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PHYSICOCHEMICAL PROPERTIES, BIOACTIVE COMPOUNDS OF DIFFERENT TYPES OF LOTUS TEA IN THUA THIEN HUE PROVINCE OF VIET NAM

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ABSTRACT

Tea is a traditional beverage. There are different types of tea, such as black, white, green, oolong, and herbal teas. In recent years, lotus tea has been increased interest. This study aimed to assess the physicochemical properties, bioactive compounds of different types of lotus tea in Thua Thien Hue, province, Viet Nam. The physicochemical properties, bioactive compounds of four kinds of lotus teas were assessed in the laboratory by measuring their pH, ^{0}Brix, sugar reducing, extract water, ash total, chlorophyll content, and some bioactive compounds consist of total tannin, polyphenol, caffeine content. The results indicated that, the physicochemical properties in lotus tea: pH: 6.17-6.25, ^{0}Brix: 1.42-4.50, total ash content: 2.87-4.95 (%dry matter), water extract content: 22.94-33.94 (%dry matter), sugar reducing content: 7.60-15.53 g/100 g, chlorophyll content in lotus teas produced from different parts of the lotus plant is different and assess the sensory color of tea. The biochemical compounds including total polyphenols: 7.46-12.97 (% dry matter), total tannin: 11.88-14.79 (% dry matter), and total caffeine: 0.261-0.354 (% dry matter). Our results indicate the high biochemical diversity of lotus tea in Thua Thien Hue province, Viet Nam. That contributes to affirming the positive role of lotus tea in human health. In conclusion, the quality of lotus tea produced in Thua Thien Hue province, Viet Nam has comparable quality characteristics with other tea-producing in the world and conformed to the international standard for other teas.

Keywords: *Biochemical compounds, Lotus tea, physicochemical properties, Thua Thien Hue province, Viet Nam.*

INTRODUCTION

Tea is one of the most popular beverages in the world, and it is the most consumed beverage next to the water. There are different types of tea, such as black, white, green, oolong, and herbal teas [1]. The major tea-producing countries are China, India, Sri Lanka, Kenya, and Japan, and is also grown in Indonesia, Vietnam, Argentina, Georgia, and other countries [2]. Much research showed that tea has many benefits for healthy for humans. They can protect against certain cancers, cardiovascular diseases, and neurodegenerative diseases [3] Besides, tea also was found to have bioactivities including antioxidant, improving immune response, anti-atherosclerosis, antihypertension, anti-infectious diseases, and anti-diabetic properties in recent studies. The chemical compositions of the tea in different forms were different and induced the change of the bioactivities [4] Tea has a very diverse chemical composition consists of polysaccharides, polyphenol, caffeine, tannin, amino acid, carbohydrate, protein, chlorophyll, minerals and trace element**,** volatile compounds, amino and organic acids, lignins, and some other compounds [4].

Lotus (*Nelumbo nucifera* Gaertn.), an ornamental aquatic plant found in Australia, China, India, and Japan, and many other countries in the world [5,6]. *N. Nucifera* is an important economic plant, not only as a dainty and ornamental flower but also as a source of herbal medicine with strong antipyretic, cooling, astringent, and demulcent properties. Virtually all parts of the lotus plant are used: Rhizome, seed, leaf, flower, seedpod, embryo [7].

In Viet Nam, Lotus is widely grown for nutritional purposes. The lotus is considered to be a symbol of purity, beauty, and good fortune. Besides, some parts of the lotus plant may be used as a tea ingredient. Lotus tea is a purely special tea of Vietnamese, and it is the best-liked and the most expensive in Vietnam. Lotus tea is recognized to be one of the most ancient teas of Vietnam which are produced by following the old and traditional processes. Vietnamese lotus tea confers a sweet and lovely aroma [8].

But, the research on lotus tea such as its biochemistry and antioxidant activity has not been interested in research and publication. Our research is aimed at clarifying the physicochemical properties and bioactive compounds of lotus tea produced from different parts of *N. Nucifera* consists of the seeds, rhizomes, leaves, and flowers. From there, it provides scientific data for tea processing technology, as well as in the exploitation of biologically active compounds highly in these teas. Knowledge of lotus tea chemical composition also helps in assessing its quality and provides orientations in the selection of lotus plant parts for effective lotus tea processing.

MATERIALS AND METHODS

Materials

Four types of lotus tea are produced from 5 different parts of lotus plants grown in Thua Thien Hue province, Vietnam including dried lotus flower tea, dried lotus leaf tea, dried lotus rhizome tea, lotus embryo tea.

Methods

Preparation of tea samples

Sample extraction

Each ground tea sample (~2 g, weighed to the nearest 0.001g) was extracted with distilled water (100 mL) at 95°C. The extraction mixture was constantly stirred with a magnetic stirrer. After 10 min., the extraction mixture was filtered through Whatman No. 4 filter paper. The residue was washed with distilled water (3x10 mL). The tea solution was cooled to room temperature [9]..

Physicochemical properties

Brix degree and pH

The pH of each tea infusion was determined using an electronic pH meter (Model 701A, Orion Research Inc., Cambridge MA, U.S.A.) at 37°C. Before the measurements, the pH meter was calibrated via test solutions of known pH (pH=4) [1]. ^{0}Brix: The number of dissolved solids was determined by the refractometric method. ATAGO brand RX-7000α model (Japan) abdominal refractometer was used for this purpose. Measurements were made at 20 °C and the results were expressed in 0Brix (AOAC, 1995) [10].

Determination of the Reducing Sugar Content

The reducing sugars content of lotus tea was extracted and estimated by the methods of Bertrand and Fehling standard procedures [11].

Determination of Total Tannin Content

The total tannin content was determined by the Titrimetric method, with minor modifications [12]. About 0.1mL of the tea sample extract was added to 1 L volumetric flask, then 750 mL of distilled water, 25 mL of indigo carmine 0.1% solution are added 0.1N aqueous solution of $KMnO_4$ is used for titration until the blue colored solution changes to green color. Then few drops at a time until the solution becomes golden yellow is added. The blank tests by titration of the mixture of 25 mL indigo carmine solution and 750 mL of distilled water are carried out. All samples were analyzed in duplicates. Each experiment was repeated 3 times to get the average result.

The tannins content (% in dry matter) in the sample is calculated as follows:

X= 100
X: The total tannins content (% in dry matter)
a: the volume of 0.1N aqueous solution of $KMnO_4$ for the titration of the sample
b: the volume of 0.1N aqueous solution of $KMnO_4$ for the blank sample
V- Volume of tea extracted from 2g of the study sample
v- Volume of tea solution taken out for the analysis
m - mass (g) of the sample taken for the analysis
k= 0.00582 (1ml $KmnO_4$ oxidizes 0.00582 g Tannin)

Determination of Total Polyphenol

The total polyphenol (TP) content was determined by spectrophotometry with gallic acid as standard [9]. About 0.2 g of the ground tea sample was put in a 10ml tube and left 1 minute at 70^0C in thermostatic tanks. The tea sample was extracted by vortex-mixing with 5 mL of 70^0C methanol for 10 min at 70^0C. and filtered Then, add 5ml of 70% methanol stabilized at 70^0C for 30 min, shake well by vortex-mixing for 10 min. and the tea solution was cooled to room temperature. The tubes were then placed in a centrifuge at 3500 rpm for 10 min. The supernatant was decanted into a graduated tube and the extraction procedure was repeated. The extracts were combined and made up to 10 ml with 70^0C of methanol. One milliliter of the sample extract was transferred into a graduated tube and diluted to 100 ml with distilled water. To a 1.0 mL sample of the diluted solution, 5.0 mL of Folin-Ciocalteu's reagent (10% v/v) and then 4.0 mL sodium carbonate (7.5% w/v) were added. The mixture was mixed and left to stand at room temperature for 60 min. before the absorbance at 765 nm was measured (UV-1800, Shimadzu - Japan). The concentration of polyphenols in the sample was derived from a standard curve of gallic acid (10-50 µg/mL). The TP was expressed as gallic acid equivalents (GAE) in g/100 g dry weight [9].

Determination of Chlorophylls

About 0.2 g of the ground tea sample was extracted by vortex-mixing with 50 mL of 80% (v/v) acetone for 2 min. and filtered. The extracted solution was analyzed spectrophotometrically at the wavelength of 663 nm (for chlorophyll a) and 645 nm (for chlorophyll b). The contents of chlorophyll a, chlorophyll b, and total chlorophyll were calculated according to the formula [9]:

Chlorophyll a (C_a, mg/g) = $\frac{(12.7A_{663} - 2.95A_{645})\times 50}{W\times 1000}$

Chlorophyll b (C_b, mg/g) = $\frac{(22.9A_{663} - 4.67A_{645})\times 50}{W\times 1000}$

Chlorophyll total (C_{a+b}, mg/g) = $C_a + C_b$

(W = weight (g) of tea sample; A663 and A645 = absorbance at 663 and 645 nm respectively)

Determination of the Total Caffeine Content

The total caffeine content is determined as described in Komes *et al.* (2009), with minor modifications [13]. Tea was prepared using an aqueous extraction. Tea samples (5 g) were poured with 200 ml of boiling water and stirred for 10 minutes. Extracts were filtered through cotton wool, cooled at room temperature, diluted to 500 ml with distilled water, and used for spectrophotometric analyses.

Caffeine determination using the lead acetate solution. This procedure is based on international standards with some modifications (Yao et al. 1992, 1993). Tea extract was treated with HCl solution (10 ml), $Pb(CH_3COO)_2$ (5ml), and H_2SO_4 solution (1.2-1.5 ml). The absorbance of obtained extracts was measured at 274 nm. The content of caffeine (mg/l) was calculated using a standard curve derived from caffeine (0–250 mg/l). All measurements were performed in triplicate.

Determination of Total Ash

The ash contents of lotus tea were determined by burning off the organic matter and to determine the inorganic matter remained [14]. Randomly collect tea lotus sample (≤ 100 g) and pass through a manual mincer twice or chop very finely and mix thoroughly. Place minced tea in a small plastic bag.

The crucible and lid are first placed in the furnace at 550°C overnight to ensure that impurities on the surface of the crucible are burnt off. Cool the crucible in the desiccator (30 mins). Weigh the crucible and lid to 3 decimal places. Weigh about 5g tea sample into the crucible. Heat over low bunsen flame with the lid half covered. When fumes are no longer produced, place crucible and lid in the furnace. Heat at 550°C overnight. During heating, do not cover with the lid. Place the lid on after complete heating to prevent loss of fluffy ash. Cool down in the desiccator. Weigh the ash with the crucible and lid to 3 decimal places. Ash must be white or light grey. If not, return the crucible and lid to the furnace for further ashing.

Ash Content (%) = Wt of ash x 100 /Wt of sample

Determination of Water Extract

The soluble matter extracted from a test portion by boiling water, under the conditions specified in this International Standard, is expressed as a percentage by mass on a dry basis [15].

Data Analysis

All tests were carried out in triplicate and mean values are presented. The chemical compositions were correlated with the color parameters of tea infusions. A linear regressive analysis was carried out using SPSS 20.0 for Windows.

RESULTS AND DISCUSSION

Physicochemical Properties

Brix degree and pH in lotus tea

After preparing the lotus tea extracts, a physicochemical analysis including the Brix and pH were determined (Table 1).

The mean value of the pH in the obtained extracts of four kinds of lotus tea ranged from 6.17-6.75, respectively. The highest values were observed in the dried lotus rhizome tea with 6.75. The results demonstrate that lotus teas are lowly acidic. The baseline acidity is a major factor for the determination of erosive potential [16]. In this study, the pH of lotus teas was found to be near neutral. Brunton and Hussain [17] reported that both conventional black tea and herbal tea eroded dental enamel. They also stated that the pH of conventional black tea and herbal tea had been measured to be lower than the critical pH (5.5) that is necessary for the demineralization of enamel. Van Nieuw *et al*. [18] investigated the erosive potential of several teas with fruit aroma and ice teas. They stated that at their drinking temperature (45°C), teas with a fruit taste typically had a pH value of between 6.2 and 7.4. Fruit teas contain organic acids, such as citric, malic, and oxalic acid [1]. Products with a pH < 5 will make it difficult for the body to absorb and unbalance the content inside, on the other hand, create a favorable environment for lactic acid bacteria to grow and damage the product. The pH value of the product should be alkaline or slightly acidic, which is better for the body. The results showed that all four types of lotus tea studied had a suitable pH value for the human body.

Brix degrees is the amount of sugar contained in an aqueous solution with one degree Brix being 1 gram of sucrose in 100 grams of solution. We can use this metric to figure out exactly how sweet, sweet tea can be [19]. The result in table 1 showed ^{0}Brix in four kinds of lotus teas ranged from 1.42-5.08. There was a significant difference between them. The highest ^{0}Brix was observed in embryo lotus tea with 5.08, next to flower lotus tea (4.50). The lowest result belongs to rhizome lotus tea and leaf lotus.

Water Extract, Total Ash, and Sugar Reducing Content in Lotus Tea

Water extract

The soluble matters such as phenolic compounds, alkaloids, amino acids, and many minor soluble substances extracted from the tea samples which determine the quality of the tea are known as water extract. The amount of water extract of tea depends on the tea and water ratio, the temperature of the tea brew, type, and size of made tea particles [14]. In this study, the water extract of four types of lotus teas ranged from 22.94% to 33.94% (w/w), with a mean of 29.18% of the dry mass (Table 2). The water extract of three of the samples of lotus tea (dried lotus rhizome tea, lotus embryo tea, and dried lotus flower tea) is near with ISO international standard for green tea. In which, dried lotus leaf tea has only exceeded the minimum requirement of ISO standards (32%) implying the presence of adequate extractable substances [14].

Total Ash Content

Ash content of tea is also an important quality parameter. Total ash content in tea correlates with the mineral content of the tea sample as well. This measures the physiological ash, which is derived from the plant tissue itself [14]. The ash content of four types of lotus tea ranged from 2.87-4.95% and is suitable with the recommended ISO 1575 standard for total ash of good quality herbal tea (maximum, 8) and good quality green tea (min, 4, maximum, 8) [20]. This result is similar to the ash content of white tea in the report of Shaha et al., [21] and green tea of Aroyeun [20].

Sugar Reducing Content

Sugar reducing content is a significant difference between the four kinds of this lotus tea. Among them, dried lotus flower tea is the highest result with 15.53 g/100 g, following is dried lotus rhizome tea with 11.73 g/100g, next to dried lotus leaf tea (10.13 g/100 g), and the lowest result is lotus embryo tea with 7.60 g/ 100 g.

The Content of Chlorophylls in Lotus Tea

Table 3 shows the concentration of chlorophyll a, b, and (a+b) in individual tea samples. The values ranged from 0.398 to 0.514 mg, 0.745-0.959 mg, 1.142-1.473 mg per 1 g of tea at chlorophyll a, chlorophyll b and (a+b) total chlorophyll. The highest amount of chlorophyll a, b, and (a+b) was found in leaf lotus tea, followed by the embryo lotus tea, and the flower lotus tea. The lowest amount of chlorophyll was found in the rhizome lotus tea.

An important coloring matter in lotus tea is chlorophyll, which gives a green or yellowish-green hue to the tea infusion [9]. From Table 3, the chlorophyll b content is higher than that of chlorophyll a in all kinds of lotus teas. Chlorophyll a is dark green and chlorophyll b is yellowish-green in color [9]. Harbowy and Balentine, 1999 showed that in tea leaves and non-fermented teas, chlorophyll is a highly important pigment as its amount determines the final color of tea infusion. The fermentation process, on the opposite hand, transforms chlorophylls into pheophorbides and pheophytins, which give rise to the dark color of black tea. Therefore, pheophorbides and pheophytins are the main products of chlorophyll degradation (Cartaxana et al. 2003). Our study aimed to investigate the type different chlorophyll content in lotus teas produced from different parts of the lotus plant. An additional objective was to assess the sensory color of tea [22].

Bioactive Compounds in the Lotus Tea

Biochemical compounds (total tannin, total caffeine, and total polyphenol content) of four types of lotus tea accessions in different parts of the lotus plant are presented in Table 4. There is a highly significant variation in all biochemical compounds was observed among accessions studied.

Total Polyphenol Content

For aqueous extract, the total polyphenol content in four types of lotus tea varies from 7.46-12.97% (Table 4). However, there was no significant difference between lotus embryo tea, dried lotus flower tea, and dried lotus leaf tea. In which, dried rhizome lotus tea has the lowest result. Polyphenolics in general have shown several biological activities such as antioxidant, antimutagenic, and anticarcinogenic as well as free-radical scavenging ability [2]. These results are lower than values detected in white tea by Shaha et al., [21] and in black tea leaf (*Camellia* L. spp.) by Kottawa-Arachchi et al., [2].

Caffeine Content (CF)

In the four lotus tea examples tested, caffeine content ranged from 0.261-0.354%. In reporter by Komes *et al*., (2009) also showed Caffeine content in tea ranged from 0.69% (black tea) to 1.33% (white tea) [13]. The results of the present study showed that the mean values of CF in lotus tea are lower than other kinds of teas such as black tea in reported of Kottawa-Arachchi (2014) [2] and clonal tea by Owuor et al.,1986 [23]. Our results since contain the lotus embryo tea is highest caffeine content, the lowest caffeine content is dried lotus leaf tea.

Caffeine is an important compound in tea brew [2]. Due to the pharmacological properties of CF which has a stimulating effect on the central nervous system, the demand for low-caffeine tea is increasing greatly from 2% of total tea consumption in 1980 to 15% in the early 21st century, especially for aged people, pregnant women, and children [2]. The decaffeination process using artificial techniques not only reduced caffeine but also reduced theaflavins, thearubigins, color, and brightness which are some important and unique characteristics of black tea. This affects consumer acceptance of decaffeinated tea and may reduce its market price. Therefore, the event of tea cultivars possessing low caffeine content through breeding and selection is more acceptable than making decaffeinated tea through artificial means. The lower caffeine-containing cultivars could be used in the engineering of caffeine-free accession and this is being attempted to produce decaffeinated tea accessions by natural means [2]. Our result shows that lotus teas are good for health especially for aged people, pregnant women, and children.

Toatal Tannin Content

The tannin content in four kinds of lotus teas ranges from 11.88 to 14.79% (Table 4). Among them, a dried lotus leaf tea is the lowest, and the highest result is dried lotus embryo tea. There was no significant difference between the remaining three kinds of lotus tea.

Tannins are a mixture of polyphenolic compounds and their derivatives. They prevent the development of oncological processes, lower blood pressure, have antimicrobial, disinfecting, antioxidant effects [24], enhance the accumulation and assimilation of acid ascorbic. White and green teas are the richest in tannins. The oxidation products of tannins - quinones, formed during the processing of tea, oxidize other substances of the tea leaf and form aromatic products involved in the creation of tea aroma [24]. Our results are higher than the tannin content in white tea was described by Shaha et al., 2014 [21] and in Gyrinops Tea was described by Wangiyana et al, [25].

The difference in the content of polyphenols, caffeine, tannin all in four types of lotus teas, is connected with the parts of the lotus plant used as raw materials for tea production.

Table 1. Brix degree and pH in lotus tea

Type of teas	pH	Brix
Dried lotus rhizome tea	6.75^{a}	1.42^{b}
Lotus embryo tea	6.51^{b}	1.32^{b}
Dried lotus flower tea	6.17^{cd}	4.50^{a}
Dried lotus leaf tea	6.25^{cd}	1.58^{b}

Table 2. Some of the biochemistry components in lotus tea in Viet Nam

Type of lotus tea	Water extract (% dry matter)	Total Ash (%dry matter)	Sugar reducing g/100g
Dried lotus rhizome tea	29.71^{b}	4.95^{a}	11.73^{b}
Lotus embryo tea	22.94^{c}	3.86^{ab}	7.60^{d}
Dried lotus flower tea	30.13^{ab}	3.52^{b}	15.53^{a}
Dried lotus leaf tea	33.94^{a}	2.87^{b}	10.13^{c}

Table 3. The content of chlorophylls in lotus tea

Type of lotus tea	Cholorophylla mg/g	Cholorophyllb mg/g	Cholorophyll (a +b) mg/g
Dried lotus rhizome tea	0.398^{bc}	0.745^{bc}	1.142^{bc}
Lotus embryo tea	0.441^{ab}	0.829^{ab}	1.270^{ab}
Dried lotus flower tea	0.420^{b}	0.790^{b}	1.210^{b}
Dried lotus leaf tea	0.514^{a}	0.959^{a}	1.473^{a}

Table 4. Some of bioactive compounds in the lotus tea

Type of lotus tea	Polyphenol (%dry matter)	Tannin (%dry matter)	Caffeine (%dry matter)
Dried lotus rhizome tea	7.46^{d}	12.61^{bc}	0.277^{c}
Lotus embryo tea	9.13^{cd}	14.79^{a}	0.354^{a}
Dried lotus flower tea	12.97^{abc}	12.85^{bc}	0.304^{b}
Dried lotus leaf tea	11.40^{bcd}	11.88^{c}	0.261^{d}

CONCLUSION

As follows from this article, the physicochemical properties, bioactive compounds of four types of lotus tea have varied significantly depending on the parts of the lotus plant used as raw materials for tea production (flower, embryo, leaf, rhizome). The physicochemical properties in lotus tea: pH: 6.17-6.25, ^{0}Brix: 1.42-4.50, total ash content: 2.87-4.95 (% dry matter), water extract content: 22.94-33.94 (% dry matter), sugar reducing content: 7.60-15.53 g/100 g, chlorophyll content in lotus teas produced from different parts of the lotus plant is different and assess the sensory color of tea. The biochemical compounds including total polyphenols: 7.46-12.97 (% dry matter), total tannin: 11.88-12.85 (% dry matter), and total caffeine: 0.261-0.354 (% dry matter). Our results indicate the high biochemical diversity of lotus tea in Viet Nam. That contributes to affirming the positive role of lotus tea in human health. In conclusion, the quality of lotus tea produced in Thua Thien Hue province, Viet Nam has comparable quality characteristics with other tea-producing in the world and conformed to the international standard for other teas.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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