Instant Drink Powder Development from Lotus Root with Probiotic Supplement using Foam Mat Drying Method

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Abstract
In this study, we developed instant drink powder of lotus root water using stevioside as a non-nutritive sweetener at different concentrations (0.01, 0.03 and 0.05% w/v) and flavor (Chrysanthemum, Pandan leaves and Roselle) addition on color, pH, %TA, TSS and organoleptic quality properties. The additional of stevioside did not show any effects on physicochemical properties. Results show that 0.03% stevioside and Pandan leaves addition exhibited the highest score of sensorial features significantly. Moreover, this formula was made as foam-mat dried to obtain lotus root supplemented with probiotics powders. The effects of composition ratio of methylcellulose: egg albumin (1:1, 1:2, 1.5:1 and 1.5:2) on the lotus root foam properties were investigated. It was observed that increase in the concentration of methylcellulose decreased foam stability and foam density. Foams were prepared from Lactobacillus plantarum M29 and Pediococcus pentosaceus MG12 with lotus root water by adding the different concentrations of foaming agents at a whipping time of 15 min. The composition ratio of methylcellulose:egg albumin (1.5:1) provided preferable foam characteristics. The greatest resulting foams were dried at 60°C for 3 h. The lotus root with probiotics instant drink was able to possess the antioxidant activity and viable probiotics count (more than 7 log CFU/g) during storage at both refrigeration and room temperature for a month.

Introduction
Lotus root (Nelumbo nucifera Gaertn.) is a widely known aquatic vegetable in China and encloses plentiful amounts of protein, amino acids, dietary fiber, starch, and vitamins (C, B1, and B2). It was explored to acquire a potent medicinal plant in Traditional Chinese Medicine that assists to medicate all manners of reproduction and vomiting blood or haematemesis. It has been described that lotus root has the activities of hypoglycemic,
anti-fungal, anti-inflammatory, antipyretic and anti-anxiety properties. Hence, an earlier study revealed that numerous extracts of lotus rhizome exhibited higher antioxidant activity. Besides, lotus root drink is extensively consumed through its favorable effects on heart and lung function.

Food probiotification is a growing concept that is gaining the probiotic cultures as food additives in developing such currently functional foods and beverages. Probiotic food products are classified as functional foods, as they have an effect to enhance the intestinal microbial balance. Probiotics have abundant health-boosting effects such as associated lactose and casein metabolism, inhibits gastrointestinal tract infections and strengthens ensimmunity, reduces cholesterol risks, vitalizes calcium absorption, biosynthesis of vitamins, enhances protein digestibility and resists the foodborne pathogens. The probiotic bacteria as profitable functional foods should be existing in significant numbers and sustain viability in the food until the date of consumption. Dairy-based probiotic products are widely accessible for consumers who encounter diet-related illnesses such as lactose intolerance. Accordingly, they do not have to renounce the prosperity of probiotics.

Foam mat drying is a process that the liquid or semifluid food is whipped to form the steady food foam by cooperating an enormous capacity of air with foaming agents and then expand into a delicate layer and subsequently dried. While this process can expense from 4 to 8 times less than freeze drying. This drying process is relatively reasonable and straight forward process. The major preferences of foam mat drying are simply, inexpensive process, lower temperatures, and rapid drying times when compared to non-foamed material. Accordingly, the similar drying process was applied in various types of foods such as fruit juices, yogurt, spirulina, beans, and yacon juice.

This research is expected to contribute to the development of non-dairy probiotic products from lotus root matrices. Therefore, this research aims to investigate the production of instant drink powder from lotus root with probiotic supplement using foam mat drying methods considering the foam properties and qualities of the product.

Materials and Methods
Lotus Root Water Preparation
Fresh lotus root (Daucus carata Linn.), dried Chrysanthemum (Dendranthamaindicum L.), dried Pandan leaves (Pandanus amaryllifolius Roxb.) and dried Roselle (Hibiscus sabdaiiffa L.) were acquired from the regional market of Pathum Thani (Thailand). Steviolglycoside was purchased from Sugavia Co., Ltd.

The lotus root was washed and cut into small pieces at the ratio of lotus root:water(1:10) after that blended and filtered. The formulation development of lotus root water using stevia sugar with the amount of 0.01, 0.03 and 0.05% (w/v) were boiled, filled into glassware, and then pressed tightly. Color analysis, total acidity (%), total soluble solid (TSS) and pH were examined. The color analysis was determined using Colorimeter (Chromameter CR-10, CIEL*a*b*). The change in pH was monitored by digital pH meter (Fisher Scientific, Instruments, Pittsburgh, PA). Titratable acidity was assessed by titrating against 0.1 N NaOH. The total soluble solid was determined by hand refractometer (Atago, Brix 0-33%). The organoleptic test on the 9-point hedonic scale, were evaluated by 30 untrained panelists. Then, the lotus rootwater was added with dried Chrysanthemum, Pandan leaves and Roselle (30 g) in 1L of juice. Color analysis, total acidity (%), total soluble solids, pH, and sensory acceptance were determined.

Preparation of Lactic Acid Bacteria (LAB) Culture and Foams
Freeze dried Lactobacillus plantarum M29 and Peddiococcus pentosaceus MG12 were taken from previously study and applied in the present study. LAB (L. plantarum M29 and P. pentosaceus MG12) were plated overnight at 37°C on MRS agar and was inoculated by double activations (1:10 v/v) in MRS broth at 37°C for 24 h. The cell suspension was centrifuged (5000 × g, 5 min), washed twice in sterilized 0.90% (w/v) of NaCl solution and resuspended to the equivalent volume (100 ml) of the final solution (1 L) were added to lotus root water.

Foam Preparation and Drying Experiments
Foams were made by mixing at different ratios of methyl cellulose: egg albumin at 1:1, 1:2, 1.5:1, and 1.5:2 with highest speed mixer for 15 min
and determined of foam characteristics. Foam density was measured as described by Abbasi and Azizpour\(^1\) and expressed as g cm\(^{-3}\). Syneresis method was adapted from Krasaekoopt and Bhatia.\(^1\)

The foam was drained into a tray with a layer 5 mm thick and placed in a hot air oven at 60°C for 3 h. After drying, the exfoliates were milled to obtain the powdered product.\(^1\) The powder was kept in the air-tight container at -20°C for further analysis.

**Characteristic Analysis of Instant Drink Powder with Probiotic Culture**

The viable starter cells were counted using the pour plate method at 37°C for 48 h with MRS-agar. The survival rate after foam mat drying process and during storage at different temperature (freeze (-5 ± 2°C), refrigerator (4 ± 2°C) and room temperature (33 ± 2°C)) were expressed as log CFU/g. The water activity (\(a_w\)) was determined using a water activity meter (Hygro-Palm HP 23, Rotronic, Bassersdorf, Switzerland). Samples were examined for antioxidant activity by DPPH assay.\(^2\) All results were expressed as μg Ascorbic equivalent antioxidant capacity per microliter sample.

The sensory attributes of instant lotus root drink powder with probiotic were conducted by 30 untrained panelists using a 9-point hedonic scale. The 10 g of powder dissolved in 1L warm water in plastic cup were served to each panelist in 50 ml white plastic cups, coded with three-digit random numbers. The sensory acceptability of samples was rated in terms of color, odor, flavor, texture, and overall acceptability.

**Statistical Analysis**

All experiments were repeated at least three times (n=3). Statistical significance (p≤0.05) was performed with ANOVA with Duncan’s multiple-range test using SPSS software. For sensory analysis, a randomized block design was used.

**Results and discussion**

**Lotus Root Water Development**

In this study, we found that no significant effect the physicochemical characteristics, the color (\(L^*\) 14.11-16.69, \(a^*\) 5.72-5.98, \(b^*\) 2.40-3.07), pH (6.19-6.26), total acidity (TA; 0.04) and total soluble solids (TSS; 2.00).

![Figure 1: Sensory evaluation of lotus root water with different steviol glycoside concentrations (SGC). Different lower case letters in the same column differ significantly (p≤0.05)](image-url)

The physicochemical and sensory quality were analyzed (Table 1 and Figure 2). The result found that the addition of dried Roselle causes the pH to decrease because it has a sour taste and is commonly used in the preparation of beverages and as a food colorant.\(^2\) The color analysis value tends to
vary naturally of herbs that are used to develop the formulation of lotus root water. The results exhibited that no significant effect the total soluble solids (TSS) of each formulation.

Table 1: Color analysis, pH, and %TA of lotus root water with different formulation development materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>Color analysis</th>
<th>pH</th>
<th>TA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L* a* b*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysanthemum</td>
<td>32.99±0.58a</td>
<td>5.50±0.53b</td>
<td>12.94±1.19a</td>
</tr>
<tr>
<td>Roselle</td>
<td>17.58±0.43c</td>
<td>22.11±0.89a</td>
<td>8.86±4.21b</td>
</tr>
<tr>
<td>Pandan leaves</td>
<td>19.48±1.02b</td>
<td>-4.99±0.45b</td>
<td>5.74±0.74c</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column differ significantly (p≤0.05) ± Standard Deviation (n=3).

For sensory evaluation, the result found that chrysanthemum and Pandan leaves scores showed no significantly in texture, but had a better color, odor, and flavor preference score than Chrysanthemum (Figure 2).

Pandan leaves have been described as the outstanding natural origins of the particular volatile compound in aromatic rice. Accordingly, Pandan leaves are generally used when providing rice recipes, dessert, and beverages as flavor enhancement in South-East Asia. So, a formulation that contains Pandan leaves with natural scents was chosen and continue testing.

Figure 2: Sensory evaluation of lotus root water with different odor development materials. Different lower case letters in the same column differ significantly (p≤0.05)

There were used egg albumin and methylcellulose as foaming agents in this experiment. All foaming agents were added into lotus root water with probiotic cultures. The combination was then mixed by using high-speed mixer for 15 min. The foam properties, as foam density and drainage volume, were examined after the foam was formed. As a result, the ratio of methylcellulose: egg albumin (1.5:1) as foaming for lotus root powder production shown incorporate air to form stable foam by less whipping time and resulting in lower foam density. There were significant (p≤0.05) differences for the foam density. These results demonstrated that the foam density of methylcellulose was higher, indicating more uniform distribution of air bubble. The properties of lotus root water foam with probiotic...
was shown in Table 2. In this study, foam density in the range of 0.36-0.46 g cm$^{-3}$ and drainage volume in the range of 5.33-15.00 ml was observed. This range observed in this study is consistent with numerous studies indicated that higher foam density in the range of 0.2 to 0.6 g cm$^{-3}$ and a drainage volume of 0-8 ml are acceptable for foam mat drying were reported.\textsuperscript{23} Syneresis (drainage volume) rate in this study may also influence the water holding capacity of the foam.\textsuperscript{19} Foam from methylcellulose:egg albumin (1.5:1) exhibited less drainage volume as compared to others.

Table 2: Foam characteristics of lotus root water with different foaming agents

<table>
<thead>
<tr>
<th></th>
<th>Methylcellulose (g/100g)</th>
<th>Egg White (g/100g)</th>
<th>Drainage Volume (ml)</th>
<th>Foam Density (g cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8.00±1.00$^b$</td>
<td>0.46±0.01$^a$</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>15.00±1.00$^a$</td>
<td>0.45±0.10$^a$</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>5.33±0.57$^c$</td>
<td>0.36±0.02$^b$</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>8.00±0.92$^b$</td>
<td>0.38±0.03$^b$</td>
<td></td>
</tr>
</tbody>
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Different superscript letters in the same column differ significantly ($p\leq0.05$) ± Standard Deviation (n=3).

The foam mat of lotus root with probiotic dried powders were carried out at 60°C drying air temperatures using methylcellulose and egg albumin as foaming agents. Quality of the reconstituted dried lotus root with probiotic powder was assessed at an interval of 1 month for $a_w$, DPPH antioxidant activity (μg Ascorbic/ml) and microbial load (probiotics). According to the results, the viable count of probiotics was ranged between 7.13 and 9.28 log CFU/g during storage time. The high rates of survival of probiotics after drying compared to before drying demonstrating less effect of foam-mat drying on the viable counts of probiotic strain.\textsuperscript{14} The powder contained viable cell count as much as 7 log CFU/g during the storage period. The counts of probiotics at 30 days had reduced 2 log cycles. No significant difference was observed between the probiotic viability at different temperatures during storage period as shown in Figure 3. No considerable changes were found on viable cell count by a foam mat drying process. It was found that methylcellulose: egg albumin (1.5:1), whipping time (15 mins), foam thickness (5 mm) and drying temperature (70°C) optimum for foam mat dried lotus root water with probiotic powder.
Table 3 shows that the antioxidant activity was decreased when compared with the fresh lotus root water, whereas $a_w$ was slightly higher than lotus root water powder with probiotic at 0 day. Hence, it was concluded that the antioxidant activity of the lotus root water compared to the probiotic lotus root powder was significant decreased from 240.83±0.34 to 23.46±0.51 µg Ascorbic/ml during the storage (p≤0.05). This result could be linked with the findings of Kha et al.\textsuperscript{24} mentioned that the antioxidant activity of powders reduced due to increase in drying time and temperature (40 to 80°C).

Table 3: $a_w$ of lotus root water with probiotic powder during storage at different conditions

<table>
<thead>
<tr>
<th>Days Storage Temperature (°C)</th>
<th>$a_w$</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.55±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>30 33 ± 2</td>
<td>0.61±0.35\textsuperscript{b}</td>
</tr>
<tr>
<td>4 ± 2</td>
<td>0.58±0.41\textsuperscript{b}</td>
</tr>
<tr>
<td>(-5) ± 2</td>
<td>0.58±0.17\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column differ significantly (p≤0.05) ± Standard Deviation (n=3).

The optimized formulation obtained was used to produce instant lotus root drink powder with probiotic for the consumer acceptance test. The consumer acceptance result shows that instant lotus root drink powder with probiotic had acceptance liking score (like very much level) in the term of appearance (56%), aroma (53%), taste (51%) and overall acceptability (51%) was performed. These results indicated the suitable potential of instant lotus root drink powder with probiotic in development of product for future uses.

Conclusion
The appropriate condition for producing lotus root water with probiotic powder was the application of methylcellulose:egg albumin (1.5:1) as a foaming agent and drying temperature at 60°C for 3 h. The foam density and drainage volume are suitable for foam mat drying. The lotus root water powder contained probiotic as high as 7 log CFU/gof the product has been approved for representation of well-being benefit. The foam mat drying process was confirmed for the production of probiotic fortified lotus root water powders which is favorable from the survivability of probiotic cells.

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Conflict of Interest
The authors state no conflict of interest.

References
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