The Effect of pH on Melatonin Stability in Commercial Grape Juices: HPLC-FD Study

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***Abstract* - Melatonin (N-acetyl-5-metoxytryptamine) is a biogenic amine largely found in various animals and plants. It is known for its various biological activities such as the regulation of circadian rhythm, antioxidant capabilities and cancer treatments. To study the stability of melatonin in three commercial grape juices varieties with pH (3.6) for the first one; and pH (2.65) for both other ones; and a storage period more than five months. The optimal conditions for a prior analysis were defined using the high-performance chromatography coupled to fluorescence detector method. The stability of melatonin standard solutions prepared in water (0.1 mg.L^{-1} / 1 mg.L^{-1}) at the same pH condition has been investigated, in order to compare and evaluate the presence of melatonin in grape juices and its stability under the pH condition. Shown in this way prior to the study design process of this compound extraction for others red fruit juices.**

The results showed that the stability of melatonin depends on pH conditions, and the most melatonin degradation resulted with pH of 2.65.

Keywords: ***Melatonin, Stability, Grape juice, Degradation.***

I. INTRODUCTION

The melatonin or N-acetyl-5-metoxytryptamine is a biogenic indoleamine. It is known for its important role in the regulation of circadian rhythm, metabolism, immune system and sleep disorders [1; 2]. Furthermore, it has been demonstrated that melatonin has an effective antioxidant activity, beneficial effects in neural disorders and inhibition activities on some cancer cells [2; 3]. Melatonin was initially discovered, in mono- and dicotyledon edible plants families in 1995 [4] including edible seeds such as rice or corn [5]. Higher levels of melatonin were identified in traditional medicinal herbs [6] such as in St.Johnswort (Hepiricumparthenium) [7]. It has been found and detected in many parts of higher plants such as roots, leaves, seeds, flowers and fruits [8]. These opened new research fields in the nutritional and food area to discover the melatonin benefits on health [9]. A great interest has been given to natural foods and drinks containing melatonin. Consequently, the need of reliable analytical methods for its measurements is even a greater challenge. Grape and its related products (e.g., wine) are considered as the main components in Mediterranean diet, where a moderate daily intake can help in the prevention of aging-related diseases. In grapes, many various substances are known for their beneficial health. For instance, anthocyanins, flavonoids and tannins are responsible for colour variation, aroma and texture characteristics. However, the isolation of new bioactive metabolites from grape, such as melatonin. In spite of the extensive knowledge about phenylpropanoids, principally polyphenols and tannins in grape wine products (e.g., grape, wine and grape juice) regular and appropriate consumption can contribute to improve the health benefits. Unfortunately, little it is known about the other compounds, such as melatonin [10; 11; 12].

This work aims to study the stability of melatonin in three varieties of commercial grape juices (GJ1, GJ2 and GJ3) at different pH conditions using HPLC-FD analysis techniques. The grape juices were stored for more than five months at room temperature (23ºC). The obtained results were compared with melatonin standard solutions prepared in water at the same pH conditions.

II. MATERIALS AND METHODS

A. Chemicals and reagents

Melatonin standard and tartaric acid were purchased from Sigma-Aldrich**TM** (Somaprol, s.a.r.l). Methanol (HPLC grade) was purchased from Fluka, glacial acetic acid for analysis was purchased from Riedel dehaën, and tartaric acid was from Aldrich. Three varieties of commercial grape juices were purchased from Lidl (supermarket in Ceuta).

B. Samples preparations

B.1. Melatonin standard solutions

A 0.1 mg.L^{-1} and 1 mg.L^{-1} of melatonin standard were prepared in Milli-Q water at three different pH of 6.9; 3.6 and 2.65. The two solutions with pH values 3.60 and 2.65 were prepared by adding tartaric acid to Milli-Q water.

B.2. Grape juice solutions

Grape juice solutions for each variety were prepared following the above procedure (i.e., preparation of melatonin standard solutions). One of the prepared grape juice solution has a pH of 3.6, while the two others solutions have almost the same pH (2.65). All samples were prepared and checked by duplicate.

B.3. Preparation samples procedure

Three melatonin standard solutions (0.1 $mg.L^{-1}$) were prepared in Milli-Q water. The first solution was prepared without adding tartaric acid (pH=6.9) and considered as a reference. For the two next solutions, tartaric acid was added to obtain the same pH values as the prepared grape juice solutions (i.e., pH of 3.6 and 2.65). In order to obtain a matrix as similar_as possible and finally, to compare the standard samples results with those of the grape juices.
Likewise, three series of melatonin standard solutions were prepared with a melatonin concentration of 1 $mg.L^{-1}$.
Following the same procedure as above, six series of spiked grape juices were prepared where the final concentrations are the same of melatonin standard solutions 0. 1 $mg.L^{-1}$ and 1$mg.L^{-1}$. The prepared solutions were filtered through a 0.45 µm membrane before adding a melatonin standard solution. The pH values of the melatonin standard solutions are obtained by adding tartaric acid using the pH meter. All samples are prepared and checked by duplicate.

C. Storage conditions

Table 1 reported different prepared solutions and their corresponding pH values and codes. All solutions have been prepared, covered and kept at room temperature (23ºC), throughout the study period, that lasted more than five months. Melatonin levels obtained at different pH values with two different melatonin concentrations 0. 1 $mg.L^{-1}$ and 1$mg.L^{-1}$ were compared to the standard solutions and real samples.

Table 1. Storage pH conditions of melatonin standard & spiked grape juices and codes used to identify them.

Samples	Codes	pH values
Melatonin standard	Std 6.9	6.9
Melatonin standard	Std 3.60	3.60
Melatonin standard	Std 2.65	2.65
Spiked grape juice 1	G.J 1	3.60
Spiked grape juice 2	G.J 2	2.65
Spiked grape juice 3	G.J 3	2.65

The experimental data showing the effect of the pH condition on the stability of melatonin standard solutions are presented in Figure 1 and 2.

D. Determination and extraction by HPLC-FD

Chromatographic analyses were carried out on an Alliance® System HPLC 2695 with pump system (Waters 600) and fluorescence detector. A C18 column used in this study as the stationary phase was Symmetry® C18 (5µm; 4,6mmx150mm) from Waters. Millennium chromatographic software was used for HPLC control and peak integration.

Two mobile phases were used: phase A (2% acetic acid and 8% methanol in water) and phase B (2% acetic acid and 8% water in methanol), applying an isocratic elution 50/50 (A: B) at a flow rate of 0.5 mL/min; and an injection volume of 20µL. For the fluorescence detector; the fixed conditions were as follows: an excitation wavelength of 290 nm and an emission wavelength of 330 nm.

HPLC mobile phases were first degassed in an ultrasonic bath and filtered through a 0.45 µm membrane before HPLC-FD analysis.

III. RESULTS AND DISCUSSIONS

The stability of melatonin in liquid solutions might be dependent in several parameters (e.g., pH) and environment conditions (e.g., temperature). Here, we focused on the influence of pH and storage period on the stability of melatonin in three commercial grape juices. In order to assess the pH effect, the stability of melatonin in three grape juice solutions were compared to three different melatonin standard solutions prepared at three different pH values (Table 1). All samples were prepared at the two concentrations: 0.1 $mg.L^{-1}$ and 1$mg.L^{-1}$ and analyzed by HPLC-FD.

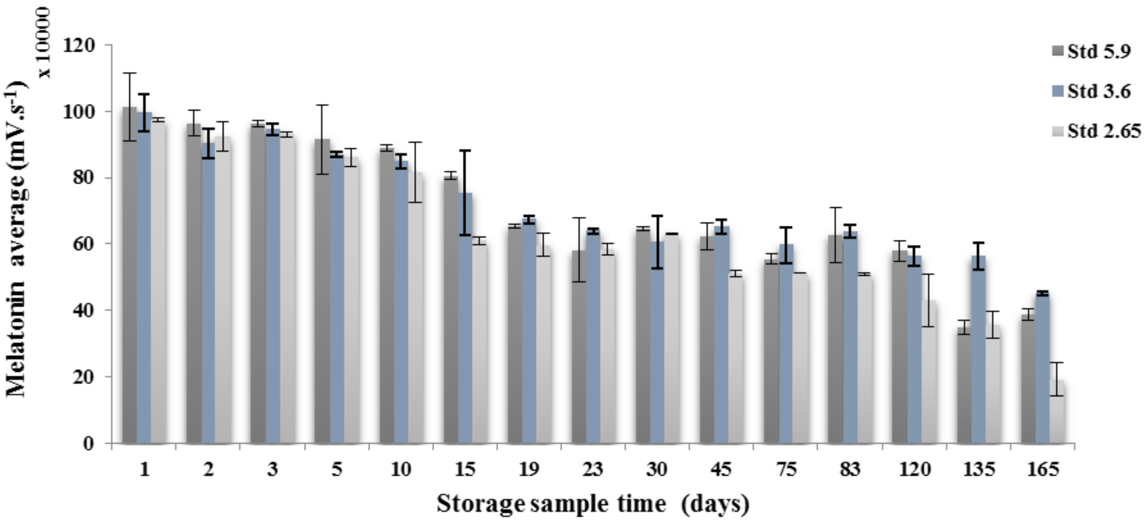

Figure 1. Average areas (mV.s^{-1}) results of three different melatonin standard solutions (0.1mg.L^{-1}) prepared at three different pH values (6.9; 3.60 & 2.65) and stored at room temperature (23ºC).

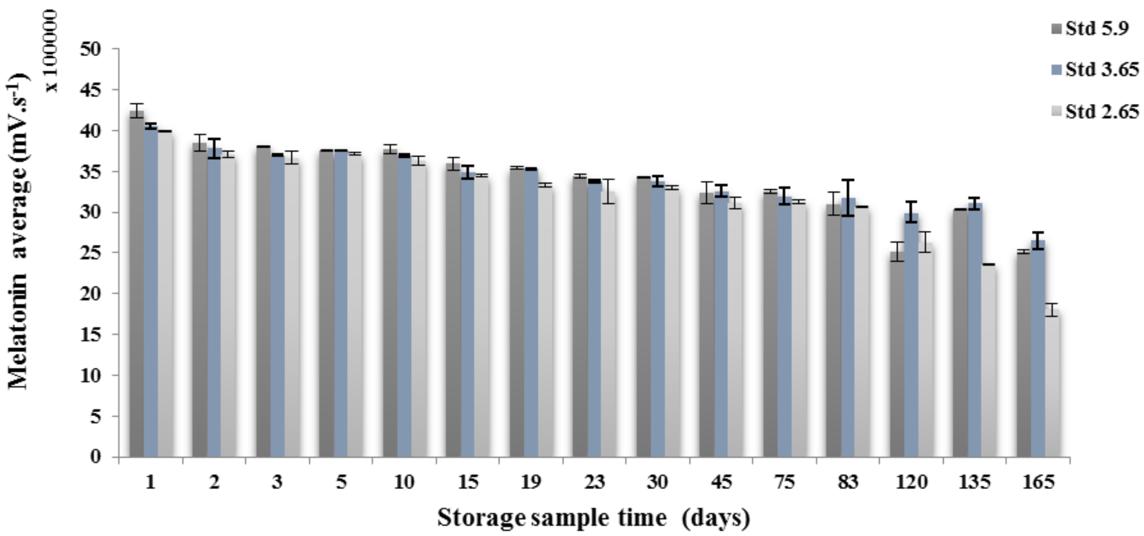

Figure 2. Average areas (mV.s^{-1}) results of three different melatonin standard solutions (1mg.L^{-1}) prepared at three different pH values (6.9; 3.60 & 2.65) and stored at room temperature (23ºC).

Figure **1** shows the results of storage time at three different pH values: pH =6.9; pH =3.6 and pH =2.65 for the melatonin concentration 0.1 mg.L^{-1} (the lowest concentration).

Similar results were obtained in the first 10 days. After that, a slight degradation of melatonin was observed for different pH, but it is more higher for pH (2.65). It degradation become more than 60% in 135 days, and more than 70% after 5 months. Consequently, the stability of melatonin decreases with the increased of the pH value (i.e., the degradation of melatonin increased by decreasing pH of solution).

Figure **2** shown the results obtained for higher concentration of melatonin standard solutions (1 mg.L^{-1}). No many differences were observed between the two levels of melatonin concentrations (0.1 and 1 mg.L^{-1}), i.e, the melatonin is degraded slightly to reach more and less 40% degradation on day 135 for the samples hat have a lower pH value (2.65). After this day, the same samples suffer more degradation (reaching more than 50%), than those have a higher pH (6.9 and 3. 60).

From the two Figures **1 & 2** can be concluded that the pH is an important variable for melatonin degradation, in low concentrations as well as in high concentrations.

Figures **3 & 4** shows the results of spiked sample of grape juices.

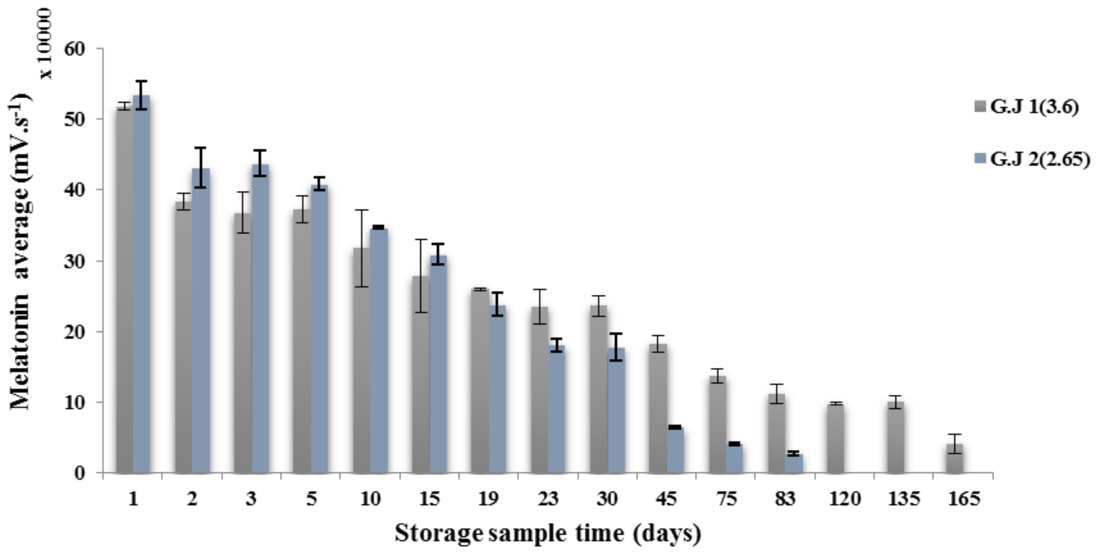

Figure 3. Average areas (mV.s^{-1}) results of spiked grape juices (0.1mg.L^{-1}) prepared and stored at room temperature (23ºC).

As can be seen from Fig. 3, the degradation of melatonin in both GJ 1 and GJ 2 **(**pH values 3.60 and 2.65**)** begin directly from the first day. It is worth to mention that the degradation of melatonin in GJ 1 is more important than GJ 2**,** with lower pH (2. 65) compared to GJ 1. A total degradation is noted after day 83. The degradation of melatonin in GJ 1 (3. 60) is slow and does not appear after 5 months. The melatonin compound has not been detected in GJ 3; most likely due to it degradation after spiking the sample.

The same results were obtained for the spiked samples prepared at 1mg.L^{-1}concentration, and the results are shown in Fig. 4. In this case, the melatonin was detected and degraded directly in the first day of storage (more than 75% in the second day). A total degradation reached in 19 day.

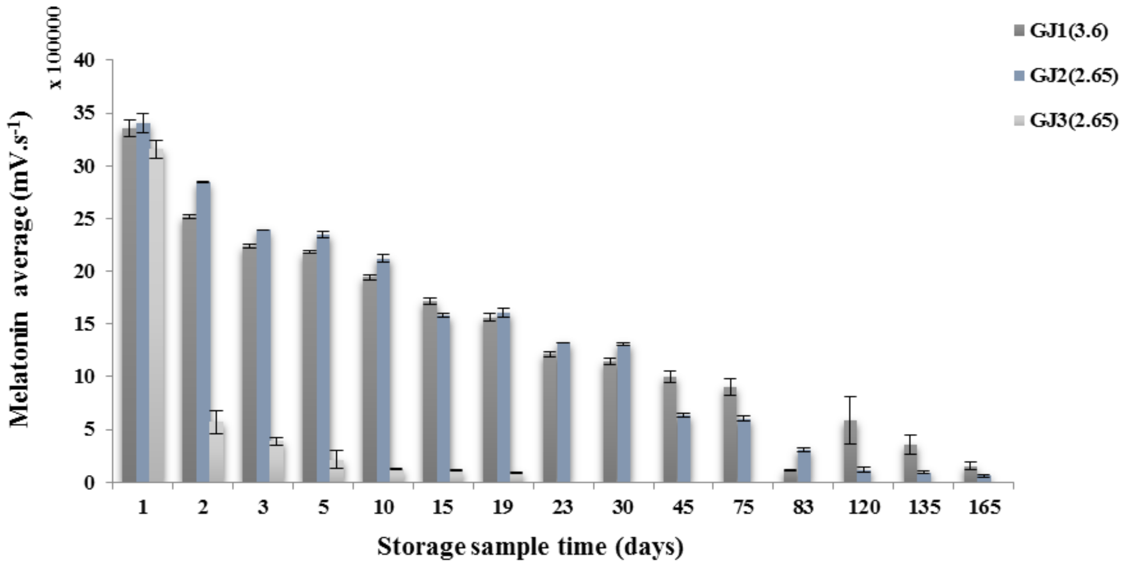

Figure 4. Average areas (mV.s^{-1}) results of spiked grape juices (1mg.L^{-1}) prepared and stored at room temperature (23ºC).

Based on the Figures (1-4), it appears that melatonin degradation is more important in spiked grape juices than in standard solutions. Therefore, the dramatic increase of melatonin degradation in grape juices might due to the existence of some components in solution, especially in low pH value. If the melatonin from grapes is present in the resulting grape juice, most likely it will be degraded in few days after juice preparation. In order to guarantee no degradation, some antioxidants and/or specific storage conditions should be used.

IV. CONCLUSION

In the present study, the stability of melatonin was investigated in standard solutions and three commercial grape juice varieties at the same conditions of pH and storage period. The results showed that the melatonin is not stable in the three different pH values of the tested solutions, and that the maximum degree of degradation reached at the lowest pH value (2.56). The degradation of melatonin is more important in grape juices than in standard solutions. Therefore, it can be conclude that components from grape juices quickly react with melatonin. It means that the determination of melatonin in samples must take into consideration these reactions in order to avoid wrong interpretations.

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