Melatonin Profile during Rice (Oryza Sativa) Production

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***Abstract*—Rice (*Oryza sativa*) is the foremost cereal crop in Southeast Asia. It serves as staple food, thus has a major contribution to the calorie intake. In addition, rice contains melatonin which is beneficial for human health. It is, therefore, essential to retain this compound by appropriate rice production processes. Melatonin profile during rice production was monitored for three varieties (IR64, *umbul-umbul* and *pandan wangi*) from conventional farming and four varieties (*batang lembang, pandan wangi*, black and red rice) from organic farming. The effect of polishing degree on melatonin content in rice was also evaluated. Melatonin level decreased throughout rice production and then remained steady at roughly 25-40% in final product. The most influential factor was polishing which led to melatonin losses of up to 50%. The results for organically cultivated varieties were similar. However, melatonin in black rice appeared to be persistent in the matrix during rice production.**

***Index Terms*—melatonin, rice, organic rice, polishing degree**

I. Introduction

Melatonin, also known chemically as N-acetyl-5-methoxytriptamine, is an indoleamine that is synthesized from the essential amino acid L-tryptophan. It has a number of important activities that include neurohormone and chronobiotic actions in biological systems. There is good evidence that melatonin provides effective treatments for cardiovascular diseases [1], sleep disorders and headache [2].

Melatonin is a naturally occurring compound that is found in humans, animals, plants and microbes. The presence of melatonin in plants has been identified in a range of species including rice [3].

Rice (*Oryza sativa*) is a major staple food and is pivotal for Southeast Asian livelihoods. Therefore, rice is exceptionally important for the nutrition of large numbers of the population in Southeast Asia. Within this region, rice dominates not only food security but also national economies. Rice production has increased since 1925 in some countries of Southeast Asia, particularly in Indonesia. It has been domesticated in Indonesia from the time of approximately 2300 B.C., thereby giving rise to a number of rice varieties.

Around 7,000 rice varieties (or lines) have been recognized and being suitable for diverse areas of Indonesia. Furthermore, hundreds of improved varieties have been developed and introduced into society since 1940. Nevertheless, merely 10 to 20 varieties are widely cultivated by Indonesian farmers [4]. These cultivated rice varieties are high-yielding types, mainly IR-64, and local varieties such as *umbul-umbul*, *batang lembang*, *pandan wangi* (aromatic rice), black and red rice (pigmented rice). The nutritional properties of rice including melatonin differ in specific classified rice varieties [5].

In addition to the rice variety in question, changes in the chemical composition of rice can also occur in the post-harvest treatment and rice processing. Nutritional losses can occur in the field, principally during processes such as improper drying or milling [6].

It has been demonstrated that changes occur in the major compounds during processing and one would also expect changes in the minor compounds i.e. melatonin. Nonetheless, research into the melatonin profile for different cultivation systems, post-harvest treatments and rice production processes has not been carried out to date.

The aim of the study described here was to assess melatonin levels throughout the rice production processes. The results from this study could help rice producers to ensure that maximum melatonin levels are present in the final rice product, which in turn would lead to some important health benefits from rice intake.

II. Materials and Methods

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A. Materials and Chemicals

HPLC-grade methanol, acetic acid and ethyl acetate were purchased from Merck (Darmstadt, Germany). Melatonin standard, M-5250, was obtained from Sigma-Aldrich (St. Louis, MO, USA). Water was purified with a Milli-Q purification system (Millipore, Billerica, MA, USA).

B. Rice Samples

Three varieties of Indonesian rice produced using conventional farming systems were obtained randomly from various smallholder rice mills in the Central Java area (*umbul-umbul*, *pandan wangi* and IR64). Samples were taken from different lines of rice production to represent each step of the production process, i.e., threshing (rough rice), drying (dried rice), hulling (whole grain rice), and polishing (polished rice).

A sample of the rice variety *pandan wangi* was then collected per degree of polishing (70%, 80%, 90% and 100% bran removal) based on the Indonesian National Standard SNI 6128:2008 [7]. Four varieties of Indonesian organic rice were obtained from CV. Green Health Agriculture (*batang lembang*, black rice, red rice and *pandan wangi*). The samples were stored in vacuum packaging.

C. Sample Preparation

A rice sample (20 g) was placed in a plastic cylinder and rice grains were milled with an Ultraturrax homogenizer (IKA® T25 Digital, Germany) for 10 min prior to extraction. The milling process was stopped every 1 min to avoid excessive heating of the sample. The fine grain was then homogenized by stirring and then stored in a closed, labelled bottle.

D. Extraction Technique

Melatonin extraction was performed in a Dionex ASE 200 extractor (Dionex, Sunnyvale, CA, USA) equipped with incorporating stainless steel extraction cells (11 mL volume) and collection vials (60 mL capacity). A cellulose filter (Dionex) was inserted at the outlet end of the extraction cell.

An accurately weighed sample of rice powder (2.5 g) and washed sea sand was loaded into the extraction cell. The Pressurized Liquid Extraction (PLE) conditions were 70% (v/v) EtOAc, static time of 5 min in 2 cycles at 200 ºC under a pressure of 200 atm, flushing 50% and purge for 60 s. The resulting extract was dried under vacuum on a rotary evaporator. The residue was reconstituted with methanol and adjusted to a final volume of 10.0 mL. The liquid was then passed through a 0.45 µm nylon filter prior to injection on the HPLC-FD system.

E. Determination of Melatonin

High performance liquid chromatography (HPLC) analyses were carried out on an Alliance HPLC 2695 system, controlled by an Empower Pro 2002 data station (Waters, Milford, MA) and coupled with a fluorescence detector (Waters 474 Fluorescence Detector). A reverse phase RP-18 Lichrospher Column (LiChroCART 250 × 4 (5 µm)) from Merck was used. A gradient elution program was used with two mobile phases: A (2% acetic acid and 5% methanol in water) and B (2% acetic acid and 88% methanol in water). The mobile phase was filtered through a 0.45 µm membrane filter (Millipore) and was degassed for 15 min prior to use. The applied gradient was as follows: (time, solvent B): 0 min, 0%; 5 min, 35%; 12 min, 40%; 15 min, 40%; 20 min, 45%; 25 min, 50%. The flow rate was fixed at 0.5 mL min^{-1}. The established conditions for fluorescence detector were as follows: excitation wavelength was set at 290 nm, emission wavelength was set at 330 nm, sensitivity was set at gain 1000, attenuation was fixed at 16, and injection volume was set to 10 µL.

F. Performance of the Method

The analytical procedure for the chromatographic method for melatonin determination was carried out according to the recommendations of ICH Guideline Q2 (R1) [8] and suggestions in ISO 17025 [9]. Linearity, range, precision, detection and quantification limits of the method were established. Linearity was assessed in order to express the ability of the method to obtain test results that are directly proportional to the concentration of melatonin in two different ranges. Appropriate dilution from a stock solution of melatonin was carried out to give concentrations ranging from 0.75 to 15 µg L^{-1} and 15 to 750 µg L^{-1}. Gnumeric 1.12.9 was used to generate the regression analysis. Calibration curves were obtained from this regression analysis and the melatonin in the extracts was quantified. The standard deviation (σ) obtained for the response and the slope (a) from the regression were then used to calculate the limit of detection (LOD) and limit of quantification (LOQ). The precision of the method was evaluated by studying repeatability (intra-day) and intermediate precision (extra-day). Precision was expressed as Coefficient of Variance (CV) of retention time (RT) and peak height. The analytical properties for the chromatographic method for the determination of melatonin are listed in Table I.

G. Data Analysis

The experimental results in single- and two-factor experiments were analysed using Gnumeric. The Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test were used to determine the significant differences ($p < 0.05$) between the means.

TABLE I. ANALYTICAL CHARACTERISTICS FOR THE DETERMINATION OF MELATONIN

Concentration range (mg L^{-1})	Observations (n)	Linear equation	R^2	LOD	LOQ	Intra-day, CV (%) n = 10		Inter-day, CV (%), n = 3 × 5	
						RT	Height	RT	Height
0.75 to 15	11	Y = 587.82X + 1722.99	0.982	1.7	5.2	0.99	0.93	1.23	2.05
15 to 750	14	Y = 614.22X + 1472.11	0.999						

III. Result and Discussion

A. Rice Production Processes

The main objective of industrial rice production is the transformation of rough rice (paddy) into polished rice. A number of production processes are necessary to provide a good quality grain for the consumer.

Three different rice varieties from conventional cultivation were used in this study. In addition to being considered as aromatic rice, together with *umbul-umbul*, *pandan wangi* represents the local rice varieties of Indonesia. IR64 is a rice variety that was developed in a major advance in rice production as it provided higher yield potential, greater yield stability and more efficient management practices.

Melatonin was determined in the samples taken at different production processes. The levels of melatonin remaining after treatments i.e. threshing (rough rice), drying (dried rice), hulling (whole grain rice), and polishing (polished rice) during rice production are demonstrated in Fig. 1. Subsequently, in order to study the effect of rice production processes and rice varieties on the level of melatonin, a two-factor ANOVA has been used (*p*.0.05). ANOVA revealed that the processing steps in rice production and the varieties of rice both have significant effects on the level of melatonin in rice and therefore LSDs for these factors were also estimated (Fig. 2).

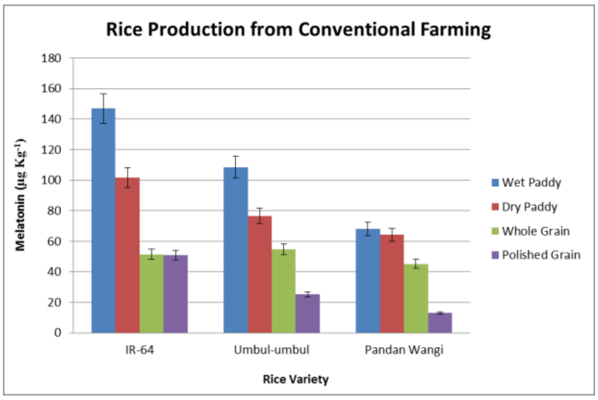

Figure 1. Melatonin levels in different rice varieties during rice production by conventional farming.

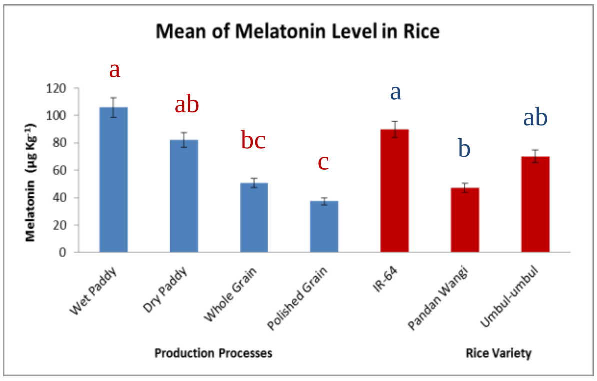

Figure 2. LSD analysis for rice production processes and rice variety. Bars followed by the same letter are indicated as not being significantly different ($p = 0.05$)

The level of melatonin for the three difference rice varieties decreased gradually during the course of processing throughout rice production, particularly after hulling (decreased around 30-50%). Nonetheless, a substantial decreasing in melatonin level by around 30% was also observed for *umbul-umbul* and IR64 due to drying process. The stability of melatonin is known to be adversely affected by light and heat. Similar results were obtained by Murch *et al*., who conducted research into melatonin levels in feverfew leaves (*Tanacetum parthenium*) and found that the levels of melatonin reduced by about 30% after drying the fresh leaves in an oven [10].

The levels of melatonin in final rice products after a set of milling processes remained as high as 55 to 65%. This is a reasonable content of melatonin bearing in mind that husk and bran removal have taken place during these processes. The bran consists of pericarp, which is known as a source of protein as it has three fibrous layers of protein. To the best of present knowledge, nitrogen-containing compounds, specially the amino acid tryptophan, are essential for the biosynthesis of melatonin in plants. Furthermore, the presence of melatonin in seeds was related to the protection of the germs to oxidative stress experienced by the pericarp of the grain [11]. Hence, the removal of this part of the grain may reduce the concentration of melatonin in the product.

Different rice varieties showed differences in melatonin losses due to the production processes. Drying was found to be an insignificant process in terms of its effect on melatonin content for the aromatic local variety *Pandan wangi*, whereas polishing was insignificant for the high-yielding variety IR64. These differences in the melatonin stability and loss for different rice varieties during the drying and polishing processes can be best explained by considering the initial level of melatonin that is naturally present in rice grains and the possible distribution of melatonin in the seed.

Allameh and Alizadeh confirmed that rice cultivars and drying days after harvesting had significant effects ($p<0.01$) on head rice yield. The results of this study demonstrated that the strength of varieties against applied stresses is different and is related to both genetics and grain physical properties [12]. Additionally, the thickness of grain contributes to rice breakage as stress is applied on the rice kernel due to moisture transfer between the grain and environment. Grains of freshly harvested paddy with a lower thickness have higher average moisture contents than the thicker ones [13].

Pandan Wangi is characterized by a round-shaped grain and it is a relatively thick grain with a lower moisture content than other rice varieties, e.g., IR64 (long thin grain). As a result, a shorter drying treatment is required to meet the standard drying process (< 14% of moisture content in rice product) and consequently melatonin degradation during the drying step can be suppressed for this rice variety.

B. Organic Farming

Consumers are inclined to pick healthier food produced from a cultivation system where the use of chemical pesticides, weed killers and fertilizers, genetically modified organisms, antibiotics and growth hormones is avoided. As a result, the most important developments in rice cultivation systems have concerned an increased awareness of organic farming.

The effect of rice production by organic farming on the melatonin content of rice is shown in Fig. 3. Comparison of the melatonin levels of rough rice (paddy) and finished rice products shows that the melatonin content decreased, especially in milled rice (*batang lembang* and *pandan wangi*). In pigmented rice, particularly black rice, the melatonin level was maintained in the final product due to the absence of a husk and bran removal process.

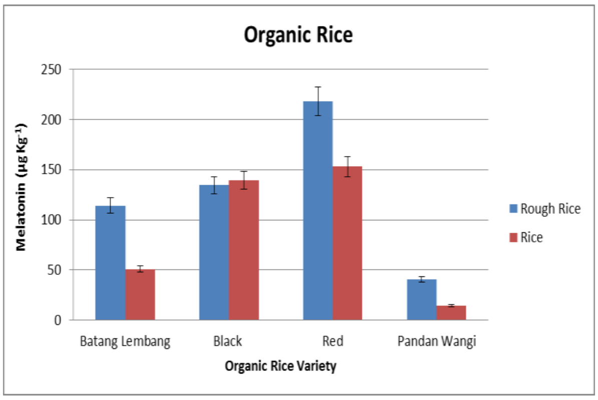

Figure 3. Melatonin levels in organic rice samples.

Hence, rice production from both organic and conventional farming produced similar results and milling was the most significant process in rice production in terms of the loss of melatonin.

C. Polishing Degree

It can be seen in Fig. 4 that an increase in bran removal leads to a gradual decrease in the melatonin content in rice. The melatonin levels present after different polishing degrees of 70%, 80%, 90% and 100% were of 28.8 µg Kg^{-1}, 23.8 µg Kg^{-1}, 20.9 µg Kg^{-1} and 16.3 µg Kg^{-1}, respectively. The significance level between polishing degree and melatonin retention was assessed by single-factor ANOVA (*p*.0.05).

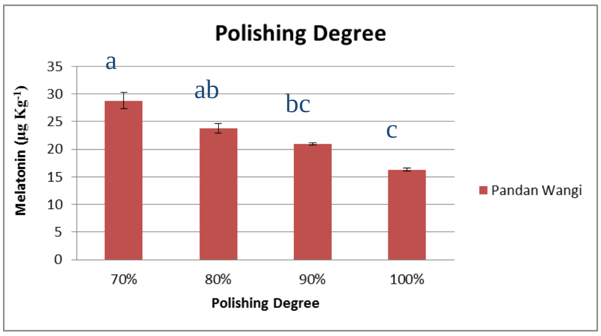

Figure 4. LSD analysis for different polishing degrees. Bars followed by the same letter indicate that parameters are not significantly different ($p = 0.05$)

Result from data analysis indicated that the polishing degree has a significant effect on the melatonin level in rice. A post-hoc test by means of LSD was then considered in order to review the significances between treatments in the polishing process. LSD showed that the percentage retention of melatonin differs significantly for every 10% unit difference in the polishing degree.

In summary, the level of melatonin decreased by as much as 9 to 16% when the polishing degree was increased. This result seems to be associated with the removal of bran in the polishing process as this component must contain a certain amount of melatonin.

IV. Conclusion

Melatonin profiles throughout the rice production process were studied. Differences in rice production processes and rice varieties have significant effects on the melatonin level in semi-finished and finished rice.

In addition to samples from conventional rice farming, the effect of processing steps during rice production on melatonin levels from organic rice farming was also studied. Similar results were obtained for each farming approach, showing that the changes in melatonin levels are due to the application of rice production processes.

The milling process involves hulling and polishing and it is considered to be a major factor that affects the retention of melatonin in rice. The degree of polishing was also investigated and the results indicated that the bran removal process decreased the melatonin content in the rice product. Hence, it is recommended that the rice production processes are carefully controlled in order to attain a high level of melatonin in rice products by efficient drying and the optimum degree of milling. The level of melatonin in pigmented black rice was successfully retained in the final product as the husk and bran removal processes were omitted.

The study reported here provides opportunities to develop advanced techniques in rice processing. Bearing in mind the importance of rice science and technology, it is envisaged that this work will significantly support further studies to improve rice production techniques in the coming decades.

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