Different Aspects Affecting To Production of Lotus (Nelumbo Nucifera) Tea

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Abstract.
Lotus (Nelumbo nucifera), a common perennial aquatic herb, is extensively cultivated in Vietnam. Lotus leaves are rich in flavonoids, alkaloids, flavonoids and their associated antioxidant activity. Lotus pollens are increasingly being used as natural health supplements. They possess antioxidant properties when brewed in hot water. It is thus highly desirable to expedite the research on how to make the best use of lotus leaves as potential products for the food or pharmaceutical products to improve human health. This present study focused on the effect of blanching, drying and storage of Nelumbo nucifera tea. Results showed that Nelumbo nucifera leaf should be blanched at 95°C in 4 seconds with the presence of 3% CaCl₂; drying at 40°C in 2.0 cm of size. This herbal tea could be preserved at 4°C in PET/AL/PE vacum bag within 6 weeks without any deterioration. Therefore, lotus pollen tea would be a good value-added product that can bring in more steady income to lotus growers in Vietnam.

Keywords: Lotus, blanching, drying, storage, herbal, vaccum

I. INTRODUCTION
Lotus (Nelumbo nucifera) is an angiosperm and classified in floating leaved plants among aquatic plants. Lotus is a perennial, large and rhizomatous aquatic herb with slender, elongated, branched, creeping stem consisting of nodal roots; leaves are membranous, peltate (60-90 cm and above), orbicular and concave to cupshaped; petioles are long, rough with small distinct prickles; flowers are white to rosy, sweet-scented, solitary, hermaphrodite, 10-25 cm diameter ripe carpels are 12 mm long, ovoid and glabrous; fruits are ovoid having nut like achenes; seeds are black, hard and ovoid. Lotus plants propagated vegetatively through rhizomes.

It has been reported that the quercetin extracted from lotus leaves may be a potential antibacterial agent for periodontitis (Li and Xu, 2008; Xiaotian Chen et al., 2014). The flavonoids, alkaloids and volatile oils from lotus leaves have been reported have strong inhibition on bacteria but no distinct inhibition on yeast and mold (Ming-Zhi Zhu et al., 2015). Lotus leaves extracted was reported to possess antioxidative quality (Anesini et al., 2008). Two anti-HIV principles have been isolated from the ethanolic extract of the lotus leaves (Kashiwada, Y., et al., 2005). Extracts of the lotus leaf have been used to treat obesity, and have had reported to possess anti-obesity and anti-hyperlipidemia effects on rodents (Ono, Y., et al., 2006). Lotus leaves are used in traditional medicine to treat hypertension, diarrhea, fever, weakness, infection, skin inflammation, and body heat imbalance (Yu and Hu, 1997; Sridhar, K.R. and Rajeev, B., 2007). The rhizome extract has anti-diabetic due to presence of asteroidal, triterpenoid (Mukherjee et al., 1997). Additionally, leaf extracts were found to modulate lipolysis-activity and decreased adipogenesis in human pre-adipocytes as well as to lower elevated cholesterol levels in mice and reducing levels of phospholipids and triglycerides (Sieglner, R. et al., 2010). They are also an effective treatment against abnormal bleeding such as hematemesis, epistaxis, hemoptysis, hematuria, and metrorrhagia (Moro, C.F. et al., 2013). The leaf of Nelumbo nucifera has potent hypoglycemic and hypolipidemic properties (Dipa Islam et al., 2017). Ogle et al. (2001) reported the use of lotus stem (consists of 6, 2.4, 0.2 mg/100 g calcium, iron and zinc respectively) as a vegetable used in salads at Vietnam. Lotus leaves are useful to treat summer heat syndrome. Petals of lotus are floated in soups or used as a garnish, while the stamens are used in flavoring the tea. The green embryos in the seeds are bitter and usually removed prior to selling in the markets as food product. The seeds can be popped like popcorn, ground into powder and eaten dry or used in bread making. The roasted seeds are good coffee substitute and possess saponins, phenolics and carbohydrates in appreciable quantities (Ling, Z.Q et al., 2005). Duangtip Hongsamoot, Suvarin Bumroongsok (2015) had a feasibility study on organic lotus pollen tea as consumer product. Nelumbo nucifera leaves may evolve as a natural drug of wonder for patients suffering from cardio-vascular diseases as it has potential antibacterial and antithrombotic activities (Ashwanti Devi and Charu Sharma, 2016). This present study focused on the effect of blanching, drying and storage of Nelumbo nucifera tea.

II. MATERIALS AND METHOD
2.1 Material
We collected lotus leaves in Dong Thap province, Vietnam. They must be cultivated following VietGAP to ensure food
2.2 Researching procedure

2.2.1 Effect of blanching temperature and time to vitamin C (mg/100g): the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, and color (sensory score) in the dried Nelumbo nucifera leaf tea

Raw Nelumbo nucifera leaves were blanched in water solution with 2% CaCl₂ at different temperature and time (100°C, 2 seconds; 95°C, 4 seconds; 90°C, 6 seconds; 85°C, 8 seconds). Then they were dried by heat pump at 60°C until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate blanching condition.

2.2.2 Effect of CaCl₂ concentration in blanching to vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried Nelumbo nucifera leaf tea

Raw Nelumbo nucifera leaves were blanched in water solution with different CaCl₂ concentration (1.0%, 2.0%, 3.0%, 4.0%, 5.0%) at 95°C, 4 seconds. Then they were dried by heat pump at 60°C until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate blanching condition.

2.2.3 Effect of Nelumbo nucifera leaf size during drying to vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried Nelumbo nucifera leaf tea

Raw Nelumbo nucifera leaves were blanched in water solution with 4% CaCl₂ at 95°C, 4 seconds. Then they were dried at different size (0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm, 2.5 cm) by heat pump at 60°C until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate blanching condition.

2.2.4 Effect of drying temperature to vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried Nelumbo nucifera leaf tea

Raw Nelumbo nucifera leaves were blanched in water solution with 4% CaCl₂ at 95°C in 4 seconds. Then these samples would be dried in 1.0 cm of size under heat pump dryer at different temperature (10°C, 20°C, 30°C, 40°C, 50°C, 60°C) until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate drying temperature.

2.2.5 Effect of storage condition to the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays in the dried leaf tea

After completion of drying treatment, the dried Nelumbo nucifera leaves were subjected to storage. They were kept in PET/AL/PE (vacuum) bag at different 4°C, 28°C. The ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays will be analyzed in 1 week interval for 6 weeks.

2.3 Physico-chemical and biological analysis

The vitamin C (mg/100g) content of the Nelumbo nucifera leaves was determined by redox titration using iodate solution. The ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays was determined with reference to the method of Benzie and Strain (1996); Brand-Williams et al., (1995). Total phenolic (TP, μg GAE/g fw) contents were measured according to the method of Singleton and Rossi (1965) with slight modifications. Total soluble solids (TSS, %) were measured by refractometry method. Color (sensory score) of Nelumbo nucifera leaves was assessed by a group of panelist. They were required to evaluate the odour, colour, taste, sweetness and overall acceptance using the 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

The ferric reducing antioxidant power (FRAP) method was conducted according to Benzie and Strain (1996). To conduct the assay, a 2.97-ml aliquot of FRAP reagent, a mixture of 0.1 mol/l acetate buffer, 10 mmol/l 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and 20 mmol/l ferric chloride (10:1:1 v/v/v) was combined with 30 μl of the extract. After incubation for 10 min, the absorbance of each solution was determined at 593 nm. The DPPH radical scavenging capacity was measured using the method of Brand-Williams et al. (1995). The radical solution was prepared by dissolving DPPH (40 mg/l) in methanol. For the assay, a 2.97-ml aliquot of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and 30 μl samples were mixed. After 10 min, the reaction absorbance was measured at 515 nm. To determine the antioxidant capacity of samples by both procedures, absorbance values were compared with those obtained from
standard curves of trolox (10–100 μmol/l). Antioxidant capacity values were expressed as trolox equivalents (TE)/g fw.

Total phenolic (TP) contents were measured according to the method of Singleton and Rossi (1965) with slight modifications. To determine the levels of TP, 1 ml of each extract was combined with Folin-Ciocalteu’s phenol reagent and water 1:1:20 (v/v) and incubated for 8 min, followed by the addition of 10 ml of 7% (w/v) sodium carbonate. After 2 h, the absorbance of each was measured at 750 nm. Values of TP were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. Results are expressed as micrograms of gallic acid equivalents on a fresh weight basis (GAE/g fw).

Total soluble solids (TSS) were measured by refractometry method.

2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan’s multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

III. RESULT & DISCUSSION

3.1 Phytochemical composition in *Nelumbo nucifera* leaves

Lotus (*Nelumbo nucifera*) is a perennial aquatic plant of economic important grown in wetlands or ponds which are widely distributed in temperate and tropical regions (J. Hu et al., 2012). Phytochemical composition in *Nelumbo nucifera* leaves was primarily analyzed. Results were elaborated in table 1.

From table 1, we clearly noticed that *Nelumbo nucifera* leaves had good antioxidant capacity which was suitable for herbal tea production. The dietary fiber rich mature leaves had good antioxidant capacity which was suitable for preparation of antioxidant beverages and tea bags in Asia (K.M. Pulok et al., 2012).

3.2 Effect of blanching temperature and time to vitamin C (mg/100g); the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried *Nelumbo nucifera* leaf tea

Raw *Nelumbo nucifera* leaves were blanched in water solution with 2% CaCl₂ at different temperature and time (100°C, 2 second; 95°C, 4 seconds; 90°C, 6 seconds; 85°C 8 seconds). Then they were dried by heat pump at 60°C until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate blanching condition. From table 2, the optimal blanching condition should be performed at 95°C, 4 seconds.

Arumugam Abirami et al., (2017) proposed to investigate the antioxidant and radical scavenging property of aqueous acetone extract of raw flower petals and raw and processed (boiling and blanching) core parts from *Nelumbo nucifera* and *Nymphaea alba*. Total phenolics and tannin content of raw and processed petals and core extracts were ranged from 173.13 - 312.81mg/g extract and 149.50- 302.43mg/g extract respectively. In general, the processed core part extracts registered greater polyphenolic contents than the raw extracts. Interestingly, among the various processing methods, boiled core sample of *N. alba* registered higher DPPH (535121.6 mmol TE/g extract) and ABTS (302642.21 mmol TE/g extract), superoxide (74.92%), hydroxyl (73.4%) radical scavenging activity, metal chelating property (0.43 mg EDTA/g extract), FRAP (6156 mmol Fe (II)/g extract). These results revealed that processing methods significantly increased the content and potential of antioxidant components of *N. nucifera* and *N. alba*.

### Table 1. Phytochemical composition in *Nelumbo nucifera* leaves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vitamin C (mg/100g)</th>
<th>FRAP (μmol TE/g fw)</th>
<th>DPPH (μmol TE/g fw)</th>
<th>Total phenolic (TP, μg GAE/g fw)</th>
<th>Total soluble solid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>33.29±0.03</td>
<td>29.57±0.01</td>
<td>47.84±0.03</td>
<td>63.45±0.02</td>
<td>13.07±0.01</td>
</tr>
</tbody>
</table>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

### Table 2. Effect of blanching temperature and time

<table>
<thead>
<tr>
<th>Blanching condition</th>
<th>Vitamin C (mg/100g)</th>
<th>FRAP (μmol TE/g fw)</th>
<th>DPPH (μmol TE/g fw)</th>
<th>Sensory score</th>
</tr>
</thead>
<tbody>
<tr>
<td>100°C, 2 seconds</td>
<td>18.59±0.01</td>
<td>20.03±0.02</td>
<td>27.43±0.01</td>
<td>4.32±0.00</td>
</tr>
<tr>
<td>95°C, 4 seconds</td>
<td>22.47±0.02</td>
<td>21.44±0.01</td>
<td>29.30±0.02</td>
<td>5.74±0.01</td>
</tr>
<tr>
<td>90°C, 6 seconds</td>
<td>17.55±0.00</td>
<td>19.05±0.03</td>
<td>24.15±0.00</td>
<td>3.12±0.04</td>
</tr>
<tr>
<td>85°C, 8 seconds</td>
<td>15.31±0.01</td>
<td>16.21±0.00</td>
<td>22.04±0.01</td>
<td>3.01±0.03</td>
</tr>
</tbody>
</table>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

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Lotus rhizomes (Nelumbo nucifera Gaertn.) were pretreated using the following 4 treatments, blanching at 40°C, blanching at 90°C, soaking in 0.5% CaCl₂, and blanching at 40 °C followed by immersion in 0.5% CaCl₂. Subsequently, the cell wall material of pretreated samples was isolated and fractioned to identify changes in the degree of esterification (DE) and monosaccharide content of each section, and the texture of the lotus rhizomes in different pre-treatments was determined after thermal processing with different time. The results showed that the greatest hardness was obtained after blanching at 40 °C in CaCl₂, possibly attributing to the formation of a pectate calcium network, which maintains the integrity of cell walls (Wenlin Zhao et al., 2016).

### 3.4 Effect of Nelumbo nucifera leaf size during drying to vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried Nelumbo nucifera leaf tea

Raw Nelumbo nucifera leaves were blanched in water solution with 4% CaCl₂ at 95°C in 4 seconds. Then those samples would be dried in 1.0 cm of size under heat pump dryer at different temperature (10 °C, 20 °C, 30 °C, 40 °C, 50°C, 60°C) until 6.5% moisture. All samples were analyzed vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays, color (sensory score) to validate the appropriate drying temperature. From table 4, the optimal Nelumbo nucifera leaf size should be 2.0 cm.

Herbs are valued for its specific aroma, taste, putative physiological effect and medicinal properties which appeal to sense of taste, smell, and sight. Herbal tea is a polyherbal formulation of different medicinal plants that is a rich source of antioxidant. However tea manufacturing processes can greatly affect the oxidation of tea polyphenols (Rabeta, M. S. and Vithyia, M., 2013).

#### 3.5 Effect of drying temperature to vitamin C (mg/100g), the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays and color (sensory score) in the dried Nelumbo nucifera leaf tea

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when stored at 25°C for 5 days, 4°C for 30 days, and −10°C for 90 days, regardless of the pretreatment used. At the same storage temperatures, pH, soluble solids, total acid content, polyphenols, and microorganisms were significantly different among the pretreatments (p < 0.05). Soluble solids, pH, and total acid contents were not significantly different for the same pretreatments at different storage temperatures. However, the polyphenol, oxalic acid, and tartaric acid contents of lotus leaves were significantly different after drying and blanching treatments. In particular, it was shown that polyphenol content of the lotus leaf was affected by both pretreatment and storage temperature.

3.6 Effect of storage condition to the ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays in the dried *Nelumbo nucifera* leaf tea

After completion of drying treatment, the dried *Nelumbo nucifera* leaves were subjected to storage. They were kept in PET/AL/PE (vacuum) bag at different 4°C, 28°C. The ferric reducing antioxidant power (FRAP, μmol TE/g fw) and DPPH (μmol TE/g fw) radical scavenging assays will be analyzed in 1 week interval for 6 weeks. From table 6, the dried *Nelumbo nucifera* leaf tea was nearly stable for 6 weeks under PET/AL/PE (vacuum) bag at 4°C. Food antioxidants often lost as a result of sterilisation, pasteurisation, dehydoration and prolonged storage (Manzocco et al., 1998).

IV. CONCLUSION

*t Nelumbo nucifera* leaves are rich in flavonoids. These flavonoids from lotus leaves possess strong antioxidant activity, and demonstrate very good potential to be explored as food supplements or even pharmaceutical products to improve human health. All parts of lotus, including the leaves, stamens, flowers, seeds and rhizomes, have been used as traditional medicines or vegetables for thousands of years. We have successfully optimized the effect of blanching, drying and storage of *Nelumbo nucifera* tea. Therefore, this is a good opportunity for lotus growers to offer lotus tea, full of beneficial flavonoids, as an alternative to imported beverages that offer no health benefit. In addition, healthy lotus tea fittingly caters to the need for healthy food products currently in high demand by the top-tiered consumer markets with high purchasing power.

### Table 6. Effect of storage condition

<table>
<thead>
<tr>
<th>Storage duration (week)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP (μmol TE/g fw)</td>
<td>24.77±0.01</td>
<td>24.73±0.02</td>
<td>24.70±0.03</td>
<td>24.61±0.00</td>
<td>24.54±0.01</td>
</tr>
<tr>
<td>DPPH (μmol TE/g fw)</td>
<td>31.97±0.03</td>
<td>31.92±0.01</td>
<td>31.85±0.02</td>
<td>31.80±0.02</td>
<td>31.76±0.00</td>
</tr>
<tr>
<td>FRAP (μmol TE/g fw)</td>
<td>24.77±0.01</td>
<td>24.64±0.00</td>
<td>24.53±0.02</td>
<td>24.45±0.02</td>
<td>24.33±0.01</td>
</tr>
<tr>
<td>DPPH (μmol TE/g fw)</td>
<td>31.97±0.03</td>
<td>31.88±0.01</td>
<td>31.80±0.02</td>
<td>31.75±0.04</td>
<td>31.69±0.01</td>
</tr>
<tr>
<td>FRAP (μmol TE/g fw)</td>
<td>24.49±0.02</td>
<td>24.26±0.03</td>
<td>24.11±0.02</td>
<td>24.11±0.02</td>
<td>31.37±0.04</td>
</tr>
<tr>
<td>DPPH (μmol TE/g fw)</td>
<td>31.55±0.04</td>
<td>31.42±0.02</td>
<td>31.46±0.02</td>
<td>31.46±0.02</td>
<td>31.46±0.02</td>
</tr>
</tbody>
</table>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

REFERENCES


