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STUDY OF THE DEGRADATION KINETICS OF CROCIN DURING STORAGE AT DIFFERENT TEMPERATURES: EFFECT OF pH VARIATION AND THE USE OF PRESERVATIVES

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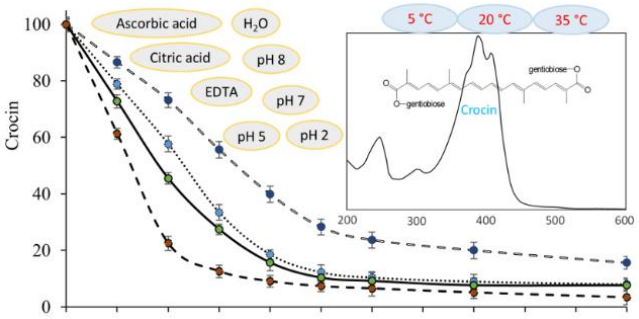
Saffron is the most expensive spice in the world, not only due to its labor-intensive production and very low yield, but also because of its multitude of therapeutic properties. Despite the development of many functional food products, maintaining the stability of saffron compounds remains a significant challenge for both researchers and manufacturers. This study focused on investigating the stability of crocin, the bioactive compound responsible for saffron's color, under various temperatures, using different media with varying pH levels and preservatives. Crocin was stored in media with different pH levels (2, 5, 7, and 8) and in the presence of preservatives (EDTA, citric acid, and ascorbic acid) at variable temperatures (5, 20, and 35 °C), with analyses performed at different time intervals. The results revealed that the degradation kinetics of crocin followed a second-order reaction. It was found that neither an acidic medium (pH 2) nor neutral or basic pH levels were suitable for crocin storage; only pH 5 provided satisfactory stability. The use of preservatives showed positive effects on crocin's stability, with ascorbic acid demonstrating the best results in terms of half-life, particularly at lower storage temperatures of 5 °C and 20 °C, with half-lives of 266.34 and 141.97 days, respectively. For optimal crocin storage, it is recommended to use ascorbic acid as a preservative in a weakly acidic medium, while avoiding high temperatures.



INTRODUCTION

Crocus sativus is a perennial plant cultivated for its floral stigmas; in fact, around 150,000 flowers must be carefully picked to produce a single kilo of saffron. Harvesting the flowers and separating the stigmas are time-consuming and require intensive labor; for this reason, it remains the most expensive spice in the world, for which it is referred to as "Red Gold". [1,2]

Saffron is highly valued around the world for its richness in secondary metabolites, including phenolic compounds, essential oils, apocarotenoids, monoterpenoids, and phytosterols.[3] The key properties of this spice are attributed to three compounds: crocin (responsible for its color), picrocrocin (responsible for its bitter taste), and safranal (the main volatile component responsible for its aroma).[4,5] Therefore, the quality of saffron is

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directly linked to the levels of these components. In fact, these bioactive compounds exhibit high bioavailability and bioaccessibility, and are even able to pass the blood-brain barrier, where they exert neuroprotective, anxiolytic, and antidepressant effects, as well as enhancing learning and memory capacity.[3,6] In addition, some scientific studies have demonstrated that these chemical components act as antiulcer and anticancer agents, improve digestion, and have anti-inflammatory properties.[7]

Saffron is used as a flavoring and coloring agent in various fields and industries, including culinary applications, the food industry, cosmetics, pharmaceuticals, and perfumery.[8,9] Saffron has been used in the development of many functional food products, such as beverages (herbal teas and a range of bitter drinks), baked goods, cookies, pasta, jam, chocolate, and dairy products, including cheese, yogurt, and milk.[10] The coloring property of saffron is primarily attributed to crocin ($C_{44}H_{64}O_{24}$), an apocarotenoid formed by the glycosylation of crocetin, which constitutes 18 to 37% of the spice's weight basis.[11]

Once extracted from the stigmas, crocin becomes highly susceptible to oxidation and degradation. This compound is sensitive to changes in pH and temperature, and is also affected by light and the presence of oxygen.[12] Several studies have investigated the stability of crocin and found that increased acidity or temperature accelerates its degradation.[13,14] In an effort to improve the stability of crocin, numerous investigations have focused on microencapsulation techniques, such as using chitosan/alginate and gelatin[15,16] or nanocomplexation with proteins (sorghum, pearl millet, or foxtail millet proteins) or casein.[17,18] However, these methods are labor-intensive, time-consuming, and expensive.

Therefore, this investigation aims to study the degradation kinetics of crocin extracted from saffron in different media with varying pH levels (2, 5, 7, and 8) during storage at different temperatures (5, 20, and 35 °C). In addition, certain preservatives commonly used in the food industry (EDTA, ascorbic acid, and citric acid) were tested to delay and reduce crocin loss, thereby increasing its shelf life.

RESULTS AND DISCUSSION

The norm ISO 3632-2[19] standard classifies saffron into three categories: I, II, and III, based on several parameters, including color intensity or coloring strength, which indicate crocin concentration. From the scanning spectrum of the aqueous solution (Fig. 1), saffron displayed the typical UV-vis spectrum with peaks corresponding to its three characteristic compounds: picrocrocin (λ_{max} = 257 nm), safranal (λ_{max} = 330 nm), and crocin (λ_{max} = 440 nm). The obtained spectrum aligned with the ISO 3632 standard and was supported by other studies,[20,21] confirming the authenticity of the sample. Based on the calculated specific extinction of 262.5, the saffron exhibits a coloring power that falls into category I (≥ 200), indicating the high quality of the studied saffron. Of the 66 saffron samples analyzed by Sánchez *et al.*,[21] only four were classified into category II and two into category III, while the remaining samples were categorized as category I. All saffron samples studied by Naim *et al.*[22] were classified into categories I and II. Determining the authenticity and category of saffron is crucial for ensuring quality and accurately estimating the product's price.

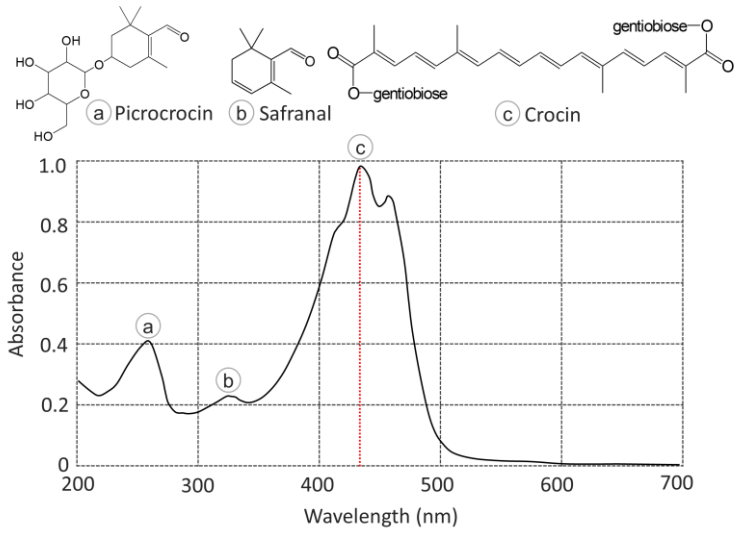

Fig. 1 – Scanning spectrum of the aqueous saffron solution with the peaks of the three characteristic compounds.

Kinetic of crocin degradation

The effect of pH on crocin degradation kinetics during storage at 5 °C is shown in Fig. 2A. Crocin levels decreased rapidly at all pH levels, but in distinct ways. Degradation was least intense at pH 5, followed by pH 7 and 8, with the most rapid degradation observed at pH 2. The degradation kinetics can be divided into two phases: an initial phase of rapid crocin degradation, which lasted for 6 days at pH 2, 12 days at pH 7 and 8, and 15 days at pH 5, followed by a second phase characterized by a lower degradation rate. By the end of the storage period, the remaining crocin levels at the different pH levels were low, ranging from 3.32% (pH 2) to 15.57% (pH 5). Crocin was highly sensitive to pH variation, and the results suggest that pH 5 is more suitable for its preservation.

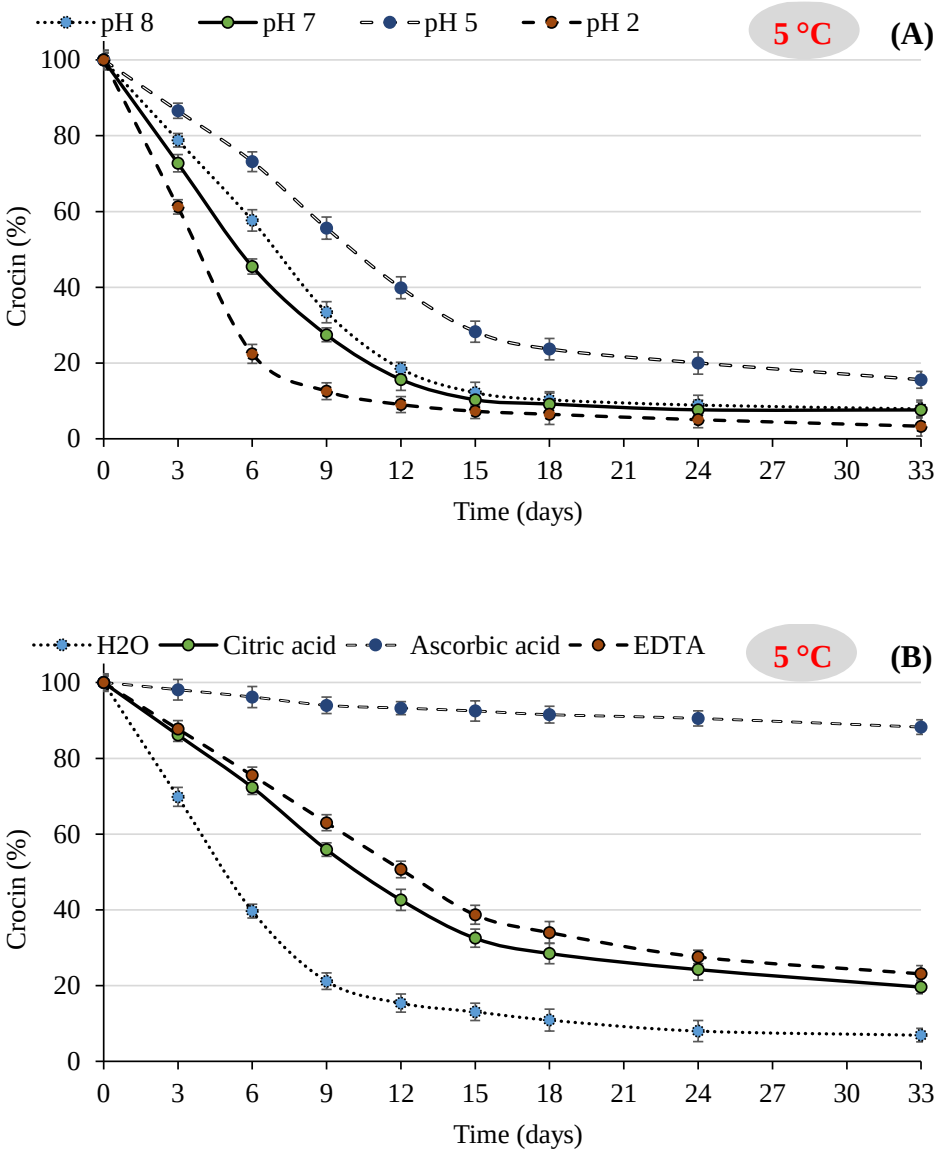

Fig. 2 – Crocin degradation kinetics during storage at 5 °C, effects of pH (A) and preservatives (B).

Figure 2B shows that crocin levels decreased very slowly in the presence of ascorbic acid, with only 12% of the initial amount degraded by the end of the storage period (33 days). The use of the other two preservatives, citric acid and EDTA, resulted in a more rapid decrease in crocin levels up to the 15th day, with reductions of 67 and 61%, respectively. After this, crocin degradation continued gradually, stabilizing at around 20% by the end of the storage period. Storing crocin in distilled water, without preservatives, led to the most rapid degradation, with an 80% loss recorded by the 9th day, after which the degradation slowed, leaving only 6% remaining. It was noted that crocin was highly protected in preservative solutions (ascorbic acid, with moderate protection from citric acid and

EDTA) at 5 °C, but its levels strongly decreased in distilled water. At 5 °C, ascorbic acid was the most effective preservative for crocin, followed by EDTA, citric acid, pH 5, pH 8 & 7, distilled water, and lastly pH 2.

The effect of pH on crocin degradation kinetics during storage at 20 °C (Fig. 3A) showed that crocin levels decreased for all four pH values tested. The order of crocin degradation was as follows: pH 5, pH 7, pH 8, and pH 2. The degradation process occurred in two phases: an initial phase of rapid decrease, observed by the 9th day for pH 2, the 12th day for pH 7 and pH 8, and the 15th day for pH 5, with respective remaining crocin percentages of 11, 18, 13, and 20%. This was followed by a stabilization phase, where the degradation slowed, reaching final crocin levels of 4–15% by the end of the storage period.

Figure 3B shows that the amount of crocin in the presence of ascorbic acid decreased slowly to 85% by the 9th day and then continued to decrease more slowly until the 33rd day, reaching 80% of the initial level, marking only 1/5 of crocin degradation. With the use of EDTA and citric acid, rapid decreases were observed until the 12th day, with remaining contents of 40% for EDTA and 30% for citric acid, and then continued to decrease until the 33rd day, with remaining levels of around 20 and 11%, respectively. In the case of distilled water, the highest intensity of degradation was observed, with only 16% of crocin left on the 9th day, and it continued to decrease and stayed around 6% in the last days of storage. Therefore, the use of preservatives (ascorbic acid, EDTA, and citric acid) stabilized the amount of crocin in saffron extract more efficiently compared to the tested pHs and distilled water.

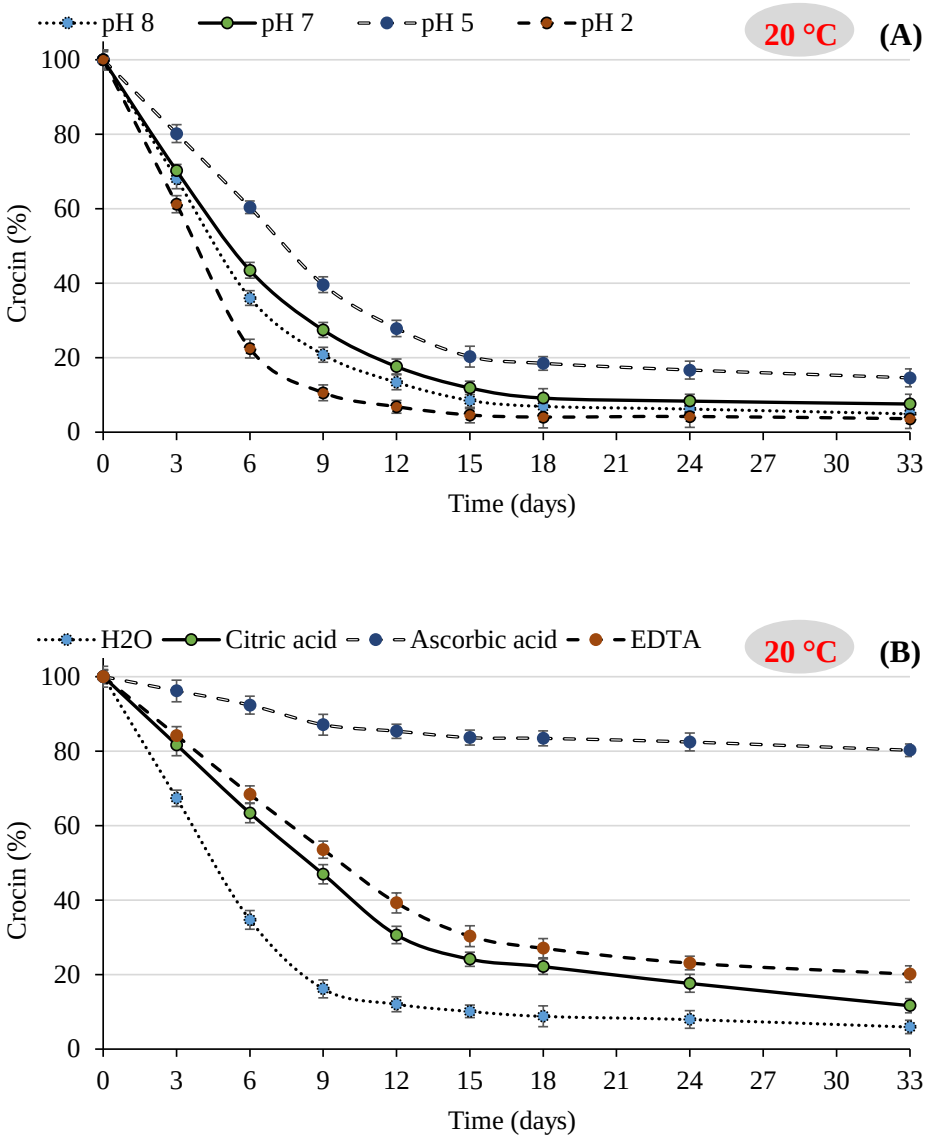

Fig. 3 – Crocin degradation kinetics during storage at 20 °C, effects of pH (A) and preservatives (B).

The effect of pH on crocin degradation kinetics during storage at 35 °C is shown in Fig. 4A. Until the sixth day, it was observed that crocin decreased rapidly for all pH levels, reaching 39% for pH 5, 33% for pH 8, 20% for pH 7, and 15% for pH 2. Degradation continued for all pHs but gradually, eventually reaching 1.42–11.13% at the end of the storage period. Statistical analysis revealed that pH 7 and pH 8 showed slightly better conservation of crocin compared to neutral (pH 7) and strongly acidic (pH 2) conditions.

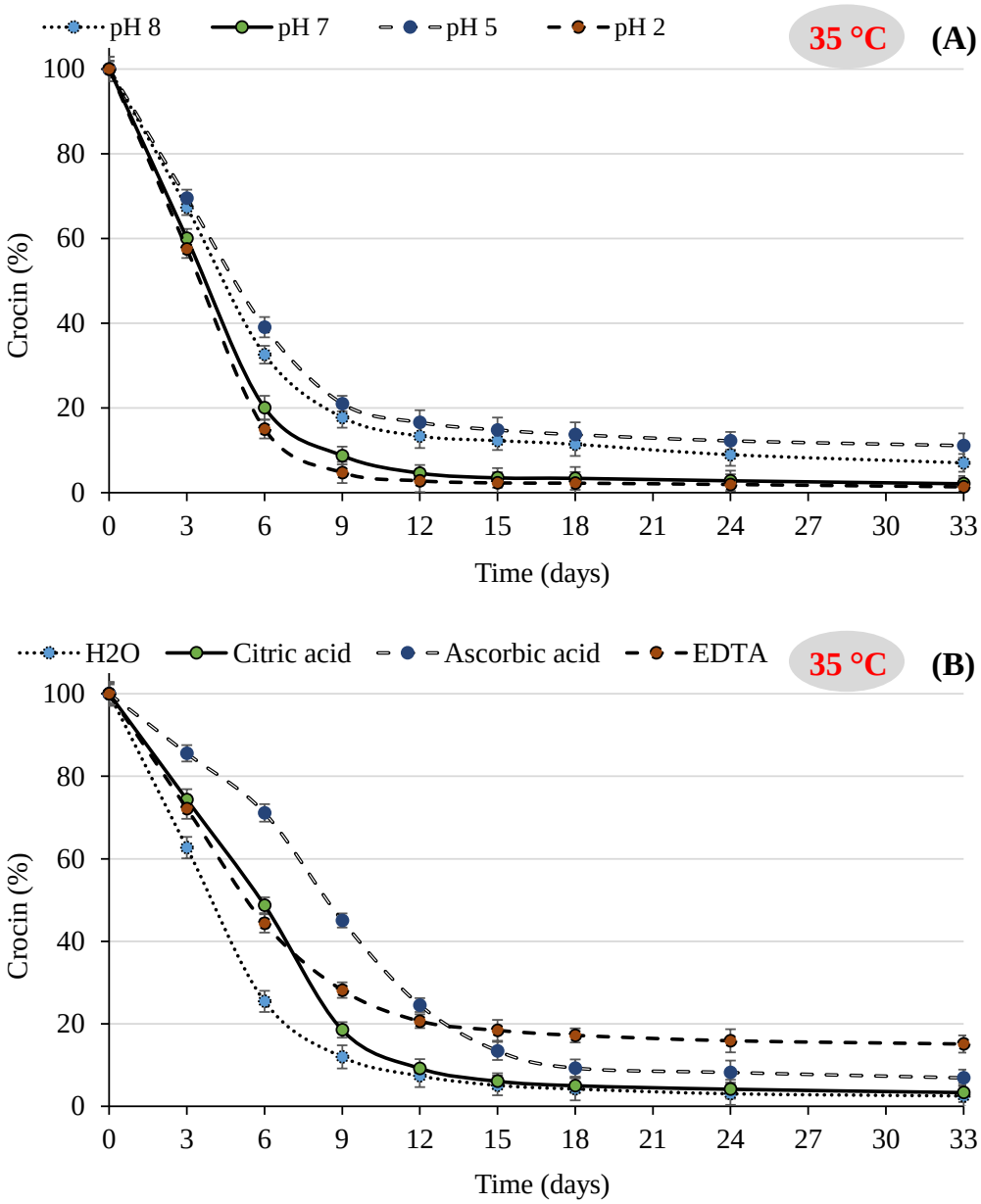

Fig. 4 – Crocin degradation kinetics during storage at 35 °C, effects of pH (A) and preservatives (B).

In the presence of preservatives, the crocin content decreased rapidly to 45% with ascorbic acid, 28% with EDTA, 19% with citric acid, and 12% with distilled water by day 9 (Fig. 4B). It continued to decrease slightly, reaching a level of 3–15% by the end of the storage period. Storage at 35 °C revealed that ascorbic acid does not preserve crocin for a long time, as its preservation potential diminishes over time. EDTA, on the other hand, showed some effectiveness at the end of the storage period. pH 5, followed by pH 8, had weaker effects on crocin but were slightly more effective than distilled water. In contrast, pH 2 and citric acid accelerated crocin degradation compared to the control. Overall, high temperatures should be avoided for crocin storage, even in the presence of preservatives.

Modeling the degradation kinetics of crocin

To study the degradation of crocin in various environments (including the presence of preservatives, pH variation, and water) and to assess the efficiency of the media used, degradation kinetics models were developed. The reaction for crocin degradation was expressed as a function of crocin concentration [*Crocin*], resulting degradation product(s) [*Degradation product*], and reaction constant (*k*) as follows:

$$[Crocin] \xrightarrow{k} [Degradation\ product]$$

The reaction rate can be expressed as a function of the product or reagent. The equation for the rate of crocin disappearance can be written as follows. Since the concentration of crocin decreases over time, the derivative is negative.

$$V = \frac{\mathrm{d}[Crocin]}{\mathrm{d}t} = -k[Crocin]^{\alpha}$$

where V was the reaction rate, d[*Crocin*] was the variation in crocin concentration, dt was the variation in time, k was the rate constant, α was the reaction order.

The reaction order of a reactant is the value of the exponent α, which is not necessarily an integer or equal to the stoichiometric coefficients of the reaction, and can only be determined experimentally. If the rate does not depend on the concentration of one of the reactants, the reaction is zero-order (α = 0); in this case, $V = -k\,[Crocin]^0$ or $V = -k$. If the reaction rate is proportional to the concentration of the reactant, the reaction is first-order: $V = -k\,[Crocin]^1$. In the case where $\alpha = 2$, the reaction is second-order and $V = -k\,[Crocin]^2$.

The order of the rate of crocin degradation kinetics was determined from the results obtained based on the various parameters shown in Table 1.

Table 1

Different reaction rate parameters for orders 0, 1 and 2

Order	Equation of reaction rate	Linearization of equation	Integral of equation	Regression slope	Time of half-life
0	$(\mathrm{d}[C]/\mathrm{d}t) = k$	[C] function of t	$[C]t = -kt + [C]_0$	−	$[C]_0/2k$
1	$(\mathrm{d}[C]/\mathrm{d}t) = k[C]$	Ln[C] function of t	$\mathrm{Ln}([C]t/[C]_0) = -kt$	−	$0.693/k$
2	$(\mathrm{d}[C]/\mathrm{d}t) = k[C]^2$	1/[C] function of t	$(1/[C]t) = -kt + 1/[C]_0$	+	$1/k[C]_0$

By exploring all the data and analyzing the three types of models (data not shown), it was found that crocin degradation followed second-order kinetics, as the coefficients of determination for the inverse of concentration versus time were the highest.

Once the order of crocin degradation, which was second-order, had been determined, the various equations showing the effect of time on crocin percentages in the presence of the different preservatives and pH levels were linearized. The linear equations, coefficients of determination, and half-life times ($t_{1/2}$) for crocin degradation during storage are summarized in Tables 2 (5 °C), 3 (20 °C), and 4 (35 °C). High coefficients of determination (R^2) of the linear models ranging from 0.828 to 0.990 were obtained, demonstrating that crocin degradation kinetics strongly fit a second-order reaction. In contrast, another investigation on microencapsulated crocin found that the degradation kinetics followed first-order behavior;[13] this variation in reaction order was likely due to the effect of encapsulation. A kinetic degradation study of anthocyanin and vitamin C in strawberry soft candy followed a first-order reaction model.[23] In another study concerning anthocyanins, the data indicated that these compounds sometimes followed first-order kinetic behavior, as observed in *Hibiscus rosa-sinensis* and *Clitoria ternatea*, but at other times fit second-order kinetics, as in the case of *Hibiscus Sabdariffa*.[24] The degradation behavior can vary depending on intrinsic factors (such as the type of compound and encapsulation) and extrinsic parameters (such as temperature, medium, presence of other compounds, and oxygen).

Table 2

Linearized equations of crocin degradation kinetics at 5 °C, coefficients of determination, and time half-lives ($t_{1/2}$)

Preservers	Linear regression equation	R^2	$t_{1/2}$ (days)	$t_{1/2}$ (hours)
H_2O	$y = 0.00445\,t + 0.00824$	0.972	2.65	63.49
Citric acid	$y = 0.00133\,t + 0.00749$	0.982	9.43	226.27
Ascorbic acid	$y = 0.00004\,t + 0.0102$	0.929	266.34	6392.16
EDTA	$y = 0.00111\,t + 0.00752$	0.978	11.24	269.70
pH 8	$y = 0.00409\,t + 0.00179$	0.943	4.45	106.73
pH 7	$y = 0.0036\,t + 0.0093$	0.935	2.98	71.42
pH 5	$y = 0.00171\,t + 0.00511$	0.965	8.72	209.18
pH 2	$y = 0.00887\,t + 0.0024$	0.990	2.52	60.58

y: 1/[Crocin] expressed as %, $t_{1/2}$: time of half-life.

Table 3

Linearized equations of crocin degradation kinetics at 20 °C, coefficients of determination, and time half-lives ($t_{1/2}$)

Preservers	Linear regression equation	R^2	$t_{1/2}$ (days)	$t_{1/2}$ (hours)
H_2O	$y = 0.00491\,t + 0.01016$	0.984	2.01	48.15
Citric acid	$y = 0.00237\,t + 0.00313$	0.977	7.12	170.83
Ascorbic acid	$y = 0.00007\,t + 0.01057$	0.828	141.97	3407.29
EDTA	$y = 0.00173\,t + 0.005$	0.975	8.66	207.82
pH 8	$y = 0.0064\,t - 0.00086$	0.978	3.26	78.20
pH 7	$y = 0.00415\,t + 0.01072$	0.909	2.23	53.62
pH 5	$y = 0.00225\,t + 0.00709$	0.967	5.74	137.77
pH 2	$y = 0.00856\,t + 0.01184$	0.958	0.95	22.88

y: 1/[Crocin] expressed as %, $t_{1/2}$: time of half-life.

Table 4

Linearized equations of crocin degradation kinetics at 35 °C, coefficients of determination, and time half-lives ($t_{1/2}$)

Preservers	Linear regression equation	R^2	$t_{1/2}$ (days)	$t_{1/2}$ (hours)
H_2O	$y = 0.00629\,t + 0.00907$	0.978	1.74	41.69
Citric acid	$y = 0.01428\,t - 0.02906$	0.933	3.44	82.46
Ascorbic acid	$y = 0.00868\,t - 0.03215$	0.939	6.01	144.15
EDTA	$y = 0.00409\,t + 0.00264$	0.990	4.25	101.95
pH 8	$y = 0.00725\,t + 0.00389$	0.987	2.22	53.30
pH 7	$y = 0.0148\,t - 0.00127$	0.963	1.44	34.50
pH 5	$y = 0.00446\,t + 0.01066$	0.984	2.10	50.29
pH 2	$y = 0.02188\,t + 0.00421$	0.947	0.72	17.32

y: 1/[Crocin] expressed as %, $t_{1/2}$: time of half-life.

The time of half-life is an important kinetic parameter that represents the time required for 50% of the crocin to degrade. This parameter directly reflects the effectiveness of the different media (preservatives and pH levels) used to preserve crocin. Based on the time of half-life results at 5 °C, the ascorbic acid solution was the most effective for crocin preservation, with a time of half-life of 266.34 days, followed by EDTA (11.24 days) and citric acid (9.43 days). However, the buffers with different pH levels (2, 7, and 8) and the distilled water exhibited shorter times of half-life, ranging from 2.52 to 8.72 days.

Based on the crocin degradation kinetics parameters at 20 °C, as shown in Table 3, crocin in ascorbic acid solution had the longest $t_{1/2}$ (142 days) compared to other preservatives and pH levels. EDTA and citric acid had relatively short half-lives of 8.66 and 7.12 days, respectively. pH has a strong influence on crocin stability. Indeed, storage at pH 5 demonstrated the highest crocin stability compared with other pH levels, followed by pH 8, then pH 7. The latter indicated a storage capacity similar to that of distilled water due to the proximity of their pH values. However, pH 2 showed the shortest half-life. It was also observed that as the temperature increased from 5 to 20 °C, crocin degradation accelerated, causing reductions in half-lives by 23 to 26%.

The storage of crocin at 35 °C showed a considerable drop in half-life times (Table 4). Ascorbic acid remained the best preservative with a

time of half-life of 6.01 days, followed by EDTA (4.25 days) and citric acid (3.44 days). Crocin preservation across various pH values (2, 5, 7, 8) and distilled water resulted in very low $t_{1/2}$ values, ranging from 0.72 to 2.22 days. According to Tsimidou and Tsatsaroni,[14] the aqueous pigments of saffron were found to be sensitive to storage. Indeed, the pigments degraded significantly as the pH decreased, with the lowest half-life recorded for pH 3 (16 h), followed by pH 5 (99 h), and the highest at pH 7 (120 h).

According to Tables 2, 3, and 4, crocin half-lives were significantly influenced by heating; increasing temperature led to a decrease in half-lives. The use of 35 °C caused a dramatic reduction in crocin preservation, resulting in a 46 and 63% decrease in $t_{1/2}$ compared to 20 °C and 5 °C, respectively.

The influence of preservatives and different pH levels on crocin storage at various temperatures (5, 20, and 35 °C) compared to the control (H_2O) revealed that the three preservatives (citric acid, ascorbic acid, and EDTA) had a highly significant positive impact on crocin preservation, regardless of the storage temperature. The use of pH 5, 7, and 8 at 5 °C and 20 °C also significantly improved crocin preservation. However, the use of pH 2 at all temperatures, as well as pH 7 at 35 °C, had a negative effect ($p < 0.05$) on crocin preservation.

In a study examining the effect of temperature (60, 70, 80, and 90 °C) and pH (2, 4.4, and 6) on the kinetics of crocin degradation, it was found that encapsulated crocin decreased with increasing temperature, particularly at 80 °C and 90 °C.[13] The authors also found that increasing the pH of the medium reduced the impact of crocin degradation. Similarly, the half-life of saffron pigments stored at 40 °C was reduced by 7 to 16 times compared to storage at 4 °C;[14] further supporting the importance of temperature during storage. In another stability study, it was revealed that crocin had weak stability under high temperatures, light exposure, and in strong alkaline and acidic media. The authors recommended a pH of 6 and the use of an appropriate amount of ascorbic acid to improve crocin stability.[12]

Crocin is the main compound in saffron, comprising up to 30% of its mass. Chemically, it is a carotenoid formed by a long carbon chain with numerous conjugated double bonds and glycosylated at each of its two extremities by gentiobiose, as seen in alpha crocin (the main form of crocin). This structure, with multiple unsaturations, makes crocin highly sensitive to heat, pH, oxygen, and storage duration. Crocin degradation occurs through various mechanisms and chemical reactions, including autooxidation and isomerization.[12,25,26]

MATERIALS AND METHODS

Plant material

Crocus sativus flowers were harvested from September to October at the research farm in the department of Iben Badis, located 40 km from the capital of the Constantin subdivision (Algeria). Saffron stigmas were manually removed from the flowers and air-dried for ten days at 20–25 °C to obtain dry saffron. Fifteen grams of this dry saffron were transported to the Laboratory of Applied Biochemistry (University of Bejaia), where they were ground using a mortar and pestle, then passed through a sieve with a 500 µm diameter mesh. The resulting powder was placed in a hermetically sealed stainless steel container and stored in the freezer at -20 °C.

Preparation of extracts

Maceration was used to prepare the saffron extract. Saffron powder was placed in a beaker with a solvent (water or buffers) and stirred at a speed of 400 rpm for 20 minutes using a magnetic stirrer, according to an optimized procedure (data not shown).

To confirm the authenticity of saffron, the water extract was subjected to spectrophotometric analysis by recording the optical density in the ultraviolet-visible (UV-vis) range between 200 and 700 nm.[19] To assess the quality of the saffron used, several parameters were measured, of which only the crocin level was reported due to its relevance to this study. The crocin concentration was expressed as the extinction absorbance at the maximum wavelength (440 nm) of a 1 g/100 ml solution of the test sample, using a 1 cm quartz cell, following Eq. 1.

$$E_{1cm}^{1\%}(\lambda_{440nm}) = \frac{A \times 200}{m(100 - Wmv)} \dots (Eq.1)$$

where $E_{1cm}^{1\%}(\lambda_{440nm})$ – the specific extinction; A – the absorbance at 440 nm; m – the used sample mass (g); Wmv – the mass fraction of moisture and volatile matter content of the sample, which was determined to be 8%, 200 – the dilution factor.

Preparation of buffers and preservatives

To test the stability of crocin in different environments, various media were prepared. Sodium phosphate monobasic acid (H_2NaO_4P) and its conjugate base, sodium phosphate dibasic (HNa_2O_4P), were separately prepared at a molarity of 0.2 M. These two solutions were mixed in variable volumes to achieve different pH levels, and the pH of the solutions was adjusted using a pH meter. The final pH levels of the prepared solutions were 2, 5, 7, and 8.

Three preservatives (ethylenediaminetetraacetic acid (EDTA), citric acid, and ascorbic acid) were also tested for crocin stability. The preservatives were prepared in a pH 7 phosphate buffer solution at a final concentration of 1 mg/ml. To avoid the influence of the acidic nature of the preservatives, the solutions were adjusted to pH 7 using either basic or acidic sodium phosphate solutions, monitored with a pH meter.

Preparation of crocin in different media and storage

The procedure involved mixing equal volumes of saffron extract (prepared in water for control or in phosphate buffer) and preservative solutions (1 mg/ml in buffer). The prepared solutions included citric acid, ascorbic acid, EDTA, neutral pH phosphate buffer (pH 7), acidic pH buffers (pH 2 and 5), basic buffer (pH 8), and distilled water (control).

After measuring the absorbance of the initial solutions, all preparations were stored at different temperatures: refrigeration (5 °C), room temperature (20 °C), and high temperature (35 °C). Analyses were performed at various time points (0, 6, 8, 9, 12, 16, 23, and 33 days). Crocin content was monitored by measuring absorbance at 440 nm, and results were expressed as the percentage of crocin degradation relative to the initial level (Eq. 2).

$$Crocin(\%) = \frac{Abs_{t0} - Abs_t}{Abs_{t0}} \dots (Eq.\,2)$$

There, Abs_{t0} – the initial absorbance of crocin, Abs_t – the absorbance of crocin at time *t*.

Statistical analysis

A descriptive analysis of the results was conducted using Microsoft Office Excel 2013 to determine the means and standard deviations of the three replicates, as well as to generate graphs of the degradation kinetics and their corresponding equations. The analysis of variance (MANOVA, LSD test: Least Significant Difference) was applied using STATISTICA 5.5 software to identify significant differences between the preparations based on temperature, medium, and storage time. The significance level for the data was set at $p < 0.05$.

CONCLUSION

The kinetics of crocin degradation extracted from saffron were investigated. It was demonstrated that crocin degradation was significantly influenced by the conservation medium and temperature. The optimal pH for crocin preservation was pH 5, while very acidic environments should be avoided. The use of preservatives had a positive effect on crocin stability. Kinetic modeling revealed that crocin degradation followed a second-order reaction rate. The calculation of the half-life showed that ascorbic acid provided the best crocin retention, particularly at 5 °C and 20 °C, with half-lives of 266.34 and 141.97 days, respectively. EDTA and citric acid also protected crocin, but with lower efficiency. Therefore, to best preserve crocin in an aqueous medium, it is recommended to use a slightly acidic environment in the presence of ascorbic acid, combined with low-temperature storage conditions.

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