Changes in Bael Tea with Aloe Vera After Thermal Processing

By
Ms. Clara Stella Renata Widianarko
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A special project summited to the Faculty of Biotechnology, Assumption University in part of fulfillment of the Requirements for the Degree of Bachelor of Science in Biotechnology

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Clara Stella Renata Widianarko

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Literature Review

Herbal drink

Herbal drink is included as a functional drink, which is defined as the drink that offers the consumer additional perceived benefits besides its primary function, which is hydration (Ashurst, 2005). Over the past 15 years, a few herbal drinks containing herbal extracts, have been put into dilutables, juice-based drinks, and bottled waters. The role of herbal extracts is primarily a marketing issue, in which consumers perceive a benefit over a drink and give reason to purchase (Ashurst, 2005).

There are several basic extraction processes used in making herbal drink:

- Infusion: steeping an herbal raw material in a liquid. Traditionally made by pouring boiling water into the dried herb, soak, and straining off the resultant liquor.
- Decoction: a variation in general infusion process, in which heat is supplied continuously to keep it at boiling point and gently simmering.
- Tincture: an ambient temperature extract that is made with a high alcohol level in the extraction liquid, 60 – 70%.
- Liquid/fluid extract: made by extracting the herb with fresh batches of extracting liquid, combining these batches, and concentrating into the original weight of herb into 1:1 yield.
- Soft extract: similar to liquid/fluid extraction, in which the resultant extract has 30% or less moisture content.
- Powdered extract: made by replacing the moisture in soft extract with an equal amount of a substrate, such as calcium phosphate, starch, or maltodextrin.
- Percolation: an alternative of infusion process, in which the solvent is trickle-fed on the top of the herb or spice material and collected as it emerges from the conical percolation vessel.

Tea is the world's most consumed beverages, surpassing the bottled water, carbonates, beer, and coffee (Euromonitor International, 2015). Herbal teas or tisanes are

usually made from herbs, fruits, seeds, or roots steeped in hot water and have lower concentrations of antioxidants than green, white, black, and oolong teas (Edgar, 2009). Tea and herbal tea consumption is largely concentrated in Asia, and there is a changing in trend of consumption, from unpackaged tea sold in rural to packaged tea sold in modern retail outlets (Euromonitor International, 2015). The tea market is mainly dominated by black tea, green tea, and followed by fruit/herbal tea.

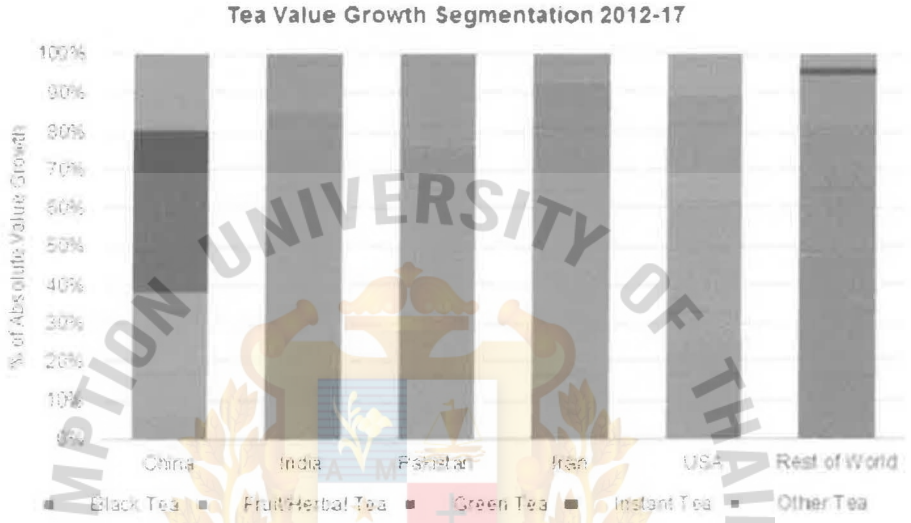

Figure 1 Tea value growth segmentation 2012-2017

(Source: http://blog.euromonitor.com/2013/08/dual-opportunities-for-tea-in-retail-and-foodservice.html)

In Thailand, the ready-to-drink (RTD) tea market consists of original tea, herbal tea, fruit tea, and premium tea. The original tea or tea from *Camellia sinensis* still dominates the market.

Estimated breakdown of Thailand's RTD tea market

	% Breakdown	THB billion
Original tea	58	8.4
Herbal tea	25	3.6
Fruit tea	15	2.2
Premium tea	2	0.3
TOTAL	100	14.4

Compiled by Mini Me Insights

Source: Bangkok Post - Oishi banks on fruit tea for growth, 13 Jan 2016

Figure 2 Thailand's RTD tea market

(Source: https://www.minimeinsights.com/2017/01/14/first-cold-activated-rtd-tea-by-oishi/)

Bael fruit

Bael fruit or *Aegle marmelos* is a native fruit from India, but widely found in southeastern Asian countries (Bhardwaj & Nandal, 2015). It is a valuable fruit which has been used since prehistoric times in India due to the nutritional and medicinal properties. In Thailand, bael fruit or "matoom kai" is mainly used for household consumption, traditional medicine, and for trade in some region (Charoensiddhi & Anprung, 2010). In food products, bael fruit is commonly cut into pieces and dried, pulverized, and packed as tea bags, also preserved in syrup as bael fruit glacé (Charoensiddhi & Anprung, 2010). In addition, bael fruit can be processed into preserves, powder, jam, syrup, toffee, and beverages (Singh *et al.*, 2014).

The peel of bael fruit is hard, and the color is green to brown depends on the ripening stage (Charoensiddhi & Anprung, 2010). The pulp is yellow to orange with slightly sweet taste and pleasant fragrant. It contains functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants. Bael fruit composed of mainly water, carbohydrate, fiber, protein, mineral, and fat (Neeraj *et al.*, 2017). Due to its beneficial properties, bael fruit may indicate that it is one of the important plants used in traditional medicine (Charoensiddhi & Anprung, 2010). In terms of bioactive compounds and characteristics flavor, bael fruit is a potential

fruit to be utilized as a functional food and value added processed products (Charoensiddhi & Anprung, 2010). The bael fruit juice has been widely used in treating diarrhea, dysentery, and gastrointestinal disorders (Bhardwaj & Nandal, 2015).

Figure 3 Dried bael slices

(Source: http://healthandbeautypages.com/wp-content/uploads/2015/02/Dried-Bael-Fruit1.jpg)

Figure 4 Bael fruit

(Source: http://stylesatlife.com/wp-content/uploads/2014/04/Bael-Fruit.jpg)

Aloe vera

Aloe vera or *Aloe barbadensis* Mill. is a perennial plant and drought-resisting plant with cactus appearance, stiff and sharp-edges thick leaf (Sánchez-Machado *et al.*, 2017). In food industry, aloe vera is introduced as a functional food by incorporating it with health drinks and beverage, such as tea (Tiwari and Deen, 2015). Aloe vera gel contains mainly water, polysaccharide, dietary fiber, ash, protein, vitamins and lipid (Sánchez-Machado *et al.*, 2017). It is widely well-known of the health benefits that aloe vera gel has, for example, promotion of wound healing, antifungal activity, hypoglycemic or antidiabetic

effects, anti-inflammatory, anticancer, immunomodulatory, and gastroprotective properties (Hamman, 2008). Some of these activities are due to the presence of polysaccharides (acemamman; glucomannan), cell wall carbohydrates (cellulose; hemicellulose) (Sánchez-Machado *et al.*, 2017; Hamman, 2008). Since it is utilized in pharmaceutical, cosmetics, and food industry, the knowledge of processing conditions that can affect aloe vera variability is important (Sánchez-Machado *et al.*, 2017). Miranda *et al.* (2009) reported that the thermal processing on aloe vera affects the physicochemical and nutritional content, in which drying aloe vera gel at 80 and 90°C resulted in the decreasing amount of crude fiber, calcium, vitamin C, and antioxidant property.

Figure 5 Aloe vera

(Source: http://www.homeremediess.com/wp-content/uploads/2015/09/aloe-vera-plant.jpg)

Figure 6 Aloe vera gel

(Source: http://cdn2.stylecraze.com/wp-content/uploads/2013/12/Is-Aloe-Vera-Good-For-Acne.jpg)

Packaging of ready-to-drink fruit juices and tea

Many fruit juices are bottled in glass bottles using hot filling system. The glass bottle has been used for a long time in fruit juice industry. It can stand the hot filling and in-bottle pasteurization for pure fruit juices or products that do not contain preservatives (Rahman & Perera, 2007). Glass bottles can maintain the product freshness for a long period and it is useful for heat sterilization and impermeable to gases and vapors. However, it is heavy and susceptible to breakages from internal pressure, impact or thermal shock (Ramos *et al.*, 2015).

Hot filling is a known method in juices industry to achieve a longer shelf life. Hot filling is used to minimized the microbial presence and to avoid reinfection of the finished product. The main spoilage organisms in juices are yeasts and molds. It is a simpler method of filling compared to aseptic packaging. In hot filling system, the product is pasteurized at 92-95°C, filled above 82°C, held hot in the pack in such a way that all parts of the pack are adequately pasteurized and cooled. The advantages of hot filling include simpler filling process compared to aseptic and no chemical sterilization of containers. On the other hands, the hot filling method only can be used for product with adequate thermal stability (Ashurst, 1999).

The headspace needed for in-bottle pasteurization and sterilization of fruit juices is 5-7% (Ashurst, 1999). The consideration of headspace is important since the fruit juice will expands during sterilization and the headspace becomes squeezed. The smaller the headspace volume, the higher the internal pressure (Ashurst, 1999).

Materials and Methods

The sliced dried bael fruits, sugar, and canned aloe vera were procured from the local market of Bangkok.

1. Bael tea preparation

The dried bael fruit was roasted in the oven for 5 to 8 minutes at 100-120°C and the fruits were crushed to small pieces. On the pot, the water was set to boil and the crushed and roasted bael fruit pieces were added while the water was boiling. The bael fruit pieces were boiled for 10 minutes at 80-100°C. The cheesecloth and strainer were sterilized before used to filter the bael tea. Then, the tea was filtered, sugar and aloe vera were added. Let the tea cool down and keep in the fridge until required.

1.1 Determination of concentration of bael in bael tea

In this experiment, different concentration of bael fruit in bael tea was determined and judged by using 9-point hedonic scale in which color, aroma, flavor, sweetness, aftertaste, and overall liking were used as attributes to measure. The amount of sugar in the tea was fixed at 2% while the amount of bael fruit was varied at 1.5%, 2%, 2.5%, 3%, and 3.5%. The data obtained from the questionnaire was analyzed using randomized complete block design and Duncan's multiple range test in SAS program.

1.2 Determination of sweetness level in bael tea

This experiment was carried out to obtain the sweetness level of bael tea that most people prefer. The bael tea contained 3% of bael fruit, 5% aloe vera, and different amount of sugar, which was 0%, 2%, 5%, 8%, and 11%. These teas were judged by panelists using 9-point hedonic scale and analyzed using randomized block design and Duncan's multiple range test in SAS program.

2. Storage test

The storage test of bael tea with aloe vera was performed for two weeks. After obtaining the final formulation from two sensory evaluations, the final formula of bael tea with aloe vera contained 84% water, 8% sugar, 5% aloe vera, and 3% bael fruit. The

samples required for storage test were prepared by autoclaving. The sensory evaluation and physicochemical analysis of bael tea was carried out every 4 days of two-weeks storage test. The physicochemical measurement consisted of pH, total titratable acidity, total soluble solids, reducing sugar, and color measurement using Hunter's Lab.

2.1 Heat treatment of bael tea with aloe vera

The bael tea was prepared and hot-filled into sterilized 300 ml-glass bottle, which was 270 ml tea per bottle, after considered 7% headspace for in-bottle sterilization. These glass bottles were sterilized for 15 minutes at 121°C and cooled down to ambient temperature using water bath. Then, the bottles were stored at ambient temperature and 4°C for further observation.

2.2 Sensory evaluation

The sensory evaluation of bael tea with aloe vera was carried out every 4 days during two-weeks storage test (day2, day6, day10, day14). The sensory evaluation was performed by 15 panelists, asking for opinion on several attributes, such as color, aroma, flavor, sweetness, aftertaste, and overall liking. Two samples were served in each time of evaluation, which were bael tea with aloe vera stored in ambient temperature and bael tea with aloe vera stored under refrigeration. Both samples were chilled before serving. The results were statistically analyzed using SAS program with RCBD, factorial RCBD design and Duncan's multiple range test.

2.3 Physicochemical analysis of bael tea

The physicochemical analysis of bael tea stored at ambient temperature and under refrigeration was carried out for two weeks as a storage test. The pH values were determined with a digital pH meter using buffers of pH 4.0 and 7.0 for calibration. The total soluble solid was measured using portable refractometer and the values were expressed as °Brix.

2.3.1 Determination of total acidity

The acidity of bael tea was determined by titration of sample against 0.1N NaOH, according to AOAC (1995). Ten ml of sample with 2-3 drops of phenolphthalein was put into Erlenmeyer flask and the titration was NaOH was taken place. The titration was carried out until the pH reached 8.2. The values were expressed as the percentage of citric acid.

2.3.2 Determination of reducing sugar

The amount of reducing sugar in the sample was determined by dinitrosalicylic acid (DNS) assay. The bael tea was diluted to 10^{-1} and 10^{-2}. On the test tubes, 0.2 ml of sample and 2 ml of DNS were boiled for 10 minutes and 1 ml of 40% potassium sodium tartrate was added to stabilize the color change. The blank was prepared by using 0.2 ml water with 2 ml of DNS and 1 ml of 40% potassium sodium tartrate, which also boiled for 10 minutes. Then, the test tubes were cooled down rapidly to room temperature using water bath. Using the spectrophotometer, the optical density was measured at 540nm.

Standard curve

A standard curve was constructed by using glucose solution at 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml. The linear regression test was performed using Microsoft Excel 2016. The obtained equation was extrapolated to allow for determination of all subsequent calculations.

2.3.3 Colorimetric analysis

The color of bael tea was determined as L*, a*, and b* values using a HunterLab Miniscan EZ. The total color differences (ΔE) was calculated by using the following equation:

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$

The color differences (ΔL*, Δa*, Δb*) was obtained from the first day of storage subtracted by the last day of storage period (day1 - day13).

Results and Discussion

1. Determination of concentration of bael in bael tea

Based on the statistical analysis, it was found that 3% of bael fruit in tea was preferred by panelists. It showed that 3% of bael fruit was significant different ($p<0.05$) in terms of color, aroma, flavor, sweetness, aftertaste and overall liking compared to other concentrations of bael tea. However, the hedonic score of sweetness was slightly low, range of 4 -5, since the amount of sugar was fixed at 2%. Thus, another sensory evaluation was performed to determine the sweetness level that meet consumers' liking. The result of this section was shown in Table 1.

2. Determination of sweetness level of bael tea

In this sensory evaluation, it was found that the preferred sugar concentration on bael tea was 8%. The 8% sugar concentration was significantly different ($p<0.05$) compared to other treatments in all attributes. Therefore, from two sensory evaluation, it was found that 3% bael with 8% sugar was preferred in the bael composition. The score for each attribute was shown in Table 2.

Table 1 Sensory evaluation result of bael tea with different concentration of bael

Treatment	Attributes					
	Color	Aroma	Flavor	Sweetness	Aftertaste	Overall liking
1.5% bael	4.4 ± 1.96^{c}	4.01 ± 1.51^{c}	3.9 ± 1.68^{c}	4.9 ± 2.10^{b}	4.9 ± 1.87^{b}	4.7 ± 1.69^{c}
2% bael	5.9 ± 1.57^{b}	$5.8 \pm 1.10^{a,b}$	$5.6 \pm 1.43^{a,b}$	5.9 ± 1.83^{a}	$5. \pm 1.52^{a}$	5.7 ± 1.45^{b}
2.5% bael	5.7 ± 1.54^{b}	5.7 ± 1.94^{b}	5.4 ± 1.55^{b}	5.5 ± 2.15^{a}	5.8 ± 1.37^{a}	$5.9 \pm 1.42^{a,b}$
3% bael	7.1 ± 1.05^{a}	6.5 ± 1.36^{a}	6.2 ± 1.64^{a}	5.6 ± 1.69^{a}	5.8 ± 1.35^{a}	6.4 ± 1.19^{a}
3.5% bael	6.9 ± 1.40^{a}	$6.2 \pm 1.65^{a,b}$	6.2 ± 1.57^{a}	5.9 ± 1.95^{a}	6.0 ± 1.62^{a}	6.5 ± 1.38^{a}

Notes: Values are mean ± S.D.
The same letter in the same column means no significant difference ($p>0.05$)

Table 2 Sensory evaluation of bael tea with different level of sugar concentration

Treatment	Attributes						
	Color	Aroma	Flavor	Sweetness	Aftertaste	Compatibility	Overall liking
0% sugar	6.3 ± 1.34^{a}	5.6 ± 1.59^{b}	5.3 ± 1.78^{c}	4.1 ± 1.92^{c}	5.3 ± 1.87^{b}	4.9 ± 1.72^{b}	5.1 ± 1.61^{c}
2% sugar	6.8 ± 1.54^{a}	$5.9 \pm 1.73^{a,b}$	6.2 ± 1.91^{b}	5.5 ± 1.91^{b}	$5.7 \pm 1.86^{a,b}$	5.6 ± 1.73^{a}	5.9 ± 1.48^{b}
5% sugar	6.4 ± 1.31^{a}	$6.13 \pm 1.62^{a,b}$	6.13 ± 1.59^{b}	$5.8 \pm 1.85^{a,b}$	6.0 ± 1.68^{a}	6.2 ± 1.37^{a}	$6.2 \pm 1.50^{a,b}$
8% sugar	6.8 ± 1.07^{a}	6.4 ± 1.45^{a}	7.0 ± 1.17^{a}	6.5 ± 1.64^{a}	6.2 ± 1.56^{a}	6.3 ± 1.24^{a}	6.7 ± 1.11^{a}
11% sugar	6.8 ± 1.28^{a}	6.5 ± 1.46^{a}	$5.8 \pm 1.90^{b,c}$	$4.9 \pm 1.81^{b,c}$	$5.6 \pm 1.69^{a,b}$	6.0 ± 1.29^{a}	5.9 ± 1.55^{b}

Notes: Values are mean ± S.D.
The same letter in the same column means no significant difference ($p>0.05$)

3. Sensory evaluation during storage test

The sensory evaluation was carried out on 9-point hedonic score with color, aroma, flavor, sweetness, aftertaste, and overall liking as the quality parameters. It was found out that bael tea with aloe vera stored at 4°C had higher overall liking score than the ones stored at ambient temperature. The decline in sensory attributes during storage period might have occurred due to the loss of structure of aloe vera in the bael tea. Figure 7-11 show the results of hedonic score on each attribute that changed over the two-week storage. The numerical data of organoleptic liking scores is given in Table 3.

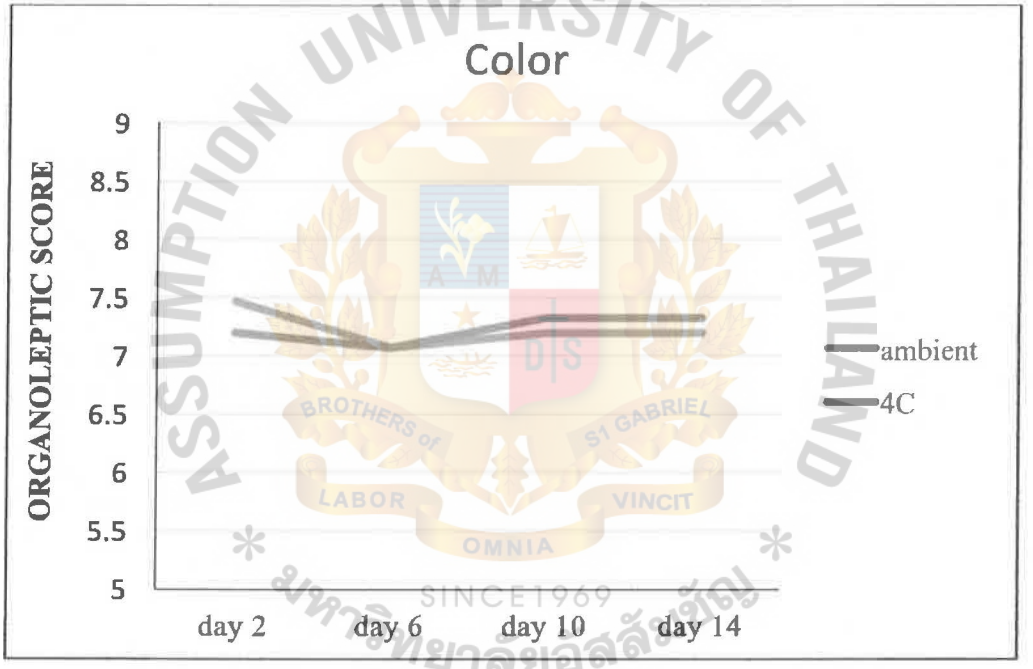

Figure 7 Color attribute of bael tea with aloe vera during storage period

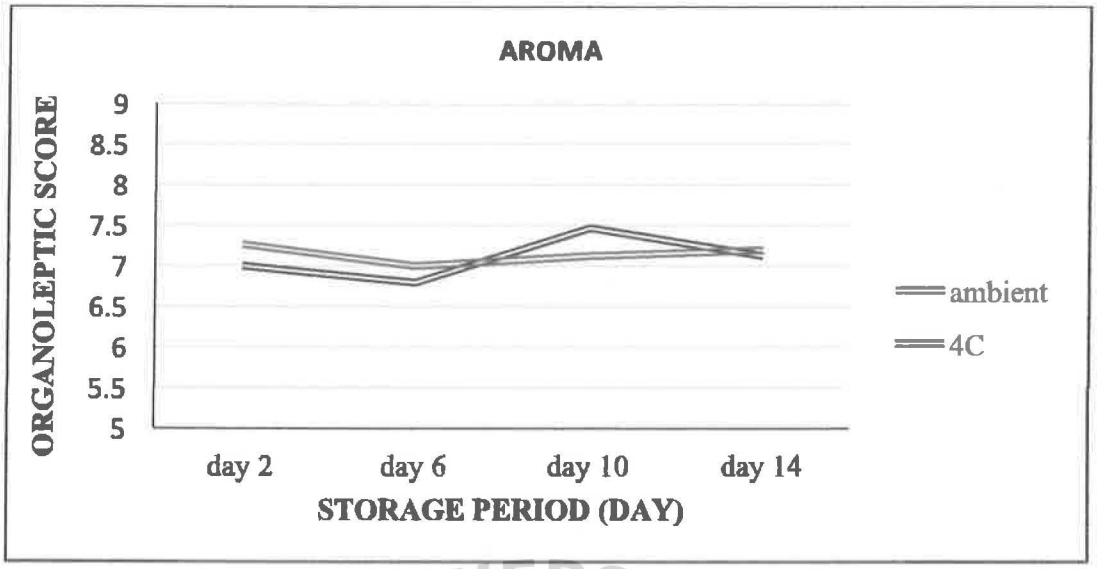

Figure 8 Aroma attribute of bael tea with aloe vera during storage test

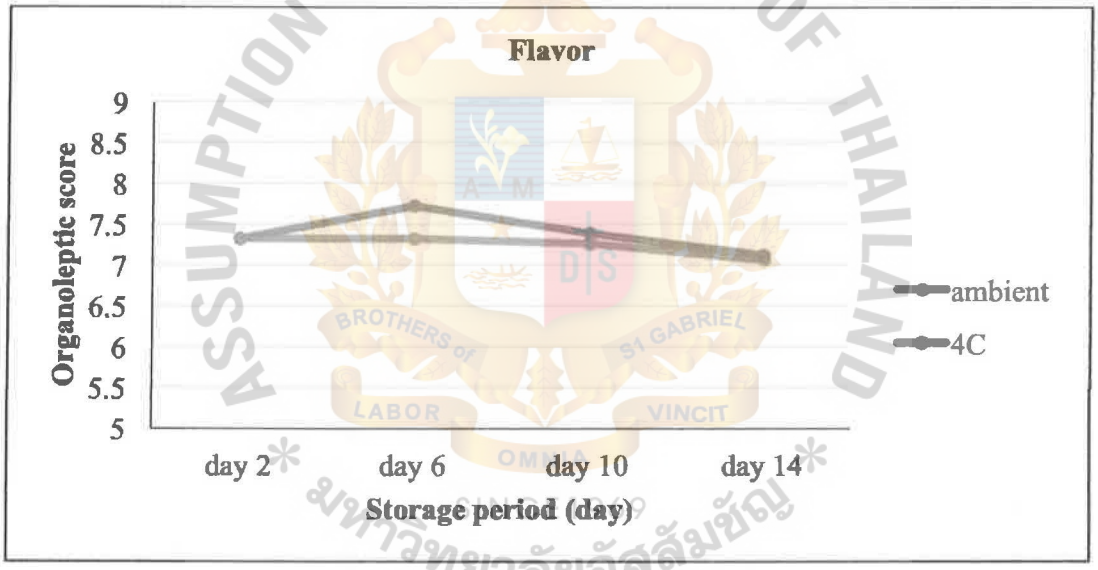

Figure 9 Flavor attribute of bael tea with aloe vera during storage test

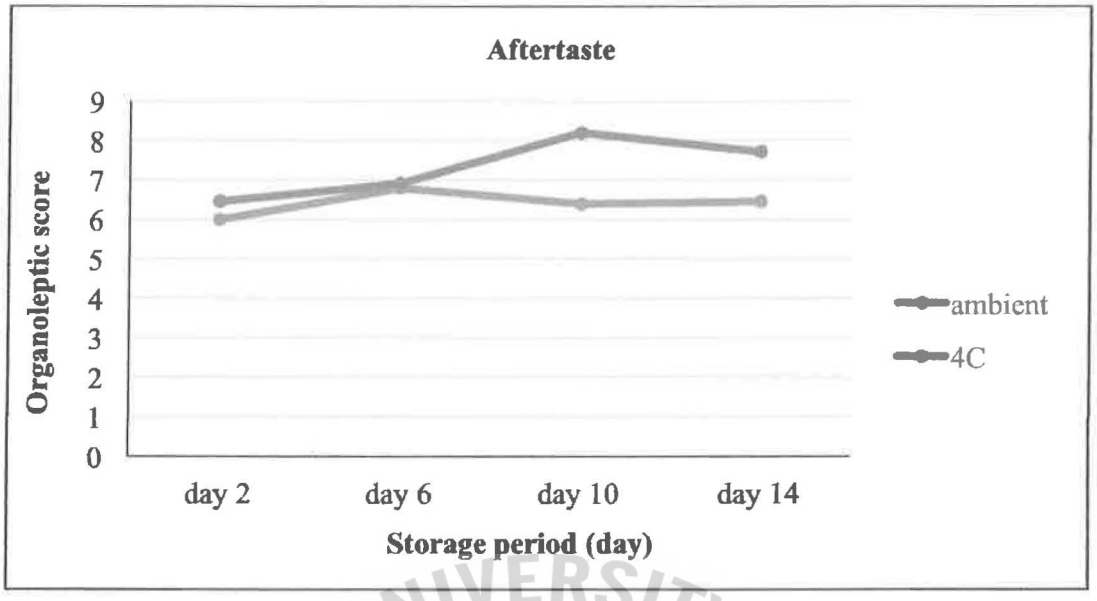

Figure 10 Aftertaste attribute of bael tea with aloe vera during storage test

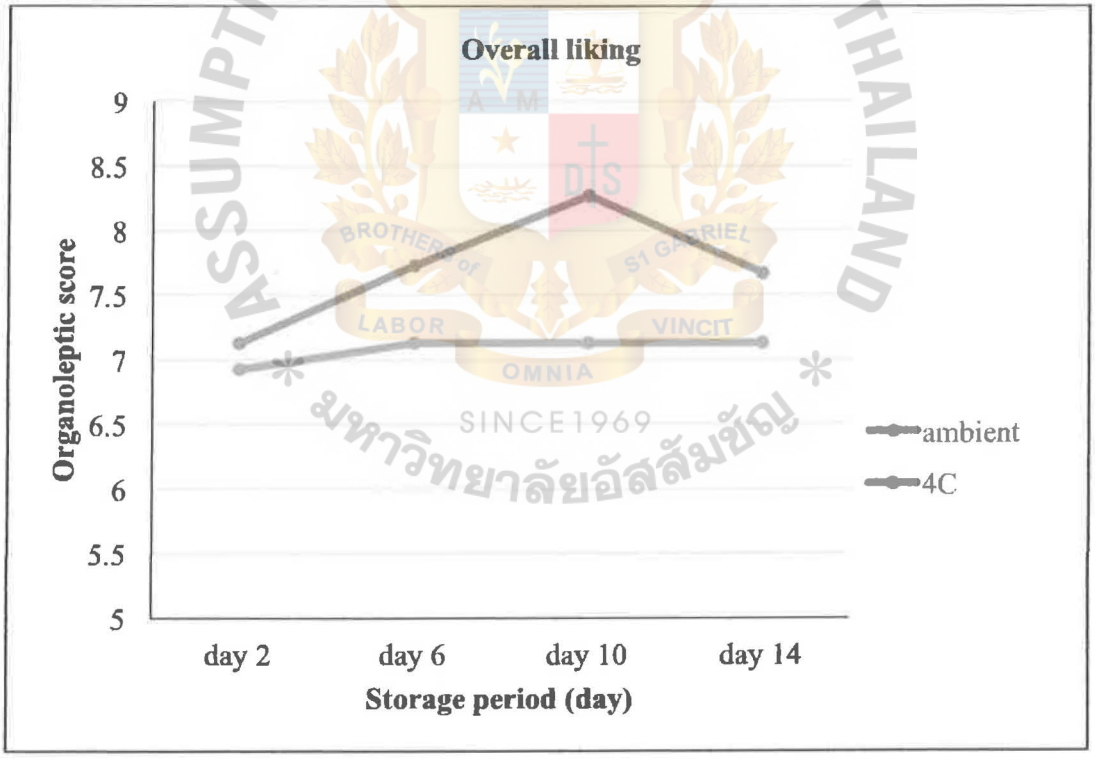

Figure 11 Overall liking attribute of bael tea with aloe vera during storage test

Table 3 Sensory quality scores during storage test

Sensory attributes	Storage condition	Storage period (day)			
		Day 2	**Day 6**	**Day 10**	**Day 14**
Color	Ambient	7.47 ± 0.99^{aA}	7.07 ± 1.28^{aA}	7.20 ± 1.1^{5aA}	7.20 ± 1.42^{aA}
	4°C	7.20 ± 1.15^{aA}	7.07 ± 1.58^{aA}	7.33 ± 1.40^{aA}	7.33 ± 1.40^{aA}
Aroma	Ambient	7.00 ± 1.25^{aA}	6.80 ± 1.57^{aA}	7.47 ± 1.30^{aA}	7.13 ± 1.92^{aA}
	4°C	7.27 ± 1.03^{aA}	7.00 ± 1.96^{aA}	7.13 ± 1.25^{aA}	7.20 ± 1.57^{aA}
Flavor	Ambient	7.33 ± 1.18^{aA}	7.33 ± 1.23^{aA}	7.27 ± 1.16^{aA}	7.07 ± 1.67^{aA}
	4°C	7.33 ± 1.05^{aA}	7.73 ± 0.88^{aA}	7.40 ± 1.24^{aA}	7.13 ± 1.81^{aA}
Sweetness	Ambient	6.80 ± 1.52^{aA}	7.80 ± 1.37^{aA}	7.40 ± 1.72^{aA}	6.93 ± 1.67^{aA}
	4°C	7.13 ± 1.46^{aA}	7.80 ± 1.08^{aA}	7.33 ± 1.76^{aA}	7.20 ± 1.57^{aA}
Aftertaste	Ambient	6.00 ± 1.60^{bB}	6.80 ± 1.90^{aB}	6.40 ± 1.64^{aB}	6.47 ± 1.55^{abB}
	4°C	6.93 ± 1.53^{bA}	7.93 ± 1.28^{aA}	8.20 ± 1.15^{aA}	7.73 ± 1.22^{abA}
Overall liking	Ambient	6.93 ± 1.16^{aB}	7.13 ± 1.19^{aB}	7.13 ± 0.83^{aB}	7.13 ± 1.30^{aB}
	4°C	7.73 ± 0.88^{aA}	8.00 ± 1.07^{aA}	8.27 ± 0.96^{aA}	7.67 ± 1.17^{aA}

Notes: Values are mean ± S.D.

The same letter (lowercase) in the same row (storage period) means no significant difference ($p>0.05$)

The same letter (uppercase) in the same column (storage condition) means no significant difference ($p>0.05$)

4. Physicochemical analysis

The physicochemical analysis of bael tea with aloe vera was carried out during 2-week storage tests to observe the stability of bael tea with aloe vera. The 4x2x3 factorial RCBD was carried out and it was found that there was no significant different in triplicates ($p>0.05$). Therefore, the 4x2 factorial CRD was used as the experimental design for all attributes measured, except for the color measurement in which 4x3x2 factorial RCBD was used.

4.1 Effect of storage on pH

Table 4 The pH value of bael tea with aloe vera during two-week storage

	Day1	Day5	Day9	Day13
Ambient	4.96 ± 0.03^{cB}	4.93 ± 0.02^{cB}	4.98 ± 0.04^{aB}	5.04 ± 0.01^{bB}
4°C	4.85 ± 0.02^{cA}	4.85 ± 0.03^{cA}	4.97 ± 0.03^{aA}	5.11 ± 0.03^{bA}

Notes: Values are mean ± S.D., analyzed by ANOVA using Duncan test.
The same letter (lowercase) in each row (storage period) means no significant difference between days.
The same letter (uppercase) in each column (storage condition) means no significant difference between storage temperature.

The pH value of the bael tea with aloe vera stored in different temperature was analyzed by ANOVA, using the factorial design to observe whether the interaction effects present between the factors. When an interaction effect is present, the impact of one factor depends on the level of another factor. From factorial CRD, the result indicated that there was significant difference in the storage period and storage conditions, along with the interaction of storage period and storage condition ($p<0.05$).

Observing from Figure 1, the pH of bael tea with aloe vera was decreasing during storage for both storage conditions, in which the sample stored at 4°C had higher fluctuation than the ambient temperature. It might be caused by the decreasing in acidity of the bael tea with aloe vera. Bael fruit is a low-acid fruit (Chavasit *et al.*, 2006), while aloe vera gel has pH of 4.6 (Sangma *et al.*, 2016). Mgaya-Kilima *et al.* (2014) reported that the pH of roselle-fruit juice blend was increasing over time, which was from 2.34-

4.37 (28°C) and 2.34-3.38 (4°C) during 6 months' storage test. On the other hand, the pH of blended bael with anola was decreasing over time, from 4.5-3.0 during 45 days' storage test (Rathod *et al.*, 2014).

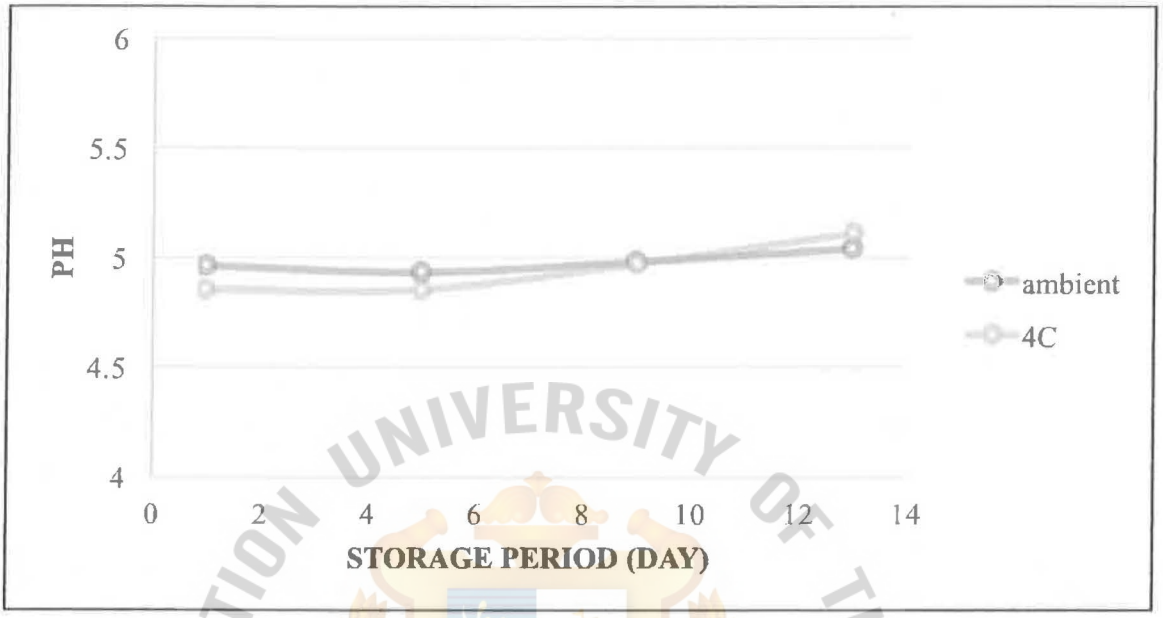

Figure 12 Effect of storage and temperature on pH of bael tea with aloe vera

4.2 Effect of storage on total acidity

Table 5 Total acidity of bael tea with aloe vera

	Day1	Day5	Day9	Day13
Ambient	0.06 ± 0.02^{bA}	0.10 ± 0.02^{aA}	0.08 ± 0.01^{abA}	0.05 ± 0.01^{bA}
4°C	0.07 ± 0.01^{bA}	0.08 ± 0.00^{aA}	0.07 ± 0.01^{abA}	0.08 ± 0.00^{bA}

Notes: Values are mean ± S.D., analyzed by ANOVA using Duncan test.
The same letter (lowercase) in each row (storage period) means no significant difference between days.
The same letter (uppercase) in each column (storage condition) means no significant difference between storage temperature.

From factorial CRD, it was found that only the storage period is significantly different ($p<0.05$). The storage condition and the interaction between storage period and

storage condition were not significantly different ($p>0.05$). The slightly decreasing in acidity at ambient temperature might be due to the acidic hydrolysis of polysaccharides where acid is utilized for converting non-reducing sugar into reducing sugar (Bhardwaj & Pandey, 2011).

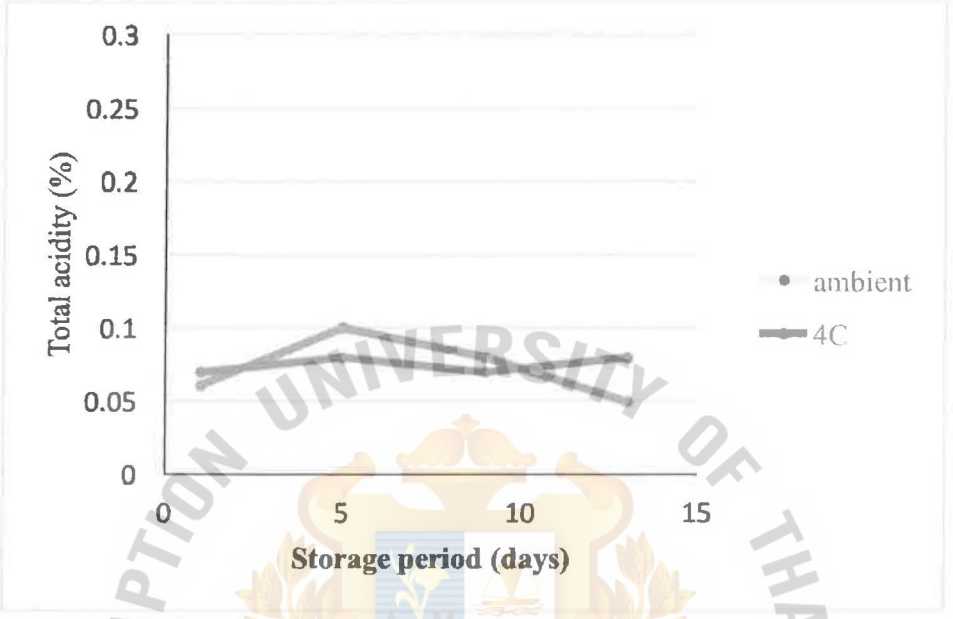

Figure 13 Total acidity of bael tea with aloe vera

4.3 Effect of storage on total soluble solid

Table 6 Total soluble solid of bael tea with aloe vera

	Day1	Day5	Day9	Day13
Ambient	11.00 ± 0.00^{aA}	9.73 ± 0.12^{cA}	10.00 ± 0.00^{bA}	9.67 ± 0.23^{bA}
4°C	10.00 ± 0.00^{aB}	8.87 ± 0.12^{cB}	9.60 ± 0.20^{bB}	9.60 ± 0.20^{bB}

Notes: Values are mean ± S.D., analyzed by ANOVA using Duncan test.
The same letter (lowercase) in each row (storage period) means no significant difference between days.
The same letter (uppercase) in each column (storage condition) means no significant difference between storage temperature.

The results in factorial CRD showed that the storage day and storage condition were significantly difference ($p<0.05$), also there was an interaction between storage period and storage condition. The total soluble solids of bael tea with aloe vera was in the range of 8 – 11. The low amount of total soluble solid might be due to the use of dried bael slices, in which usually made from unripe bael fruit (Reddy, 2012). Unripe bael fruit has difference biochemical properties with ripe bael fruit. Ripe bael fruit has higher total soluble solid, which is 39.50% as reported by Charoensiddhi & Anprung (2008). The decreasing trend in total soluble solid was observed during the storage period. This finding was similar to the study on the bael and aloe vera blended beverage (Sunita & Ananya, 2015).

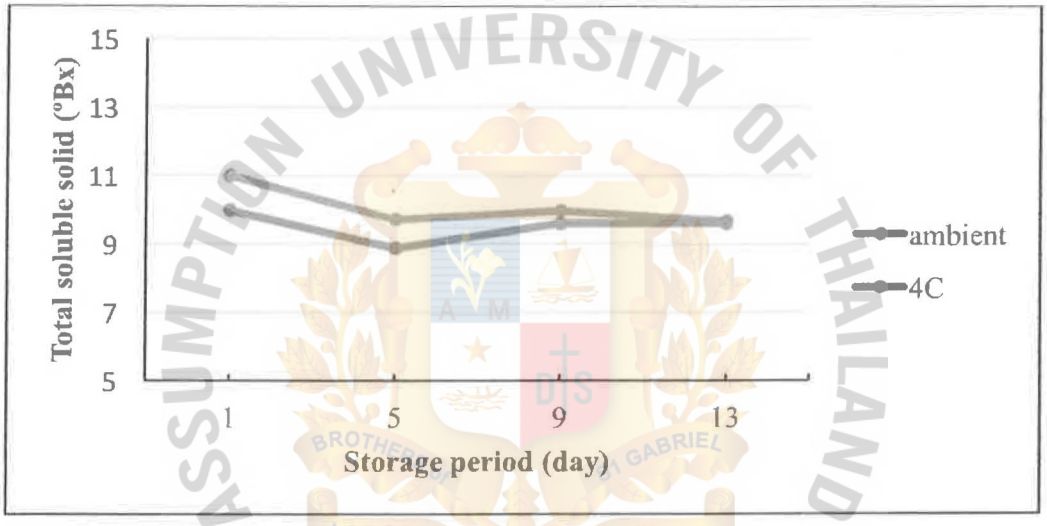

Figure 14 Total soluble solid of bael tea with aloe vera

4.4 Effect of storage on reducing sugar

Table 7 Reducing sugar content of bael tea with aloe vera

	Day1	Day5	Day9	Day13
Ambient	7.37 ± 2.43^{aA}	4.98 ± 0.24^{bA}	5.52 ± 1.09^{bA}	3.77 ± 0.77^{bA}
4°C	7.59 ± 2.03^{aA}	5.03 ± 0.88^{bA}	5.13 ± 0.70^{bA}	3.84 ± 1.10^{bA}

Notes: Values are mean ± S.D., analyzed by ANOVA using Duncan test.
The same letter (lowercase) in each row (storage period) means no significant difference between days.
The same letter (uppercase) in each column (storage condition) means no significant difference between storage temperature.

The results from factorial CRD showed that there is no significant difference in the storage temperature and interaction between storage period and storage temperature ($p>0.05$). The reducing sugar was decreasing during the storage period. However, this result did not correspond to the other researches, in which the reducing sugar content would increase over time, as reported by Sangma *et al.* (2016) of ready-to-serve of blended aloe vera, sweet lime, amla, and ginger. The reducing sugar would increase over time due to the hydrolysis of starch component from aloe vera into simple sugar, and the formation of invert sugar from sucrose (Sangma *et al.*, 2016).

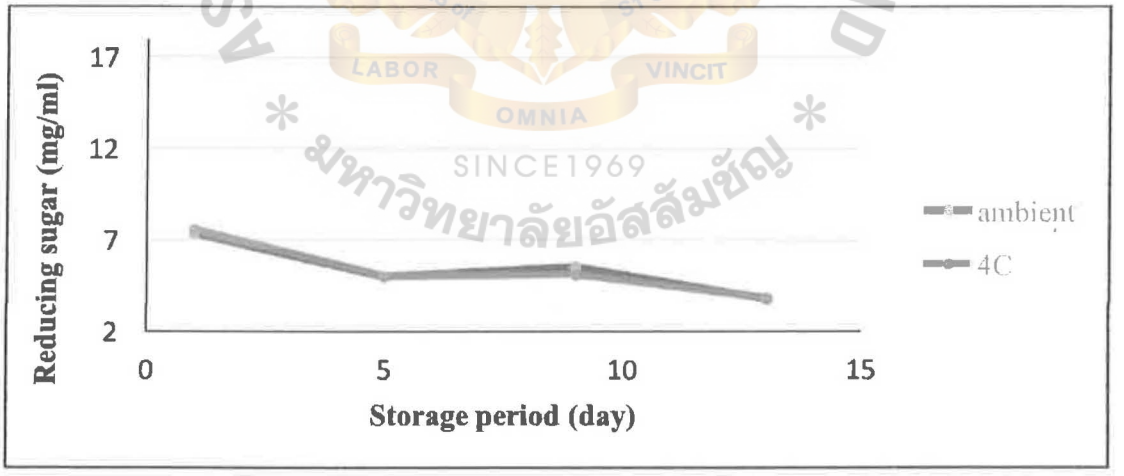

Figure 15 Reducing sugar of bael tea with aloe vera

4.5 Effect of storage on color of bael tea

Table 8 Colorimetric analysis of bael tea with aloe vera

Attributes	Storage condition	Storage period (day)			
		Day1	Day5	Day9	Day13
L*	Ambient	52.43 ± 0.69^{bA}	45.97 ± 2.96^{cA}	51.40 ± 0.52^{aA}	37.57 ± 0.14^{dA}
	4°C	40.89 ± 2.84^{bB}	38.83 ± 1.28^{cB}	53.62 ± 0.50^{aB}	40.63 ± 0.08^{dB}
a*	Ambient	6.12 ± 1.79^{cB}	10.54 ± 1.94^{bB}	6.92 ± 0.27^{dB}	18.38 ± 0.03^{aB}
	4°C	11.89 ± 0.59^{cA}	17.70 ± 1.29^{bA}	6.44 ± 0.05^{dA}	13.93 ± 0.02^{aA}
b*	Ambient	38.82 ± 2.73^{cB}	45.41 ± 1.72^{aB}	37.90 ± 0.48^{dB}	50.66 ± 0.11^{bB}
	4°C	47.65 ± 2.29^{cA}	64.99 ± 1.46^{aA}	39.37 ± 0.29^{dA}	48.59 ± 0.01^{bA}

Notes: Values are mean ± S.D., analyzed by ANOVA using Duncan test.
The same letter (lowercase) in each row (storage period) means no significant difference between days.
The same letter (uppercase) in each column (storage condition) means no significant difference between storage temperature.

The L* parameter was analyzed using factorial CRD. From factorial CRD, it was found out that there was significant difference of storage period, storage condition, and interaction between storage period and storage condition ($p<0.05$). The lightness of bael tea with aloe vera decreased during storage period. Sterilization of samples by autoclaving might lead into the development of pale color, in which occurred in coconut water (Awua *et al.*, 2011). Also, the decrease in reducing sugar might influence the paler color of tea at the end of storage period. The bael tea with aloe vera stored in ambient temperature had darker color of tea. The changes in the lightness might be due to oxidation of the tea since the tea was packed in glass bottles, which was susceptible to light oxidation and Maillard reaction from phenol.

The a* parameter and b* parameter, which indicated the redness and yellowness, were analyzed using factorial RCBD since there were significant differences between triplicates ($p<0.05$). The a* values were extremely fluctuated, between the range of 6-19, while the b* values range from 38-65. Table 9 showed the color differences of bael tea with aloe vera during the storage period. The changes in sample stored in ambient

temperature was very distinct (ΔE>3.0), while the changes of sample in 4°C was distinct (1.5< ΔE<3.0; Pathare *et al.*, 2012). From Table 9, the ambient sample had higher color differences than samples at 4°C, thus, the sample stored at 4°C was preferable in terms of color changes since color was the first attribute that people use during making the decision of either buying or consuming a product.

Table 9 Color difference measurement during storage period

	Ambient	**4°C**
ΔL*	-14.86	-0.26
Δa*	12.26	2.04
Δb*	11.84	0.94
ΔE	8.35	2.23

Figure 16 Bael tea with aloe vera

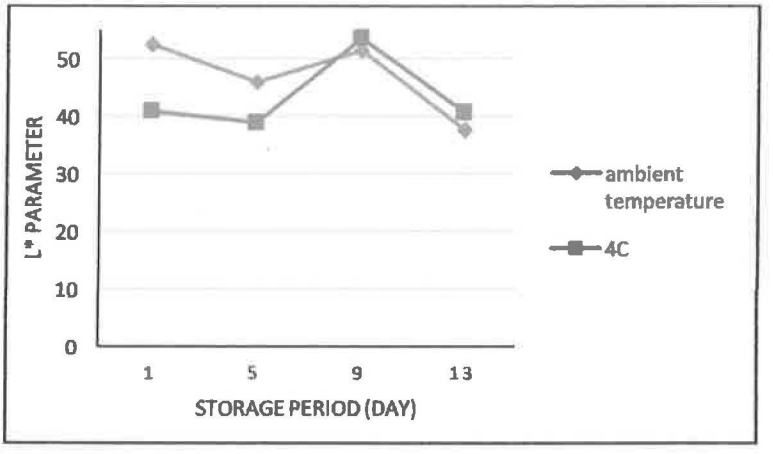

Figure 17 L parameter of bael tea with aloe vera*

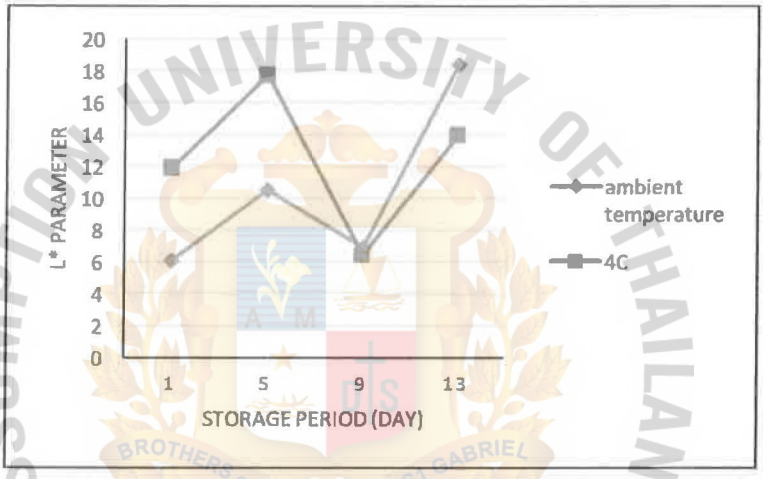

Figure 18 a parameter of bael tea with aloe vera*

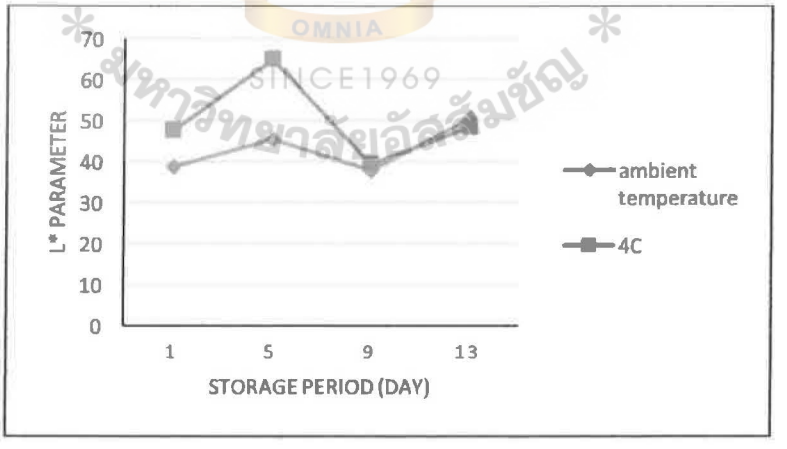

Figure 19 b parameter of bael tea with aloe vera*

Conclusion

The bael tea with aloe vera was made from 84% water, 8% sugar, 5% aloe vera, and 3% dried bael slices. The storage test was carried out for two weeks and several parameters, such as pH, total acidity, total soluble solids (TSS), reducing sugar, and color changes were measured, including the sensory evaluation. The measurements were taken every 4 days. During the storage period, samples were passed through thermal processing and stored at different temperature, ambient and under refrigeration (4°C). The pH, TSS, and color measurement were significant different ($p<0.05$) for both storage period and storage condition, range from 4.85 – 5.2, 11° - 9.6°Brix, ($L^* = 37.57, 40.63$; $a^*=18.38, 13.93$; $b^*= 50.66, 48.59$) at the end of storage period. The total acidity and reducing sugar were significantly different on storage period ($p<0.05$). The sensory evaluation during storage test resulted in significant difference in aftertaste and overall liking score ($p<0.05$), in which most attributes scores were increasing during day2 to day10, and decreasing at day 14. From the sensory evaluation, the bael tea with aloe vera showed that it can be stored up to 10 days and preferably stored under refrigeration. It is suggested that the shelf life determination should be conducted, along with the acceptance test and microbial test. Also, adjusting proper headspace in the glass bottle played important role in the efficiency of sterilization.

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Appendix

Score sheet for acceptance test of bael tea with aloe vera

Sensory Analysis of Bael Tea

Please test the samples and score them based on the following preference test of 9-point hedonic score.

The 9-point hedonic score of preference test

9 = Like extremely	6 = Like slightly	3 = Dislike moderately
8 = Like very much	5 = Neither like nor Dislike	2 = Dislike very much
7 = Like moderately	4 = Dislike slightly	1 = Dislike extremely

Sample number **Attributes**	**405**	**238**	**158**	**207**	**357**
Color (สี)					
Aroma (กลิ่นหอม)					
Bael Flavor (รสมะตูม)					
Sweetness (ความหวาน)					
After taste: Cooling effects (ความรู้สึกเย็นหลังดื่ม)					
Overall liking					

Raw data

All raw data and statistical analysis were kept as electronic file and stored in the attached thumb drive. To retrieve this data, please contact attitayatnd@au.edu.