

Report to American Pistachio Growers
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Title: Melatonin contents of raw and roasted American pistachios

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Summary of Progress to date

Two samples, raw and roasted, American pistachios were analyzed for melatonin content by two methods including UHPLC and spectrofluorometry. The two methods are described below and results reported.

1. Melatonin Analysis by UHPLC

Pistachio samples were frozen in liquid nitrogen and finely ground, one gram was extracted with 10 mL of acetonitrile at 4°C for 12 h in the dark. In some samples melatonin (1 ng/g) was added as an internal standard. After centrifugation at 12,000 rpm and 4°C for 10 min, the supernatants were sequentially evaporated under mild nitrogen stream. The evaporated samples were redissolved in 1 mL of 0.1 M HCl, and then extracted with ether (1 mL). The ether phase was collected, dried under nitrogen gas and reconstituted in 1 mL of H₂O/MeOH (80/20, v/v) for analysis by UHPLC.

Similar to liquid nitrogen pistachio samples were dried in acetone, ground to obtain acetone powder and extracted and analyzed as described above.

Results

There was no difference between liquid nitrogen and acetone extraction. Raw pistachios, dried with liquid nitrogen contained 660 ± 12 ng/g. Roasted pistachios dried with acetone contained 656 ± 6 ng/g of pistachio. These results are not statistically different.

These values are higher than walnut which contain 10 ng/g melatonin.

When the same samples were analyzed by spectrofluorometry at 275nm/366 nm, melatonin was not detectable.

2. Melatonin Analysis by Spectrofluorometry

The method of Oladi et al. (2014) was followed. Five hundred milligram samples of pistachio powder prepared as described above were extracted with 26 ml of methanol in a 50-mL Erlenmeyer flask by sonication for 20 min. The mixture was centrifuged at 3000 rpm for 15 min. 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. The fluorescence was read at 275 nm/366 nm excitation/emission.

Results

Fluorescence readings gave very high values of melatonin in both raw and roasted samples. Roasted samples had more melatonin than raw samples regardless of the method of moisture removal whether liquid nitrogen or acetone was used (see table below). Roasting may have contributed to increased fluorescent compounds and the resulting high readings may reflect that.

However, these values are lower than (almost half) values reported by Oladi et al. (Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 132: 326-329, 2014).

Melatonin in raw or roasted pistachios as measured by spectrofluorometry

Sample	Treatment/Drying	Treatmen/Drying
	Liquid Nitrogen	Acetone
Raw	$34 \pm 4 \mu\text{g/g}$	$72 \pm 8 \mu\text{g/g}$
Roasted	$128 \pm 12 \mu\text{g/g}$	$92 \pm 6 \mu\text{g/g}$