Encapsulated Cannabis Oil Oolong Tea Formulation

Anon Pongtanavong
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A Special project submitted to the School of Biotechnology,
Assumption University in part of the fulfillment of the requirement
for the degree of Bachelor of Science in Biotechnology

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Title	**:**	**Encapsulated Cannabis Oil Oolong TeaFormulation**
By	**:**	**Anon Pongtanavong**
Advisor	**:**	**Dr. ChurdchaiCheowtirakul**
Level of study	**:**	**Bachelor of Science**
Department	**:**	**Food-technology**
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Dr. ChurdchaiCheowtirakul
(Advisor)

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Anon Pongtanavong

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ABSTRACT

Today, infused teas are attracting much attention in younger generations and older. The infusion itself means adding or dispersed any nutrition into the water to create solutions. On the other side, cannabis or marijuana is also getting more opinion and becoming popular, not only for being drugs, but its medicinal purpose itself exceeds its harmfulness as psychedelic drugs. Cannabis comes in many forms, but the most popular in terms of medicinal use is the oil form. This project aims to formulate and to create a new type of infused tea. To formulate tea that contains high in antioxidants and high in nutritive value along with the addition of cannabis medicinal properties, which can be developed further to play in an essential role in the medicinal industry. Content in cannabis oil can be controlled easily to vary the illegal psychoactive substances. In cannabis, there are two primary active compounds, which are CBD (cannabidiol) and THC (Tetrahydrocannabinol). THC or tetrahydrocannabinol is a psychoactive substance that makes the user psychoactive high. In Thailand, THC content of more than 2% is illegal by law. THC is also the intoxicant, such as uncontrollable vomiting, or psychosis. CBD, on the other hand, is the medicinal compound that acts as a relaxant. Both compounds use cannabinoid receptors as an active site. Infusing Cannabis oil along with tea, not only to improve benefits but to innovate new types of products. In this project, Cannabis Sativa or marijuana strain is processing into cannabis oil by ethanol extraction, which will later be encapsulated and added into a loose-leaf tea with the weight of 0.6 grams to 2.5g weight of Oolong tea leaves. At first sensory analysis of Jasmine, Oolong, and green tea with varieties of encapsulated oil were given to find the most suitable formula, the daily dosage of cannabis oil is measured and used as the highest sample. However, because cannabis oil cannot be dissolved in the water itself, the oil molecule is needed to be coated to make oil molecule soluble in water. With the help of Beta-cyclodextrin, the oil molecule is bound to the hydrophobic sites inside the beta-cyclodextrin molecules and will be dissolvable in water due to the hydrophilic site. Via chemical analysis with the average GAE of 5.233mg/ml and 87% antioxidant activity, the final product shows significant differences in both GAE and antioxidant activity of 1.5miligrams per milliliters and 14% respectively, therefore shows that the final product increases in nutrition and health.

Keyword: *Cannabis Sativa,* cannabidiol, extraction, formulation

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INTRODUCTION

1.1 Opening Statement

Today, drinking water is getting replaced by other beverages such as soft drinks, tea, coffee and so forth. However, recently, tea is becoming one of the most consumed drinks in the world. Not only it beats coffee and coca-cola in drink per capita but history as well. Human has been consuming tea for over 3000 years and thought to have first been harvested in 2700 BC by emperor Shen Nung in China. However, today, there is the further development of tea to fit today's world or adaptation to become one of the beverages. Infusion, or adding other ingredients into a tea to create and add flavor to the drink. By definition, the infusion is a drink made by adding flavor or any ingredient into a liquid. Today, green tea, oolong tea, or Pu-erh teas are not the only default flavors you can drink anymore. Raspberry, strawberry, lemon, earl grey, peppermint, or jasmine teas are the excellent tea flavor loved by everyone around the world at this moment. Currently, with the sone be to legal law of cannabis in Thailand, infusing cannabis, which has a medicinal purpose with a medicinal drink such as tea could be an excellent innovation for current herbal and beverage market thus it would bring out more of its medicinal purpose by infusion than any other ways. Teas and cannabis both have many active compounds such as antioxidants and phenolic compounds, which are essential to our health and for our body. When combined, both antioxidants and phenolic compounds will be more significant and healthier at the same time.

1.2 Information on *Cannabis Sativa*

Cannabis Sativa or cannabis is a plant where we could use in fresh, dried, or essential oil form. It grows just like any plants in the wild of the tropical and temperate areas of the world. It can be grown in almost any climate and is increasingly cultivated utilizing indoor hydroponic technology. Cannabis usually is used in three primary forms. Grounded, Hashish, and hash oil. Each product uses varieties of cannabis plant parts. Grounded cannabis typically uses dried flowers and leaves which grounds by whether the herb grinder or any blender to reduce the size, increasing surface area, and breaking of cell walls. Hashish is obtained by collecting trichomes of the cannabis Sativa plant. Trichome is a hair-like structure that grown on a plant which its function is to protect the plant from external harm such as UV ray, insects, or microbiology. However, trichome produces a high amount of CBD, terpenes, so forth. Moreover, lastly, hash oil can be obtained by heat pressing cannabis Sativa flower. Cannabis is produced and consumed in virtually all countries of the world. Because illicit cannabis cultivation is so widespread and carried out both indoors and outdoors, it is challenging, if not impossible, to estimate how much cannabis is produced worldwide annually.

Europe continues to be one of the world's largest consumer markets for cannabis resin (hashish), the majority of which is from Morocco. The Middle East, North Africa, and South-West Asia also have large consumer markets for resin, and significant production occurs in these regions (UNODC, 2015a). The world's two largest producers and exporter countries of cannabis resin appear to be Afghanistan and Morocco, although resin is also produced in and exported from, but probably to a lesser extent, India, Lebanon, Nepal, and Pakistan. Afghanistan is becoming more prominent in global resin production, with the UNODC suggesting that it has overtaken Morocco in terms of quantities of resin produced. Despite this, Afghan resin does not currently seem to be widely available in most of Europe (Chouvy, 2016). In 2013, some 1 416 tons of cannabis resin were seized worldwide, confirming an increasing trend since 2011. After peaking at 7 060 tonnes in 2010, worldwide herbal cannabis seizures declined to reach 5 764 tons in 2013 (UNODC, 2015a)

1.3 Information on *Camellia Sinensis*

Camellia Sinensis or tea leaves is a plant that is native to China. Approximately 600,000 tons of green tea is consumed every year in the world. It is about 1/5 of total consumption on all kinds of tea. However, interest in green tea has led to increased consumption. Green tea consumption report in 1998 tells that the total green tea consumption has grown by approximately 20 % in the last 10 years. Major green tea consumption countries are located in East Asia. Only a few percentages of the people in Europe, the U.S., Africa drink green tea. Chinese are the most significant green tea-drinking nation. People in China consume approximately 50 % of the total green tea consumption in the world. Japan is the secondly largest green tea-drinking country. About 80,000 tons of green tea is consumed in Japan every year. In Indonesia, 30,000 tons and in Vietnam 20,000 tons of green tea are consumed each year.

Last 10 years the consumption amount has not increased or decreased much in Japan, but in China and Vietnam, the number has increased slightly. The amount of green tea consumption of a year by each person is 650g in Japan, 380g in Vietnam, 320g in China, and 200g in Indonesia. In any other country, people drink much less green tea than those listed countries. In Thailand, ready to drink bottle is the most popular among all types with most of the styles were originated from Japanese influence. (Chacko, S. M., 2010)

1.4 Situation in Thailand

In Thailand, people have not developed a tea culture of their own until rather recently. While the tea culture in Thailand is driven by Chinese tea tradition and Chinese tea preference, teas in Thailand seems to be flourishing at global levels in recent years too. Driven by Chinese, Europeans, and Japanese, Thais started to like and embraced the idea of tea time or coffee break.

Cannabis, however, is legalizing, which is an excellent opportunity to be used to brew tea. Typically, cannabis in the form of oil is used medicinally due to pure CBD and THC extraction is needed. The main psychoactive chemical in marijuana responsible for most of the intoxicating effect that people seek is THC. The chemical is found in resin and is produced by the leaves and buds primarily of the female cannabis plant. Marijuana use is widespread among adolescent and young adults. (Asia,n.d)

OBJECTIVE

The purpose of this research and development is because Cannabis is relatively old, but its unique medicinal effect is new to Thailand. Cannabis is considered as herbal medicine a long time ago until recreational use was abused and illegalized by Thai government. Creating new innovative product opportunities in which cannabis could be one of the keys for herbal medicine in Thailand. Thailand is also considerably slow in terms of accepting changes. This project is to be open-minded and accepting cannabis as a new medicine. Infused tea popularity is rising along with original tea itself. Cannabis-infused tea is a relatively new and unknown product. Also, mainly, the purpose of this formulation is to formulate and create new tea infusion.

2.1 Scope

This study was performed under a controlled environment such that the experiment, raw material, and final product is clean and is safe to consume. Such as; all lab equipment was sterile and is separated from laboratory equipment as much as possible. Moreover, the working environment is well separated from the microbiology lab and hazardous chemicals room as much as possible.

2.2 Hypothesis

The encapsulated cannabis oil is successfully formulated with the right tea and the right amount of encapsulated cannabis oil added as a formula.

LITERATURE REVIEW

3.1 Green tea

Green tea is one of the most popular beverages consumed worldwide. Tea, Camellia Sinensis is consumed in different parts of the world as green, black, or Oolong tea. Its effect on human health has been observed with the consumption of green tea. Green tea, however, started in India and was exported to Japan during the 17th century. Estimated about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea and the rest as other teas. Moreover, it's mainly consumed in Asia and some parts of North Africa, the United States, and Europe.

To achieve green tea, freshly harvested tea leaves are immediately steamed to prevent any further chemical change and to keep the tea leaves from the fermentation process, preventing the yielding loss, and also to stabilize or to keep the leafs fresh. The steaming process also destroys and inhibits enzymes in the leaves that could change the leaf's color and to maintain the green color. The leaves also maintain its phenolic compound and preserve natural polyphenols that are used to promote healthy properties.

Consuming Green tea or Camellia Sinensis' shows many benefits, including the prevention of cancer, cardiovascular disease, anti-inflammatory, antarthritics, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, and cholesterol-lowering effects. However, adding green tea to the diet may cause serious health concerns. The health-promoting effects of green tea mainly come from its polyphenol content, particularly antioxidants and flavanols, which are massively presented in leaf. Roughly 30% of freshly dried leaves weigh. Many of the beneficial effects of green tea were attributed to catechin (epigallocatechin-3-gallate). However, few herbal medicines have been well characterized, and their efficacy demonstrated in systematic clinical trials as compared to Western drugs. Chemical composition of green tea is composed of 15-20% dry weight, amino acids 1-4%, carbohydrates 5-7% mineral and trace element 5%, lipids, sterols, xanthicbases(caffeine, theophylline, pigments(chlorophylls, carotenoids), volatile compounds. Green tea's most crucial element is polyphenols, which include, flavonols, flavonoids, and phenolics, which made up 30% of dry weight.

3.2 Cannabis

Green tea consumption can be linked to the prevention of many types of cancers, which include the lungs, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands. Also, it can reduce the risk of many chronic diseases due to polyphenols, which are potent antioxidants. Green tea could lower blood pressure, thus reduce the risk of stroke and coronary heart disease. Moreover, studies in some animals show that green tea reduced blood glucose levels and body weight. The effectiveness of green tea in treating diarrhea and typhoid due to catechins have an inhibitory effect on Helicobacter pylori infection. It's also useful for herpes simplex's virus. In human, it also can act as antifungal via catechins which convenience of combined treatment with catechins and of antimycotics lower doses is which reduces dosage and prevent side effects of antimycotics. Green tea also helps increase bone density, which has been identified as an independent factor protecting against hip fractures. Green tea also strengthens the immune system action which protects against oxidants and radicals.

Cannabis contains more than 400 neuroactive chemicals that worked with cannabinoid receptors where it contains delta-9-tetrahydrocannabinol and cannabidiol or so forth. It's also most widely used as illicit drugs in the world, and its use has been associated with various mental health, particularly young. How cannabis active compound works is to bind with CB1 located in the brain and CB2 which located in the brain and peripheral immune system. This compound has powerful and indirect effects on these receptors. Cannabis is a complex plant with delta-9-tetrahydrocannabinol and cannabidiol. (Volkow, N. D., 2014)

The clinical or therapeutic benefit of cannabis is significant. Research regarding these benefits of cannabis has become non-existent in the US. The response rate was 94% of mean and medians age were 49.3 and 51 years old, respectively. Study shows that cannabis helps people slept, most consistent results showed a decreased time spent in slow-wave sleep and increase to time spent in random eye movement. Cannabis uses to assist ranged from 45% to 93% of people on average use it to assist sleeping disorder ranged from 0-43% which those who claim there's improvement ranged from 50-92% of the sample. (Asia, 1. (n.d.).)

There is strong evidence that cannabis can help with muscle spasms. Cannabis also could lead to solving and helping Alzheimer's disease. Some research in laboratory settings has suggested that there are effects on some of the hallmarks of Alzheimer's disease but does not cure it.

Some studies also found that taking cannabis can manage some of the behavioral symptoms of dementia, like agitation and aggression though more experiment is needed on this receptor. This is a molecular switch that activates neurons Endocannabinoids system is located in the brain which made up of cannabinoid which can regulate and help thinking function. This indeed helps to treat tremors. Some researcher thinks that cannabis might be neuroprotective, which save neurons from damage caused by Parkinson disorder from research, cannabinoid helps to treat bradykinesia and dyskinesia, but there are no conclusions for these benefits.

3.3 Beta cyclodextrin

Beta cyclodextrin is one of the most studied of all cyclodextrins. Results from numerous investigations have demonstrated that it is safe and metabolism. Beta-cyclodextrin however, is not attacked by salivary or pancreatic a-amylases. As a result, it passes through the digestive tract to the colon intact. In the market, many brands of teas are using beta-cyclodextrin to encapsulate the essential oil of flowers or fruits for their fruit-flavored and flower-scented tea.

3.4 Kneading Method

The kneading method is one of the most used encapsulation processes with Beta-cyclodextrin. This method used mechanical force to push hydrophobic substances such as fat or oil into the hydrophobic site of the compound, which ended up in the encapsulation of essential oil.

MATERIALS AND METHODS

4.1 MATERIAL

4.1.1Experimental location

All experiments were performed at the faculty of biotechnology, assumption university, Huamak campus, Thailand.

4.1.2 Raw Material

- Cannabis Sativa
- Food grade 95% ethanol
- Distilled water
- Tap water
- Beta-cyclodextrin
- Rice Bran Oil

4.1.3 Equipment

- Funnel
- Graduated cylinder
- Beaker
- Hot air dryer
- Sieve
- Ring stand
- Tray
- Distillation condensation chamber

4.1.4 Information on raw material

Cannabis plant material in this experiment was of a variety "lemon skunk" strain, which is one of the awards winning in 2009. In this case, the flower, leaves, stems, and seeds are used in this experiment. The cannabis sample, however, passed the drying process, which is for storage and to prevent any microbiological activity from happened during the storage period.

Figure 1: Alfa One Rice Bran Oil

Rice bran oil

The oil chosen to use as dilutor in this project is Rice bran oil form the brand 'Alfa one'. Because rice bran contains high in nutritional value as well as mono, poly, and saturated fat which is considered as heart-friendly oil. The reason this oil is chosen is also due to the healthy properties of these compounds inside.Moreover, Rice bran oil is available and is easy to find in south east asia such as Thailand which produces rice as their original crops. This is also to help Thai farmer

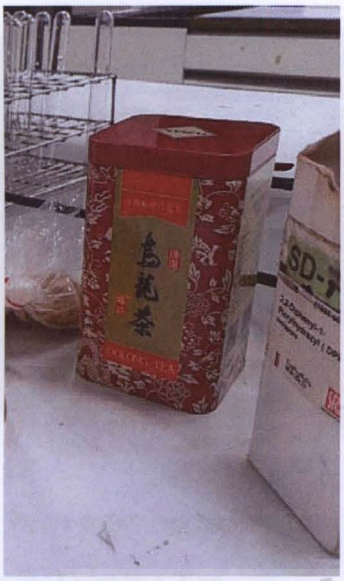

Figure 2: Fujian Oolong Tea

Oolong tea

Fujian Oolong tea was chosen for this project by the quality of arom and taste itself. From researching along isle in the supermarket, the highest quality of oolong tea will have high amount of antioxidant which originates from china itself. Varieties of oolong tea in supermarket come from northern Thailand, which the condition of growth is not as clean and right for the tea as china. However, tea that is produced in Thailand could be used instead of imported tea to reduce cost.

Figure 3: TWG Lung Ching tea

TWG lung Ching Tea

Lung ching green tea is one of the top quality produced from china. Lung ching tea is one of the oldest tea found in china which fave great scent and aroma both before and after brewing. This tea however is quite expensive and scarce in the amount of production.

Figure 4: Twinings Jasmine Green Tea

Twinings Jasmine Tea

Jasmine green tea is currently popular in infused current tea market. Because of its scent and soothing aroma, many are drinking this type of tea during lunch break or dinner.

4.2Methodology

4.2.1 Cannabis sample preparation

Cannabis sample is obtained in the dried bricked formin order to prevent any pests, microbiological activity, and internal decomposition. The sample is then, extracted roughly by using a bread knife to cut the brick into asmaller chunks for the ease of use and weighing. The smaller the chunks are the easier it is to weigh specifically. Once the sample is weighed, high power blender is used to grind the cannabis sample coarsely. This is to introducemechanical grinding is to break the cell walls, increasing surface area, and help to diffuse while added into ethanol. Moreover, the finer the cannabis breaks, the more compound yields as well. 1 kilograms of cannabis sativa was initially obtained and later on, the brick was cut and collected in a repetition of 100 grams at a time.

Figure 5: Bricked Cannabis sample

Figure 6: Grounded Cannabis sample

4.2.2 Oil extraction

Once the cannabis is finely grounded, 95% ethanol was then poured into the 2000ml beaker with ground cannabis until it is 1 inch above the ground cannabis, which is at 400ml of ethanol.The mixture is continuously stirred with metal fork or any stirrer to keep and to prevent sedimentation for 3-5 minutes before removing ground cannabis. Once 5 minutes over, figure 2 shown the method of filtration. By using funnel with coffee filter, the solution s finely filtered which leave only a fine solution without any grounded cannabis. At 3-5 minutes is the best time to leave due to the longer the grounded cannabis stays submerge, the more indesirable compound is being released as well, which leaving ground cannabis for longer than 5 minutes allows more THC release. The extracted shows desired solution which is fine opaque brown and viscous. The solution is then put into the condenser chamber. The temperature setting is at 90°C for 18 hours of condensing time. The ethanol evaporates, leaving a thick dark tar-like paste in the beaker. Yield is at around 10% of the total initial amount. To make sure that unevaporated or remained ethanol is 100% evaporated, the extract is left inside 500ml beaker with punched foil wrap and 2000ml beaker covered in order to prevent any insects or debrees and microbes from entering the extracted cannabis oil.

4.2.3 Cannabis Oil Dilution

Figure 7: Crude Cannabis oil extract

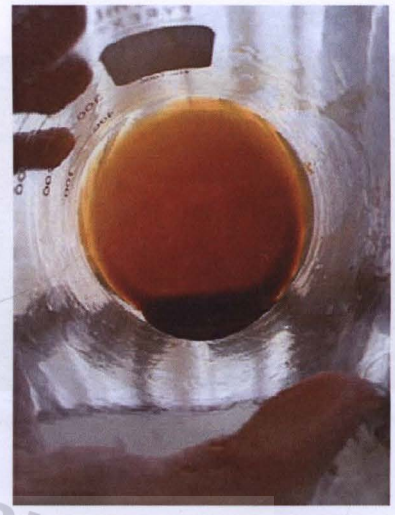

Figure 8: Diluted Cannabis oil extract

Once the cannabis oil is successfully extracted, figure 1 shows the crude extraction is viscous and unable to work with, dilution is required. 25ml of cannabis oil is archieved per experiment.ground cannabis to rice bran oil ratio is 1:2. In this case 25ml of cannabis oil to 50 ml of rice bran oil. Figure 2 shows that the viscosity of extraction oil dropped which is desired to be able to work with in this project. Once the dilution is complete, the oil is now 75ml of yields. Moreover, the cannabis oil is diluted also to be able to work with beta-cyclodextrin.

4.2.4 Cannabis oil Encapsulation

Figure 9: Cannabis oil in Beta-cyclodextrin

Figure 10: Encapsulated Cannabis oil powder

The beta-cyclodextrin powder added with at the ratio of 1:2 until toothpaste-like consistency is achieved. The cannabis extract is then, added into the paste with the ratio of 85:15 as seen in figure 1. By using dough whipper to create a mechanical force to force cannabis extract into the hydrophilic site of Beta-cyclodextrin for 15 minutes.After 15 minutes passed, figure 2 shows the final encapsulation result.The paste is then put properly into plate which will later on dry in the tray drier which shown in figure 3. The tray is then put into the drier in figure 4 in a metal tray and blow-dry at 40°C for 24 hours. Once the paste is dried, using a high-velocity blender to blend the paste into powder. Figure 5 shows the high power blender which is used for herb and powder blender. In figure 6 shows the final powdered encapsulation which is desired.

4.4 Sensory Analysis

Tea samples was prepared by using standard tea brewing method. At first, 200ml of drinking water were boiled at 100°C, then letting it cooled down at 85°C. Tea samples were then added into drinking water. 2.5 grams each of Oolong, Jasmine, and Green tea, and variation of 0.4grams, 0.6 grams, and 0.8grams of encapsulated cannabis oil were added in each samples. As for control, the encapsulated cannabis oil was not added.

4.4Chemical analysis

4.4.1 Tea sample preparation

First, 200ml of water is boiled at 89°C to achieve the best tea brewing temperature, the hotter the water is, the more undesirable taste is released to the solution as well. Then, 2.5 grams of both tea sample and tea with encapsulated cannabis oil is added for 2 minutes before using a coffee filter to remove any residue left inside the tea. Once both tea sample is infused and filtered, the sample is being cooled in room temperature until it reaches room temperature. Figure 11 shows 2 samples of tea. On the left shows oolong tea without any cannabis add and on the right is the tea sample with encapsulated cannabis added.

Figure 9: Tea samples for chemical analysis

4.4.2 DPPH solution preparation

To prepare 1mM of DPPH, 0.01 grams of DPPH are weighed and added into a volumetric flask along with methanol until it reaches 25ml. Then 1mM is diluted 10 times by adding 10ml of 1mM DPPH with 90ml of methanol in the volumetric flask. The flask is then wrapped in foil and keep in dark area to prevent any photoreaction.

4.4.3 DPPH

The sample is pipette 100 microliters of the sample into 10 times 1 micromolar of DPPH solution then keep in dark place for 30 minutes. Once the sample is ready, measure the absorbance of the sample at 517 nm. Then find the scavenging effect percentage of the sample.

4.4.4 Preparation of gallic acid

The gallic stock solution is prepared by adding 250 grams of gallic acid powder into 1ml of methanol. The sample is then centrifuged for 5 minutes to prevent any clumps and inhomogeneous. Thereafter, 5 concentration of gallic acid is prepared. At concentration of 50, 75, 100, 125, 150 mg/ml, 0.4, 0.6, 0.8, 1.0, 1.2 ml of gallic acid are added to 1.6, 1.4, 1.2, 1.0, 0.8 ml of deionized water respectively.

4.4.5 Preparation of Folin-Ciocalteu reagent

Folin-ciocalteu reagent in this experiment is diluted 1:10 or ten times because tea and cannabis contain a high number of antioxidants. 10ml of Folin-Ciocalteu reagent is added to 90 milliliters of water in a 100ml volumetric flask which is then wrapped by tin foil and keep in the dark to prevent any photoreaction.

4.4.6 Preparation of Sodium Carbonate

Sodium carbonate is prepared at 7.5% to deionized water. 7.5 gram of sodium carbonate is added to deionized water. Then it is kept in reasonable condition.

4.4.7 Phenolic

In order to prepare the sample, Gallic acid is used as a standard and prepared at 50, 100, 150, 250, and 500 mg/L. 20 microliter is then, mixed with 1.58 mL of distilled water and 0.1 ml of Folin-Ciocalteu reagent. The solution is mixed thoroughly and waited for 9 minutes and 30 seconds. Then, 300 microliters of a saturated sodium carbonate solution are added to the mixture and incubated at room temperature for 30 minutes. The mixture is then, used to measure the absorbance at 765 nm.

4.4.8 Encapsulated cannabis oil Oolong tea

The process of preparing the product, 2.5 grams of loose Oolong tea added with 5% of encapsulated cannabis oil before brewing.

4.4.9 Tea infusing

At 89°C 200ml of drinking water, prepared encapsulated cannabis oil Oolong tea is added and stir for 10 seconds to loosen oolong curled leaves then let the tea infused for 2 to 3 minutes before removing.

RESULT AND DISCUSSION

5.1 Cannabis Oil extraction

Cannabis was extracted by ethanol extraction. Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in cannabis are hydrophobic, therefore 95% ethanol is used as a solvent for hydrophobic compounds. In this project, the only desired compounds are THC and CBD to control then impurity. In order to achieve this, the extraction was timed at 3 minutes because during is period of time, CBD and THC releases due to its molecular weight, these 2 compounds are the fastest compounds to be released. Once the solutions of grounded cannabis and ethanol were filtered, the solutions were then distilled for 16 hours at the temperature of 80°C to reduce ethanol as much as possible. From 100 grams of ground cannabis and 400ml ethanol, the final yield of cannabis oil was at around 25ml or 6.25%. 25ml of crude extract was then diluted with the ratio of 1:2 or 25ml cannabis oil to 50ml of rice bran oil. The dilution was done because of the crude extract mixture was too viscous and have a consistency of toothpaste. After dilution, the final consistency becomes less viscous with vegetable cooking oil-like consistency. In the end, once the crude extract is diluted, the final volume of cannabis oil was 75ml.

5.2 Encapsulation of Cannabis Oil

In order to dissolve cannabis oil in water, beta-cyclodextrin was used to encapsulate the oil molecule. After encapsulation, the mixture was dried and blended into a powdered form. From 50ml encapsulated oil with 50grams of beta-cyclodextrin, the final weight yieldswas 60grams. Encapsulated cannabis oil was then kept in a bag with moisture control satchels. Encapsulation via beta-cyclodextrin, however, since beta-cyclodextrin is partially dissolved in tea solution, the cannabis oil will be slowly dispersed or slowly release via melting mechanism of beta-cyclodextrin. The longer the beta-cyclodextrin stays inside the tea, the more cannabis oil is getting released.

5.3 Cannabis tea preparation

Once the extraction, encapsulation, and infusing were done, the tea was then brewed with 80°C drinkingwater for 3 minutes for teas to reach its maximum infusing time. Then the tea was poured out of the pot. Tea preparation must be at the perfect temperature and time, because there are high chances that the leaves might get burned if using boiling temperature, which would results in a bitter taste of tea. By using 80°C as a boiling temperature, it was the perfect and most suitable temperature which would not burned the tea leaves. Moreover, 3 minutes of infusing leaves due to the releasing of phenolics and antioxidant, because the longer soaking time will let more phenols and antioxidants releases. This is up to the preferences of others.

5.4 Sensory Analysis of Cannabis infused teas

Table 1: Results of sensory analysis

Treatment	Color	Tea aroma	Tea flavor	Cannabis aroma	Bitterness	Overall
Jasmine with 0.4 g cannabis oil	6.96±1.098^{a}	5.24±1.508^{a}	6.08±1.579^{a}	5.04±1.619^{a}	5.28±1.4^{a}	5.28±1.173^{a}
Jasmine with 0.6 g cannabis oil	6.4±1.041^{a}	5.32±1.406^{a}	5±1.607^{ab}	5.6±1.779^{ab}	5.24±1.363^{ab}	5.52±1.327^{a}
Jasmine with 0.8 g Cannabis oil	2.52±1.327^{ab}	5.24±1.155^{b}	2.6±1.225^{ab}	2.08±1.038^{b}	1.92±0.997^{b}	1.32±0.476^{b}
Oolong with 0.4 g Cannabis oil	6.44±0.914^{ab}	5.16±1.179^{b}	5.64±1.254^{ab}	3.68±1.145^{b}	6.2±1.414^{c}	5.4±1.190^{b}
Oolong with 0.6 g cannabis oil	6.8±1.190^{ab}	6.28±1.242^{b}	6.2±1.155^{bc}	6.52±1.045^{b}	7±1.041^{c}	7.12±0.726^{b}
Oolong with 0.8 g cannabis oil	4.08±0.909^{b}	4.72±1.173^{b}	3.56±1.294^{c}	3±1.291^{c}	2.48±1.159^{d}	1.68±0.690^{b}
Green with 0.4 g cannabis oil	6.52±1.358^{c}	6.32±1.249^{b}	6.56±1.158^{d}	5.44±1.387^{c}	4.44±1.325^{e}	5.84±1.248^{c}
Green with 0.6 g cannabis oil	6.04±1.338^{d}	5.16±1.106^{c}	5.96±1.513^{e}	5.8±1.5^{d}	6.6±1.354^{e}	7.04±1.207^{cd}
Green with 0.8 g cannabis oil	2.88±1.394^{d}	2.2±1.225^{c}	1.88±1.013^{e}	1.92±1.038^{d}	2.28±1.275^{e}	1±0^{d}

The sensory analysis was conducted firstly to find the best formulation of study. According to table 3., the results showed that in every sample that contains 0.8 grams of encapsulated cannabis achived the least score. Ranking in terms of color are oolong (4.08±0.909^{b},) green tea (2.88±1.394^{d}), jasmine (2.52±1.327^{ab}), the reasons commented by panelist for the low score was that the cloudy caused by undissolved powder affects tea appearances, because tea should have clear and golden hue. In terms of aroma Jasmine (5.24±1.155^{b}), oolong (4.72±1.173^{b}), green tea (2.2±1.225^{c}). Panesists commented that cannabis oil totally overcomes every tea aroma except

jasmine tea, which results in poor score for every samples. For tea flavor attribute,Oolong (3.56 ± 1.294^{c}), jasmine (2.6 ± 1.225^{ab}), green tea (1.88 ± 1.013^{e}) 0.8 grams of encapsulated cannabis oil completely mask the tea flavor, also, it was too bitter to consume in any way. For cannabis aroma in the sample, oolong (3 ± 1.291^{c}), jasmine (2.08 ± 1.038^{b}), green tea (1.92 ± 1.038^{d}). Many said that the aroma resembles pepper which was unpleasant to smell or taste. The taste of cannabis in 0.8 grams was totally bitter, by ranking, oolong (2.48 ± 1.159^{d}), green (2.28 ± 1.275^{e}), jasmine (1.92 ± 0.997^{b}). Overall liking score ranking are, Oolong (2.48 ± 1.159^{d}), jasmine (1.32 ± 0.476^{b}), green tea (1 ± 0^{d}) of this section was the lowest in all attributes and in all samples. Many panelists rejected this sample due to the appearance and aroma alone due to the unattractive look and aroma.

At 0.4 grams of encapsulated cannabis oil added, in terms of color, jasmine tea shows the best appearance (6.96 ± 1.098^{a}), follow by Oolong (6.44 ± 0.914^{ab}) and green tea (6.52 ± 1.358^{c}). Because at this concentration, all of the powdered cannabis oil completely dissolved into the solution, there are no differences in control and formulated sample. However in terms of tea aroma, green tea showed the highest score (6.32 ± 1.249^{b}), followed by Oolong (5.16 ± 1.179^{b}) and jasmine tea (5.24 ± 1.508^{a}). Most of panelists does not like the scent of jasmine tea due to personal preferences. At 0.4 grams of encapsulated oil added, cannabis aroma was mild and does not affect tea flavor as much. But the highest score given was green tea (6.56 ± 1.158^{d}), followed by jasmine (6.08 ± 1.579^{a}) and Oolong (5.64 ± 1.254^{ab}) at last. In terms of cannabis aroma, since the flavor at this concentration was mild, the mean score was relatively low. Again, the highest mean score was green tea (5.44 ± 1.387^{c}), follow by jasmine (5.04 ± 1.619^{a}) and oolong tea (3.68 ± 1.145^{b}). Overall mean score in this attribute was relitavely high. By ranking, green tea(5.84 ± 1.248^{c}), Oolong(5.4 ± 1.190^{b}) and jasmine(5.28 ± 1.173^{a}) at the last place. Since most of panelists did not enjoy the aroma of jasmine tea, which effected to the score achived was relatively low.

Lastly at 0.6 grams of encapsulated cannabis oil added showed highest score in almost every attributes. First of all, The highest mean score in color attribute is Oolong (6.8 ± 1.190^{ab}), Jasmine (6.4 ± 1.041^{a}), then Green tea (6.04 ± 1.338^{d}). At this concentration, the color of oolong and jasmine tea were aided by the powdered cannabis oil. Most panelists said the golden hue becomes more beautiful. The following category which is tea aroma, Oolong (6.28 ± 1.242^{b}), Jasmine (5.32 ± 1.406^{a}), Green tea (5.16 ± 1.106^{c}) panelist commented that encapsulated cannabis aroma aids tea aroma which created new complex aroma that is desirable in this category. Moreover, the panelists commented that jasmine scent becomes more acceptable than before adding encapsulated cannabis powder. The highest mean score in tea flavor attribute is green tea (6.56 ± 1.158^{d}), oolong (6.2 ± 1.155^{bc}), and jasmine (5 ± 1.607^{ab}). Panelists commented that the cannabis oil flavor, which is bitter with herbal flavor, help aids the bitterness in tea which many panelists commented that each tea should be bitter and concentrated. For cannabis aroma attribute ranking, Oolong (6.52 ± 1.045^{b}), green tea (5.8 ± 1.5^{d}), and Jasmine tea (5.6 ± 1.779^{ab}) are ranked as follow.The most important attribute in this sensory analysis which is cannabis aroma, panelists commented that cannabis oil aroma helps not only created great complicated aroma but also helps increasing herbal aroma in infused tea which is desired for this infused tea to become one. For bitterness attribute ranking, oolong (7 ± 1.041^{c}), green tea (6.6 ± 1.354^{e}), Jasmine (5.24 ± 1.363^{ab}). Many panelists said that in oolong tea sample, The bitterness or the concentration was mild, by adding encapsulated cannabis oil helps boosting bitterness and make oolong taste even better, thus, the reason of high score. And finally, overall mean score ranking are Oolong, green tea, and jasmine.

According to the sensory analysis results, the best out of 9 samples is oolong tea with 0.6 grams of encapsulated cannabis oil in powdered form with the overall score of 7.12 ± 0.726^{b}. The reason described by panelist is that, the cannabis aroma and its bitterness aids the flavor of oolong tea well. From panelists perspective, oolong tea must be bitter with a touch of astringency. Moreovoer, the aroma of cannabis oil which is herbal aroma aids the aroma of oolong tea to become more complex which is desired by panelists.

5.5 DPPH phenolic analysis

Table 2: The average of phenolic compounds

Sample	Average (mg/ml)	Standard deviation
Tea	3.731	0.277
Tea + Cannabis	5.223	0.195
Difference	1.492	-

In this DPPH phenolic analysis, the best formula out of 9, or oolong tea with 0.6 grams of encapsulated cannabis oil was used to analyzed for this analysis. According to table 1, the average amount of phenolic compound in original Oolong tea was at 3.731mg/ml which was relatively high.Since oolong tea was left to dry instead of heating to stop the enzymesactivites. Moreover, Oolong tea is famous for having high amounts of polyphenols such as EGCG, epigallocatechin, epicatechin gallate, epicatechin, and flavonols such as kaempferol, quercetin, and cyricitin(Chacko and S. M., 2010). After the addition of encapsulated cannabis oil, the average amount of the sample increased to 5.223 grams, which increased significantly due to the high amount of CBD in the cannabis plant as well.

5.6Folin-ceocalteuAntioxidant Assay

Table 3: The result of antioxidant activity assay

Sample	Antioxidant activity Percentage
Tea	73.068
Tea + Cannabis	87.439
Difference	14.371

In this antioxidant assay analysis, the best formula out of 9, or oolong tea with 0.6 grams of encapsulated cannabis oil was used to analyzed for this analysis. According to table 2., the results of DPPH antioxidant assay showed that after the encapsulated cannabis oil was added, there was a significant variation of antioxidant activity inside the sample. In Oolong tea sample alonewas used as control,the result shows that the antioxidant activity was at73.068%, which was high in itself due tooolong tea contained a relatively high amount of antioxidants.The examples of antioxidant compounds contained in Oolong tea are Thearubigin, epicatechins, and catechins (Pollastro F. and Minassi A. 2018).These three compounds can be found in high amounts in the tea in general. Once encapsulated cannabis oil was added, the antioxidant activity rose significantly to 87.371%. This is because in cannabis, there are 26 flavonoids such as vitexin, isovitexin, apigenin, luteolin, kaempferol, orientin,quercetin and etc (Atakan, 2012). The increasing of antioxidant was measured by the scavenging effect, which oxidize radicals in the mixture. This decolorizes and clarifies the mixture. The antioxidant donates a hydrogen atom to the ferric complex in the DPPH mixture. With the spectrophotometer, the final refraction could be measured and the percentage would be calculated. Which in this case, highly increases in percentage.

CONCLUSION

In conclusion, the cannabis oil is successfully encapsulated by using paste method with beta-cyclodextrin, thus the methods of preparation is successfully created. Moreover, in term of formulation the best formula of encapsulated cannabis oil in Oolong tea was at 0.6 grams of encapsulated cannabis oil in 2.5 grams of Oolong tea for 200ml of water brewing the second place was at 0.4 grams of encapsulated cannabis added, and lastly, 0.8 grams of cannabis added into 2.5 grams of sample. From chemical analysis, the phenolic and antioxidants, whichwere essential and healthy to the human body, was increased significantly as well. Not only become better in taste and aroma but also increases in medicinal and becoming healthier as well.

6.1 Suggesstions for further study

This study has found that at 0.6 grams of encapsulated cannabis oil added to oolong tea creates the best formula for infused tea which verified by sensory analysis and commented by panelist. Moreover, the benefits and chemical analysis has been tested for possible heath benefit. To suggest further study for this formulation, isolating only desirable active compounds from cannabis plant to prevent any side effects from undesirable compound and to prevent any illegal substances present in the extract. Moreover, after the extraction of cannabis active compound, the extraction could be furtheranalyze to find the ingredients inside the extract which would advanced this study. Lastly, sensory analysis and effect analysis should be conduct with real patients in order to verify the effectiveness for the medicinal purposes.

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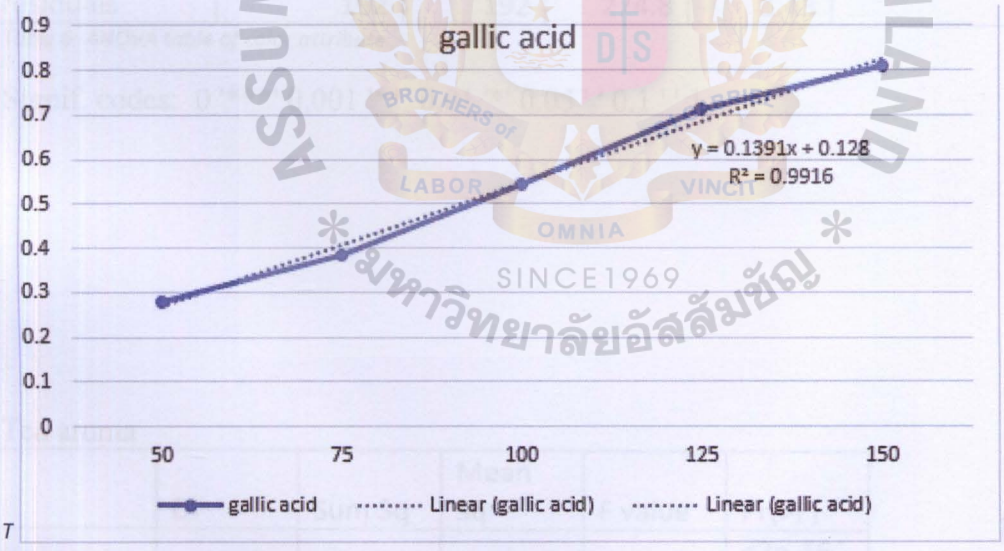
APPENDIX

6.1 Determination of total phenolic compounds

Table 6.1.1: Absorbance of standard gallic acid

Std Gallic Acid (mg/ml)	Absolute amount (mg)	OD 765			Average
		1	2	3	
50	10	0.689	0.763	0.787	0.746
75	15	0.836	0.731	0.733	0.767
100	20	0.687	0.845	0.834	0.789
125	25	0.755	0.725	0.814	0.765
150	30	0.727	0.842	0.857	0.809

Figure 6.1.1: Standard curve of gallic acid



T

Sample	OD 765			Average
	1	2	3	
Tea	0.504	0.459	0.478	0.480
Tea + Cannabis	0.474	0.466	0.524	0.488

6.2 Determination of Antioxidant Activity

Sample	OD 517			Average	%AA
	1	2	3		
Control	0.835	0.795	0.854	0.828	-
Tea	0.221	0.205	0.242	0.223	71.860
Tea +cannabis	0.261	0.282	0.253	0.265	67.995

Table 5: Absorbance of sample in determination of antioxidant activity

$$\%\text{Inhibition of DPPH radical } \left(\frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance}\right) \times 100$$

6.3 Sensory Analysis

ANOVATable

Color

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
trt	8	612.8	76.6	53.523	<2e-16 ***
rep	24	30.6	1.27	0.891	0.614
residuals	192	192	274.8	1.43	

Table 6: ANOVA table of color attribute

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Tea aroma

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
trt	8	443.3	36.287	36.287	<2e-16 ***
rep	24	47.1	1.285	1.285	0.178
residuals	192	293.2	1.53	1.53	

Table 7: ANOVA table of tea aroma attributes

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Tea Flavor

	Df	Sum sq	Mean Sq	F value	Pr(>F)
trt	2	16.64	8.32	7.983	0.00102 **
rep	24	27.41	1.142	1.096	0.38286
residuals	48	50.03	1.042		

Table 8: ANOVA table of tea flavor attribute

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Cannabis aroma

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
trt	8	585	73.12	41.288	<2e-16 ***
rep	24	47.6	1.98	1.121	0.324
residuals	192	340	1.77		

Table 9: ANOVA table of cannabis aroma attribute

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Bitterness

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
trt	8	756.9	94.62	59.125	<2e-16 ***
rep	24	39.6	1.65	1.031	0.429
residuals	192	307.3	1.6		

Table 10: ANOVA table of bitterness attribute

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Overall

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
trt	8	1197.2	149.65	154.68	<2e-16 ***
rep	24	25	1.04	1.079	0.371
residuals	192	185.8	0.97		

Table 11: ANOVA table of Overall attribute

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Duncan Statistics

Treatment	Color	Tea aroma	Tea flavor	Cannabis aroma	Bitterness	Overall
Jasmine 0.4	6.96±1.098^{a}	5.24±1.508^{a}	6.08±1.579^{a}	5.04±1.619^{a}	5.28±1.4^{a}	5.28±1.173^{a}
Jasmine 0.6	6.4±1.041^{a}	5.32±1.406^{a}	5±1.607^{ab}	5.6±1.779^{ab}	5.24±1.363^{ab}	5.52±1.327^{a}
Jasmine 0.8	2.52±1.327^{ab}	5.24±1.155^{b}	2.6±1.225^{ab}	2.08±1.038^{b}	1.92±0.997^{b}	1.32±0.476^{b}
Oolong 0.4	6.44±0.914^{ab}	5.16±1.179^{b}	5.64±1.254^{ab}	3.68±1.145^{b}	6.2±1.414^{c}	5.4±1.190^{b}
Oolong 0.6	6.8±1.190^{ab}	6.28±1.242^{b}	6.2±1.155^{bc}	6.52±1.045^{b}	7±1.041^{c}	7.12±0.726^{b}
Oolong 0.8	4.08±0.909^{b}	4.72±1.173^{b}	3.56±1.294^{c}	3±1.291^{c}	2.48±1.159^{d}	1.68±0.690^{b}
Green 0.4	6.52±1.358^{c}	6.32±1.249^{b}	6.56±1.158^{d}	5.44±1.387^{c}	4.44±1.325^{e}	5.84±1.248^{c}
Green 0.6	6.04±1.338^{d}	5.16±1.106^{c}	5.96±1.513^{e}	5.8±1.5^{d}	6.6±1.354^{e}	7.04±1.207^{cd}
Green 0.8	2.88±1.394^{d}	2.2±1.225^{c}	1.88±1.013^{e}	1.92±1.038^{d}	2.28±1.275^{e}	1±0^{d}

Appendix A-1: Example of Sensory Analysis questionnaires for Tea sample choosing

Questionnaire

This survey is a part of final project or thesis of Faculty of Biotechnology. Assumption University. This survey also would like to study about consumer's behavior, acceptance, and opinion towards the new development of encapsulated cannabis oil in oolong tea. Please check or cross in the provided box below in order to answer the question.

Part 1

Do you like to drink tea?

☐Like ☐dislike ☐neither like nor dislike

How often do you drink tea?

☐Daily
☐1-3 times a week
☐Less than once a week
☐Once per month or less

Where do you normally drink tea?

☐Home
☐Tea shop
☐Restaurant
☐Workplace

Which type do you prefer?

☐Teabag
☐Loose leaves
☐Powder tea
☐Dried powdered tea

Which tea aroma do you prefer?

☐Oolong
☐Green tea
☐Apple tea
☐Jasmine tea
☐other: _____

What is your favorite brand of tea?

☐TWG ☐Twining

☐Lipton ☐Fuji

How much do you spend for tea per session?

☐50-100 ☐100-150

☐150-200 ☐200-250

☐More than 250

Part 2 Sensory test

9-point hedonic scale

This product is Encapsulated cannabis oil Oolong Tea, in order to create new types of food trend in current market. This product is packed with phenolic compound and anti-oxidant with many medicinal effect from cannabis plant and Tea itself.

How would you rate this product by using 9 point Hedonic scale when:

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 1 = Dislike extremely

Sample No. _____

attribute	**Liking score**
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Sample No. _____

attribute	**Liking score**
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Sample No. _____

attribute	Liking score
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Preferred Sample: _____

Reason

_____

_____

Do you accept this product?

☐Yes ☐No

Is the tea aroma present Tea acceptable?

☐Yes ☐No

Part 3 Demographic

Gender

☐Male ☐female

Age

☐Under 15 years old ☐16-20 years old

☐21-30 years old ☐31-40 years old

☐41-50 years old ☐51-60 years old

☐More than 60 years old

Occupation

☐Student ☐freelance

☐Businessman ☐Housewife

☐Teacher ☐Others

Nationality

☐Asian ☐European

☐American ☐African

☐Others

Appendix A-2: Example of Sensory Analysis questionnaires for Encapsulated cannabis oil in Oolong tea formation

Sensory Analysis of encapsulated cannabis oil

Questionnaire

This survey is a part of final project or thesis of Faculty of Biotechnology. Assumption University. This survey also would like to study about consumer's behavior, acceptance, and opinion towards the new development of encapsulated cannabis oil in oolong tea. Please check or cross in the provided box below in order to answer the question.

Part 1

Do you like to drink tea?

☐Like ☐dislike ☐neither like nor dislike

How often do you drink tea?
☐Daily
☐1-3 times a week
☐Less than once a week
☐Once per month or less

Where do you normally drink tea?
☐Home
☐Tea shop
☐Restaurant
☐Workplace

Which type do you prefer?
☐Teabag
☐Loose leaves
☐Powder tea
☐Dried powdered tea

Which tea aroma do you prefer?

☐Oolong
☐Green tea
☐Apple tea
☐Jasmine tea
☐other: _____

What is your favorite brand of tea?

☐TWG ☐Twining

☐Lipton ☐Fuji

How much do you spend for tea per session?

☐50-100 ☐100-150

☐150-200 ☐200-250

☐More than 250

Part 2 Sensory test

9-point hedonic scale

This product is Encapsulated cannabis oil Oolong Tea, in order to create new types of food trend in current market. This product is packed with phenolic compound and anti-oxidant with many medicinal effect from cannabis plant and Tea itself.

How would you rate this product by using 9 point Hedonic scale when:

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
1 = Dislike extremely

Sample No. _____

attribute	**Liking score**
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Comments:

_____
_____
_____
_____
_____
_____
_____

Sample No. _____

attribute	**Liking score**
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Comments:

_____
_____
_____
_____
_____
_____
_____
_____

Sample No. _____

attribute	**Liking score**
color	
Tea aroma	
aroma	
bitterness	
After taste	
Tea flavor	
overall	

Comments:

_____
_____
_____
_____
_____
_____
_____

Preferred Sample: _____

Reason

Do you accept this product?

☐Yes ☐No

Is the tea aroma present Tea acceptable?

☐Yes ☐No

Part 3 Demographic

Gender

☐Male ☐female

Age

☐Under 15 years old ☐16-20 years old

☐21-30 years old ☐31-40 years old

☐41-50 years old ☐51-60 years old

☐More than 60 years old

Occupation

☐Student ☐freelance

☐Businessman ☐Housewife

☐Teacher ☐Others

Nationality

☐Asian ☐European

☐American ☐African

☐Others

Monthly Income

☐Less than 10,000 Baht ☐10,000 – 20,000 Baht (บาท)

☐20,000 – 30,000 Baht ☐30,000 – 40,000 Baht (บาท)

☐40,000 – 50,000 Baht ☐More than 50,000 Baht (บาท)

Appendix A-3: Example of Sensory Analysis questionnaires for Encapsulated cannabis oil in Oolong tea formation

Acceptance Sensory analysis Of formulated Encapsulated cannabis oil in Oolong tea.

Acceptance test

Sample No. 405

Do you accept this product?

☐Yes ☐ No