III. RESEARCH METHODOLOGY

A. MATERIALS AND INSTRUMENTS

1. Materials

Dried green tea (var. Oolong No 12) was supplied by Boonrod Tea Factory (Thailand). Chemical reagents with analytical grade such as folin-ciocalteu (10% v/v) and gallic acid were supplied by Fluka (Buchs, Switzerland), anhydrous sodium carbonate and potassium hexacyanoferrate \([\text{K}_3\text{Fe(CN)}_6]\) were purchased from Merck (Darmstadt, Germany), standard HPLC of caffeine and catechins were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), acetonitrile, trifluoroacetic acid (TFA) and methanol (HPLC-grade) were purchased from Fluka (Buchs, Switzerland), trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Aldrich (Steinheim, Germany). monosodium phosphate monohydrate, Disodium phosphate heptahydrate and trichloroacetic acid (TFA) were purchased from Fluka (Buchs, Switzerland), citric acid (food grade), potassium mitrute salt (food grade). Then, distilled water, filter paper No 1 and No 4.

2. Instruments

The main instruments were JMC-miniLAB spray dryer (Euro Best Technology, ltd, Thailand), ice cream maker and centrifuge (March Cool Industry Co.ltd, Thailand), hydrolic press (Owner Food Machinery Co.ltd, Thailand), hand refractometer (1-32˚Brix ATAGO Model N-2E, Japan), color analyzer (Colorquest XE HunterLab, Hunter Associates Laboratory, Inc, Virginia-USA), a spectrophotometer (UV Vis. Biochrom/Libra S22, England), HPLC C18, oven, disc mill, analytical balance, pH meter, vacuum pump.

B. EXPERIMENTAL DESIGN

This research was divided into two parts. The preliminary research were investigation on the chemical properties of raw material (dried green tea leaves) involved moisture content, total polyphenol content, antioxidant activity, catechins, and caffeine. In experiment I (Figure 6), production of concentrated green tea was made from extract green tea and increased its concentration with ice cream maker, and then determine its chemical properties. In experiment II (Figure 7), production of green tea powder was made from concentrated green tea which dried with spray dryer, and then determine its physical and chemical properties.

1. Production of Concentrated Green Tea

Dried green tea was milled with a disc mill into the small size of green tea. Milled green tea was extracted dissolved in the temperature of the hot water: 90°C and with regarding water: 1: 20 (w / v). The time of extraction used was 60 minutes, and pH value was 5.0 (Butsoongnern, 2006). Tea that has been extracted was filtered by clothes sheet and pressed by press machine to obtain the pure extract of green tea. Green tea extracts were analyzed TDS (total dissolved solids) with Refractometer and oven method. After that, green tea extract was concentrated with Freeze concentration method and it used ice maker...
machine. The way of that machine working is turn on the power, paddle, and compressor buttons. After 10 minutes, compressor was turned off and wait the ice was released from the wall. Repeat this step for several times and it will resulting in a slurry of ice crystals in a fluid concentrate. The ice crystals were then removed in some way, in this study it used centrifuge machine for separate the ice crystals and a concentrated product. This step was continued until concentrated tea contain of total solid about 3, 6, and 9%, which was measured with refractometer and confirmed with oven method.

2. Production of Green Tea Powder

Production of green tea powder with spray drying method is shown at Figure 7.
Green tea extract that was concentrated by ice maker until its concentration reached 3, 6 and 9%, were dried with spray drier. The operational conditions of the spray drying were as follows: inlet air temperatures were 180, 200, 220°C, outlet air temperatures is controlled about 75°C, blower speed was adjust in 2500 rpm. To control outlet temperature at 75°C, the pressure air and feed rate were increased or decreased. The pressure air and feed rate were affected by inlet air temperature, an increase inlet air temperature, the presssure air and feed rate increased. After spray drying process has done, characteristic of final product should be analyzed. The physical and chemical characteristics of final products were evaluated.

3. Method of Analysis

3.1. Bulk density (Bhandari et al., 1992)

Bulk density was determined by the tapping method. Two grams of powder were loosely weighed into 10 mL graduate cylinder. The cylinder containing the powder was tapped on a flat surface to a constant volume. The final volume was recorded and bulk density was calculated by dividing the sample weight by the volume.

3.2. Color Analysis (Quek et al., 2007)

Color values of dried samples (L, a, and b) were measured by using ColorQuestXE/Hunter Lab (USA).

3.3. Solubility (%) (Sanphakdee, 2007)

Weigh powder sample 0.5 g and mixed with 50 mL of distilled water (25°C) in an 100mL beaker glass. Then it was agitated using a magnetic stirer (size 2 mm X 7mm) at a speed of 600rpm. The residue was filtrated on a filter paper No 4 and using the vacuum pump. The filter paper with an insoluble solid was placed in an oven set at 102 ± 2°C until the weight was constant. The solubility(%) was calculated by using the following equation:

\[
\text{Solubility(\%)} = \left(1 - \frac{m_1 - m_2}{m}\right) \times 100
\]

Where \(m_1\) is weight of filter paper and insoluble solid after dried by oven, \(m_2\) is weight of dried filter paper, and \(m\) is weight of powder sample.

3.4. Hygroscopicity (%) (Jaya and Das, 2004, modification)

A saturated solution of potassium nitrite salt (equilibrium relative humidity = 79.5±2% at 20°C) was kept in glass wash bottle having two passage for air inlet and outlet. A diaphragm type vacuum pump was used to suck the air through the salt solution. Take filter paper in pump and weight it until constant, then add powder sample 0.5 g and it was spread uniformly in the filter paper. The increase in weight of the sample at every 15 min was noted. This measurement was continued till the difference between two successive weighings not exceed by 0.5%. The entire operation was carried out in a room maintained at 20°C.
The hygroscopicity, HG (%) was calculated by using equation as following.

\[
\text{Hygroscopicity} = \frac{b + \frac{Wi}{100}}{1 + \frac{b}{a}}
\]

Where \( b \) (g) is the increase in weight of powder, \( a \) (g) weight of powder sample, and \( Wi \) (% wb) is moisture content of powder.

### 3.5. Moisture content (AOAC, 2000)

Moisture content of the sample was determined according to the oven method. The moisture can was cleaned and dried in hot air oven for 12 hr, then cooled in desiccators and the weight which was measured by digital balance was recorded. The sample was weighed and placed into the moisture can then dried in a hot air oven at 105°C overnight until the weight was constant. The can containing sample was cooled in a desiccators. The weight of the can and sample was determined by using digital balance. The weight of dried sample was also calculated to determine its moisture content.

\[
\text{% M.C. (wet basis)} = \frac{\text{sample weight (g)} - \text{dried sample weight (g)}}{\text{sample weight}} \times 100
\]

\[
\text{% M.C. (dry basis)} = \frac{\text{sample weight (g)} - \text{dried sample weight (g)}}{\text{dry weight}} \times 100
\]

### 3.6. Total polyphenol content

The total polyphenol content was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 mL of the diluted sample extract (50-100 fold dilution) was transferred in duplicate to separated tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu’s reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperatures for 60 min before absorbance at 765nm was measured against water. The total polyphenol was expressed as gallic acid equivalents (GAE) in g/100g material. The concentration of
polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 100 µg/mL.

\[
\text{Total polyphenols content (g/100g db) } = \frac{C \times V \times DF \times 100}{1000000 \times W \times \%DM}
\]

- \(C\) = gallic acid concentration (µg/mL) obtained from calibration curve
- \(V\) = Volume of tea extract solution (mL)
- \(DF\) = Dilution factor
- \(\%DM\) = % dry matter
- \(W\) = Weight of tea sample (g)

### 3.7. Antioxidant Activity using DPPH

Antioxidant activity (DPPH free radical scavenging activity) was determined with DPPH scavenging activity and slightly modification according method by Talcott et al 2003. Pipette extracted sample 50 µL through test tubes, then add with 1950 µL of 1,1-diphenyl-2-picrylhydrazil (DPPH) methanolic solution. The mixture is thoroughly vortex-mixed and kept in dark for 30 min. The absorbance is measured later, at 517 nm. A calibration curve was prepared using a standard solution of Trolox (0, 200, 400, 600, 800, 1000µM) and the results were expressed on both fresh weight basis (fw) and dry weight basis (dw) as mmol Trolox Equivalent/100g.

### 3.8. Caffeine and Catechins Analysis

#### Preparation of sample

Add to the instant tea (0.500±0.001) g in the flask approximately 25 mL of hot water (max 50ºC). The sample was mixed in room temperature. After that, add 5.0 mL acetonitrile and it was mixed again.

#### Preparation of Standards

Use the % purity from the certificate to prepare the stock standard solution. The individual standard solution of GC, EGC, C, EC, EGCG, CF, GCG, ECG, CG were prepared by dissolving them in a small volume of metanol, to generate a stock concentration of 999.0, 313.6, 412.0, 880.0, 911.8, 1036, 1000, 469, 832.0 and 514.8 µg/mL respectively. The mixed stock standard solution was prepared by mixing an equal volume of each stock standard. Working standard solutions were prepared by dilution of the mixed stock solution and then filtered through a 0.45µm PTFE filter before HPLC analysis.

#### HPLC analysis

HPLC analysis of standards and samples was conducted on Water 966 high performance liquid chromatography comprising vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, and photo diode array detector. The column used was a Platinum EPS C18 reversed phase, 3µm (L 53 x i.d 7mm). Mobile phase eventually adopted for this study was water/acetonitrile (87:13) containing 0.05% (v/v) trifluoroacetic acid (TFA) with the flow rate of 2 mL/min. Absorption wavelength was
selected at 210 nm. The column was operated at 30°C. The sample injection was 20 µL. Peaks were identified by comparing their retention times and UV spectra in the 190-400 nm range with standards. The standard was injected before sample for made calibration curves. The caffeine and catechins content were calculated using their respective calibration curves.

3.9. Product Recovery (%) (Sanphakdee, 2007)

Product recovery is mainly determined by powder collection efficiency. Material loss in a spray drying system is due mostly to the attachment of sprayed droplets and dry powder to the wall of the apparatus and the cyclone’s poor efficiency in collecting fine particle. Product recovery was calculated from the total solid content that determined form moisture content that heat in the oven at 103±2°C for 6 hours.

\[
\text{Product Recovery} = \frac{a}{b \times \left(\frac{c}{100}\right)} \times 100\%
\]

Where a (g) is weight of powder product (dry basis), b (mL) is volume of feed, and c (total solid content of tea extract concentrated).

3.10. Energy Consumption (Kamaruddin et al, 1989)

Energy analysis is used to calculate the amount of energy at each stage in the production system. Analysis of this energy can be used to understand and improve how, where and when energy that used efficiently and effectively. This analysis can be used for identify networks and processes to obtain the final product. Energy consumption especially at spray drying stage was calculated by using the equation as following

\[
\text{Energy} = P \times t
\]

Where P (kilowatt) is power of spray dryer and t (hour)

3.11. Statistical Analysis

Data were analyzed by one way ANOVA using the application of SPSS software. Mean value were compared using The Duncan’s Multiple Range Test.