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Effect of Shelf life on Germination Percentage and Some Nutritional Value of Germinated Native Black Rice

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***Abstract*:** *Native black rice(KhaoHawm Mae Paya Tong Dam) was studied on germination percentage (%) and length of roots when storage time was increased at 1, 2, 3, 4, 5 and 6 months, respectively. The result found that % germination and length of roots exhibited a decrease at the end of storage (93.92 ± 0.63, 89.00 ± 0.70 % and 1.54±0.07, 1.42±0.53 mm at 1 and 6 months of storage, respectively). Then, some nutritional values of germinated native black rice werealso evaluated. The result indicated that γ-aminobutyric acid had increased slightly when the storage time was longer (9.01±0.43,9.09±0.07and10.82 ±0.14 mg/100 g at 1, 3, and 5 months of storage, respectively). However, vitamin B1, protein content, antioxidant activity (DPPH scavenging activity assay) and phenolic compounds decreased when the shelf lifewas longer (0.78±0.01,0.73±0.04 and0.54±0.00 mg/100 g; 13.40±0.03, 12.95 ±0.11 and12.99±0.05 g/100 g; IC_{50}22.15 ±0.17,36.34±5.47 and 36.47±4.59mg/ml; 16.07±0.14, 15.91±0.32 and 14.57±0.10 mg GAE/ml at shelf life of 1, 3, and 5 months, respectively). In contrast, the moisture content still increased significantly($p \leq 0.05$)(7.68±0.08and 8.74±0.07% at 1 and 6 months of storage, respectively). In conclusion, the finding from this research could be used as a suggestion guideline forrice consumptionas well as to control the quality of germinated nativeblack rice.*

Keywords: *Native black rice, γ-aminobutyric acid (GABA), antioxidant activity*

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population including Thailand [1]. In fact, Thai farmers grow rice in many areas of Thailand, particularly in Chanthaburi province. Native black rice (Khao Hawm Mae Paya Tong Dam) is a traditional rice plant variety in Amphur Kao Kitchakut, Chanthaburi province. The pigment of this rice is black. In pigmented rice, there is a natural colorant; anthocyanin, which is reported to possess a free radical scavenging activity [2]. In addition, black rice contains more nutritional components such as dietary fibers, phytic acid, vitamin E, and vitamin B than the ordinary milled rice [3]. Moreover, Sangkitikomon *et al.*(2008) reported that anthocyanin from black rice was found to have higher antioxidant activity than red rice and rice berry [4]. From the nutritional point of view, black rice is the most famous and generally used ingredient in snack and desserts [5]. Germinated black rice offers more considerable benefit. Especially that it had an increasingin GABA, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor. GABA is a neurotransmitter in the brain and the spinal cord of mammals. This substance can lower hypertension, promote sleepiness and has the benefit for human health [6]. Additionally, the germination of black rice frees it bound minerals, making them more absorbable by the body and the rice is tenderer and tastier since black rice is a rich source of nutritional value and high antioxidant activity[7]. On the other hand, no information regarding the effect of shelf life on germinated percentage and some nutritional values of germinated native black rice has been reported yet. Therefore, the purpose of this study was to investigate the effectof shelf lifeon germinated percentage, length of roots, and some nutritional values of germinated native black rice.

2. Materials and method

2.1 Materials

Native black rice (Khao Hawm Mae Paya Tong Dam) was purchased from a local farmer in Amphur Kao Kitchakut, Chanthaburi province in Thailand and transported to the laboratory.

2.2 Germinated native black rice preparation

Germinated native black rice was prepared according to modified methods described by Panyanak *et al.* (2010) [8]. Briefly, the sample was selected and soaked in water at the ratio of rice and water(1:10) at 40˚C for 6 hours in the tray. The water was drained and the samples were incubated for 24 hours. The germination was stopped by drying using a hot air oven, at 55˚C for 4.5 hours. Then, the obtained germinated native black rice wasstored at room temperature (30˚C) and monitored forgermination percentage, length of roots, moisture content, and phenolic compounds as shelf life was increased at 1, 2, 3, 4, 5, and 6 months, respectively. Finally, the obtained germinated native black rice wasalso investigated for γ-aminobutyric acid, vitamin B1, protein content and antioxidant activity (DPPH scavenging activity assay) when shelf life was increased at 1, 3, and 5 months, respectively.

2.3 Moisture content determination

Moisture content evaluation was performed using the method of drying method (AOAC,1990) [9]. Germinated native black rice sample (3 g) was used in the assessment.

2.4 Phenolic compound determination

Phenolic compounds evaluation was investigated according to modified methods described by Wolfe *et al.* (2005)[10]. Samples (1 g) were mixed with 500 ul of distilled water and 125 ul of folin reagent. The solution was incubated for 6 min, before adding 1,250 ul of 7 % sodium carbonated and 1,000 ul of distilled water and then the solution was incubated for 90 min. Finally, the absorbance were determined by using spectrophotometer at the wavenumber 760 nm. The phenolic compound were calculated by using standard curve of standard gallic acid.

2.5 γ-Aminobutyric acid (GABA)determination

GABA content were sent to analyze by the Institute of Food Research and Product Development (IFRPD), Kasetsart University in Bangkok, Thailand. Briefly,a sample of 2.5 gwas added to 18 ml of distilled water and 2 ml of 3% sulfosalicylic acid, respectively. Then, the mixture was stirred for 30 minutes and centrifuged. The supernatant (0.1 ml) was mixed with 0.1 ml of $NaHCO_3$ and 0.40 ml of debsyl-C andthese solutions were mixed together. The solution was heated in water bath at 70°C for 10 minutes. The obtained solution wasthen mixed with 0.25 ml of ethanol and 0.25 ml of 0.025 M KH_2PO_2. The γ-aminobutyric acid (GABA)wasvaluated using a High Performance Liquid Chromatography (HPLC)(HPLC-UV detector:agilent, 1,200 serice; column:supercosil LC-DABS, 15 cm x 4.6 cm,3 um; flow rate 1 ml/min; mobile phase: gradient 80 % CH_3COONa pH 6.80, acetonitrile inject volume 5 ul; column temperature 40 °C, detector: uv 465 nm and standard GABA ≥ 99 %)[11].

2.6 Vitamin B1 determination

Vitamin B1 wasalso sent to analyze by the Institute of Food Research and Product Development (IFRPD) at Kasetsart Universityin Bangkok, Thailand. Briefly,thiamin was extracted according the method of Ndaw *et al.* (2000)[12]. Before analysis, the samples were filtered through a 0.45 um Millex filter (Millipore). The filtrate was used for chromatographic analysis. The HPLC liquid chromatographic system was a 2690 Alliance Model (waters) with a 2475 fluorescence detector (waters). A uBondapakC_{18} column (15 cm x 3.9 mm, waters) with a mobile phase (0.05 M sodium acetate-methanol(30/70; v/v, pH 6) was used. The separation was evaluated at 30°C at a flow rate of 1 ml/min. The injection volume was 20 ul[13].

2.7 Protein content determination

Protein content of germinated native black rice was estimated using the Kjeldahl method as reported by AOAC, 1990[9].

2.8 DPPH radical scavenging activity determination

The free radical scavenging activity wassent to analyze by Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) in Bangkok, Thailand. DPPH radical scavenging activity procedure was performed according to the methods described by Zhu *et al.* (2006)[14]**.** Briefly, one gram of sample was extracted with 10 ml ethanol. The solution was separated by centrifugation at 6,000 rpm. The obtained supernatant was tested by mixing with ethanol at various concentrationsof 10, 20, 30, 40, and 50 µg/ml. The sample(1 ml) was mixed with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 95% ethanol (1ml) and incubated in dark condition for 30 minutes. The absorbance was determined using a spectrophotometer at 517 nm. Vitamin C (L-ascorbic acid), Vitamin E (Tocopherol), and BHT (Butylatedhydroxytoluen) were used in the reference standard compound. The percentage of radical scavenging activity was calculated as the following equation:

DPPH radical scavenging activity (%) = $[(A_0 – A_1)/ A_0] \times 100$

A_0 = the absorbance of control reaction

A_1 = the absorbance of test compound

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against the sample concentration.

2.9 Data analysis

Physicochemicalcharacteristics analysis was carried out in three replicates while some nutritional values were carried out in duplicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$). Mean with significant differences was separated by Duncan's multiple range test (DMRT) using the computer software.

3. Results and Discussion

From determination, the effect of shelf life ongermination percentage, length of roots, moisture content, and phenolic compounds of germinated native black rice every month until 6 monthsarepresented in Table1. The level of %germination of germinated native black ricehad a slightly significant($p \leq 0.05$) decrease when the shelf life was longer.The number of germination percentage (93.92±0.13 %) of germinated native black rice at 1 month was higher than at 6 months (89.00±0.71%). In addition, the number of length of roots of germinated native black rice had also slowly decreasesignificantly ($p \leq 0.05$) when the shelf lifewas longer. The number of length of roots (1.54±0.07 mm) of germinated black rice at storage time of 1 month was higher than of 6 months (1.42±0.53 mm). Moreover, the level of phenolic compounds of germinated native black ricewas significantly reduced when the storage time was longer. The level of phenolic compounds(16.07±0.14 mg GAE/100 ml) of germinated black rice at storage timeof 1 month was higher than of 6 months (14.00±0.28 mg GAE/100 ml).The finding in this study was in agreement with the earlier reports. Zhou *et al*. (2004) also found that a reduction in the content of bound phenolic acids in brown and white rice was higher at 37°C than at 4°C during the storage [15].In contrast, the value of moisture content of germinated native black rice had significantly ($p \leq 0.05$) increased during longer storage period. The value of moisture content (7.68±0.08%w/w) of germinated native black rice at 1 month showed lower than at 6 months(8.74±0.07%w/w)

TABLE 1 .Effect of Shelf Life on Germination Percentage, Length of roots, Moisture content and Phenolic compounds of Germinated Native Black Rice

Shelf life (month)	%Germination	Length of roots (mm)	Moisture content (%w/w)	Phenolic compounds(mg GAE/100 ml)
1	93.92±0.13[a]	1.54±0.07 [a]	7.68±0.08 [b]	16.07±0.14[a]
2	93.50±1.41 [a]	1.51±0.44 [a b]	8.36±0.45 [a]	16.02±0.11 [a]
3	93.25±1.76 [a]	1.46±0.42[bc]	8.69±0.50 [a]	15.92±0.32 [a]
4	93.25±0.35 [a]	1.44±0.40[c]	8.76±0.43 [a]	14.90±0.10 [b]
5	90.25±1.76 [b]	1.42±0.48[bc]	8.77±0.11 [a]	14.57±0.10 [b]
6	89.00±0.71 [b]	1.42±0.53[bc]	8.74±0.07 [a]	14.00±0.28 [c]

Mean values ± standard deviation (n = 3) with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test (DMRT)

From determination, the effect of shelf life on some nutritional values of germinated native black riceat 1,3, and 5 months, respectively are exhibited in Table2.The level of γ-aminobutyric acid (GABA) had increased with a significant difference ($p \leq 0.05$) when the storage time was longer. GABA content (10.82 ± 0.14 mg/100 g) of germinated native black rice at 5 months was higher than at 1 month (9.01±0.43 mg/100 g). However, the value of vitamin B1 of germinated native black rice had significantly ($p \leq 0.05$) decreased when the storage time was longer. The level of vitamin B1 (0.54±0.00 mg/100 g) of germinated native black rice at 5 months was lower than at 1 month (0.78±0.01 mg/100 g).The results of this study agreed with Rehman (2006), who had studied the effect of storage on nutrition quality of commonly consumed cereals. He had also found that the thiamin content of rice had gradually declinedin six month's storage[16]. In addition, the level of protein content of germinated native black rice also decreased when the storage time was longer. On the other hand, non-significant ($p \leq 0.05$) difference was exhibited. The level of protein content (13.04±0.03 g/100 g) of germinated black rice at storage of 1 month was a little higher than at 5 months (12.99 ±0.05 g/100 g). Moreover, the antioxidant activity (DPPH scavenging activity) decreased when the storage time was longer. The number of IC_{50}(concentrationthat inhibited the growth by 50%)(22.15±0.17 mg/ml) of germinated black ricestoredat 1 month was significantly lower than at 5 months (36.47±4.59 mg/ml). From these results, it could be explained that phenolic compounds were mainly responsible for the antioxidant activity of rice grains [17]since the level of phenolic compounds decreased during prolonged storage period. Therefore, the antioxidant activity of germinated black rice had reduced as found in this research.

TABLE 2.Effect of Shelf Life on Some Nutritional Value of Germinated Native Black Rice

Shelf life (month)	γ-Aminobutyric acid (mg/100 g)	Vitamin B1(mg/100 g)	Protein content^{ns} (g/100 g)	IC_{50} (mg/ml)
1	9.01±0.43 ^{b}	0.78±0.01 ^{a}	13.04±0.03	22.15±0.17^{b}
3	9.09±0.07 ^{b}	0.73±0.04 ^{a}	12.95±0.11	36.34±5.47^{a}
5	10.82±0.14 ^{a}	0.54±0.00 ^{b}	12.99±0.05	36.47±4.59^{a}

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test (DMRT)

Bars represent standard deviation from duplicate determination and triplicate determination in the value of IC_{50}

4. Conclusion

The results indicated that the germination percentage, length of roots, phenolic compounds, vitamin B1, protein content, and antioxidant activity of germinated native black rice decreased whilethe level of moisture content and γ-aminobutyric acidshown a significant increase when shelf life was prolonged. In summary,the finding from this research could be used as a suggestion guideline for rice consumptionas well as to control the quality of germinated native black rice. Based on the potential beneficial effects on health, the consumer should eat germinated native black rice that had been stored for a short period of time. However, if they would like to receive high amount of γ-aminobutyric acid, native rice that had been stored longer should be consumed instead.

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6. References

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