Characteristic and functional properties of Thai lotus seed
(Nelumbo nucifera) flours

Singthong, J. and Meesit, U.

Department of Agro-Industry, Faculty of Agriculture, Ubon Ratchathani University, Warinchamrap, Ubon-Ratchathani, 34190

Abstract

Thai lotus (Nelumbo nucifera) seeds have been widely used for both food and medicine. The goals of this study were to investigate the characteristics and the functional properties of flours from four varieties of lotus seeds in Thailand (Patoom, Boontric, Chatkaw and Chatchompoo). The results showed that all Thai lotus seed flours contained a high content of carbohydrate, protein and amylose. These flours have good water and oil absorption capacity, protein solubility, emulsion and foaming properties. The pasting properties were slightly altered when heated. The gelatinization temperature was high, but the enthalpy was low. These flours have a high syneresis during storage at low temperature and a low freeze-thaw stability. When incorporating 10% lotus seed flour (Patoom) in bread, both the bread quality and consumer acceptance were improved. The results of this study could provide basic information to promote lotus seed flour utilization in other food products.

Introduction

Lotus plants provide numerous bioactive ingredients such as alkaloids, flavonoids, antioxidants, antisteroids, antipyretics, anticancer substances, antiviral substances and substances that have anti-obesity properties (Sridhar and Bhat, 2007). Lotus seeds contain high levels of flavonol compounds with high antioxidant potential and are rich in protein as well as minerals (Bhat and Sridhar, 2008; Kreydy et al., 2010). These seeds are beneficial in the treatment or prevention of a variety of diseases and have nutraceutical potential. (Sridhar and Bhat, 2007; Kreydy et al., 2010). Lotus seeds are reported to be rich in protein and nutritional value (containing amino acids, vitamins B1, B2, B6, C, and E, and phospholipids) (Wu et al., 2007). In Thailand, cultivation of lotus (Nelumbo nucifera Gaertn.) is an important economic activity for farmers. Four cultivars are popular: Sacred lotus (Patoom), Hindu lotus (Boontric), Magnolia lotus (Chatkaw) and Roseum Plenum (Chatchompoo) (Leichakul, 1992). Many researchers have investigated lotus seed to improve the nutritional value in various food products and reported on the nutritional value of lotus seed (Wu et al., 2007; Bhat and Sridhar, 2008; Paiyarach and Punbusayakul, 2009). The high amount of carbohydrates, protein and the nutritional value of lotus seed indicate its potential as a new source of flour in food applications, especially in the development of value-added products from flours that depend on a thorough knowledge of their functional properties. However, no information on Thai lotus seed flours is available in the literature.

Flours and starches are important ingredients in the food industry. As new food products are developed, starches or flours with specific properties are necessary to impart functionally desirable attributes. Starch gelatinization refers to the disruption of the molecular order within starch granules when they are heated in the presence of water. Evidence for the loss of an organized structure includes irreversible granule swelling, loss of birefringence and crystallization (Freitas et al., 2004). Thermal and pasting properties are the most important functional properties related to the evaluation and estimation of process design, unit operation and the quality of the final starch-based products. Pasting behaviour is usually studied by observing changes in the viscosity of a starch system based on rheological principles. From the pasting curve, several parameters can be derived that indicate the extent of disintegration and whether there is retrogradation (Huang et al., 2006).

The objective of this work was to evaluate the functional properties of flours from lotus seeds in Thailand, investigate possible applications in bread products and study changes occurring in breads, as well as determine customer acceptance. This study is expected to provide useful information that can give further support to the consideration of lotus seed
as an alternative source of flour for the food industry.

Materials and Methods

Materials

Three lotus seed cultivars (Patoom, Chatkaw and Chatchompoo) were obtained from Bueng Boraphet in Nakornsawan province, Thailand. The Boontaric cultivar was procured from the local farm of Tansum in Ubon Ratchathani province, Thailand. The lotus seeds were boiled at 75 ± 5°C in 2% (w/v) sodium hydroxide solution for 2 minutes, and the uncoated seeds and embryos were washed in water and dried in a hot air oven at 40°C for 14 hr. Seeds were milled and ground through a 100 mesh sieve to produce whole flour. The flour was packaged in polyethylene bags and stored in a desiccator until required for further analysis.

Physicochemical compositions of lotus seed flours

The moisture, ash, protein, lipid and fibre contents of the lotus seed flours were determined according to the AOAC method (1995). The amylose content was determined by using an amylose-iodine complex following Juliano’s method (1971). Colour changes in samples were determined by the Hunter Lab (Color Flex Model 45/0, USA) in the L*, a*, and b* colour parameters.

Water and oil absorption capacity (WAC and OAC)

WAC and OAC were determined according to Bhat and Sridhar (2008). One gram of the seed flour was vortexed with 10 ml distilled water or oil for 30 s in centrifuge tube. The solution was allowed to stand at room temperature for 30 min, centrifuged and the volume of supernatant was measured in a 10 ml graduated cylinder.

Emulsion properties

Emulsifying activity (EA) and emulsion stability (ES) were determined according to Bhat and Sridhar (2008) with slight modification. Fifty milligrams of seed flour dispersion in distilled water (5 ml) were homogenised with 5 ml oil. The emulsions were centrifuged and the height of emulsified layer and total contents in the tube were determined as EA. ES was determined by heating the emulsion before centrifuging.

Foaming properties

Foaming properties of the seed flour was determined according to the method of Bhat and Sridhar (2008). Two grams of the flour were dispersed in 100 ml of distilled water and whipped vigorous for 2 min. The volumes were recorded before and after whipping and the percentage volume increase was calculated as foam capacity. Foam stability was determined as the volume of foam that remained after 8 h at room temperature and expressed as the percentage of initial foam volume.

Swelling power and solubility index

The swelling power and solubility index were evaluated according to the method of Wang et al. (2010). Sample suspension (2% w/v) was heated in water bath at 90°C for 30 min. The samples were centrifuged for 15 min. The supernatant was removed and the sediment was weighted. Aliquots of supernatant were dried in an oven at 105°C to constant weight. The swelling power (g/g) and solubility (%) was calculated.

Pasting properties

The pasting properties were measured using a Rapid Visco Analyser (RVA-4D, Newport Scientific, Warriewood, Australia) following the method described by Stevenson et al. (2006) and Sandhu and Singh (2007). Flour (3 g at 14 % moisture basis) was weighed directly into an RVA canister, and distilled water was added to obtain a sample weight of 28 g. The sample was held at 50°C for 1 min, heated to 95°C in 7.5 min, and then held at 95°C for 5 min. The sample was cooled to 50°C in 7.5 min, and then held at 50°C for 2 min. The rotating speed was maintained at 160 rpm during the process. Parameters including peak viscosity, final viscosity, breakdown and setback were recorded. Three replicate samples were analysed. Freeze-thaw stability was analysed using the method of Pal et al., (2002).

Thermal properties

Thermal properties were determined using a differential scanning calorimeter (DSC, Mettler Toledo, DSC1/400W, Japan) (Sandhu and Sing, 2007). Flour (3 mg) and distilled water were loaded into an aluminium pan, which was hermetically sealed. The sample pans were allowed to stand for 1 hr at room temperature to attain an even distribution of water before heating the calorimeter. An empty aluminium pan was used as the reference, and the calorimeter was calibrated with indium. The scanning temperature range was 20-120°C at a heating rate of 10°C/min. The onset (To), peak (Tp) and conclusion (Tc) temperatures and the enthalpy (ΔH) of gelatinization of the starch were calculated automatically.

Preparation of bread

The ingredients for making the control bread were wheat flour, salt, sugar, yeast, butter and water.
Thai lotus seed flour was added as a substitute for wheat flour at 0, 10, 20, 30 and 40% while the other ingredients were kept constant for all formulae.

For bread preparation, wheat flour, Thai lotus seed flour and yeast were mixed well and passed through a 100 mesh sieve. Simultaneously, sugar, salt and water were mixed well in the other bowl. After combining the two mixtures, butter was added. The mixture was kneaded to form dough. The dough was left at ambient temperature for 10 min, then transferred to one lb rectangular bowl and left for a further 180 min in the bread dough incubator. The bowl was placed in an oven set at 190-200°C for 35 min or until the surface turned yellow. The product was removed from the oven and the bowl and left to cool before further analysis.

**Analytical methods for bread**

Gas retention, loaf weight, loaf volume and the specific volume of the bread were determined by the method of Shittu et al., (2009). The colour of the bread was determined using a Hunter Lab (Color Flex Model 45/0, USA). The texture of bread was evaluated in terms of TPA by a texture analyser (LLOYD texture analyser model LRSK, UK) with a 50x50 mm compression plate and a test speed of 60 mm/min. The proximate analysis of the bread was done according to the method of AOAC (1995).

The sensory evaluation of the bread was conducted by 40 panellists, who were students and staff members in the Department of Agro-Industry, Ubon-Ratchathani University. Randomly coded bread samples were served individually to the panellists. Six sensory attributes were evaluated (appearance, colour, odour, taste, texture and overall acceptability) using a 9-point hedonic scale for each trait, where 9=excellent and 1=extremely poor.

Scanning electron micrographs (SEM) were obtained using a JEOL, JSM-5410LV scanning electron microscope (JEOL Ltd., Tokyo, Japan). Samples were coated with gold using a sputter coater (model SPI-MODULETM Sputter Coat). An accelerating potential of 20 KV was used during electron micrography.

**Data analysis**

All experiments and analytical measurements were run in triplicate. The means of each parameter were analysed by analysis of variance (ANOVA). Differences between treatments at the 5% (p≤0.05) level were considered significant.

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**Results and Discussion**

**Physicochemical characteristics of lotus seed flours**

The proximate compositional profiles for four cultivars of Thai lotus seed flours are given in Table 1. The results showed that the moisture, ash, fat, protein, crude fibre, carbohydrate and amylose contents were 4.46-9.68%, 3.30-3.79%, 1.99-2.41%, 17.16-21.41%, 1.70-2.20%, 62.90-66.48% and 21.27-26.09%, respectively. The lotus seed flours were mainly composed of carbohydrate but also had a high protein content that emphasize their value as a vital source of nutrients. The quantity of ash in lotus seed flours is also important, as it determines the nutritionally minerals. The amylose content of flours from different lotus seed cultivars was significantly different. The amylose content of the starches was different from that reported for other flours: 17% for tapioca and 28% for corn (Whistler and BeMiller, 1997); 28.5 and 19.8% for yam and tapioca, respectively (Gunaratne and Hoover, 2001); 27.2% for wheat (Zaidul et al., 2008); and 25.3 and 19.2% for potato and rice, respectively, and 30% in yam by Mali et al., (2002). The differences in amylose content could be related to cultivar, growing zone and environment. (Wang et al., 2010). Amylose content is an important factor to the functional properties. The swelling behaviour is primarily property of its amylopectin, but amylose acts as both diluent and an inhibitor to swelling (Wang et al., 2010). The colour
Characteristics are all of the same order of value. This result indicated that the flours had a slight yellowish colour and an oval shape.

Functional properties of lotus seed flours

DSC was used to study the thermal properties of lotus seed flours (Table 2). It showed a single symmetrical endotherm during gelatinization. The onset, peak and conclusion temperatures of gelatinization (To, Tp, and Tc) and enthalpy of gelatinization from the different cultivars of lotus seed flours ranged from 71.62-75.37°C, 76.28-79.04°C, 80.48-82.37°C and 2.76-3.95 J/g, respectively. The flour from lotus seed with a high amylose content gelatinizes at a high temperature (Huijbrechts et al., 2008). The same phenomenon was found for gelatinization temperature in rice flour (72.86-80.15°C), which is higher than tapioca starch (62.99-79.47°C), corn starch (68.41-77.14°C) or waxy rice starch (60.49-71.67°C). Lotus seed flours required a high temperature to ensure complete gelatinization and pasting, which is potentially useful in products for which delayed pasting is desirable, such as retorted canned foods (Torre-Gutiérrez et al., 2008).

The results from the Rapid Visco Analyser (RVA) for the lotus seed flours are summarized in Table 2. The pasting temperatures of the different lotus seed flours ranged from 80.31 to 83.47°C, with the highest observed for CP and the lowest for PT. The minimum pasting temperature is required to cook the flour, and a high pasting temperature of the flour indicates a high resistance to swelling and rupturing. Significant differences were observed in the peak viscosity of flours from different lotus seed cultivars. The highest peak viscosity was found for PT (2607 cP) and the lowest for CP (1409 cP). A lower enthalpy for a,b,c,d

Values with different letters in the same row are significantly different (p≤0.05). Means ± SD, each value in the table is the mean of three replicates. PT=Patoom, BT=Boontric, CW=Chatkaw, CP=Chatchompoo

Table 2. Functional properties of Thai lotus seed flours

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PT</th>
<th>BT</th>
<th>CW</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal Properties</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>To (°C)</td>
<td>73.62 ± 0.49</td>
<td>71.62 ± 0.18</td>
<td>72.96 ± 0.09</td>
<td>75.37 ± 0.13</td>
</tr>
<tr>
<td>Tp (°C)</td>
<td>77.91 ± 0.40</td>
<td>76.28 ± 0.15</td>
<td>78.90 ± 0.21</td>
<td>79.04 ± 0.94</td>
</tr>
<tr>
<td>Tc (°C)</td>
<td>81.72 ± 0.89</td>
<td>80.67 ± 0.31</td>
<td>80.48 ± 0.55</td>
<td>82.37 ± 0.74</td>
</tr>
<tr>
<td>Enthalpy (J/g)</td>
<td>2.76 ± 0.09</td>
<td>3.86 ± 0.24</td>
<td>2.88 ± 0.29</td>
<td>3.17 ± 0.94</td>
</tr>
<tr>
<td>Pasting properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak viscosity (cP)</td>
<td>2607.75±105.8</td>
<td>2173.17±56.38</td>
<td>1840.25±16.04</td>
<td>1409.33±32.53</td>
</tr>
<tr>
<td>Final viscosity (cP)</td>
<td>3601.25±122.68</td>
<td>3458.33±16.97</td>
<td>3205.25±82.38</td>
<td>2232.59±45.42</td>
</tr>
<tr>
<td>Breakdown</td>
<td>1144.75±45.61</td>
<td>1310.00±57.71</td>
<td>1396.08±123.63</td>
<td>907.32±11.31</td>
</tr>
<tr>
<td>Pasting temperature (°C)</td>
<td>80.31±0.50</td>
<td>81.98±0.39</td>
<td>81.27±0.31</td>
<td>83.47±0.76</td>
</tr>
<tr>
<td>Swelling power (g/g)</td>
<td>11.02 ± 0.14</td>
<td>10.76 ± 0.30</td>
<td>10.54 ± 0.50</td>
<td>11.20 ± 0.32</td>
</tr>
<tr>
<td>Solubility index (%)</td>
<td>36.09 ± 0.52</td>
<td>32.90 ± 0.49</td>
<td>20.20 ± 0.03</td>
<td>34.76 ± 0.25</td>
</tr>
<tr>
<td>WAX (mg/g)</td>
<td>1.59 ± 0.06</td>
<td>0.92 ± 0.09</td>
<td>0.92 ± 0.07</td>
<td>1.33 ± 0.08</td>
</tr>
<tr>
<td>OAC (mg/g)</td>
<td>1.55 ± 0.04</td>
<td>1.54 ± 0.06</td>
<td>1.54 ± 0.06</td>
<td>1.5 ± 0.00</td>
</tr>
</tbody>
</table>

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The swelling power and solubility index of flours from different lotus seed cultivars are presented in Table 2. The swelling power of flours from different lotus seed cultivars ranged from 10.54 to 11.20 (g/g) and were not significantly different. Starch granules become increasingly susceptible to shear disintegration as they swell. Starch with a lower amylose content swells more than starch with higher amylose content. Amylopectin contributes to the swelling of starch granules and pasting, while amylose and lipids inhibit the swelling (Singh et al., 2008, Zaidul et al., 2008). Starch granules with
a low total amylose content are less rigid and swell freely when heated. The swelling power of starch depends on the water holding capacity of the starch molecules and is influenced by hydrogen bonding (Adebooye and Singh, 2008). The solubility indexes of flours from different lotus seed cultivars ranged from 28.20 to 36.29%. The solubility index of PT was higher than CP, BT and CW, respectively. Solubility provided evidence of interactions between water molecules and starch chains in amorphous and crystalline domains. The solubility of starch is dominated by the amylose content and amylopectin mainly influences the swelling power (Wang et al., 2010). The solubility index was low due to the high amylose content and low gelatinization temperature (Huijbrichts et al., 2008).

The results of WAC were similar as solubility index of Lotus seed flours (Table 2). Retention of liquid is an index indicating the ability of protein to absorb and retain water. The WAC of flours from different lotus seed cultivars ranged from 0.93 to 1.59 (ml/g). PT gives the highest WAC, which suggests that this flour is more hydrophilic due to the higher protein content (Sirivongpaisal, 2008). The high solubility index and WAC of lotus seed flours would provide better aqueous flour dispersion in food applications, as well as higher water absorption and retention (Torre-Gutiérrez et al., 2008). WAC values of 1.33-1.47 g/g were observed for chickpea flour (Kaur and Singh, 2005), 1.24-1.25 g/g for field pea flour and 1.37-1.39 g/g for pigeon pea flour (Kaur et al., 2007). The OAC of the Thai lotus seed flours was not significantly different (Table 2). All of the flours ranged from 1.5-1.55 (ml/g). Kaur and Singh (2005) reported an OAC of 1.24 g/g for soybean flour, 1.0 g/g for great northern bean flour and 1.05-1.17 g/g for chickpea flour. Variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil in the flours, might possibly explain the difference in the oil binding capacity of the flours (Adebawale and Lawal, 2003). The WAC and OAC values of seed flours indicate the ability of the protein to absorb and retain water or oil and may help to improve binding of the structure, flavour retention, and mouthfeel and reduce moisture and fat losses in extended meat products and baked products (Kaur et al., 2007; Bhat and Sridhar, 2008).

The emulsion activity (EA) and emulsion stability (ES) of flours from different lotus seed cultivars are shown in Figure 1(A). The EA of flours from different lotus seed cultivars ranged from 52.00 to 61.17%; The EA of PT was significantly higher than the others. The ES of flours from different lotus seed cultivars was not significantly different, ranging from 79.58 to 83.81%. The efficiency of emulsification by flour varies with the type, concentration and the solubility of the protein (Akubor et al., 2000). Lotus seed flours may be most useful as an additive for the stabilization of fat emulsion in the production of sausage, soup, mayonnaise, salad dressing and bakery products.

The foaming capacity (FC) and foaming stability (FS) of flours from different lotus seed cultivars are shown in Figure 1 (B) and (C), respectively. The FC of flours from different lotus seed cultivars differed significantly; the FC ranged from 22.66 to 49.99%, and PT had the highest value. The FS of flours from different lotus seed cultivars was not significantly different, but an increased correlation between FS and time was observed. Increased protein concentration has been found to facilitate enhanced protein-protein interactions at the air-water interface and promote the formation of a highly viscoelastic multilayer film that offers resistance to the coalescence of bubbles (Adebawale and Lawal, 2003). The foaming properties of lotus seed flours may be improved by the addition of sodium chloride because it enhances the ionic strength of water and solubilised protein (Bhat and Sridhar, 2007).

Syneresis of starches is an undesired property for the use of starch in both the food and non-food industries, an also is an index for degree of starch retrogradation at low temperature (Wand et al., 2010). The syneresis values of flours from different lotus seed cultivars are shown in Figure 1(D). The syneresis of CP was lower than PT, BT and CW, respectively according to syneresis increased with increasing amylose. The syneresis from the gelatinized lotus seed flour paste increased with increasing number of syneresis cycles. Lotus seed flours had high syneresis...
and low gel stability and thus were not adequate for use in food systems involving refrigeration or freezing processes. Syneresis or loss of water is an important parameter that is critical to the stability of a gel system and indicates the storage stability at low temperatures. Lower syneresis is probably due to high intracellular and intermolecular hydrogen bonding (Phimolsiripol et al., 2011).

Application of Thai lotus seed flour in bread

The results concerning the physicochemical and functional properties of Thai lotus seed flour indicate the possibility of developing various applications for these flours in food processing, due to the high protein content and functional properties, such as their enhanced ability to absorb water and oil. These flours also have high viscosities and viscosity stabilities when the temperature changes and good emulsion properties and stability. Lotus seed flours may be most useful as additives for the stabilization of fat emulsion in the production of sausage, soup, mayonnaise, salad dressing and bakery products.

The amount of Thai lotus seed flour incorporated into a bread recipe was a key factor examined in this study. Increased lotus seed flour resulted in a decrease in gas retention. All lotus seed flour-added breads had significantly lower loaf volumes and a firmer texture than the control bread and bread with 10% lotus seed flour added (Table 3). It suggests that added lotus seed flour reduces loaf volume by diluting the gluten content and changing the crumb structure, which in turn impairs CO₂ retention (Shittu et al., 2009). According to Siddiq et al. (2009), increased substitution of wheat flour with defatted maize starch (5, 10, 15 and 20%) resulted in decreased loaf volume and specific volume of breads. Wang et al. (1997) found that when the partial substitution of wheat flour with defatted soy flour was increased, the resultant volume of muffins decreased. The darkness of the crumb and crust was directly related to the lotus seed flour content increased with increasing yellowish colour (b*) and decreasing lightness (L*). The protein and moisture content, as well as the fibre and ash content of bread were increased as the amount of lotus seed flour added was increased because lotus seed flour is a good source of protein, fibre, vitamins and minerals.

Sensory evaluation results are shown in Table 3. Generally, all the samples were found acceptable to the panelists. Hedonic scores of appearance, colour, taste, softness and overall acceptance for 10% Lotus seed flour added bread were not different from the control. Hedonic scores of all attributes for 20, 30 and 40% Lotus seed flour added bread were significantly lower when compared with the control. The darker colour and unfamiliar taste to panelists of some treated samples could be contributed to lower hedonic scores when compared with the control bread. All lotus seed flour added breads had significantly

<table>
<thead>
<tr>
<th>Lotus seed flour (%)</th>
<th>Appearance</th>
<th>Colour</th>
<th>Taste</th>
<th>Softness</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.28±1.05²</td>
<td>7.33±1.07²</td>
<td>7.20±1.16²</td>
<td>7.33±1.60²</td>
<td>7.53±0.60²</td>
</tr>
<tr>
<td>10</td>
<td>7.28±1.39²</td>
<td>7.30±1.40²</td>
<td>7.03±1.40²</td>
<td>7.08±1.19²</td>
<td>7.36±0.60²</td>
</tr>
<tr>
<td>20</td>
<td>6.78±1.27²</td>
<td>8.43±1.31²</td>
<td>6.23±1.07²</td>
<td>6.25±1.27²</td>
<td>6.28±1.01²</td>
</tr>
<tr>
<td>30</td>
<td>5.20±1.82²</td>
<td>5.60±1.56²</td>
<td>5.18±1.63²</td>
<td>4.33±1.67²</td>
<td>4.89±1.47²</td>
</tr>
<tr>
<td>40</td>
<td>4.25±1.76²</td>
<td>4.55±1.73²</td>
<td>4.08±1.91²</td>
<td>3.50±1.73²</td>
<td>4.90±1.76²</td>
</tr>
</tbody>
</table>

Table 3. Effect of lotus seed flours on physical properties and sensory evaluation of bread
number of crumb cells was reduced after the addition of lotus seed flour. The decreased number of cell in lotus seed flour breads reflects the lower loaf volume and specific volume, which in turn had a significant relationship with crumb softness (Addul-Hamid and Luan, 2000; Hu et al., 2009).

Conclusion

The present study showed that lotus seed flours were important sources of protein and carbohydrates. The lotus seed flours had better functional properties especially in solubility, swelling power, WAC, OAC and emulsion properties than many conventional grains or legumes, indicating the potential for their addition to food systems, especially in bakery products, meat products, and as nutrient supplements as well as functional agents. The results of this study could provide basic information to promote the utilization of flour from lotus seeds in food products, including functional and health food supplements.

Acknowledgement

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