PRODUCT DEVELOPMENT OF HERBAL TEA BAG FROM *CENTELLA ASIATICA* AND GOJI BERRY FOR ELDERLY PEOPLE

BY

MS. PREEYAPA SRIPROMMA
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A Special Project Submitted to School of Biotechnology, Assumption University, In Part Fulfillment of the Requirements of the Degree of Bachelor of Science in Biotechnology
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Title	: Product Development of Herbal Tea Bag from *Cemtella Asiatica* and Goji Berry for Elderly People
By	: Ms. Preeyapa Sripromma
Advisor	: Dr. Aussama Soontrunnarudrungsri
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_____ **Advisor**

(Dr. Aussama Soontrunnarudrungsri)

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ABSTRACT

Centella asiatica has known as medical plant that used for treating bruises and reducing swelling. From the previous research lately, it reported that *C.asiatica* has properties to improve memory recognition and promote healthy skin. Then, it is extracted for using with skin care for elderly people who have a problem with their wrinkle. In term of food, herbal tea from *C.asiatica* has already available in the market. But it has been developed to enhance the amount of antioxidant. Therefore, this study was aimed to develop new herbal tea from *C.asiatica* with goji berry in the ratio of 1:2 and study the amount of antioxidant in tea bag. To achieve the aim, dried *C.asiatica* leaves were brewed to find the most efficient temperature and time for extracting antioxidant. The best method to process dried leaves also determined as well among four methods. Those were no roasting and no kneading leaves, no roasting but kneading leaves, roasting and no kneading leaves, and roasting and kneading leaves. The formation of consuming tea was determined by 30 target panelists and the result showed that the most preference of formation was tea bag. Also, the temperature and time for brewing are the important parameters used to control when brewing tea. The temperature was studied was 80°C, 85°C, and 90°C and time was 2, 3, 4, and 5 minutes. And the result showed that there was no significant difference among all temperature and time that were determined. The best temperature and time for brewing is 80°C at 5 minutes because it saves energy and can extract the highest amount of antioxidant from herbal tea. *C.asiatica* tea can be enhanced the amount of antioxidant by mixing with the fruit that has high antioxidant properties. Goji berry is one of the fruits that contain high antioxidant. Moreover, it helps improve neurosystem and memory like C.asiatica. From the result, C.asiatica tea mixed with golji berry had much higher amount of antioxidant than pure C.asiatica tea. Therefore, the ratio between C.asiatica tea and goji berry was determined in the ratio of 1:1. 1:1.5, and 1:2. The result showed that at ratio 1:2 had the highest preference score due to it had less green aroma and flavor and satisfied sweetness.

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INTRODUCTION

World population right now is an increasing median age. Meaning, it is a shift in the distribution of a country's population towards old ages. There are two demographic effects that affecting the world population. First, increasing in longevity causes the average age of the population by increasing the numbers of elderly people. Another factor is decreasing in fertility which reducing the population of babies. (Brunborg H., 2012) According to data from World Population Prospects in the 2015 Revision (United Nations, 2015), they expect that between 2015 and 2030, the number of people in the world aged 60 years or over is predicted to grow by 56 percent from 901 million to 1.4 billion by 2050. (United Nations, 2015) In Thailand, it is currently ranked the third most rapidly ageing population in the world. By 2040, Thailand's aging population is expected to increase to 17 million, according for 25 percent of the population. This means that out of every four Thais, one will be a senior citizen. So, Thailand has begun the challenging process of meeting the needs of an ageing society. In practical terms, this demographic transition translates to challenges with care and support of older people especially in term of foods. Food is the important thing that provides nutritional support for everyone. Elderly people need food for providing energy, maintenance, and repair their metabolisms. Consuming good and healthy foods is the major concern in this age of people. Plus, Thailand is the land of agricultural which has a lot of herb that used as a traditional medicine. It is very easy to find the herb and Thai people also familiar with it. Normally, herb and spice contain many health benefits. For example, ginger used to improve respiratory conditions, aid digestion, and reduce excess gas and so on. Also, those herbal extracts can improve the emotional of consumers by increasing the happiness and relaxation

According to the food technology and industry, product development of herbal food is focused for this group of people by researching and developing the new beverage product that comes from herb and spice.

LITERATURE REVIEW

World population

Human population has grown very slowly for the most of its existence on earth due to global economic recession. The main factors that cause low population are low fertility rate and life expectancy. Fertility is driven by the socio-economic development of the population. Poverty and lack of education cause lower in population in develop counties. (Future Population Growth: Max Roser). For life expectancy is increasing rapidly due to the improvement of health and safety living conditions.

World population growth in the 21st century will be different from previous decades. People are living longer and having fewer children so that the number of older people (age 60 and above) will increase more than young people (Human Population Dynamics, Annenberg Learner).

Population aging

Population aging refers to changes in the entire age distribution. From the study of population aging, it is driven by a concern of retirement systems which measured by increases in the percentage of elderly people of retirement ages. The world aging population right now is excess 8 to 10%. By this standard, the percentage of elderly people in the United States stood at 12.6% in 2000 and it is predicted to increase to 20% by the year 2030 (United Nations 2001).

Population aging in Thailand

The number of older people (defined as aged 60 and over) in Thailand has grown rapidly and will continue to do so in future decades. Since 1960 the number of older people in the Thai population has increased seven-fold from approximately 1.5 million to 10.7 million by 2015 or 16% of the total population. Future population ageing will occur even more rapidly with the number of older persons projected to increase to over 20 million by 2035, at which point they will constitute over 30% of the population. Moreover, within the next few years, persons 60 and older will outnumber children under age 15 for the first time in Thai history (John Knodel, 2015).

Elderly Health

As people continue to lead longer lives, they become more likely to develop different kinds of health problems. One challenge older adults in particular are likely to face is living with multiple health problems. More than half of all adults 65 and older have three or more ongoing medical problems, such as heart disease, diabetes, cancer, or arthritis (HealthinAging, 2017). One of important disease for elderly is Alzheimer's Disease. It causes injury to nerve cells in the brain. It results in disrupted memory, thinking and functioning.

- Everyone who has Alzheimer's Disease has dementia
- Not everyone who has dementia has Alzheimer's Disease
- Not the same progression for everyone but the stages are predictible
- Somewhat greater risk for people with family history of Alzheimer's Disease
- Race or ethnicity does not seem to be a factor

Behavior and emotions of aging

When people becomes older age, they change and loss something from usual. For example, they change in physical like vision, hearing, and the senses. All of senses tend to change with age. Their eyes have more difficulty focusing on near objects changes in light intensity. Those things can make many problems for living. Moreover, memory is another thing that will be change when people get older. They will process and retrieve information in different ways. They slightly take longer to recall information. Also elderly emotions will change as well, They will feel depression, loneliness, and sense of isolation in a normal stage of grief that difficulty in moving on after the loss or in this stage the individual may withdraw, sleep more than usual, overeat or not eat enough (John Knodel, 2015).

Herbal Tea

A beverage made from steeping or boiling herbs, or the herbs used to make such a beverage. Herbal tea made of a variety of plants, including leaves of certain flowers, herbs, barks, and grasses. Some herbs used in these teas have been demonstrated to have pharmacological properties. Herbal tea benefits are as numerous as there are herbs. The health benefit of the tea is dependent on the plant, root, or herb being used. Now, people consume herbal tea to support and healthy mind and body (American Journal of Clinical Nutrition). There are many advantages of drinking herbal tea. For example, antioxidant in all herbal teas help fight cell-damaging free radical in body. It can lower bad cholesterol, herbal tea can reduce blood cholesterol around 2.19mg/dL (American Journal of Clinical Nutrition). Additionally, it can also interact negatively with blood pressure medication or diabetes treatments such as insulin (Alan Carter, 2016).

Herbal tea market in Thailand

Nowadays, herbal tea becomes more popular due to the healthy trend. People focus and concern more about health and safety. Herbal tea is one of healthy beverage that helps reduce weight and reduce risk for getting some diseases. Herbal tea is classified as functional beverages or neutraceuticals. In the industrial, producing herbal tea is also popular among manufacturers. Some industries add some herbal into their product and add some flavor and color to create more variety of teas. In addition, herbs are some parts of plant that give medical properties. These kinds of plant normally grow in Thailand so that producing herbal tea in Thailand will have less cost of raw materials. Target consumers normally are elderly people who love to drink tea with herbs so that herbal tea is chosen as souvenir. The market of herbal tea right now is expanding into middle age people in Thailand who concern about the health and love to consume organic stuffs.

***Centella Asiatica* (*Asiatic Pennywort*)**

Centella Asiatica is a plant from India and Southeast Asia known as Asian medicine. It prefers in riverbank and humid area. It comes up with spherical shape of leave and has long stem. (Vine Vera, 2014) *Centella Asiatica* is a creeping annual herb with soft tender leaves. The roots and leaves develop from the nodes. *Centella Asiatica* is a very popular herb in India for longevity and mental function. It is used to decrease fatigue and depression. It energizes the central nervous system. The plant is also useful in repairing skin and connective tissues. *Centella Asiatica* is used in Indian system of disease treatment as diuretic, nerve tonic, alternative, memory enhancer and blood purifier (Dr. Arvind Singh, 2015). Chemically been identified leading to therapeutic properties. Asiatic acid, asiaticoside, and madecassoside form the major constituents responsible for pharmacological value apart from being rich in flavonoids and terpenoids. other volatile compounds was found to be in a prominent amount in the essential oil of *C. asiatica* on analysis with gas chromatography-mass spectrometry (GC-MS). It contains bioactive compounds that can be identified by using high-performance liquid chromatography (Nasir MN, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J., 2011)

- **COGNITIVE FUNCTION**

  Centella Asiatica enhances synaptic differentiation which can improve cognitive function. It carries aluminum-induced cognitive function. From the previous study, it has proved to carry neuroprotective potential. Also, *C.asiatica* helps prevent impairment of cognitive system by damage cochicine.

- **ANTIBACTERIAL ACTIVITY**

  Methanol hot extract from C. asiatica leaves was taken to check the antibacterial activity which was assessed by zone of inhibition and minimum inhibitory concentration (MIC) value (2 μg/disc) by disc diffusion method (Francis SC, Thomas MT, 2016) Antioxidants provide protection to the cells of organism.

- **ANTIDEPRESSANT**

  Compared to diazepam C. asiatica possesses antianxiety effect but has no effect on behavioral despair (Pitinidhipat N., 2015). It was evaluated for antidepressant activity using forced swimming test, the result showed a reduction in stillness duration and regulated amino acid levels (Thomas TN, Thomas PM, 2015)

Goji Berry (*Lycium barbarum*)

Goji berry is known as superfood-berries because it has high-antioxidant compounds such as vitamin A, vitamin C, beta-carotene, lutein, and lycopene. Those antioxidant compounds can help protect the cells from damaging of free radical which can lead into chronic diseases. Chinese people use goji berries with foods to make a meal that can enhance longevity properties as a medicine. (Michael Tierra, 1998) It has ability to nourish and toxify liver and kidney, and improve life activities. (Junkuan Wang, 2014) Additionally, it gives benefit to eye vision. (Cheng et al. 2004; Potterat 2010)

There are many phytochemicals that provide benefits to human body as followings;

1. **Polysaccharides**
   - It is primary source of dietary fiber for internal system. It stimulates the immune system and provides antioxidant protection.
2. **Beta-carotene**
   - It contains high content of vitamin A which can help improve eye vision, cell structure, and healthy skin.
3. **Zeaxanthin**
   - It is a powerful vision protector that allows you to clearly distinguish fine detail. It generates protector against macular degeneration.

From the previous studies, university of Hong Kong has studied about anti-aging and its effects to neuroprotective effects against Alzheimer's disease. The result showed that goji berry extract can protect human brain from the toxin effects of beta amyloid protein. (Bernstein, 2002) Homocystein is a non-protein amino acid that can cause inflammation marker of aging and bad health. High level of homocystein is the major factor of aging and bad health. Also, the high level of plasma homocystein can cause higher risk of developing Alzheimer's disease. Too much homocysteine in the blood can cause damage to health cells. (Awakening from alzheimer's, 2017)

Aim

To develop herbal tea from *Centella asiatica* and goji berry (Lycium barbarum) for elderly people

Objectives

1. To study the proper production process of *Centella asiatica t*ea
2. To study effect of proper brewing time and temp on antioxidant activity and consumer acceptance
3. To develop herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)
    a. To study proper dried fruit that best paired with *Centella asiatica* tea
    b. To optimize ratio of *Centella asiatica* and goji berry (Lycium barbarum) for herbal tea
    c. To conduct acceptance test of the herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)

MATERIALS AND METHODS

Objective 1: To study the proper production process of *Centella asiatica* tea

Experimental design

To achieve this objective, ANOVA Randomized Complete Block Design (RCBD) was used as an experimental design with Latin Square Design as a multiple comparison. ANOVA RCBD was analyzed by the follows:

Factor : Attributes

Treatment : 4 formulas of tea (for analyzing formulations of tea)

3 forms of tea (for analyzing formation of tea)

Block : Consumer

Response : Liking score

Preparation of *C. asiatica* leaves

C.asiatica leaves and stems are separated from each other and weight. Then, leaves are washed for 3 times and steamed over boiled water at 100°C for 1 minute. Next, the leaves are dried for 1.30 hours and roasted on iron pan at 45°C for 25 minutes. For leaves need to be concerned for determine %yield. For *C.asiatica* preparation is varying about the different process of making tea as table 1.

Table 1 Formulation of preparation *C.asiatica* leaves

Formulas	Kneading for 5 minutes	Roasting on iron pan at 45°C for 25 minutes
1.NKNR	✘	✘
2.KNR	✔	✘
3.NKR	✘	✔
4.KR	✔	✔

Figure 1 De-stemming and washing

Figure 2 Steaming

Figure 3 Draining

Figure 4 Roasting

Figure 5 Dried *C.asiatica* leaves

Preparation of *C.asiatica* matcha powder

Dried *C.asiatica* leaves are grinded into 200 mesh matcha powder by using 200 g Thaigrinder (WF-04) for 1.30 minutes. The *C.asiatica* powder is filtered again and kept in aluminum foil zip lock.

Figure 6 Grinder

Figure 7 Top side of grinder

Figure 8 Matcha powder

Preparation of *C.asiatica* tea bag

2 g of dried *C. asiatica* leaves are weighted into tea bag. One tea bag is soaked into 150 ml of boiled water for 5 minute s. Tea is served at 65°C.

Figure 9 Weighting

Figure 10 Tea bags

Figure 11 Boiling water at 90°C

Figure 12 Soaking in hot water

Objective 2: To study effect of proper brewing time and temp on antioxidant activity and consumer acceptance

Experimental design

To determine brewing temperature and time for brewing tea, ANOVA Factorial 3x4 Randomized Complete Block Design was used to analyze the difference of temperature and time of brewing *C.asiatica* tea. Duncan's multiple comparison was used as multiple comparisons. ANOVA Factorial RCBD was analyzed by follows:

Factor : Attributes

Treatment : 3 temperature and 4 brewing time

Block : Consumers

Response : Liking score

Table 2 Temperature and time for determining the brewing tea

Temperature (°C)	Time (minutes)
80	2
80	3
80	4
80	5
85	2
85	3
85	4
85	5
90	2
90	3
90	4
90	5

Antioxidant Efficiency

DPPH radical scavenging activity

C.asiatica tea is determined antioxidant activity by taking 20 µl of diluted 1:10 tea sample mixed with 2 ml methanol in a tube and mix well by using vortex. DPPH solution is prepared by weighting 0.001972 g DPPH in 100 ml volumetric flask and filled up with methanol up to 100 ml mark. The different intensities of DPPH and sample are prepared as table 2. The mixtures are mixed vigorously and leave in dark room at room temperature for 30 minutes. The measurement of the reaction is taken by using UV-VIS spectophotometer at 517 nm. The results will be shown in the unit of µg/mL of gallic acid equivalent (GAE) per 1 ml of sample.

Table 3 Praparing DPPH solution and sample

50 μM DPPH solution in μL	Sample in μL
2000	0
1960	40
1920	80
1880	120
1840	160
1800	200

Ferric reducing (FRAB) antioxidant power

FRAP assay is used to determine antioxidant activity of *C.asiatica* tea. The sample needs to dilute into 1:10 before using. FRAB reagent is prepared from mixing 300 mmol of sodium acetate buffer pH 3.6, 20 mmol of ferric chloride, and 10 mmol of 2,4,6-tripyridyl-s-triazine (TPTZ). The FRAB reagent is prepared in the ratio of 10:1:1 (v:v:v:) and mixed with 20 μL of sample. The mixture is kept in the dark room at room temperature for 30 minutes. The absorbance of the mixture is performed by using UV-Vis spectrophotometer at 593 nm. The result is calculated in the unit of mmol of $FeSO_4$ equivalent per 1 mL of sample.

Total Phenolic Compounds

Total phenolic compound assay is used to determine phenolic content in *C.asiatica* tea. The sample needs to dilute into 1:10 before using. Total phenolic mixture is prepared by mixing 1.58 mL of distilled water, 100 μL of Follin-Ciocalteu reagent, and 20 μL of tea sample. The mixture is shaken vigorously and left at room temperature for 8.30 minutes. The, 300 μL of sodium carbonate solution is added into the mixture and mixed well. The mixture is incubated at room temperature without light for 30 minutes. The absorbance of the mixture is measured by using UV-VIS spectrophotometer at 765 nm. The result is calculated in the unit of μg/mL of gallic acid equivalent (GAE) per 1 mL of sample.

Objective 3: To develop herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)

a. To study proper dried fruit that best paired with *Centella asiatica* tea

Preparation of fruit and *C.asiatica* tea bag

Experimental design

This experiment is conducted in three replications and statistical analysis is accomplished by using t-test.

- **Goji berry**

  2 g of dried *C.asiatica* leaves are weighted with 2 g of dried goji berry in the ratio of 1:1. One tea bag is soaked into 150 ml of boil water for 5 minutes. Tea is served at 65°C.

- **Cranberry**

  2 g of dried *C.asiatica* leaves are weighted with 2 g of dried cranberry in the ratio of 1:1. One tea bag is soaked into 150 ml of boil water for 5 minutes. Tea is served at 65°C.

b. To optimize ratio of *Centella asiatica* and goji berry (Lycium barbarum) for herbal tea

Experimental design

This experimental was analyzed by ANOVA Randomized Complete Block Design with Latin Square Design. ANOVA RCBD was analyzed by follows:

Factor : Attributes

Treatment : 3 ratio of goji berry

Block : Consumers

Response : Liking score

Ratio of 1:1, 1:1.5, 1:2 of dried tea leaves and dried goji berry were weighted into tea bag and asked consumers to test the product.

c. **To conduct acceptance test of** the herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)

Sensory Test

The sensory tests were performed with 30 consumers with 9-hedonic scale. Herbal tea bag was brewed with 150 mL of water at 90°C for 5 minutes. The sample was served at 65°C and served at room temperature.

RESULTS AND DISCUSSION

Objective 1: To study the proper production process of *Centella asiatica* tea

Formulations of *C.asiatica* tea and its properties

Table 4 Sensory attributes and liking score of *C.asiatica* tea

Sample	Overall liking	Color [ns]	Green aroma	Roast aroma [ns]
No roasting, no kneading	6.20±1.37[b]	6.60±1.40	6.17±1.51[b]	6.10±1.40
No roasting, kneading	7.07±1.51[a]	7.40±1.30	7.10±1.12[a]	6.60±1.52
Roasting, no kneading	6.93±1.26[a]	7.13±1.01	6.60±1.48[ab]	6.27±1.70
Roasting, kneading	6.30±1.47[b]	7.07±1.26	6.30±1.62[b]	6.27±1.11

Table 5 Sensory attributes and liking score of *C.asiatica* tea

Sample	Sweetness [ns]	Bitterness [ns]	Green flavor [ns]	Roast Flavor [ns]
No roasting, no kneading	6.17±1.70	6.27±1.53	6.47±1.48	6.17±1.56
No roasting, kneading	6.80±1.21	6.50±1.25	6.87±1.57	6.67±1.35
Roasting, no kneading	6.67±1.37	6.83±1.34	6.77±1.38	6.60±1.33
Roasting, kneading	6.40±1.30	6.10±1.24	6.40±1.45	6.23±1.30

Table 6 Sensory attributes and liking score of *C.asiatica* tea

Sample	Green Aftertaste	Astringent [ns]
No roasting, no kneading	6.00±1.62[c]	6.07±1.86
No roasting, kneading	6.87±1.55[a]	6.97±1.38
Roasting, no kneading	6.73±1.23[ab]	6.73±1.31
Roasting, kneading	6.13±1.48[bc]	6.47±1.59

Figure 13 Characteristics of tea in different formulations

Table 7 Amount of antioxidant of each formula

Sample	FRAP (mmol Fe2+/mg dried weight)	TPC ns (µg GAE/mg dried weight)	DPPH (% reduction)
No roasting, no kneading	0.23 ± 0.01	0.15 ± 0.01	0.07 ± 0.01[ab]
No roasting, kneading	0.20 ± 0.03	0.07 ± 0.05	0.07 0.00[a]
Roasting, no kneading	0.21 ± 0.03	0.13 ± 0.06	0.07 ± 0.00[ab]
Roasting, kneading	0.17 ± 0.01	0.06 ± 0.01	0.06 0.00[b]

Processes of making *C.asiatica* tea were classified into four processes. The difference among those four processes were kneading and roasting technique. Kneading technique helps to improve its color, aroma, and flavor. (Nagatani Soen, 2009) Roasting technique is performed to induce the roasted aroma and flavor. Also, roasting can help reduce moisture content in *C.asiatica* leaves before air drying. (Ayumi Eguchi and Shouhei Eguchi) As the result, the sensory test was performed with 15 students in the age of 18-25 years old and 15 target consumer in the age of 50-65 years old. In table 3,4, and 5, the liking scores of overall liking, green flavor, and green aroma flavor after taste showed significant difference. The highest scores for those three attributes went to no roasting but kneading tea. However, the most preference among all formulas was roasting and no kneading tea. Also, the amount of

antioxidant of roasting and no kneading tea was higher than roasting and kneading tea. Therefore, roasting and no kneading tea was picked up to determine the formation of tea. The purpose of roasting tea is for improving flavor of tea by marking green flavor. Also, roasting technique helps destroy polyphenol oxidase to stop fermentation and reduce moisture of tea. (Agnieszka Kosinska, 2014). The purpose of kneading or rolling is for breaking cells and letting compounds inside tea leaves spreading through all tea leaves. Withering is for letting water come out from tea. The oxidation of polyphenol oxidase is reacted and produced more flavor and aroma. (Micheal J. Coffey, 2013) The purpose of drying is for reducing moisture content and prolongs its shelf life. (Haslam, 2003)

Yield Percentage

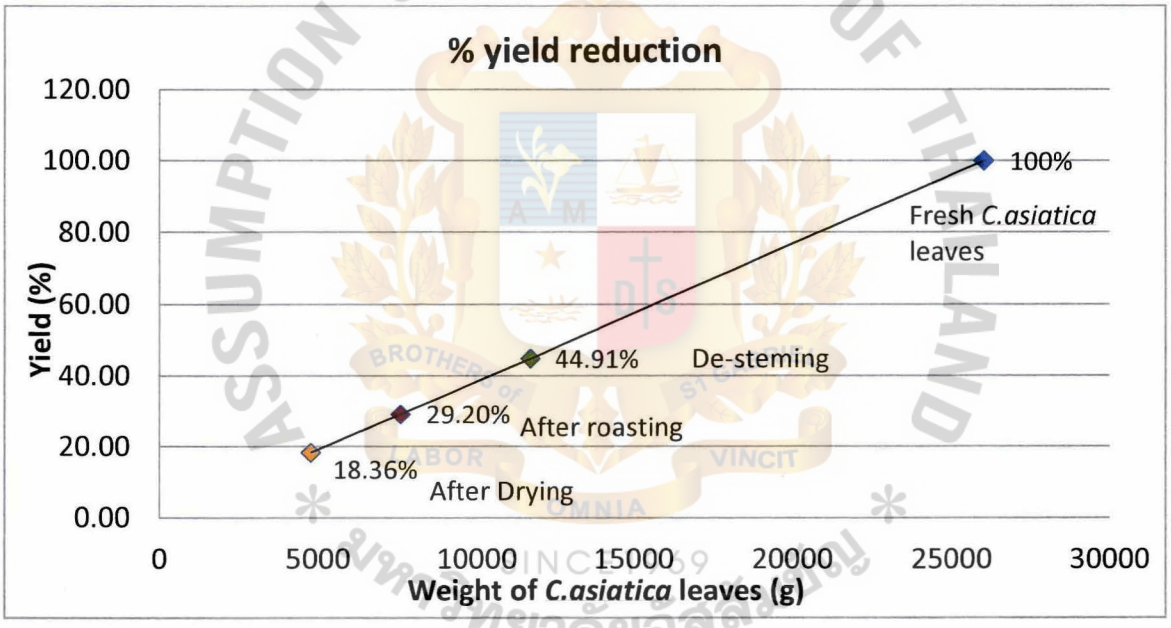

Figure 14 Yield Reduction for Making Dried *C.asiatica* tea leaves

From figure 14, the yield reduction showed that there were only 18.36% left from making dried tea leaves. The yield percentage was reduced by de-stemming, roasting, kneading, withering, and drying. These tea leaves need to remove stem because stem produces very strong of grassy aroma and flavor which make consumer do not like it. Also, the color of tea will turn to more yellow instead of green color.

Formation of consuming *C.asiatica* tea and its properties

Table 8 Sensory attributes and liking score of *C.asiatica* tea

Samples	Overall liking	Color	Green Aroma^{ns}	Roast Aroma^{ns}
Tea Bag	7.1±1.2^{a}	7.2±1.4^{a}	6.4±1.5^{a}	6.4±1.5^{a}
Matcha latte	5.3±1.7^{b}	6.0±1.8^{b}	6.4±1.6^{a}	6.2±1.5^{a}
Matcha Powder	5.1±2.0^{b}	6.0±1.7^{b}	6.0±2.1^{a}	6.1±2.2^{a}

Table 9 Sensory attributes and liking score of *C.asiatica* tea

Samples	Sweetness	Bitterness	Green Flavor	Roast Flavor
Tea Bag	6.8±1.5^{a}	6.7±1.3^{a}	6.4±1.3^{a}	6.3±1.4^{a}
Matcha latte	5.1±2.1^{b}	4.6±1.9^{b}	5.5±1.8^{b}	5.3±1.7^{b}
Matcha Powder	5.0±2.5^{b}	4.3±2.5^{b}	5.2±2.0^{b}	5.3±1.9^{b}

Table 10 Sensory attributes and liking score of *C.asiatica* tea

Samples	Green Aftertaste	Astringent
Tea Bag	6.2±1.8^{a}	6.2±1.6^{a}
Matcha latte	4.7±2.0^{b}	5.2±1.8^{b}
Matcha Powder	4.4±2.3^{b}	4.5±2.0^{b}

The following figures are example of *C.asiatica* tea in different forms except in Matcha latte form

Figure 15 *C.asiatica* tea in Matcha powder form

Figure16 *C.asiatica* tea in tea bags

Table 11 Amount of antioxidant for each form of tea

Sample	FRAP (mmol Fe2+/mg dried weight)	TPC (µg GAE/mg dried weight)	DPPH (% reduction)
Tea Bag	1.86 ± 0.14^{b}	46.94 ± 2.43^{b}	0.16 ± 0.01^{a}
Matcha latte	6.80 ± 0.43^{a}	278.59 ± 50.87^{a}	0.08 ± 0.00^{b}
Matcha Powder	2.89 ± 0.91^{b}	68.50 ± 3.40^{b}	0.04 ± 0.02^{c}

From table 7, 8, and 9, it showed that there were significant differences among all attributes except green and roast aroma liking scores. According to the results, tea bag got the highest liking scores in every attribute and also it got the most preference. For matcha latte and matcha powder tea, they had low liking scores because *C.asiatica* tea leaves were grinded into small particle size at 200 mesh or 74 microns. The powder was not small enough to dissolve in water. (David Michaud, 2014) The appropriated particle size of matcha should be 500 mesh and above or 24 microns so that it needed to reduce size into suitable size for matcha powder. (Hirofumi Tachibana, 2011) Once the particle size of matcha powder could not dissolve in the water, consumers did not like the texture because it was sandy and feel irritated in the throat instead of feeling moisten. So, tea bag was picked to determine which types of fruit that can go along with. For the amount of antioxidant in table 10, tea bag got the lowest amount of antioxidant due to the size of tea leaves but the was no significant difference between tea bag and matcha powder tea in FRAP and total phenolic compounds assays. (Banthawan Turapra, 2017) To improve the preference score of matcha powder and matcha latte, stone miller is the best grinder for making matcha. Stone miller generates less heat which will not destroy antioxidant compounds in the *C.asiatica* tea leaves. So, matcha that grinded by stone miller will be classified as high quality of tea and contains high antioxidant compounds. (Mizuba, 2018) If matcha powder can be improved to finer particles, it might be the most preference form of consumption tea because it has good taste and aroma. Also, it contains very high of antioxidant compounds.

Objective 2: To study effect of proper brewing time and temp on antioxidant activity and consumer acceptance

Temperature and time for brewing *C.asiatica* tea

Table 12 Sensory attributes and liking score of *C.asiatica* tea

Temperature (°C)	Time (minute)	Overall liking ns	Color ns	Green Aroma ns	Roast Aroma ns
80	2	7.0 ±0.9	6.9 ±1.2	6.8 ±1.0	6.6 ±1.0
80	3	6.9 ±1.2	7.0 ±1.1	7.0 ±1.1	6.8 ±1.3
80	4	6.8±1.2	6.9±1.3	6.6±1.3	6.9±1.2
80	5	6.6 ±1.4	6.8 ±1.1	6.6 ±1.1	6.3 ±1.2
85	2	6.7 ±1.3	6.7 ±1.4	6.7 ±1.1	6.6 ±1.2
85	3	6.4 ±**1.0**	7.0 ±**1.1**	6.9 ±**1.0**	6.9 ±0.9
85	4	6.5 ±1.1	7.0 ±1.0	6.6 ±1.1	6.3 ±1.0
85	5	6.7 ±1.1	7.0 ±1.3	6.9 ±1.2	6.6 ±1.0
90	2	6.4±1.2	8.8 ±13.3	6.1 ±1.4	5.8 ±1.3
90	3	6.6 ±1.0	6.8 ±1.2	6.4 ±1.1	6.5 ±0.8
90	4	6.8 ±1.0	7.0 ±1.0	6.9 ±1.1	6.7 ±0.8
90	5	2.4 ±1.1	6.7 ±1.0	6.7 ±1.0	6.6 ±0.8

Table 13 Sensory attributes and liking score of *C.asiatica* tea

Temperature (°C)	Time (minute)	Green Flavor ns	Roast Flavor ns	Sweetness ns	Bitterness ns
80	2	6.9±1.1	7.1±0.6	6.9±1.0	7.2±0.8
80	3	7.3±1.2	6.8±1.2	7.3±0.84	7.0±0.8
80	4	6.4±1.1	6.4±1.0	6.6±1.3	6.6±1.4
80	5	6.2±1.4	6.3±1.4	6.0±1.7	6.5±1.5
85	2	6.4±1.4	6.4±1.3	6.9±1.5	6.9±1.5
85	3	6.6±4.0	6.6±1.0	6.3±1.3	6.8±1.3
85	4	6.6±1.3	6.66.5±1.1	6.56.5±1.3	6.5±1.4
85	5	6.6±1.1	6.8±1.1	6.8±1.0	6.7±1.0
90	2	6.0±1.3	6.1±1.0	6.2±1.2	6.5±1.0

90	3	6.5±1.2	6.4±1.4	6.5±1.1	6.7±1.0
90	4	6.5±1.1	6.6±1.3	6.6±1.2	6.9±1.2
90	5	6.7±1.1	6.7±0.8	6.6±1.0	6.6±1.0

Table 14 Sensory attributes and liking score of *C.asiatica* tea

Temperature (°C)	Time (minute)	Green Aftertaste ^{ns}	Astringent ^{ns}
80	2	7.0±0.9	7.2±0.9
80	3	7.3±0.6	7.3±0.9
80	4	6.6±1.2	6.8±1.4
80	5	6.5±1.5	6.7±1.5
85	2	6.6±1.4	6.7±1.5
85	3	6.8±1.1	7.0±1.1
85	4	6.5±1.4	6.5±1.3
85	5	6.8±0.9	6.8±0.9
90	2	6.9±0.9	6.8±1.0
90	3	7.1±0.8	7.0±0.7
90	4	6.8±1.0	6.9±1.0
90	5	6.6±1.2	7.7±4.2

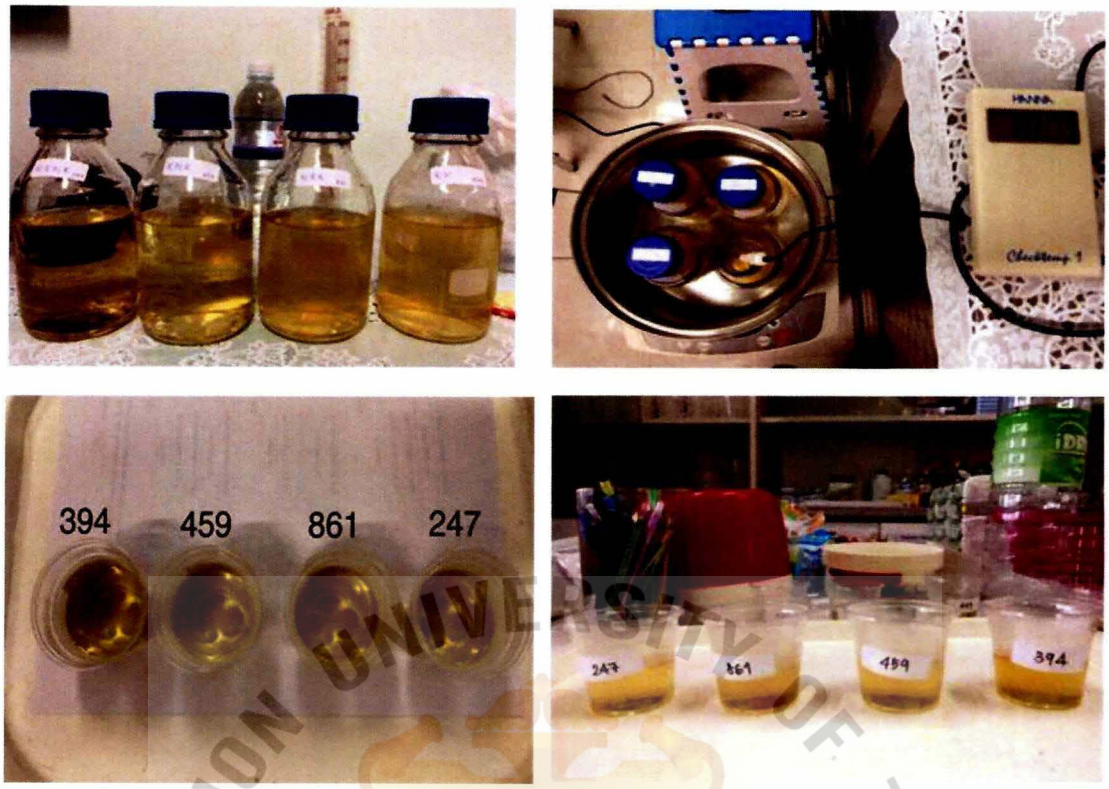

Figure 17 Example of determination of temperature and time for brewing tea

Table 15 The relationship between temperature and time of brewing tea

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Temp	2	0.39795000	0.19897500	0.25	0.7822
Time	3	6.69074167	2.23024722	2.78	0.0648
Temp*Time	6	0.14998333	0.02499722	0.03	0.9998
Rep	2	6.71280000	3.35640000	4.19	0.0287

Table 16 The relationship between temperature and time of brewing tea

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	0.39795000	0.19897500	0.25	0.7822
Time	3	6.69074167	2.23024722	2.78	0.0648
Temp*Time	6	0.14998333	0.02499722	0.03	0.9998
Rep	2	6.71280000	3.35640000	4.19	0.0287

Table 17 Amount of antioxidant for temperature and time of brewing tea

Temperature (°C)	Time (minute)	FRAP ^{ns} mmol Fe^{2+}	TPC ^{ns} µg/ml	DPPH ^{ns} µg/ml
80	2	1.4±0.98	1.4±0.9	0.1±0.0
80	3	1.0±0.3	1.0±0.3	0.2±0.0
80	4	1.3±0.2	1.3±0.2	0.2±0.1
80	5	2.2±2.3	2.2±2.3	0.6±0.1
85	2	1.5±0.3	1.5±0.3	0.4±2.5
85	3	1.3±0.4	1.3±0.3	0.15±0.0
85	4	1.6±0.3	1.6±0.3	0.2±0.0
85	5	2.5±1.8	2.5±1.8	1.8±0.5
90	2	1.2±1.1	1.2±1.1	0.3±0.1
90	3	1.1±0.2	1.1±0.2	2.6±1.9
90	4	1.6±0.6	1.6±0.6	0.8±0.4
90	5	2.2±0.6	2.2±0.6	0.5±0.2

According to table 11, 12 and 13, it was the result of sensory test about temperature and time for brewing tea. The result showed that there was no significant difference ($p>0.05$) among temperature and time. Also, there was no significant difference in amount of antioxidant in the table 14. Therefore, at temperature 80-85°C and time at 2-5 minutes for brewing tea would not affect the amount of antioxidant, taste, aroma, and flavor of tea. As the result, temperature at 80°C and time at 5 minutes were chosen to brewing tea in further process

because it used less energy and suitable time for getting highest flavor of tea. From the previous research, the average temperature and time for brewing commercial tea bag is 80°C for 3 minutes. However, the result of commercial tea bag compared with tea bag that used in the experiment showed that there was the same. Brewing for 5 minutes can extract more amount of antioxidant and the intensity of tea. (Lelia Nikniaz, 2016)

Objective 3: To develop herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)

a. To study proper dried fruit that best paired with *Centella asiatica* tea

Table 18 Sensory attributes and liking score of fruits with *C.asiatica* tea

Sample	Overall liking ^{ns}	Color ^{ns}	Green Aroma ^{ns}	Roast Aroma ^{ns}	Fruit Aroma ^{ns}
Cranberry	7.03 ± 1.12	6.83 ± 1.58	7.23 ± 1.41	6.57 ± 1.55	6.33 ± 1.65
Golji berry	7.53 ± 1.36	7.27 ± 1.39	7.23 ± 1.14	6.78 ± 1.25	6.03 ± 1.30

Table 19 Sensory attributes and liking score of fruits with *C.asiatica* tea

Sample	Green Flavor Aftertaste ^{ns}	Astringent ^{ns}
Cranberry	6.27 ± 1.95	6.53 ± 20.03
Golji berry	6.63 ± 1.38	6.83 ± 1.66

Table 20 Sensory attributes and liking score of fruits with *C.asiatica* tea

Sample	FRAP (mmol Fe2+/mg dried weight)	TPC ^{ns} (µg GAE/mg dried weight)	DPPH (% reduction)
Cranberry	1.92 ± 1.09^{a}	29.89 ± 8.76	0.08 ± 0.00^{a}
Golji berry	0.08 ± 0.06^{b}	29.89 ± 5.90	0.07 ± 0.00^{b}

From the table 15, 16, and 17, it showed that there was only bitterness of tea that was significant different ($p < 0.05$). According to the result, the most preference went to Golji berry tea. The consumers said that it had mild sweet, less bitterness, and less green aroma. They said that it can be consumed much easier than pure *C.asitatica* tea. From the previous researches, golji berry itself has many benefits. It is classified as high antioxidant and fiber food. It has benefits like a cross between cranberries and cherries. Additionally, it helps promote healthy skin because it loads with beta-carotene and it acts like a natural skin cancer treatment. (Dr. Axe, 2014) Once golji berry was mixed with *C.asiatica* tea, it helps increasing antioxidant content of each other. It is neuroprotective effect due to antioxidant enzyme level. (Ponnusamy, 2010)

***b.* To optimize ratio of *Centella asiatica* and goji berry (Lycium barbarum) for herbal tea and to conduct acceptance test of the herbal tea product from *Centella asiatica* and goji berry (Lycium barbarum)**

Ratio of Goji Berry and *C.asiatica* tea

Table 21 Sensory attributes and liking score of Goji berry with *C.asiatica* tea

Sample (*C.asiatica*:Golji berry)	Fruit Flavor	Sweetness	Astringent
1:1	5.2 ± 1.3^{b}	5.5 ± 1.8^{b}	6.3 ± 0.9^{b}
1:1.5	4.8 ± 2.1^{b}	6.5 ± 1.4^{a}	6.7 ± 1.5^{ab}
1:2	6.9 ± 0.9^{a}	7.1 ± 1.4^{a}	7.1 ± 1.0^{a}

Table 22 Sensory attributes and liking score of Goji berry with *C.asiatica* tea

Sample (*C.asiatica*:Golji berry)	Overall liking	Color	Green Aroma
1:1	6.1 ± 1.2^{b}	5.9 ± 1.2^{b}	6.1 ± 1.1^{b}
1:1.5	6.5 ± 1.1^{b}	6.8 ± 1.5^{a}	6.8 ± 1.3^{a}
1:2	7.8 ± 0.9^{a}	7.0 ± 1.1^{a}	7.1 ± 1.3^{a}

Table 23 Amount of antioxidant of the ratio of Goji Berry and *C.asiatica* tea

Sample (*C.asiatica*:Golji berry)	**FRAP** (mmol Fe2+/mg dried weight)	**TPC** ^{ns} (µg GAE/mg dried weight)	**DPPH** (% reduction)
1:1	1.86 ± 0.14^{b}	46.94 ± 2.43^{b}	0.16 ± 0.01^{a}
1:1.5	2.89 ± 0.91^{b}	68.50 ± 3.40^{b}	0.04 ± 0.02^{c}
1:2	6.80 ± 0.43^{a}	278.59 ± 50.87^{a}	0.08 ± 0.00^{b}

Figure 18 Characteristics of different ratio of dried goji berry and tea

The differences in ratio of goji berry and *C.asiatica* were determined in the ratio of 1:1, 1:1.5, and 1:2. The result in table 19 and 20 showed that the most preference tea was goji berry and *C.asiatica* tea at the ratio at 1:2. There were significant different in all attributes ($p<0.05$). The overall liking score of tea at ratio 1:2 was the highest score. Also, it had the highest in amount of antioxidant in three assays. The result of this study showed that the more goji berry was added, the higher antioxidant content was increased. Since it was the most preference among consumers and high amount of antioxidants. Therefore, it is effective

herbal tea from *C.asiatica* leaves. Since goji berry has vitamin A helps in reduce the risk of neurodegenerative decline. (Dr.Axe, 2014) While *C.asiatica te*a has Acetylcholinesterase inhibitors (AChEIs) which helps enhance memory and mental disorder. (Anchalee Chuthaputti, et al, 2017) Once goji berry and *C.asiatica* are combined, the amount of antioxidant is multiplied to three times of original *C.asiatica* tea.

CONCLUSION

C.asiatica tea with goji berry was developed to be functional herbal tea which improved for the elderly people. People in the age of 50-65 is the target of this product. Only *C.asiatica* tea can help to promote skin and improve memory and intelligence. But *C.asiatica* tea mixes with goji berry can enhance its amount of antioxidant content. The most preference method for making tea for elderly people is roasting but no kneading tea which has higher antioxidant content than roasting and kneading method. As the result, the formation that will be acceptable by consumers is tea bag. Tea bag is the most preference score because of its texture and taste. It is convenient and easy for consuming. Also, Thai people are familiar to tea bag. The temperature and time for brewing tea do not affect the amount of antioxidant much so that brewing at temperature 80°C and time at 5 minutes is the most appropriate method for getting the most effective tea. At 80°C and 5 minutes for brewing tea can save energy and less time consuming. The fruit that can go along well with *C.asiatica* tea is goji berry. Goji berry enhances the taste and sweetness of *C.asiatica* tea. It makes tea can be consumed easier and mark green aroma and flavor of tea. The ratio of goji berry and *C.asiatica* that consumers prefer is 1:2. Because of its fruit flavor, sweetness, and color are satisfied consumers. Plus, the amount of antioxidant in goji berry tea is very high amount of antioxidant comparing to without goji berry. Thus, the most effective for elderly people in the age of 50-65 years old in term of health skin, eyes sight, and neuro system is goji berry and *C.asiatica* tea bag in the ratio of 1:2.

REFERENCES

Arisun. Mesh Powder for Green Tea Powder.

Availale at: https://arisunchem.en.alibaba.com/product/60367723073-801822797/Matcha_Powder_Matcha_Tea_Matcha_Green_Tea_Powder.html

[Access May, 2018]

Agnieszka Kosinska and Wilfried Andlauer. Antioxidant Capacity of Tea: Effect of Processing and Storage. pp 113-115.

Anchalee C., et al. 2017. Effects of *Centella asiatica* (L.) Urb. on cognitive function and mood related outcomes: A Systematic Review and Meta-analysis. Version 7. pp 54-57.

Awakening from alzheimer's. Goji Berries Possess a Unique Brain Nutrient.

Available at: https://www.awakeningfromalzheimers.com/goji-berries-possess-a-unique-brain-nutrient/ [Access May,2018]

Ayumi Eguchi and Shouhei Eguchi. Methods of Manufacture of Japanese Tea and The Newly Developed Automatic Leaf-Roasting Device. Session II, pp 262.

Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. Resonance Raman measurement of macular carotenoids in normal subjects and in agerelated macular degeneration patients. Ophthalmology 2002 Oct;109(10):1780.

Berendschot TT, Broekmans WM, Klopping-Ketelaars IA, Kardinaal AF, Van Poppel G, Van Norren D. Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. Arch Ophthalmol 2002 Dec;120(12):1732-7.

Brunborg, H. (2012). Increasing life expectancy and the growing elderly population. Volume 22.

Cao GW, Yang WG, Du P. [Observation of the effects of LAK/IL-2 therapy combining with Lycium barbarum polysaccharides in the treatment of 75 cancer patients] Zhonghua Zhong Liu Za Zhi. 1994 Nov;16(6):428-31.

Cheng C.Y, Chung W.Y, Szeto Y.T, Benzie I.F.F. Fasting plasma zeaxanthin response to Fructus barbarum L. (wolfberry; Kei Tze) in a food-based human supplementation trial. Brit J Nutr. 2005;93:123–30.

Chin YW, et al., "Hepatoprotective pyrrole derivatives of Lycium chinense fruits," Bioorg Med Chem Lett. 2003 Jan 6;13(1):79-81.

David Michaud. Mesh to Micron Conversion Table.

Available at: https://www.911metallurgist.com/blog/mesh-to-micron-conversion-table

[Access May, 2018]

Deng HB, et al., "Inhibiting affects of Achyranthes bidentata polysaccharide and Lycium barbarum polysaccharide on nonenzyme glycation in D-galatose induced mouse aging model," Biomed Environ Sci. 2003 Sep; 16(3):267-75.

Dr. Axe. Goji Berry Benefits: Antioxidant & Anti-Inflammatory Superfruit.

Available at: https://draxe.com/goji-berry-benefits/ [Access May, 2018]

Hammond BR, et al. Preservation of visual sensitivity of older subjects; association with macular pigment density. Inv Ophthalmol 1996;93:54-8.

Haslam E., et al. 2009. Moisture Measurements in an End-Tipped Embankment. pp 8-13.

Izzo AA, Di Carlo G, Borrelli F, Ernst E. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. Int J Cardiol. 2005 Jan;98(1):1-14.

Junkuan Wang, et al. 2014. Biomolecular and Clinical Aspects of Chinese Wolfberry. Edition 2. pp 141-150.

Kim SY, et al., "New antihepatotoxic cerebroside from Lycium chinense fruits," J Nat prod. 1997 Mar;60(3);274-6.

Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE.

Micheal J. Coffey. More Thand Mid-oxidized.

Micheal Tierra. 1998. Chinese Traditional Herbal Medicine. Version 2.

Available at: www.tibetgojiberry.com [Access May,2018]

Available at: http://www.teageek.net/blog/ [Access May,2018]

Misuba. What Using a Stone Mill Matcha is All About!.

Available at: https://www.mizubatea.com/blogs/news-1/the-ishi-usu-what-using-a-stone-matcha-mill-is-all-about [Access May, 2018]

Gan L, Wang J, Zhang S. [Inhibition the growth of human leukemia cells by Lycium barbarum polysaccharide] Wei Sheng Yan Jiu. 2001 Nov;30(6):333-5.

Haleagrahara N., and Ponnusamy K., 2010, Neuroprotective effect of Centella asiatica extract (CAE) on experimentally induced parkinsonism in aged Sprague-Dawley rats, The Journal of Toxicological Sciences, 35: pp 41–47.

Haslam, E. (2003). Thoughts on thearubigins. *Phytochemistry, volume 64*, pp 61-73.

Huang X, Yang M, Wu X, Yan J. [Study on protective action of lycium barbarum polysaccharides on DNA imparments of testicle cells in mice] Wei Sheng Yan Jiu. 2003 Nov;32(6):599-601.

Leila N., et al., 2016. Effect of Different Brewing Times on Antioxidant Activity and Polyphenol Content of Loosely Packed and Bagged Black Teas (*Camellia sinensis L.).* 6(3). pp 313-321.

Hirofumi, M. M., et al., 2011. Effect of green tea powder (*Camellia sinensis* L. cv. Benifuuki) particle size on *O*-methylated EGCG absorption in rats, pp 5-7.

Potterat O, Hamburger M. Goji juice: A novel miraculous cure for longevity and well-being? A review of composition, pharmacology, health-related claims and benefits. Schweiz Zeitschr Ganzheits Medizin. 2008;20:399–405.

Rock CL, Thornquist MD, Neuhouser ML, Kristal AR, Neumark-Sztainer D, Cooper DA, Patterson RE, Cheskin LJ. Diet and lifestyle correlates of lutein in the blood and diet. Nutr 2002 Mar;132(3):525S-530S.

Shaban H, Richter C. Biol Chem 2002 Mar-Apr;383(3-4):537-45 A2E and blue light in the retina: the paradigm of age-related macular degeneration. Biol Chem 2002 Mar-Apr;383(3-4):537-45.

Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. Acta Ophthalmol Scand 2002 Aug;80(4):368-71.

Ujitawara, 2017. Hand-Kneadinf Techniques Producing Japreen Tea.

Available at: http://tea-meister.com/en/greentea/hand-kneading-techniques-for-producing-japanese-green-tea [Accessed May, 2018]

United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Ageing 2015 (ST/ESA/SER.A/390).

Vine Vera. Ingredient Spotlight*: Centella Asiatica.*

Available at: http://vineveracosmetics.com/tag/history-of-centella-asiatica/

Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. Prog Retin Eye Res 2002 Mar;21(2):225-40.

Wu H, Guo H, Zhao R. Effect of Lycium barbarum polysaccharide on the improvement of antioxidant ability and DNA damage in NIDDM rats. Yakugaku Zasshi. 2006 May;126(5):365-71.

Wu SJ, Ng LT, Lin CC. Antioxidant activities of some common ingredients of traditional Chinese medicine, Angelica sinensis, Lycium barbarum and Poria cocos. Phytother Res. 2004 Dec;18(12):1008-12.

Young, G, Lawrence, R., Schreuder, M. Discovery of the Ultimate Superfood. Essential Science Pub, July 2005.

Zhao R, Li Q, Xiao B. Effect of Lycium barbarum polysaccharide on the improvement of insulin resistance in NIDDM rats. Yakugaku Zasshi. 2005 Dec;125(12):981-8.

APPENDIX

SAS Program version 9.4

1. Formulation of *C.asiatica* tea

Code

```
Title 'Randomized Complete Block';

Data RCB;

Input block treatment OL Color GA RA GF RF Sweet Bitter GAF Astringent@@;
datalines;

   1  861  6  8  8  4  5  6  6  6  5  6   1  247  5  8  8  6  5  6  6  6  5  6   1  459  6  8  8  6  5  6  6  6  5  6  6  6  6

ods rtf;
proc anova data=RCBD;
      class block treatment;
      model OL Color GA RA GF RF Sweet Bitter GAF Astringent=block treatment;
means treatment/lsd;
run;
ods rtf close;
```

2. Formation of *C.asiatica* tea

Code

```
Title 'Randomized Complete Block';
Data RCB;
Input block treatment OL Color GA RA GF RF Sweet Bitter GAF Astringent@@;
datalines;
 1  732  7  9  8  8  7  7  8  5  7  6   1  164  7  9  7  8  8  8  8  7  8  7   1  590  6  5  6  5  4  4  3  2  1  1
 2  732  7  7  8  9  7  7  8  5  7  6   2  164  8  8  8  8  8  8  8  8  8  7   2  590  6  5  6  6  5  5  7  7  5  8
30  732  4  5  5  5  4  3  1  1  2  2  30  164  8  7  8  8  8  8  7  8  7  8  30  590  5  6  7  7  6  4  6  6  6  6
;
ods rtf;
proc anova data=RCBD;
        class block treatment;
        model OL Color  GA      RA      GF      RF      Sweet   Bitter GAF      Astringent=block treatment;
means treatment/lsd;
run;
ods rtf close;
```

3. Temperature and time for brewing *C.asiatica* tea

```
    data    Anti_temp_time;
    input   Temp$ Time$  Rep     TPC FRAP DPPH;
    cards;

    80          2           1     112.26      0.65        0.10
    90          5           3      39.86      2.82        0.54
;
proc glm data= Anti_temp_time;
class       Temp Time Rep;
model       TPC=Temp|Time Rep;
means Temp|Time;
```

```
means Temp|Time/Duncan;
ods rtf;
proc glm data= Anti_temp_time;
class          Temp Time Rep;
model          FRAP=Temp|Time          Rep;
means Temp|Time;
means Temp|Time/Duncan;
ods rtf;
proc glm data= Anti_temp_time;
class Temp Time Rep;
model DPPH=Temp|Time Rep;
means Temp|Time;
means Temp|Time/Duncan;
ods rtf;
run;
ods rtf close;
```

4. Ratio of Goji berry and *C.asiatica* tea

```
Title 'Randomized Complete Block';
Data RCB;
Input block treatment OL Color GA FR Astringent@@;
datalines;
 1   800  6  9  8  5  6  8  1   321  8  8  9  7  8  9  1   144  6  7  7  6  4  5
 30  800  5  7  6  6  6  7  30  321  9  9  9  9  9  9  30  144  5  5  4  3  5  7
;
ods rtf;
proc anova data=RCBD;
        class block treatment;
        model OL Color GA FR Astringent =block treatment;
means treatment/lsd;
run;
ods rtf close;
```

SAS results

The ANOVA Procedure

Sensory Test

1. **Formulation for making *C.asiatica* tea leaves**

Overall liking score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	40	206.0950944	5.1523774	7.00	<.0001
treatment	14	18.2733922	1.3052423	1.77	0.0419

Color score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	80.04166667	2.76005747	1.48	0.0832
treatment	3	0.82500000	0.27500000	0.15	0.9309

Green aroma score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	40	191.8362736	4.7959068	6.09	<.0001
treatment	14	17.9648998	1.2832071	1.63	0.0700

Roast aroma score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	151.9666667	5.2402299	5.87	<.0001
treatment	3	4.3000000	1.4333333	1.60	0.1941

Green flavor score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	126.9666667	4.3781609	2.69	0.0002
treatment	3	0.6666667	0.2222222	0.14	0.9381

Roast flavor score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	149.9666667	5.1712644	4.91	<.0001
treatment	3	0.3000000	0.1000000	0.09	0.9627

Sweetness score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	247.8000000	8.5448276	8.36	<.0001
treatment	3	0.5666667	0.1888889	0.18	0.9065

Bitterness score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	115.3750000	3.9784483	2.98	<.0001
treatment	3	0.4250000	0.1416667	0.11	0.9564

Green aftertaste score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	40	164.1317703	4.1032943	5.28	<.0001
treatment	14	31.2002569	2.2285898	2.87	0.0004

Astringent score of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	134.3666667	4.6333333	5.82	<.0001
treatment	3	2.2666667	0.7555556	0.95	0.4204

2. **Formation of consuming *C.asiatica* tea**

Overall liking core among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	141.8333333	4.8908046	2.95	0.0002
treatment	2	72.4666667	36.2333333	21.85	<.0001

Color score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	90.26666667	3.11264368	1.22	0.2595
treatment	2	28.80000000	14.40000000	5.62	0.0059

Green aroma score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	134.2666667	4.6298851	2.06	0.0095
treatment	2	3.2000000	1.6000000	0.71	0.4944

Roast aroma score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	149.9555556	5.1708812	2.51	0.0014
treatment	2	1.3555556	0.6777778	0.33	0.7206

Green flavor score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	138.4888889	4.7754789	2.19	0.0056
treatment	2	25.4888889	12.7444444	5.84	0.0049

Roast flavor score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	124.7222222	4.3007663	2.02	0.0113
treatment	2	21.3555556	10.6777778	5.02	0.0097

Sweetness score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	239.2888889	8.2513410	3.34	<.0001
treatment	2	64.0222222	32.0111111	12.96	<.0001

Bitterness score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	187.8222222	6.4766284	2.51	0.0015
treatment	2	101.4222222	50.7111111	19.62	<.0001

Green aftertaste score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	217.7888889	7.5099617	3.06	0.0001
treatment	2	54.4222222	27.2111111	11.10	<.0001

Astringent score among three formations of *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	161.9555556	5.5846743	2.51	0.0014
treatment	2	40.2888889	20.1444444	9.05	0.0004

3. **Temperature and time for brewing *C.asiatica* tea**

Overall liking score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	141.8333333	4.8908046	2.95	0.0002
treatment	2	72.4666667	36.2333333	21.85	<.0001

Color score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	90.26666667	3.11264368	1.22	0.2595
treatment	2	28.80000000	14.40000000	5.62	0.0059

Green aroma score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	134.2666667	4.6298851	2.06	0.0095
treatment	2	3.2000000	1.6000000	0.71	0.4944

Roast aroma score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	149.9555556	5.1708812	2.51	0.0014
treatment	2	1.3555556	0.6777778	0.33	0.7206

Green flavor score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	138.4888889	4.7754789	2.19	0.0056
treatment	2	25.4888889	12.7444444	5.84	0.0049

Roast flavor score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	124.7222222	4.3007663	2.02	0.0113
treatment	2	21.3555556	10.6777778	5.02	0.0097

Sweetness score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	239.2888889	8.2513410	3.34	<.0001
treatment	2	64.0222222	32.0111111	12.96	<.0001

Bitterness score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	187.8222222	6.4766284	2.51	0.0015
treatment	2	101.4222222	50.7111111	19.62	<.0001

Green aftertaste of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	217.7888889	7.5099617	3.06	0.0001
treatment	2	54.4222222	27.2111111	11.10	<.0001

Astringent score of *C.asiatica* tea bag

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	161.9555556	5.5846743	2.51	0.0014
treatment	2	40.2888889	20.1444444	9.05	0.0004

4. **Types of fruit that match with *C.asiatica* tea**

Overall liking score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	134.5575397	10.3505800	0.49	0.9196
treatment	6	140.6714286	23.4452381	1.11	0.3690

Color score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	4380.778968	336.982998	0.24	0.9964
treatment	6	4912.152381	818.692063	0.58	0.7447

Green aroma score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	209.3230159	16.1017705	0.68	0.7758
treatment	6	34.9380952	5.8230159	0.24	0.9592

Roast aroma score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	277217.2968	21324.4074	0.46	0.9357
treatment	6	211873.0095	35312.1683	0.76	0.6013

Green flavor score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	169.9611111	13.0739316	0.54	0.8907
treatment	6	162.3261905	27.0543651	1.11	0.3704

Roast flavor score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	9704.97302	746.53639	0.64	0.8126
treatment	6	11259.23810	1876.53968	1.60	0.1675

Sweetness score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	133.1714286	10.2439560	0.35	0.9788
treatment	6	146.2666667	24.3777778	0.83	0.5492

Bitterness score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	238967.3635	18382.1049	0.41	0.9605
treatment	6	302118.3881	50353.0647	1.12	0.3669

Green aftertaste score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	304.6742063	23.4364774	0.87	0.5870
treatment	6	118.4380952	19.7396825	0.73	0.6249

Astringent score of two fruits and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	13	11608.50873	892.96221	0.72	0.7314
treatment	6	6054.08333	1009.01389	0.82	0.5613

5. Ratio of goji berry and *C.asiatica* tea

Overall liking score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	26.40000000	0.91034483	0.68	0.8662
treatment	2	50.86666667	25.43333333	19.12	<.0001

Color score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	58.76666667	2.02643678	1.43	0.1251
treatment	2	18.86666667	9.43333333	6.63	0.0025

Green aroma score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	34.32222222	1.18352490	0.67	0.8834
treatment	2	18.15555556	9.07777778	5.10	0.0091

Fruit aroma score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	71.82222222	2.47662835	1.09	0.3825
treatment	2	71.35555556	35.67777778	15.68	<.0001

Sweetness score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	48.62222222	1.67662835	0.63	0.9114
treatment	2	37.75555556	18.87777778	7.10	0.0017

Astringent score of three different ratio of goji berry and *C.asiatica* tea

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	29	34.05555556	1.17432950	0.79	0.7572
treatment	2	11.35555556	5.67777778	3.80	0.0281

Antioxidant Properties

1. **Formulation of *C.asiatica* tea**

   DPPH radical scavenging activity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	2	0.00011217	0.00005608	1.13	0.3833
treatment	3	0.00075892	0.00025297	5.10	0.0435

Ferric reducing (FRAB) antioxidant power

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	2	0.00130217	0.00065108	1.47	0.3014
treatment	3	0.00527667	0.00175889	3.98	0.0707

Total Phenolic Compounds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
block	2	0.00115550	0.00057775	0.32	0.7353
treatment	3	0.01714225	0.00571408	3.20	0.1047

2. **Formation for consuming *C.asiatica* tea**

DPPH radical scavenging activity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	0.02403467	0.01201733	89.53	<.0001

Ferric reducing (FRAB) antioxidant power

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	40.80970022	20.40485011	58.48	0.0001

Total Phenolic Compounds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	98265.62109	49132.81054	56.59	0.0001

3. **Temperature and time for brewing *C.asiatica* tea**

DPPH radical scavenging activity

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Temp	2	5.59775556	2.79887778	3.06	0.0670
Time	3	2.04010000	0.68003333	0.74	0.5370
Temp*Time	6	20.09040000	3.34840000	3.67	0.0112
Rep	2	0.30020556	0.15010278	0.16	0.8495

Ferric reducing (FRAB) antioxidant power

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Temp	2	0.39795000	0.19897500	0.25	0.7822
Time	3	6.69074167	2.23024722	2.78	0.0648
Temp*Time	6	0.14998333	0.02499722	0.03	0.9998
Rep	2	6.71280000	3.35640000	4.19	0.0287

Total Phenolic Compounds

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Temp	2	216.20857	108.10429	0.10	0.9055
Time	3	4002.14773	1334.04924	1.23	0.3224
Temp*Time	6	9463.18125	1577.19688	1.45	0.2394
Rep	2	14852.18801	7426.09400	6.85	0.0049

4. **Types of fruit that match with *C.asiatica* tea**

DPPH radical scavenging activity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	1	0.00016017	0.00016017	120.13	0.0004

Ferric reducing (FRAB) antioxidant power

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	1	5.07104267	5.07104267	8.52	0.0433

Total Phenolic Compounds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	1	0.00001667	0.00001667	0.00	0.9996

5. **Ratio of goji berry and *C.asiatica* tea**

DPPH radical scavenging activity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	0.02403467	0.01201733	89.53	<.0001

Ferric reducing (FRAB) antioxidant power

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	40.80970022	20.40485011	58.48	0.0001

Total Phenolic Compounds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	98265.62109	49132.81054	56.59	0.0001