Distribution of Melatonin and Serotonin in Germinated Rice

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Abstract:

Melatonin is a hormone synthesized in the pineal gland of the brain. It plays an important role in regulating the biological rhythm of the body, improve the sleeping quality, and exhibits strong antioxidant activity in animals and human. Melatonin can also be found in plants and plays a significant role in seed germination and regulates plant growth. This study was conducted to investigate the effect of germination on changes of melatonin and its derivative i.e. serotonin content distributed in different milling fractions of germinated rice including rice bran, polished rice, and brown rice. The paddy rice of non-waxy rice (Red Mali) and waxy-rice (RD 6) were soaked in water for 24 h and then germinated in room temperature for 1, 2, 3, and 4 days. The samples were dried and milled to obtain brown rice prior to polishing to obtain polished rice and rice bran. The results showed that germination improved melatonin content approximately 2 and 3 folds in both rice types after germinated from 2 to 4 days. In non-waxy rice, all milling fractions were a rich source of melatonin when germinated for 2 and 3 days whereas highest level of melatonin of waxy rice was found in brown rice and polished rice after germinated for 3 to 4 days. These results suggested that all fractions of germinated rice are a rich source of melatonin and rice bran fraction is a good source of serotonin revealing the potential use of each milling fraction of rice grain.

Keywords: Melatonin, Serotonin, Tryptophan, Germinated rice, Brown rice

Introduction

Germination has been widely used to enhance the concentration of bioactive compounds and various nutrients in seed plants. During germination, chemical compositions change dramatically, due to the chemical activity of the seeds for energy use in germination causing the degradation of large molecules to be smaller, such as starch molecules to be monosaccharide. In addition, germination can also improve the level of bioactive compounds such as ascorbic acid, tocopherols, tocotrienols, phenolic compounds, γ-aminobutyric acid (GABA), dietary fiber, ferulic acid, magnesium, potassium, zinc, gamma-oryzanol [1], [2], [3]. Moongngarm et al. (2010) [4] compared the bioactive compounds of un-germinated rice, germinated paddy rice, and germinated brown rice and found that the concentration of bioactive substances in germinated paddy rice was higher than in germinated brown rice and un-germinated rice. However, currently, there is little information about the effect of germination on melatonin and serotonin. Melatonin is a hormone in the nervous system that synthesized at the pineal gland in the brain [5]. Melatonin in humans plays an important role in controlling the biological rhythm and treating insomnia [6]. The structural formula melatonin, N-acetyl-5-methoxytryptamine or N-acetyl- 3- (2-aminoethyl) -5-methoxyindole is C_{13} H_{16} N_2 O_2, the molecular mass of 232.278 g/mol with a melting point at 116-118 °C, and the chemical structure is shown in Fig.1 [7]. It has amphipathic properties, which causes melatonin to dissolve with solutions containing polar and non-polar solutions**.** In addition, melatonin also has highly effective antioxidant [8], [9]. Therefore, melatonin can be used as a supplement. In plants, melatonin is a signaling hormone which influences development processes during germination, vegetative and reproductive growth. This study was carried out to study the effect of germination on melatonin and serotonin in rice. Including the study of the distribution of melatonin in various parts of rice obtained from rice milling.

Materials and methods

Materials

Paddy rice (Oryza *sativa* L.) cultivar RD 6 (waxy rice) and Red Mali (non-waxy rice) were purchased from a local rice milling factory in Mahasarakham province, Thailand. Un-germinated rice was prepared by removing a husk to obtain brown rice using a rice milling machine. Some portions of brown

rice were further polished to obtain polished or white rice and rice bran. Germinated rough rice was prepared by following the method reported by [4] with some modifications. Paddy rice seeds were soaked in tap water at room temperature for 1 day (40% moisture content) and water was changed every 7-8 h. The steeped kernels were placed in plastic baskets and covered by the cheesecloth. Germination took place in a germinating basket from 1 to 4 days at 28-30°C. The germinated seeds from each germination day were dried at 50°C to approximately 10% of moisture content. The hull, root, and shoot were separated. The rice grains were de-husked to obtain brown rice which divided into two portions, one for brown rice sample and the other for polishing to obtain rice bran and white rice using a rice milling machine. All samples were performed in triplicates. Germinated rice samples were stored at -20°C and finely ground (40 mesh) prior to analyses.

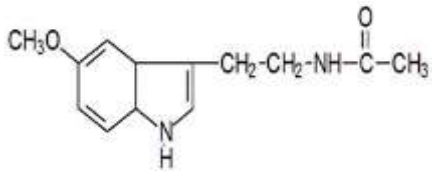

Figure 1 Chemical structure of melatonin [7]

Sample extraction

Dried sample (10 g) was extracted with 50 mL of 80% methanol (MeOH) then shaking incubated for 16-22 h, at 150 rpm and room temperature. The extract was filtered and the supernatant was collected for melatonin and serotonin.

Determination of melatonin and serotonin

Melatonin and serotonin content were determined according to the method of Kocadağlı, Yılmaz, & Gökmen, (2014) [10], and Pothinuch & Tongchitpakdee, (2011) [11]. The extracted sample was purified using Solid Phase Extraction (SPE). SPE was activated with 10 ml of methanol (MeOH) followed by 10 ml of deionized (DI) water. The extracted sample (5 ml) was loaded and the impurity in SPE was washed with 10 ml of 5% MeOH and then 5 ml of 80% MeOH was eluted. The eluted sample was filtered using a 0.2 µm filter before analysis using liquid chromatography-tandem mass spectrometry (LC/MS-MS). The mobile phase was prepared as (A) 0.45% formic acid in DI water and (B) acetonitrile in the ratio (50:50, v/v). InertSustain® C18 column (2.1×150 mm i.d., 3 µm) with the column oven set at 40°C was used. The isocratic elution was performed with 2 µl of injection volume, the flow rate at 0.2 ml/min and total running time for 10 min. Acquire of mass spectral data were positive mode and identification of melatonin using multiple reaction monitoring (MRM) with ESI settings. Nitrogen gas was used nebulizing and drying, flow rate 3L/min and 15L/min with 4.5kv of the interface voltage at 250°C and 400°C for the desolvation line (DL), and the heat block. The MS (Q3 scan) was selected for product ion scans using argon as the collision-induced dissociation (CID) gas at 230 kPa. The transition at 233.0→174.0 (collision energy of -15ev) was used to detect the melatonin and dwell time at 100 ms.

Results and discussion

Comparison of melatonin content in various milling fractions of germinated waxy rice and non-waxy rice

The study found that melatonin content in polished rice and brown rice of waxy rice (RD 6), germinated for 0, 1, and 2 days, had no significant increase whereas the melatonin in rice bran significantly increased after germinated for 1 day prior to decreased when germinated from 2 to 4 days, as shown in Figure 1. There was a significant increase in the amount of melatonin in polished rice and brown rice germinated for 3 and 4 days, with the content of 553.35ng/g and 507.69 ng/g, respectively. The highest melatonin content was observed in white rice and brown rice whereas there was no significant change in rice bran had, while the white rice obtained from the rice that grew 3 days had the highest amount and in the brown rice obtained from the germinated rice 4 The day has the highest volume.

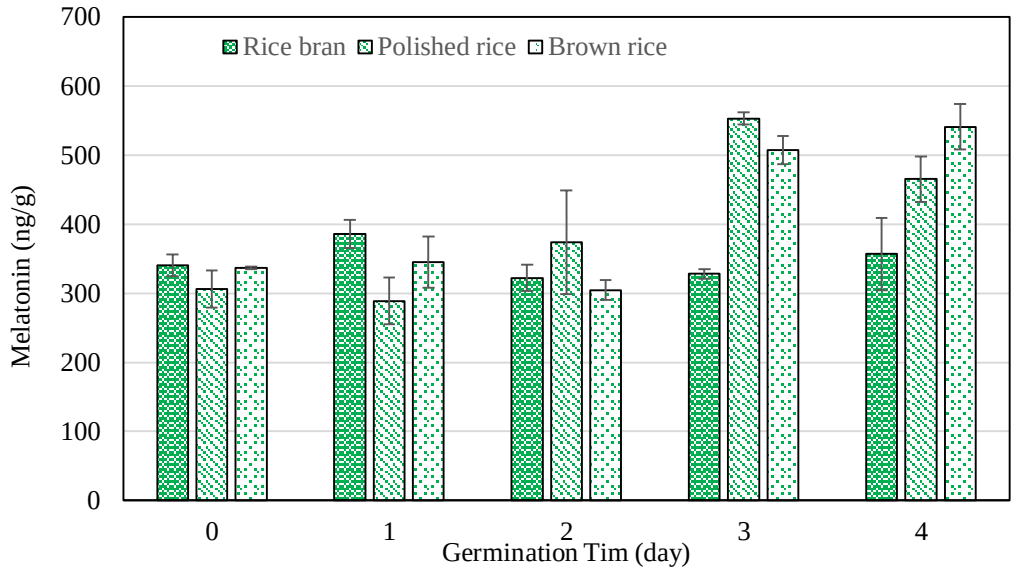

Figure 2 Melatonin content in various milling fraction of waxy rice

From the analysis of melatonin content in various milling fractions of non-waxy rice (Red Mali rice), it showed that day 0 and 1 of germination did not affect the increase in melatonin content in all milling fractions. The germination day of 2, 3, and 4 days showed that melatonin in all parts of the rice kernel increased significantly (Fig. 3). The rice bran obtained from germinated rice for 2 days, germinated polished rice and brown rice obtained from the 4th day of germinated rice had the highest amount of melatonin with the amount of 685.95, 704.71 and 681.76 ng/g, respectively. The melatonin in germinated brown rice and polished rice trended to be increased on day 3 and 4 of germination while the amount of melatonin in rice bran revealed somewhat reduced but non-significant difference from the day 2 of germination.

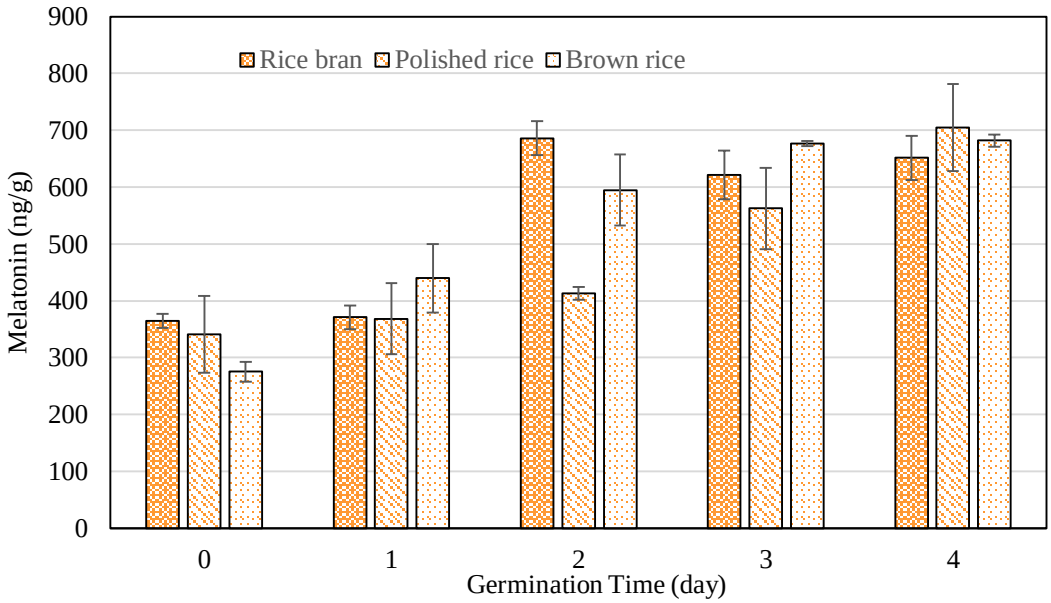

Figure 3 Melatonin content in various milling fraction of non-waxy rice (Red Mali rice)

Comparison of serotonin content in various milling fractions of germinated waxy rice and non-waxy rice

The effect of germination on the serotonin content and distribution in rice milling fraction in RD 6 cultivars, it was found that germination affected the serotonin content in all milling fractions of germinated rice as shown in Fig. 4. The highest content of serotonin was observed in rice bran fraction of day 1 and 2 germinated rice (76.10 and 71.40 ng/g, respectively), which increased as highs as 114.77% compared with rice bran fraction from un-germinated rice. The serotonin content also significantly increased in polished rice and brown rice of waxy rice but with a lower level than the rice bran fraction.

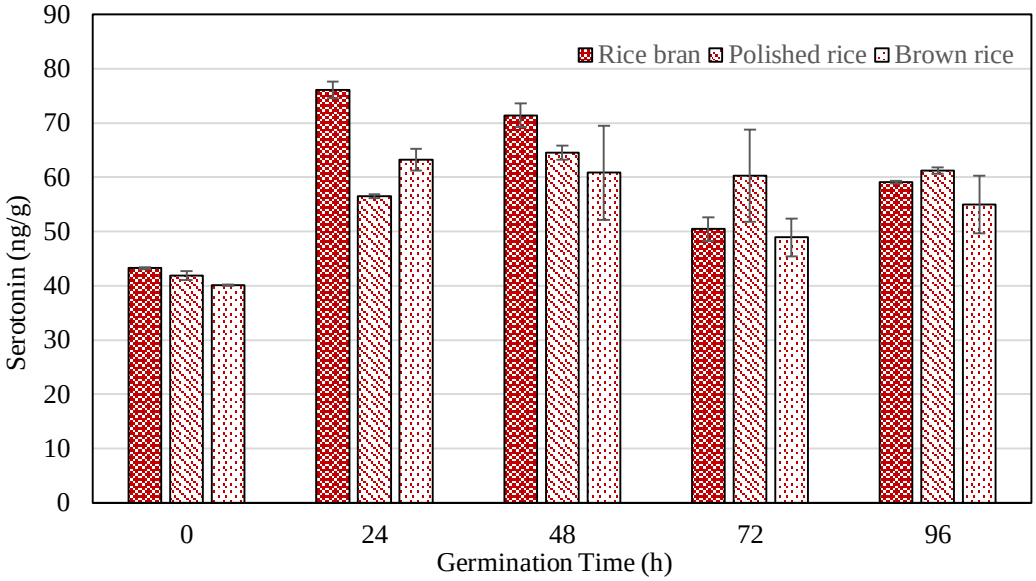

Figure 4 Serotonin content in various milling fraction of waxy rice (RD 6)

The un-germinated red Mali had the serotonin content of 41.19 ng/g and the maximum serotonin content was found in the day 2 of germinated rice with the amount of 61.41 ng/g which increased approximately 49.09% of un-germinated rice. The serotonin in rice bran fraction of Red Mali significantly reduced when germinated for 4 days. The germination time did not affect the content of serotonin in polished rice and brown rice during germination for 1 to 2 days, however, the serotonin content slightly decreased after germination for 3 to 4 days.

Comparison of melatonin content of two germinated rice

The comparison of melatonin and serotonin content of two germinated rice cultivars, namely, RD 6 (waxy) and Red Mali (Non-waxy), obtained from the most suitable day of germination are indicated in Table1. It was found that melatonin and serotonin content in Red Mali were higher than that of RD 6 and found that melatonin content distributed in all three fractions of the rice milling. For the waxy rice RD 6, it was found that melanin content is high in white polished rice and brown rice fraction while the serotonin was mainly distributed in rice bran fraction.

Discussion

Melatonin is a signaling hormone in plants which influence development processes during germination, vegetative and reproductive growth. It plays an important role on seed germination, therefore its level increase significantly during the germination process. The serotonin is a precursor of melatonin, even though it was found in a lower amount than melatonin, it also significantly increased during germination. These findings of this study was supported by several studies. Previous investigations have proved that exogenously applied melatonin had the potential to enhance seed germination and plant development. Many studies have suggested that melatonin can act as a plant growth regulator [12], [13] and biostimulator in stressful situations [14], [15], [16], [17]. Pre-treatment with melatonin has increased seed germination of *Brassica oleracea rubrum* [15], *Cucumis sativus* [18], and *Phacelia tanacetifolia* [19]. The use of melatonin in seed pre-treatment has also influenced the further growth of plants such as the study of Tan et al. (2007) in soybean seed priming, they found that melatonin increased leaf size, plant height, and seed number.

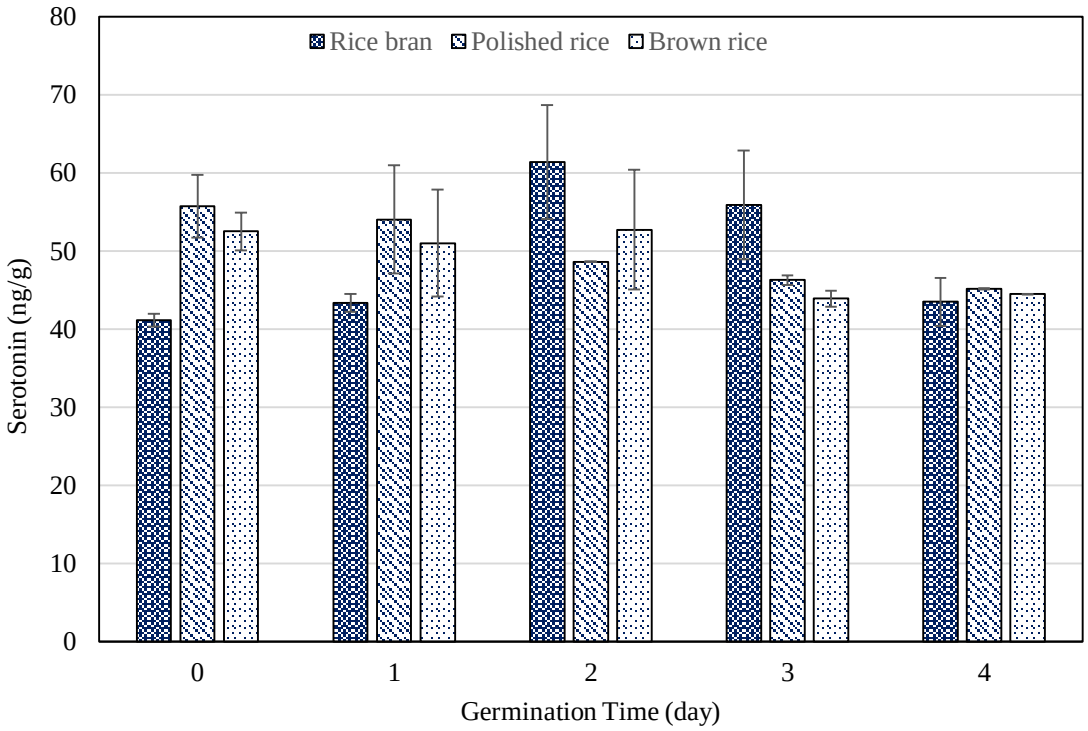

Figure 5 Serotonin content in various milling fraction of non-waxy rice (Red Mali rice)

Table 1 Comparison of melatonin and serotonin content (ng/g) of two germinated rice cultivars obtained from the suitable germination time of each cultivar

Milling fractions	Melatonin		Serotonin	
	Waxy (RD 6)	Non-waxy (Red Mali)	Waxy (RD 6)	Non-waxy (Red Mali)
Rice bran	385.66 ± 20.40^{Bb}	685.95 ± 9.49^{Aa}	76.10 ± 10.04^{Aa}	61.41 ± 7.32^{Aa}
Polished rice	553.35 ± 8.86^{Ba}	704.71 ± 76.41^{Aa}	64.49 ± 13.02^{Ab}	48.65 ± 4.01^{Ab}
Brown rice	541.26 ± 32.83^{Ba}	681.76 ± 10.64^{Aa}	63.23 ± 1.96^{Ab}	52.75 ± 7.69^{Aab}

Results are expressed as mean value ±SD
Results in the same column with the same superscript (A, B) are not significantly ($p<0.05$)
Results in the same row with the same superscript (a, b) are not significantly ($p<0.05$)

Conclusions

The melatonin content was the highest in polished rice and brown rice fraction in waxy rice germinated for 3-4 days whereas in non-waxy rice (Red Mali) the melatonin content was highest in the bran fraction after germinated for 2 to 4 days. The serotonin content was highest in rice bran fraction in both rice cultivars germinated for 1 to 2 days in waxy rice and germinated for 2 to 3 days in Red Mali rice. These results suggested that each rice milling fractions germinated rice is a rich source of melatonin and derivatives depending on cultivar revealing the potential use of each milling fraction of rice grain.

Acknowledgements

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