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Original Article

Effect of pre-germination at varying stages of embryonic growth length on chemical composition and protein profile of Thai rice (*Oryza sativa* L.)

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ABSTRACT

The effect of pre-germination at varying stages of embryonic growth length (EGL) (0.5–7 mm, for 30–56 h of pre-germination time) on the chemical composition and protein profiles were investigated in three different Thai rice cultivars—SPT1 (waxy), PTT1 (low amylose), and PNL2 (high amylose). Pre-germination at 0.5–1 mm of EGL (first-stage) of all rice led to a significant change in most of the total starch, crude protein, and crude fat contents except in the crude protein content of PNL2 and the crude fat content of SPT1, which significantly changed at 3–7 mm of EGL (malted-stage) and at 2–3 mm of EGL (third-stage), respectively. Analysis of protein profiles from all rice samples indicated that glutelin molecules existed in subunits linked by disulfide bonds. Pre-germination to the second-stage of SPT1 and PTT1, and to the first-stage of PNL2 caused the cleavage of proglutelin (~51.6 kDa) into acidic-glutelin (~31.9 kDa) and basic-glutelin (~17.8 kDa) and also increased the relative concentration of 13 kDa prolamins. Pre-germination of these three rice cultivars at 30 °C for 32–48 h clearly indicated the function as bio-modification of rice proteins, particularly in the reduction of glutelin disulfide bonds.

Introduction

Rice (*Oryza sativa* L.) is one of the staple foods of the world, with approximately 80% of rice being the *Indica* subspecies, which is the major rice grown in Thailand and many Asian countries (Mackill, 1995). Thai rice cultivars can be grouped on the amylose content as waxy (<5%), very low amylose (5–12%), low amylose (12–20%), intermediate amylose (20–25%), and high amylose (>25%) (Juliano et al., 1981). During rice development, nutrients reserves in the form of carbohydrates, proteins and lipids are synthesized and accumulated (Juliano, 1972).

Rice proteins are mainly stored in the endosperm amounting to 6.2–7.9% as storage proteins (Wadchararat et al., 2006). Glutelins are the major storage proteins having two subunits of acidic-glutelin, with a molecular weight (MW) of 28.5–30.8 kDa and basic-glutelin, with MW 20.6–21.6 kDa (Wen and Luthe, 1985). Both glutelin subunits are cross-linked with a disulfide bond acting as the glutelin precursor also known as proglutelin, with MW 50–51 kDa (Krishnan and Okita, 1986; Likitwattanasade and Hongsprabhas, 2010). The glutelin subunits were also speculated to be cross-linked by disulfide bonding, resulting in glutelin molecules with MW 64–500 kDa (Sugimoto et al., 1986). Prolamins were reported to be encoded into three groups—10, 13 and 16 kDa—with the major group of 13 kDa prolamin (Muench et al., 1999). Alpha-globulins have been shown to have MW 25–26 kDa (Tanaka et al., 1980).

The qualitative and quantitative components of rice proteins have been reported to affect the physicochemical properties of rice flour, which are related to the quality of rice-based products. Disulfide-linkage in the glutelin molecule can form a macromolecular protein, which restricts heat-induced swelling of rice starch (Derycke et al., 2005), resulting in a barrier to forming the structure of brown rice bread (Renzetti and Arendt, 2009; Yano, 2010).

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Additionally, an increase in prolamins in rice starch was negatively correlated to a decrease in the hardness, adhesiveness and gumminess of starch gel (Baxter et al., 2004).

The germination process has been widely reported to induce chemical changes in rice due to a complex biochemical process of reserves being considerably activated, particularly during rice paddy growth at 0.5–3 mm of embryonic growth length (EGL), which is called pre-germination process (Panchan and Naivikul, 2009; Pinkaew et al., 2016). Veluppillai et al. (2009) reported that proteolytic activity increased from 0 to 0.12 U/g dry weight during 5 d of brown rice germination. The significant increase of protease activity after 2 d of brown rice germination showed the hydrolysis of rice proteins to provide soluble protein, small peptides and free amino acids (Li et al., 2011). Partial hydrolysis of high molecular weight polypeptides of rice between 45 and 97 kDa were found after 5 d of germination (Mohan et al., 2010). However, there has been limited work on both the qualitative and quantitative changes of rice proteins during the pre-germination stage of paddy in different rice cultivars.

The objectives of this study were to investigate the dynamic changes in the pre-germinated rice chemical composition, particularly of proteins, in three different Thai rice cultivars and EGL in terms of the crude protein content, protein profile and relative concentration of each polypeptide size. This should provide useful information depicting the pre-germination stage of rice protein bio-modifications and their application for rice products.

Materials and methods

Materials

Matured paddy of three Thai rice (*Oryza sativa* L.) cultivars—San-Pah-Tawng1 (SPT1; waxy: 4.89% amylose); Pathum-Thani1 (PTT1; low amylose: 15.10% amylose); and Phitsanulok2 (PNL2; high amylose: 26.78% amylose)—were used as representatives of three different groups of rice cultivars. They were obtained from the Rice Research Center (Bangkok, Thailand) after 2 mth of dormancy and then stored at −18 °C.

Preparation of pre-germinated paddy cultivars and their flour samples

Pre-germinated paddy (PGP) was prepared using a slightly modified method from Panchan and Naivikul (2009) and Pinkaew et al. (2016). Rice paddy was soaked in warm water at 30 °C for 0–18 h. The soaking water was changed every 4 h to avoid fermentation. The moisture content of paddy was determined every 6 h according to (American Association of Cereal Chemists, 2000). Optimally soaked paddy (500 g) was placed on a plastic tray, covered with a wet cloth to retain moisture (85% RH), and incubated at 30 °C in the dark. The EGL and germination percentages of the paddy samples were evaluated every 2 h up to 50 h. The incubated paddy samples (5 ± 0.5 g) in each tray were randomly observed for EGL and then the amount of germinated paddy per total amount of observed paddy was multiplied by 100 as the pre-germination percentage. The native rice paddy (A) was compared to four stages of PGP (Fig. 1), which were defined by the EGL: first (B), 0.5–1 mm EGL (60–70% pre-germination); second (C), 1–2 mm EGL (71–80% pre-germination); third (D), 2–3 mm EGL (81–90% pre-germination); and malted (E), 3–7 mm EGL (more than 90% pre-germination). After reaching the desired pre-germination stages, the PGP was dried at 40 °C for 12 h, dehusked, ground using a pin mill (Alpine Model 160Z, Augsburg, Germany), and sieved through a 100-mesh sieve.

Chemical composition analysis

The moisture contents of rice flour samples were determined using the air-oven method according to method 44-15A (American Association of Cereal Chemists, 2000). The total starch content of samples was enzymatically analyzed using a Megazyme total starch content assay kit (Megazyme International Ireland Ltd.; Wicklow, Ireland). The crude protein contents of samples were determined using the improved Kjeldahl method which involves copper catalyst modification according to approved method 46-12 with a conversion factor of 5.95 to convert the nitrogen content to crude protein (American Association of Cereal Chemists, 2000). The crude fat contents of samples were determined according to approved method 30-25 (American Association of Cereal Chemists, 2000).

Protein profile

The protein profiles of rice flour samples were evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with the slightly modified method of Kubota et al. (2010). Each rice flour sample was weighed to contain 4.5 mg of crude protein on a dry basis and then suspended in 525 μL of sample buffer (0.125 M Tris-HCl pH 6.8, 4 %w/v SDS, 8 M urea, and 0.2 mg/mL bromophenol blue) with or without 75 μL of 5 %v/v β-mercaptoethanol (B-ME) as reducing and non-reducing conditions, respectively. Each sample was then heated in boiling water for 3 min and then cooled down; the pH of the mixture was adjusted to 6.8 ± 0.1 by adding 1 M HCl and then centrifuged at 7000 × *g* and 25 °C for 5 min to remove any insoluble materials. The solubilized proteins (19 μL) were fractionated using SDS-PAGE (using stacking gel containing 4.5 %w/v acrylamide and resolving gel containing 15 %w/v acrylamide) using a Bio-Rad Mini-Protean II cell (Bio-Rad Laboratories; Hercules, CA, USA) at 20 mA per gel for 210 min. A series of molecular weight markers (MW = 10–250 kDa; Bio-RAD control 35,000,216 Precision Plus Protein™ Kaleidoscope™; Bio-Rad Laboratories; Hercules, CA, USA) was used as standards. After electrophoresis, the gels were fixed, stained simultaneously using Bio-Rad Coomassie blue R-250 stain solution for 20 min, and then destained using Bio-Rad Coomassie blue R-250 destain solution for 20 h.

Relative concentrations of each polypeptides band

Based on molecular weight, each polypeptide band of protein profile was presumably identified into six considered polypeptides bands, consisting of: 1) greater than 50 kDa proglutelin; 2) around 50 kDa proglutelin; 3) around 30 kDa for acidic-glutelin; 4) around 25 kDa for alpha-globulin; 5) around 20 kDa for basic-glutelin; and 6) around 13 kDa for 13 kDa prolamin. The intensity and molecular weight of each polypeptide band in the protein profile were measured using the Image-J version 1.51k software (available from https://imagej.nih.gov/ij/, March 2017). The relative concentration (RC) of each polypeptide band was calculated as the percentage ratio of the intensity of each polypeptide band to the intensity of total polypeptide band (total protein) in the protein profile.

Statistical analysis

All sample measurements were expressed as a mean ± SD. Data were analyzed using the SPSS 11.0 software (SPSS Inc.; Chicago, IL, USA) for one-way ANOVA. Tukey's multiple comparison test was used for detection of statistically significant differences among the investigated groups of samples using a 95% confidence interval.

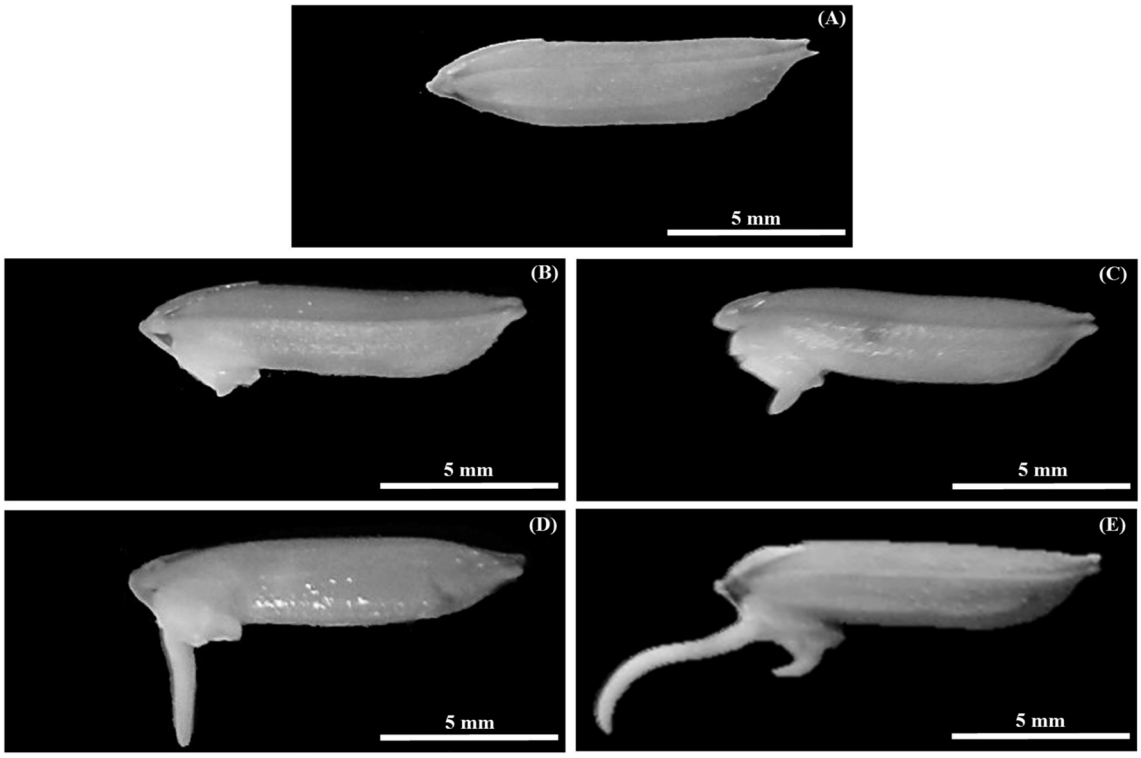

Fig. 1. Characteristics of pre-germinated paddy (PGP) from Pathum-Thani1 at different stages of pre-germination with varying embryonic growth length (EGL): (A) native rice paddy; (B) first-stage of PGP, 0.5–1 mm EGL; (C) second-stage of PGP, 1–2 mm EGL; (D) third-stage of PGP, 2–3 mm EGL; and (E) malted-stage of PGP, 3–7 mm EGL.

Results and discussion

Optimum pre-germination process for the four stages of EGL

Table 1 shows the conditions of the four stages of pre-germination for the three Thai paddy cultivars. Before soaking, the moisture content of paddy based on a wet weight basis (wb) was the highest at 11.3% wb for SPT1 followed by 10.8% wb for PTT1 and 10.2% wb for PNL2. The moisture contents of the three paddy cultivars rapidly increased to 27.3–29.0% wb after 6 h of soaking and reached approximately 30% wb at 12 h of soaking; these amounts were not significantly different among the three paddy cultivars. At 18 h of soaking, the moisture contents of three paddy cultivars increased to 36.1% wb for SPT1, which was significantly higher than the 33.6% wb for PTT1 and 33.2% wb for PNL2. These results indicated that all three Thai paddy cultivars required 12 h soaking time to obtain a moisture content of 30% wb. As indicated from previous studies, the suitable moisture content for Thai paddy

Table 1
Pre-germination process—soaking and incubation—for four stages of pre-germinated paddy (PGP) from three Thai rice cultivars: San-Pah-Tawng1 (SPT1); Pathum-Thani1 (PTT1); and Phitsanulok2 (PNL2).

Pre-germination process	Parameter		Rice cultivar		
			SPT1	PTT1	PNL2
Soaking	Moisture content (% wet basis)[a]	0-hr (before soaking)	11.3 ± 0.2^{dA}	10.8 ± 0.0^{dB}	10.2 ± 0.1^{dC}
		6-hr	29.0 ± 0.0^{cA}	27.3 ± 0.2^{cB}	28.9 ± 0.2^{cA}
		12-hr	30.2 ± 0.1^{bNS}	30.4 ± 0.2^{bNS}	30.1 ± 0.1^{bNS}
		18-hr	36.1 ± 0.1^{aA}	33.6 ± 0.2^{aB}	33.2 ± 0.2^{aB}
Incubation	Time (h)[b]	First-stage PGP	28	18	36
		Second-stage PGP	32	20	40
		Third-stage PGP	36	22	42
		Malted-stage PGP	40	24	44
Total pre-germination	Pre-germination percentage (%)[a]	First-stage PGP	60.7 ± 4.5^{dNS}	66.3 ± 3.5^{dNS}	65.1 ± 3.0^{dNS}
		Second-stage PGP	74.5 ± 3.8^{cNS}	78.0 ± 1.4^{cNS}	77.6 ± 1.9^{cNS}
		Third-stage PGP	85.3 ± 2.5^{bNS}	85.9 ± 3.6^{bNS}	87.4 ± 2.1^{bNS}
		Malted-stage PGP	92.3 ± 2.8^{aNS}	95.7 ± 2.7^{aNS}	92.6 ± 1.3^{aNS}
	Time (hr)[c]	First-stage PGP	40	30	48
		Second-stage PGP	44	32	52
		Third-stage PGP	48	34	54
		Malted-stage PGP	52	36	56

[a] Results are expressed as mean ± SD ($n = 4$). Values with different lowercase superscript letters in the same column within each parameter and values with different uppercase superscript letters in the same row being significantly different ($P < 0.05$). ^{NS} refers to no significant difference in the same row ($p \geq 0.05$).
[b] Incubation time was investigated after optimum soaking time (12 h).
[c] Total pre-germination time is the sum of optimum soaking time (12 h) and optimum incubating time to achieve each stage of pre-germination.

pre-germination is 30% wb, which is essential for the mobilization of rice reserves during pre-germination (Panchan and Naivikul, 2009; Pinkaew et al., 2016). Therefore, 12 h soaking time is highly recommended to be used in paddy before the incubating process to provide a suitable moisture content of paddy for germination and also as a standard moisture content for the three experimental paddy cultivars.

After optimal soaking (12 h), the soaked paddy was incubated to obtain the four stages of PGP at a specific incubation time for each EGL and paddy cultivar. The incubation time to the first-stage of PGP was 18 h for PTT1 (the shortest incubation time) followed by 28 h for SPT1 and 36 h for PNL2. Additionally, the progressive incubation times for the subsequent desired EGLs of PGP were different in terms of the time and germination rate depending on the rice cultivar and the period of the pre-germination stage. Therefore, the incubation time plays a key role during germination before the process of embryonic growth. However, the pre-germination percentages of the three rice cultivars within the same stage of pre-germination were not significantly different. Previous works have shown that pre-germination times largely depended on rice sub-species—*Oryza sativa* var. *Indica* or var. *Japonica* (Mohan et al., 2010), the storage time and pH of soaked water (Songtip et al., 2012), and the moisture content of soaked paddy before incubation for pre-germination (Pinkaew et al., 2016).

Effect of pre-germination stages on chemical composition

The changes in the chemical compositions during pre-germination were investigated after each sample had been de-husked and dry-milled into flour (Table 2). The range of moisture contents of all samples was 8.06–11.11% wb. A significant decrease in the total starch content was found at each prolonged stage of pre-germinated brown rice (PGBR) for all three rice cultivars. The activation of amylolytic enzymes degrades native starch molecules during pre-germination resulting in decreased starch molecules (Mohan et al., 2010; Xu et al., 2012). Therefore, the degradation of starch molecules is the predominant mechanism to produce the necessary energy during rice growth at 0.5–1 mm of EGL.

The crude protein contents of PGBR were significantly changed at 0.5–1 mm of EGL (first-stage) for the SPT1 and PTT1 cultivars but at 3–7 mm of EGL (malted-stage) for the PNL2 cultivar. The significant changes in the crude fat contents of PGBR were mostly found at 0.5–1 mm of EGL (first-stage) for the PTT1 and PNL2 cultivars but at 2–3 mm of EGL (third-stage) for the SPT1 cultivar. Therefore, there were several activated biochemical compounds affecting the changes in starch, protein and lipid from the start of 0.5–1 mm of EGL.

In addition, pre-germination showed different effects on the crude protein and crude fat contents of the three rice cultivars, which were increased for SPT1 but decreased for PTT1 and PNL2. The different changes in the crude protein and crude fat contents for each rice cultivar were probably related to the metabolism of rice during pre-germination, which involved the degradation of protein by proteases (Li et al., 2011) and fat by lipases (He et al., 2011), while amino acids and fatty acids were also bio-synthesized to promote embryonic growth (Moongngarm and Saetung, 2010; He et al., 2011).

Effect of pre-germination stages on protein profile

Qualitative changes in the rice protein profiles during pre-germination were investigated under non-reducing and reducing conditions (Fig. 2). The protein profiles from the non-reducing conditions without B-ME of all rice cultivars and their pre-germination indicated that rice protein contains many MWs (bands) of polypeptides, particularly that of PNL2 cultivar. The rice proteins mostly contained polypeptides with a high MW (greater than or equal to 50 kDa), which was glutelin. In the presence of B-ME, disulfide bonds were eliminated, resulting in decreasing intensities of polypeptides bands at greater than or equal to 50 kDa and subsequent increasing intensities of two polypeptides—the glutelin subunits—at a band between 25 kDa and 37 kDa and another band between 15 kDa and 20 kDa. These results indicated that the glutelin molecule existed in two subunits linked by disulfide bonds which were similar to those reported by Likitwattanasade and Hongsprabhas (2010).

The number of polypeptide bands of protein profiles was unchanged throughout the different stages of pre-germination in all rice cultivars, but the intensity of some polypeptide bands initially changed, especially for the six considered polypeptides bands, as identified by molecular sizes of greater than 50 kDa proglutelin (Sugimoto et al., 1986), around 50 kDa proglutelin (Krishnan and

Table 2
Chemical composition of brown rice (BR) and four stages of pre-germinated brown rice (PGBR).

Rice sample	Moisture content* (% wet basis)	Chemical composition* (% dry basis)		
		Total starch	Crude protein	Crude fat
San-Pah-Tawng1				
BR	11.11 ± 0.06^{a}	81.40 ± 0.34^{a}	7.83 ± 0.01^{c}	3.23 ± 0.04^{b}
First-PGBR	9.32 ± 0.04^{b}	80.64 ± 0.37^{b}	8.02 ± 0.06^{b}	3.24 ± 0.05^{b}
Second-PGBR	8.74 ± 0.03^{d}	79.15 ± 0.31^{c}	8.25 ± 0.05^{a}	3.37 ± 0.03^{b}
Third-PGBR	8.73 ± 0.02^{d}	78.66 ± 0.20^{d}	8.26 ± 0.06^{a}	3.42 ± 0.08^{a}
Malted-PGBR	8.83 ± 0.04^{c}	78.24 ± 0.18^{e}	8.26 ± 0.04^{a}	3.44 ± 0.01^{a}
Pathum-Thani1				
BR	10.00 ± 0.10^{a}	81.67 ± 0.27^{a}	8.72 ± 0.07^{a}	3.10 ± 0.01^{a}
First-PGBR	8.06 ± 0.03^{d}	80.98 ± 0.36^{b}	8.57 ± 0.04^{b}	3.02 ± 0.04^{b}
Second-PGBR	8.36 ± 0.08^{b}	80.10 ± 0.38^{c}	8.57 ± 0.03^{b}	2.63 ± 0.00^{c}
Third-PGBR	8.09 ± 0.11^{cd}	79.39 ± 0.41^{d}	8.46 ± 0.10^{b}	2.60 ± 0.09^{c}
Malted-PGBR	8.15 ± 0.03^{c}	78.87 ± 0.22^{e}	8.31 ± 0.00^{c}	2.29 ± 0.03^{d}
Phitsanulok2				
BR	10.70 ± 0.01^{a}	81.24 ± 0.34^{a}	9.07 ± 0.00^{a}	2.69 ± 0.02^{a}
First-PGBR	9.07 ± 0.00^{b}	79.65 ± 0.24^{b}	9.03 ± 0.05^{a}	2.53 ± 0.01^{b}
Second-PGBR	8.22 ± 0.01^{e}	79.26 ± 0.25^{c}	9.08 ± 0.11^{a}	2.39 ± 0.02^{c}
Third-PGBR	8.43 ± 0.04^{d}	78.87 ± 0.21^{d}	9.07 ± 0.00^{a}	2.34 ± 0.07^{c}
Malted-PGBR	8.72 ± 0.02^{c}	78.58 ± 0.14^{e}	8.92 ± 0.01^{b}	2.25 ± 0.00^{d}

* Results are expressed as mean ± SD ($n = 6$) with different lowercase superscript letters in the same column within the cultivar being significantly different ($p < 0.05$).

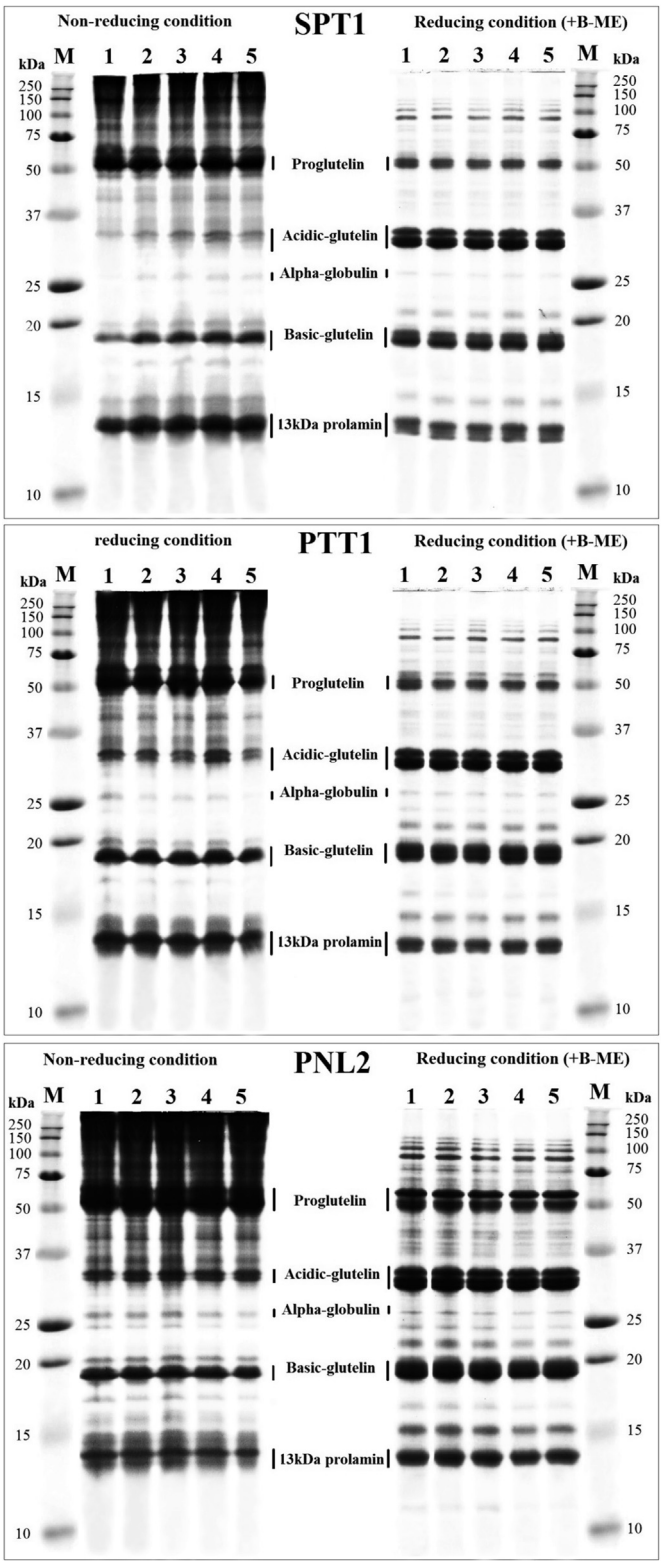

Fig. 2. Protein profiles of brown rice (BR) and four stages of pre-germinated brown rice (PGBR) from San-Pah-Tawng1 (SPT1), Pathum-Thani1 (PTT1), and Phitsanulok2 (PNL2) under non-reducing (left) and reducing (right) conditions, where M = standard molecular weight marker; 1 = brown rice; 2 = first-stage of PGBR; 3 = second-stage of PGBR; 4 = third-stage of PGBR; and 5 = malted-stage of PGBR.

Okita, 1986; Likitwattanasade and Hongsprabhas, 2010), around 30 kDa for acidic-glutelin (Wen and Luthe, 1985), around 25 kDa for alpha-globulin (Tanaka et al., 1980), around 20 kDa for basic-glutelin (Wen and Luthe, 1985), and around 13 kDa for 13 kDa prolamin, respectively. Therefore, the changes in these six polypeptide bands should be closely investigated in terms of their relative concentration (RC).

Effect of pre-germination stages on relative concentration of rice protein

Changes in the RC of rice proteins—the six considered polypeptide bands—during pre-germination were observed. Under non-reducing conditions as the natural stage during pre-germination (Table 3), the average MWs of proglutelin, acidic-glutelin, alpha-globulin, basic-glutelin, and 13 kDa prolamin were 51.6 kDa, 31.9 kDa, 25.5 kDa, 17.8 kDa and 13.4 kDa, respectively. A significant decrease in the RC of proglutelin (51.6 kDa) was found at 0.5–1 mm of EGL (first-stage) in all rice cultivars. The RC of proglutelin at greater than 50 kDa was also significantly decreased at 0.5–1 mm of EGL (first-stage) in the PNL2 cultivar and at 1–2 mm of EGL (second-stage) in the SPT1 and PTT1 cultivars. On the other hand, there was a significant increase in pre-germination at 0.5–1 mm of EGL (first-stage) in most of both subunits of glutelin—acidic-glutelin and basic-glutelin—of the three rice cultivars except for the acidic-glutelin of SPT1, which significantly increased at 1–2 mm of EGL (second-stage). These results clearly indicated that disulfide bond cleavage took place between acidic-glutelin and basic-glutelin subunits, which might be related to the activity of glutathione S-transferase, protein disulfide-isomerase, thioredoxi, and peroxiredoxin thioredoxins as disulfide reductases in germinated brown rice (Yano et al., 2001; He et al., 2011). These results indicated the activities of disulfide reductases enzymes for PGBR. Furthermore, this information would be useful to reduce the disulfide-linked macromolecular protein in brown rice, which acts as a barrier to the structural form of brown rice bread (Renzetti and Arendt, 2009; Yano, 2010).

In addition, the RC of alpha-globulin of SPT1 significantly increased at 3–7 mm of EGL (malted-stage), while those of PTT1 and PNL2 were not significantly different throughout the stages of pre-germination. Pre-germination led to a significant increase in the RC of 13 kDa prolamin at 0.5–1 mm of EGL (first-stage) for the SPT1 cultivar and at 3–7 mm of EGL (malted-stage) for the PTT1 and PNL2 cultivars. The increased RC of alpha-globulin and 13 kDa prolamin may also be related to the homeostatic mechanism for maintaining the total protein content during rice pre-germination (Kawakatsu et al., 2010).

Under reducing conditions (Table 4), the average MWs of proglutelin, acidic-glutelin, alpha-globulin, basic-glutelin and 13 kDa prolamin were 52.8 kDa, 31.6 kDa, 25.8 kDa, 18.6 kDa and 13.0 kDa, respectively. The RC values of proglutelin at greater 50 kDa in all rice cultivars were not significantly different throughout the stages of pre-germination, while a significant decrease in the RC of proglutelin (52.8 kDa) was found at 0.5–1 mm of EGL (first-stage) in the SPT1 and PTT1 cultivars but at 1–2 mm of EGL (second-stage) in the PNL2 cultivar. The RC of acidic-glutelin significantly increased at 0.5–1 mm of EGL (first-stage) in the three rice cultivars, while that of basic-glutelin also significantly increased but at different stages depending on the rice cultivar, being at 0.5–1 mm of EGL (first-stage) in the PNL2 cultivar, at 1–2 mm of EGL (second-stage) in the SPT1 cultivar and at 3–7 mm of EGL (malted-stage) in the PTT1 cultivar. These changes in the RC of proglutelin and subunits under reducing

Table 3
Relative concentration of rice protein in brown rice (BR) and four stages of pre-germinated brown rice (PGBR) under non-reducing conditions.

Rice protein sample	Relative concentration (% of total protein)*					
	Proglutelin (>51.6 kDa)	Proglutelin (51.6 kDa)	Acidic-glutelin (31.9 kDa)	Alpha-globulin (25.5 kDa)	Basic-glutelin (17.8 kDa)	13 kDa Prolamin (13.4 kDa)
San-Pah-Tawng1						
BR	43.7 ± 0.1^{a}	21.9 ± 0.2^{a}	2.3 ± 0.1^{c}	1.5 ± 0.1^{b}	8.6 ± 0.2^{c}	15.2 ± 0.1^{d}
First-PGBR	43.3 ± 0.4^{ab}	20.5 ± 0.3^{b}	2.4 ± 0.3^{c}	1.7 ± 0.2^{ab}	9.3 ± 0.4^{b}	15.7 ± 0.0^{c}
Second-PGBR	42.5 ± 0.4^{b}	19.9 ± 0.4^{bc}	3.6 ± 0.2^{b}	1.7 ± 0.3^{ab}	10.0 ± 0.6^{ab}	15.9 ± 0.3^{bc}
Third-PGBR	42.2 ± 0.5^{bc}	19.6 ± 0.4^{c}	4.0 ± 0.4^{ab}	1.7 ± 0.1^{ab}	10.3 ± 0.5^{a}	16.0 ± 0.1^{b}
Malted-PGBR	41.5 ± 0.5^{c}	19.2 ± 0.4^{c}	4.2 ± 0.3^{a}	1.8 ± 0.2^{a}	10.5 ± 0.2^{a}	16.4 ± 0.2^{a}
Pathum-Thani1						
BR	43.9 ± 0.3^{a}	18.2 ± 0.3^{a}	3.5 ± 0.1^{d}	2.2 ± 0.2^{NS}	11.2 ± 0.3^{c}	16.1 ± 0.2^{b}
First-PGBR	43.5 ± 0.3^{a}	16.7 ± 0.2^{b}	4.2 ± 0.3^{c}	2.0 ± 0.3^{NS}	12.1 ± 0.1^{b}	16.0 ± 0.3^{b}
Second-PGBR	42.3 ± 0.4^{b}	16.6 ± 0.3^{b}	5.1 ± 0.3^{b}	2.0 ± 0.2^{NS}	12.5 ± 0.1^{a}	16.1 ± 0.2^{b}
Third-PGBR	41.4 ± 0.3^{c}	16.4 ± 0.3^{b}	6.0 ± 0.2^{a}	2.0 ± 0.0^{NS}	12.7 ± 0.3^{a}	16.2 ± 0.0^{b}
Malted-PGBR	41.2 ± 0.3^{c}	16.3 ± 0.4^{b}	6.1 ± 0.2^{a}	1.8 ± 0.3^{NS}	12.8 ± 0.3^{a}	16.5 ± 0.2^{a}
Phitsanulok2						
BR	45.9 ± 0.3^{a}	17.4 ± 0.2^{a}	7.0 ± 0.3^{c}	2.1 ± 0.2^{NS}	10.1 ± 0.2^{b}	10.1 ± 0.2^{b}
First-PGBR	45.0 ± 0.1^{b}	16.9 ± 0.3^{b}	7.5 ± 0.2^{b}	2.2 ± 0.2^{NS}	10.6 ± 0.3^{a}	10.2 ± 0.2^{ab}
Second-PGBR	44.8 ± 0.3^{bc}	16.2 ± 0.4^{c}	7.7 ± 0.3^{b}	2.3 ± 0.4^{NS}	10.7 ± 0.1^{a}	10.4 ± 0.1^{ab}
Third-PGBR	44.4 ± 0.5^{c}	16.0 ± 0.3^{c}	7.9 ± 0.4^{ab}	2.0 ± 0.4^{NS}	10.9 ± 0.3^{a}	10.5 ± 0.3^{ab}
Malted-PGBR	44.0 ± 0.3^{c}	15.7 ± 0.4^{c}	8.2 ± 0.2^{a}	1.9 ± 0.2^{NS}	11.0 ± 0.3^{a}	10.6 ± 0.3^{a}

* Results are expressed as mean ± SD ($n = 6$) with different lowercase superscript letters in the same column within the cultivar being significantly different ($p < 0.05$). ^{NS} refers to no significant difference ($p \geq 0.05$).

Table 4
Relative concentration of rice protein in brown rice (BR) and four stages of pre-germinated brown rice (PGBR) under reducing conditions.

Rice protein sample	Relative concentration (% of total protein)*					
	Proglutelin (>52.8 kDa)	Proglutelin (52.8 kDa)	Acidic-glutelin (31.6 kDa)	Alpha-globulin (25.8 kDa)	Basic-glutelin (18.6 kDa)	13 kDa Prolamin (13.0 kDa)
San-Pah-Tawng1						
BR	7.6 ± 0.2^{NS}	11.1 ± 0.2^{a}	28.7 ± 0.1^{d}	0.8 ± 0.0^{b}	22.8 ± 0.0^{d}	18.7 ± 0.2^{c}
First-PGBR	7.6 ± 0.2^{NS}	9.5 ± 0.4^{b}	29.0 ± 0.2^{c}	0.9 ± 0.0^{a}	23.0 ± 0.3^{cd}	19.1 ± 0.2^{b}
Second-PGBR	7.5 ± 0.4^{NS}	8.8 ± 0.3^{b}	29.3 ± 0.0^{b}	0.9 ± 0.0^{a}	23.1 ± 0.2^{c}	19.5 ± 0.0^{a}
Third-PGBR	7.4 ± 0.2^{NS}	9.0 ± 0.2^{b}	29.6 ± 0.2^{a}	0.9 ± 0.0^{a}	23.4 ± 0.0^{b}	19.3 ± 0.2^{ab}
Malted-PGBR	7.4 ± 0.2^{NS}	9.0 ± 0.3^{b}	29.6 ± 0.2^{a}	0.9 ± 0.0^{a}	23.8 ± 0.3^{a}	19.3 ± 0.2^{ab}
Pathum-Thani1						
BR	7.4 ± 0.2^{NS}	12.7 ± 0.2^{a}	28.2 ± 0.4^{b}	1.1 ± 0.0^{NS}	23.6 ± 0.1^{b}	14.3 ± 0.0^{c}
First-PGBR	7.3 ± 0.3^{NS}	11.3 ± 0.3^{b}	29.1 ± 0.2^{a}	1.1 ± 0.1^{NS}	23.7 ± 0.2^{b}	14.5 ± 0.0^{b}
Second-PGBR	7.3 ± 0.2^{NS}	11.0 ± 0.3^{bc}	29.3 ± 0.0^{a}	1.2 ± 0.1^{NS}	23.9 ± 0.2^{ab}	14.8 ± 0.1^{a}
Third-PGBR	7.3 ± 0.2^{NS}	10.7 ± 0.3^{c}	29.3 ± 0.0^{a}	1.2 ± 0.1^{NS}	23.8 ± 0.3^{ab}	14.7 ± 0.1^{a}
Malted-PGBR	7.3 ± 0.3^{NS}	10.8 ± 0.1^{c}	29.2 ± 0.2^{a}	1.1 ± 0.0^{NS}	24.0 ± 0.2^{a}	14.8 ± 0.1^{a}
Phitsanulok2						
BR	8.3 ± 0.1^{NS}	18.8 ± 0.4^{a}	23.9 ± 0.1^{d}	1.0 ± 0.1^{NS}	19.9 ± 0.1^{c}	13.3 ± 0.1^{b}
First-PGBR	8.3 ± 0.1^{NS}	18.4 ± 0.6^{ab}	24.6 ± 0.1^{c}	1.0 ± 0.0^{NS}	20.2 ± 0.2^{b}	13.5 ± 0.3^{ab}
Second-PGBR	8.3 ± 0.1^{NS}	18.1 ± 0.1^{b}	25.0 ± 0.1^{b}	1.0 ± 0.1^{NS}	20.4 ± 0.0^{b}	13.6 ± 0.1^{a}
Third-PGBR	8.3 ± 0.2^{NS}	17.9 ± 0.1^{b}	25.6 ± 0.1^{a}	1.0 ± 0.0^{NS}	20.5 ± 0.2^{ab}	13.8 ± 0.1^{a}
Malted-PGBR	8.4 ± 0.2^{NS}	18.0 ± 0.4^{b}	25.6 ± 0.1^{a}	1.0 ± 0.0^{NS}	20.6 ± 0.1^{a}	13.8 ± 0.2^{a}

* Results are expressed as mean ± SD ($n = 6$) with different lowercase superscript letters in the same column within the cultivar being significantly different ($p < 0.05$). ^{NS} refers to no significant difference ($p \geq 0.05$).

conditions were probably due to the preparation and utilization of glutelin molecules during pre-germination (Liu et al., 2011).

The increases of alpha-globulin and 13 kDa prolamin during rice pre-germination, in terms of the RC under reducing conditions were rather similar to the changes under non-reducing conditions in all rice cultivars. However, significant changes were found under reducing conditions at the shortened stage of pre-germination compared to analysis under the non-reducing conditions.

In conclusion, different rice cultivars and different stages of pre-germination, in relation to EGL, had distinct effects on the partial degradation of starch and resulted in significant changes in the crude protein and crude fat contents. The changes in the protein profiles and the RC of polypeptides indicated the biochemical effects on the bio-modification of rice proteins during pre-germination, namely, the reduction of disulfide bonds of glutelin molecules and the increased RC of 13 kDa prolamin. These were significant and occurred after the second-stage of SPT1 (44 h of pre-germination) and PTT1 (32 h of pre-germination) and after the first-stage of PNL2 (48 h of pre-germination), which could be further used for textural development of specific rice products such as rice bread.

Conflict of interest

The authors declare that they have no conflicts of interest.

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