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# Impact of pre-germination on amylopectin molecular structures, crystallinity, and thermal properties of pre-germinated brown rice starches

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ABSTRACT

Ungerminated brown rice (UGBR) and pre-germinated brown rice (PGBR) obtained from different pre-germination durations were studied to investigate the changes in total starch contents of flour, amylopectin molecular structures, crystallinity, and thermal properties of starches as affected by pre-germination. Each paddy of three rice cultivars with different amylose contents (RD6, waxy; KDML105, low amylose; and RD31, high amylose) was soaked in water at 30°C for 12 h and incubated over different periods until the three stages of embryonic growth length (EGL) were achieved. The total starch contents of three-stage PGBR flour from all rice cultivars decreased when pre-germination durations were increased. The three-stage PGBR starches from the three rice cultivars had lower weight-average molecular weight (Mw) and number-average molecular weight (Mn) than UGBR starches. All starches from the three rice cultivars displayed an A-type X-ray diffraction pattern (XRD). Isolated UGBR starch from RD6 had the highest (31.33%) relative crystallinity (RC), while RD31 showed the lowest RC (26.79%). The slight increases in the RC of three-stage PGBR starches from three rice cultivars were found after pre-germination. Isolated PGBR starches from the three rice cultivars had higher gelatinization temperatures and enthalpy, but lower retrogradation enthalpy and %retrogradation than UGBR starches.



## 1. Introduction

Rice starch consists of two polysaccharides: amylose (low molecular weight; $10^5$–$10^6$) and amylopectin (high molecular weight; $10^7$–$10^8$) (Hizukuri, 1986). Rice starch properties are influenced by the amylose/amylopectin ratios, amylopectin fine structures, and the crystalline structures (Jane et al., 1999).

Pre-germinated brown rice (PGBR) was produced by soaking paddy in controlled temperature water at 30°C for 12 h and incubating over different periods until the three stages of embryonic growth length (EGL) were achieved (Pinkaew and Naivikul, 2012; Pinkaew et al., 2016). During pre-germination, hydrolytic enzymes were activated to break down high molecular weight rice starch and other components to supply energy for germination (Kaneko et al., 2002).

Germination induced important changes in starch fine structures (Xu et al., 2012; Wu et al., 2013; Pinkaew et al., 2016) and chemical compositions, including starch yields (Xu et al., 2012; Pinkaew et al., 2016), total starch contents (Xu et al., 2012; Wu et al., 2013), and apparent amylose contents (AACs) (Xu et al., 2012; Wu et al., 2013; Pinkaew et al., 2016). Both the starch degradations and the reductions of the AACs affected the pasting properties of germinated brown rice (GBR) starches. Germination also affected amylopectin branch chain length distributions, which led to the changes in the pasting properties of GBR starches (Xu et al., 2012; Pinkaew et al., 2016).

*Abbreviations:* AAC, Apparent amylose content; EGL, Embryonic growth length; GBR, Germinated brown rice; ΔHgel, Gelatinization enthalpy; HPSEC-MALLS-RI, High performance size-exclusion chromatography with multi-angle laser light scattering and refractive index detector; ΔHret, Retrogradation enthalpy; KDML105, Khao Dawk Mali105; Mn, Number-average molecular weight; Mw, Weight-average molecular weight; Mw/Mn, Polydispersity index; PGBR, Pre-germinated brown rice; RC, Relative crystallinity; RD31, Rice Division31; RD6, Rice Division6; Rz, Average radius of gyration; Tc, Conclusion temperature; To, Onset temperature; Tp, Peak temperature; UGBR, Ungerminated brown rice; XRD, X-ray diffraction patterns.

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A few studies have reported the effects of germination on starch molecular structures. After a regular long-grain brown rice was soaked in distilled water at room temperature for 12 h and then germinated at 30°C for 24 h with 65% relative humidity (RH) under biological oxygen demand (BOD), the GBR starch had a much lower number average molecular weight (Mn; $3.76\times10^5$ g/mol) than that of ungerminated brown rice (UGBR) starch ($7.06\times10^5$ g/mol). Thus, the GBR starch showed a higher polydispersity (Mw/Mn) value (3.18) than that of UGBR starch (2.48), which indicated that starch molecules became more non-uniform (Xu et al., 2012). In addition, after the three Chinese brown rice cultivars (medium-grained waxy, medium-grained japonica and long-grained indica) were soaked in deionized water at 25°C and germinated in the dark for 5 days, both the weight-average molecular weight (Mw) and Mn values differed significantly ($P < 0.05$) among three rice cultivars. The Mw values of isolated UGBR starches from medium-grained waxy, medium-grained japonica and long-grained indica were 2.83, 2.39, and $1.80\times10^8$ g/mol, respectively, while the GBR starches at the 1st to 5th days of germination from those three rice cultivars were in the range of $2.84–2.86\times10^8$ g/mol, $2.41–2.48\times10^8$ g/mol, and $1.78–1.87\times10^8$ g/mol, respectively (Wu et al., 2013).

The crystallinity of brown rice starch was also affected by germination. The differences in major reflections and relative crystallinity (RC) between UGBR and germinated Malaysian brown indica rice starches have been reported (Musa et al., 2011).

Germination has been reported to affect the thermal properties of brown rice starch. The decreases in the gelatinization temperatures and enthalpy of germinated long-grain brown rice starch have been reported (Xu et al., 2012). The gelatinization temperature was not affected by germination for medium-grained waxy while there was a slight decrease for medium-grained japonica and long-grained indica after 2 days of germination (Wu et al., 2013).

Although many studies have investigated the effect of germination on chemical compounds, fine structures, and physicochemical properties of brown rice starches, there is less information on the impact of germination on starch molecular structure properties, crystallinity, and thermal properties. In addition, the information of germinated paddy was less pronounced than those of germinated brown rice. Thus, studies in the effect of pre-germination by using paddy could fulfill the knowledge gap between pre-germinated brown rice and paddy.

In the current research, the amylopectin molecular structures, RC, and thermal properties of UGBR and three-stage PGBR starches from three Thai paddy cultivars with different amylose contents were determined to provide useful information regarding the potential uses of PGBR as raw material for specific food products.

## 2. Materials and methods

### 2.1. Materials

Three Thai paddy cultivars (*Oryza sativa* L.): Rice Division6 (RD6, waxy, 6.2–6.5% amylose content); Khao Dawk Mali105 (KDML105, low amylose, 16.6–17.4%); and Rice Division31 (RD31, high amylose, 29.7–31.6%) rice from the 2011 crop year were obtained from the Rice Research Center (Thailand). Analytical grade chemicals were used in the study unless otherwise noted.

### 2.2. Preparation of pre-germinated paddy and pre-germinated brown rice flour

The pre-germination process was conducted as described in our previous work (Pinkaew and Naivikul, 2012; Pinkaew et al., 2016). Paddy was soaked in 1.7% (w/v) sodium chloride solution for 30 min to suppress mold growth, followed by washing 4 times with tap water. The cleaned paddy grains were soaked in tap water at a controlled temperature of 30°C for 12 h. The soaking water was changed every 6 h to prevent microorganism fermentation. Soaked paddy was removed and allowed to incubate at a controlled temperature of 30°C and 85% RH until the three stages of EGL were achieved,: first stage (0.5–1 mm, 60–70% of pre-germination), second stage (1–2 mm, 71–80% of pre-germination), and third stage (2–3 mm, more than 80% of pre-germination). The pre-germination times for 1st to 3rd stages of each rice cultivar were: RD6 (28, 32, and 36 h); KDML105 (36, 44, and 48 h); and RD31 (32, 36, and 44 h), respectively. After that, each pre-germinated paddy was dried at $45 \pm 10$°C until the moisture content was less than 12%. Samples of ungerminated and the three-stage pre-germinated paddy were dehulled prior to being finely ground by a pin mill (Alpine Ausburg 160 Z 1979, Germany). The flour from UGBR and the three-stage PGBR were passed through a 100-mesh sieve, packed in plastic bags, and stored at −18°C for further use.

### 2.3. Determination of total starch content

The total starch content of flour was determined by Megazyme assay kit (Megazyme Intl. Ireland Ltd., Wicklow, Ireland) in accordance to Approved Method 79-13 (AACC, 2000). Duplicate measurements were taken.

### 2.4. Rice starch isolation

Samples of UGBR and the three-stage PGBR flour (200 g each) were defatted with 800 mL hexane and dried under a fume hood at room temperature for at least 24 h (Agboola et al., 2005). The defatted flour was then used for starch isolation using alkali extraction involving steeping defatted flour in 0.2% NaOH solution (Suksomboon and Naivikul, 2006; Pinkaew et al., 2016). The dried isolated starch (moisture content 12%) was finely ground using a hammer mill grinder with a 0.5-mm sieve, then passed through a 100-mesh sieve, packed in plastic bags, and stored at −18°C for further analyzes.

### 2.5. Experimental design

A 3×4 full factorial in completely randomized design (CRD) was used. The main effects were three rice cultivars (RD6, KDML105, and RD31) and four pre-germination durations (ungerminated, pre-germinated at the first, second, and third stage). Each treatment combination had three replicates.

### 2.6. Amylopectin molecular structures

Amylopectin molecular structures were analyzed in duplicate using high-performance size-exclusion chromatography (HPSEC) with multi-angle laser light scattering (MALLS) and refractive index (RI) detector (HPSEC-MALLS-RI) (Patindol et al., 2007). Ten mg of isolated starch was dispersed in 2 mL of LiBr (50 mM) in 90% DMSO, and heated in a boiling water bath for 30 min, cooled, and stirred overnight. A 0.5 mL aliquot was precipitated with 10 mL methanol, allowed to stand for 30 min, and centrifuged at 3000 rpm for 10 min. The supernatant was carefully discarded, and the precipitated amylopectin was re-dispersed in 5 mL of LiBr in 90% DMSO, heated in a boiling water bath for 30 min, cooled for 5 min, centrifuged at 3000 rpm for 10 min, and then 200 μL was injected into HPSEC-MALLS-RI system.

The HPSEC-MALLS-RI system consisted of a 515 HPLC pump with a 200 μL sample loop (Waters, Milford, MA, USA), an inline degasser, a DAWN-EOS 18-angle light scattering detector (Wyatt Technology, Sta. Barbara, CA, USA) and an Optilab rEX refractive