International Food Research Journal 24(6): 2563-2571 (December 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Changes of nutritional value, bioactive compounds and antioxidant activity of primed white rice, Chainat 1, during seedling

Rattanapon, R., *Siripongvutikorn, S., Usawakesmanee, W. and Thongraung, C.

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

Article history

Received: 13 July 2016
Received in revised form: 28 October 2016
Accepted: 29 October 2016

Keywords

Ricegrass
Seedling time
Bioactive compounds
Antioxidant

Abstract

Seedling time is an important determinant factor for agricultural practice involves with costing and further growing step of all plant seeds. To improve seeding time, growth character, nutritional value and bioactive compounds relating to biological activity as antioxidant property, priming process is necessary for many plants. This study aims to investigate the nutritional value, bioactive compounds and antioxidant activity of ricegrass cv. Chainat 1 primed with fish protein hydrolysate (FPH) during seedling process. The results found that the nutritional value of ricegrass including protein and ash content was highest at 14 d while fat content increased when seedling time increased. Moreover, ricegrass aged 21 d had the highest content of chlorophyll and carotenoids compared with 7 and 14 d. Phytic acid content was lowest in ricegrass aged 7 d thereafter, it was increased. While, total phenolic content (TPC) was highest in ricegrass aged 7 d which also revealed greater antioxidant activity evaluated by ABTS radical scavenging activity, ferric reducing antioxidant power (FRAP) value and iron chelating activity than ricegrass aged 14, 21 d and primed seed. Therefore, seedling time had a significant impact on TPC and antioxidant activity of ricegrass, especially 7 d. In addition, the ricegrass aged 7 d could be possible to produce functional food products in the future.

Introduction

Seedlings, young plants grown from any plant seed such as wheat, oats, barley and rice are claimed to be rich in nutrient, vitamins, minerals, bioactive compounds and antioxidant activity (Hattori, 2002) which are interesting because of their beneficial to human health. It has been preclaimed to help blood flow, digestion and general detoxification of the body (Padalia *et al*., 2010). Rice seedling or ricegrass is a young grass stage of rough rice (*Oryza sativa* L.). Because of high mineral contents beside the higher of antioxidant activity compared with rice grain (Benjawan *et al*., 2011), ricegrass is more focusing in Thailand.

Recently, Chainat 1, a white rice, is a high productive type as it is non-sensitive to photoperiod, resistant to insects (Vetayasuporn, 2012). In addition, with high amylose content, the cooked rice is hard and crumble therefore Chainat 1 is famous for producing cooked rice, noodle and germinated rice. Kaosa-ard and Songsermpong (2012) reported that germinated Chainat 1 yielded higher gamma aminobutyric acid (GABA) content compared with un-germinated rice. Rattanapon *et al.* (2016) stated that ricegrass of Chainat 1 primed with FPH yielded greater TPC and antioxidant activities. In addition, ricegrass juice from colored rice cultivar Kum Doisaket possessed a high antioxidant activity and exhibited DNA protective effect (Khanthapok *et al*., 2015).

Priming process is a method for improving seedling development due to dormant enzyme activation increasing phenolic compounds and reducing anti-nutrient agent (Zhang *et al*., 2015). Moreover, the secondary metabolite compounds and antioxidant activity could be changed during seedling growth (Jiang et al., 2007). Liu *et al*. (2010) addressed that TPC of pigeon pea seedlings was enhanced during initial germination thereafter, the content was decreased. Sharma and Gujral (2010) reported that TPC of barley *(Hordeum vulgare)* sprouts was higher than that of barley seeds.

Fish protein hydrolysate (FPH) which was produced from byproduct of fishery industry was applied in many plant seeds such as corn, soy bean and tomato (Horii *et al*., 2007). Actually, FPH is now used in wide spectrum range for food and food ingredients as well as fertilizer (Randhir and Shetty, 2005). Randhir and Shetty (2003) reported an increase of germination rate, plant height, fresh weight and total phenolic content of fava bean primed with mackerel fish protein hydrolysate. In addition, Horii

*Corresponding author.
Email: *sunisa.s@psu.ac.th*

et al. (2007) reported that seed vigour (weight and height), phenolic compounds, chlorophyll content and antioxidant activity of soybean and tomato were different and depended on FPH concentration during priming process. Using 5 ml/l of FPH had higher these values in soybean compared with using 2.5 ml/l FPH. In contrast to tomato, seed vigour was higher when using 2.5 ml/l FPH compared to 5 ml/l. Thomson *et al.* (2015) reported that FPH produced from silver carp with papain at degree of hydrolysis (DH) 23.4% and used as a seed primer, stimulated growth and increased phenolic compounds of soybean seedlings. Rattanapon *et al*. (2016) addressed that the rough rice of Chainat 1 primed with 10 ppm FPH significantly enhanced growth character, TPC and antioxidant activity of ricegrass. Though, there are several factors such as rice varieties, moisture content and storage time which determine biochemical compositions and bioactive compounds of ricegrass, seedling time is one of the most important factors affecting on the accumulation of bioactive compounds. To sum up, the changes of phenolic compound contents during seedling depend on plant species and the conditions of germination. Therefore, this study aimed to investigate the optimum seedling time of ricegrass primed with FPH. Additionally, the chemical compositions, bioactive compounds and antioxidant activity of ricegrass were also monitored.

Materials and Methods

Materials

The rough rice of Chainat 1 variety with moisture content less than 14% obtaining from Phattlung rice research center located in Phattalung, Thailand in June, 2014 was packed in plastic bag and kept in plastic box with a lid at room temperature (28±2ºC) used as the primary sample within 6 mo. Fish protein hydrolysate (FPH) prepared from tilapia *(Oreochromis niloticus)* by papain with DH 14.23% was provided from Department of Food Technology, Prince of Songkla University.

Preparation of ricegrass

FPH solution at 10 mg/l was prepared from FPH powder containing 74.00±1.86 g/100 g of protein content (dry basis). The conductivity of the solution was 20.00±0.20 µS/cm and total nitrogen, nitrate-N, ammonia-N and nitrate-N content were 4, 0.22, 0.64 and 0.05 mg/l, respectively. The rough rice seeds were soaked in the FPH solution (10 mg/l) with a ratio of rice seed to FPH solution as 1:5, stored for 24 h at room temperature (28±2ºC) before subjected to drain and wash with tap water (2 times). Thereafter, the seeds were spread on surface of the soil filled in plantation bed. The experiment was done for 4 replications per treatment (100 g of seed per replication). Five-hundred milliliter of water were sprayed on the plantation bed size 35.5 x 55 x 4.5 cm every day. The ricegrass aged 7, 14 and 21 d was cut above the soil 0.5 cm before brought to wash with tap water 2 times and drain 2 min on the sieve and keep at 4ºC for further study.

Nutritional value

Nutritional values of ricegrass including moisture, protein, fat, fiber, ash and carbohydrate contents were analyzed following the AOAC (2000).

Chlorophyll and carotenoid content

Chlorophyll a, b content and carotenoid content of the shoots were analyzed following the AOAC (1990).

Phytic acid content

Phytic acid content was determined by using the method of Haug and Lantzsch (1983). Briefly, the sample (0.8-1.0 g) was extracted with 25 ml of 0.2 N hydrochloric acid (HCl) in flask and shaken for 1 h on shaker at 30ºC, thereafter mixture was added with 1 ml acidic ammonium iron-III sulphate ($FeNH_4(SO_4)_2$) solution and heated at 105ºC for 30 min. After cooled down, the solution was centrifuged at 1600 x g for 30 min, then the supernatant (1 ml) was transferred to test tube and 1.5 ml of 2,2-bipyridine solutions was added. The absorbance was measured at 519 nm. A decrease in absorbance of iron content, in the supernatant was measured and taken to calculate for phytic acid contents.

Preparation of ricegrass extract

The ricegrass from each treatment was homogenized with water at ratio of ricegrass to water as 1:2 for 5 min, centrifuged at 10000 x g for 20 min, and the supernatant was freeze dried to obtain the powder and stored at -20ºC until further analysis.

Total phenolic content (TPC)

The powder of ricegrass extract (0.1g) was dissolved in distilled water (10 ml) to obtain stock solution for measurement TPC (Calzuola *et al*., 2004). The total phenolic content of the ricegrass was measured using a modified Folin-Ciocalteu method (Tan and Kassim, 2011). Briefly, the ricegrass extract 0.5 ml was put into volumetric flask, then added with 10% (v/v) Folin-Cioculteu reagent 5 ml and allowed to stand at room temperature for 5 min. Thereafter 4 ml of 1 M sodium carbonate (Na_2CO_3) was added

and adjusted final volume to 10 ml, kept in dark for 90 min. The absorbance was measured at 750 nm. The measured values was compared with a standard curve of gallic acid prepared in the range of 0-0.3 mg/ml and expressed as milligram of gallic acid equivalents/g ricegrass.

Radical scavenging assay by using DPPH

The ricegrass extract (1.5 ml) from the right concentration was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed and allowed to stand for 30 min at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm using spectrophotometer. A standard curve was prepared using Trolox in the range of 10-60 µM. The activity was expressed as µmol Trolox equivalents (TE)/g ricegrass (Kulkarni *et al*., 2006).

ABTS radical scavenging activity

The stock solutions including 7.4 mM 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonicacid (ABTS) solution and 2.6 mM potassium persulphate ($K_2S_2O_8$) solution were prepared. The working solution was prepared by mixing the two stock solutions in equal quantities and allowed them to react for 12 h at room temperature in the dark. The extract (150 µl) was mixed with 2850 µl of ABTS solution and left at room temperature for 2 h in the dark. The absorbance was measured at 734 nm using the spectrophotometer. A standard curve of Trolox ranging from 50 to 600 µM was prepared. The activity was expressed as µmol Trolox equivalents (TE)/g rice grass (Kulkarni *et al*., 2006).

Ferric reducing antioxidant power (FRAP)

A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution and 2.5 ml of 20 mM iron (III) chloride ($FeCl_3$) solution then incubated at 37ºC for 30 min and be referred as FRAP solution. The rice grass extract (150 µl) was mixed with 2850 µl of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 50 to 600 µM. The activity was expressed as µmol Trolox equivalents (TE)/g rice grass (Kulkarni *et al*., 2006).

Iron chelating activity

The ricegrass (277.5 µl) from the right concentration was added with 7.5 µl of 2 mM iron (II) chloride ($FeCl_2$) solution and 15 µl of 5 mM ferrozine and the mixtures were incubated for 5 min. The absorbance was measured at 562 nm. The chelating activity of a sample was expressed in terms of µmol equivalents of ethylenediaminetetraacetic acid (EDTA) per gram of sample (Ebrahimzadeh *et al*., 2009).

Statistical analysis

The data was subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test.

Results and Discussion

Nutritional values

It was found that nutritional values including fat, ash and protein of any aged ricegrass were higher than primed rice seed (Table 1). During seedling period, the metabolic rate in the seed increased rapidly, due to the active enzymes, which lead to the utilization of fat, protein and carbohydrate for energy and growth (Evelyn and Juliano, 1972). Moreover, the catabolism of primary metabolites including lipids and carbohydrates were utilized to produce energy for the plant development. Protein content of aged ricegrass seemed to be increased when seedling time increased. In general, an increasing of protein content may be affected by activated enzymes which related to protein synthesis at the period of germination (Zhang *et al*., 2015). Therefore, the changes of protein content during seedling period may due to storage proteins present in cotyledon were hydrolyzed to be peptides which involve in embryo axis development through proteins synthesis, thereby elevating protein concentration in seedling (Onwuka *et al*., 2009). Maneemegalai and Nandakumar (2011) reported that the protein content of Vigna radiata and Vigna mungo increased after germination 2 d. In addition, Inyang and Zakari (2008) also noted that germination may increase the protein content. In cereals and legumes, this increase is due to the presence of protein hydrolysis as well as the results of protease enzyme activity during germination the seeds. Nzelibe and Nwasike (1995) suggested that the mobilization of nitrogen was increased during germination lead to increase protein content. In addition, Suhaidi (2003) addressed that the seedling induced changes in the biology of the breakdown of the components in plants. Moreover, during plantation, protease are active and which involved in degradation of peptide to amino acids, thus protein may increase. Fat content of ricegrass increased and highest when seedling time increased and reached the peak at 21 d. An increasing of fat content during

Table 1. Nutritional values of ricegrass primed with FPH at difference germination time

Compositions	Germination time (d)			
	Primed			
(%)*	seed	7	14	21
Crude protein	8.44±0.31^{b}	18.36±1.45^{a}	19.64±0.51^{a}	19.47±0.62^{a}
Crude fat	5.51±0.20^{d}	9.21±0.25^{c}	15.13±1.50^{b}	19.24±0.22^{a}
Ash	11.99±0.15^{c}	18.25±0.83^{b}	19.40±0.42^{a}	19.24±0.22^{a}
Crude fiber	5.56±0.97^{c}	17.77±0.67^{b}	17.90±0.48^{a}	18.10±0.25^{a}
Carbohydrate	74.06±0.60^{a}	54.18±2.02^{b}	45.83±2.20^{c}	41.76±0.47^{d}

Each value was expressed as the mean ± standard deviation (n=3). Different little letters in the same row indicate significant differences ($p<0.05$). *Each value was expressed as g/100 g dry basis.

Table 2. Chlorophyll and carotenoids content of ricegrass primed with FPH at difference germination time

Content	Germination time (d)			
	primed			
(mg/g)	seed	7	14	21
Chlorophyll a	ND	0.48±0.02^{c}	0.59±0.05^{b}	0.81±0.07^{a}
Chlorophyll b	ND	0.28±0.01^{b}	0.28±0.06^{b}	0.45±0.08^{a}
Total chlorophyll	ND	1.03±0.04^{c}	1.21±0.12^{b}	1.70±0.16^{a}
Carotenoids	ND	0.21±0.01^{b}	0.23±0.03^{b}	0.32±0.03^{a}

Each value was expressed as the mean ± standard deviation (n=3). Different little letters in the same column indicate significant differences ($p< 0.05$). ND: not detected

seedling may due to triacylglycerol (TAG) was hydrolyzed by enzyme to fatty acid, which enzymes were lipase and lipoxygenese. Moreover, Kornberg and Beevers (1957) addressed that fatty acid have been mobilized to provide energy for seedling growth. Ash content was lowest in primed seed while the content significantly increased during plantation. It may due to inorganic compounds as minerals was released by active enzyme during germination, and utilized for plant growth. El-Adawy *et al.* (2003) reported that the ash content significantly increased during sprouting in mungbean and pea. Moreover, Hussain *et al.* (2010) addressed that ash content of wheat was increased during growth and related to mineral content. In addition, an increasing of ash content due to mineral bioavailability of plant during germination and development (Rao and Prabhavathi, 1982). Carbohydrate content of primed seed was higher compared with ricegrass at aged 7, 14 and 21 d. While crude fiber seemed to increase during seedling growth. The amount of crude fiber in the primed rice seed may explain the presence of bran layer, outer layer of rice and hull. However, an increasing of crude fiber during seedling growth may due to polysaccharides in the cell wall including cellulose, glucose and mannose were changed and led to increase in the cellular structure of the plant during plant growth (Martin-Cabrejas *et al*., 2003). Peer and Leeson (1985); Cuddeford (1989) reported an increase of cell wall which mainly consists of cellulose and hemicelluloses, are synthesized from carbohydrate during plant growth. This present result also showed the decreased carbohydrate content in aged ricegrass may due to activated α-amylase. The α-amylase breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing of seedlings during the early stages of germination (Lasekan, 1996). Generally, it pointed out that there were various changed in each proximate compound during priming and seedling period.

Chlorophyll and carotenoids content

It was found that chlorophyll and carotenoid content of ricegrass increased as increased seedling time ($p<0.05$) as showed in Table 2. Therefore, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content in ricegrass aged 21 d were highest followed by 14 and 7 d. Chlorophyll and carotenoids are photosynthetic pigments in plants. An increasing of chlorophyll due to chlorophyll biosynthesis, which is essential in the formation of photosystems to provide more energy for plant growth. Horii *et al.* (2007) addressed that total chlorophyll content of soybean increased during late germination (12 d). In addition, chlorophyll a and total chlorophyll content were highest at 9 d of tomato seedling (Horii *et al*., 2007). Lefsrud *et al*. (2007) stated that chlorophyll and carotenoids content of kale leaves (*Brassica oleracea* L. var. *acephala*) at leave development stage (7-14 d) significantly increased before decreased afterword. There was reported a good relationship between chlorophyll and carotenoid content due to the chlorophyll biosynthesis regulate the transcription of light-harvesting chlorophyll-binding proteins which are also responsible for carotene and xanthophyll binding (Lohr *et al*., 2005). Therefore, it could be stated that the increasing of photosynthetic pigment determined as chlorophyll and carotenoid compounds of ricegrass significantly increased as increased plantation time for 21 d.

Phytic acid content

It was found that phytic acid content of ricegrass was highest at 14 of seedling time while the content

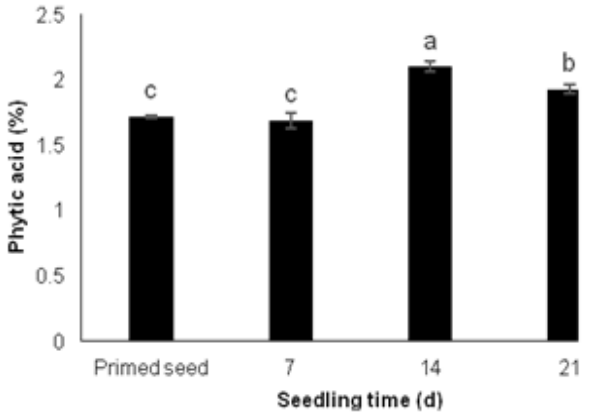

Figure 1. Phytic acid content of ricegrass primed with FPH at difference germination time. Each value was expressed as the mean ± standard deviation (n=3). Different little letters indicate significant differences ($p< 0.05$).

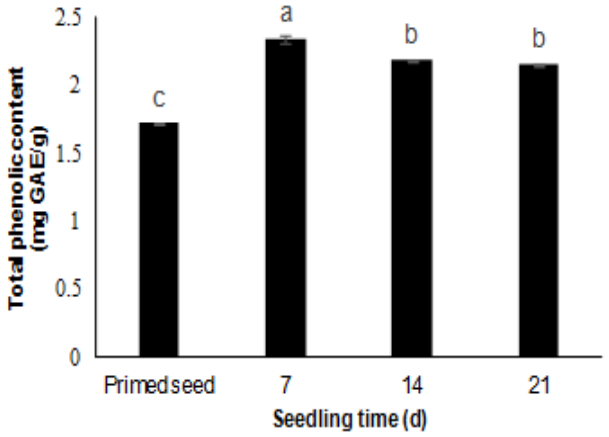

Figure 2. Total phenolic content of ricegrass primed with FPH at difference germination time. Each value was expressed as the mean ± standard deviation (n=3). Different little letters indicate significant differences ($p<0.05$). GAE: Gallic acid equivalent.

was lowest in primed seed and ricegrass aged 7 d as showed in Figure 1. Kumar *et al*. (2010) mentioned that a decrease of phytic acid is a function of phytase enzyme during germination of cereal seeds. The biological function of phytase is to produce free inorganic phosphorus (Debnath *et al*., 2005). However, excessive of phosphorus could inhibit phytase activity (Sung *et al.* 2005), thus phytic acid would be increased. Moreover, an increasing of phytic acid content after 7 d may due to the degradation of the enzyme by active protease (Houde *et al.* 1990) or expression of phytase delayed relatively even when other proteins are being synthesized. This result was agreement in the result of Azeke *et al*. (2011) who reported that phytic acid content of rice seedling was lowest at 7 d of germination then increased. In addition, stage of seedling, genetics, environmental and fertilizer application were determinant factors affecting to phytic acid content (Wu *et al*., 2009).

Total phenolic content

It was found that the content of total phenolic compounds (TPC) of ricegrass significantly increased at seedling for 7 d before decreased after word as showed in Figure 2. The total phenolic content of ricegrass aged 7 d was highest, before slightly decreased as timing increased to be 14 and 21 d but still higher than primed seed. As know that phenolic compounds are important secondary metabolized in plant during seedling growth. Those compounds are mainly produced via shikimate pathway and phenylpropanoid biosynthesis pathway (Zhang *et al*., 2015). During soaking or priming process, the amount of free form of phenolic compounds in primed rice seed was increased due to enzyme hydrolysis (Cornejo *et al*., 2015). While, the bound phenolic content was increased at seedling stage due to enzymes involved in the phenylpropanoid pathway and degradation of the cell wall polysaccharides and proteins leading to release of bound phenolics (He *et al.*, 2011). Moreover, during seedling growth, L-phenylalanine is transformed to cinnamic acid under the catalysis of phenylalanine ammonialyase (PAL). Thereafter, many phenolic compounds are synthesized to be flavonoids, tannins, lignins, and other compounds (Zhang *et al*., 2015). Moreover, phenylalanine ammonialyase (PAL) could be enhanced during germination (Tang and Zhao, 1998). Kong *et al.* (2007) addressed that the phenolic contents of malting barley increased along with an increase of PAL. Thus, it could be assumed that the increasing of TPC in the ricegrass in this experiment may due to the increase of PAL activity during the initial stage of ricegrass germination. Since FPH are rich in amino acid or nitrogen element which increased accumulation of PAL (Stewart *et al*., 2001). To prove that PAL activity of primed germinated rice plays a role for TPC, therefore PAL activity needs to be further investigated. In addition, Liu *et al.* (2008) explained that the phenolic compound contents increased rapidly at the early stage of seedling growth (1-3 d). It may due to the seedlings require the some compounds for protect itself from environmental stress and microorganism or insects (Anguiano *et al*., 2015). However, in this experiment the levels of phenolic compounds in the aged ricegrass 14 d declined gradually and eventually reached steady levels during the late-germination, 21 d. Gujral *et al.* (2011) stated that a decrease of total phenolic content of germinated rice was attributed to polyphenol oxidase activity during germination. Moreover, Randhir *et al.* (2004) also reported that the total phenolic content of green mung decreased during

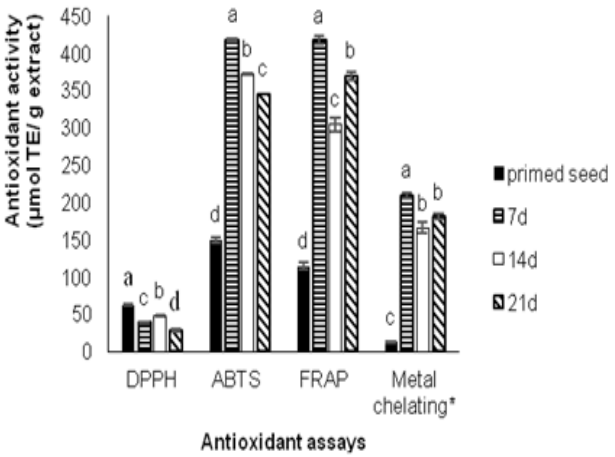

Figure 3. Antioxidant activity of ricegrass primed with FPH at difference germination time. Each value was expressed as the mean ± standard deviation (n=3). Different little letters in the same column indicate significant differences ($p< 0.05$). TE: Trolox equivalent. *: the value was expressed as the unit µmol EDTA equivalent/g.

germination. It pointed out that an increasing or a decreasing of phenolics depended on plant variety, germination/seedling time, priming condition and planting condition and so on.

Antioxidant activity

The antioxidant activity of ricegrass primed with FPH during seedling time showed in Figure 3. It was found that ricegrass aged 7 d exhibited greatest antioxidant activities determined as ABTS and FRAP compared with 14, 21 d and primed seed. As know that hydroxyl groups in the phenolic compounds structure play a role for a radical scavenger by giving hydrogen atom or electron to react with free radicals to convert them to more stable products then blocked the radical chain reaction (Zou *et al.*, 2004). The higher of ABTS and FRAP in ricegrass aged 7 d may due to a high oxygen demand during early germination as a result, the plants produced phenolic compounds to protect the cell from oxidation-induced deterioration. Zhang *et al*. (2015) explained that during seedling growth, total phenolic content increased then antioxidant activity was significantly improved. Moreover, antioxidant activity was related to the biochemical metabolism of seedling during growth, which resulted in raising the antioxidant compounds such as phenolic acid and polyphenolic. Zinca and Vizireanu (2013) suggested that phenolics compounds of seedling during initial germination stages may serve as radical scavengers or antioxidants. However, in this experiment the greatest DPPH radicals scavenging activity was showed in primed seed before it decreased at seedling stage. This may due to enzyme was activated and produced free form phenolics during seed priming process (Cornejo *et al*., 2015). Moreover, type of phenolic in primed seed may be a small phenolic molecule, which have better activity to access the DPPH radical site. Regardless primed seed, DPPH radical scavenging activity of ricegrass aged 14 d was highest compared with 7 and 21 d which differed from ABTS and FRAP values. It pointed out that type and amount of phenolic compounds in each stage of growth may differ which should be furthermore investigated. In addition, it was observed that the DPPH value was lower than the ABTS and FRAP value. It may due to the steric effect inside the DPPH radical molecule as resulted in difficulty for the large reactive antioxidant compounds to react with its radical site (Prior *et al.*, 2005). In the other hand, an increasing of dietary fiber during seedling may correspond to reduce phenolic content, and hence reduced radical scavenging activity (Maisuthisakul *et al.*, 2008).

The iron chelating activity in primed seed was lowest and highest in ricegrass aged 7 d before it slightly decreased at 14 and 21 d. The result indicated that seedling time had affected on iron chelating ability of ricegrass. From the literature review, it was found that not only phenolic compounds and/or flavonoids can be chelators but also chlorophyll, phytic acid and fiber play a role in chelating activity (Jacobsen and Ellingsen, 1983; Hsu *et al.*, 2013). Chlorophyll act as free radical scavengers and Fe (II) chelators, which may prevent the catalysis of lipid peroxidation. Moreover, chlorophyll would require substitution of Fe (II) for Mg atom (Hsu *et al*., 2013). In addition, phytic acid is claimed as the iron chelator and exhibited a high ability of antioxidation. There was proposed that negative charge of phosphate group in phytic acid molecule would react with positive charge of iron and block the formation of hydroxyl radicals as well as suppress lipid peroxidation. (Graf *et al*., 1987). Zha *et al.* (2009) suggested that fiber exhibited reducing power and chelating ferrous ion. Moreover, it can against the hydroxyl free radical and superoxide radical.

Conclusion

Significant changes in nutritional values, bioactive compounds and antioxidant of primed Chainat 1 rice were affected by seedling period. It was suggested that both phenolic compounds and antioxidant activity were significantly great in the ricegrass grown for 7 d even the ricegrass aged 21 d was a good source of chlorophyll, carotenoids and nutritional values. Therefore, based on less phytic acid content but high total phenolic content and antioxidant activity

(ABTS and FRAP value), rice primed with FPH and grown for 7 d must be chosen to obtain a better use for primary ingredients in food supplements or functional food products and pharmaceuticals in the further work. Moreover, these results can be used as a prototype for improving bioactive compounds and biological activity for other rice varieties.

Acknowledgements

Authors would like to thank Faculty of Agro-Industry, the Phatthalung Rice Research Center and the Graduate School of Prince of Songkla University, Thailand for the financial support.

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