



## Research article

## Influence of pH, temperature, and light on the stability of melatonin in aqueous solutions and fruit juices

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## ARTICLE INFO

## Keywords:

Food science  
Melatonin  
Serotonin  
Tryptophan  
Thermal degradation  
Light  
pH  
Stability  
Neuroendocrine hormone  
Circadian rhythm  
Antioxidant

## ABSTRACT

The ability to predict melatonin stability during food processing or storage is important. Therefore, the degradation of melatonin in both aqueous solutions and fruit juice samples was investigated. The pH values of aqueous solutions were set over a pH range from 1 to 13 and at four different temperatures (60, 70, 80 and 90 °C). The highest remaining melatonin ( $C_R$ ) was observed in the lowest pH solution (pH = 1,  $C_R > 65\%$ ). Melatonin concentrations decreased with rising pH levels from pH 4 to 13 during storage time. The thermal degradation rate constant of melatonin ( $k$ ) values obtained followed the order:  $k_{90^\circ\text{C}} (0.175) > k_{80^\circ\text{C}} (0.123) > k_{70^\circ\text{C}} (0.082) > k_{60^\circ\text{C}} (0.027)$ . Thermal degradation kinetics followed the first-order reaction model with a high range of coefficients of determination ( $0.9744 < R^2 < 0.995$ ). The temperature also affected on melatonin degradation in fruit juices which the degradation rate was increased with the presence of light and high temperature. Our results can be used as guidelines to develop a processing method that predicts melatonin degradation during thermal processing of food products.

## 1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone produced primarily by the pineal gland in the brain through the tryptophan metabolic pathway with the immediate precursor, serotonin (Murch et al., 2000). Melatonin structure is composed of an indole ring with an alkyl amide side chain and a methoxy side chain on a benzene ring (Kim et al., 2019) as a potent free-radical scavenger which also stimulates activity of the antioxidant enzymes and has an influence on the immune system (Mercolini et al., 2012). Melatonin can inhibit sleep disorders such as insomnia (Murch et al., 2000; Xie et al., 2017). Furthermore, melatonin is an effective antioxidant as an inhibitor for the treatment of some cancer cells with beneficial effects in neural disorders (Jan et al., 2009). Therefore, melatonin may have an important clinical role in many disorders.

The melatonin level in humans declines with age and lifestyle (Dopfel et al., 2007). However, several researchers have reported higher circulating melatonin levels in humans through an increase in dietary melatonin from several external sources (Garrido et al., 2010; Johns et al., 2013; Oba et al., 2008; Reiter et al., 2005). Melatonin is also present in different parts of plants including the flowers, roots,

leaves, fruits, and seeds. Typical ranges of melatonin concentration in plants vary from picograms to micrograms per gram (Burkhardt et al., 2001; de la Puerta et al., 2007; Paredes et al., 2009; Reiter et al., 2005). Consequently, natural sources of melatonin in plants have attracted increased research interest in the context of human diets (Huang and Mazza, 2011; bib\_citation\_to\_be\_resolvedKolář and Macháčková, 2005; Paredes et al., 2009; Posmyk and Janas, 2009; Reiter et al., 2007; Tan et al., 2012). Many studies have reviewed methods of extraction and analysis of melatonin as well as its biosynthetic pathway, distribution, and functions in different plant sources. However, some aspects such as melatonin stability are only briefly addressed in previous studies. Process and storage conditions including light, pH, temperature, air and storage time are all considered to be key factors of melatonin degradation in products and solutions (Erland et al., 2016). Appropriate control of these factors and conditions during extraction, separation, purification, processing, and storage are crucial for the stability of melatonin molecules.

Accordingly, more information is required to investigate melatonin stability under the influence of varied pH, temperatures and light using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Data obtained could be used as guidelines to develop a processing method and

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predict the remaining concentration of melatonin during thermal processing of melatonin-rich food products.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All solvents and reagents used were of analytical grade or higher. Melatonin standard was purchased from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA). Five juices including apple juice, lychee juice, guava juice, tomato juice, and orange juice were purchased from a local supermarket. Solvents used included HPLC-grade acetonitrile and methanol (VWR Scientific, Mississauga, Canada). Analytical-reagent grade formic acid, NaOH and HCl were purchased from BDH Chemicals (Poole, UK). HPLC grade water was prepared by reverse osmosis with purification through a Barnstead™ Pacific TII Water Purification System and filtered through a 0.22 μm membrane filter (Whatman, USA) before use.

### 2.2. Preparation of melatonin standard solutions

A standard stock solution of melatonin was prepared by dissolving 0.001 g of melatonin standard in 10 ml methanol (final concentration: 100 μg/ml) (Wójciak-kosior & Woźniak, 2008). This was then diluted to ten different concentrations from 0.002–1 μg/ml for calibration purposes. All standard stock solutions were freshly prepared before analysis.

### 2.3. Influence of pH on melatonin stability in solution

Melatonin solutions were prepared as a previous study (Daya et al., 2001), with slight modifications. One milliliter of standard stock solution of melatonin (0.1 μg/ml) was added in 100 ml phosphate buffer solutions (final concentration: 0.001 μg/ml) at pH 1, 4, 7, 10 and 13 and adjusted by 0.1 M HCl and 0.1 M NaOH. Ten milliliter aliquots of melatonin solution were placed in 20 ml amber glass bottles with screw cap to minimize the amount of light and stored at room temperature for 28 days. Each solution was randomly taken out every 7 days (7, 14, 21, 28 days) at the same time and assayed for melatonin concentration. An aliquot of each sample was taken up in a syringe and filtered through a 0.22 μm nylon syringe filter (Whatman plc) and the filtered solution was injected into a liquid chromatography-tandem mass spectrometry system to determine melatonin concentration.

### 2.4. Influence of process temperature on melatonin stability in solution

To study the effect of temperature, 1 ml standard stock solution of melatonin (1 μg/ml) was dissolved in 100 ml phosphate buffer solution pH 1 (final concentration: 0.01 μg/ml). Hydrochloric solution was used to adjust the pH value of the aqueous solutions. Ten milliliter aliquots of melatonin solutions were placed in 20 ml amber glass bottles with screw cap to minimize the amount of light and the bottles were placed in a thermoelectric water bath at the desired temperature (60, 70, 80 and 90 °C). At regular intervals (6, 9, and 12 h), samples were removed from the hot water bath and immediately transferred to an ice water bath to prevent further degradation. All sets of samples were taken up in a syringe and filtered through a 0.22 μm nylon syringe filter (Whatman plc) and the filtered solutions were injected into a liquid chromatography-tandem mass spectrometry system to determine melatonin concentration.

### 2.5. Stability of melatonin in fruit juices

The pH of fruit juices was measured using a pH meter before the study. The stability of melatonin in fruit juices was conducted according to the procedure as indicated in 2.4. Ten milliliters of the juices (apple juice, lychee juice, guava juice, tomato juice, and orange juice) were

added with 0.1 ml of melatonin standard (1 mg/ml) where the final concentrations were 10 μg/ml. Then, the prepared juices were placed in 20 ml amber glass bottles which stored in a refrigerator (4 °C) and room temperature (with the presence of light and without light) for 14 days. Each juice sample was randomly taken out every 7 days (7, and 14 days) and used for melatonin determination using a liquid chromatography-tandem mass spectrometry system.

### 2.6. Liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS) of melatonin

The concentration of melatonin was measured using LC-MS/MS performed on a Shimadzu 8030 system (Shimadzu Corporation, Kyoto, Japan) coupled with an MS/MS system consisting of a triple quadrupole mass spectrometer. LC solution software was used for instrument control and data acquisition. Melatonin was detected and quantified with electrospray ionization (ESI), operated in positive ion mode by monitoring the transition of the protonated molecular ions of melatonin 233.15 u to the production 174 u in ESI.

Melatonin was separated following the method reported by Cao, Murch, O'Brien and Saxena (2006) with an InertSustain® C18 HPLC column 150 × 2.1 mm, 3.5 μm (GL Science Inc., Tokyo, Japan). Mobile phase A was constituted using 0.45% formic acid (HCOOH) in water (H<sub>2</sub>O) and mobile phase B was acetonitrile (CH<sub>3</sub>CN). After filtration through a 0.22 μm membrane filter (Whatman plc) and sonification for at least 15 min, the degassed mobile phase was pumped through the column. The gradient pump program was 0.45% formic acid: acetonitrile (0–5 min, 80:20% (v/v); 5–6 min, 50:50% to 0:100% (v/v); 6–9 min, 0:100% (v/v), and 9–10 min, 80:20% (v/v)). The compounds were eluted from the column with 0.25 ml/min for a total flow time of 10 min.

Quantification of melatonin was determined by comparing the retention time. The recorded chromatograms and peak areas were used to calculate the melatonin concentration against the calibration curve of external standards. All experiments were triplicated and values were expressed as mean ± SD.

### 2.7. Calculations for melatonin remaining

The remaining concentration of melatonin ( $C_R$  value) was expressed as Eq. (1).

$$C_R(\%) = \frac{C_t}{C_0} \times 100 \quad (1)$$

where  $C_t$  is the melatonin concentration at time  $t$ , and  $C_0$  is the initial concentration of melatonin.

### 2.8. Mathematical model for thermal degradation kinetics

Thermal degradation of melatonin in solution was confirmed to follow first-order kinetics with a coefficient of determination, while the degradation rate constant of melatonin was calculated using Eq. (2).

$$C_t = C_0 \exp(kt) \quad (2)$$

where  $C_t$  is the melatonin concentration at time  $t$ ,  $C_0$  is the initial concentration of melatonin, and  $k$  is the first-order rate constant (hours<sup>-1</sup>).

Half-life values ( $t_{1/2}$ , the amount of time required for a quantity to decrease to half of its initial value) of melatonin degradation were calculated according to Eq. (3).

$$t_{1/2} = -\ln 0.5/k \quad (3)$$

### 2.9. Statistical analysis

A linear regression model was utilized to describe melatonin stability using the coefficient of determination ( $R^2$ ), degradation rate constant ( $k$ ),

and half-life values ( $t_{1/2}$ ). Results obtained from triplicate experiments were analyzed using analysis of variance (ANOVA) with a confidence level of 95% ( $P < 0.05$ ) to assess statistical significance.

### 3. Results and discussion

Temperature, pH, light and storage time are considered to be key degradation factors. Thus, this study focused on investigating the stability of melatonin in solutions and juices with the presence of light, different pH levels, and thermal degradation kinetics. The concentration of melatonin was measured using liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS). The conditions applied for LC-MS/MS study included protonated molecular ions (parent ions) monitored at 233 m/z. The production scan spectra showed a high abundance of fragment ion (daughter ion) at m/z 174.0 using electrospray ionization (ESI) (Cao et al., 2006). Therefore, multiple reaction monitoring (MRM) using transition at m/z 233.15 to 174.0 was used to quantify melatonin. The retention time of melatonin was  $6.398 \pm 0.029$  min. A melatonin standard curve with a high coefficient of determination ( $r^2 = 0.9996$ ) was obtained (data not shown).

#### 3.1. Influence of pH on melatonin stability in solutions

To evaluate the effect of pH on the stability of melatonin, results were compared to the concentration of melatonin in aqueous solutions with pH values of 1, 4, 7, 10, and 13 as illustrated in Figure 1. Melatonin content in all pH solutions decreased with increasing storage time, and degradation of melatonin in pH solutions demonstrated two rates. A slower degradation rate was observed in solution pH 1 during storage. The remaining melatonin ( $C_R$  value) was 65% compared with the initial concentration which reduced from 567.83 to 374.58 pg/ml after storage for 28 days (Table 1). On the other hand, melatonin solutions pH 4, 7, 10 and 13 showed a dramatic rate of degradation after storage for 21 days and the rate further increased until day 28 with the  $C_R$  values of 0–4%. The highest acidic solution (pH 1.0) showed higher melatonin remaining content than the other solutions, indicating that melatonin was more stable at lower pH and its stability decreased with rising pH. This may be due to the deprotonation of amide functional groups of the indole group in the melatonin structure to an aqueous solution (Zafra-Roldán et al., 2018), relative to the acidity constant values, ( $pK_a$ ). A lower  $pK_a$  value indicates a greater ability to donate a proton to a base in aqueous solution (Helmenstine and Marie, 2018). These findings were in agreement with observations by Andrisano et al. (2000) who found that melatonin was rapidly degraded at pH 9.0 by photodegradation while the degradation rate decreased at lower solution pH owing to oxidation of the indole ring to give a formyl amine group. Similarly, melatonin structure was shown to be stable in acidic environments but unstable at basic or neutral pH

(Baker & Attala, 2005; Lee, 1989). By contrast, Daya et al. (2001) studied melatonin stability in solutions over a pH range of 1.2–12 and reported that pH did not play a significant role in melatonin stability.

#### 3.2. Influence of process temperature on melatonin stability in solutions

The above results revealed that melatonin was more stable in a lower pH solution. Consequently, we chose the pH 1 solution to evaluate the melatonin stability for 12 h with four different temperatures (60, 70, 80 and 90 °C). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to trace the melatonin content throughout the study. Results showed a continuous decrease in melatonin content with time for all temperatures studied.

However, at six hours of incubation, melatonin content significantly decreased ( $P < 0.05$ ) at different temperatures. The incubation of melatonin solution at 60 °C for 12 h revealed relative stability when compared with the other temperatures (Figure 2). The  $C_R$  value of melatonin in solutions at 60 °C after 6 h was approximately 85% of the initial value, whereas at 70 °C, 80 °C and 90 °C,  $C_R$  values decreased to approximately 64%, 52%, and 37% respectively. After incubating for twelve hours, Table 2 showed that solutions kept at 60 °C retained melatonin concentration 6.7 pg/ml of melatonin, about eight times more than at 90 °C (0.80 pg/ml of melatonin). These results indicated that melatonin concentration decreased with increasing temperature and time. Similar values were investigated by El Moussaoui and Bendriss (2014) who found that concentration of melatonin standard stored at four different temperatures (-32, 4, 25 and 50 °C) under exposure to light for 15 days showed high degradation at up to 16%, 17%, 22%, and 22%, respectively. Moreover, concentration of melatonin in solutions kept at room temperature (25 °C) under exposure to light and air significantly decreased to 43% (Moussaoui et al., 2015; El Moussaoui and Bendriss, 2014). Therefore, temperature might not be the most important variable for melatonin degradation. Air was determined as a critical variable related to melatonin stability, and exposure to air plus light increased melatonin degradation. This finding concurred with Zafra-Roldán et al. (2018) who found that melatonin in acidic pH solution was stable when protected from the environment but the solution became unstable when exposed to light and oxygen.

#### 3.3. Thermal kinetic studies

To understand the kinetics of melatonin degradation during heating at 60, 70, 80, and 90 °C, logarithms of melatonin contents were plotted as a function of time (Figure 3a). The linear relationship in Figure 3b demonstrated that thermal degradation of melatonin in pH 1 solution followed first-order reaction kinetics with a high range of the coefficient of determination ( $0.9744 < R^2 < 0.995$ ). The equation well described the

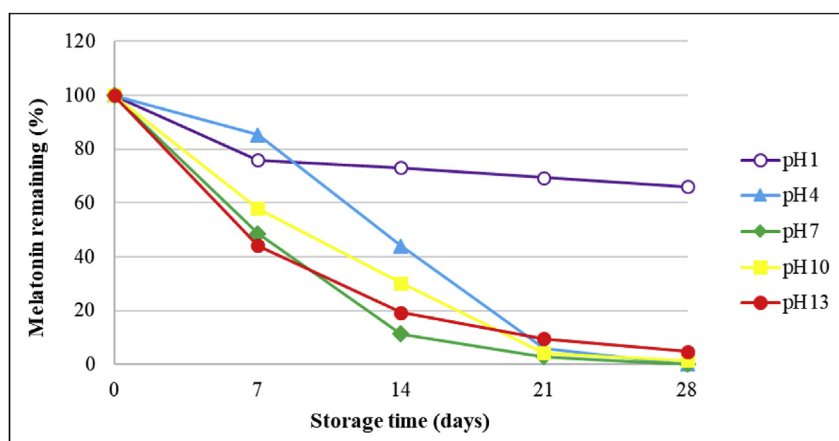


Figure 1. Degradation of melatonin in solution with different pH levels during storage for 28 days at room temperature.

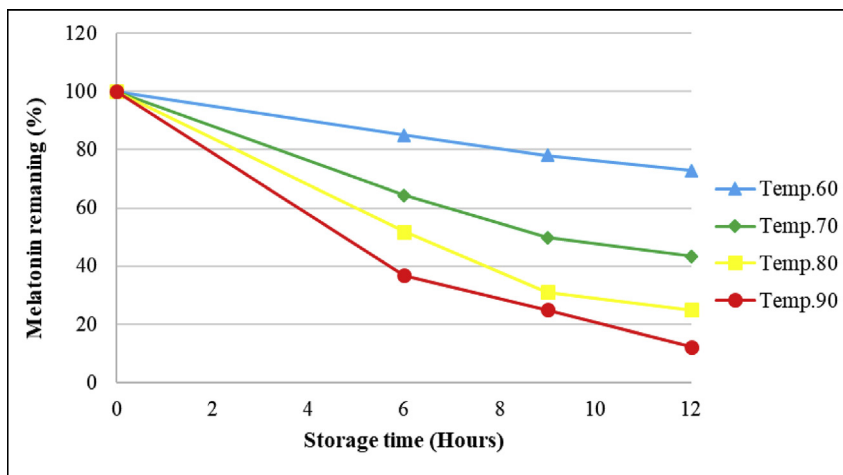
**Table 1.** Concentrations of melatonin in varied pH solution.

pH	Melatonin concentration (pg/ml)				
	Day 0	Day 7	Day 14	Day 21	Day 28
1	567.83 ± 39.18 <sup>a</sup>	430.72 ± 14.36 <sup>a</sup>	414.66 ± 47.25 <sup>a</sup>	394.59 ± 6.08 <sup>a</sup>	374.58 ± 17.34 <sup>a</sup>
4	295.23 ± 14.16 <sup>bc</sup>	251.68 ± 29.5 <sup>b</sup>	129.99 ± 36 <sup>b</sup>	17.13 ± 3.24 <sup>c</sup>	ND
7	235.98 ± 40.9 <sup>c</sup>	114.51 ± 38.51 <sup>c</sup>	26.75 ± 2.31 <sup>c</sup>	6.75 ± 0.04 <sup>d</sup>	ND
10	299.59 ± 38.29 <sup>bc</sup>	173.46 ± 48.64 <sup>c</sup>	90.49 ± 46.09 <sup>bc</sup>	12.66 ± 3.36 <sup>cd</sup>	6.32 ± 0.167 <sup>bc</sup>
13	339.59 ± 30.07 <sup>b</sup>	149.97 ± 13.63 <sup>c</sup>	65.57 ± 7.77 <sup>bc</sup>	32.32 ± 6.34 <sup>b</sup>	16.32 ± 5.49 <sup>b</sup>

ND represents an amount that was not detected.

Values represent mean ± standard deviation of replicate readings (n = 3).

Values with the same letters along the same columns are not significantly different (P < 0.05).



**Figure 2.** Thermal degradation of melatonin in pH1 solution at varied temperature.

**Table 2.** Concentrations of melatonin at varied temperatures.

Temperature (°C)	Melatonin concentration (pg/ml)			
	0 Hours	6 Hours	9 Hours	12 Hours
60	9.21 ± 0.14 <sup>a</sup>	7.83 ± 0.09 <sup>a</sup>	7.19 ± 0.64 <sup>a</sup>	6.7 ± 0.11 <sup>a</sup>
70	7.58 ± 2.82 <sup>a</sup>	4.88 ± 2.25 <sup>bc</sup>	3.78 ± 1.49 <sup>b</sup>	3.29 ± 1.61 <sup>b</sup>
80	10.55 ± 2.49 <sup>a</sup>	5.46 ± 0.95 <sup>ab</sup>	3.27 ± 1.30 <sup>bc</sup>	2.63 ± 0.75 <sup>b</sup>
90	6.51 ± 2.92 <sup>a</sup>	2.39 ± 1.15 <sup>c</sup>	1.62 ± 0.03 <sup>c</sup>	0.80 ± 0.40 <sup>c</sup>

Values represent mean ± standard deviation of replicate readings (n = 3).

Values with the same letters along the same columns are not significantly different (P < 0.05).

degradation of melatonin in pH 1 solution over the entire temperature range. Although no previous research related to thermal degradation kinetics of melatonin was available, our results agreed with degradation studies of melatonin under different oxidation conditions such as UV, UV/H<sub>2</sub>O<sub>2</sub>, Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>, and UV/H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>. All these conditions followed first-order reaction kinetics (Xu et al., 2009). The photodegradation process (250, 350, 450 and 550 W/m<sup>2</sup>) of melatonin solutions was also well fitted by a first-order reaction (De Luca, Tauler, Ioele and Ragno, 2013; Luca and Ragno, 2009).

This kinetic type was expressed by the Eqs. (4) and (5):

$$C_t = C_0 \exp(kt) \tag{4}$$

$$t_{1/2} = -\ln 0.5/k \tag{5}$$

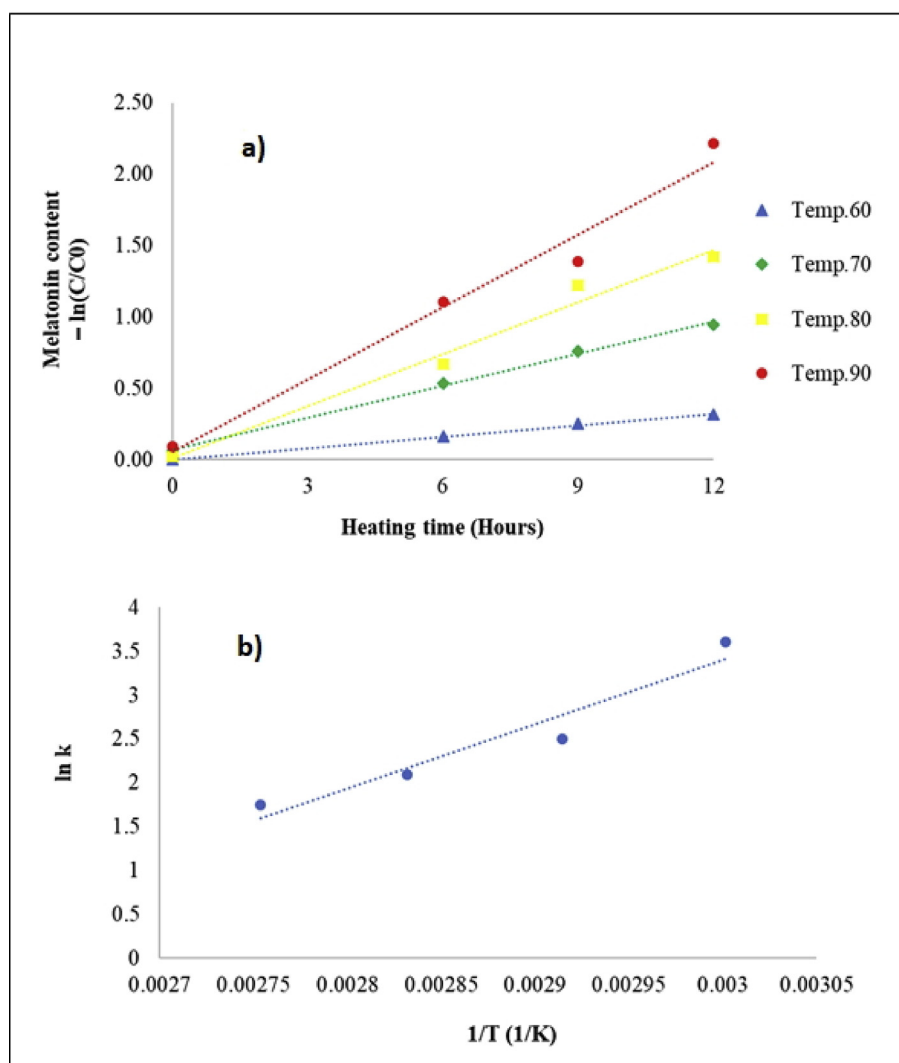
where C<sub>0</sub> is initial melatonin content and C<sub>t</sub> is melatonin content after heating time t (hours) at the given temperature, and k is the first-order

kinetic constant. The Arrhenius equation was used to present the thermal degradation rate constant

$$k = k_0 \times e^{-E_a/RT} \tag{6}$$

where k<sub>0</sub> is the frequency factor (per min), E<sub>a</sub> is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol/K) and T is the absolute temperature (in Kelvin, K).

In addition to the coefficient of determination (R<sup>2</sup>), the reaction rate constant (k) and half-life value (t<sub>1/2</sub>) as the amount of time required for a quantity to decrease to half of its initial value were also indicators used to predict melatonin degradation (Table 3). Degradation rate constants were 0.027, 0.082, 0.123 and 0.175 at 60, 70 80 and 90 °C, respectively. In other words, melatonin degradation increased with increasing temperature. The time required for 50% degradation of melatonin at 60 °C was about 6 times longer than that at 90 °C. Thus, temperature had a significant effect on melatonin degradation. The reaction rate usually increases with temperature due to higher kinetic



**Figure 3.** Degradation of melatonin in solution (pH1) during heating at 60, 70, 80, and 90 °C (a), and Arrhenius degradation plots (b).

**Table 3.** Kinetic parameters for melatonin degradation in solutions at varied temperatures.

Temperature (°C)	k (hours <sup>-1</sup> )	t <sub>1/2</sub> (hours)	R <sup>2</sup>
60	0.027 ± 0.003 <sup>c</sup>	25.957 ± 2.875 <sup>a</sup>	0.9985
70	0.082 ± 0.057 <sup>bc</sup>	11.068 ± 5.702 <sup>b</sup>	0.9837
80	0.123 ± 0.031 <sup>ab</sup>	5.872 ± 1.378 <sup>bc</sup>	0.9814
90	0.175 ± 0.042 <sup>a</sup>	4.101 ± 0.876 <sup>c</sup>	0.9744

Values represent mean ± standard deviation of replicate readings (n = 3).

Values with the same letters along the same columns are not significantly different (P < 0.05).

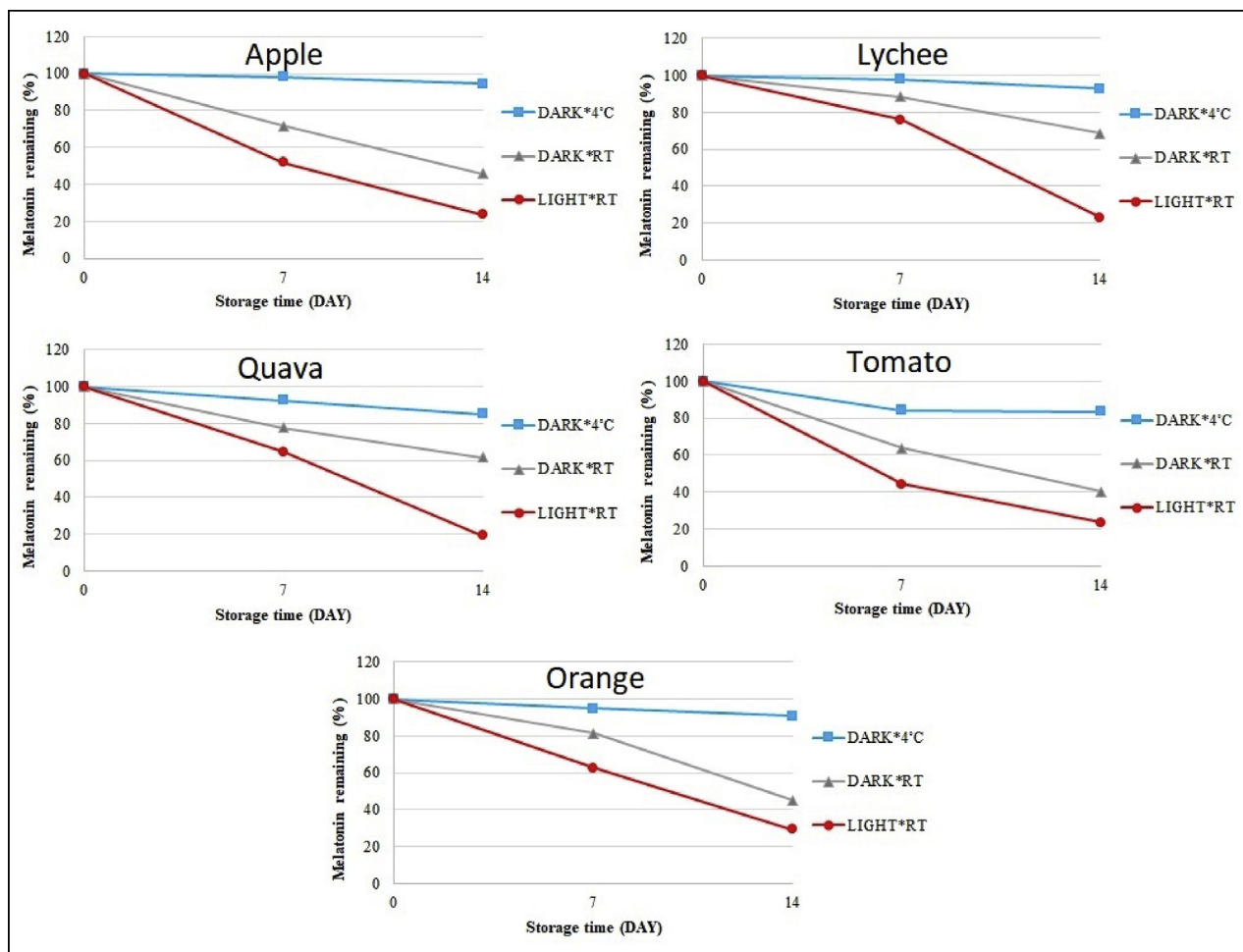
energy of the reactant molecules (Key and Ball, 2015). For each temperature increase by 10 °C, the rate of reaction doubles (Helmenstine & Marie, 2018). Long storage time also increases melatonin degradation. Consequently, temperature and storage time are powerful factors for melatonin degradation.

### 3.4. The stability of melatonin in juices

The study of melatonin stability could not be completed without testing a real food sample. Previous results (3.1) suggested that melatonin was more stable at lower pH and its stability decreased with rising

pH. Therefore, juices having low pH were selected to investigate the stability of melatonin during storage at different temperatures, with light and without light. Moreover, these juices were divided into two groups. Guava and orange juices were high acid (pH: 4.24 and 4.43, respectively), while low acid juices included tomato, apple, and lychee (pH: 4.68, 4.71 and 4.73, respectively).

Our results (Figure 4) showed that although the juices had varied pH values, this did not have different effects on melatonin stability. All juice samples showed a gradual decrease in melatonin during storage without light in the refrigerator (4 °C). After storage for 14 days, the melatonin remaining (C<sub>R</sub> value) in all juice samples ranged from 83.5-94.5%, while



**Figure 4.** Degradation of melatonin in juices (apple juice, lychee juice, guava juice, tomato juice and orange juice) stored in two different temperatures (4 °C and Room temperature) with light and without light for 14 days.

melatonin content of samples stored at room temperature decreased, varying from 40 to 68.5%. This may be due to the double reaction with increasing temperature (Helmenstine & Marie, 2018). This finding concurred with the previous result in pH1 solution which indicated that melatonin degradation rate increased with rising temperature. Nonetheless, lowest melatonin remaining was observed in fruit juice stored in the presence of light at room temperature for 14 days (19.5–29% remaining). These results indicated that temperature plus light had more intensity on the degradation rate than temperature alone. A similar result was reported by El Moussaoui and Bendriss (2014) who found that melatonin solution stored at 25 °C under exposure to light for 15 days showed degradation at 22%, while the solution kept at the same temperature (25 °C) under exposure to light and air showed higher degradation at 43%. Thus, exposure to both light and air affected the degradation of melatonin.

#### 4. Conclusions

Our findings demonstrated that pH, temperature, and light significantly affected the stability of melatonin. Melatonin was most stable at pH1 and its concentration decreased with rising pH levels (pH 4–13) during storage time. Thermal degradation kinetics followed the first-order reaction model with a high range of the coefficient of determination ( $0.9744 < R^2 < 0.995$ ). The  $k$  values obtained followed the order:  $k_{90^{\circ}\text{C}} (0.175) > k_{80^{\circ}\text{C}} (0.123) > k_{70^{\circ}\text{C}} (0.082) > k_{60^{\circ}\text{C}} (0.027)$ . Similarly, half-life value decreased as heating time increased. Temperature and

light also affected melatonin degradation in five fruit juices. Our results can be used to develop a method that predicts melatonin degradation during thermal processing of food products.

#### Declarations

##### Author contribution statement

Thorung Pranil: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anuchita Moongngarm, Patiwit Loypimai: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

##### Funding statement

This work was supported by Thailand Research Fund, Thailand (TRF; Royal Golden Jubilee Ph.D. (RGJ-PHD) Program [PHD/0113/2558]) and Mahasarakham University, Thailand [6203001/2562].

##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

## References

- Andrisano, V., Bertucci, C., Battaglia, A., Cavrini, V., 2000. Photostability of drugs : photodegradation of melatonin and its determination in commercial formulations, 23, 15–23.
- Baker, Max T., Attala, Mohamed Naguib, 2005. Melatonin analogue prodrugs. US007381740B2 1 (9), 1–6.
- Burkhardt, S., Dun Xian, Tan, Manchester, L.C., Hardeland, R., Reiter, R.J., 2001. Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (*Prunus cerasus*). *J. Agric. Food Chem.* 49 (10), 4898–4902.
- Cao, J., Murch, S.J., O'Brien, R., Saxena, P.K., 2006. Rapid method for accurate analysis of melatonin, serotonin and auxin in plant samples using liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1134 (1), 333–337.
- Daya, S., Walker, R.B., Glass, B.D., Anoopkumar-Dukie, S., 2001. The effect of variations in pH and temperature on stability of melatonin in aqueous solution. *J. Pineal Res.* 31 (2), 155–158.
- de la Puerta, C., Carrasco-Salmoral, M.P., García-Luna, P.P., Lardone, P.J., Herrera, J.L., Fernández-Montesinos, R., et al., 2007. Melatonin is a phytochemical in olive oil. *Food Chem.* 104 (2), 609–612.
- De Luca, M., Tauler, R., Ioele, G., Ragno, G., 2013. Study of photodegradation kinetics of melatonin by multivariate curve resolution (MCR) with estimation of feasible band boundaries. *Drug Test. Anal.* 5 (2), 96–102.
- Dopfel, R.P., Schulmeister, K., Schernhammer, E.S., 2007. Nutritional and lifestyle correlates of the cancer-protective hormone melatonin. *Canc. Detect. Prev.* 31 (2), 140–148.
- El Moussaoui, N., Bendriss, A., 2014. The influence of storage conditions on melatonin stability. *Int. J. Eng. Res.* 3 (3), 2243–2246.
- Erland, L.A.E., Chattopadhyay, A., Jones, A.M.P., Saxena, P.K., 2016. Melatonin in plants and plant culture systems: variability, stability and efficient quantification. *Front. Plant Sci.* 7 (November).
- Garrido, M., Paredes, S.D., Cubero, J., Lozano, M., Toribio-Delgado, A.F., Muñoz, J.L., Rodríguez, A.B., 2010. Jerte valley cherry-enriched diets improve nocturnal rest and increase 6-sulfatoxymelatonin and total antioxidant capacity in the urine of middle-aged and elderly humans. *J. Gerontol. Series A Biol. Sci. Med. Sci.* 65 A (9), 909–914.
- Helmenstine, Marie, Anne, 2018. pH and pKa Relationship: the Henderson-Hasselbalch Equation. Retrieved from. <https://www.thoughtco.com/the-ph-and-pka-relationship-603643>.
- Huang, X., Mazza, G., 2011. Application of LC and LC-MS to the Analysis of Melatonin and Serotonin in Edible Plants Application of LC and LC-MS to the Analysis of Melatonin and Serotonin, p. 8398.
- Jan, J.E., Reiter, R.J., Wasdell, M.B., Bax, M., 2009. The role of the thalamus in sleep, pineal melatonin production, and circadian rhythm sleep disorders. *J. Pineal Res.* 46 (1), 1–7.
- Johns, N.P., Johns, J., Porasuphatana, S., Plaimée, P., Sae-Teaw, M., 2013. Dietary Intake of Melatonin from Tropical Fruit Altered Urinary.Pdf.
- Key, Jessie A., Ball, D.W., 2015. Introductory Chemistry- 1st Canadian Edition (1st Canadian). 2015. Retrieved from. <https://opentextbc.ca/introductorychemistry/chapter/dilutions-and-concentrations-2/>.
- Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Bolton, E.E., 2019. PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res.* 47 (D1), D1102–D1109. Retrieved from.
- Kolář, J., Macháčková, I., 2005. Melatonin in higher plants: occurrence and possible functions. *J. Pineal Res.* 39 (4), 333–341.
- Lee, Ya-Ling, 1989. Development of a Transdermal Delivery System for Melatonin. Oregon State University.
- Luca, M. De, Ragno, G., 2009. Application of Multivariate Curve Resolution (MCR) to the Photodegradation Study of Melatonin. CAMO Software AS, Oslo, Norway.
- Mercolini, L., Mandrioli, R., Raggi, M.A., 2012. Content of melatonin and other antioxidants in grape-related foodstuffs: measurement using a MEPS-HPLC-F method. *J. Pineal Res.* 53 (1), 21–28.
- Moussaoui, E., El Hassane, A., Bendriss, A., Science, C., Box, P.O., Arabia, S., 2015. Melatonin stability at different storage conditions and during the ultrasound assisted extraction. *Int. J. Curr. Res.* 7 (5), 15526–15530.
- Murch, S.J., KrishnaRaj, S., Saxena, P.K., 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in vitro regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. *Plant Cell Rep.* 19 (7), 698–704.
- Oba, S., Nakamura, K., Sahashi, Y., Hattori, A., Nagata, C., 2008. Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration. *J. Pineal Res.* 45 (1), 17–23.
- Paredes, S.D., Korkmaz, A., Manchester, L.C., Tan, D.X., Reiter, R.J., 2009. Phytomelatonin: a review. *J. Exp. Bot.* 60 (1), 57–69.
- Posmyk, M.M., Janas, K.M., 2009. Melatonin in plants. *Acta Physiol. Plant.* 31 (1), 1–11.
- Reiter, R.J., Manchester, L.C., Tan, D.X., 2005. Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. *Nutrition* 21 (9), 920–924.
- Reiter, R., Tan, D., Manchester, L., Simopoulos, A., Maldonado, M., Flores, L., Terron, M., 2007. Melatonin in edible plants (phytomelatonin): identification, concentrations, bioavailability and proposed functions. *World Rev. Nutr. Diet.* 97 (October 2016), 211–230.
- Tan, D.X., Hardeland, R., Manchester, L.C., Korkmaz, A., Ma, S., Rosales-Corral, S., Reiter, R.J., 2012. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J. Exp. Bot.* 63 (2), 577–597.
- Wójciak-kosior, M., Woźniak, A., 2008. Quantitative determination of melatonin in *Lamium album* flos. *Herba Pol.* 54 (3), 13–18.
- Xie, Z., Chen, F., Li, W.A., Geng, X., Li, C., Meng, X., Yu, F., 2017. A review of sleep disorders and melatonin. *Neurol. Res.* 39 (6), 559–565.
- Xu, X.R., Li, X.Y., Li, X.Z., Li, H. Bin, 2009. Degradation of melatonin by UV, UV/H<sub>2</sub>O<sub>2</sub>, Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> and UV/Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> processes. *Separ. Purif. Technol.* 68 (2), 261–266.
- Zafra-Roldán, A., Corona-Avenidaño, S., Montes-Sánchez, R., Palomar-Pardavé, M., Romero-Romo, M., Ramírez-Silva, M.T., 2018. New insights on the spectrophotometric determination of melatonin pKa values and melatonin-βCD inclusion complex formation constant. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 190, 442–449.