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Spectrofluorimetric determination of melatonin in kernels of four different Pistacia varieties after ultrasound-assisted solid–liquid extraction

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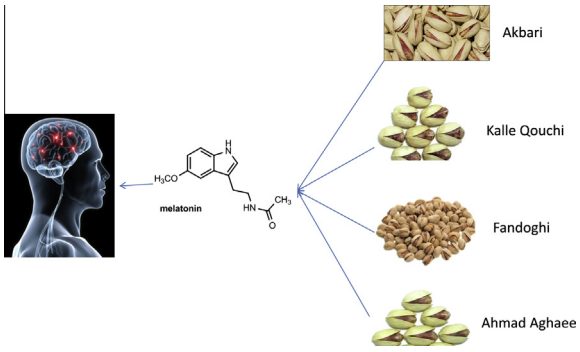
HIGHLIGHTS

- We identified melatonin in pistachio kernels using GC/MS analysis.
- Ultrasound-assisted solid–liquid extraction was used for melatonin extraction.
- Fluorescence of melatonin was measured for its determination in the extract.
- We determined the melatonin contents of four different pistachio varieties.

GRAPHICAL ABSTRACT



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ABSTRACT

Melatonin is normally consumed to regulate the body's biological cycle. However it also has therapeutic properties, such as anti-tumor, anti-aging and protects the immune system. There are some reports on the presence of melatonin in edible kernels such as walnuts, but the extraction of melatonin from pistachio kernels is reported here for the first time. For this, the methanolic extract of pistachio kernels was exposed to gas chromatography/mass spectrometry analysis which confirmed the presence of melatonin. A fluorescence-based method was applied for the determination of melatonin in different extracts. When excited at $\lambda = 275$ nm, the fluorescence emission intensity of melatonin was measured at $\lambda = 366$ nm. Ultrasound-assisted solid–liquid extraction was used for the extraction of melatonin from pistachio kernels prior to fluorimetric determination. To achieve the highest extraction recovery, the main parameters affecting the extraction efficiency such as extracting solvent type and volume, temperature, sonication time and pH were evaluated. Under the optimized conditions, a linear dependence of fluorescence intensity on melatonin concentration was observed in the range of 0.0040–0.160 μg mL^{-1}, with a detection limit of 0.0036 μg mL^{-1}. This method was applied successfully for measuring and comparing the melatonin content in the kernels of four different varieties of Pistacia including Ahmad Aghaei, Akbari, Kalle Qouchi and Fandoghi. In addition, the results obtained were compared with those obtained using GC/MS. A good agreement was observed indicating the reliability of the proposed method.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indole amine hormone that in mammals is synthesized mainly in the pineal

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gland and the retina and is regulated by the photoperiodic environment. It plays an important role in circadian rhythmicity [1] and photoperiodic responses, not only among various members of the animal kingdom [2], but also in plants [3]. The role of melatonin as a radical scavenger [4] and the relationship between health and well-being on one hand and melatonin level in human blood [5] on the other, has been the subject of many investigations.

Recent investigations suggest that melatonin treatment could improve sex life [6], fight the ravages of AIDS [7], and decelerate the evolution of Alzheimer's disease [8] and aging [9]. The presence of melatonin has been confirmed in fruits including tomatoes, pineapples and apples [10].

For its therapeutical properties, melatonin has been praised as a "panacea", leading to a great increase in pharmaceutical preparations containing this drug, and consequently, there is a great need for analytical methods for quality control [11].

Due to the importance of melatonin in mammalian life, several analytical methods were developed for its determination based on antibody radioimmunoassay [12], enzyme immunoassay [13] and voltammetry [14] or instrumental techniques such as reversed phase high performance liquid phase chromatography with electrochemical [15] or fluorescence detection [16], electrophoresis [17], gas chromatography coupled with negative ion chemical ionization [18] or electron impact mass spectrometry [19,20].

The presence of melatonin in some edible nuts such as walnuts [21,22] has been reported. The presence of melatonin in some edible nuts such as walnuts (Harandi et al., 2013; has been reported. In the present work, the presence of melatonin in pistachio nuts is reported for the first time. In addition, an optimized ultrasound-assisted extraction coupled with spectrofluorimetry detection is proposed for the extraction and determination of melatonin in pistachio kernels.

Experimental

Plant materials

Four different varieties of *Pistacia vera* L. (Ahmad Aghaei, Akbari, Kalle Qouchi and Fandoghi) were obtained from Rafsanjan in Kerman province, Iran.

Reagents

Melatonin was purchased from Sigma (Saint Louis, MO, USA). A 100 μg mL^{-1} stock solution of melatonin in methanol was prepared and stored at 4 °C in appropriate vials protecting the solution from sun light. Fresh working standard solutions were supplied daily by diluting the stock solution. The GC-grade methanol used for the extraction, as well as HCl and Na_2CO_3 were obtained from Merck (Darmstadt, Germany).

Instrumentation

For the spectrofluorimetric assays, a Shimadzo RF_5301 spectrofluorimeter (Kyoto, Japan) was used. When excited at $\lambda = 275$ nm the fluorescence emission intensity of melatonin was measured at $\lambda = 366$ nm. The extraction was performed by a Sonorex RK255 ultrasonic water bath obtained from Bandelin (Berlin, Germany). The pH values were measured with a Metrohm 827 pH meter (Herisau, Switzerland) supplied with a combined glass electrode.

Gas chromatography/mass spectrometry (GC/MS) analysis was carried out on a Hewlett–Packard HP 6890 (Palo Alto, California, USA) connected with a mass detector HP 5793 using an HP-1 column (30 m × 0.25 mm, film thickness 0.25 μm). The experimental conditions were as follows: oven temperature programmed from 40 °C (1 min) to 250 °C (60 min) at 3 °C/min; injector and detector temperature 250 °C and 230 °C, respectively; the carrier gas helium 99.999% at a flow rate of 1 mL/min. The mass spectrometer was operated at 70 eV with the mass range of 40–350 amu and the scan time of 1 s.

Ultrasound-assisted solid–liquid extraction of melatonin from pistachio kernels (UASLE)

Samples of pistachio kernels were carefully crushed and accurately weighed at 0.5 g and transferred to a 50-mL Erlenmeyer flask. Then 26 mL methanol was added to each sample, extraction was performed by sonication in an ultrasonic bath for 20 min and the mixture was then centrifuged at 3000 rpm for 15 min. Now the resulting extract was filtered through a 0.2 μm filter and the solution obtained was diluted to 50.0 mL with methanol. For fluorescence measurement, 100 μl of the final solution was diluted to 5.0 mL with distilled water. When excited at 275 nm the fluorescence intensity of melatonin was measured at 366 nm.

Results and discussion

Identification of melatonin

Identification of melatonin was based on the comparison of retention index and mass spectrum with those of an authentic sample and with NIST MS library. The standard of melatonin in methanol was also analyzed using the same GC/MS conditions. The peak of melatonin was observed at 41.2 min. As is shown in Fig. 1, the MS chromatogram of the peak also appeared at 41.2 min in the extract chromatogram and this confirmed the presence of melatonin in the extract of pistachio kernels.

Optimization of UASLE procedure

To obtain high extraction efficiency, the main extraction parameters such as pH, sonication time, extraction temperature, type and volume of extracting solvent were optimized. The results of the optimization are reported in Table 1.

Effect of extracting solvent

For investigating the effect of extracting solvent, 0.5-g samples of pistachio kernels were exposed to the extraction procedure using 26 ml of different solvents including methanol, acetonitrile and chloroform. After measuring the fluorescence intensity of the obtained extracts, it was observed that the fluorescence of methanol extract was 1.5 times that of acetonitrile and 3.5 times that of chloroform extract. So, methanol was chosen as extraction solvent in further experiments.

Effect of extracting solvent volume

In this experiment, the extraction procedure was applied on similar kernel samples using different volumes of methanol (10–60 ml). As one can see from the results shown in Fig. 2, the fluorescence intensity increased sharply with increasing methanol volume from 10 to 26 mL and then leveled off with further increasing up to 60 mL. So, 26 mL methanol was selected as optimal volume.

Effect of sonication time

Sonication time plays an important role in the extraction procedure. Enough time will make the crushed kernels disperse more finely into the extracting solution and result in an excellent extraction efficiency. So, the effect of sonication time was evaluated in the range of 5–50 min. It was observed that, increasing the time