# Physicochemical properties of blends from tapioca starch and waxy rice for use as functional food

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**Abstract**
*The study aims to investigate the starch blends of Tapioca starch with pigmented waxy rice flours (Niawdangkarmrad and Dummor varieties) for developing to Thai pudding prepared with steaming. A 0.5:1 blend with Niawdangkarmrad had high amylose, low swelling power and high solubility index. This indicates that amylose in the crystalline regions of Niawdangkarmrad can inhibit swelling. The 0.5:1 (w/w) blend with Niawdangkarmrad was chosen because of having the highest total phenolic and anthocyanin contents. The pasting temperature of this blend was also the highest, and similar its 66.34°C glass transition temperature was highest. The retrogradation properties including breakdown, final and setback viscosity were lowest, among the starch blends. It was considered the preferred starch blend for Thai pudding prepared with steaming due to high total phenolic and anthocyanin contents.*

**Keywords:** Native tapioca starch, waxy rice flour, amylose, swelling power, total anthocyanin content

## 1. Introduction

Thailand is the most important exporter of tapioca products derived from cassava tuber (Moorthy, 2004). Tapioca starch and sticky rice flour are usually employed to make various traditional Thai pudding desserts, such as Khanom Thein, Sakoo Piek, Bua Loy and Khanom Keng among others. The native starches are unstable in functionality and suffer from molecular fragmentation, and their physicochemical properties in native form are not optimal for the various applications in food industry. The native tapioca starches tend to have no ionic charge, and their functionality can be modified by phosphorylation that gives a negative charge, to make the molecules repel each other. Phosphorylated tapioca starch has reduced breakdown during cooking, reduced swelling power and solubility, and is more heat and shear resistant than native tapioca starch (Muhammad et al., 1999). A current market trend is to try to replace chemical treatments of food substances by the use of natural components (Ortega-Ojeda & Eliasson, 2001). Starch modifications are accomplished in various ways, including enzymatic, chemical, and physical treatment that may alleviate the disadvantages of native starches in their degradability or physicochemical properties. However, native tapioca starch can alternatively be modified by blending it with another starch, which is easy to do and safe for the environment, without the use of chemicals. A starch blend can provide improved properties over the native starches, in pasting behavior (Chen, Lai, & Lii, 2003), texture and freeze-thaw stability (Karam et al., 2005). Important factors affecting properties of the blend include the concentrations of starches (Liu, & Lelievre, 1992), their mixing ratio (Ortega-Ojeda, & Eliasson, 2001, Chen, Lai, & Lii, 2003,), their chemical compositions, especially amylose contents (Ortega-Ojeda, Larsson, & Eliasson, 2004, Sasaki, Yasui, Matsuki, & Satake, 2007), their swelling-solubility power (Chen, Lai, & Lii, 2003) and interactions between the granules (Obanni, & BeMiller, 1997).

The composition of rice granules varies depending on the variety of rice. Amylose content is approximately15-27% in non-waxy rice, whereas waxy rice varieties have nearly 100% amylopectin content. Such difference in compositions contributes to different properties (Guy, 1994). Hence, waxy rice flour has lower gelatinization temperature and lower retrogradation than non-waxy rice flour. The uses of native rice flours are limited by physical and chemical properties (Manson, 2009). Correlation between starch structure and its gelatinization temperature have been reported, and the important structural features include amylose content, molecular size, average degree of polymerization and chain length distribution. This affect the various physicochemical properties of starch granules, such as swelling power, thermal, rheological and textural properties (Varavinit et al., 2003, Vandeputte et al., 2003a, Vandeputte et al., 2003b, Singh et al., 2006, Jayakody et al., 2007, Sandhu, & Singh, 2007).

The objective of this study was to improve the molecular properties of native tapioca starch with the amylopectin supplementation in the form of pigmented waxy rice flour. The pigmented rice flour should be beneficial to human health and nutrition, as the bioactive substances in pigmented rice reduce the risk of various chronic diseases (Kay, Kroon, & Cassidy, 2009, He, & Giusti, 2010). The blends of native tapioca starch with the pigmented waxy rice flour are introduced as an alternatively to actual starch modifications, in order to change the chemical and physical properties of importance in food uses of starch. The blend ratio is here chosen with a view to functional starch applications in Thai pudding desserts prepared with steaming.

## 2. Materials and Methods

### 2.1. Materials

Commercial native tapioca starch was kindly supplied by Siam Modified Starch Co., Ltd. (Thailand). The varieties of waxy rice used were Niawdangkarmrad and Dummor, and the samples were ground with dry mill to obtain flours. These varieties were supplied from Pattani Rice Research Center, Pattani province, Thailand. A control waxy rice flour was purchased from the local supermarket. After milling, the flours were passed through a sieve with mesh No.10 (1.65 mm holes). The native tapioca starch (Niawdangkarmrad and Dummor) flours were dried at 105°C overnight, in a hot air cabinet, which gave constant weight indicating complete drying. Amylose standard from potato was purchased from Sigma-Aldrich (Steinheim, Germany), for use in determinations of amylose content in the control starch and in the pigmented waxy rice flours.

**2.2. The preparation of starch blends**

The blends of native tapioca starch and waxy rice flours (Niawdangkarmrad and Dummor) were prepared on weight basis, following the method described by Zaidul et al. (2007). The tapioca/rice weight ratios in the blends were 0.5:1, 1:1, 2.5:1, 5:1 and 10:1.

**2.3. Determination of swelling power and solubility index**

Swelling power and solubility index were determined with the method of Rosell et al. (2011). A 0.25 g of the blend was dissolved in 1 mL of the distilled water. The slurry was heated in water bath at 70°C for 20 min. Then it was centrifuged at 3,500 rpm for 20 min. The supernatant obtained was transferred into a pre-weighed evaporating dish and dried at 105°C overnight and the solid residue was weighed ($W_s$) for the solubility. The swelling power was obtained by weighing the residue after centrifugation ($W_r$), and dividing this by the initial dry weight of the starch ($W_i$). The determinations were done in triplicates, and the data are reported here as mean±SD. The swelling power and solubility index were calculated as following equations;

$$\text{Swelling power (g/g)} = \left(\frac{W_r}{Wi(100-SI)}\right) \quad (1)$$

$$\text{\%Solubility index (SI)} = \left(\frac{W_s}{W_i}\right) \text{x } 100 \quad (2)$$

**2.4. Determination of apparent amylose content**

The apparent amylose content was measured by using the iodine absorption procedure of Hoover and Ratnayake (2002). Briefly, 2 mL of 1 M Sodium hydroxide was added into the aliquot of suspended starch (0.2 g of the blend in 4 mL of the distilled water). The starch solution was mixed thoroughly and heated to 100°C for 10 min. Then 5 mL of 0.5%trichloroacetic acid (TCA) was added to the solution and mixed by vortex, after which 50 mL of Iodine reagent (0.32 g $I_2$ and 0.75 g potassium iodide (KI) were mixed and the volume adjusted to 250 mL with distilled water) was added. Then the sample was incubated in the dark for 30 min, and the absorbance was measured at $\lambda_{max}$ = 620 nm on a UV-Vis spectrophotometer. Amylose from potato was used as the standard for the calibration curve for the concentration range with 1-10% of potato amylose (w/w). The determinations were done in triplicates and data were expressed as mean±SD.

**2.5. Phase transitions of starch blends**

**2.5.1. Gelatinization temperature**

The thermal properties determined were gelatinization temperature, retrogradation properties and amylose-lipid complexation for control starch and the starch blends.

The gelatinization temperature was determined using a Differential Scanning Calorimeter (DSC7, PerkinElmer, USA). Before the operation of DSC7, it was calibrated with indium. The system was purged with ultra-high purity nitrogen gas, and the temperature set at 25°C. Each sample was weighed to 10 mg dry weight in a silver pan. Then distilled water was added into the pan in order to make a suspension (starch:water = 1:3, w/v). The DSC was first heated at a rate of 5°C $min^{-1}$ from 25 to 150°C, and the gelatinization temperature was determined along with the enthalpy change ($\Delta H_{gel}$, in j $g^{-1}$) of the blend.

The second DSC scan assessed the formation of amylose-lipid complexes. After the first scan was completed, the sample was held at 150°C for one minute. The annealing was by cooling from 150°C to 30°C at a rate of 5°C $min^{-1}$. Finally, a heating re-scan was run from 30°C to 150°C at a rate of 5°C $min^{-1}$. All determinations were done in triplicates.

### 2.5.2. The characteristic of pasting properties

The pasting properties of the control and the starch blends were measured using a Rapid Visco-Analyzer (RVA, Newport Scientific Pvt. Ltd., Australia). Each sample was suspended in 25 mL distilled water (6% w/w, dry basis). The suspensions was heated to 50°C and held at this temperature for 1 min, then the temperature was increased to 95°C at 5°C $min^{-1}$ and held for 10 min. After that, the sample was cooled down to 50°C at 3°C $min^{-1}$ and held at this temperature for 10 min. The stirring speed was at 160 rpm. The pasting temperature is the onset temperature of increased viscosity. Breakdown is defined as the difference between the peak viscosity and the minimum viscosity during the holding phase at 95°C. Setback is defined as the difference between the minimum viscosity at 95°C and the final viscosity. The determinations were done in triplicates and results were expressed as mean±SD.

## 2.6. Determination of total phenolic contents

Total phenolic content was determined by the Folin-Ciocalteu colorimetric method (Chlopicka et al., 2012). A 200 µL of sample solution was added to 1.90 mL of the Folin-Ciocalteu reagent. After 5 min, the solution was mixed with 1.90 mL of 6% sodium carbonate. The mixture was allowed to stand at room temperature for 120 min. The absorbance of the mixture was measured at 725 nm. The standard curve for ferulic acid covered the range of 5-100 µg $mL^{-1}$ and the results were expressed as mg ferulic acid equivalent per gram of sample.

## 2.7. Determination of total anthocyanin contents

The total anthocyanin content was determined by the pH differential method (Giusti, & Worlstad, 2001). Two fractions of the same sample were prepared in 0.025 M potassium chloride solution (in 0.4 M sodium acetate solution) adjusted to pH 1.0 (to pH 4.5) with 5 M hydrochloric acid. The absorbances of each fraction were measured at 520 and 700 nm against a distilled water blank using UV-Vis spectrophotometer. The measured absorbances were used as following equations:

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \qquad (3)$$

$$\text{Anthocyanin content (mg } L^{-1}) = (A \times MW \times DF \times 1000) / (\varepsilon \times 1) \qquad (4)$$

Whereas MW is the molecular weight of cyanidine 3-*O*-glucoside (449.2 g $mol^{-1}$), DF is the dilution factor, and ε is the molar extinction coefficient of cyanidine-3-*O*-glucoside (ε = 26,900 L $cm^{-1}$ $mol^{-1}$)

## 2.8. Statistic analysis

All determinations were done in triplicates and expressed as mean±SD. The mean and standard deviations were calculated using Microsoft Excel spreadsheet.

# 3. Results and Discussion

## 3.1. Proximate analysis and chemical compositions

The native tapioca starch and waxy rice flours were dried at 105°C overnight prior to the experiments. Each measurement is referenced to dry sample weight. According to Table 1, the contents of Niawdangkarmrad and Dummor flour were higher than that of native tapioca starch. This confirms the expectation that waxy rice flours are comparatively rich in minerals and inorganic components. Also the protein contents of the waxy rice flours were higher than that in native tapioca starch. The degree of milling of these waxy rice flours was about 75% prior to all analyses, and they were produced by dry milling. Only a small amount of lipid remained in the endosperm after milling, and the low <1% lipid contents of Niawdangkarmrad (0.40±0.01%) and Dummor (0.39±0.01%) were complexed with the mainly amylopectin starch and the small contents of amylose.

**Table 1**. The chemical composition of native tapioca starch and the waxy rice flours.

| Starch/flour | %Moisture | %Ash | %Lipid | %Protein | %Carbohydrate |
|---|---|---|---|---|---|
| Niawdang | 8.63 ± 0.13 | 0.53 ± 0.01 | 0.40 ± 0.01 | 5.69 ± 0.79 | 84.75 ± 1.33 |
| Dummor | 7.54 ± 0.24 | 0.37 ± 0.02 | 0.39 ± 0.01 | 5.27 ± 0.82 | 86.43 ± 1.54 |
| Tapioca | 5.89 ± 0.59 | 0.11 ± 0.01 | 0.44 ± 0.06 | 0.44 ± 0.06 | 93.12 ± 1.02 |

The waxy rice flours were from Niawdangkarmrad and Dummor varieties.
“Tapioca” indicates native tapioca starch.
All measurements were done in triplicates.

### 3.2. Apparent amylose content

Prior to studying swelling power and solubility index, the amylose contents of the starch blend were investigated, including pure (unblended) native tapioca starch, commercial sticky rice starch (Baiyok), and pure Niawdangkarmrad and Dummor flours. Generally, the smallish amylose content in waxy rice flour depends on the variety.

The results in Table 2 indicate a lower 0.73 ± 0.10% amylose content in Dummor than the 2.18±0.10% for Niawdangkarmrad. In the starch blends, the amylose content of native tapioca starch acted as a diluents (Tester, & Morrison, 1990) and the amylose contents of the blends with Niawdangkarmrad were not higher than of this rice flour alone. At the blend ratio 0.5:1 for Tapioca : waxy rice, the 1.38 ± 0.06% amylose content with Niawdangkarmrad was higher than the 1.23 ± 0.11% content in the similar blend with Dummor flour.

**Table 2**. Apparent amylose contents of the starch blends, including blends with commercial sticky rice starch (Baiyok) for baseline reference.

| Source | Ratio of blending | Amylose content (%) |
|---|---|---|
| Tapioca* | | 22.09 ± 3.15 |
| Baiyok | | 1.43 ± 0.10 |
| Niawdangkarmrad | | 2.18 ± 0.10 |
| Dummor | | 0.73 ± 0.10 |
| Tapioca:Niaw | 0.5:1 | 1.38 ± 0.06 |
| | 2.5:1 | 0.90 ± 0.12 |
| | 5:1 | 0.51 ± 0.02 |
| | 10:1 | 0.38 ± 0.07 |
| Tapioca:Dum | 0.5:1 | 1.23 ± 0.11 |
| | 2.5:1 | 0.18 ± 0.03 |
| | 5:1 | 0.48 ± 0.02 |
| | 10:1 | 0.43 ± 0.13 |
| Tapioca:Baiyok | 0.5:1 | 1.49 ± 0.60 |
| | 2.5:1 | 0.21 ± 0.06 |
| | 5:1 | 5.93 ± 0.92 |
| | 10:1 | 2.75 ± 0.83 |

Tapioca = native tapioca starch, Baiyok = commercial sticky rice starch,
Niaw = Niawdangkarmradwaxy rice flours, Dum = Dummorwaxy rice flours
%Amylose is the apparent amylose content determined by a colorimetric method.
* %Amylose in native tapioca starch has been reported as 15-21% in prior literature [34].
All measurements were done in triplicates.

### 3.3. Swelling power and solubility index

Swelling power and solubility index indicate interactions between the water molecules and the amylose and amylopectin in starch granule (Sodhi, & Singh, 2003). Cui and Oates reported that amylose-lipid complexes restrict the swelling and solubilization of starch (Hoover, 2001). Consequently, the leaching of amylose out from starch granule decreases at elevated temperatures where amylose-lipid complexes are formed (Cui, & Oates, 1999). From Table 1, the lipid contents of these starches were smallish. Therefore, the amylose-lipid complexation did not influence the swelling of these starch blends. The results in Table 3 indicate that the comparatively high amylose content in native tapioca starch may have leached out from the starch granule at 70°C temperature used to test swelling. The excess water accessed and hydrated, along with amylose leaching, also amylopectin region in the amorphous structure. Therefore, the swelling power of native tapioca starch was higher than of the waxy rice flours. However, the amylose in the crystalline regions is strongly bound within the starch granules, possibly causing the low solubility of native tapioca starch relative to the waxy rice flours. The swelling power of blends with Niawdangkarmrad flour was the lowest at blend ratio of 0.5:1, and the swelling slightly increased with the portion of native tapioca starch while solubility decreased. Dummor had the opposite pattern of behavior in the blends. The swelling power of blends with Dummor flour did not tend to increase, because of the 0.73%amylose content in Dummor flour being much lower than the 2.18% in Niawdangkarmrad. Therefore, the leached amylose did not influence the swelling power of blends with Dummor flour, and the solubility did not change much either because of the small amount of leached amylose. The amylose and amylopectin leached from the amorphous region of starch granules tend to affect the swelling behavior of waxy rice flour. The intermolecular forces between water molecules and these amorphous domains include hydrogen bonding, and impact the swelling and solubility of starch granules (Hoover, 2001), so that the solubility index correlates with only the leached amylose.

**Table 3**. Swelling power and %solubility of starch blends.

| | | Swelling power, $gg^{-1}$ | %Solubility |
|---|---|---|---|
| Tapioca | | 3.45 ± 0.64 | 0.44 ± 0.14 |
| Niaw | | 2.47 ± 0.08 | 4.74 ± 0.78 |
| Dum | | 2.58 ± 0.24 | 5.52 ± 0.82 |
| Tapioca:Niaw | 0.5:1 | 2.76 ± 0.21 | 3.11 ± 0.49 |
| | 1:1 | 3.47 ± 0.22 | 2.64 ± 0.45 |
| | 2.5:1 | 3.48 ± 0.21 | 1.37 ± 0.12 |
| | 5:1 | 4.58 ± 0.15 | 0.28 ± 0.03 |
| | 10:1 | 4.49 ± 0.16 | 0.96 ± 0.14 |
| Tapioca:Dum | 0.5:1 | 3.88 ± 0.22 | 1.79 ± 0.55 |
| | 1:1 | 4.56 ± 0.06 | 0.78 ± 0.18 |
| | 2.5:1 | 4.30 ± 0.08 | 0.68 ± 0.04 |
| | 5:1 | 3.88 ± 0.14 | 1.06 ± 0.08 |
| | 10:1 | 3.82 ± 0.18 | 1.01 ± 0.10 |

All measurements were done in triplicates.

### 3.4. Total phenolic and anthocyanin contents in the blends

The 75% degree of milling still retains some pigment of the waxy rice endosperm. The pigmented waxy rice flours in human nutrition may help prevent some chronic disorder. The mechanism underlying such cardio- and neuroprotection may relate to the antioxidant properties of dietary phenolics, including flavonoids and anthocyanins (Chong, Macdonald, & Lovegrove, 2010). The blends of native tapioca starch with Niawdangkarmrad had higher total phenolic and anthocyanin contents than the blends with Dummor, as shown in Table 4. Particularly, the blend ratios 0.5:1 and 1:1 with the waxy rice flours demonstrated high total phenolic and anthocyanin contents. These bioactive compounds were considered the main criteria of a beneficial functional starch ingredient in Thai pudding desserts, so that the blends with listed anthocyanin contents in Table 4 were further assessed for their gelatinization thermal behavior and retrogradation properties.

**Table 4**. Total phenolic and anthocyanin contents in the starch blends.

| Source | Blending | Total phenolic $mgL^{-1}g^{-1}$ samples | Total anthocyanincontents, $mgL^{-1}g^{-1}$ samples |
|---|---|---|---|
| Tapioca | - | - | - |
| Niaw | - | 468.21 ± 22.31 | 6.17 ± 0.55 |
| Dum | - | 246.67 ± 33.33 | 2.03 ± 0.68 |
| Tapioca:Niaw | 0.5:1 | 384.69 ± 22.09 | 5.48 ± 0.79 |
| | 1:1 | 303.21± 18.64 | 4.54 ± 0.13 |
| | 2.5:1 | 169.88 ± 10.20 | - |
| | 5:1 | 90.86± 2.14 | - |
| | 10:1 | 37.16± 8.75 | - |
| Tapioca:Dum | 0.5:1 | 234.11 ± 21.05 | 1.17 ± 0.22 |
| | 1:1 | 160.89 ± 11.15 | - |
| | 2.5:1 | 113.27 ± 11.48 | - |
| | 5:1 | 78.15 ± 2.73 | - |
| | 10:1 | 63.27 ± 12.16 | - |

All measurements were done in triplicates.

**3.5. Phase transition behavior and retrogradation properties**

The transformation in the granules under heating or cooling in excess water is associated with the molecular mobility of amylopectin and their order-disorder molecular processes. The variation in starch composition (amylose to amylopectin ratio) and the presence or absence of lipids to complex with amylose affect both gelatinization behavior and swelling properties (Hoover, 2001). However, the amylose-lipid complexation effects were small in this study. Futhermore, in waxy rice the glass transition is dominated by amylopectin in amorphous domain, with its phase transition from rubbery to glassy state. The melting peaks were not detected in the DSC heating scans, as they were below 25°C due to low amylose contents in the blends. The phase transitions of amylopectin domain were represented by glass transition temperature shown in Table 5. Adding tapioca to the pure waxy rice flours reduced their glass transition temperatures consistently. The pasting temperature is a technically important characteristic of the viscous paste with starch granules, and its behavior was similar to the glass transition temperature: tapioca addition consistently reduced it. The low amylose content in Dummor (0.73±0.10%) caused the comparatively high final viscosities of its blends, because the amylopectin leached out of starch granules creates crosslinks on network formation. The amylose crystalline domains in the granules also impact the retrogradation properties. It has been noted that swelling power may contribute to peak viscosity and breakdown viscosity (BeMiller, 2011). As shown in Table 5, the blends with Niawdangkarmrad that had low swelling power also had low peak viscosities and retrogradation properties. This might relate to the weak hydrogen bonds with water or other starch granules. The blends with Niawdangkarmrad had high pasting and glass transition temperature, making them sensitive to break down by shear forces at elevated temperatures. Meanwhile, the tapioca starch had the highest swelling power and the highest breakdown value, and it is known to be resistant to heat and shear forces (Saeleaw, & Schleining, 2010).

**Table 5**. Glass transition temperature, pasting temperature andretrogradationproperties of the blends

| Sources | $T_g$ (°C) | Pasting temp. (°C) | Peak viscosity (cP) | Breakdown viscosity (cP) | Final viscosity (cP) | Setback viscosity (cP) |
|---|---|---|---|---|---|---|
| Tapioca | 61.16 | 69.73±0.54 | 4,370.67±25.54 | 2,537.0±37.75 | 2,411.33±37.07 | 577.67±46.61 |
| Niaw | 66.85 | 75.10±0.05 | 1,330.0±14.0 | 263.33±12.50 | 1,295.67±6.03 | 229.0±1.0 |
| Dum | 66.34 | 75.33±0.49 | 1,925.33±18.15 | 510.33±25.70 | 1,654.0±8.0 | 239.0±1.0 |
| Tapioca:Niaw 0.5:1 | 66.34 | 72.70±0.10 | 1,830.0±6.0 | 606.0±11.27 | 1,759.0±3.46 | 535.0±9.54 |
| Tapioca:Niaw 1:1 | 63.37 | 71.62±0.54 | 2,242.0±31.13 | 942.67±22.81 | 1,840.33±19.55 | 551.33±15.95 |
| Tapioca:Dum 0.5:1 | 63.54 | 72.67±0.06 | 2,307.67±10.02 | 821.33±16.20 | 2,050.67±9.71 | 564.33±14.01 |

All measurements were done in triplicates.

## 4. Conclusions

A 0.5:1 blend of tapioca starch and Niawdangkarmrad flour was considered the preferred starch blend for Thai pudding prepared with steaming. This starch blend can act as a functional ingredient due to its high 384.69±22.09 mg $L^{-1}$ $g^{-1}$ sample total phenolic and 5.48±0.79 mg $L^{-1}$ $g^{-1}$ sample anthocyanin contents. This blend had comparatively low crystallinity, high swelling power, and low retrogradation temperature, but high pasting temperature. These physicochemical characteristics of a starch blend were dominantly determined by the waxy rice component of the blend. The starch blend is proposed as a novel functional ingredient for Thai pudding prepared with steaming, with potential nutrition and health benefits.

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