CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journalhomepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

OPTIMIZING THE AQUEOUS EXTRACTION OF CROCIN FROM SAFFRON AND MODELING THE KINETICS OF ITS DEGRADATION DURING STORAGE AND HEAT TREATMENT

Mostapha BACHIR-BEY[1]✉, Yazid KHALED KHODJA[2], Aicha BENBOURICHE[3], Malika CHOUALI[1], Salima DJEBELLANNOUAR[1], Yassine BENCHIKH[4], Djamel Edine KATI[1]

[1] Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria

[2] Laboratory of Exploration and Valorization of Steppe Ecosystems, Faculty of Natural Sciences and Life, University of Djelfa, PO Box 3117, Djelfa 17000, Algeria

Algeria[3] Laboratoire de Biochimie Biophysique Biomathématique et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria

[4] Laboratory of Biotechnology and Food Quality, Institute of Nutrition, Food and Agro-Food Technologies, University of Constantine 1, Constantine, Algeria

✉*mostapha.bachirbey@univ-bejaia.dz*

https://doi.org/10.34302/crpjfst/2024.16.2.11

Article history:
Received: September 1st, 2023
Accepted: April 25th, 2024

Keywords:
Crocin;
Extraction method;
Degradation kinetic;
Storage;
Heat treatment.

ABSTRACT

This study aims to optimize the extraction of crocin from saffron through various methods and energy levels, and to investigate its stability during storage and heat treatment. Three extraction techniques−maceration, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE)−were evaluated at different energy levels to determine the most efficient method. The resulting extracts were then subjected to stability tests under varying storage temperatures (-12 to 35 °C) and heat treatment conditions (100 to 200 °C). Our findings indicate that MAE and UAE, particularly at higher energy levels for 5 minutes, yielded the most efficient extraction, with an average coloring strength of 265. During storage, crocin degradation followed a zero-order kinetic model, with the degradation rate increasing with higher storage temperatures. The shortest half-life was observed at freezing temperature (100 hours), while the shortest half-life at 35 °C was less than 10 hours. Similarly, during heat treatment, crocin degradation followed a zero-order kinetic model at 100 and 150 °C, with half-lives of 260 and 74 minutes respectively. At 200 °C, the degradation kinetics shifted to first order, with a half-life of 20 minutes. Our results suggest that MAE and UAE at high energy levels are optimal for crocin extraction, and highlight the impact of temperature on crocin stability during storage and heat treatment.

1.Introduction

Crocus sativus L. is a highly prized species within the Iridaceae family, renowned for producing saffron, the world's most expensive spice. Saffron is derived from the carefully harvested stigmas of the flowers, which are meticulously dried to yield the coveted red filaments. With a history dating back to ancient times, saffron has been used not only as a spice but also as a medicinal plant and a natural coloring and flavoring agent in culinary applications. Extensive research has highlighted the myriad health benefits of saffron, including its anti-inflammatory, antioxidant, anti-carcinogenic, anti-

proliferative, and cardioprotective properties. Moreover, saffron exhibits neuroprotective qualities and has been associated with alleviating depression and stress (Dhiman and Kharkwal, 2020).

The therapeutic attributes of saffron stem from three primary compounds: crocin, picrocrocin, and safranal, which impart its distinctive coloring, bitter taste, and aromatic qualities, respectively. Beyond its traditional role as a coloring and flavoring agent in home cooking, saffron finds applications in various industries, including food, pharmaceuticals, cosmetics, and perfumery (Karbasi and Zandi Dareh Gharibi, 2022; Mzabri *et al.*, 2019). Due to its high value, saffron is incorporated into a wide range of dietary formulations to enhance the nutritional profile and sensory appeal of various functional food products. These include dairy items such as milk, cheese, and yogurt, as well as pasta, jams, baked goods, cookies, chocolates, and beverages like herbal teas and bitter drinks (Lage and Cantrell, 2009).

Crocin, also known as crocins, comprises a group of water-soluble compounds formed through the esterification of a fat-soluble dicarboxylic acid carotenoid known as crocetin with one or two molecules of glucose and/or gentiobiose (El Khoudri *et al.*, 2021; Poma *et al.*, 2012). This yellow coloring compound is highly concentrated in saffron, comprising up to 37% of its total weight (Lage and Cantrell, 2009).

The extraction of crocin, along with other bioactive compounds present in saffron, employs various methods and extraction durations. Originally, a standardized 24-hour extraction period was employed, utilizing the maceration technique as prescribed by (ISO.3632, 1980). This method has been used in previous studies for the extraction of secondary compounds from saffron (Esfanjani *et al.*, 2015; Oukhrib *et al.*, 2015). Other extended extraction durations, such as overnight (Escribano *et al.*, 1996) or 5 hours (Najafi *et al.*, 2021), have been investigated. However, these prolonged periods were subsequently reduced to one hour after observing the degradation of saffron's bioactive compounds (Orfanou and Tsimidou, 1996). The one-hour extraction method has been widely adopted by several authors (Masoumi *et al.*, 2021; Moradi *et al.*, 2022). Additionally, sonication at various durations (1, 3, 5, and 10 minutes) has been explored for extraction purposes (Kadkhodaee and Hemmati-Kakhki, 2006). Hence, these variations in extraction methods and conditions may contribute to differences in results.

Once crocin is extracted from its original stigma tissues, this highly water-soluble compound becomes remarkably susceptible to degradation, with various factors accelerating its decomposition. Studies on crocin stability have shown that variations in medium pH, whether acidic or basic, can induce its decomposition. Temperature is another crucial parameter, as it can facilitate oxidation processes. Furthermore, the presence of oxygen and exposure to light also contribute to crocin degradation (Karasu *et al.*, 2019; Tsimidou and Tsatsaroni, 1993).

This investigation seeks to establish the optimal extraction conditions using three methods set at varying energy levels: maceration (at 0, 200, 600, and 1000 rpm), microwave-assisted extraction (at powers of 100, 300, and 500 W), and ultrasound-assisted extraction (using amplitudes of 20, 60, and 100%). Subsequently, the stability of the extract was evaluated through a kinetics modeling study conducted during storage at different temperatures (−12, 4, 22, and 35 °C) and heat treatment at 100, 150, and 200 °C.

2. Materials and methods

2.1. Saffron sampling

Saffron flowers (*Crocus sativus*) were procured from the Safran Tariki Association after their harvest at the Constantine research farm in Algeria. The stigmas were manually extracted from the flowers and subsequently air-dried in the shade at an average temperature of 23 °C for 10 days. Upon drying, the samples were transported to the Laboratory of Applied Biochemistry (LBA) at the University of

Bajaia. The stigmas were then ground using a mortar and sieved, with particles smaller than 250 nm retained for subsequent analyses.

2.2. Optimization of crocin extraction with different methods

To investigate the optimization of crocin extraction, three distinct methods were employed: one conventional method involving maceration, and two non-conventional methods, namely microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). The extraction process entailed mixing 10 mg of saffron powder with 50 ml of distilled water. Extractions were carried out at various time intervals (0, 0.5, 1.5, 3, 5, 10, 15, and 20 minutes). The resultant extracts were recovered via centrifugation at 5000 rpm for 5 minutes using a Növe NF 200 centrifuge. Crocin content was determined by measuring the absorbance at 438 nm using a spectrophotometer (UvLine 940, Secomam, France). Crocin results were expressed as the coloring strength of the solutions using a 1-cm quartz cell, as per the following equation (Eq. 1).

$$E_{1cm}^{1\%}(\lambda_{440nm}) = \frac{Abs \times 200}{\mathrm{W}(100 - \mathrm{Wmv})} \qquad (1)$$

Where, $E_{1cm}^{1\%}(\lambda_{440nm})$ is the specific extinction, Abs is the absorbance at 438 nm, W is the used sample weight (g), Wmv is the weight of moisture and volatiles in the sample (8%), 200 was the dilution factor.

For each method, different levels were tested. Maceration was conducted using a magnetic stirrer (Multistirrer Digital 15, VELP Scientifica, Italy) at three rotation speeds: 200, 600, and 1000 rpm. Additionally, extraction without agitation (0 rpm) was evaluated. The microwave (Maxipower, China) was operated at three power levels: 100, 300, and 500 W. The sonicator (Sonics Vibra Cell VCX 130, Sonics, USA) was equipped with a probe, and three amplitudes were tested: 20, 50, and 100%.

2.3. Effect of storage temperature on the stability of crocin

To assess the stability of the aqueous crocin extract during storage, various temperatures were examined. Crocin extract obtained under optimal conditions was divided into 20 ml aliquots and sealed in test tubes to prevent water evaporation. These test tubes were then placed in different environments: a freezer at −12 °C, a refrigerator at 4 °C, and ovens set to 20 °C and 35 °C. Crocin levels were measured at different time intervals throughout the storage period (0, 2, 5, 10, 15, and 20 days). The results were expressed as percentages relative to the initial crocin levels.

2.4. Effect of heating on the stability of crocin

To evaluate the thermal stability of the crocin aqueous extract, various temperatures were applied during heat treatment. The optimized crocin extract was subjected to different temperatures for varying durations. Specifically, 20 ml of the aqueous extract was placed in test tubes and exposed to temperatures of 100, 150, and 200 °C for durations ranging from 1 to 90 minutes. Crocin levels were quantified as percentages relative to the absorbance measured at the initial time point (t = 0).

2.5. Degradation kinetics

The evaluation of three reaction models (orders 0, 1, and 2) for predicting crocin degradation indicated that only the zero and first-order models effectively represented the degradation rate (Eq. 2 and Eq. 3).

$$[\mathrm{C}]_t = -kt + [\mathrm{C}]_0 \qquad (2)$$

$$\mathrm{Ln}\left(\frac{[\mathrm{C}]_t}{[\mathrm{C}]_0}\right) = -\mathrm{kt}. \qquad (3)$$

Where, $[C]_t$ is the concentration of crocin at the time *t*, $[C]_0$ is the initial concentration of crocin, t is time, and k is constant rate of degradation.

The half-life time ($t½$) for the zero-order reaction is calculated as $[C]_0/2k$, while for the first-order reaction, it is calculated as $Ln(2)/k$.

2.6. Statistical analysis

The data underwent processing using Microsoft Office Excel 2013 for calculating the means and standard deviations of the triplicates, graph plotting, and kinetic modeling. The analysis of variance (ANOVA) along with the HSD test was conducted using Statistica software (StatSoft, Inc., version 7.0.61.0).

3. Results and discussion

3.1. Effect of extraction method and time on crocin yield

The extraction kinetics of crocin from saffron powder varied according to the extraction method, as illustrated in Figure 1. The initial extraction, occurring immediately upon contact of the solvent with the powder (t = 0 min), yielded an average value of 74.64 across all the three methods. This initial phase represents the rapid dissolution of crocin molecules present on or near the surface of the powder particles, requiring minimal extraction time. In the case of maceration, crocin concentration increased quickly at all the three rotation speeds (200, 600, and 1000 rpm) during the initial minutes of extraction, followed by a gradual decrease in extraction rate until stabilization. Statistical analysis revealed that maximum crocin extraction values were achieved after 10 minutes at all rotation levels, reaching a value of 205.80. However, extraction without agitation showed a continuous increase in crocin concentration throughout the extraction period, albeit resulting in a lower value of 178.06 at the end of extraction compared to agitation-assisted methods. Stirring facilitated the diffusion of crocin particles from the plant matrix into the solvent, explaining the slower extraction rate observed in the absence of agitation. While the speed of agitation did not significantly affect the crocin extraction rate, minimal agitation proved necessary for effective extraction.

In a study investigating the optimization of bioactive compound extraction from apricot kernel shells, it was observed that the extraction rate increased with the stirring speed from 300–700 rpm to 1100 rpm, after which it stabilized at 1500 rpm (Teffane *et al.*, 2022). Similarly, research on the extraction of polyphenols from mango seed kernels demonstrated that higher agitation speeds enhanced extraction, although speeds of 400–450 rpm resulted in a detrimental effect (Anta *et al.*, 2020). It's noteworthy that elevated stirring speeds do not invariably translate to improved yields; in fact, they can sometimes lead to reduced yields because of compound degradation caused by oxygen dissolution, which triggers the oxidation of already extracted molecules, particularly during prolonged extractions and at high temperatures. Moreover, exceeding a certain speed threshold represents unnecessary energy consumption.

The kinetics of Microwave-Assisted Extraction (MAE), depicted in Figure 1, illustrated an escalating crocin recovery rate over time during the initial extraction phase across all the tested power levels. Crocin concentration stabilized after 5 minutes at 500 W, reaching 257.61. However, extraction stability was achieved after 10 minutes for 300 W and 100 W. While 300 W attained a similar extraction level as 500 W, it necessitated twice the duration. Statistical analysis encompassing the entire extraction period identified 500 W as the optimal power for crocin extraction, followed by 300 W and then 100 W. Nonetheless, during the latter phase of extraction (post-10 min), no discernible difference in crocin yield was observed between 300 W and 500 W. Consequently, for energy efficiency considerations, a microwave power of 300 W was deemed preferable for saffron crocin extraction, notwithstanding the longer extraction duration.

Numerous studies have highlighted the substantial impact of microwave power on the extraction rate of target molecules. For instance, in the recovery of carotenoid pigments from *Citrus clementina* peels, the extraction rate surged with increasing power until reaching 560 W; beyond this threshold, the yield notably decreased (Kadi *et al.*, 2022).

Similarly, the extraction efficiency of antioxidant compounds from *Opuntia ficus-indica* seeds exhibited an increase in yield between 100 and 500 W, with no observed effect beyond this range (Boudjouan *et al.*, 2021). Conversely, the extraction of phenolic compounds from *Bellis perennis* flowers revealed that escalating microwave power led to a significant reduction in yield and degradation of the compounds (Bouallag *et al.*, 2022).

The use of reasonable microwave levels improved extraction yields. Indeed, during microwave extraction, the waves generated penetrate the particles and interact with polar molecules having a positive dipole moment, in particular water, to induce magnetic interactions and rotational movements, which cause molecular friction and an increase in heat at the center of the particle. The difference in heat between the variable levels of the granule creates a pressure gradient, which causes a draining force of the compounds from the inside of the particle to the outside (Khaled Khodja *et al.*, 2020). However, the choice of microwave power for the extraction of the desired compounds must be made with care, as compounds can be decomposed by the effects of irradiation and increased temperature, which accelerate chemical reactions, especially with the use of extended extraction times and in the presence of oxygen (Bachir-bey *et al.*, 2013; Ismail-Suhaimy *et al.*, 2021).

The ultrasound-assisted extraction results for crocin are depicted in Figure 1. The extraction process exhibited two distinct phases. Initially, there was a rapid acceleration phase lasting from 0 to 5 minutes for 100% amplitude and from 0 to 10 minutes for 60% and 20% amplitudes. Subsequently, a stationary phase ensued until crocin extraction reached stability. Statistical analysis encompassing the entire extraction period revealed significantly different efficiencies among the three amplitudes, with 100% exhibiting the highest efficiency, followed by 60%, and then 20%. However, during the stationary phase, the last two amplitudes exhibited comparable crocin yields, averaging around 243.57. Consequently, the 100% amplitude, with a 10% higher efficiency compared to the other two, is recommended for optimal crocin extraction from saffron.

The findings from the ultrasound-assisted extraction (UAE) align with previous research by Kadkhodaee and Hemmati-Kakhki (2006), demonstrating that increasing the sonication amplitude enhances the extraction rate while significantly reducing the required time. The authors concluded that an amplitude of 100% and a sonication duration of 10 minutes provided optimal conditions for crocin extraction, yielding an estimated 239.3.

The efficacy of ultrasound-assisted extraction (UAE) in extracting target compounds has been highlighted in various studies. For instance, in the extraction of phenolic compounds from carob and date pulps, it was observed that yields increased with the amplitude up to 85%; however, concentrations decreased beyond that threshold (Benkerrou *et al.*, 2018a; Saci *et al.*, 2018). Similarly, optimal amplitudes of 74% and 65% were identified during the extraction of bioactive compounds from other plant resources using ultrasound extractors (Benkerrou *et al.*, 2018; Zemouri-Alioui *et al.*, 2018).

The efficiency of extraction is significantly enhanced with the amplification of mechanical action resulting from increased ultrasonic amplitude. This phenomenon triggers cavitation bubbles, which collapse on the particle surface, releasing substantial energy that ruptures cell tissue and facilitates compound release. However, at certain amplitudes, extraction efficiency may decline due to elevated temperatures and the production of free radicals through sonochemical reactions, leading to compound oxidation. Additionally, cavitation efficiency may decrease as bubbles are destroyed upon formation (Benkerrou *et al.*, 2018; Tiwari *et al.*, 2010; Zemouri-Alioui *et al.*, 2018).

Statistical analysis revealed that crocin extraction stabilized between 5 and 10 minutes

for all methods except for maceration without agitation (Table 1). Initially, extraction commenced with crocin molecules diffusing from the matrix, driven by concentration gradients between particles and solvent. However, this diffusion slowed over time until equilibrium was reached between the interior and exterior of the powder particles. Molecules nearer the surface were more accessible to the solvent compared to those in deeper layers, resulting in a gradual slowdown of extraction until stability was attained. This equilibrium could be influenced by the extraction method and energy level employed.

Notably, the most efficient crocin extraction was achieved with 100% ultrasound amplitude, as well as microwave powers of 300 W and 500 W. Conversely, maceration proved to be the least efficient method, regardless of agitation level. Maceration without agitation and microwave extraction at 100 W exhibited a significant loss of approximately one-third of the expected crocin amount and should thus be avoided.

The selection of an appropriate extraction duration is pivotal for achieving optimal results. Traditionally, a prolonged extraction period of 24 hours was advocated in the ISO 3632 standard ISO.3632 (1980). However, this extended timeframe often resulted in a notable loss of color intensity due to the rapid degradation of saffron pigments. Recognizing this, subsequent revisions in ISO 3632-2010 (ISO.3632-2, 2010) reduced the recommended extraction time to just 1 hour. Supporting this adjustment, a study by (Orfanou and Tsimidou, 1996) demonstrated a 15% increase in crocin yield when utilizing a 1-hour extraction compared to the conventional 24-hour period. In line with these findings, our investigation determined that a duration of 5 to 10 minutes proved optimal for achieving maximum crocin extraction efficiency.

This study underscores the significant influence of extraction method, energy level (such as agitation, power, or amplitude), and extraction duration on the efficiency of crocin extraction from saffron.

3.2. Effect of storage temperature on crocin stability

Saffron is not only valued for its therapeutic compounds but also cherished for its vibrant yellow hue attributed to crocin. Given its widespread use in functional foods, products containing saffron are often subjected to various storage conditions and temperatures, which may accelerate the degradation of bioactive compounds. Thus, this section delves into the kinetics of crocin degradation during storage at different temperatures.

To investigate, a crocin extract obtained under optimal extraction conditions (UAE at 100% for 5 minutes) was divided into four aliquots and stored at varying temperatures (-20, 4, 22, and 35 °C) to simulate freezing, refrigeration, room temperature, and warm conditions, respectively. Crocin levels were measured at different intervals (0, 2, 5, 10, and 20 days), and the results were expressed as percentages (Figure 2).

Analysis of the extract over 20 days of storage revealed significant crocin degradation across different temperatures. Notably, degradation intensified with higher temperatures. At freezing temperatures (-12 °C), crocin degradation was minimal, with only a 10% loss observed at the end of the storage period. Preservation of crocin was satisfactory at 4 °C, albeit with gradual degradation over time. Conversely, room temperature (22 °C) led to rapid crocin degradation, with an 80% reduction by the end of the storage period. Lastly, storage at 35 °C resulted in swift crocin degradation, nearly depleting its levels entirely within 20 days.

Similar findings were noted in studies investigating the stability of saffron aqueous extract, wherein crocin degradation escalated with rising temperatures (Tsimidou and Tsatsaroni, 1993). Notably, crocin degradation was observed even during the storage of saffron stigma and powder (Atyane *et al.*, 2017; Chaouqi *et al.*, 2018).

To enhance comprehension of crocin degradation kinetics during storage, modeling of the degradation reaction rate was

undertaken. Statistical analysis revealed that this rate conforms to a zero-order reaction, as described by the following model:

$$V = -k[C]^0 \text{ or } V = -k \qquad (4)$$

Where V is the reaction rate, $-k$ is the reaction constant, the minus sign indicates the decrease in crocin, and the number in superscript indicates the order of the degradation rate.

The concentration of crocinat a time "t" ($[C]_t$) is given by the following equation:

$$[C]_t = -kt + [C]_0 \qquad (5)$$

Where $[C]_0$ is the initial concentration of crocin.

The parameters used for modeling crocin degradation kinetics are presented in Table 2. The negative sign signifies crocin depletion during storage, with degradation becoming more pronounced as the value of k escalates. A minimal degradation rate was observed at −12 °C, whereas degradation at 4 °C was fourfold that of freezing. Elevating the temperature from 4 to 22 °C and from 22 to 35 °C increased the degradation rate by 150%. Moreover, the high coefficients of determination (R^2), ranging from 0.976 to 0.996, indicate a strong agreement between the experimental results and the calculated values by the models. The low probability values (P-values) suggest the significant fit of the four models, indicating that crocin degradation conforms to the zero-order reaction rate.

To determine the shelf life of crocin at various temperatures, the half-lives were computed for the zero-order reaction rate using the formula: $t_{1/2} = [C]_0/2k$. As per the results depicted in Table 2, the longest half-life (100 days) was observed at freezing temperature, followed by refrigeration (4 °C), room temperature, and finally 35 °C, where the estimated half-life was less than 10 days.

In a study examining the stability of microencapsulated crocin in aqueous environments, crocin degradation followed first-order kinetics, with increasing k values observed at higher storage temperatures (Karasu *et al.*, 2019). The researchers reported a half-life of 52 hours for crocin microencapsulated at pH 6 when stored at 60 °C. However, this duration decreased notably at higher temperatures of 70, 80, and 90 °C, with corresponding half-lives of 27, 17, and 11 hours, respectively. Another investigation showed that the aqueous extract of crocin at pH 7 experienced degradation during storage that was 20 times more pronounced than at 40 °C (Tsimidou and Tsatsaroni, 1993).

The degradation of bioactive compounds can fellow different pathways. The degradation kinetics of ascorbic acid were investigated in an intermediate moisture model food system, varying with water activity (0.69-0.90) and temperature (61-105°C). The degradation of ascorbic acid in each scenario adhered to a zero-order kinetic model (Laing *et al.*, 1978). Whereas, the study on the stability of anthocyanin extracts from mangos teen peel under varying storage temperatures revealed that the extracts exhibited also first-order kinetics, with the highest half-life observed at 5°C (4006 hours), followed by 28°C (370 hours), 40°C (125 hours), and 50°C (93 hours) (Chisté *et al.*, 2010).

The findings from the storage experiments underscore the importance of storing food products containing saffron or saffron extracts at lower temperatures to maintain the coloring power of crocin over extended periods.

3.3. Heating effect on crocin stability

During food processing or culinary preparation, heat treatments are frequently employed to eliminate microorganisms, inhibit enzymes, and develop desired organoleptic characteristics. Consequently, this section addresses the effect of heating on aqueous crocin extract to study its impact on crocin degradation. Three temperatures were tested: 100, 150, and 200 °C, to simulate cooking in an aqueous medium at atmospheric pressure, under pressure, and in the oven, respectively. The remaining crocin content was measured as a percentage at various time intervals over a

total duration of 90 minutes, corresponding to the maximum cooking time.

The results of the study on crocin thermal degradation kinetics in aqueous extracts are depicted in Figure 3. Minimal degradation was observed during the heating treatment at 100 °C, with only one-fifth degraded during the 90-minute heating period. At 150 °C, crocin degradation occurred gradually, while at 200 °C, an accelerated degradation was observed initially, followed by a slowdown over time.

To comprehend crocin degradation behavior during heating, a kinetic study was conducted. Modeling of the three kinetics revealed that crocin degradation at temperatures of 100 and 150 °C followed zero-order reaction kinetics, indicating a linear decrease in concentration over time, similar to storage conditions as described previously.

However, treatment at 200 °C fitted the first-order degradation kinetics according to the equation: $V = -k\,[C]^1$. The half-life time ($t_{1/2}$) is equal to 0.693/k. The kinetic parameters for the three temperatures are summarized in Table 3. Based on the R^2 and P-values, the models of crocin degradation kinetics showed a strong fit with the established kinetic orders. Moreover, at 100 °C, the longest half-life was observed, followed by 150 °C, while 200 °C revealed very rapid crocin degradation.

It has also been observed that the degradation of aqueous crocin extracts increases with rising temperatures, from 5 to 70 °C (Sánchez *et al.*, 2008). Since crocin is a carotenoid derivative, heat treatment induces its isomerization from *trans* to *cis* form, oxidation, and degradation (Atencio *et al.*, 2022; Meléndez-Martínez *et al.*, 2023). Therefore, the high temperatures applied during various food preparations lead to crocin degradation; consequently, incorporating saffron filaments or infusions in food preparations should occur towards the end of heat treatments, approximately five to ten minutes before processing is completed.

The kinetics of color degradation in pineapple puree were examined during heat treatment within the range of 70-110 °C by Chutintrasri and Noomhorm (2007). The alterations in L and b values conformed closely to the first-order kinetic model, whereas a value and Browning index adhered to the zero-order kinetic model. The order of reaction can even vary for the same compounds, depending on the medium. For instance, the decolorization reaction of carotenoids, including β-carotene, di-esterified capsanthin, and capsanthin, was examined. It was observed that reactions occurring in an anhydrous medium follow zero-order kinetics, whereas those occurring in an aqueous medium adhere to first-order kinetics (Minguez-Mosquera and Jaren-Galan, 1995).

Table 1. Crocin amounts at optimal times according to the extraction method and energy level

Extraction method	Energy level	Crocin	Required time for extraction (min)
Maceration	0 rpm	178.06 ± 5.12 ^{e}	20
	200 rpm	208.70 ± 4.09 ^{d}	10
	600 rpm	204.57 ± 7.16 ^{d}	10
	1000 rpm	204.13 ± 7.16 ^{d}	10
MAE	100 W	168.04 ± 8.18 ^{e}	10
	300 W	264.78 ± 9.21^{a}	10
	500 W	257.61 ± 4.09 ^{ab}	5
UAE	20%	238.04 ± 7.16 ^{c}	10
	60%	242.09 ± 6.14 ^{bc}	10
	100%	270.65 ± 5.74 ^{a}	5

The results of crocin with different letters are statistically different (ANOVA, HSD test, $p < 0.05$, a>b>c>d>e).

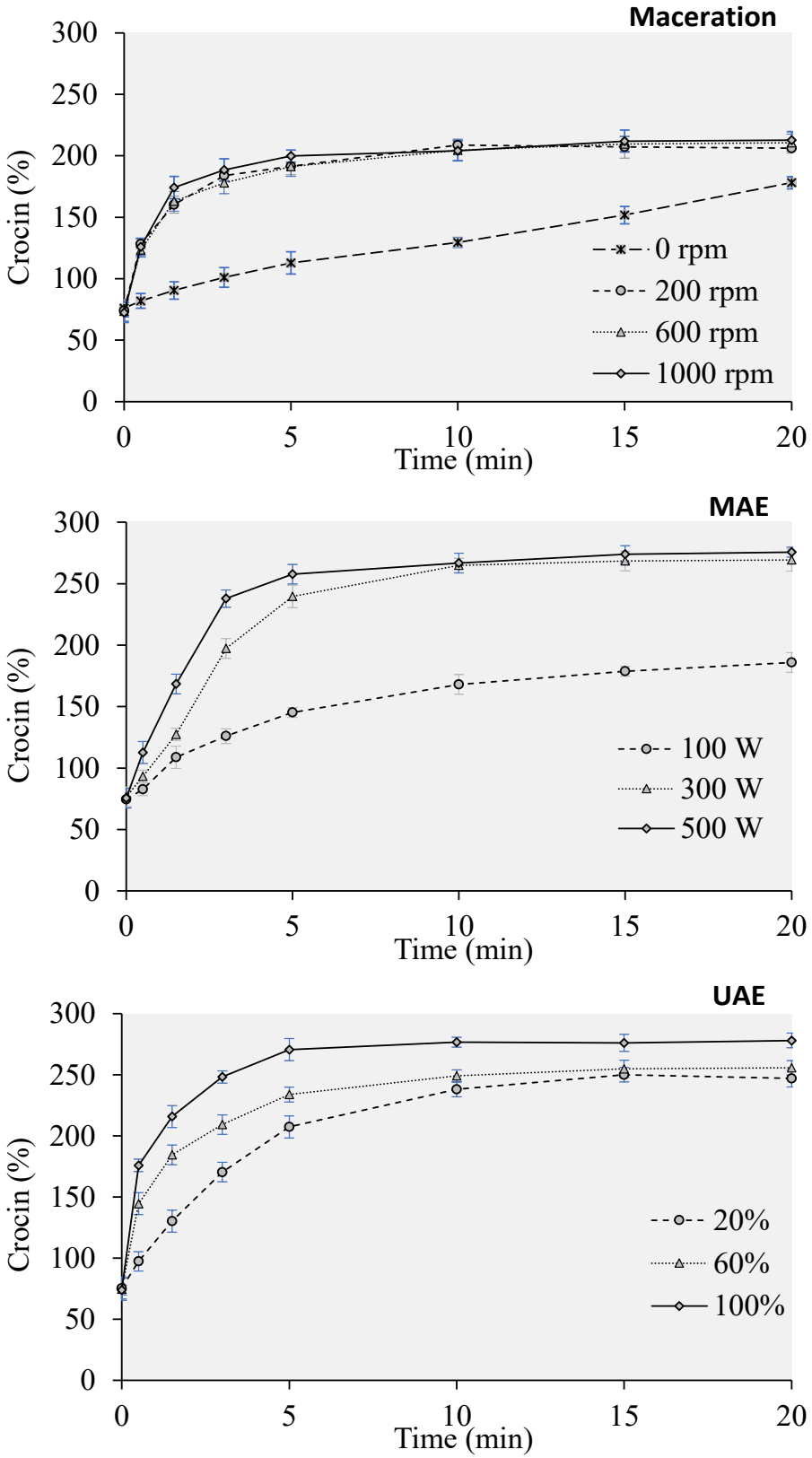

Figure 1. Evolution of crocin extraction using maceration

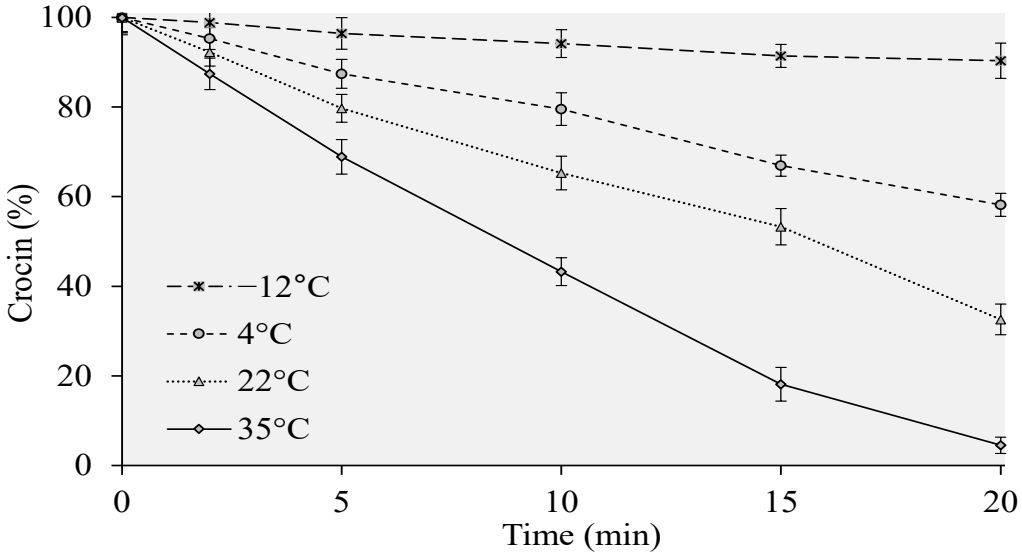

Figure 2. Evolution of crocin percentage during storage of aqueous extract at different temperatures

Table 2. Kinetic parameters of crocin degradation at different storage temperatures

Parameter Temperature	Crocin concentration	R^2	P-value	$t_{1/2}$ (days)
−12 °C	$[C]_t = -0.497\ t + 99.505$	0.976	0.0002	100.050
4 °C	$[C]_t = -2.087\ t + 99.315$	0.996	< 0.0001	23.791
22 °C	$[C]_t = -3.232\ t + 98.543$	0.993	< 0.0001	15.244
35 °C	$[C]_t = -4.874\ t + 95.939$	0.986	< 0.0001	9.842

$[C]_t$, amount of crocin; t, any time in the studied interval period; R^2, coefficients of determination; P-value, the level of significance of the model; $t_{1/2}$, the half-life of crocin degradation expressed in days.

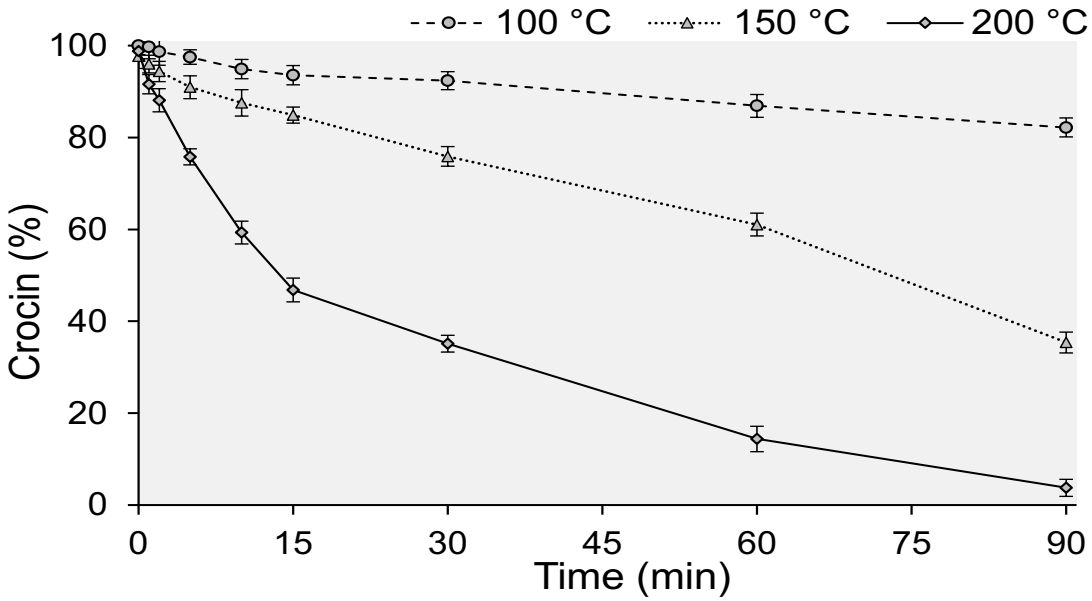

Figure 3. Evolution of crocin percentage during heat treatment

Table 3. Kinetic parameters of crocin degradation at different treatment temperatures

Parameter Temperature	Crocin concentration	Reaction Order	R^2	P-value	$t_{1/2}$ (min)
100 °C	$[C]_t = -0.189\ t + 99.505$	0	0.958	< 0.0001	260.051
150 °C	$[C]_t = -0.647\ t + 99.315$	0	0.990	< 0.0001	74.018
200 °C	$Ln[C]_t = -0.034\ t + 1.509$	1	0.989	< 0.0001	20.211

$[C]_t$, amount of crocin; t, any time in the studied interval period; R^2, coefficients of determination; P, the level of significance of the model; $t_{1/2}$, the half-life of crocin degradation expressed in minutes.

4. Conclusion

Saffron contains numerous bioactive compounds, particularly crocin, responsible for sensory characteristics and medicinal properties but which are sensitive to different handling during extraction, storage, and heat treatment. The experiments regarding the method and conditions of crocin extraction revealed considerable variations. Microwave (500 W) and ultrasound (100%) extractions for 5 minutes were the best procedures for crocin recovery. The storage of the aqueous extract of crocin demonstrated its sensitivity as the temperature rose and adopted the zero-order degradation rate. The heat treatment revealed good resistance of crocin at 100 °C, but the degradation was high at 150 °C and more pronounced at 200 °C. The degradation rate follows a zero-order kinetics at 100°C and 150°C, while at 200°C, it exhibits first-order kinetics. Consequently, the temperature during storage and heat treatment of the aqueous saffron extract must be taken into account, and the use of low temperatures is recommended to reduce crocin degradation.

5. References

Anta, J. F. E. A., Biloa, D. M., & Raghavarao, K. (2020). Optimization of extraction parameters of polyphenols from mango seed kernel through response surface methodology. *European Journal of Engineering and Technology Research,* 5(8), 877-883. https://doi.org/10.24018/ejeng.2020.5.8.2069

Atencio, S., Verkempinck, S. H., Reineke, K., Hendrickx, M., & Van Loey, A. (2022). Heat and light stability of pumpkin-based carotenoids in a photosensitive food: A carotenoid-coloured beverage. *Foods,* 11(3), 485. https://www.mdpi.com/2304-8158/11/3/485#

Atyane, L., Ben El Caid, M., Serghini, M., & Elmaimouni, L. (2017). Influence of different extraction methods and the storage time on secondary metabolites of saffron. *International Journal of Engineering Research and Technology,* 6, 65-69.

Bachir-bey, M., Louaileche, H., & Zemouri, S. (2013). Optimization of phenolic compound recovery and antioxidant activity of light and dark dried fig (*Ficus carica* L.) varieties. *Food Science and Biotechnology* 22, 1613-1619. https://doi.org/10.1007/s10068-013-0258-7

Benkerrou, F., Bachir-bey, M., Amrane, M., & Louaileche, H. (2018). Ultrasonic-assisted extraction of total phenolic contents from *Phoenix dactylifera* and evaluation of antioxidant activity: Statistical optimization of extraction process parameters. *Journal of Food Measurement Characterization,* 12, 1910-1916. https://doi.org/10.1007/s11694-018-9805-5

Benkerrou, F., Louaileche, H., & Bachir-bey, M. (2018a). Optimized ultrasonic-assisted extraction of total phenolics and antioxidant activity of date (*Phoenix dactylifera* L.) using response surface methodology. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology,* 42(2), 9-22.

Bouallag, O., Zeghichi-Hamri, S., Bachir-bey, M., Brahmi-Chendouh, N., Bouallag, N., Takka, M., et al. (2022). Optimization of phenolic compounds extraction from *Bellis perennis* flowers and assessment for antioxidant properties. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology,* 46(1), 125-139. https://doi.org/https://doi.org/10.35219/foodtechnology.2022.1.10.

Boudjouan, F., Zeghbib, W., Bachir-bey, M., Djaoudene, O., Elothmani, D., Bououdina, M., et al. (2021). Extraction methods comparison for polyphenols recovery and antioxidant activity from *Opuntia ficusindica*'s seeds. *Studia Universitatis Vasile Goldis Seria Stiintele Vietii,* 31(4).

Chaouqi, S., Moratalla-López, N., Lage, M., Lorenzo, C., Alonso, G. L., & Guedira, T. (2018). Effect of drying and storage process on Moroccan saffron quality. *Food Bioscience,* 22, 146-153. https://doi.org/10.1016/j.fbio.2018.02.003

Chisté, R. C., Lopes, A. S., & De Faria, L. J. (2010). Thermal and light degradation kinetics of anthocyanin extracts from mangosteen peel (*Garcinia mangostana* L.). *International Journal of Food Science Technology,* 45(9), 1902-1908. https://doi.org/10.1111/j.1365-2621.2010.02351.x

Chutintrasri, B., & Noomhorm, A. (2007). Color degradation kinetics of pineapple puree during thermal processing. *LWT-Food Science Technology,* 40(2), 300-306. https://doi.org/10.1016/j.lwt.2005.11.003

Dhiman, N., & Kharkwal, H. (2020). Biosynthesis and derivatization of the major phytoconstituents of saffron. In *Saffron* (pp. 83-92): Elsevier.

El Khoudri, M., Ouahhoud, S., Lahmass, M., Khoulati, A., Benyoussef, S., Mamri, S., et al. (2021). Biological effects and pharmacological activities of saffron of *Crocus sativus*. *Arabian Journal of Medicinal Aromatic Plants,* 7(2), 254-268. https://doi.org/10.48347/IMIST.PRSM/ajmap-v7i2.22962

Escribano, J., Alonso, G.-L., Coca-Prados, M., & Fernández, J.-A. (1996). Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells *in vitro*. *Cancer Letters,* 100(1-2), 23-30. https://doi.org/10.1016/0304-3835(95)04067-6

Esfanjani, A. F., Jafari, S. M., Assadpoor, E., & Mohammadi, A. (2015). Nano-encapsulation of saffron extract through double-layered multiple emulsions of pectin and whey protein concentrate. *Journal of Food Engineering,* 165, 149-155. https://doi.org/10.1016/j.jfoodeng.2015.06.022

Ismail-Suhaimy, N. W., Gani, S. S. A., Zaidan, U. H., Halmi, M. I. E., & Bawon, P. (2021). Optimizing conditions for microwave-assisted extraction of polyphenolic content and antioxidant activity of *Barleria lupulina* lindl. *Plants,* 10(4), 682. https://www.mdpi.com/2223-7747/10/4/682#

ISO.3632-2. (2010). Spices − Saffron (*Crocus sativus* L.) − Part 2: Test methods. The International Organization for Standardization: Geneva, Switzerland. Available online: https://www.iso.org/standard/44526.html (accessed on 04 September 2023). .

ISO.3632. (1980). Saffron−Specification, The International Organization for Standardization: Geneva, Switzerland. .

Kadi, A., Boudries, H., Bachir-bey, M., Teffane, M., Taibi, A., & Boulekbache-Makhlouf, L. (2022). Optimization of microwave-assisted extraction of carotenoids from *Citrus clementina* peels. *Current Bioactive Compounds,* 18(6), 63-73. https://doi.org/10.2174/1573407218666211230152122

Kadkhodaee, R., & Hemmati-Kakhki, A. (2006). *Ultrasonic extraction of active compounds from saffron.* Paper presented at the II International Symposium on Saffron Biology and Technology.

Karasu, S., Bayram, Y., Ozkan, K., & Sagdic, O. (2019). Extraction optimization crocin pigments of saffron (*Crocus sativus*) using response surface methodology and determination stability of crocin microcapsules. *Journal of Food Measurement Characterization,* 13, 1515-1523. https://doi.org/10.1007/s11694-019-00067-x

Karbasi, A., & Zandi Dareh Gharibi, B. (2022). Economic aspects of saffron in the world. In J. Vakhlu, S. Ambardar, S. A. Salami & C. Kole (Eds.), *The saffron genome* (pp. 275-287). Cham: Springer International Publishing.

Khaled Khodja, Y., Dahmoune, F., Bachir-bey, M., Madani, K., & Khettal, B. (2020). Conventional method and microwave drying kinetics of *Laurus nobilis* leaves: effects on phenolic compounds and antioxidant activity. *Brazilian Journal of Food Technology,* 23, e2019214. https://doi.org/10.1590/1981-6723.21419

Lage, M., & Cantrell, C. L. (2009). Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. *Scientia horticulturae,* 121(3), 366-373. https://doi.org/10.1016/j.scienta.2009.02.017

Laing, B., Schlueter, D., & Labuza, T. P. (1978). Degradation kinetics of ascorbic acid at high temperature and water activity. *Journal of Food Science,* 43(5), 1440-1443. https://doi.org/10.1111/j.1365-2621.1978.tb02515.x

Masoumi, A., Anwari, S., Rahimi, B. A., & Shankarapppa, T. (2021). Effects of microwave, ultraviolet light and low temperature on khapra beetle mortality and quality of saffron. *International Journal of Agricultural Natural Sciences,* 14(2), 78-84.

Meléndez-Martínez, A. J., Esquivel, P., & Rodriguez-Amaya, D. B. (2023). Comprehensive review on carotenoid composition: Transformations during processing and storage of foods. *Food Research International,* 169, 112773. https://doi.org/10.1016/j.foodres.2023.112773

Minguez-Mosquera, M. I., & Jaren-Galan, M. (1995). Kinetics of the decolouring of carotenoid pigments. *Journal of the Science of Food Agriculture,* 67(2), 153-161. https://doi.org/10.1002/jsfa.2740670203

Moradi, S., Kafi, M., Aliniaeifard, S., Moosavi-Nezhad, M., Pedersen, C., Gruda, N. S., et al. (2022). Monochromatic blue light enhances crocin and picrocrocin content by upregulating the expression of underlying biosynthetic pathway genes in saffron (*Crocus sativus* L.). *Frontiers in Horticulture,* 1, 960423. https://doi.org/10.3389/fhort.2022.960423

Mzabri, I., Addi, M., & Berrichi, A. (2019). Traditional and modern uses of saffron (*Crocus sativus*). *Cosmetics,* 6(4), 63. https://www.mdpi.com/2079-9284/6/4/63#

Najafi, Z., Kahn, C. J. F., Bildik, F., Arab-Tehrany, E., & Şahin-Yeşilçubuk, N. (2021). Pullulan films loading saffron extract encapsulated in nanoliposomes; preparation and characterization. *International Journal of Biological Macromolecules,* 188, 62-71. https://doi.org/10.1016/j.ijbiomac.2021.07.175

Orfanou, O., & Tsimidou, M. (1996). Evaluation of the colouring strength of saffron spice by UV—Vis spectrometry. *Food Chemistry,* 57(3), 463-469. https://doi.org/10.1016/S0308-8146(96)00003-9

Oukhrib, R., El Issami, S., Chaouay, A., El Mouaden, K., Jmiai, A., Ibrahimi, B., et al. (2015). The inhibitory effect of Saffron extract (*Crocus sativus*., L) on copper corrosion in seawater. *Chemical Science Review Letters,* 4(13), 241-251.

Poma, A., Fontecchio, G., Carlucci, G., & Chichiricco, G. (2012). Anti-inflammatory properties of drugs from saffron crocus. *Anti-Inflammatory Anti-Allergy Agents in Medicinal Chemistry,* 11(1), 37-51.

https://doi.org/10.2174/18715231280347628 2

Saci, F., Benchikh, Y., Louaileche, H., & Bachir-bey, M. (2018). Optimization of ultrasound-assisted extraction of phenolic compounds and antioxidant activity from carob pulp (*Ceratonia siliqua* L.) by using response surface methodology. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology,* 42(1), 26-39.

Sánchez, A. M., Carmona, M., A. Ordoudi, S., Z. Tsimidou, M., & Alonso, G. L. (2008). Kinetics of individual crocetin ester degradation in aqueous extracts of saffron (*Crocus sativus* L.) upon thermal treatment in the dark. *Journal of Agricultural Food Chemistry,* 56(5), 1627-1637. https://doi.org/10.1021/jf0730993

Teffane, M., Boudries, H., Bachir-bey, M., Kadi, A., & Boukhalfa, F. (2022). Effect of solvent type, extraction temperature, agitation speed and microwave power on phenolic compound extraction and antioxidant activity of apricot kernels (*Prunus armeniaca* L.). *Current bioactive compounds,* 18(1), 24-32. https://doi.org/10.2174/1573407217666210215085507

Tiwari, B., Patras, A., Brunton, N., Cullen, P., & O'donnell, C. (2010). Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrasonics Sonochemistry,* 17(3), 598-604. https://doi.org/10.1016/j.ultsonch.2009.10.009

Tsimidou, M., & Tsatsaroni, E. (1993). Stability of saffron pigments in aqueous extracts. *Journal of Food Science,* 58(5), 1073-1075. https://doi.org/10.1111/j.1365-2621.1993.tb06116.x

Zemouri-Alioui, S., Bachir-bey, M., Kurt, B. Z., Sonmez, F., & Louaileche, H. (2018). Optimization of ultrasound-assisted extraction of total phenolic contents and antioxidant activity using response surface methodology from jujube leaves (*Ziziphus jujuba*) and evaluation of anticholinesterase inhibitory activity. *Journal of Food Measurement and Characterization,* 13(1), 321-329. https://doi.org/10.1007/s11694-018-9947-5

Acknowledgment

We are very grateful to the Algerian Ministry of Higher Education and Scientific Research for the financial support, particularly the DGRSDT.

Authors contribution

Methodology: BACHIR-BEY M., BENCHIKH Y.; Data collection: CHOUALI M., DJEBELLANNOUAR S.; Data processing: KHALED KHODJA Y., BENCHIKH Y.; Writing – original draft preparation: BACHIR-BEY M.; Writing – review and editing: BENBOURICHE A., BELLIK Y.; Supervision: KATI D. E.

Conflicting interests

The authors have no competing interests to declare that are relevant to the content of this article.