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Optimization of the ultrasound-assisted extraction of melatonin from red rice (*Oryza sativa*) grains through a response surface methodology

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ABSTRACT

An analytical ultrasound-assisted extraction (UAE) technique has been optimized and validated for the extraction of melatonin from rice grains. A Box–Behnken design in conjunction with a response surface methodology based on six factors and three levels was used to evaluate the effects of the studied factors prior to optimizing the UAE conditions. The significant ($p < 0.05$) response surface models with high coefficients of determination were fitted to the experimental data. Solvent composition and extraction temperature were found to have very significant effects on the response value ($p < 0.005$). The optimal UAE conditions were as follows: extraction time of 10 min, ultrasound amplitude of 30%, cycle of 0.2 s^{-1}, extraction temperature of 40 °C, 50% methanol in water as the extraction solvent at pH 3.5 and a solvent/solid ratio 2.5:1. The method validation ensured right values for linearity, LOD, LOQ, precision and recovery. Furthermore, the method was successfully applied in the analysis of a number of rice samples throughout the rice production process. Hence, it was demonstrated that this particular UAE method is an interesting tool for the determination of melatonin in rice grain samples.

1. Introduction

Since the beginning of this decade melatonin has been considered to have potent antioxidant properties and anti-inflammatory effects [1]. Melatonin mitigates neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases [2] and it also acts as an anticancer agent [3]. However, research into the role that melatonin plays in biological systems has been limited by a number of factors, including the very low levels present in samples, the dearth of analytical methods and the complexity of the biological matrices. It is therefore particularly important to be able to extract and quantify accurately the levels of melatonin present in food in the human diet. This goal is challenging given the complex chemistry of plant tissues, which contain a diverse range of primary and secondary metabolites.

Cultivated rice (*Oryza sativa* L.) is one of the most important cereal crops in the world since more than half of the world's population subsists wholly or partially on this grain [4]. Besides the contribution of rice to the total human calorie intake, rice contains some specific compounds that have demonstrated benefits for human health, including melatonin and phenolic compounds [5] .

The development of an optimal procedure for the extraction of melatonin from food presents some difficulties due to its potent antioxidant activity, which leads to rapid reaction with other constituents in the matrix. Ultrasound-Assisted Extraction (UAE) appears to offer a solution to this problem as it is a technology that can accelerate mass transfer and enhance the extraction kinetics [6]. The ultrasound method is cheaper and easier to operate than other novel extraction techniques such as pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE) [7]. Additionally, like Soxhlet extraction, UAE is not restricted by the solvent and type of matrix used. The UAE technique is therefore suitable for the extraction of a wide variety of natural compounds including melatonin in a complex matrix of a biological system, e.g., rice.

The UAE method is a very interesting technique to extract natural compounds from food matrices due to the cavitation effect, which enhances mass transport by disrupting the plant cell walls [8]. Consequently, ultrasonic power is considered to be one of the factors that leads to enhancement of the extraction [9]. In addition, several factors govern the efficiency of ultrasound and these include frequency, temperature, type of solvent, and sonication time.

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Various factors can affect the extraction process and these have to be optimized in order to extract quantitatively the analytes of interest. The chemometric approach based on the advantages of the Box–Behnken design (BBD) have been successfully applied in the optimization of UAE [10]. The BBD is compatible with the response surface methodology (RSM) because it allows an estimation of the parameters of the quadratic model, the building of sequential designs, the detection of lack of fit of the model and the use of blocks [11]. The particular focus of the study described here was the optimization and validation of the UAE method for the extraction of melatonin in rice grains by BBD in conjunction with RSM.

2. Materials and methods

2.1. Materials and chemicals

HPLC-grade methanol, acetic acid and acetonitrile 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).

2.2. Rice sample preparation

Red rice samples from Thailand were obtained from a regular market. Each rice sample (20 g) was placed in a plastic cylinder and the 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 in order to avoid excessive heating of the sample. The fine powder of rice grain was then homogenized by stirring and the sample was stored in a closed container. The final extraction method was applied to two Indonesian rice varieties (*umbul-umbul* and IR-64) taken at different stages of the production process, i.e. drying (dried paddy), hulling (whole grain rice) and polishing (polished rice), and these were obtained randomly from various smallholder rice mills in Central Java (Indonesia). Additionally, the suitability of the developed method was also evaluated by analyzing a number of organic pigmented rice samples from Indonesia and pigmented rice from Thailand.

2.3. Extraction of melatonin

UAE was carried out using a 200 watts and 24 kHz UP200S ultrasonic system (Hielscher Ultrasonics GmbH, Teltow, Germany). A 7 mm diameter probe was used for the experiments. This compact ultrasonic system is designed to be mounted on a stand and is equipped with a water bath coupled to a temperature controller (Frigiterm, J.P. Selecta, Barcelona, Spain) to maintain the desired extraction temperature in the range from −10 °C to 100 °C. Rice powder (2 g) was accurately weighed and then placed in an extraction tube. Based on the experimental design, a set volume and type of solvent was added into the extraction vessel and the extraction was performed under controlled UAE conditions. After extraction, the solid material in the extract was removed using a centrifuge (J.P. Selecta, Barcelona, Spain) at 8000 rpm at 4 °C for 5 min. The centrifuge cake was subsequently washed using fresh solvent and the liquids were collected with the extract and adjusted to a certain volume based on the design of experiment (DOE). The extract was filtered through a nylon filter (0.22 μm) prior to injection into a UPLC-FD system.

2.4. Determination of melatonin

Analyses were carried out on an ACQUITY UPLC® H-Class system coupled to an ACQUITY UPLC® Fluorescence Detector (FD) and controlled by Empower™ 3 Chromatography Data Software (Waters Corporation, Milford, MA, USA). Separations were performed at a temperature of 47 °C on a reverse phase RP 18 Acquity UPLC® BEH Column (Acquity UPLC® BEH 100 × 2.1 (1.7 μm) from Waters Corporation, Ireland).

The mobile phase was a binary solvent system consisting of phase A (water with 0.01% acetic acid) and phase B (acetonitrile with 2% acetic acid). The flow rate was 0.7 mL min^{-1}. The 4.0 min programmed gradient was as follows (%B): 0–1 min, 0%; 1–1.1 min, 0–10%; 1.1–2 min, 10%; 2–3 min, 10–20%; 3–3.5 min, 20–60%; 3.5–4 min, 60–100%. The column was subsequently

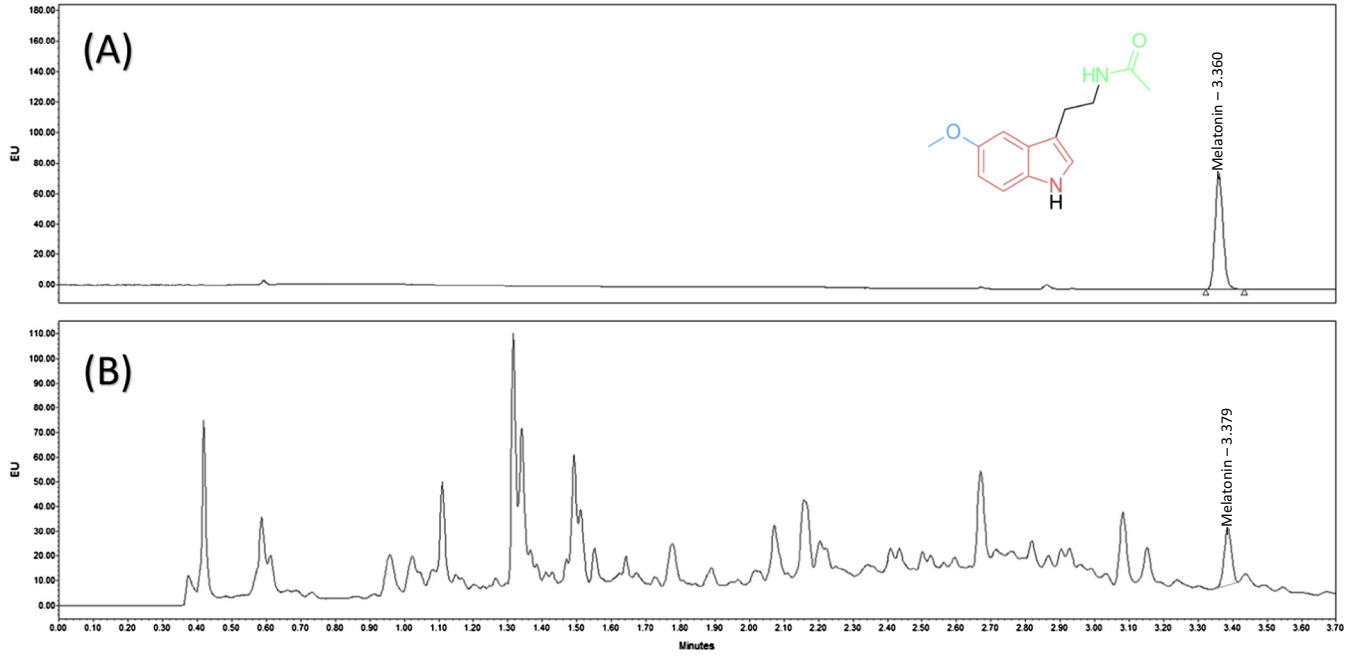

Fig. 1. Chromatogram of melatonin in standard solution (A) and in rice extract (B).