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Short Communication

# Determination of Melatonin in Azorean Bovine Milk by RP-HPLC as a Supplement for Sleepless Senior Population

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## Abstract

Melatonin (N-acetil-5-metoxitriptamine) is an indoleamine that is synthesized from the essential amino acid tryptophan via 5-hydroxytryptophan, serotonin and N-acetylserotonin in the vertebrate's pineal gland, particularly during the nocturnal period (light suppress its biosynthesis) [1,2]. This neurohormone decreases with advancing age, paralleled by a deterioration of normal sleeping pattern and changes in circadian rhythmicity. It is frequently used as a sleep prescribed drug to reduce the symptoms associated to jetleg or shift work [3]. Furthermore, melatonin mediates many neuro-physiological, neuro-endocrinological and behavioral processes, and also participate in several other cellular functions, such as: cancers, Alzheimer`s disease, and depressive syndrome [4].

The objective of this study was the determination of melatonin in Azorean bovine milk (in the range of ppt), in order to make a good use of milk's melatonin to supplement senior people that, generally, presents low level of this neurohormone, fact that increases with the progression of the ageing process. The results shows that Azorean milk melatonin level change according to the season of the year and also according to the nocturnal or diurnal milked periods, showing an average of 40.2 and 78.9 pg/mL for diurnal and nocturnal periods, during winter period, respectively, and the values of 13.20 and 28.7 pg/mL for diurnal and nocturnal during summertime, respectively. The results also show that the heating treatment affect the melatonin content in the milk, revealing a decreasing level from the raw (46 pg/mL) to the pasteurized (31 pg/mL) and ultra-pasteurized (decreasing from 27 pg/mL at 120°C to 6.6 pg/mL at 150°C).

**Keywords:** Melatonin; azorean bovine milk; pineal gland; HPLC melatonin determination; sleep patterns.

## Methodology, Results and Discussion

The melatonin level was determined in milk collected in three different locations farms in São Miguel Island (Azores) from the nocturnal and diurnal periods, following the Egoshi methodology with some modifications. This methodology includes the precolumn oxidation of melatonin into a highly fluorescent compound N-[(6-methoxy-4-oxo1,4-dihydroquinolin-3-yl)methyl]acetamide(6-MOQMA), and the determination of 6-MOQMA was achieved by high pressure liquid chromatography (HPLC) in an Omni Sphere C18-5µm reversed-phase column, using the following mobile phase: A – 50 mm NaH2PO4 (pH 7.0) and B – CH3CN. Gradient: t=0 min - 15% B; t=15 min - 18% B; Flow rate - 0.6 mL/min.; Injection volume: 100 µL and Fluorescence Detector: 245 nm (excitation) and 380 nm (emission) (Figure 1).

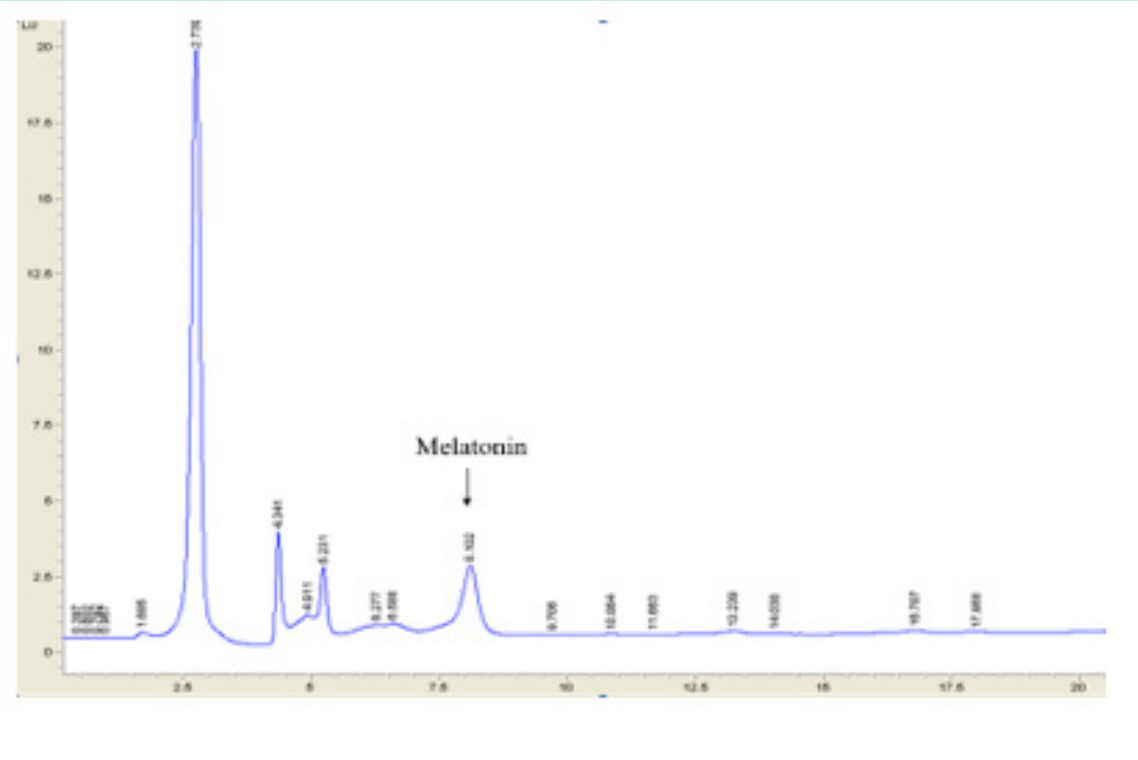


**Figure 1:** Chromatogram of the Azorean milk melatonin by HPLC.

The results show that the Azorean bovine milk melatonin levels change according to the season of the year, to the nocturnal or diurnal milked periods, and also to the heating treatment. The literature reported values of melatonin milk content (36.72 pg/mL [5] and 41.94 pg/mL [6]) were significantly lower with respect to our results, 13.2 pg/mL (summer) and 45.1 pg/mL (winter) for diurnal period and 28.7 pg/mL (summer) and 92.5 pg/mL (winter) for nocturnal period, that can be explained by the longer winter nights (Table 1). These results may be related to differences in terms of extraction/analysis methodologies, and by genetics, geographical location, time of milking collection, variables related with illuminance, storage conditions, and particularly the lactating stage that stimulate higher melatonin production (local B). The melatonin thermal stability was determined at different temperatures from raw milk to pasteurized milk at 60°C, following the ultra-pasteurized (UHT) milk from 120 - 150°C, that show melatonin´s stabilization decrease with increased temperature (Figure 2).

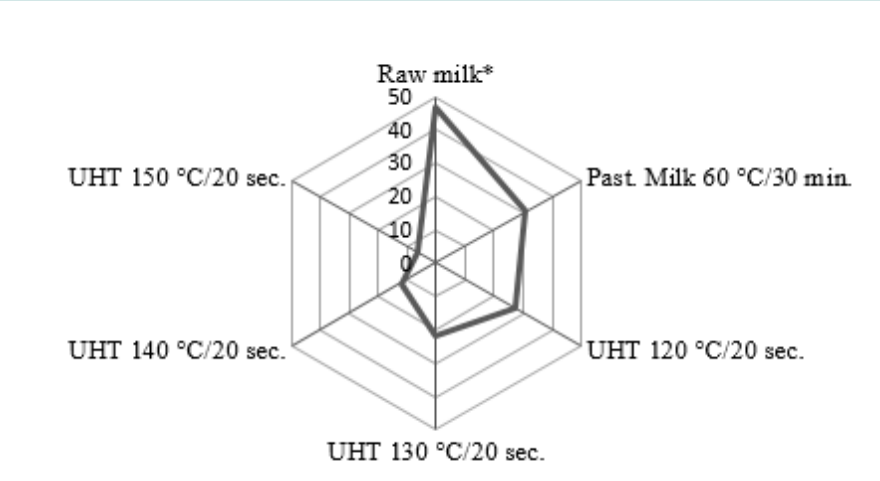


**Figure 2:** Thermal stability of Azorean milk melatonin *Milk from a cow during lactating stage.

**Table 1:** Determination of melatonin in nocturnal and diurnal Azorean raw milk.

| Local (season) | Samples of raw milk | Time of milk collection | Melatonin content (pg/mL of milk) |
|---|---|---|---|
| Local A (winter) | Nocturnal milk | 7:00 AM | 65.3 ± 2.31 |
| | Diurnal milk | 7:00 PM | 35.2 ± 1.56 |
| Local B* (winter) | Nocturnal milk | 5:00 AM | 92.5 ± 2.57 |
| | Diurnal milk | 5:00 PM | 45.1 ± 1.12 |
| Local C (summer) | Nocturnal milk | 6:00 AM | 28.7 ± 1.08 |
| | Diurnal milk | 6:00 PM | 13.2 ± 1.11 |

*Milk from a cow during lactating stage.

## Conclusion

The determination of milk melatonin was successfully achieved by HPLC in the range of ppt. The Azorean bovine milk melatonin level changes according to the weather/season of the year, to the nocturnal or diurnal milked periods, and also according to the heating treatment, from the raw, pasteurized and ultra-pasteurized milk. The variables related to illuminance have greater effects on the melatonin concentration in cow's milk than other factors, particularly the time of milking. The melatonin concentrations obtained from the night milk will be a beneficial supplement for human health, particularly for senior people.

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