

Effect of pre-germination process on molecular weight degradation and retrogradation of pre-germinated brown rice starches

Warunee Kupkanchanakul¹ and Onanong Naivikul^{1*}

ABSTRACT

Paddy from two Thai rice cultivars as San-Pah-Tawng1 (SPT1; 5.25%db amylose) and Pathum-Thani1 (PTT1; 17.77%db amylose) were soaked at 30°C for 12 hours before pre-germinated in an incubator at 30°C, 85% relative humidity (RH) to get embryonic growth length at their first (0.5-1mm), second (1-2mm), third (2-3mm) and malted (3-7mm) stages. Then the chemical properties, starch molecular weight distribution and thermal properties, especially retrogradation, of pre-germinated brown rice starch (PGBRS) were determined by using brown rice starch (BRS) as a control. The total starch of brown rice from both rice cultivars (80.94-81.13%db) were significantly decreased (78.16-79.01%db) but the damaged starches were increased during the four stages of pre-germination. After pre-germination process, the amylose content of SPT1 was decreased to 4.75%db but that of PTT1 was increased to 18.73%db. When the average molecular weight and the molecular weight distribution of PGBRS molecules were revealed by using gel permeation chromatography (GPC), it was found that the rice starch molecule fractions, low molecular weight (MW) fraction (F2) ($10^{2.5}$ - $10^{6.5}$ g/mol) and high MW (F1) ($10^{6.5}$ - $10^{8.5}$ g/mol), were occurred on both rice cultivars. Increasing of low MW starch fractions after rice pre-germination process was observed from SPT1 (45.07 to 50.23%) and PTT1 (54.59 to 72.95%) which indicated that rice starch molecules were degraded as the embryonic growth length increased. The results from the differential scanning calorimeter (DSC) showed that the gelatinization temperatures of PGBRS from both rice cultivars had higher values than that of BRS. Then all gelatinized samples were kept at 4°C for 7days, the results showed that the delta enthalpy (ΔH_{ret}) of PGBRS from SPT1 was significantly decreased ($p<0.05$) until the third stage (from 0.51 to 0.28J/g) but the malted stage was increased from the third stage to 0.40J/g, while that of PGBRS from PTT1 was significantly decreased ($p<0.05$) from 1.24 to 0.72J/g. This result indicated that the starch molecules after pre-germination process were retrograded less than native starch molecules. Therefore, the pre-germination process could be applied to develop various rice products, such as rice bread.

Keywords: rice pre-germination process, pre-germinated brown rice starch, starch molecular weight, retrogradation

*E-mail address: fagionn@ku.ac.th

¹ Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, 10900.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of staple food in the world. Approximately 80% of rice is Indica type, which is the major rice planted in Thailand and many Asian countries. Thai rice has a very wide diversity which could be grouped by using cooked texture as waxy and non-waxy. Moreover, the non-waxy could be sub-grouped by using the ratio of two types of alpha-glucan, amylose and amylopectin, in starch granule as low amylose rice (12–20%), medium amylose rice (20–25%) and high amylose rice (>25%) (Bao and Bergman, 2001).

Germination process, especially the early stage of germination as pre-germination, could be considered as bio-modification or enzymatic modification of starch within rice grain. During germination process, amylolytic enzymes (alpha-amylase, beta-amylase, alpha-glucosidase and debranching enzymes) were activated, which degrade rice starch molecules to change the characteristic of PGBRS (Xu *et al.*, 2012). Therefore, the pre-germinated brown rice flour (PGBRF) has received much attention to promote for improvement the soften texture, an increase flavor components (Ohtsubo *et al.*, 2005; Wu *et al.*, 2011), nutritional components changed to get better health benefits (Wu *et al.*, 2013) and the decreasing of viscosity, which are benefits to apply the PGBRF for the new rice products as rice cake (Chaichaw *et al.*, 2011) and rice bread (Kupkanchanakul and Naivikul, 2012).

Two Thai rice cultivars as SPT1 and PTT1, are represented the two group of rice cultivars, which are categorized by the ratio of starch molecules, amylose and amylopectin. Therefore, the aim of this study was to investigate effect of pre-germination process on molecular weight degradation and retrogradation behavior between waxy and non-waxy Thai rice starches.

MATERIALS AND METHODS

Materials

The two Thai rice cultivars, San-Pah-Tawng1 (SPT1; 5.25%db amylose) and Pathum-Thani1 (PTT1; 17.77%db amylose), were purchased from Rice Department; Thailand, packed in plastic bag and kept in freezer before being used.

Methods

1. Determination the condition of pre-germinated paddy (PGP)

1.1 The soaking time

One kilogram of paddy was sorted to remove any foreign matters and to separate the immature (float) paddy out of the mature paddy (submergence) by soaking in three liters of 1.7%w/v sodium chloride solution (1.08 specific gravity). After that, the mature paddy was soaked in the warm water at 30°C for 18 hr. The moisture content of soaked paddy was measured every 2 hr. The soaking time to reach the paddy moisture content higher than 30% for each cultivar was selected to prepare four stages of the pre-germinated paddy (PGP).

1.2 The incubation time

The moist-paddy was incubated in an incubator at 30°C, 85%RH to get embryonic growth length (more than 80% of germination rate) at their first (0.5-1mm), second (1-2mm), third (2-3mm) and malted (3-7mm) stages. The incubation time to reach each stage of PGP was detected.

2. Pre-germinated brown rice flour and starch preparation

The PGP was dehusked and milled by using pin miller. Then, the milled pre-germinated brown rice was sieved pass through a 100-mesh sieve. All the samples were stored at -18 °C in hermetically sealed plastic containers before analyzed. The PGBRS were isolated by using alkaline method following Lumdubwong and Seib (2000).

3. Chemical characteristics of rice starch

The moisture content was determined by the AACC method 44-31A (AACC, 2000). The total starch (AACC method 76.13) and starch damage (AACC method 76-31.01) were determined enzymatically with a Megazyme Assay Kit (Megazyme International Ireland Ltd., Bray, Ireland). The amylose content was determined according to Juliano (1971). The results were expressed as %dry weight.

4. The molecular weight distribution of rice starch by using gel permeation chromatography (GPC)

Rice starch molecular weight distribution was determined by using a GPC system (PL-GPC 220, Polymer Laboratories Varian, Inc. Amherst, MA). The PL-GPC 220 system is equipped with differential refractive index detector and Phynogel 00H-0646-KO, 00H-0644-KO, 00H-0642-KO columns (Phenomenex, Torrance, CA) connected in series. The mobile phase in the column was dimethyl sulfoxide (DMSO) with 5 mM NaNO₃, at a flow rate of 0.8 ml/min. The column oven temperature was controlled at 80 °C. A series of dextran standards were used to compare the retention time with the test samples for the molecular weight calculations. For GPC analysis, 4 mg (db) of starch was dissolved by 4 ml DMSO solution in a boiling water bath for 24 hr. with constant stirring. Each rice starch solution was filtered through a 2.0 µm filter, and then the filtrate was injected into a GPC system by an autosampler (Zhu *et al.*, 2010).

5. Thermal properties

The gelatinization and retrogradation temperatures of rice starches (8mg) were measured by a differential scanning calorimeter (DSC Q100, TA Instruments, New Castle, DE) at 66.7% water. Gelatinization was determined by heating the pan in the DSC from 10 to 95°C at a 10°C/min heating rate. The transition temperatures, namely the onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) and the delta enthalpy (ΔH) were determined. For retrogradation, the gelatinized rice starches were stored at 4°C for 7 days and then rescanned with the same conditions, which temperature range exhibits the retrogradation of amylopectin molecules (Zhu *et al.*, 2010).

6. Statistical analysis

All sample measurements were carried out in replicates and expressed as means \pm standard deviations. One-way analysis of variance by using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA) was used to assess the significance of the differences between means at $p < 0.05$.

RESULTS AND DISCUSSIONS

The condition for PGP preparation

The moisture contents of soaked paddy from SPT1 and PTT1 at warm water (30°C) every 2 hr. until 18 hr. were measured and showed in Table1.

Table1 Effect of soaking time on the moisture content of soaked paddy from two Thai rice cultivars, as SPT1 and PTT1, at warm water (30°C) until 18 hr.

Soaking time (hr)	Moisture content (%) ¹	
	San-Pah-Tawng1	Pathum-Thani1
0	11.32 ⁱ ± 0.15	10.27 ⁱ ± 0.02
2	24.74 ^h ± 0.31	25.37 ^h ± 0.25
4	25.91 ^g ± 0.33	26.02 ^g ± 0.33
6	28.95 ^f ± 0.04	27.29 ^f ± 0.18
8	29.12 ^f ± 0.09	28.63 ^e ± 0.09
10	29.74 ^e ± 0.14	29.71 ^d ± 0.32
12	30.33 ^d ± 0.09	30.45 ^c ± 0.25
14	32.59 ^c ± 0.15	30.80 ^c ± 0.11
16	33.10 ^b ± 0.33	32.41 ^b ± 0.38
18	36.14 ^a ± 0.14	33.63 ^a ± 0.20

¹Values are means of 2 replicate and 3 duplicate measurements ± standard deviation. Means for each characteristics followed by the different letter within the same column and cultivars are significantly different (p<0.05).

The moisture content of soaked paddy from both rice cultivars were significantly increased (p<0.05) every 2 hr. until 18 hr., excepting the soaking period on 6th to 8th for SPT1 and the 12th to 14th for PTT1. Komatsuzaki *et al.* (2007) suggested that the suitable paddy moisture content to rice germinate should be more than 30 percentages. The soaked paddy moisture contents from both cultivars (30.33-30.45%) was higher than 30 % at 12 hr. Therefore, the suitable soaking times to germinate for both rice cultivars were 12 hr.

After soaking, the moist-paddy was incubated in the incubator at 30°C, 85%RH to get embryonic growth length at their first (0.5-1mm), second (1-2mm), third (2-3mm) and malted (3-6mm) stages (Table2).

Table2 The incubation time to reach four stages of pre-germinated paddy from two Thai rice cultivars.

Cultivars	Incubation time (hr.)			
	First	Second	Third	Malted
San-Pah-Tawng1 (SPT1)	28	32	36	40
Pathum-Thani1 (PTT1)	18	20	22	24

The moist-paddy from SPT1 was reached the first-PGP at 28 hr. incubation time and continued to the next stages of PGP every 4 hr. The moist-paddy from PTT1 was the least incubation time to reach the first-PGP (18 hr.) and the fastest rate of germination, which was continued to the next stages of PGP every 2 hr.

Effect of pre-germination process on the starch characteristics

The rice starch characteristics in BRF and four stages of PGBRF from SPT1 and PTT1 were measured by using the chemical methods as total starch, damaged starch and amylose content which were shown in Table 3.

Table3 The rice starch characteristics of brown rice flour (BRF) and PGBRF from two Thai rice cultivars.

Treatments	Rice starch characteristics (%db) ¹			
	Moisture content	Total starch content	Starch damage content	Amylose content
San-Pah-Tawng1				
- BRF	11.23 ^a ± 0.03	80.94 ^a ± 0.13	12.41 ^d ± 0.18	5.25 ^a ± 0.06
- 1 st PGBRF	9.69 ^b ± 0.03	80.55 ^a ± 0.23	12.63 ^c ± 0.02	5.13 ^b ± 0.08
- 2 nd PGBRF	9.27 ^{cd} ± 0.00	79.08 ^b ± 0.23	12.74 ^c ± 0.06	4.95 ^c ± 0.08
- 3 rd PGBRF	9.24 ^d ± 0.06	78.46 ^c ± 0.21	13.37 ^b ± 0.09	4.79 ^d ± 0.08
- Malted BRF	9.33 ^c ± 0.00	78.16 ^c ± 0.02	14.01 ^a ± 0.07	4.75 ^d ± 0.06
Pathum-Thani1				
- BRF	10.19 ^a ± 0.02	81.13 ^a ± 0.01	12.16 ^c ± 0.34	17.77 ^c ± 0.19
- 1 st PGBRF	8.50 ^c ± 0.08	80.59 ^b ± 0.06	12.28 ^c ± 0.09	17.83 ^c ± 0.07
- 2 nd PGBRF	8.61 ^{bc} ± 0.03	79.91 ^c ± 0.15	12.43 ^c ± 0.08	18.00 ^c ± 0.29
- 3 rd PGBRF	8.62 ^b ± 0.05	79.55 ^c ± 0.27	12.79 ^b ± 0.02	18.45 ^b ± 0.37
- Malted BRF	8.65 ^b ± 0.02	79.01 ^d ± 0.24	13.30 ^a ± 0.02	18.73 ^a ± 0.18

¹Values are means of 2 replicate and 3 duplicate measurements ± standard deviation. Means for each characteristics followed by the different letter within the same column and cultivars are significantly different (p<0.05).

The moisture content of BRF from each rice cultivar (10.19-11.23%) was higher than the PGBRF (8.50-9.69%) because the PGBRF was prepared from the PGP, which was dried to stop the enzymatic reaction in each stage of pre-germination. Therefore, the moisture content of PGBRF was depended upon the duration and the temperature of drying. The BRF from both rice cultivars (80.94-81.13%db) was significantly higher total starch content than PGBRF (78.16-79.01%db). After pre-germination, the total starch of PGBRF from SPT1 was significantly decreased (p<0.05); however the third and malted stage-PGBRF were not significantly different (p≥0.05). For the PTT1, the significant decrease of total starch were occurred, excepting the total starch of second and third-PGBRF were not significantly different (p≥0.05). The decrease of total starch content was related to the increase of damaged starch. During pre-germination, the increasing of damaged starch content of BRF from SPT1 (14.01% from 12.41%db) was higher than that from PTT1 (13.30% from 12.16%db). After pre-germination, the amylose content of SPT1 was significantly decreased (p<0.05) in each stage from 5.25 to 4.75%db but it was not significantly different (p≥0.05) between the third to malted stages, while that of PTT1 was significantly increased (p<0.05) from 17.77 to 18.73%db at malted stage.

During pre-germination process, the amylolytic enzyme, as alpha-amylase, which is randomly degraded starch molecules at the amorphous lamellae of amylopectin clusters (Murata *et al.*, 1968). The decreasing of amylose content of PGBRF from SPT1 could be similar result from the research of Wu *et al.* (2013) using a waxy brown rice flour (Yannuo cultivar; YN) contained the amylose content 5.20%db (calculated from the percentage ratio between 3.97%db of amylose and 72.37%db of amylopectin) which was decreased to 5.17%db amylose during two days germinated YN brown rice flour. While the increasing of amylose content of PGBRF from non-waxy rice (PTT1) is in agreement with the result obtained by Mohan *et al.* (2010) using non-waxy brown rice, which contained 24.1%db

amylose content. This amount of amylose was increased due to alpha-amylase was partially breakdown the long chain branches of amylopectin during brown rice germination for 2 days to be 25.0%db amylose.

The average molecular weight and the molecular weight distribution of PGBRS molecules were revealed by using gel permeation chromatography (GPC) (Table4).

Table 4 The structural characteristic of starch molecules from two Thai rice cultivars during pre-germination.

Treatments	Structural characteristic of starch molecules ¹				
	Fraction (%)		Molecular Weight; MW (x10 ⁵ g/mol)		
	F1(high MW)	F2(low MW)	F1(high MW)	F2(low MW)	Average
San-Pah-Tawng1					
- BRS	54.93 ^a ±0.84	45.07 ^d ±0.84	292.47 ^a ±9.89	11.93 ^{ns} ±0.38	182.04 ^a ±3.71
- 1 st PGBRS	54.06 ^{ab} ±2.92	45.94 ^{cd} ±1.82	270.85 ^b ±6.29	12.63 ^{ns} ±0.46	166.20 ^b ±9.94
- 2 nd PGBRS	53.41 ^{bc} ±2.58	47.59 ^{bc} ±1.60	267.65 ^b ±17.00	11.87 ^{ns} ±0.63	156.66 ^b ±14.5
- 3 rd PGBRS	50.56 ^{cd} ±0.78	49.44 ^{ab} ±0.48	251.96 ^c ±2.19	12.39 ^{ns} ±0.28	140.96 ^c ±0.89
- Malted BRS	49.77 ^d ±0.34	50.23 ^a ±0.34	240.75 ^c ±8.98	11.84 ^{ns} ±0.13	131.56 ^c ±3.38
Pathum-Thani1					
- BRS	45.05 ^a ±4.56	54.95 ^c ±4.56	273.59 ^a ±3.19	6.82 ^a ±0.25	109.07 ^a ±8.78
- 1 st PGBRS	43.28 ^a ±6.62	56.72 ^c ±6.62	269.71 ^a ±18.93	6.51 ^a ±0.42	100.83 ^{ab} ±15.23
- 2 nd PGBRS	39.11 ^a ±2.96	60.89 ^c ±2.96	261.13 ^{ab} ±16.44	6.43 ^a ±0.05	90.21 ^b ±1.17
- 3 rd PGBRS	29.54 ^b ±0.35	70.46 ^b ±0.35	243.70 ^b ±5.99	5.87 ^b ±0.19	64.17 ^c ±1.49
- Malted BRS	27.05 ^c ±0.46	72.95 ^a ±0.46	240.25 ^b ±2.58	5.72 ^b ±0.29	60.37 ^c ±1.47

¹Values are means of 2 replicate and 2 duplicate measurements ± standard deviation. Means for each characteristics followed by the different letter within the same column are significantly different (p<0.05).

^{ns} refers to the mean values in the same column and cultivars are not significantly different (p≥0.05).

It was found that the rice starch molecules fractions were categorized into high MW fraction (F1; MW 10^{6.5}-10^{8.5} g/mol) as amylopectin and low MW fraction (F2; MW 10^{2.5}-10^{6.5} g/mol) as amylose and intermediate materials because of their differences in molecular size. Increasing of low MW starch fractions after pre-germination was observed in SPT1 (45.07 to 50.23%) and PTT1 (54.59 to 72.95%). For the waxy rice as SPT1, the MW of amylopectin from malted BRS (240.75x10⁵g/mol) was significantly smaller than that from brown rice starch (BRS) (292.47x10⁵g/mol) which indicated the presence of degradation of amylopectin by hydrolytic enzyme during rice germination process. However, the MW of amylose and intermediate materials (11.84-12.63x10⁵g/mol) in starch of SPT1 was not significantly different (p≥0.05) while the average MW of all starch molecules from PGBRS of SPT1 were significantly decreased (p<0.05), The MW in both fractions of PGBRS from PTT1 were significantly decreased at third stage of pre-germination (p<0.05) which indicated that the hydrolytic enzyme could degrade not only the amylopectin but also the amylose and intermediate materials in non-waxy rice starch during rice germination process.

The differences of heat flow pattern from two Thai rice starches are shown in Table 5. The gelatinization pattern showed the T_g of BRS from both rice cultivars were lowered than that of PGBRS while the range of transition temperature (T_c-T_g) of PGBRS from both rice starches showed narrower than that of BRS. The increasing of T_p of PGBRS from SPT1 rice cultivars was significantly different from BRS at the second stage. The delta enthalpy as the energy to gelatinized starch granules (ΔH_{gel}) in PGBRS was significantly decreased (p<0.05) from BRS to the malted stage of SPT1 while it was not significantly different (p≥0.05) between BRS and each stage of PGBRS from PTT1. Then

all gelatinized samples were kept at 4°C for 7 days to stimulate the re-association of rice starch molecules and measured the thermal properties again, the results showed that the delta enthalpy as the energy to melt the retrograded amylopectin (ΔH_{rel}) in PGBRS from SPT1 was significantly decreased ($p < 0.05$) until the third stage (from 0.51 to 0.28 J/g) but the malted stage was increased from the third stage to 0.40 J/g while that of PGBRS from PTT1 was significantly decreased ($p < 0.05$) from 1.24 to 0.72 J/g until the malted stage. This result indicated that the amylopectin molecules after pre-germination process were retrograded less than native amylopectin in starch molecules.

Table 5 The differences of heat flow pattern from two Thai rice starches during pre-germination.

Flour samples	Gelatinization ¹					Retrogradation ^{1*}				
	T _o	T _p	T _c	T _c -T _o	ΔH	T _o	T _p	T _c	T _c -T _o	ΔH
San-Pah-Tawng1										
- BRS	60.60 ^d	70.60 ^b	81.06 ^{cd}	20.61 ^a	13.15 ^{ab}	37.74 ^a	48.74 ^a	62.20 ^d	24.45 ^c	0.51 ^a
	±0.06	±0.28	±0.11	±0.17	±0.18	±0.01	±0.75	±0.18	±0.17	±0.00
- 1 st PGBRS	61.48 ^c	70.80 ^b	80.84 ^d	19.36 ^b	13.87 ^a	37.86 ^a	48.06 ^a	61.33 ^e	23.44 ^d	0.47 ^a
	±0.00	±0.10	±0.00	±0.00	±0.17	±0.02	±0.71	±0.04	±0.08	±0.01
- 2 nd PGBRS	62.22 ^b	71.36 ^a	81.41 ^{bc}	19.20 ^b	13.31 ^{ab}	36.83 ^b	46.00 ^b	62.72 ^c	25.62 ^b	0.30 ^c
	±0.02	±0.18	±0.00	±0.02	±0.27	±0.31	±0.34	±0.30	±0.36	±0.00
- 3 rd PGBRS	62.82 ^a	71.51 ^a	81.76 ^{ab}	18.76 ^c	13.09 ^{ab}	37.68 ^a	48.04 ^a	63.14 ^b	25.27 ^b	0.28 ^c
	±0.04	±0.03	±0.30	±0.08	±0.41	±0.01	±0.16	±0.10	±0.03	±0.01
- Malted BRS	62.91 ^a	71.70 ^a	82.05 ^a	18.96 ^c	12.81 ^b	37.70 ^a	48.20 ^a	64.63 ^a	26.98 ^a	0.40 ^b
	±0.22	±0.22	±0.10	±0.06	±0.52	±0.14	±0.08	±0.00	±0.06	±0.04
Pathum-Thani1										
- BRS	65.38 ^c	71.62 ^{c±}	81.70 ^{ab}	16.32 ^a	10.58 ^{NS}	41.44 ^b	55.99 ^b	67.44 ^d	26.27 ^c	1.24 ^a
	±0.01	0.08	±0.00	±0.01	±0.01	±0.11	±0.21	±0.06	±0.06	±0.03
- 1 st PGBRS	65.99 ^b	72.08 ^{bc}	81.98 ^a	15.71 ^b	10.52 ^{NS}	40.27 ^c	56.08 ^b	67.63 ^{cd}	27.38 ^b	0.85 ^b
	±0.01	±0.01	±0.60	±0.20	±0.23	±0.01	±0.08	±0.01	±0.02	±0.02
- 2 nd PGBRS	66.07 ^{ab}	72.48 ^{ab}	82.08 ^a	15.69 ^b	10.25 ^{NS}	39.71 ^d	56.54 ^a	68.13 ^b	28.70 ^a	0.78 ^c
	±0.01	±0.22	±0.13	±0.24	±0.26	±0.02	±0.04	±0.32	±0.05	±0.02
- 3 rd PGBRS	66.47 ^{ab}	72.64 ^{ab}	81.89 ^a	15.49 ^b	10.11 ^{NS}	44.46 ^a	56.48 ^a	68.05 ^{bc}	23.71 ^d	0.77 ^c
	±0.04	±0.00	±0.06	±0.00	±0.26	±0.01	±0.01	±0.21	±0.03	±0.02
- Malted BRS	66.59 ^a	72.86 ^a	81.07 ^b	15.25 ^b	10.09 ^{NS}	44.66 ^a	56.40 ^a	68.61 ^a	23.20 ^a	0.72 ^d
	±0.47	±0.61	±0.28	±0.32	±0.01	±0.23	±0.02	±0.00	±0.05	±0.00

¹ Values are means of 2 replicate and 2 duplicate measurements ± standard deviation. Means for each characteristics followed by the different letter within the same column are significantly different ($p < 0.05$).

^{NS} refers to the mean values in the same column and cultivars are not significantly different ($p \geq 0.05$).

* The retrogradation of amylopectin molecules.

CONCLUSION

The PGBRF from both rice cultivars had lower total starch content and higher damaged starch than that of BRF. However, the decreasing amylose content during pre-germination of PGBRF from waxy rice as SPT1 was conversely occurred in non-waxy rice as PTT1 which might be related to the hydrolytic enzyme activity on waxy rice and non-waxy rice starches during pre-germination. Moreover, the starch molecules after pre-germination process were retrograded less than native starch molecules. Furthermore, each germination stage-PGBRF was changed rice starch characteristics, which could be applied to produce various kinds of rice products.

ACKNOWLEDGEMENT

This research was financially supported by the Royal Golden Jubilee Ph.D. program and Graduate School, Kasetsart University, Bangkok, Thailand. The authors would like to sincerely acknowledge Prof. Yong-Cheng Shi from the Department of Grain Science and Industry at Kansas State University, USA for his research consulting this research.

LITERATURE CITED

- AACC. 2000. **AACC International Approved Methods of Analysis**, 11th ed. AACC international, St. Paul, MN, U.S.A.
- Bao, J. and C.J. Bergman. 2001. The functionality of rice starch. In: **Starch in Food: Structure, Function and Applications**. A.-C. Eliasson, Ed., Woodhead Publishing Limited. Cambridge, pp. 250-294.
- Chaichaw, C., H. Pinkaew, W. Kupkanchanakul and O. Naivikul. 2011. Effect of modified rice Flours for Banana Rice Cake Production, pp. 316-323. *In The Proceeding of the 49th Kasetsart University Annual Conference*. Kasetsart University, Bangkok.
- Juliano, B.O. 1971. A simplified assay for milled-rice amylose. **Cereal Sci. Today**. 16: 334-338.
- Komatsuzaki, N., K. Tsukahara, H. Toyoshima, T. Suzuki, N. Shimizu and T. Kimura. 2007. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. **J. Food. Eng.** 78: 556-560.
- Kupkanchanakul, W. and O. Naivikul. 2012. Effect of pre-germination process on the qualities of rice bread. *In The Proceeding of AACC International Annual Meeting 2012. Supplement to Cereal Foods World*. 57(4): A25.
- Lumdubwong, N. and P.A. Seib. 2000. Rice Starch Isolation by Alkaline Protease Digestion of Wet-milled Rice Flour. **J. of Cereal Sci.** 31: 63-74.
- Mohan, B.H., N.G. Malleshi and T. Koseki. 2010. Physico-chemical characteristics and non-starch polysaccharide contents of Indica and Japonica brown rice and their malts. **LWT-Food Sci. Technol.** 43: 784-791.
- Murata, T., T. Akazawa and S. Fukuchi. 1968. Enzymic Mechanism of starch Breakdown in Germinating Rice Seeds. **Plant Physiol.** 43: 1899-1905.
- Ohtsubo, K.I., K. Suzuki, Y. Yasui and T. Kasumi. 2005. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. **J. Food Compos. Anal.** 18: 303-316.
- Wu, F., N. Yang, H. Chen, Z. Jin and X. Xu. 2011. Effect of germination on flavor volatiles of cooked brown rice. **Cereal Chem.** 88: 497-503.
- Wu, F., N. Yang, A. Touré, Z. Jin and X. Xu. 2013. Germinated brown rice and its role in human health. **Crit. Rev. Food Sci. Nutr.** 53: 451-463.
- Xu, J., H. Zhang, X. Guo and H. Qian. 2012. The impact of germination on the characteristics of brown rice flour and starch. **J. Sci. Food Agric.** 92:380-387.
- Zhu, L.J., Q.Q. Liu, Y.J. Sang, M.H. Gu and Y.C. Shi. 2010. Underlying reasons for waxy rice flours having different pasting properties. **Food Chem.** 120: 94-100.