasts in chaptalized tangerine must

The fermentation profiles of three types of culture in chaptalized 100% tangerine juice (must) are shown in Fig 1. The result showed that *S. bayanus* performed the fastest fermentation rate which the fermentation completed to 12% alcohol within 5 days, while *S. cerevisiae* and the mixed culture similarly reached alcohol 12% within 7 days (Figure 1a). The increasing of population number of *S. bayanus* at the initial stage of the fermentation was significantly larger than *S. cerevisiae* and mixed culture and showed the fastest sugar consumption rate (Figure1b and 1c). As shown in Table 2, fermentation with *S. bayanus* consumed 3.70% sugar per day and rapidly generated alcohol at rate 2.61% per day. Whereas the alcohol production rate of mixed culture and *S. cerevisiae* batches were much slower. The sugar content in the finish wine fermented with *S. cerevisiae* also higher than mix culture and *S. bayanus*, respectively (Figure 1b). This was inconsistent with the previous studies (Chanprasartsuk and Prakitchaiwattana, 2015). 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* (Baker's yeast) during the fermentation. Therefore, it is possible that this yeast could be also a species specific for tangerine juice fermentation since it could perform an efficient fermentation which indicated by performing efficient sugar consumption and rapid alcohol generation. This observation indicated that yeast species and culture types performed different fermentation profiles.

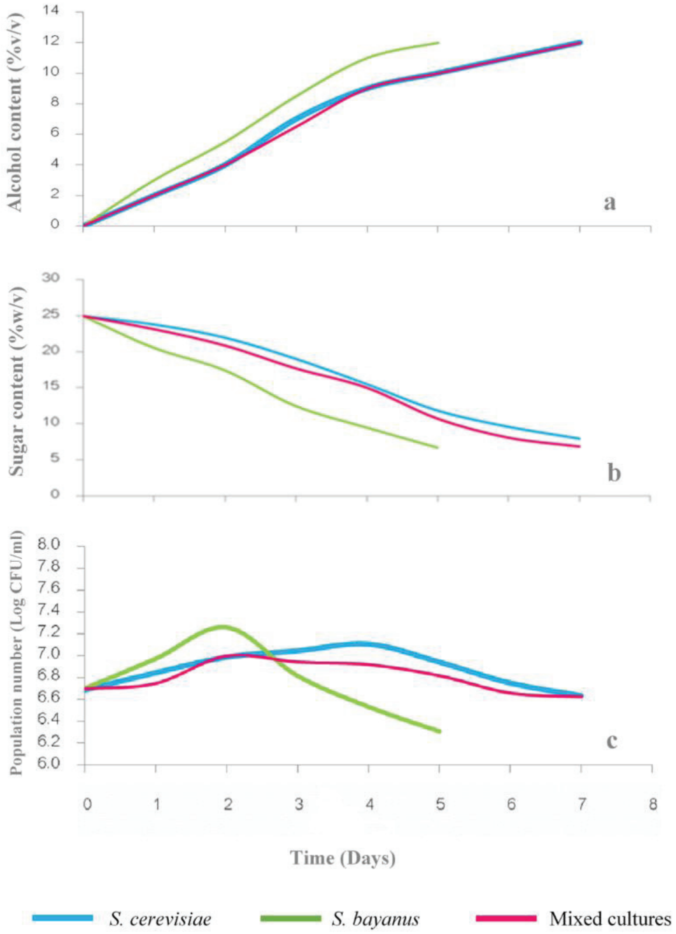

Figure 1. The changes of alcohol content (a) sugar content (b) yeast population number (c) during fermentation of tangerine juice

Table 2. Fermentation efficiency and properties of tangerine wine fermented with 3 types of yeast culture to reach 12%v/v alcohol content

Fermentation characteristics	Starter cultures		
	S. cerevisiae	***S. bayanus***	**Mixed cultures**
Fermentation time (day)	7	5	7
% reducing sugar	5.12[a] ± 0.18	3.55[b] ± 0.15	3.47[b] ± 0.22
% titratable acidity [NS]	0.32 ± 0.07	0.32 ± 0.05	0.32 ± 0.00
alcohol production rate (per day)	1.91[b] ± 0.00	2.61[a] ± 0.00	1.90[b] ± 0.00
sugar consumption rate (per day)	2.66[b] ± 0.00	3.70[a] ± 0.13	2.81[b] ± 0.06
% sugar conversion [NS] (% sugar / % alcohol)	1.42 ± 0.04	1.52 ± 0.05	1.51 ± 0.07

[a,b,...] value with significantly difference in each row are indicated by different letters. ($p \leq 0.05$)
[NS] value with non-significant difference in each rate are indicated ($p > 0.05$)

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, 2020). 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. For appearance of the wine, the color of 3 basic wine was not different from the tangerine juice must (data was not shown).

As the most efficient fermentation profile, the single culture *S. bayanus* was used to make wine from tangerine must with different initial sugar content. The result showed in Table 3.

Table 3. Fermentation efficiency and properties of tangerine wine from must contained different initial sugar concentrations fermented by *S. bayanus*

Fermentation characteristics	Initial sugar content in must			
	16%	19%	22%	25%
Fermentation time (day)	3	5	5	5
Maximum obtained alcohol content	8%	10%	11%	12%
% reducing sugar	$1.16^{d} \pm 0.03$	$1.41^{c} \pm 0.05$	$1.93^{b} \pm 0.11$	$3.86^{a} \pm 0.41$
% titratable acidity	$0.32^{b} \pm 0.05$	$0.32^{b} \pm 0.13$	$0.38^{a} \pm 0.07$	$0.38^{a} \pm 0.13$
alcohol production rate (per day)	$2.67^{a} \pm 0.00$	$2.00^{d} \pm 0.00$	$2.20^{c} \pm 0.00$	$2.40^{b} \pm 0.00$
%sugar conversion (%sugar / %alcohol)	$1.77^{a} \pm 0.03$	$1.64^{b} \pm 0.06$	$1.75^{a} \pm 0.07$	$1.57^{c} \pm 0.07$

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

The fermentation profiles from must with different initial sugar content were significantly different ($p \leq 0.05$). 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, 2020). From (Table 3), wine from the must with 25 % initial sugar had the highest % sugar content but has lower % sugar conversion than the must with 22% initial sugar. 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.

The percentage of total titratable acidity in wine was lower than the tangerine juice must (Table 1 & Table 3). This 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. 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; 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.

Therefore, the tangerine juice chaptalized to have 22% reducing sugar which 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 primary sensory evaluation.

3.2. Evaluation of an acceptability of formulated tangerine wine

The sensory acceptance of the 9 formulated wines, evaluated by 108 fruit alcohol and fruit wine consumers (54 females and 54 males) by BIB design, were shown in Table 4., the result by all panelists of showed that the formulas containing higher reducing sugar content were more accepted than the formulas with lower levels. The statistical analysis showed that there were significant effects of the sugar and the interaction between sugar content and acidity ($p \leq 0.05$) on the "overall liking" score, and the effect of acidity was not significant ($p > 0.05$). Interestingly, significantly difference in the acceptance between gender groups was shown. ($p \leq 0.05$). The overall liking score of the tangerine wine from female panelists was higher than from male panelists. Therefore, the data were separately analyzed by gender.

Table 4. The means of overall liking scores of the 9 formulas (using 9-points hedonic scale)

Formulated wine			All panelists (n = 24)*	Female Panelists (n_1 = 12)*	Male Panelists (n_2 = 12)*
No.	%reducing sugar	%acidity			
1	2%	0.50%	$4.6^{c} \pm 1.9$	$5.1^{de} \pm 1.8$	$4.1^{c} \pm 1.9$
2		0.70%	$4.8^{c} \pm 1.7$	$4.6^{e} \pm 1.5$	$5.0^{abc} \pm 1.8$
3		0.90%	$4.8^{c} \pm 1.6$	$4.6^{e} \pm 1.7$	$5.0^{abc} \pm 1.4$
4	4%	0.50%	$5.6^{ab} \pm 1.7$	$6.1^{bc} \pm 1.3$	$5.1^{ab} \pm 1.9$
5		0.70%	$5.1^{bc} \pm 1.9$	$5.6^{cd} \pm 2.0$	$4.6^{bc} \pm 1.7$
6		0.90%	$5.7^{ab} \pm 1.8$	$6.1^{bc} \pm 1.9$	$5.3^{ab} \pm 1.7$
7	6%	0.50%	$6.1^{a} \pm 1.8$	$7.1^{a} \pm 1.33$	$5.0^{abc} \pm 1.5$
8		0.70%	$5.9^{a} \pm 1.5$	$6.6^{ab} \pm 1.10$	$5.1^{ab} \pm 1.5$
9		0.90%	$6.1^{a} \pm 1.6$	$6.4^{b} \pm 1.50$	$5.8^{a} \pm 1.7$

* Based on BIB design, each sample was evaluated by 24 panelists (each person evaluated 4 samples)
[a,b,c,….] value with significantly difference in each column are indicated by different letters. ($p \leq 0.05$)

For male, the acidity was only one factor that significantly affected on the "overall liking" score (p≤0.05). However, all formulated 9 wine was not well accepted, none had the liking score over than 6. For female group, the result showed that % reducing sugar was the factor affecting overall liking as the sweeter wine (6% reducing sugar) was preferred to the 4% sugar. The semi-sweet wine (2% reducing sugar) was not accepted (the means of overall liking were lower than 6, from the 9-point hedonic scale).

Table 5 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 panelists. Appearance of all 9 formulas was accepted, with the means of "liking of color" and "liking of clarity" were higher than 6 (like slightly). For liking of aroma and flavor, 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, as shown significantly effects of the interaction effect between reducing sugar and acidity (p≤0.05). The liking score of flavor, might be a key driver of the overall like, as it showed a similar trend. The formula no 7. with 6% reducing sugar and 0.5% acidity had the highest score for "liking of flavor" (p≤0.05).

Table 5. The means of liking scores of the 9 formulas from the female panelists (using 9-points hedonic scale)

Formulated wine			mean liking scores			
No.	**%reducing sugar**	**%acidity**	**color**	**clarity**	**aroma**	**flavor**
1	2%	0.50%	$7.1^{ab} \pm 1.2$	$7.3^{a} \pm 1.4$	$5.5^{d} \pm 1.3$	$4.8^{d} \pm 1.7$
2		0.70%	$6.9^{bc} \pm 1.3$	$6.8^{cd} \pm 1.4$	$6.1^{abc} \pm 1.3$	$5.0^{cd} \pm 1.4$
3		0.90%	$6.5^{cd} \pm 1.1$	$6.6^{d} \pm 1.2$	$6.3^{ab} \pm 1.1$	$4.5^{d} \pm 1.5$
4	4%	0.50%	$6.4^{d} \pm 1.8$	$6.9^{bcd} \pm 1.5$	$5.8^{bcd} \pm 1.3$	$5.5^{c} \pm 1.4$
5		0.70%	$6.6^{cd} \pm 1.4$	$6.8^{cd} \pm 1.3$	$6.0^{bcd} \pm 1.4$	$5.5^{c} \pm 1.9$
6		0.90%	$7.3^{a} \pm 1.1$	$7.1^{ab} \pm 1.2$	$6.1^{abc} \pm 1.2$	$6.3^{b} \pm 1.7$
7	6%	0.50%	$7.3^{a} \pm 1.1$	$7.0^{abc} \pm 1.3$	$6.6^{a} \pm 1.3$	$7.0^{a} \pm 1.6$
8		0.70%	$6.6^{cd} \pm 1.2$	$6.8^{cd} \pm 1.4$	$6.3^{ab} \pm 1.5$	$6.4^{b} \pm 1.4$
9		0.90%	$6.6^{cd} \pm 1.2$	$6.6^{d} \pm 1.3$	$5.6^{cd} \pm 1.2$	$6.3^{b} \pm 1.3$

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

According to this study, suggesting that female consumers could be a potential target market for the tangerine wine. The most accepted formulated tangerine wines was the tangerine wine with 11% alcohol, contained 6% of reducing sugar with 0.5% acidity. Comparing 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. Conclusion

The optimum condition for 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 *S. 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% per day and generated the alcohol at rate 2.57% per day 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.

The acceptance of formulated tangerine wines by female panelists were higher than male assessor. 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 panelists whereas the sweetness and flavor property were the key that played role the liking scores. The most accepted formula was the wine contained 6% sugar and 0.5% acidity. It could be a potential alcoholic beverage for lady market.

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