A Study of Lime Beer Formulations

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Abstract

The suitable method to produce low-hopped ale with a lime flavor formulation and the optimum formulation for lime juice beer production was studied in this experimental work. The experiment was divided into three stages. In stage 1, the lime juice concentrations of 0.3, 0.6, 1, 2, and 3% (v/v) were added before and after the primary fermentation, respectively, to determine the optimum lime juice content and time of lime juice addition. In stage 2, hop additions were set to 0.12, 0.14, 0.1% 0.18 and 0.20% to determine the optimum hop addition. In stage 3, the wort boiling temperatures were set to 85-90, 90-95 and 95-100°C to determine the optimum wort boiling temperature. The alcohol content, pH, sugar content, specific gravity, color, total acidity and vitamin C content were tested for each sample and sensory evaluation was held to determine the optimum fermentation factors. Our results showed that the best lime beer in this study was produced by adding 0.3% (v/v) lime juice after primary fermentation. The optimum hop addition was 0.18% w/v and the wort boiling temperature was 95-100°C. The final product of lime juice beer had a bright pale color, with a color of 12 EBC, and it had a pleasant aroma and taste.

Keywords: *Lime beer, lemon beer, fruit beer, juice beer, brewing process, beer innovation.*

1. Introduction

More and more research is made to study the technology on juice beer. Recently, "Bud Light have just pushed innovation to the next level with its latest offering, a drink that combines the taste of its Bud Light Lime Beer with the classic margarita cocktail to give customers a brand new alcoholic drink" (DBN Editor 2012). Fruit beers are any beers using fruit or fruit extracts as an adjunct and/or flavoring agent in either primary or secondary fermentation, "providing obvious, yet harmonious, fruit qualities" (Protz 2004; GABF 2007). According to The Gourmet Retailer (2007), "Fruits have been used as a beer adjunct or flavoring for centuries, especially with Belgian lambic styles. Cherry, raspberry, and peach are a common addition to this style of beer." Also, "beers with dash of fruit or spirits become a runaway success as customers seek imaginative alternatives" (Smithers 2012). However, "fruit qualities should not be overpowered by hop character" (GABF 2007). As stated by Zhang and Zhao (2007), "It was shown that the fruit beer contained more flavors and had better constitution proportions compared with conventional beer. The content of long-chain alcohol is lower in fruit beer, while the contents of organic acids were higher, especially for citric acid. Besides, the content of sucrose was decreased in fruit beer with an increasing in the contents of fructose and glucose."

There are many kinds of commercial fruit beer in the market. Juice beers mostly popular in the Occident market such as Henninger and Bavaria - St. Pauli mainly have pineapple taste, lemon taste, apple taste, strawberry taste, coke taste, etc. The technologies of sweet potato beer (Hare 1827), fragrant pear juice beer (Magerramov *et al*. 2007), kiwifruit and strawberry mixed fruit beer (Huang 2008) have also been reported.

Lime is a common citrus fruit in Thailand. Limes are usually smaller and contain more Vitamin C than lemons. Also (eHow Contributor 2013), "Lemons are too acidic to be able use to brew a true beer. Most so-called lemon beer is really lemonade that has been allowed to ferment slightly or beer mixed with lemonade."

This study intended to develop a suitable method for the production of low-hopped ale with a lime flavor formulation and determine the optimum condition for lime juice beer production. The optimum fermentation parameters such as optimum wort boiling temperature, fermentation period, ratio of lime juice addition and amount of hops added were also studied. Beers fermented at different treatments were held for optimum pH, alcohol percentage and °Bx analysis.

2. Materials and Methods

2.1 Methods

The experiment was separated into three stages. In the first stage, different ratios of lime juice were added to the wort and the time of lime juice addition was tested. In this stage, the hop addition was set to 0.16(w/v) and the boiling temperature was set to 90-95°C. The optimum hop addition level was tested in the second stage and the optimum wort boiling temperature was tested in the third stage. Galena hop pellets were used as bitter hops and cascade hop pellets were used as aroma hops.

2.1.1 Water analysis: Tap water was used in this experiment. The pH, hardness and alkalinity of tap water were tested before the brewing process.

2.1.2 pH: The pH of water was measured by a pH meter.

2.1.3 Calcium hardness: The amount of 3.7224 g of disodium ethylene diamine tetraacetate dihydrate was dissolved in distilled water and diluted to 1.0 liter to make 0.01 M ethylene-diamine-tetraacetic-acid (EDTA) solution. Then 50 ml of water sample was taken and 1N NaOH was used to adjust pH to 12-13. Two drops of Eriochrome Black-T (EBT) indicator were added. The mixture was titrated slowly by EDTA solution, until the color changed from wine-red to a clear blue. EDTA formed a complex with calcium in a one-to-one molar ratio. (Clesceri *et al*. 1998)

2.1.4 Total hardness: A 50-ml water sample was added into a 250-ml Erlenmeyer flask followed by the addition of 1-2 ml of pH 10 ammonia buffer. EBT was used as indicator and the mixture was titrated slowly by 0.01 M EDTA solution, until the color of the solution changed from wine-red to a clear blue. EDTA formed a complex with calcium and magnesium in a one-to-one molar ratio.

2.1.5 Alkalinity: A 100-ml water sample was titrated with 0.1 N HCl to pH 4.3 using methyl orange as indicator. The total alkalinity of the sample was calculated using the relation

$$\text{Alkalinity (mg } CaCO_3\text{/L)} = A \times N \times 50{,}000 \text{ / (mL of sample)},$$

where: A is the total volume in mL of the standard acid used; N is the normality of the standard acid used; and 50,000 is a conversion factor for the change of the normality into units of mg $CaCO_3$/L.

2.1.6 Lime juice preparation: The fresh whole limes were washed and dried and then each one was sliced in half with a sharp knife. The lime halves were pressed or squeezed by using a citrus juicer. The freshly squeezed lime juice was stored in a tightly sealed container in a refrigerator (Hardwick 1995).

2.1.7 Brewing process:

2.1.7.1 Malt preparation: Commercial malt (Fig. 1) was milled using a grinding machine and then mixed with a certain quantity of water to achieve the desired solid-liquid ratio. The ratio of malt: water was equal to 1: 4.

Fig. 1. Malt used in brewing (provided by Boon Rawd Brewery Co. Ltd., Bangkok, Thailand).

2.1.7.2 Mashing: The wort was gone through a single temperature infusion mashing process in this experiment. The mixture was boiled at 50°C for 30 min. Then the temperature was slowly increased to 65°C in

10 minutes and the mixture was boiled for 40 min. After that, the temperature was increased to 75°C within 20 min. The mashing process is described in Table 1.

Table 1. Mashing process.

Temperature (°C)	Holding time (min)
50	30
50 to 65	10
65	40
65 to 75	20

Iodine tests and °Bx measurements were needed. The final °Bx was adjusted to 12°Bx by adding malt syrup or sugar. Before boiling, the wort was filtered by a cheese cloth.

2.1.7.3 Wort boiling: Initially, 0.11% of ground bitter hop was added to the wort. The wort was boiled to 90°C for 60 min. Then 0.05% of ground aroma hop was added and the wort was boiled vigorously. After that, the whirlpool was done and the residue let to precipitate. The wort was further placed into a ferment container and rapidly cooled down to 20°C by immersing the container into ice water. Galena hop (Fig. 2) was used as bittering hop being an excellent high alpha acid hop with balanced bittering profiles paired with acceptable hop aroma, and containing 12-14% of alpha acid and 7-9% of beta acid. Galena hop gave a citrusy aroma to the beer, while cascade hop (Fig. 3) containing 4.5-7% of alpha acid and 4.5-7% of beta acid was used as aroma hop, giving a pleasant, flowery and spicy, citrus-like aroma (Goldwaite 1995).

Fig. 2. Galena hop pellets.

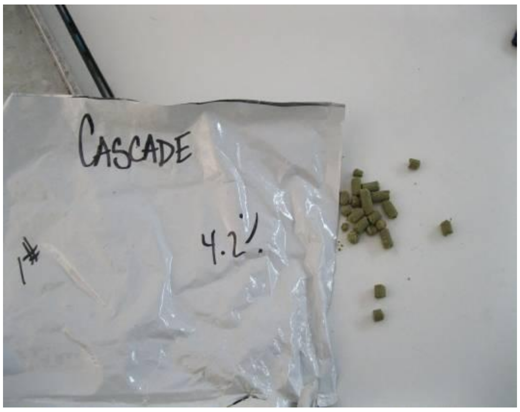

Fig. 3. Cascade hop pellets.

2.1.7.4 Yeast pitching and wort aeration: Dry yeast of *Saccharomyces cerevisiae* (Lavin RC 212 Bourgorouge Selection Vivb *Saccharomyces cerevisiae*, Fig. 4) was used in this experiment and 0.5% yeast starter culture was pitched and added into the wort. The container was shaken to entrap air and promote the growth of yeast.

Fig. 4. Lalvin RC 212 Bourgorouge Selection Vivb *Saccharomyces cerevisiae.*

2.1.7.5 Lime juice addition and fermentation: Different ratios of lime juice to wort (0.3, 0.6, 1, 2 and 3% juice addition) were tested. Two groups were set for the experiment. For Group 1, wort and juice were mixed in each ratio, fermented at room temperature for around five days, and then the containers were transferred to a cold room (~0°C) for 2 weeks. For Group 2, lime juice was added in each ratio after primary fermentation. Before fermentation, the volume of wort and the pH of the mixture were measured. The original gravity was measured by using a hydrometer (Bamforth 2002).

2.1.7.6 Filtration, carbonation and package: The beer was filtered by using diatomaceous earth (DE) as filtration aid. After filtration, the beer was transferred to a keg. Then the beer was carbonated for two days (Fig. 5). The beer was carbonated for one hour during the first day and also for half an hour during the second day at the pressure of 3 bars.

2.1.7.7 Test of optimum hop addition level and wort boiling temperature: After determining the optimum juice content and time of juice addition, the optimum wort boiling temperature and hop addition were tested in further stages. The hop addition levels were set to 0.12, 0.14, 0.16, 0.18 and 0.20% in stage 2 and the experiment was repeated. After identifying the optimum hop addition, the wort boiling temperatures were set to 85-90, 90-95 and 95-100°C in stage 3, and the experiment was repeated again.

Fig. 5. Carbonation equipment.

2.1.8 Beer analysis: For each sample, the alcohol content, pH, sugar content, specific gravity, color, total acidity and vitamin C content were tested.

2.1.8.1 Alcohol content: The alcohol content was measured by a Dujardin-Salleron Ebulliometer.

2.1.8.2 pH: The pH of the beer was measured by a pH meter.

2.1.8.3 Sugar content: The sugar content (degrees Brix) was converted from specific gravity (SG) according to the specific gravity - degrees Brix (°Bx) conversion (Table 2),

$$°Bx = 261.3 \times (1 - 1/SG).$$

2.1.8.4 Specific gravity: The specific gravity was measured by a hydrometer.

2.1.8.5 Color: The beer color (Fig. 6) was determined by a beer color reference guide.

2.1.8.6 Total acid: The total acid was measured by titration with 0.1N standard sodium hydroxide solution (NaOH). NaOH solution was standardized by titration with 0.1N potassium hydrogen phthalate (KHP). Before titration, the samples were put in 50°C water bath for 30 min and shaken regularly.

A 10-ml sample was dispensed with a pipette into a 125-ml Erlenmeyer flask and titrated with standard NaOH solution using phenolpthalein as indicator, so that

$$\%\text{Total acid (mg/100 ml)} = (V_2 \times M_2 \times MW) \times 10^{-2} / V_1,$$

where: V_1 and V_2 are volumes of sample and NaOH used, respectively; M_2 is concentration of NaOH; and MW is the molecular weight of predominant acid.

2.1.8.7 Determination of vitamin C: Vitamin C was measured by titration with standard 0.01N iodine solution.

Table 2. Gravity-°Bx conversion (Fermsoft 2013).

Specific gravity	°Bx
1.010	2.56
1.011	2.81
1.012	3.07
1.013	3.32
1.014	3.57
1.015	3.82
1.016	4.08
1.017	4.33
1.018	4.58
1.019	4.83
1.020	5.08
1.021	5.33
1.022	5.57
1.023	5.82
1.024	6.07
1.025	6.32
1.026	6.57
1.027	6.81
1.028	7.06
1.029	7.30

RM/ Lovibond	Example	Beer color	EBC
2	Pale lager, Witbier, Pilsener, Berliner Weisse		4
3	Maibock, Blonde Ale		6
4	Weissbier		8
6	American Pale Ale, India Pale Ale		12
8	Weissbier, Saison		16
10	English Bitter, ESB		20
13	Biere de Garde, Double IPA		26
17	Dark lager, Vienna lager, Marzen, Amber Ale		33
20	Brown Ale, Bock, Dunkel, Dunkelweizen		39
24	Irish Dry Stout, Doppelbock, Porter		47
29	Stout		57
35	Foreign Stout, Baltic Porter		69
40+	Imperial Stout		79

Fig. 6. Beer colour based on standard reference method (SRM) (Wikipedia 2013).

2.1.9 Preparation of standard iodine solution: A 100-mL beaker was used to weigh 5 g of potassium iodide (KI) and then 5 ml of distilled water and 1.2691 g of iodine (I_2) were added and mixed until all reagents were completely dissolved. The reagents were further diluted with distilled water to scale in a 1,000-ml volumetric flask.

A 10-ml iodine solution was dispensed with a pipette into a 100-ml Erlenmeyer flask followed by the addition of 25 ml of distilled water. It was titrated with standard sodium thiosulfate ($Na_2S_2O_3$) solution until the solution turned pale yellow. After adding 2 ml of 1% (w/v) starch solution, the titration with $Na_2S_2O_3$ solution reached the endpoint when the solution became colorless. The titration above was repeated and the concentration of iodine solution was found.

2.1.10 Determination of vitamin C: Before titration, the samples were put in 50°C water bath for 30 min and shaken regularly. A 10-ml sample was dispensed with a pipette into an Erlenmeyer flask followed by: addition of 25 ml of distilled water, 10 ml of 1.0 M sulfuric acid (H_2SO_4), and 3 ml of starch solution; and titration with standard 0.01N iodine solution. The titration above was repeated and the vitamin C content was found.

2.2 Sensory Test of Beer

Ten experienced panelists were invited to the sensory test. There were four descriptive factors for evaluation of stage 1 and stage 3: clarity, flavor, taste and general impression. One more factor was added into the evaluation of stage 2: bitterness.

Distilled water was provided for rinsing of the palate during the testing. Evaluations took place in the afternoon between 14:00 and 16:00 hours and were conducted at room temperature (25°C) under white light. The amount of beer in each sample was 50 ml and the temperature of the samples was controlled around 10°C. The mean score for each quality attribute of beer was also computed.

2.3 Statistical Analysis

The data was collected under Randomized Complete Block Design (RCBD) and analyzed by using SPSS® (2003) for Microsoft® Windows® (2009).

3. Results and Discussion

3.1 Water Analysis

The water used in this experiment was tap water. The result of water analysis (Table 3) shows that the pH of tap water was 7.05, calcium hardness was 67.4 mg $CaCO_3$/L, total hardness was 72.0 mg $CaCO_3$/L and alkalinity was 62.5 mg $CaCO_3$/L.

According to the quality criteria of brew water (Eßlinger 2009), the pH of brew water should be within the range of 7.0-8.0. The ideal range of calcium is 50-100 mg/L, and alkalinity varies by beer style depending on the lime treatment or acid treatment (Briggs *et al*. 1981). As can be seen from the result, water adjustment was not needed in this experiment. Tap water can be used directly.

Table 3. Analysis of tap water.

pH	7.05
Calcium hardness ($mgCaCO_3/L$)	67.4
Total hardness ($mgCaCO_3/L$)	72.0
Alkalinity ($mgCaCO_3/L$)	62.5

3.2 Beer Properties of the First Stage

Due to the differences in fruit composition, yeast strains used for this fermentation experiment were added to adapt to the appropriate environments. The lime beer in this stage was a brownish red, limpid and transparent beer with unique lime fragrance and moderately sour. The alcohol content, pH, sugar content, specific gravity, color, total acidity and vitamin C content were obtained and the results are shown in Table 4.

Usually, the pH of beer for most styles is between 3.90 and 4.20. According to Murphy & Son Ltd. (2012), "The acceptable range of pH for cask conditioned beers is 3.7-4.1. For a given brand the pH should not vary by more than ±0.15 units." In stage 1, the pH of the samples varied from 3.77 to 4.05. Figure 7 shows samples of 1% A and 1% B in bottles and Fig. 8 shows the differences of the pH between Group A and Group B in said stage.

For both Group A and Group B, the pH of the beer was decreasing while increasing the lime juice content. Lime juice usually contains a pH of 2.00-2.35 (US FDA/CFSAN 2007), which is lower than that of the normal beer. So when it is mixed with beer, the pH of the liquor would decrease. Besides, in carbonated beer, the pH value is influenced by the presence of carbon dioxide (CO_2). The entire CO_2 only appears in free form with pH values smaller than 4. At higher pH level, the dissolved CO_2 partially changes into HCO_3^-. The HCO_3^- rate is proportional to the CO_2 concentration and the pH value of beer. The pH value of carbonated beer is up to 0.3 pH units lower than the value of the degassed beer sample (Beneš and Trinkel 2005; Basařová *et al*. 2010).

In Fig. 8, the pH of the sample of group B is clearly higher than that of group A. This indicated that yeast might be able to metabolite sugar more efficiently in the acidic condition.

The total acidity of the samples was increasing while increasing the lime juice content. Fig. 9 shows that when lime juice was added before fermentation (Group B) the total acidity was affected by the fermentation and became lower compared to the sample with added lime juice after fermentation (group B). However, the difference is not significant.

Table 4. Analysis of samples in stage 1.

Group A					
Juice content	0.003	0.006	0.01	0.02	0.03
pH	4	3.88	3.86	3.77	3.68
Total acidity as citric acid (mg/100ml)	118.6	136.39	144.1	190.95	246.1
sugar content (°Bx)	5.57	5.08	5.57	5.57	5.08
SG (°Bx)	1.022	1.02	1.022	1.022	1.02
alcohol content (%vol)	5.12	4.2	5.82	5.55	6.4
vitamin C content (mg/L)	381.28	485.46	625.05	693.81	937.58
Color (EBC)	35	40	35	45	40
Group B					
Juice content	0.003	0.006	0.01	0.02	0.03
pH	4.05	3.98	3.92	4	3.77
Total acidity as citric acid (mg/100ml)	115.64	128.68	161.89	127.24	247.87
sugar content (°Bx)	5.57	5.57	6.07	6.07	6.07
SG (°Bx)	1.022	1.022	1.024	1.024	1.024
alcohol content (%vol)	4.2	4.6	6.52	6.5	6.4
vitamin C content (mg/L)	347.94	520.88	660.47	729.23	833.4
Color (EBC)	40	35	50	40	45

Notes: Group A: Wort and lime juice are mixed after primary fermentation. Group B: Wort and lime juice are mixed before primary fermentation.

Fig. 7. Samples of 1% A and 1% B in bottles.

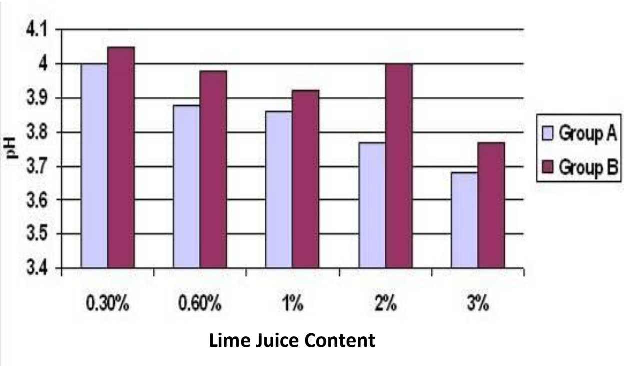

Fig. 8. The comparison of pH for samples of Group A and Group B in stage 1.

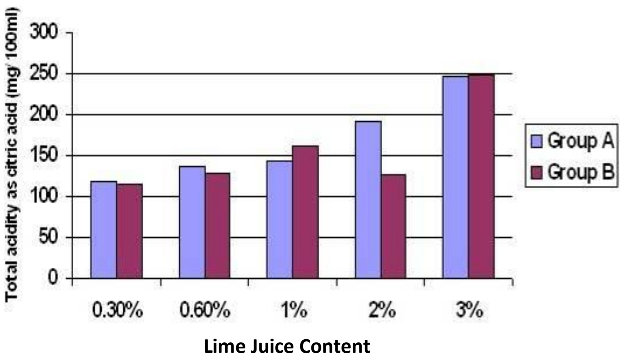

Fig. 9. The comparison of total acidity for samples of Group A and Group B in stage 1.

The sugar content of Group B beer varies from 5.57 to 6.07 °Bx and it is slightly higher than that of Group A beer (from 5.08 to 5.57°Bx). This difference in sugar value can be attributed to the variation of boiling time during the saccharifying process at 65°C during mashing. In general, a longer boiling time at the saccharifying temperature (65°C) should be preferred for the enzyme breakdown from polysaccharide to dextrin. The increase of the percentage of malt to water (15% to 25% w/v) results in an increased reduction of sugars and proteins. If the starting specific gravity of wort is too high, it will cause an unbalanced flavor profiles in the final beer product (Brown and Hammond 2003). The result in Fig. 10 shows that the alcohol contents of Group B are higher than that of Group A. These may indicate that the yeast used in the experiments might prefer the acidic condition during the fermentation process. Therefore the sugar substrate can be converted to alcohol with a higher efficiency during fermentation. This assumption remains to be further investigated. Zheng (1999) reported that the loss of vitamin C in fermentation is related to the fermentation temperature. High fermentation temperature accelerates the loss of vitamin C. According to his research, the best fermentation temperature range is 27-31°C. The difference of vitamin C content is shown in Fig. 11.

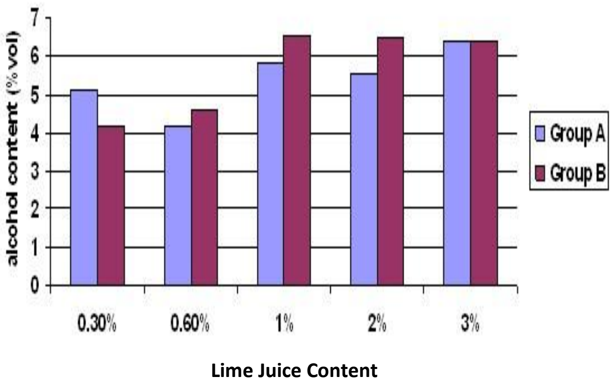

Fig. 10. The comparison of alcohol contents (%vol) for samples in stage 1.

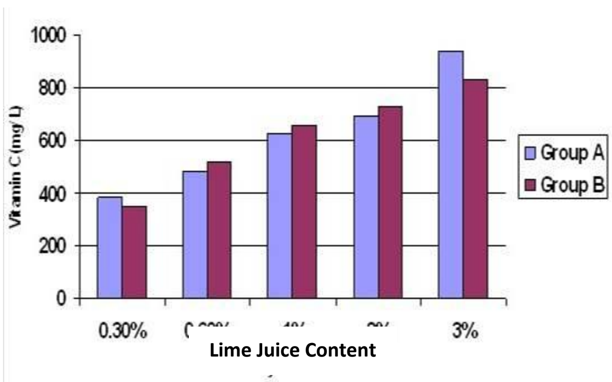

Fig. 11. The comparison of Vitamin C content (mg/L) for samples in stage 1.

The essential oils in lime are very important flavor components. Espina *et al.* (2011) indicated that terpenes, such as limonene, and terpenoids, such as neral or geranial, may be found in many common citrus fruits. Terpenes and terpenoids in lime from their research are shown in Table 5.

Table 5. Common terpenes and terpenoids found using GC/MS analysis of citrus-fruit extracts (Espina *et al.* 2011).

Component	Retention time (min)	Grapefruit	Lemon	Lime	Navel orange	Valencia orange
α-Thugene	6.050		X	X		
α-Pinene	6.183	X	X	X	X	X
Sabinene	6.600	X	X	X	X	X
β-Pinene	6.658		X	X		
β-Myrcene	6.700	X	X	X	X	X
p-Cymene	6.842	X			X	X
d-Limonene	7.133	X	X	X	X	X
γ-Terpinene	7.358		X	X		
Terpinolene	7.575		X	X	X	
Linalool	7.642	X	X	X	X	X
Citronellal	8.008	X	X		X	
α-Terpineol	8.350	X	X	X	X	X
Neral	8.592		X	X	X	X
Geranial	8.758		X	X	X	X
Neryl acetate	9.208		X	X		
Geranyl acetate	9.308		X	X		
α-Farnesene	9.692	X	X	X		
β-Bisabolene	10.042		X		X	X

Note: An X denotes the presence of a particular terpene or terpenoid in the indicated citrus-fruit extract.

The major flavor components of the fresh lime fruit have been reported as follows: limonene, α-terpineol, 4-terpineol, 1,4-cineole, 1,8-cineole, ρ-cymene, β-pinene, β-bisabolene, citral, geranial and neral (Ranganna *et al.* 1983).

Zhang and Zhao (2006) reported that the organic acids in juice are important taste substances of juice beer which can reduce the unpleasant taste and enhance the aftertaste. Moderate acidity gives a mellow fragrance in beer, and it can also reduce the bitterness of beer.

Ten panelists were invited to evaluate ten lime juice beer samples. They were focused on the clarity, flavor, taste and general impression of the beer. The results are shown in Table 6.

Table 6. Sensory evaluation of samples, Groups A and B, in stage 1.

Group (%)	Clarity (10 points)	Flavor (40 points)	Taste (40 points)	General impression (10 points)	Total (100 points)
0.0	6.6^{c}	15.2^{d}	23.7^{d}	5.6^{b}	54.1^{d}
0.1 A	7.2^{a}	17.8^{b}	25.2^{a}	5.6^{b}	58.0^{d}
0.1 B	7.4^{a}	15.0^{d}	23.1^{d}	5.2^{b}	53.2^{b}
0.3 A	7.2^{a}	20.1^{a}	27.0^{a}	6.8^{a}	61.1^{a}
0.3 B	8.0^{b}	16.9^{bd}	21.1^{bd}	5.8^{b}	51.8^{bd}
0.6 A	7.5^{ab}	20.7^{a}	21.7^{bd}	6.6^{a}	56.1^{d}
0.6 B	6.7^{c}	17.5^{b}	20.7^{b}	6.4^{a}	51.3^{bd}
1 A	7.1^{a}	12.5^{c}	13.4^{c}	4.4^{c}	37.4^{c}
1 B	7.2^{a}	17.8^{b}	23.1^{d}	5.6^{b}	53.7^{b}
2 A	8.4^{bd}	20.0^{a}	23.1^{d}	6.5^{a}	58.0^{d}
2 B	7.0^{a}	19.0^{ab}	25.2^{a}	6.2^{d}	57.4^{d}
3 A	7.8^{b}	15.0^{d}	21.2^{bd}	5.8^{b}	49.8^{b}
3 B	8.6^{d}	15.3^{d}	20.6^{b}	6.3^{ad}	50.8^{b}

Note: same alphabet in the same column means not significant difference ($p \geq 0.05$). Group A: Wort and juice are mixed after primary fermentation. Group B: Wort and juice are mixed before primary fermentation.

The sample in group A that contained 0.3% of lime juice (v/v) obtained the highest score in all attributes. Therefore, 0.3% lime juice addition after main fermentation was used in the next 2 stages. Too small an amount of lime juice may cause insufficient fruit flavor of the beer. When the volume of lime juice was increased very high to 6%, the sour taste of the beer was very strong. The panelists found it hard to accept. The addition of lime juice after main fermentation can retain the better lime flavor, but may have some effect on the clarity of beer. The clarity of beer also has a great impact on the sensory acceptability of beer. However, most of the panelists preferred the lime beer rather than beer without lime.

In this study, sucrose was used as adjunct. Engan (1972) reported that worts containing higher glucose and fructose produce more esters, which are responsible for the fruity character of fermented beverages.

Huang (2008) reported that the most important factor to impact sensory evaluation in beer is alcohol content, then sugar content and pH. Alcohol content and sugar content were therefore determined in stage 1.

The color of all the samples was brownish red and within an EBC range from 35 to 50. It was different from that of the next 2 stages (range from 8 to 15) which can be explained by the different malt used (Seaton and Cantrell 1993). The malt used in stage 1 was stored in a cold room for more than a year, while fresh malt was used in the next 2 stages. It remains unclear whether there is a direct relationship between the lime juice and the color development during the fermentation stage. However, according to Carvalho *et al*. (2009), it is very likely that enzymes present in the fruits (such as polyphenol oxydase) will contribute to the appearance of a darker color (enzymatic browning).

3.3 Beer Properties of the Second Stage

Following the experiment of the first stage, different hop additions were tested in the second stage to find out the optimum hop addition. For fruit beer, in order to avoid the fruit flavor been covered by hops, the amount of hops added was lower than the average level in regular beer. The results of chemical tests and sensory evaluation are shown in Table 7.

Beer in this stage was pale yellow, limpid and transparent, with unique lime fragrance and moderately sour. The averages of the results in triplicate are shown in Table 7. The lime juice beer had pH of 4.18-4.39 on the average. The total acidity varied from 104.57 to 114.18 mg/100 ml. No significant differences were found in pH, total acidity, SG and vitamin C content. The alcohol content varied from 6.2 to 6.8%. The color (EBC) of the beer was much lighter than that of stage 1 because new malt was used. Samples of the beer for this stage are shown in Fig. 12.

The beverage was subjected to sensory analysis to assess its acceptance among the panelists. The results are shown in Table 8.

Hop addition is an important factor for fermentation. A low hop addition causes an insufficient bitterness and flavor of the beer, and a high hop addition covers the fruit taste.

According to Table 8, samples which contained 0.18% of hop pellets got the highest score in sensory evaluation, so the hop addition was set to 0.18% in the next stage. According to the results of the sensory test, the panelists preferred a bitter taste than a sour taste in beer. The samples with high hop content had a higher score in bitterness.

3.4 Beer Properties of the Third Stage

Three wort boiling temperatures (80-90, 90-95 and 95-100°C) were tested in this stage. Samples of the beer for this stage are shown in Fig. 13. The results of chemical tests are shown in Table 9.

The lime juice beer in this stage had an average pH of 4.17-4.22. The total acidity varied from 120.13 to 137.20 mg/100 ml and the alcohol content varied from 6.1% to 6.7%. The sugar content was in the range from 3.57 to 5.08°Bx. Vitamin C content varied from 474.40 to 517.73 mg/L. There was little variation of pH, total acidity, vitamin C content and color among the samples.

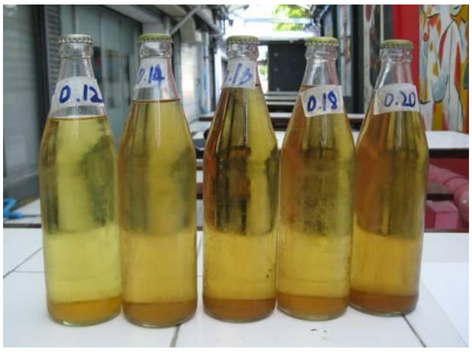

Fig. 12. Samples of stage 2 in bottles.

Table 7. Chemical analysis of samples in stage 2.

Hops content (%)	0.12	0.14	0.16	0.18	0.2
pH	4 .39	4.34	4.37	4.18	4.23
Total acidity as citric acid (mg/100 ml)	109.10	104.57	105.14	114.18	111.36
Sugar content (°Bx)	3.82	4.58	3.07	3.57	4.08
SG (°Bx)	1.015	1.018	1.012	1.014	1.016
Alcohol content (%vol)	6.40	6.20	6.80	6.40	6.40
Vitamin C content (mg/L)	433.34	428.78	426.50	403.69	405.97
Color (EBC)	8	12	13	13	15

Table 8. Sensory evaluation of stage 2.

Hop addition (kg/L)	Clarity (10 points)	Bitterness (10 points)	Flavor (30 points)	Taste (40 points)	General impression (10 points)	Total (100 points)
0.12	6.1^{a}	5.4^{a}	17.5^{a}	19.1^{a}	5.0^{a}	53.1^{a}
0.14	8.1^{b}	6.2^{b}	20.3^{b}	25.8^{b}	6.5^{b}	66.9^{b}
0.16	8.0^{b}	6.5^{bc}	20.7^{bc}	27.8^{bc}	6.4^{b}	69.4^{bc}
0.18	8.2^{b}	7.0^{c}	22.1^{c}	31.2^{d}	8.1^{c}	76.6^{d}
0.20	8.5^{b}	7.2^{c}	20.9^{b}	29.5^{c}	7.4^{d}	73.5^{cd}

Note: Same alphabet in the same column means that the difference is not significant ($p \geq 0.05$)

Table 9. Chemical analysis of samples in stage 3.

Boiling temperature (°C)	85-90	90-95	95-100
pH	4.17	4.22	4.20
Total acidity as citric acid (mg/100 ml)	120.13	121.40	137.20
Sugar content (°Bx)	5.08	3.57	4.58
SG (°Bx)	1.020	1.014	1.018
Alcohol content (%vol)	6.7	6.1	6.4
Vitamin C content (mg/L)	490.36	474.40	517.73
Color (EBC)	11	12	12

According to Table 10, the optimum wort boiling temperature range is 95-100°C.

The results from this study are sufficient to formulate a reasonable recipe for lime beer. The derived formula is summarized in the conclusion.

Table 10. Sensory evaluation of stage 3.

Boiling temp. (°C)	Clarity (10 points)	Flavor (40 points)	Taste (40 points)	General impression (10 points)	Total (100 points)
85-90	6.30^{a}	25.00^{a}	29.60^{a}	6.55^{a}	67.45^{a}
90-95	6.60^{a}	26.20^{a}	25.00^{b}	5.70^{b}	63.50^{a}
95-100	7.75^{b}	31.60^{b}	30.00^{a}	7.30^{c}	76.65^{b}

Note: Same alphabet in the same column means that the difference is not significant ($p \geq 0.05$).

Fig. 13. Samples of stage 3 in kegs.

4. Conclusion

Traditionally, the raw materials for beer production are barley, hop pellets, water, and yeast. However, most brewers also use different adjuncts. During the alcoholic fermentation, the contribution of aroma compounds from other ingredients to the final beer flavor depends on the composition of raw material, the yeast strain, and the process conditions. On the basis of this study, it can be concluded that lime juice beer can be produced by adding juice during the beer fermentation process.

Adding lime juice during fermentation can produce a beer having high sensory acceptability, high quality, good taste and unique flavor. According to this study, the best formula for lime beer production is to add 0.3% (v/v) lime juice after the main fermentation. The optimum hop addition is 0.18% and the wort boiling temperature is 95-100°C. The color of the final product is bright pale yellow with a color of 12 EBC. The lime juice beer has a pleasant aroma and taste. The results of the chemical analysis show that the pH of lime juice beer is 4.2, the sugar content is around 4.5°Bx and it contains 6.4% alcohol. The vitamin C content is around 517.73 mg/L.

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