

Comparison of phenolic compounds and  
antioxidant activities between Thai tea and  
Black tea Kombucha over the course of  
fermentation

By

Mr. Panuvit Sirivoranak

5915271

A special project submitted to the Faculty of  
Biotechnology, Assumption University in part of  
fulfillment of the Requirements for the Degree of  
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2019

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Faculty: Biotechnology

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Mr. Panuvit Sirivoranak



## Abstract

Kombucha is a fermented tea that has been consumed all around the world as a healthy drink for a long time. The tea relies on the fermentation by microbial consortium which are a symbiotic culture of acetic acid and lactic acid bacteria and yeasts. The aim of this experiment was to compare the antioxidant compound and activity between kombucha tea which made Thai tea and black tea and to survey the liking scores on sensory attributes of both kombucha teas. In the present study, Thai tea and black tea kombucha were analyzed for total phenolic content, DPPH radical scavenging method and sensory evaluation. On day 12, black tea kombucha had the higher total phenolic content than Thai tea, 971.13 $\mu$ g/ml and 886.51 $\mu$ g/ml, respectively. However, the total phenolic content of both kombucha teas were found to be not significantly different on day 15. The DPPH radical scavenging of black tea kombucha was higher than that of Thai tea kombucha over the course of fermentation (61.38% GAE to 90.58% GAE). For the sensory evaluation, Thai tea kombucha received a higher liking score when compared with black tea kombucha (7.5 and 6.1, respectively). In conclusion, black tea kombucha possessed higher antioxidant activity but Thai tea kombucha was more preferred in terms of sensory testing.

## Table of Content

<b>Acknowledgement.....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>Abstract.....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>Table of Contents.....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>List of Tables .....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>List of Figures .....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>Introduction .....</b>	ผิดพลาด! ไม่ได้กำหนดน้กมาร์ก
<b>Objectives.....</b>	<b>3</b>
<b>Literature Review .....</b>	<b>4</b>
1. History of kombucha.....	4
2. Health benefits .....	5
3. Marketing of Kombucha tea.....	6
4. The protection of kombucha tea .....	7
5. Ingredient of kombucha.....	8
5.1 Tea.....	8
5.2.1 Thai tea.....	8
5.2.2 Black tea.....	9
5.2.3 Nan Chao Wei.....	10
5.2.4 Jiaogulan.....	11
5.2.5 Indian Marsh Fleabane.....	13
5.2 SCOBY.....	14
6. Previous literatures on Kombucha.....	15
<b>Materials and Methods.....</b>	<b>17</b>
Materials.....	17
Methods.....	17
1. Preparation of kombucha.....	17
2. Antioxidant measurement.....	18
2.1 DPPH radical scavenging assay.....	18
2.2 Determination of total phenolic content.....	18
3. Sensory evaluation.....	19

4. Statistical analysis.....	19
<b>Result and Discussion.....</b>	<b>20</b>
1. Kombucha characteristics.....	20
2. DPPH radical scavenging assay.....	21
3. Determination of total phenolic content.....	24
4. Sensory evaluation.....	25
<b>Conclusion.....</b>	<b>28</b>
<b>References.....</b>	<b>29</b>
<b>Appendix.....</b>	<b>32</b>



## List of Tables

Table		Page
1	Table 1: Result of scavenging effect (% GAE) of Thai tea and black tea kombuchas on day 0, 3, 6, 9, 12, and 15 measured by spectrophotometer at OD 517 nm	22
2	Table 2: Total phenolic content of Thai tea and black tea kombuchas on day 0, 3, 6, 9, 12, and 15 measured by spectrophotometer at OD 750 nm	23
3	Table 3: Sensory evaluation of Thai tea and black tea kombuchas	26





## List of Figures

Figure		Page
1	Figure 1: Kombucha tea	4
2	Figure 2: The origin of kombucha	4
3	Figure 3: Income Statement Evolution	7
4	Figure 4: Thai Tea	8
5	Figure 5: Black tea	9
6	Figure 6: Nan Chao Wei	10
7	Figure 7: Jiaogulan	11
8	Figure 8: Indian Marsh fleabane	13
9	Figure 9: SCOBY	14
10	Figure 10: Preparation of kombucha	17
11	Figure 11: Thai tea fermented after 15 days	20
12	Figure 12: Black tea fermented after 15 days	20
13	Figure 13: Scavenging effect of Thai tea and black tea kombuchas	22
14	Figure 14: Total phenolic content of Thai tea and black tea kombuchas	23
15	Figure 15: Tannin structure	24
16	Figure 16: Sensory evaluation of Thai tea and black tea kombuchas	26

## Introduction

Kombucha tea, or its other name, is tea fungus. This tea is a fermented tea that has been consumed all around the world as a healthy drink for a long time especially in China, Russia and Germany (Dipti *et al.*, 2003). It is a beverage of Manchuria origin that obtains the composition of yeast and bacteria by microbial consortium. They are symbiotic culture of acetic acid and bacteria (AAB; *Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species) lactic acid bacteria (LAB; *Lactobacillus*, *Lactococcus*) and yeasts (*Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*) (Villarreal-Soto *et al.*, 2018) which obtain in Kombucha tea, and give general sweet medium.

The lifestyle of modern people nowadays tends to prefer the products that are good for health. Kombucha tea is more prevalent in the community to prevent of disease because it can boost the body's immune system. Kombucha tea is the most popular fermented tea healthy drink in the world, that kombucha tea has been led into the western market because many consumers want to try to taste fermented tea which is good for health such as treating AIDS, cancer, aging, diabetes, etc.

In the world's market, kombucha tea is gaining popularity in an emerging market like Middle East, Eastern Europe, South East Asia, and other in the recent years. Kombucha tea shows the fastest growth in the tea market because of the disposable income and urbanization has been increased among consumers. In the Global Kombucha Tea Market Report and Forecast 2019-2024, the kombucha tea market makes a value of USD 1.5 billion by 2018, growing strongly at a CAGR of 23% between 2014 -2018. The market is further expected to grow at a CAGR of 20.6% in the forecast period of 2019-2024, reaching USD 4.6 billion by 2024 (Unilever, 2019)

Thai tea is a popular traditional beverage in Thailand because it can be served either cold or hot by adding milk and sugar. Moreover, people all around the world like to drink Thai tea because it can make them fresh and boost the energy. Furthermore, Thai tea is the best seller in Thailand since it can be made into many forms of food products such as ice cream, snack, etc. It is interesting to use Thai tea to make kombucha because there are several benefits of Thai tea that it can reduce cancer risk,

help in weight loss management, provide good mineral source such vitamin A, vitamin C and calcium, help people who have problem about excrete the waste and can lower the risk of heart attack, stroke, and arteriosclerosis (Staughton, 2020).

Therefore, this study is aimed to compare the antioxidant compound and activity between kombucha tea which made Thai tea and black tea and to survey the liking scores on sensory attributes of both kombucha teas.



## Objectives

1. To determine and compare the amount of antioxidant compounds and activity of kombucha tea that made from black tea and Thai tea over the course of fermentation.
2. To survey the liking scores on sensory attributes of kombucha made from black tea and Thai tea.





## Literature Review

### History of kombucha tea



**Figure 1: Kombucha tea**

(Source: <https://en.wikipedia.org/wiki/Kombucha>)

Kombucha tea is a fermented tea that tastes slightly alcoholic, lightly effervescent, sweetened black or green tea and this tea is consumed commonly for health benefits (Figure 1). The microorganism that obtains in kombucha is bacteria and yeast.



**Figure 2: The origin of kombucha**

The origins of Kombucha have become lost in the mists of time. It is thought to have originated in the Far East, probably China, and has been consumed there for at least two thousand years. The first recorded use of kombucha comes from China in 221 BC during the Tsin Dynasty. It was known as "The Tea of Immortality". It has been used in Eastern Europe, Russia, and Japan for several centuries. It's from Japan in 415

AD that the name kombucha is said to have come. A Korean physician called Kombu or Kambu treated the Emperor Inyko with the tea and it took his name, "Kombu" and "cha" meaning tea. Russia has a long tradition of using a healing drink called "Tea Kvass" made from a "Japanese Mushroom". (James Roche and Taoist Philosopher, 2008)

### **Health benefit**

There are many benefits of using kombucha that can help to improve in energy level, metabolic disorders, allergies, cancer, digestive problems, candidiasis, hypertension, HIV, chronic fatigue and arthritis. Moreover, Kombucha has contained antioxidants that can kill harmful bacteria and help fight several diseases. (Leech, 2018)

First of all, kombucha is a potential source of probiotics because of a large number of bacteria that grow in the mixture which contain several species of lactic-acid bacteria that may have a probiotic function. Probiotics provide consumer's gut with healthy bacteria and these bacteria can improve many aspects of health, digestion, inflammation and weight loss (Marsh, 2014).

Secondly, kombucha contains antioxidants which are substances that fight free radicals, reactive molecules that can damage human cells. Also, in study in rats it was found that drinking kombucha helped to reduce liver toxicity caused by toxic chemicals (Ames *et al.*, 1993).

Thirdly, kombucha can kill bacteria because one of the main substances produced during the fermentation of kombucha is acetic acid that can be able to kill many potentially harmful microorganisms. Kombucha made from black or green tea show the strong antibacterial properties, and these antimicrobial effects destroy the growth of undesirable bacteria and yeasts but they do not affect the benefit of probiotic bacteria and yeast (Sreeramulu *et al.*, 2000).

Besides, kombucha can help reducing heart disease risk because in rat studies, it was shown that kombucha can greatly improve "bad" LDL and "good" HDL cholesterol levels, in as few as 30 days. Furthermore, the tea protects LDL cholesterol particles from oxidation, which is thought to contribute to heart disease (Yang and Koo, 2000).



Lastly, kombucha can help protect against cancer because, in test-tube studies, kombucha helped preventing the growth and spread of cancerous cells due to its high concentration of tea polyphenols and antioxidants.

## **Marketing of Kombucha tea**

Kombucha tea has been consumed for a long time, but the commercialization process of kombucha tea arises in the last decade year. As a functional beverage, kombucha tea is a mainstream contender in retail and commercial restaurants. The future market outlook is very promising, especially in emerging markets such as Asia-Pacific and South America.

The researcher has studied the global market size of Kombucha in key regions like North America, Europe, Asia Pacific, Central & South America, and Middle East & Africa focuses on the consumption of Kombucha in these regions. The North America is leading region in this market and it is followed by increasing demand for probiotic and fortified beverages, developing region, and trend of consuming natural and healthy products. (XYZ Research, 2019)

In the world's market, kombucha tea is gaining popularity in an emerging market like Middle East, Eastern Europe, South East Asia, and other in the recent years. Kombucha tea shows the fastest growth in the tea market because of the disposable income and urbanization has been increased among consumers. In the Global Kombucha Tea Market Report and Forecast 2019-2024, the kombucha tea market makes a value of USD 1.5 billion by 2018, growing strongly at a CAGR of 23% between 2014 -2018. The market is further expected to grow at a CAGR of 20.6% in the forecast period of 2019-2024, reaching USD 4.6 billion by 2024 (Unilever, 2019)

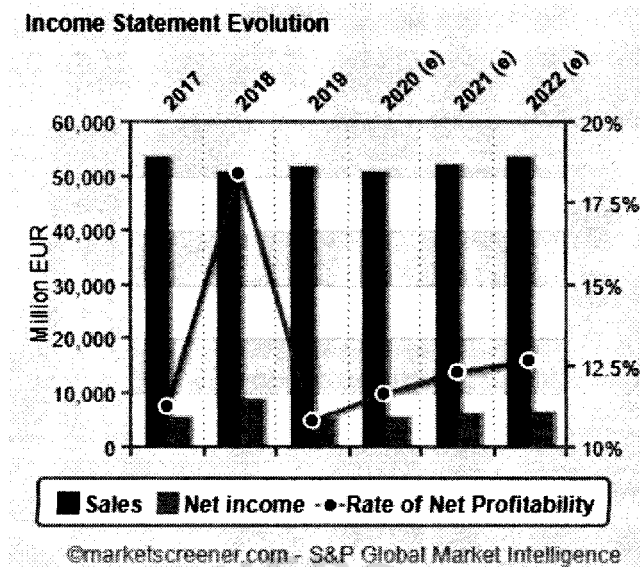


Figure 3: Income Statement Evolution

(source: <https://www.marketscreener.com/UNILEVER-N-V-61006158/news/Unilever-Kombucha-Tea-Market-Expected-to-Grow-at-a-CAGR-of-20-6-in-the-Forecast-Period-of-2019-20-29519595/>)

### The protection of kombucha

In addition to solve the poor sanitary of kombucha tea to be safe for people would be brewing kombucha tea correctly because the product will not be contaminated and in every step of brewing, all equipment should be sterile in a sanitary condition and proper preparation and fermentation technique to ensure that the product will be safe. Besides, choose high quality, low-calorie, low sugar products stored in dark glass containers. This packaging protects the probiotics from light damage. Moreover, intake of kombucha tea to one or two serving per day is the best.

## Ingredients of kombucha

### 1. Tea

Kombucha cannot be kombucha without tea. However, there are many different types of tea and forms to choose from. Several type of tea can be mixed together to create a unique kombucha flavor base. Some examples of Thai herbal tea that are common in Thai market include the following.

#### 2.1 Thai Tea

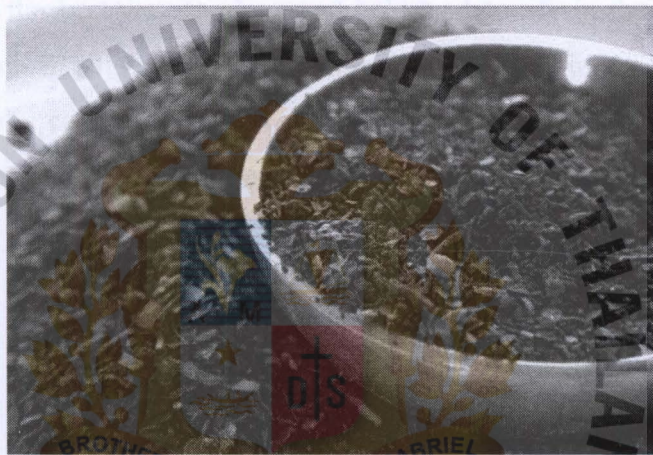


Figure 4: Thai Tea

(Source: <https://www.thaitable.com/thai/ingredient/thai-tea>)

Thai tea is a popular traditional tea from Asia, and people in Thailand like to consume this tea conveniently because Thai tea is delicious and it can be consumed as hot or cold beverage and people can add milk, and sugar. The major ingredient of Thai tea is made from Ceylon tea or local people from Assam called Bai Miang. Furthermore, Thai tea contains caffeine which is about 50 mg – 60 mg per 8-ounce cup of this beverage (Zuzunetra, 2020).

Thai tea is rich in antioxidants and it has a good source of vitamins and minerals such as vitamin A, vitamin C, and calcium. However, the calories of Thai tea are between 180 to 300 but these calories are for Thai tea that mixed with milk and sugar, without these, Thai tea is no calories and good for health.



There are several health benefits of Thai tea which include reducing cancer risk because Thai tea is made from black tea and it contains antioxidants that can reduce the risk of many different forms of cancer such as lung, skin, stomach, etc. Next, people, who consume Thai tea their arteries will be healthy because Thai tea can help to expand arteries, so their important roles are to prevent heart attacks and strokes. Moreover, Thai tea can help people with weight loss because it has caffeine that can stimulant, so this can help people to focused and energetic. The combination of caffeine and antioxidants in Thai tea can help to support the metabolism, so human body will burn calories more quickly. Furthermore, Thai tea can be used as a detoxification to help people who have a problem with the passing out of the metabolic waste which is painful and difficult, so drinking Thai tea is the best way to clean and help to detoxify in their body system. Next, Thai tea is rich in vitamins and minerals such as vitamin A, vitamin C, and calcium. For example, calcium supports strong bones and teeth and vitamin A is a supplement to the cornea that helps to protect eye infections and weaken eye vision.

## 2.2 Black tea

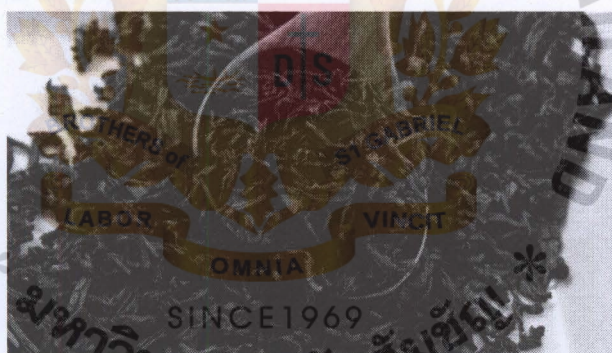


Figure 5: Black tea

(Source: <https://www.thespruceeats.com/black-tea-infused-vodka-recipe-766113>)

Black tea is a type of tea that is more oxidized than oolong, green, and white teas. Black tea is generally stronger in flavor than other teas. It comes from the *Camellia sinensis* plant and is often blended with other plants for different flavors, such as Earl Grey, English breakfast, or chai.



Black tea is the best choice for people who want to lose their weight because it has low calories and non-sweetened beverages with less caffeine. Besides, black tea contains a substantial antioxidant that can provide a lot of benefit to health.

Black tea has offered a variety of health benefits because it contains antioxidants and compounds that can help to reduce inflammation in the body. First of all, black tea contains a group of polyphenols that have antioxidant properties. Consuming antioxidants may help decrease the risk of chronic disease and improve your overall health. Moreover, black tea contains flavonoids that can help to reduce the risk of heart disease and it can help to decrease LDL levels. Furthermore, black tea can improve gut health because polyphenol and antimicrobial properties which found in black tea can help to improve gut health and immunity. Besides, black tea can reduce blood pressure and the risk of stroke because drinking black tea on regular basis can help decrease systolic and diastolic blood pressure, and studies have found that drinking black tea may also help to reduce the risk of stroke. Lastly, black tea may reduce the risk of cancer because polyphenol that contains in black tea which can help to fight cancer cells in the body (Enloe, 2018).

### 2.3 Nan Chao Wei

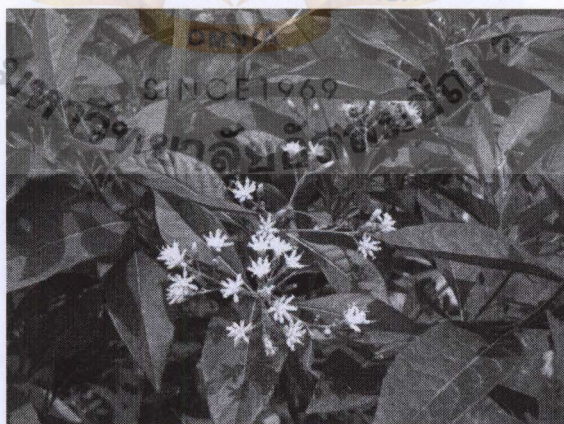


Figure 6: Nan Chao Wei

(Source: [https://en.wikipedia.org/wiki/Gymnanthemum\\_extensum](https://en.wikipedia.org/wiki/Gymnanthemum_extensum))

*Gymnanthemum extensum*, Nan Chao Wei or Nan Fui Chao, is originated from China and has been planted and propagated in Thailand for many years. Popularly planted only in the Chinese herb garden and Thai herb garden to be used as a medicinal



drug. The fresh leaves of "Nan Chao Wei" are bitter and flavorful when chewing and eating fresh. But after eating for a while, it feels like it has a sweet taste in the mouth and throat.

There are several benefits of Nan Chao Wei tea for human health. First of all, they help to reduce blood sugar levels and blood pressure. Moreover, Nan Chao Wei is believed to treat diabetes and gout. Furthermore, this tea can help to release toxic in the human body and they can treat people who have problems with thyroid but it should be consumed at low level. Thus, Nan Chao Wei is not recommended for people who have a high level of the thyroid. (Jurairat N., 2018)

#### 2.4 Jiaogulan

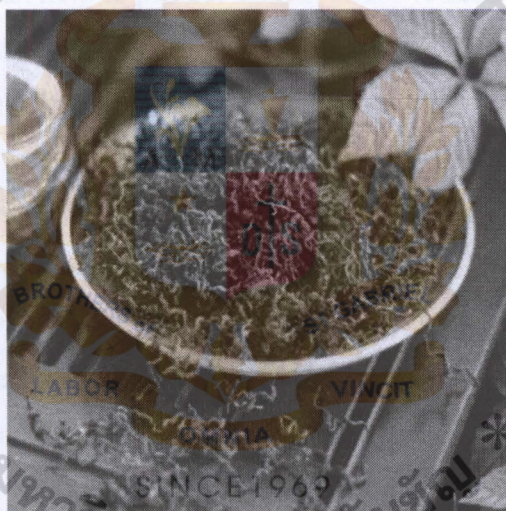


Figure 7: Jiaogulan

(Source: <http://www.orientalteastore.com/herbal-tea-c-7/jiaogulan-tea-gynostemma-pentaphyllum-p-95.html>)

Jiaogulan is also called *gynostema pentaphyllum*. They belong in the family *Curcubitaceae*, which includes melons, cucumbers, and gourds. Jiaogulan is dioecious that means each plant can be either male or female and it is also herbaceous climbing vine of the family *Cucurbitaceae* and it can attach itself to support by using tendrils. Moreover, Jiaogulan is used to make it into traditional medicine. Although there is no evidence-based medicine that demonstrates any effect on human health. Furthermore,



the vine's leaves are used as food and medicine, and tea made from the leave is caffeine-free and a bit bittersweet taste. (Morris, 2017)

The Chinese people called jiaogulan as an immortality herb and claim it has rejuvenating properties. There are a lot of people who say that jiaogulan has several benefits to their health such it can boost cardiovascular health and help the body to withstand stress.

First of all, jiaogulan can help to control diabetes and from the previous research on jiaogulan which have tested on diabetes from rats and the test has shown that the herb's effect on diabetes rat and found that it helped to decrease blood sugar levels and lower LDL cholesterol (Megalli *et al.*, 2006), and jiaogulan can help control blood sugar by repairing activity in certain liver enzymes.

Next, jiaogulan can help to protect against stress-related anxiety disorders, so it can balance the mind and take the anxiety away out of the head. In the test on mice, the research has observed that jiaogulan can help inhibit stress and induced anxiety, there is influencing activity in the certain brain to control mood. Furthermore, jiaogulan can protect against asthma and according to from the previous research, the study's authors have observed the effect of jiaogulan on mice, and it was found that the herb can reduce the inflammation of airway that associate with asthma. Moreover, jiaogulan can help to control weight in long term like other healthy tea and it can help to reduce calories that will be benefits for human waistline. According to a clinical study, jiaogulan can help people who have fatty liver disease and also help to decrease their body mass (Wong and Forgos, 2020).

### 2.3 Indian Marsh Fleabane (ใบขี้เหล็ก)



Figure 8: Indian Marsh Fleabane

(Source: <https://medthai.com/%E0%B8%82%E0%B8%A5%E0%B8%B9%E0%B9%88/>)

Indian Marsh Fleabane is a species of flowering plant in the aster family, Asteraceae. The plant contains the compounds  $\beta$ -sitosterol and stigmasterol, which have antidiabetic properties. The  $\beta$ -sitosterol isolated from the root extract can also neutralize the venom of Russell's viper (*Daboia russelii*) and the monocled cobra (*Naja kaouthia*) (Wikipedia, 2019)

Indian Marsh Fleabane offers a number of health benefit. First of all, this plant is helpful to decrease the level of sugar in diabetes patients and reduce fat in the blood. Moreover, Indian Marsh Fleabane improves the maintenance of eyes and nerve cells. Besides, they help the reduce workload of liver functioning, release kidney stones and also help to release diuretic. (MedThai, 2017)



## 2. SCOBY

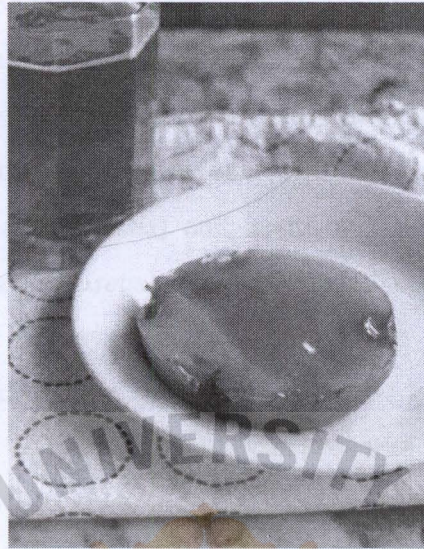


Figure 9: SCOBY

(source: <https://www.thekitchn.com/how-to-make-your-own-kombucha-scooby-cooking-lessons-from-the-kitchn-202596>)

**SCOBY** is the main component of kombucha tea. Scoby is grayish-white, squishy mass floating within the brewed culture, and it takes the responsibility on vinegar-like flavor, trivial alcohol content, and characteristic carbonation of kombucha. On the other hand, SCOBY is the symbiotic of multiple bacteria and yeast species that live together with a cellulose mix and SCOBY is also a biofilm which is the colony of many microbial attach each other on the surface. The type of bacteria and yeast which is found in SCOBY: *Acetobacter*, *Saccharomyces*, *Brettanomyces*, *Lactobacillus*, *Pediococcus*, *Gluconacetobacter*, and *Zygosaccharomyces*. Other fermented foods also use other symbiotic culture which is kefir, ginger beer, vinegar, and sourdough. (Phung, 2015)

## Previous literatures on Kombucha

Sun (2015) had studied about the effect of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage. In this experiment, sweetened black tea (10% w/v) and wheatgrass juice (WGJ) were mixed together in various ratios and fermentation substrate was used for enhancing phenolic compound and antioxidant activity which was DPPH. The starter included yeast (*Dekkera bruxellensis*) and acetic acid bacteria (*Gluconacetobacter rhaeticus* and *Gluconobacter roseus*), was inoculated at 20% (v/v), and fermented statically at  $29 \pm 1^\circ\text{C}$  for 12 days. For the result in this experiment, total phenolic, flavonoid contents and antioxidant activity of modified kombucha were higher than traditional kombucha. All WGJ-blend kombucha preparation had higher concentration of various phenolic compounds as parallel to traditional ones. Addition of WGJ resulted in the DPPH scavenging ability of kombucha was  $> 90\%$ , whereas the oxygen radical absorbance capacity was increased from 5.0 mmol trolox equivalents/mL to 12.8 mmol trolox equivalents/mL equally the ratio of WGJ increased from 0% to 67% (v/v). The highest antioxidant activity was attained using 1:1 (v/v) black tea decoction to WGJ ratio and 3 days fermentation, producing several types of phenolic acids. These results had suggested intake of fermented black tea advanced with wheatgrass juice is valuable over traditional kombucha formulas in term of giving several complementary phenolic and might have more potential to decrease oxidative stress. (Sun, 2015)

Gramza-Michałowska (2016) studied on the effect of culture time on the kombucha beverage's antiradical capacity and sensory value. Four kinds of dried tea leaves which were white, green, black, and yellow and the kombucha were analyzed by total phenolic content (TPC), DPPH radical scavenging method and sensory value. The result had shown that TPC content and DPPH radical scavenging capacity values were valued in yellow tea samples, both fermented and unfermented, that did not contrast within the storage time. The result of sensory evaluations of kombucha tea brews relied on the tea leaf variety used for preparing drink. In conclusion, researcher was specified the fermentation process of tea brews with kombucha microbiota did not affect significantly its polyphenol content and antiradical capacity. (Gramza-Michałowska, 2016)

Pure (2016) studied about the antioxidant and antibacterial activity of kombucha beverages prepared using banana peel, common nettles and black tea infusions. Kombucha was made by using nettles leaf and banana peel infusion to ferment. For materials and methods, herbal infusions were fermented by kombucha fungi. Folin-Ciocalteu assay was used to evaluate total phenolic content, and free radical scavenging activity was estimated by using DPPH. The result showed that black tea had the highest amount of phenolic and fermentation decomposed approximately 50% of phenolic contents to 265.5 ppm though total phenolic of nettles infusion and fermented beverage were 173 gAE and 188 gAE respectively and for banana peel, 136.5 gAE and 155 gAE; it showed increase of phenolic contents due to fermentation that may be cause of protein contents of nettles and banana peel gone under fermentation by lactic acid bacteria. Fermented beverage of three herbs had higher antioxidant effective than infusions. Kombucha from banana peel showed the highest antioxidant activity by inhibiting 94.62% of DPPH. Whereas, antioxidant of black tea and nettles leaf were more related to their acidic acid content and it was found that significant part of antioxidant activity of banana peel kombucha due to other acid and phenolic (Pure, 2016).



## Materials and Methods

**Materials:** Thai tea, black tea and sugar were purchased from supermarket. SCOBY was ordered from USA by Dr. Atittaya Tandhanskul. Glass Jars were purchased from IKEA and thermometer was provided by Dr. Atittaya Tandhanskul. DPPH, gallic acid, Sodium carbonate, methanol, Follin-Ciocalteu reagent were already provided from Assumption university lab.

### 1. Preparation of kombucha

For each tea, four grams of tea (5 tea packs) and 1 liter of drinking were mixed and brought to boil within 5 minutes. After boiling the tea mixture, cooling down to room temperature was achieved by a measurement using a thermometer. When the tea mixture was at room temperature, 30-35 grams of SCOBY was added which was from the previously fermented kombucha. The top of the glass jar was covered by using a sterile cheesecloth and the cloth was secured with rubber band to protect contamination. Kombucha was left fermented at room temperature for 15 days. During the course of fermentation, the kombucha was collected from each fermentation day: Day 0, Day 3, Day 6, Day 9, Day 12, and Day 15. Kombucha samples were preserved at  $-20^{\circ}\text{C}$  until needed.

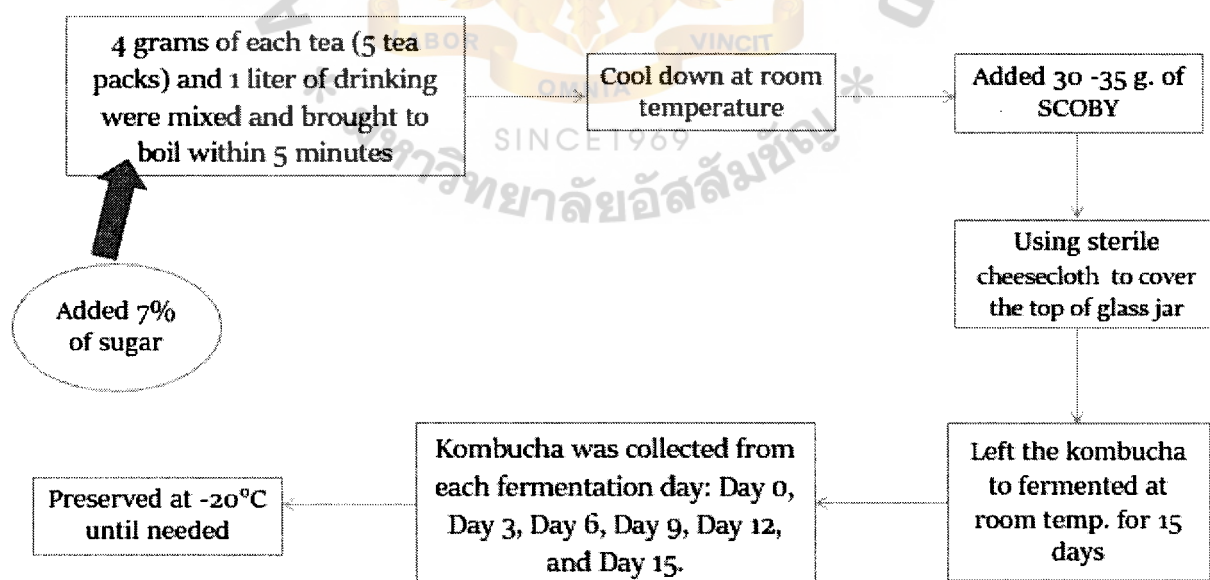


Figure 10: Preparation of kombucha



## 2. Antioxidant measurement

### 2.1 DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol (Huang *et al.*, 2005). DPPH needs to be freshly prepared before the DPPH radical scavenging assay. A 1mM solution of the radical was prepared by dissolving 0.0392 g DPPH in 100 ml of methanol and then the mixture had to be shaken vigorously. When DPPH dissolved, the mixture was covered with aluminum foil and kept in the dark at room temperature.

The assay was performed by adding 0.2 ml of kombucha with 3.8 ml methanol and then 0.6 ml of 1mM DPPH solution was added. The mixture was incubated at room temperature for 30 min in the dark. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV spectrophotometer. The mixture of methanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging effect (%) was determined as below.

$$\% \text{scavenging effect} = \left[ 1 - \left( \frac{A_{517 \text{ sample}} - A_{517 \text{ blank}}}{A_{517 \text{ control}}} \right) \right] \times 100$$

### 2.2 Determination of total phenolic content

Fifty  $\mu\text{l}$  of Kombucha was added to 2 ml of 7% sodium carbonate (prepared by dissolving 7.5 g of  $\text{NaCO}_3$  into 100 ml of water). After 2 min, 0.1 ml Follin-Ciocalteu reagent was mixed with the solution, the absorbance at 750 nm was measured after 30-min incubation.

For preparation of a standard curve, 0.01 g of gallic acid was mixed with 100 ml of water and then diluted to make into the concentration of 1000, 500, 250, 125 and 75  $\mu\text{g/ml}$  and then the method above was performed by using gallic acid instead of tea. The total phenolic content values were determined via a calibration curve prepared with a series of gallic acid standard.

### 3. Sensory evaluation

The sensory evaluation of kombucha was carried out when kombucha tea finished fermentation for 15 days. The sensory evaluation was performed by 22 panelists who were Assumption University students. Several attributes, such as color, bitterness, fermented flavor, sweetness, sparkling, sourness, and overall liking were asked on 9-point hedonic scale.

### 4. Statistical Analysis

In this experiment, RCBD has been used for evaluated Thai tea and black tea kombucha and comparing for each treatment. All data from the sensory evaluation, DPPH radical scavenging assay and total phenolic content that have been collected by the SAS 9.4 program with RCBD, and Duncan's multiple range tests and analysis of variance (ANOVA) has been used also.





## Result and Discussion

### 1. Kombucha characteristics

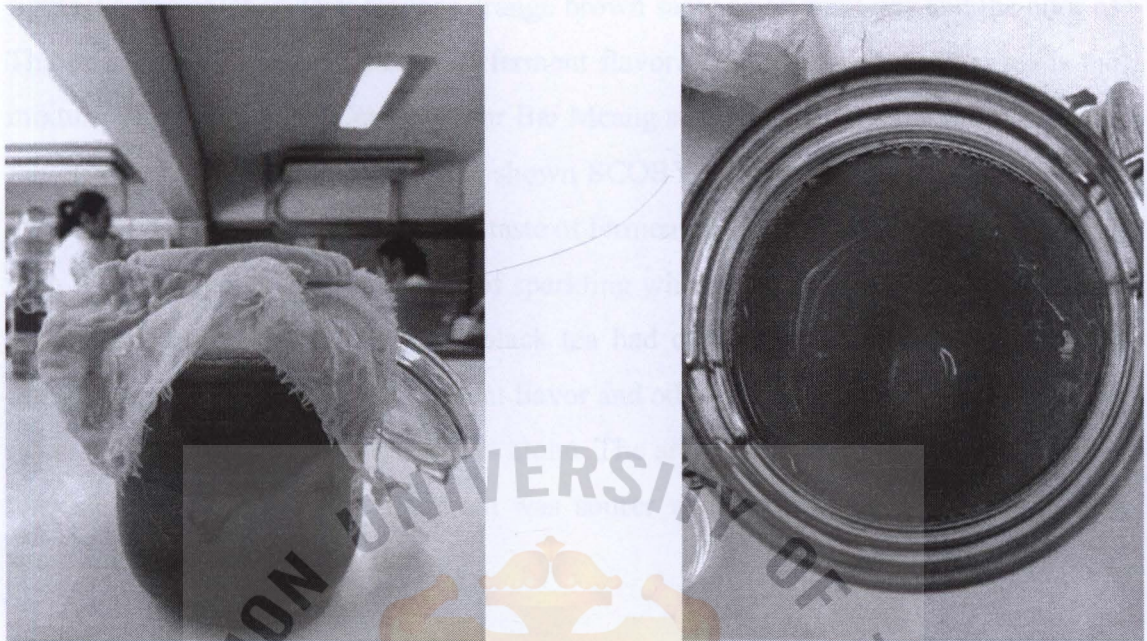


Figure 11: Thai tea fermented after 15 days



Figure 12: Black tea fermented after 15 days



The characteristic of Thai tea and black tea after fermenting for 15 days, from figure 11, the color of Thai tea was orange brown same as original one and the odor of Thai tea was good not too strong of ferment flavor. This was because Thai tea is the mixture of black tea and Ceylon tea or Bai Meang so its aroma covered the fermented flavor. Next, fermented Thai tea had shown SCOBY which was grow during 15 days (The right picture, Figure 11) and the taste of fermented Thai tea is a bit of sweet mixed with sourness and it had a moment of sparkling when swallow through the neck. For black tea (Figure 12), the color of black tea had change from black to brown after fermenting for 15 days and the ferment flavor and odor was too strong because of black tea is made from fermented tea leaves alone. The aroma received was purely from the tea. Moreover, the taste of black tea was sourer without any sweetness and higher sparkling sensation after swallow.

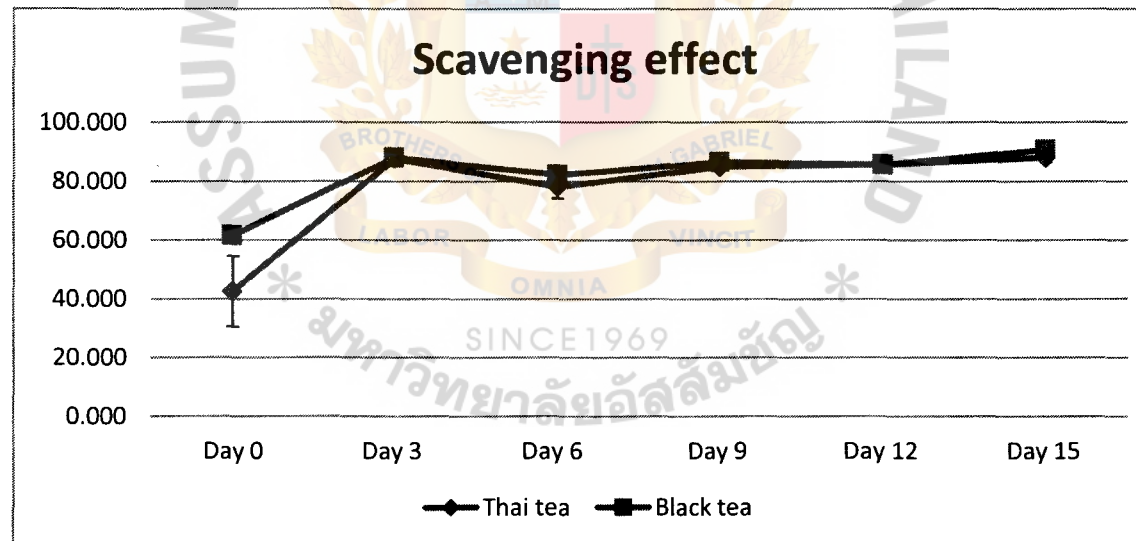
## **2. DPPH radical scavenging assay**

As it can be seen from Table 1, the scavenging effect of black tea and Thai tea on day 3 to day 12 had significantly different but except day 0 and day 15 which was not significantly different. From the graph (Figure 13), it shows the DPPH scavenging abilities of Thai tea and black tea (control) over the 15-day fermentation. Percent scavenging of both teas showed an increase from day 0 to day 3 and reached values which were scavenging effect of Thai tea is 42.43% to 87.62% and for black tea 61.38% to 87.91%. However, the scavenging effect of Thai tea and black tea decreased on day 6 and then continued to slightly increase at day 9, day 12, and day 15. This maybe explained that different kombucha sample had shown diverse radical scavenging abilities which were possibly participated with their normal microbiota (*Acetobacter* species, *Lactobacillus*). They showed the difference relating to the sources and affected the metabolic fate of culture broth (Chu and Chen, 2006). Black tea had more scavenging effect since day 0 - day 15 than Thai tea because black tea contained higher antioxidants than Thai tea. Black tea is renowned for composing of a large group of polyphenols which include catechins, theaflavins and thearubigins (Khan and Mukhtar, 2007). These antioxidants were preserved throughout the fermentation and even increased as a result of microbial metabolism.

**Table 1:** Result of scavenging effect (% GAE) of Thai tea and black tea kombuchas on day 0, 3, 6, 9, 12, and 15 measured by spectrophotometer at OD 517 nm

Type of tea	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Thai tea	42.43 ± 11.97 <sup>b</sup>	87.62 ± 1.13 <sup>a</sup>	77.84 ± 3.80 <sup>a</sup>	84.91 ± 0.93 <sup>a</sup>	85.65 ± 1.63 <sup>a</sup>	87.99 ± 1.55 <sup>b</sup>
Black tea	61.38 ± 2.61 <sup>a</sup>	87.91 ± 2.50 <sup>a</sup>	82.08 ± 0.62 <sup>a</sup>	86.37 ± 2.25 <sup>a</sup>	85.58 ± 2.12 <sup>a</sup>	90.58 ± 2.13 <sup>a</sup>

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

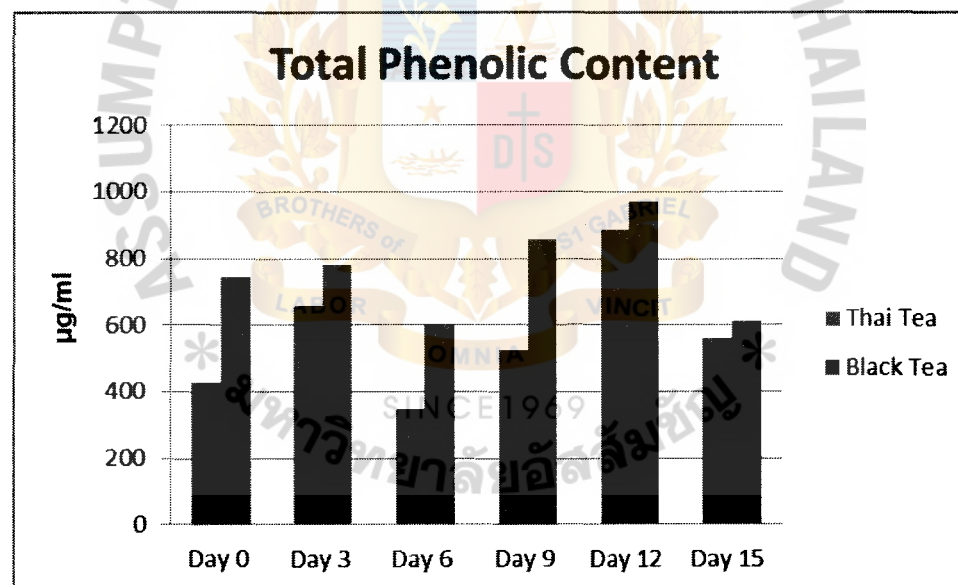


**Figure 13:** scavenging effect of Thai tea and black tea kombucha

**Table 2:** Total phenolic content of Thai tea and black tea kombuchas on day 0, 3, 6, 9, 12, and 15 measured by spectrophotometer at OD 750 nm

Type of Tea	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Thai tea	427.28 ± 24.57 <sup>b</sup>	656.76 ± 45.53 <sup>b</sup>	349.33 ± 10.61 <sup>b</sup>	524.2 ± 10.61 <sup>b</sup>	886.51 ± 54.23 <sup>a</sup>	561.123 ± 5.56 <sup>b</sup>
Black tea	745.99 ± 24.5 <sup>a</sup>	781.383 ± 7.81 <sup>a</sup>	603.43 ± 23.74 <sup>a</sup>	859.07 ± 30.1 <sup>a</sup>	971.13 ± 29.8 <sup>a</sup>	610.356 ± 7.7 <sup>a</sup>

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

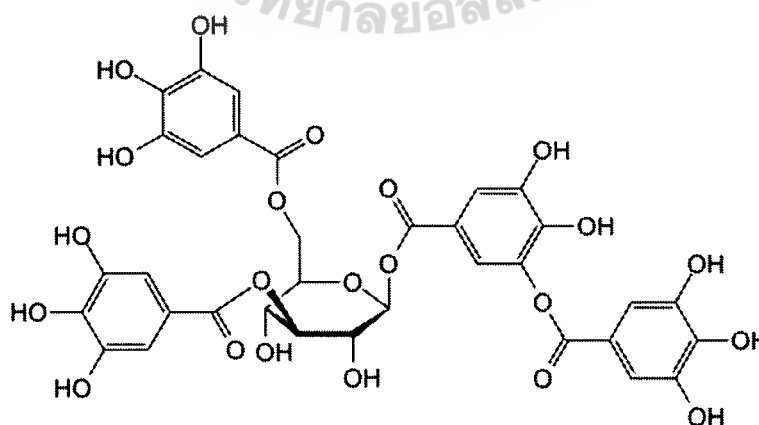


**Figure 14:** total phenolic content of Thai tea and black tea kombuchas



### 3. Determination of total phenolic content

From Table 2, the total phenolic content of Thai tea and black tea from day 0 to day 9 and day 15 were significantly different but day 12 was not significantly different. As shown in Figure 10, the total phenolic content (TPC) of Thai tea and black tea increased with the fermentation time (day 0 to day 3). However, TPC of Thai tea and black tea decreased on day 6 and then continued to increase again on day 9. This data is in agreement with the percent scavenging effect as shown in previous section. After that, the total phenolic content of samples increased significantly on day 12 before declining almost half of the amount on day 15 because when the color of kombucha broths got lighter, it indicated the polyphenols did undergo the microbial transformation (Chu and Chen, 2006). However, kombucha can be kept longer for 18 -21 days of fermentation. Therefore, the total phenolic content of Thai tea and black tea were expected to increase from day 15. Furthermore, it shows that black tea contained higher total phenolic content from the beginning which was more than Thai tea throughout the fermentation. This is because the brown color of black tea came from the chromophoric group of thearubigins which consisted of a planar structure formed by oxidative oligomerization of the flavonol precursors as derivatives of catechins (Chu and Chen, 2006). It was suggested that the changes of total phenolic content and antioxidant activity in both kombuchas were result of tannin breakdown. The breakdown of tannin into smaller phenolic and flavonoid compounds, subsequently, increased the antioxidant activity as found in this study. Further investigation on monitoring of the breakdown products is encouraged.



**Figure 15: Tannin structure**

(Source: <https://en.wikipedia.org/wiki/Tannin>)

## Sensory Evaluation

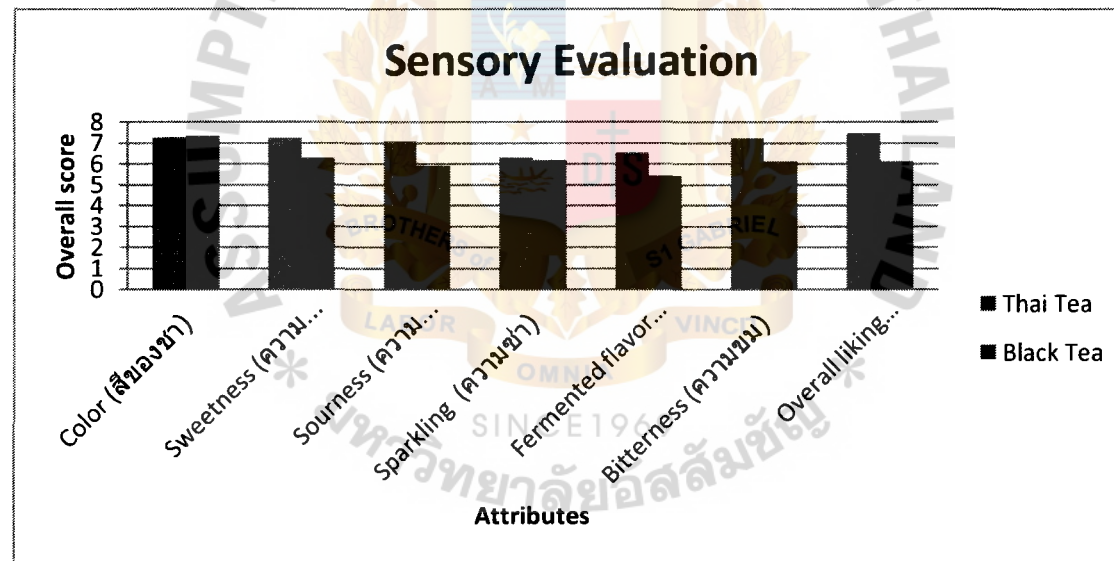
Using 9 points hedonic score to test the sample and score to prefer liking product (9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, and 1 = Extremely Dislike). Twenty-two panelists were asked to evaluate 2 type of kombucha which made from Thai tea and black tea. The panelists had to score each attribute for both samples. The results of attribute (color, sweetness, sourness, sparkling, fermented flavor, bitterness) and overall liking score were shown in Table 3 and comparison of 2 samples of kombucha from each attributes in Figure 16.



**Table 3:** Sensory evaluation of Thai tea and black tea kombuchas

Type of tea	Color (สีของชา)	Sweetness (ความหวาน)	Sourness (ความเปรี้ยว)	Sparkling (ความซ่า)	Fermented flavor (รสชาติหมัก)	Bitterness (ความขม)	Overall liking (ความชอบโดยรวม)
Thai Tea	7.3 ± 0.77 <sup>a</sup>	7.2 ± 0.81 <sup>a</sup>	7.1 ± 1.37 <sup>a</sup>	6.3 ± 1.76 <sup>a</sup>	6.5 ± 1.63 <sup>a</sup>	7.2 ± 1.44 <sup>a</sup>	7.5 ± 1.05 <sup>a</sup>
Black Tea	7.3 ± 1.25 <sup>a</sup>	6.3 ± 1.45 <sup>b</sup>	5.9 ± 1.41 <sup>b</sup>	6.2 ± 1.46 <sup>a</sup>	5.4 ± 1.71 <sup>b</sup>	6.1 ± 1.42 <sup>b</sup>	6.1 ± 1.16 <sup>b</sup>

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



**Figure 16:** Sensory Evaluation of Thai tea and black tea kombuchas

According to Table 3, color and sparkling of Thai tea and black tea kombuchas were not significantly different but the other attributes sweetness, sourness, fermented flavors, bitterness and overall liking were significantly different. Figure 16 shows the satisfaction of consumer in 2 samples of kombucha made from Thai tea and black tea, the color liking score was similar in each other (7.3). For the sweetness of Thai tea and black tea kombuchas, liking score of Thai tea (7.2) was higher than black tea kombucha according from the table 3. Moreover, the consumer liking score of sourness and fermented flavor of Thai tea kombucha were 7.1 and 6.5 respectively and higher than black tea kombucha because the consumer commented that black tea kombucha was sourer while Thai tea kombucha was less sour and tasted better. Furthermore, Figure 16 shows that the liking score of sparkling was nearly comparable which score was 6.2 – 6.3 but consumer had mentioned that black tea kombucha should be carbonated with no fermented tea taste. Additionally, bitterness of black tea kombucha liking score (6.1) was lower than the liking score of Thai tea (7.2), since consumer had noted that black tea kombucha was too bitter and strong.

Consequently, the consumers preferred Thai tea kombucha than black tea kombucha (7.5 to 6.1) which was like moderately to like very much. This could be because the masking effect of Thai tea that has their own flavor and odor in themselves to cover the ferment flavor. Besides, Thai tea kombucha tasted much better than black tea kombucha after fermentation since black tea does not have their own flavor like Thai tea. As a result., the taste after fermentation was too sour and yielded too strong fermented flavor.



## Conclusion

Black tea kombucha showed higher antioxidant activity in DPPH and total phenolic content according to the result. For DPPH radical scavenging assay, black tea kombucha had higher scavenging effect than Thai tea kombucha since day 0 to day 15 which was 61.38% GAE to 90.58% GAE but scavenging effect of Thai tea on day 15 was almost equal with black tea which were 87.99% GAE so it almost equal with black tea that got 90.58% GAE . For the total phenolic content, black tea also had greater total phenolic content than Thai tea but they both increased on day 12 which were 971.13 $\mu$ g/ml and 886.51 $\mu$ g/ml respectively and then decreased on day 15 to almost the same amount. For sensory evaluation, Thai tea kombucha got overall liking score (7.5) more than black tea kombucha (6.1) which was like moderately to like very much. This was because the consumers did not like the fermented flavor of black tea that was too strong, too bitter and sourer but Thai tea kombucha had their own flavor and odor, the fermented flavor was reduced with a hint of sweetness. It was suggested that the changes of total phenolic content and antioxidant activity in both kombuchas were result of tannin breakdown. The breakdown of tannin into smaller phenolic and flavonoid compounds, subsequently, increased the antioxidant activity as found in this study. Further investigation on monitoring of the breakdown products is encouraged.

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## Appendix



## Appendix

### DPPH radical scavenging assay

Type of tea		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Thai tea	rep 1	32.06	88.83	82.22	84.29	86.06	86.56
	rep 2	39.69	86.59	75.95	85.97	87.03	87.78
	rep 3	55.53	87.45	75.35	84.45	83.86	89.64
	Mean	42.43	87.62	77.84	84.90	85.65	87.99
	S.D.	11.97	1.126	3.80	0.930	1.62	1.54

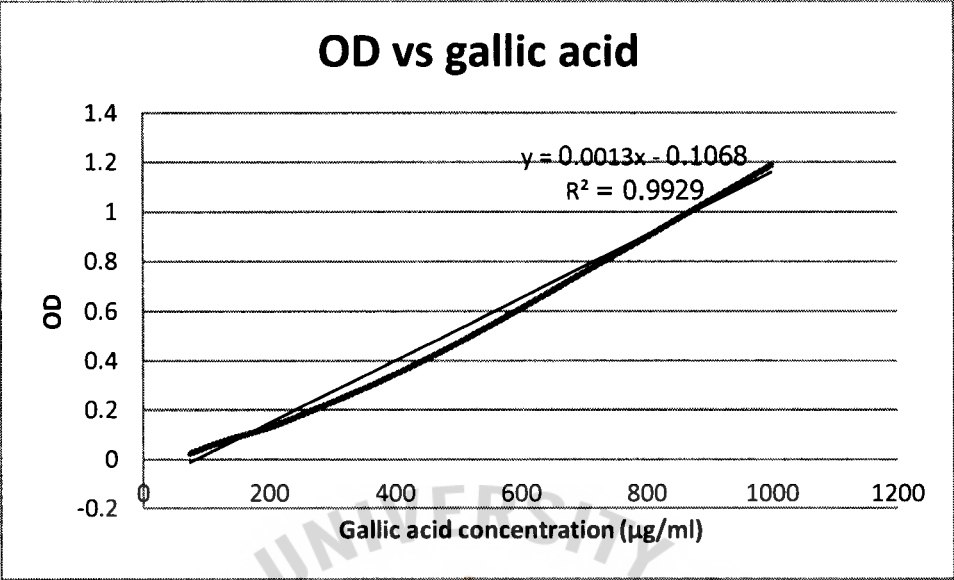
Type of tea		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Black tea	rep 1	61.83	89.69	82.22	88.85	87.86	88.18
	rep 2	58.58	85.05	81.41	85.81	84.96	91.34
	rep 3	63.74	89.01	82.62	84.45	83.72	92.23
	Mean	61.38	87.91	82.08	86.37	85.51	90.58
	S.D.	2.60	2.50	0.62	2.25	2.12	2.13

$$\% \text{scavenging effect} = \left[ 1 - \left( \frac{A_{517} \text{ sample} - A_{517} \text{ blank}}{A_{517} \text{ control}} \right) \right] \times 100$$

$$= \left[ 1 - \frac{0.356 - 0}{0.524} \right] \times 100$$

$$= 32.06 \%$$

**Total Phenolic Content**



$$y = 0.0013x - 0.1068$$

$$0.485 = 0.0013x - 0.1068$$

$$0.485 + 0.1068 = 0.0013x$$

$$0.5918 = 0.0013x$$

$$X = \frac{0.5918}{0.0013} = 455.23 \text{ µg/ml}$$

Type of tea		Day 0 (µg/ml )	Day 3 (µg/ml )	Day 6 (µg/ml)	Day 9 (µg/ml)	Day 12 (µg/ml)	Day 15 (µg/ml)
Thai tea	rep 1	455.23	678.3	345.23	512.15	949.07	567.53
	rep 2	409.07	687.53	341.4	528.3	852.92	557.53
	rep 3	417.53	604.46	361.38	532.15	857.53	558.31
	Mean	427.27	656.76	349.34	524.2	886.51	561.12
	S.D.	24.57	45.53	10.6	10.61	54.23	5.56



Type of tea		Day 0 ( $\mu\text{g/ml}$ )	Day 3 ( $\mu\text{g/ml}$ )	Day 6 ( $\mu\text{g/ml}$ )	Day 9 ( $\mu\text{g/ml}$ )	Day 12 ( $\mu\text{g/ml}$ )	Day 15 ( $\mu\text{g/ml}$ )
Black tea	rep 1	749.84	779.84	586.76	842.92	945.23	619.07
	rep 2	768.31	789.85	630.61	893.7	964.46	604.46
	rep 3	719.84	774.46	592.92	840.61	1003.69	607.54
	Mean	745.99	781.38	603.43	859.07	971.12	610.35
	S.D.	24.46	7.81	23.74	30.01	29.8	7.702

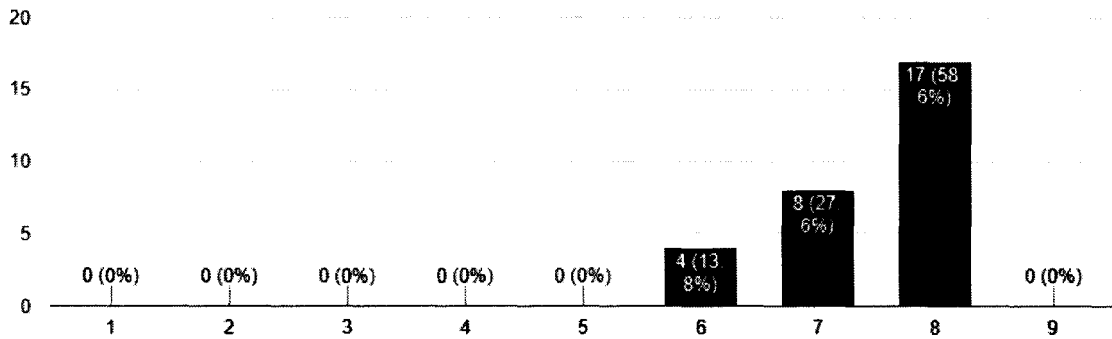
## Sensory Evaluation

### Thai tea (447)

Type of tea	Color (สีของชา)	Sweetness (ความหวาน)	Sourness (ความเปรี้ยว)	Sparkling (ความซ่า)	Fermented flavor (รสหมัก)	Bitterness (ความขม)	Overall liking (ความชอบโดยรวม)
Thai tea	8	8	7	7	8	7	8
	7	7	7	4	6	7	7
	6	6	7	7	5	8	7
	8	7	8	8	7	8	9
	7	8	8	7	8	9	8
	7	7	6	2	6	8	7
	8	8	9	9	9	8	8
	7	7	7	6	8	7	8
	8	7	7	6	6	6	8
	8	7	7	6	6	6	8
	8	9	9	9	8	9	9
	8	7	9	9	7	8	8
	7	7	4	3	5	6	7
	6	7	4	6	2	7	4
	7	8	8	6	6	8	8
	8	8	5	5	7	2	7
	8	7	8	6	9	7	7
	7	6	7	6	7	7	6
	7	8	8	6	6	8	8
	6	6	7	7	5	8	7
	6	6	7	7	5	8	7
	8	8	7	7	8	7	8
Mean	7.27	7.23	7.1	6.32	6.54	7.23	7.45
SD	0.76	0.81	1.37	1.75	1.62	1.44	1.05

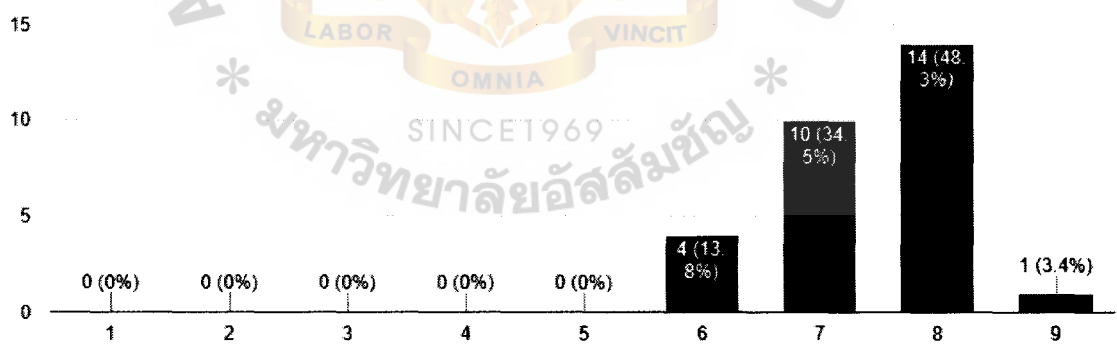
### Color (สีของชา)

29 responses



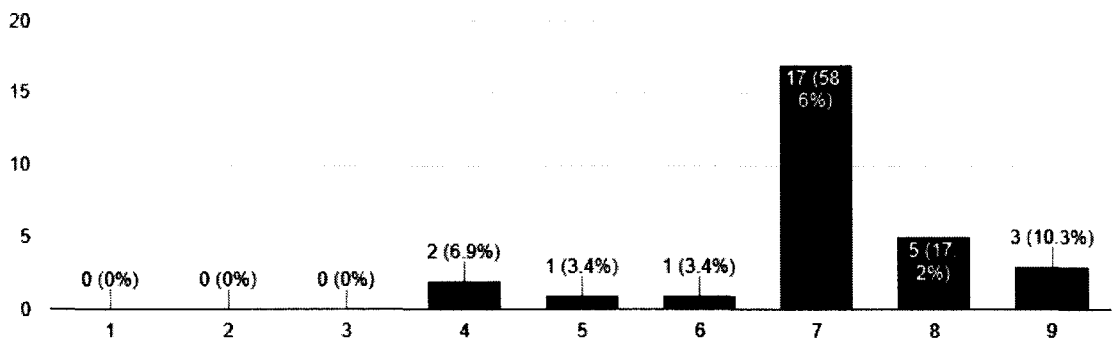
### Sweetness (ความหวาน)

29 responses



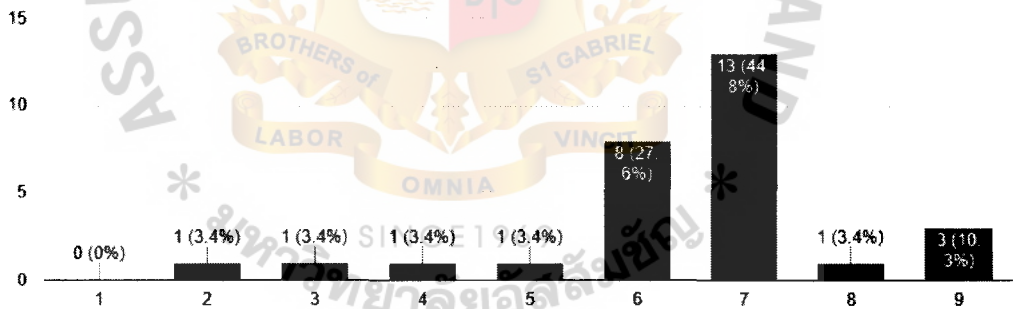
Sourness (ความเปรี้ยว)

29 responses



Sparkling (ความซ่า)

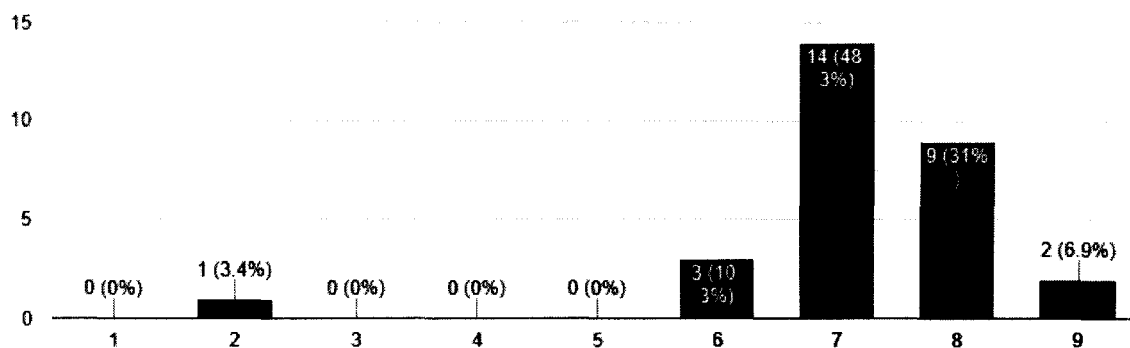
29 responses





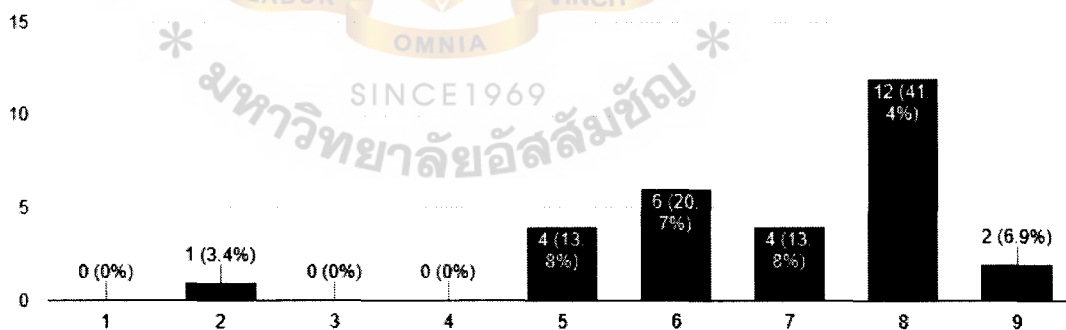
## Bitterness (ความขม)

29 responses



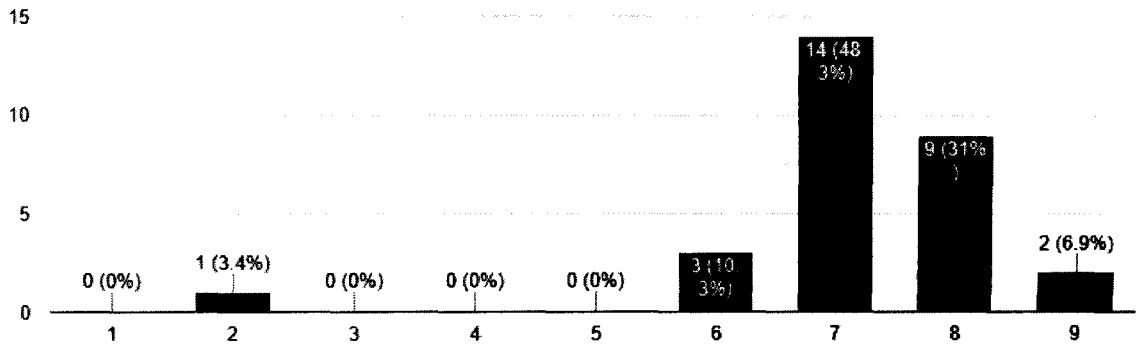
## Fermented flavor (รสหมัก)

29 responses



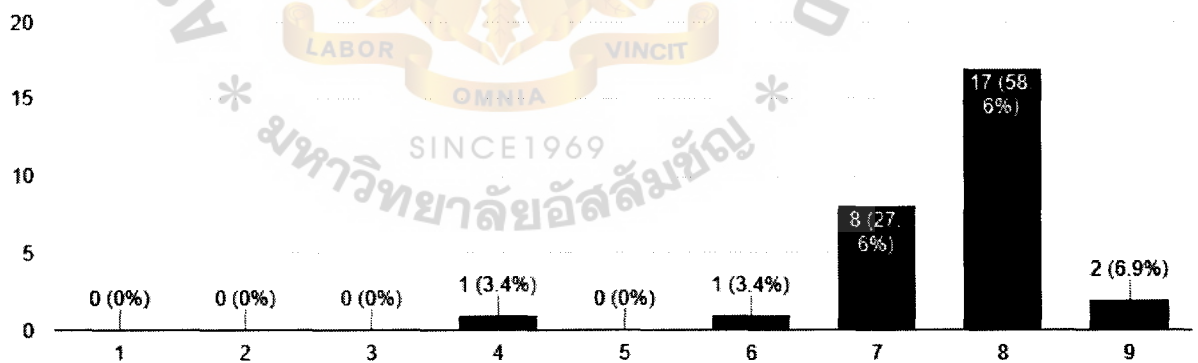
### Bitterness (ความขม)

29 responses



### Overall liking (ความชอบโดยรวม)

29 responses



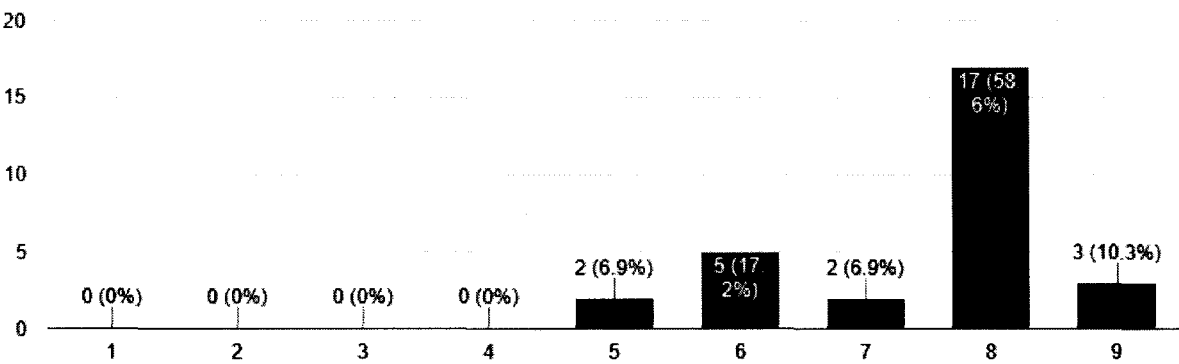
# Black tea (556)

Type of tea	Color (สีของชา)	Sweetness (ความหวาน)	Sourness (ความเปรี้ยว)	Sparkling (ความซ่า)	Fermented flavor (รสหมัก)	Bitterness (ความขม)	Overall liking (ความชอบโดยรวม)
Black tea	8	7	6	7	6	6	7
	6	4	4	4	4	5	5
	8	6	6	7	7	7	6
	8	7	5	7	7	8	8
	8	6	5	5	4	7	6
	6	7	6	5	6	3	5
	7	6	7	7	7	6	7
	6	5	5	5	4	6	6
	8	6	4	6	4	6	6
	8	6	4	6	4	6	6
	9	7	7	7	6	8	7
	7	8	8	8	7	8	7
	5	7	4	6	3	3	5
	5	4	6	3	5	6	4
	9	9	8	8	4	6	8
	8	3	8	3	9	8	5
	6	6	4	7	2	4	4
	6	6	7	6	6	6	6
	9	9	8	8	4	6	8
	8	6	6	7	7	7	6
	8	6	6	7	7	7	6
	8	7	6	7	6	6	7
Mean	7.32	6.27	5.91	6.2	5.41	6.14	6.13
SD	1.25	1.45	1.41	1.47	1.71	1.42	1.16



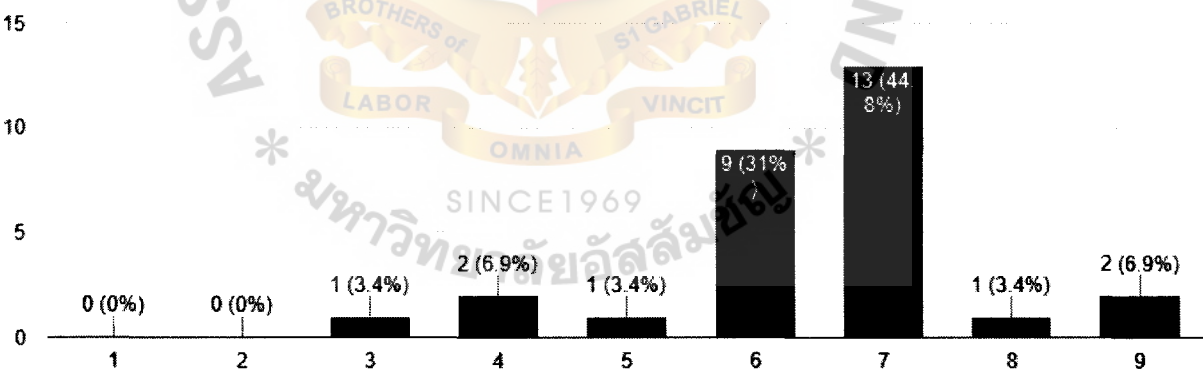
color (สีของชา)

29 responses



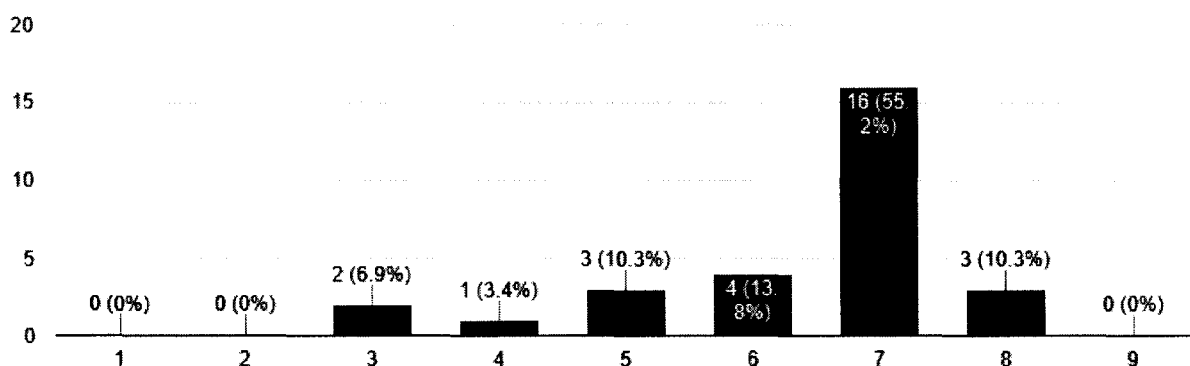
Sweetness (ความหวาน)

29 responses



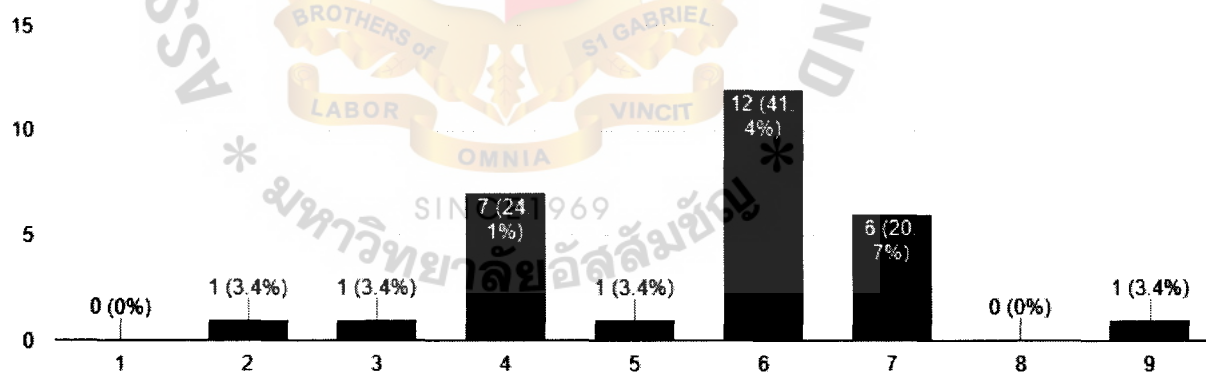
## Sparkling (ความซ่า)

29 responses



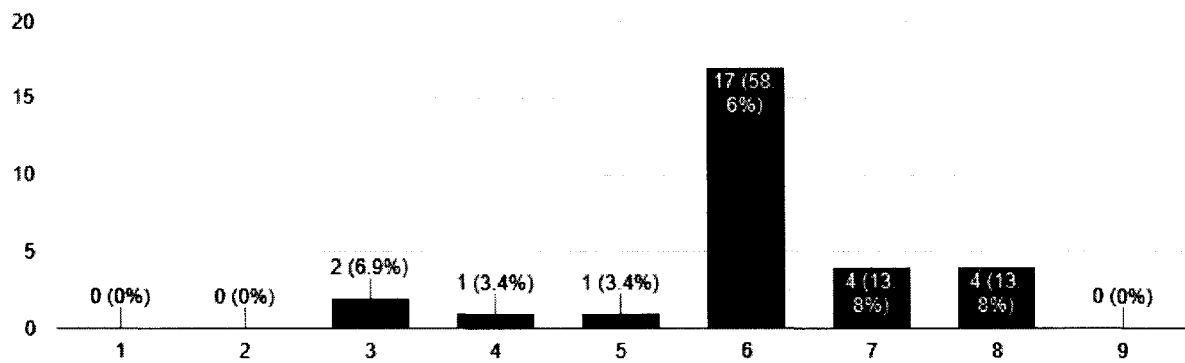
## Fermented flavor (รสหมัก)

29 responses



Bitterness (ความขม)

29 responses



Overall liking (ความชอบโดยรวม)

29 responses

