JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EVALUATING THE VOLATILE COMPOUNDS DURING ALCOHOLIC FERMENTATION OF DIFFERENT *SACCHAROMYCES CEREVISIAE* STRAINS IN MIXED JUICES OF CASHEW APPLE AND LONGAN

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doi: 10.15414/jmbfs.2021.10.4.598-603

ARTICLE INFO

Received 22. 9. 2019
Revised 12. 10. 2020
Accepted 15. 10. 2020
Published 1. 2. 2021

Regular article

OPEN ACCESS

ABSTRACT

The purpose of this research was to evaluate the microbiological, chemical, and volatile compounds of mixed juices comprised of cashew apple and longan fermented with two yeast strains of *Saccharomyces cerevisiae* (C12 and EC1118). The two fermentations in the mixed juices were similar physicochemical parameters (the growth of yeast, pH, total acidity, total soluble solids, and total sugar). The most abundant of the volatile compounds in these wines were ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, ethanol, isobutanol, isoamyl alcohol, 2-phenylethanol, β-ocimene, and acetic acid. Seven volatile compounds in the wines had odor activity values (OAVs) higher than 1, and also were selected and quantified (ethyl octanoate, ethyl hexanoate, ethyl decanoate, isoamyl acetate, isoamyl alcohol, 2-phenylethanol, and linalool). Ethyl octanoate (fruity, floral, and pineapple) had the highest OAVs in both of the mixed juices fermented with the yeast strains C12 and EC1118. The *S. cerevisiae* strain EC1118 can significantly produce the highest total volatile contents (359.23 mg/L), and we also found a higher level of total ester (95.50 mg/L) in the wines. Additionally, the *S. cerevisiae* strain EC1118 can provide a higher content of fruity aroma in wines, as indicated by the calculation of OAVs. The sensory analysis of the wines was acceptable to all the panelists, who gave color, clarity, taste, flavor, and overall acceptance scores as "like slightly" to "like moderately."

Keywords: *Saccharomyces cerevisiae,* cashew apple, longan, odor activity values, aroma

INTRODUCTION

Cashew (*Anacardium occidentale* Linn var. *nanum*) is a tropical fruit that was first grown in South America, and then spread to countries such as Thailand, Vietnam, and India **(Deenanath *et al.*, 2015; Kaewbutra *et al.*, 2016)**. The peduncle of the cashew fruit, known as the cashew apple. The composition of the peduncle is rich in tannins, reducing sugars (fructose and glucose), minerals such as calcium, iron and phosphorus, vitamin C, and some amino acids **(Pereira and Rodrigues, 2012)**. It has also been reported to exhibit many medicinal properties, such as antitumor, antimicrobial, and antioxidant activity **(Kaewbutra *et al.*, 2016)**. Cashew apples can be served fresh or processed as canned fruits, syrup, juice, pickles, jam, chutney, or candy **(Gawankar *et al.*, 2018)**. So, they can serve as a good raw material for wIinemaking. Various compounds have been found during cashew apple wine fermentation, such as ethyl butyrate, ethyl 3-methyl butyrate, methyl butyrate, methyl 3-methyl pentanoate, methyl 3-methyl butyrate, and trans-ethyl crotonate **(Garruti *et al.*, 2006)**. However, there have only been a few studies on fermented-beverage products from cashew apples. Longan (*Dimocarpus longan* Lour) is an important plantation crops in Thailand. It can be used for value-added products such as dried and canned longan. The main nutritional components of dried longan are fructose, sucrose, glucose, amino acids (proline, alanine, aspartic acid, tyrosine, serine, leucine, isoleucine, valine, and glycine), vitamins, and minerals. The major volatile compounds from dried longan include ocimenes, furfural, 5-methyl furfural, benzenemethanol, isoamyl alcohol, 2-furancarboxylic acid ethyl hexadecanoate, and linalool. However, caramelization and the Millard reaction have an effect on the formation of aromatic substances during longan drying process (**Chang *et al.*, 1998**).

Cashew apple and longan are agricultural products in Thailand, and are suitable to produce wine because they have a rich amount of amino acids and sugar for yeast culture. Cashew is widely cultivated in many regions of Thailand. It has been recognized as one of the new economic plants **(Kaewbutra *et al.*, 2016)**. Longan is interesting to winemaking because it's a processed economic agricultural product in Thailand. Previous studies have also shown that dried longan wine has a pleasant aroma **(Sanoppa *et al.*, 2019; 2020)**. *Saccharomyces cerevisiae* is one of the most important yeast strains used in winemaking, which play role fermentation in juices and convert sugar ethanol, carbon dioxide, and others compounds and also are tolerant to high ethanol, high sugar, acidic, and anaerobic conditions **(Chen and Liu, 2016)**. *S. cerevisiae* is one of the yeast strains suitable for producing quality fermented foods and beverages **(Carrau *et al.*, 2008)**. Yeasts produce secondary metabolites which play as an important wine flavor, including esters, carbonyls, sulfur compounds, and higher alcohols. These are important in determining the aroma and flavor profiles of the wine (**Walker and Stewart, 2016**).

In this work, the mixed juices of cashew apple and longan were used as raw material for wine fermentation; this had never previously been reported in wine. The aim of this work was to evaluate the chemical, yeast growth and volatile compounds of the mixed juices of cashew apple and longan fermented with two yeast strains of *S. cerevisiae* (C12 and EC1118). This research was to investigate the use of different strains of yeast to aromatic profiles of wine especially, wine that is made from a mixture of fruit. The results will indicate the information to select *S. cerevisiae* strains for aroma profiles formations of wine, and to develop this industry.

MATERIALS AND METHODS

Microorganism and culture media

Two strains of *Saccharomyces cerevisiae* var. *bayanus*, C12 and EC1118 were obtained from Blue H_2O Filtration Pty Ltd. (Australia) and Lallemand Inc. (Australia), respectively. The freeze-dried yeasts were propagated in a sterile nutrient broth (0.25% w/v yeast extract, 2% w/v glucose, 0.25% w/v malt extract, and 0.25% w/v bacteriological peptone at pH 5.0; Y) under static conditions at 25 ^{O}C without agitation for 48 h, and finally stored at -80 ^{O}C in YM medium with adding glycerol 20%v/v before use. Yeast was grown in Yeast Malt broth (YM broth) (Difco, USA) and maintained on Yeast Malt agar (YM agar) (Difco, USA).

Substrate preparation and fermentation

Cashew apples and dried longans were obtained from Thailand, respectively. Dried longan was further dried in a hot-air dryer at 70 ^{O}C for 8 hours. First, cashew apples (1 kilogram) were cleaned in tap water. The cashew apples were crushed in a blender and then passed through a filter (The initial Brix of cashew apples juices was 12 ^{O}Brix). 100 grams of dried longans were mixed with 300 mL of distilled water. The sample was homogenized to form longan juice using a blender, and then it was passed through a filter (The initial Brix of dried longans juices was 14 ^{O}Brix) **(Mohanty *et al*., 2006; Sanoppa *et al*., 2019; 2020)**. The cashew apples juices and dried longans juices were mixed at a ratio of 1:1 (v/v). Then, 200 mL of the mixed juices of cashew apple and longan were put into 500 mL Erlenmeyer conical flasks and cotton plugged. Both the cashew apple and longan juices were adjusted to pH 3.5 with 50% w/v malic acid and the Brix adjusted to 20.0% (The initial Brix of mixed juices was 13 ^{O}Brix) with pure sucrose, and then 100 ppm of potassium metabisulfite (KMS) was added in the juices **(Chen and Liu, 2014; 2016)**. Fermentations of the sterile mixed juices of cashew apple and longan were conducted under static conditions at 25 ^{O}C for 14 days. *S. cerevisiae* was inoculated with 1×10^6 CFU/mL.

Microbiological and physicochemical analysis

During fermentation, wine samples were taken at these intervals: Day 0, 2, 4, 6, 8, 10, 12 and 14, and these samples were stored at freezes (−20 °C) until the analysis was done. Yeast enumeration was done by spread-plating on YM agar. The wines were analyzed pH value by pH meter, assessing total acidity content by titration to the pH endpoint with 0.05 M NaOH **(A.O.A.C, 2005)**, total soluble solids (°Brix value) was estimated by a hand refractometer and the phenol-sulfuric acid method was used to measure the total sugar content **(Agrawal *et al*., 2015)**.

Volatile compounds

The identification of volatile compounds was performed using the headspace solid-phase microextraction (HS-SPME) technique with a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm, Supelco, Bellefonte, PA, U.S.A.) The determination was performed in combination of Gas chromatography mass spectrometry (GC/MS) (Agilent 7890B, detector on a DB-Wax UI column. The volatile compounds were performed based on a previously published method by **Sanoppa *et al*. (2019; 2020).**
The odor activity values are defined as the ratio between c is the total concentration (mg/L) of each volatile compound in the wines, and t is the odor threshold value (mg/L) (OAVs =c/t) **(Cheng *et al*., 2015)**.

Sensory analysis

The sensory attributes of the mixed fruit wines (such as color, clarity, flavor, taste, and overall acceptance) were evaluated using a 9-point Hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely) among 30 panelists (10 women, 20 men, aged 20-45) selected from staff and the postgraduate students in the Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok who are familiar with wine consumption. The wine samples (50 mL) were evaluated using transparent glasses which were labeled with random three-digit numbers **(Ray *et al*., 2012)**. Another set of the mixed fruit wines and grape wine (commercial wine) were evaluated as a second replication the following day **(Mohanty *et al*., 2006)**.

Statistical analysis

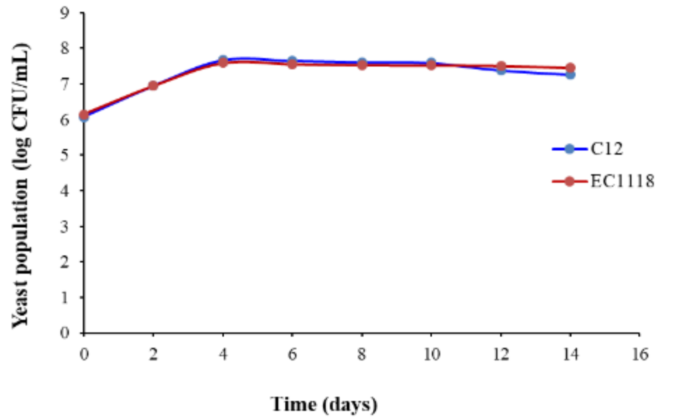
All experiments were performed in triplicate. Data are expressed as the mean ± standard deviation (SD). The results were compared by ANOVA using the SPSS Statistics package version 20.0 at a significance level of p <0.05 (SPSS Inc., Chicago, IL). Principal component analysis (PCA) was used to analyze the data of volatile compounds in wines with the software Minitab 18.

RESULTS AND DISCUSSION

Microbiological and chemical parameters

During the fermentation of the mix of the cashew apple and longan juices, the two strains of *S. cerevisiae* yeasts exhibited similar yeast population (Fig. 1A). Both strains' yeasts culture achieved yeast population of approximately 1.0×10^{-6} – 5.0×10^{-7} CFU/mL, which was maintained until the end of the process. The maximum yeast populations in the wines of strains C12 and EC1118 were 7.67 and 7.59 log CFU/mL, respectively, on the 4th day. The growth of yeast in the wines during the first four days of fermentations rapidly increased, and gradually decreased as the wine fermentation time increased.
The chemical compositions of the wines produced by two strains of *S. cerevisiae* are presented in Figures 1B, 1C, 1D, and 1E. As shown in Figure 1E, the pH of the wines by the two strains increased slightly after fermentation, ranging from pH 3.5–3.9. Similar results in papaya wines were published by **Lee *et al.* (2010; 2013)** found in durian wine and **Chen and Liu (2014)** for lychee wine In addition, the total acid content remained relatively constant during fermentation (0.11–0.12%) (Fig 1D), which was similar to previous results reported by **Okeke *et al.* (2015)**, who found during the fermentation period, the pH of mixed pineapple and watermelon wine was in the acidic range, which was also similar to research published by **Balogun *et al.* (2017).** As the result, the pH values of the wines were correlated with total acidity values. The total soluble solid decreased initially and then tended to stabilize. The Brix decreased from about 20% to approximately 9% for all wines after fermentation (Fig 1C). The Brix value was reduced most quickly in both strains of the *S. cerevisiae* cultures. Figure 1E shows the change in total sugar during fermentation in both strains *S. cerevisiae* cultures. The total sugar of the wines decreased slightly during fermentation, from around 6% to 0.4%. The total sugar value was reduced more quickly in wine fermented with EC1118 than with C12.

(A)



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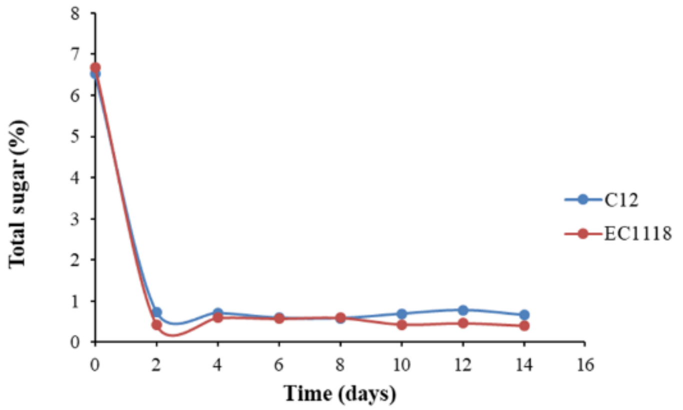

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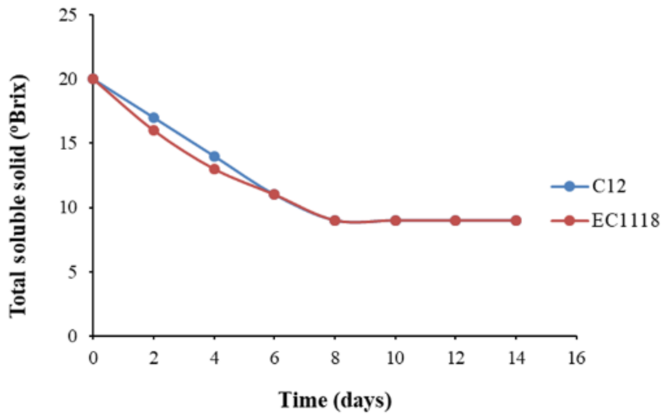

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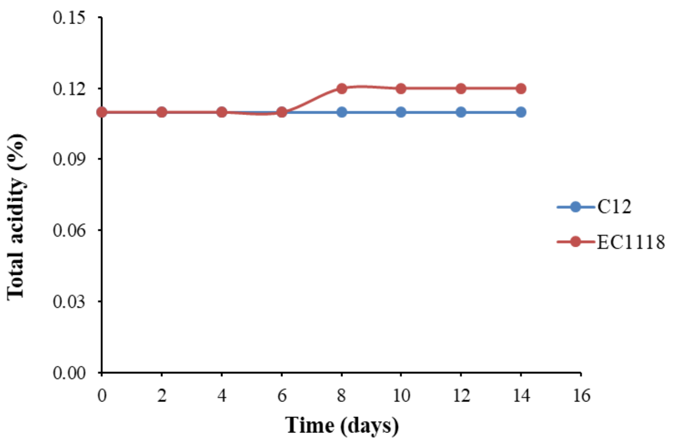


(E)

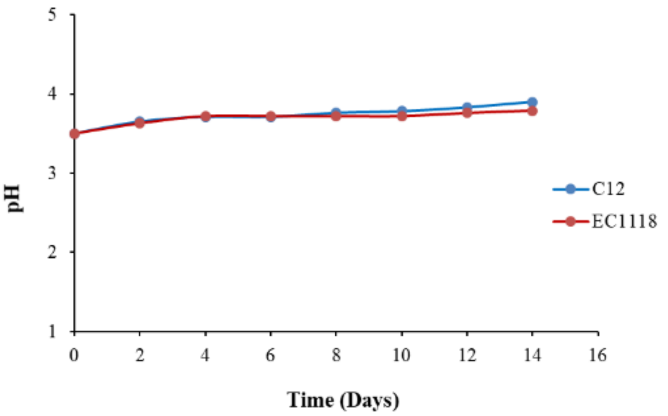


Figure 1 The growth of yeasts (CFU/mL) (A), the total sugar content (%) (B), the total soluble solids (^{O} Brix) (C), the total acidity (%) (D) and pH value (E) of the mixed juices of cashew apple and longan fermented with two *S. cerevisiae* yeasts.

Volatile compounds

We determined the aromatic profiles of wines produced by different yeast strains. During the fermentation of the mixed juices of cashew apple and longan, volatile compounds were produced by yeast. Twenty-one volatile compounds were identified in the wines, including nine esters, five alcohols, three terpenes, one acid, and three others (Table 1). Alcohols comprised the largest group of volatiles in the mixed wines (240 mg/L to 260 mg/L). Higher alcohols (fusel alcohols) are quantitatively major volatile by yeast, and also can have an aromatic effect in wines. Thus, such alcohols are the most important group of compounds that *S. cerevisiae* produces during fermentation **(Swiegers *et al*., 2005; Parapouli *et al*., 2020)**. Ethanol, isobutanol, isoamyl alcohol, γ-methylmercaptopropyl alcohol, and 2-phenylethanol were the main alcohols produced by the two strains of *S. cerevisiae*. Ethanol was the most abundant of the alcohols in the wines. The ethanol content produced by strain C12 importantly exhibited the highest in the wines. Similar studies have found that the concentration of ethanol in dried longan wines ranged from 130-160 mg/L **(Sanoppa *et al*., 2019; 2020)**, whereas the alcohol content of cashew apple wine was 5.0% (v/v) **(Mohanty *et al*., 2006)**. In addition, isobutanol, isoamyl alcohol, γ-methylmercaptopropyl alcohol, and 2-phenylethanol can be synthesis by two strains, with no significant differences among the cultures. Isoamyl alcohol and 2-phenylethanol can contribute to the flowery and sweet notes of wine, and isobutanol was detected above their threshold **(Zhang *et al*., 2018)**. The Ehrlich products of leucine and valine derived from isoamyl alcohol and isobutanol. These are considered as cheap "fusel alcohols", whereas the phenylalanine derived 2-phenylethanol **(Swiegers *et al*., 2005; Etschmann *et al*., 2008)**. Higher alcohol synthesis is correlated with α-keto acid synthesis. This occurs via the catabolic or Ehrlich pathway or anabolic pathway, which involves amino acids synthesis via their biosynthetic pathway from glucose. The catabolism process of branched-chain amino acid is transaminated to form α-keto acid (α-ketoisocaproic acid from leucine; α-ketoisovaleric acid from valine; and α-keto-β methylvaleric acid from isoleucine), and then decarboxylation reaction involves to the synthesis of acids, aldehydes, and alcohols **(Dickinson and Norte, 1993; Swiegers *et al*., 2005)**. The higher alcohols concentrations are reached, however, are strongly related to the strain used **(Carrau *et al*., 2008)**.

Esters are the important volatiles in the mixed fruit wines. Most of the esters were synthesized during fermentation from acyl-CoA and alcohol via yeast-alcohol acyltransferase, and this can also can have an effect on the fruity flavors in wine **(Swiegers *et al*., 2005)**. Esters are catalyzed by an acyl transferase or ester synthase. The reaction requires energy provided by the thioester linkage of the acyl-CoA co-substrate. There are two main groups of flavor-active esters in wines: the ethyl esters and the acetate esters **(Saerens *et al*., 2010)**. ethyl decanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, and 2-phenylethanol were the main esters in the wines (Table 1). Similar results were reported by **Zhang *et al.* (2018)**, who found five esters above the threshold and also found to be consistent with commercial wine found their esters, which have a potential impact on the aroma profile **(Swiegers *et al*., 2005)**. Among the two treatments, the mixed juices culture with *S. cerevisiae* EC1118 generated the highest total esters that there was no significant difference among the culture with *S. cerevisiae* C12 inoculation.

The first group comprises the ethyl esters (the alcohol group is ethanol; the acid group is a medium-chain fatty acid). The biosynthesis of fatty acid ethyl esters (FAEEs) proceeds by alcoholysis or esterification. Alcoholysis mechanism is the synthesis of esters from acylglycerols (alcohols and fatty acyl-CoAs derived from the metabolism of fatty acids) and alcohols. Alcoholysis is important for transferase reaction in which fatty acyl groups from acylglycerols or acyl-CoA derivatives are directly transferred to alcohols. Esterification mechanism is the synthesis of esters from carboxylic acids and alcohols, and is catalyzed by enzymatic mechanisms (FAEE synthases/carboxylesterases) **(Saerens *et al*., 2010).** The FAEEs synthesis by mechanism of alcoholysis is catalyzed by enzymatic mechanisms (acyl-CoA:ethanol O-acyltransferases; AEATases), *EEB1*, and *EHT1*. *ATF1* and *ATF2* are not involved in ethyl ester synthesis. *EEB1* is the important enzyme for the synthesis of ethyl ester, while *EHT1* plays a minor role. However, the deletion of *EEB1* and *EHT1* resulted in a severe decrease in the ethyl ester synthesis **(Saerens *et al*., 2005). Trinh et al. (2010**) Depending on the length of the carbon chain of fatty acids, the short-chain ones are more desirable. Studies have shown that the ethyl esters in wines are ethyl heptanoate (grape), ethyl hexanoate (apple), ethyl octanoate (sweet soup), ethyl nonanoate (wax, fruity aroma), ethyl decanoate (floral, soup aroma), and ethyl dodecanoate (sweet, floral, fruity aroma) (Table 1). The concentration of ethyl dodecanoate, ethyl nonanoate, and ethyl heptanoate in the mixed juices cultures with strain C12 had a higher level than in the strain EC 1118. Meanwhile, ethyl octanoate, ethyl decanoate, and ethyl hexanoate produced by yeast strain EC1118 had higher than strain C12, but all of the esters in the wines showed no significant difference in either of the strains.

The second group is acetate esters (the acid group is acetate, and the alcohol group is ethanol or a complex alcohol derived from amino acid metabolism). The acetate esters are produced at much higher levels, so they can have a more significant impact on wine flavor than ethyl esters **(Saerens *et al*., 2010)**. The formation of acetate esters is catalyzed by alcohol acetyltransferases, and encoded by the genes *ATF*1 and *ATF*2, which use an alcohol and acetyl-CoA as substrates **(Swiegers *et al*., 2005)**. As seen in Table 1, the acetate esters in wines were isoamyl acetate (banana and pear aroma) and ethyl acetate (fruity, solvent-like). The results of acetate esters in all the wines correlated with the alcohol content. The formation of acetate ester dependent on two factors, the concentration of the two substrates (acetyl-CoA and alcohol) and also the activity of the Alcohol acetyltransferase (AATase) **(Zhang *et al*., 2014)**, which was consistent with the findings in **Procopio *et al.* (2015),** who reported acetate esters synthesis that depends on the concentration of their corresponding higher alcohols, except for ethyl acetate, whose synthesis depends on the ethanol concentration. Acetate esters synthesis in *S. cerevisiae* (isoamyl acetate and ethyl acetate) is ascribed to three enzymes: isoamyl acetyltransferase, ethanol acetyltransferase, and alcohol acetyltransferase **(Lilly *et al*., 2000)**. The concentration of ethyl acetate and isoamyl acetate were higher in cultured with yeast strain EC 1118, there was no significant difference in the culture with *S. cerevisiae* C12 inoculation.

Additionally, acids and terpenes were detected in the wines. Terpenes provide many floral, fruity, herbal, and spicy odor in wine, which have low odor thresholds **(Zhang *et al*., 2018)**. Monoterpenes were synthesized from terpene-synthase. The terpene synthase gene family has expanded in grapevines, which underlines the importance of terpenoids in this species **(Ilc *et al*., 2016)**. Terpenes, including β-ocimene, α-ocimene, and linalool, were identified in wines (Table 1). β-ocimene and linalool were found in wine inoculated with yeast C12 higher level than inoculated with yeast strain EC1118. On the contrary, α-ocimene in wines produced by yeast strain EC1118 had higher than for that produced by yeast strain C12.

Table 1 Volatile compounds (mg/L) in mixed juices of cashew apple and longan fermented with two *S. cerevisiae* yeasts.

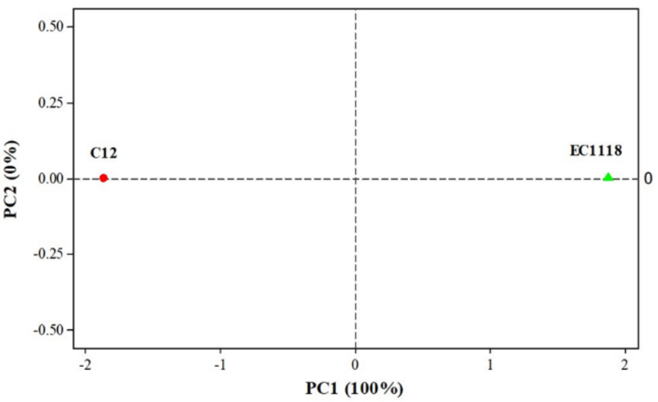
Compounds	Mixed juices		*S. cerevisiae* C12		*S. cerevisiae* EC1118		Odor Threshold value (mg/L)	Odor description
	Total values (mg/L)	OAVs	Total values (mg/L)	OAVs	Total values (mg/L)	OAVs		
Esters								
Ethyl acetate	ND	-	5.47 ± 0.12 ^{NS}	0.66	5.67 ± 0.94 ^{NS}	0.81	7.5*	Fruity, pineapple, sweet
Ethyl decanoate	ND	-	9.19 ± 1.00 ^{NS}	45.95	10.24 ± 0.57 ^{NS}	51.20	0.2**	Fruity, floral, waxy
Ethyl dodecanoate	ND	-	1.98 ± 0.10 ^{NS}	-	1.95 ± 0.11 ^{NS}	-	-	-
Ethyl heptanoate	ND	-	2.03 ± 0.05 ^{NS}	-	1.77 ± 0.07 ^{NS}	-	-	-
Ethyl hexanoate	ND	-	6.22 ± 0.21 ^{NS}	1088.00	14.89 ± 0.37 ^{NS}	1504.00	0.005***	Fruity, apple, strawberry
Ethyl octanoate	ND	-	21.39 ± 0.78 ^{NS}	10255.00	28.59 ± 1.25 ^{NS}	15370.00	0.002*	Fruity, floral, pineapple
Ethyl nonanoate	ND	-	1.44 ± 0.10 ^{NS}	-	0.87 ± 0.04 ^{NS}	-	-	-
Isoamyl acetate	ND	-	13.71 ± 0.88 ^{NS}	375.67	30.56 ± 3.79 ^{NS}	396.00	0.03****	Fruity, banana, sweet
Isobutyl acetate	ND	-	1.04 ± 0.16 ^{NS}	0.65	0.96 ± 0.08 ^{NS}	0.60	1.6**	Fruity, banana, sweet
Sum	ND		62.46 ± 1.18^{NS}		95.50 ± 4.59^{NS}			
Alcohols								
Ethanol	12.38 ± 2.45^{c}	-	160.89 ± 1.89^{a}	-	146.98 ± 3.22^{b}	-	-	-
Isobutanol	ND	-	6.62 ± 0.08 ^{NS}	0.14	9.37 ± 0.58^{NS}	0.07	40*	Fruity, banana
Isoamyl alcohol	ND	-	76.76 ± 3.77 ^{NS}	1.65	61.67 ± 2.84 ^{NS}	1.32	30*	Banana, sweet, rancid, rubber
γ-Methylmercaptopropyl alcohol	ND	-	0.55 ± 0.10^{NS}	-	0.96 ± 0.13^{NS}	-	-	-
2-Phenylethanol	ND	-	15.90 ± 0.34^{NS}	1.47	24.87 ± 1.12^{NS}	1.55	10*	Floral, rose, sweet
Sum	12.38 ± 2.45^{c}		260.72 ± 2.22^{a}		243.85 ± 2.38^{b}			
Miscellaneous								
β-Ocimene	0.39 ± 0.13^{c}	-	10.65 ± 0.36^{a}	-	5.71 ± 0.45^{b}	-	-	-
α-Ocimene	ND	-	0.90 ± 0.05 ^{NS}	-	0.58 ± 0.02 ^{NS}	-	-	-
Styrene	ND	-	2.13 ± 0.09 ^{NS}	-	3.63 ± 0.45 ^{NS}	-	-	-
Linalool	ND		1.29 ± 0.06 ^{NS}	86.00	0.74 ± 0.07 ^{NS}	49.33	0.015*	Floral, herbal, rosewood
Methyl N-hydroxybenzene-carboximidate.	ND	-	2.10 ± 0.06^{a}	-	1.69 ± 0.02^{b}	-	-	-
Acetic acid	0.69 ± 0.15^{c}		8.56 ± 0.06^{a}	0.04	7.54 ± 0.17^{b}	0.04	200*	Vinegar
2,3-Butanediol	0.18 ± 0.10	-	ND	-	ND	-	-	-
Sum	1.27 ± 0.27^{c}		25.62 ± 0.30^{a}		19.89 ± 0.90^{b}			
Total	13.65 ± 0.10^{c}		348.81 ± 2.86^{b}		359.23 ± 6.04^{a}			

[a, b, c, d] Statistical analysis ANOVA (n=3) at 95% confidence level with same letters indicating no significant difference.
ND, not detected; NS, not significant; OAVs, Odor activity values; * **Murnane *et al.* (2013); ** Cullere *et al.* (1997); *** Chen and Liu (2014); **** Chen and Liu (2016); ***** Casassa *et al.* (2019)**

Principal component analysis of volatile compounds in the mixed juices of cashew apple and longan fermented with two *S. cerevisiae* yeasts and Odor activity values

The odor activity values (OAVs) are important for food products, which indicates the contribution of volatile compounds to the aroma. OAVs of the odorants were calculated using the total concentration and the threshold concentration **(Cheng *et al*., 2015).** As shown in Table 1, although a total of 21 volatile compounds were detected in the wines. Generally, volatile aromatic compounds with the OAVs level greater than 1 may potentially contribute to the distinctive aroma of a wine. **(Chen and Liu, 2014)**. Table 1 indicates that seven aroma compounds with OAVs greater than 1 were detected in the two fermentation of wines. As seen in Table 1, seven odorants were selected and quantified: ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, isoamyl alcohol, 2-phenylethanol, and linalool. Ethyl octanoate had the highest total OAV value, followed by ethyl hexanoate, isoamyl acetate, linalool, ethyl decanoate, isoamyl alcohol, and 2-phenylethanol, respectively (Table 1). This finding was in agreement with previous research which demonstrated that the lychee wines had higher OAVs values of ethyl octanoate, and ethyl hexanoate, ethyl octanoate **(Chen and Liu, 2014, 2016)**. Ethyl hexanoate had a high OAVs value. Therefore, ethyl hexanoate is an important volatile compound in four wine varieties **(Cheng *et al.*, 2015)**, as was also confirmed by **Sanoppa *et al.* (2019)**. The OAVs of ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, and 2-phenylethanol in the *S. cerevisiae* EC1118 culture were higher than in strain C12, but there was no significant difference. Regarding these results of this research, there were responsible for the fruity and floral aroma in the mixed fruit wines. Principal component analysis was applied to compare the differences among different fermentations and the volatile compounds of each wine. As shown in Figure 2, the first two principle components accounting for 100% of the overall variance, with PC1 contributed to 100% of the overall variance. The wines obtained from both the *S. cerevisiae* strain C12 and EC1118 cultures could be clearly separated. As a result, the wine cultured with yeast strain EC1118 had higher diversified aroma profiles. The mixed juices cultured with yeast EC1118 was associated with the positive part of PC1, and had high levels of ethyl octanoate, ethyl hexanoate, Isoamyl acetate, ethyl decanoate, and 2-phenylethanol. The fermented wine by yeast C12 was exhibited in the negative part of PC1, which was related to higher contents of linalool and isoamyl alcohol.

(A)



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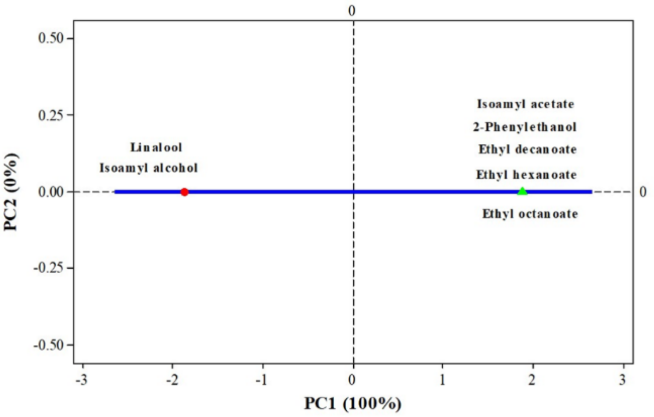

Figure 2 The principal component analysis (PCA) of mixed juice of cashew apple and longan fermented with *S. cerevisiae* C12 and EC1118.
The PCA score plots of *S. cerevisiae* strains (A), Biplot of the PCA between *S. cerevisiae* strains and volatile compounds in wines (B).

Influence of different inoculation yeast strains on Odor activity values of the mixed juices of cashew apple and longan

As a result, seven odorants had OAVs higher than 0.1 (ethyl octanoate, ethyl hexanoate, isoamyl acetate, linalool, ethyl decanoate, isoamyl alcohol, and 2-phenylethanol) (Table 1). The odorants could be defined as an odor group of aromatic series, including the floral, fruity, sweet, and chemical odors groups, as follows: the floral odor (2-phenylethanol, ethyl octanoate, and ethyl decanoate); the fruity odor (isoamyl alcohol, ethyl decanoate, ethyl hexanoate, isoamyl acetate, and ethyl octanoate); the sweet odor (isoamyl acetate, 2-phenylethanol, and isoamyl alcohol); and the chemical odor (isoamyl alcohol) **(Zhang *et al*., 2018)**. As shown in Figure 3, the fruity odor was prominent in the wines, followed by the floral, sweet, and chemical odors, respectively. The ethyl ester of straight-chain fatty acids (ethyl octanoate, ethyl hexanoate, and ethyl decanoate) can provide pleasant fruity and floral odors in the wines **(Englezos *et al*., 2018)**. The strain EC1118 possessed a higher level of fruity, floral, sweet, and chemical odors in the wines. The chemical odor had a lower concentration than 5. The results demonstrated that the desired aromatic quality of the mixed fruit juices was fermented by *S. cerevisiae* EC1118.

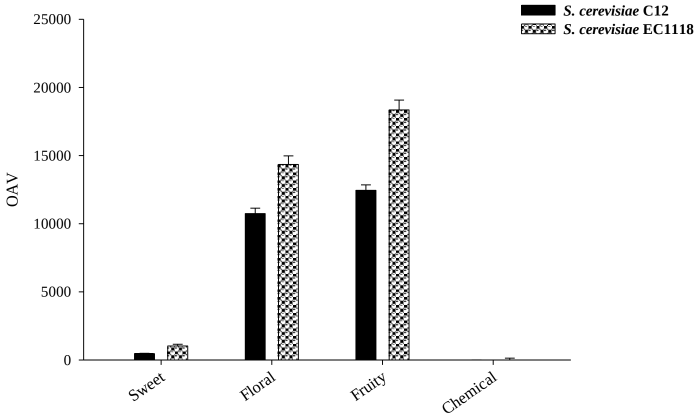

Figure 3 Aroma groups in mixed of cashew apple and longan wines fermented with *S. cerevisiae* C12 and EC1118.

Sensory analysis

We conducted a preference testing for color, clarity, flavor, taste, and overall acceptance (Table 2). There were no significant differences (p<0.05) in terms of these categories among the mixed fruit wines. However, the commercial wine presented significantly higher color, clarity, flavor, taste, and overall acceptance than the mixed fruit wines. The attributes of commercial wines like color, clarity, taste, flavor, and overall acceptance were scored at roughly 8.0 ("like very much"). However, the panelists rated color, clarity, flavor, taste, and overall acceptance scores 6.0-7.3 ("like slightly" - "like moderately"), possibly because the cashew wine had a high tannin content, which imparted a somewhat astringent flavor, as was also confirmed by **Mohanty *et al*. (2006)** in cashew apple wines. Furthermore, ethyl octanoate, isoamyl acetate, and isoamyl alcohol were found in the mixes of cashew apple and longan wines, and were also responsible for a fruity flavor (**Zhang *et al*., 2018)**. Nevertheless, the mixed fruit wines were acceptable to all the panelists. Their overall acceptance score was "like slightly." Similar research was reported by **Mohanty *et al*. (2006)**, who found the panelists accepted the cashew apple juice fermented with *S. cerevisiae*

Table 2 Sensory evaluation of the mixed of cashew and longan wines and commercial wine

Attributes	Commercial wine	The mixed of cashew apple and longan wines	
		S. cerevisiae C12	*S. cerevisiae* EC1118
Color	8.47 ± 0.51[a]	7.23 ± 0.86[b]	7.27 ± 0.64[b]
Clarify	8.43 ± 0.50[a]	6.27 ± 0.58[b]	6.30 ± 0.65[b]
Flavor	8.23 ± 0.50[a]	6.37 ± 0.67[b]	6.33 ± 0.48[b]
Taste	8.43 ± 0.50[a]	6.07 ± 0.69[b]	6.10 ± 0.55[b]
Overall acceptance	8.40 ± 0.67[a]	6.27 ± 0.78[b]	6.37 ± 0.85[b]

CONCLUSION

Cashew apple and dried longan are important economic crops in Thailand and are also an interesting option to produce wine because they contain a wide variety of nutrients. Our purpose was to investigate the effects of the culture of two yeast strains of *S. cerevisiae* on the microbiological, chemical, and volatiles content in the mixed fruit wines. These results demonstrated that both the *S. cerevisiae* strain C12 and EC1118 can increase the aroma intensity and complexity of the wines. Seven odorants had OAVs higher than 1, which can make an active contribution to the wine's aroma. The mixed juices of cashew apple and longan fermented with the *S. cerevisiae* strain EC1118 significantly produced the highest total volatile contents, and we also found a higher total level of esters, but there was no statistically significant difference when compared with the fermentation inoculated with *S. cerevisiae* strain C12. On the contrary, *S. cerevisiae* C12 produced significantly higher concentrations of total alcohol and miscellaneous elements than with *S. cerevisiae* EC1118. As the result, the volatile compounds in the mixed fruit wines correlated with the panelists' sensory evaluations. Those evaluations indicate that the mixed of cashew apple and longan wines imparted the characteristic cashew apple and dried longan and astringency. Therefore, the panelists rated their overall acceptance of the product as "like slightly." However, both the mixed juices of cashew apple and longan cultured with yeast strain C12 and EC1118 exhibited the highest of the fruity odorants group in the mixed fruit wines. The *S. cerevisiae* EC1118 culture positively affected the aroma characteristic.

Acknowledgment: This research was funded by King Mongkut's University of Technology North Bangkok. Contract no. KMUTNB-GEN-57-40.

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