Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

# Extraction of bioactive compounds from saffron stigma into sunflower oil: A comparative study of various solid-liquid extraction methods

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**ARTICLE INFO**

**Article History:**

Received: 2024/5/5
Accepted: 2024/10/27

**Keywords:**

Maceration,
Microwave-assisted extraction,
Oil aromatization,
Sunflower oil,
Saffron (Crocus sativus L.) stigma,
Ultrasound-assisted extraction.

DOI: **10.22034/FSCT.22.160.142.**

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**ABSTRACT**

Flavoring edible oils is aimed at enhancing oxidative stability and creating desirable sensory properties to expand their market. This study aimed to produce saffron-flavored sunflower oil using various solid-liquid extraction techniques including stirring-assisted maceration (SAM), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE). The study aims to optimize the effective parameters in each extraction method and characterize the physicochemical properties of the flavored oils. The UAE method effectively extracted bioactive compounds of saffron stigma in a shorter time frame. However, the high energy input during the UAE process led to the deterioration of its physicochemical and sensory properties. Similarly, in the MAE method, sensitive and volatile compounds of saffron stigma were degraded. Alternatively, the SAM method, in a gentle and continuous process, managed to dissolve a high concentration of saffron compounds in the oil while preserving its physicochemical and sensory properties. The optimal concentrations of picrocrocin, safranal, and crocins were determined to be 317, 56, and 160 mg $L^{-1}$, respectively. The aromatized sunflower oil presents a promising novel product for diverse food applications and market expansion.



[ DOI: 10.22034/FSCT.22.160.142 ]

## 1- Introduction

Production of edible oils flavored with different herbs and spices has been increasing in recent years [1]. The global practice of flavoring oils serves several purposes, including the enhancement of oxidative stability in edible oils [2], which are prone to degradation through exposure to air, light, and heat. This oxidative process can lead to unfavorable odors, decreased nutritional quality, and potential health risks for consumers. Artificial antioxidants like TBHQ, BHT, BHA, and PG are commonly used in commercial settings to prevent this degradation, yet their safety and effectiveness are under scrutiny [3]. Therefore, there is a critical need to explore alternative methods, such as incorporating natural antioxidants from vegetables, herbs, spices, and fruits, to improve the quality and stability of edible oils [4]. In response to consumer demand for products with enhanced sensory qualities and health benefits, the practice of aromatizing edible oils with natural aromatic and flavoring substances, such as herbs, spices, vegetables, and fruits, has gained prominence [5, 6]. These substances, in various forms like powders, extracts, or essential oils, are added to oils to create products with unique sensory attributes that cater to the preferences of consumers seeking gourmet experiences. Furthermore, the incorporation of bioactive substances into oils not only enhances their nutritional value [7] but also brings about additional benefits, including antimicrobial, anticancer, and antioxidant effects, along with improvements in sensory perception and stability [5, 8].

Various methods exist for aromatization of edible oils. These methods include direct addition of essential oils and oleoresins [8, 9], maceration [10], malaxation [11], and liquid-liquid extraction (LLE) [6]. In maceration, solid parts of plants or fruits directly contact the oil [12]. Sometimes, maceration is coupled with stirring, heating [13], ultrasound [14, 15] or microwave [9] treatment to accelerate extraction.

Iran is the world's leading producer of saffron (*Crocus sativus* L.) [16], with its stigma containing bioactive compounds such as safranal, picrocrocin, and crocins, known for their aroma, bitter taste, and color respectively. Saffron has been historically used for medicinal and culinary purposes due to its unique composition [17]. Modern research has uncovered its various health benefits, including anticarcinogenic, antimutagenic, immunomodulating, neuroprotective, and antioxidant properties [18]. In Iran, saffron is widely utilized in coloring and flavoring various food products [19]. However, limited literature exists on incorporating saffron stigma into edible oils. A study by Sena-Moreno et al. blended olive oil with aqueous saffron extract, but it led to a decrease in oxidative stability due to the back-extraction of polyphenolic compounds [6]. Thus, there's a need for suitable methods to flavor edible oils with saffron stigma. Additionally, no research has explored flavoring sunflower oil (extracted from sunflower plant seeds (*Helianthus annuus*)) with saffron stigma, presenting a potential opportunity for creating a unique and valuable product.

In this study, sunflower oil was aromatized with saffron stigma for the first time.





[ DOI: 10.22034/FSCT.22.160.142 ]

Different methods such as stirring-assisted maceration (SAM), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) were explored and optimized to extract saffron stigma's bioactive compounds into sunflower oil. Furthermore, the physicochemical characteristics of the resulting flavored sunflower oils were evaluated.

## 2 Experimental section

### 2.1 Materials and chemicals

Saffron stigma was obtained from Ilia's company (Iran). Sunflower oil, obtained through the cold-press method, was purchased locally in Iran without any antioxidant additives. Cyclohexane and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA), while ethanol (96%) was supplied by Ameretat Co (Iran). Methanol, Folin Ciocalteu's reagent, acetic acid, potassium iodide, potassium hydroxide, starch, and phenolphthalein were obtained from Merk (Germany). Sodium carbonate was sourced from AppliChem (Germany), and isooctane was from Supelco (Germany). Deionized water was produced using the ZU101 water purification system (Zolalan Pars Co., Iran).

### 2.2 Aromatization of sunflower oil by saffron stigma

#### 2.2.1 Ultrasound-assisted extraction (UAE)

Initially, saffron stigma was ground into a fine powder using a mortar. Then, 0.03 g of the powder was added to 4.5 mL of sunflower oil. The extraction process was conducted using either an ultrasonic probe (p-UAE) (UHP-400, Topsonics, Iran) or an ultrasonic bath (b-UAE) (vCLEAN 1- L2, Backer, Iran). p-UAE operated with a duty cycle of 7s on/3s off. After 10 min of operation, a 10-min resting period was implemented, followed by resumption until extraction completion. The b-UAE process involved continuous operation for 10 min followed by a 5-min resting period, with optional stirring using a magnetic stirrer during intervals.

#### 2.2.2 Microwave-assisted extraction (MAE)

0.03 g of saffron powder was mixed with 4.5 mL of sunflower oil. The sample underwent microwave heating (M245, Butane, Iran) for 30 s at different power levels, followed by stirring on a magnetic stirrer for 5 min. This cycle of microwave heating and stirring was repeated sequentially until a total time of 60 min was reached (total 5 min microwave).

#### 2.2.3 Stirring-assisted maceration (SAM)

0.03 g of saffron powder was mixed with 4.5 mL of sunflower oil. The mixture was then stirred using a magnetic stirrer (MR3001 K, Heidolph, Germany) at 500 rpm for one week.

### 2.3 Determination of saffron stigma bioactive compounds in aromatized sunflower oil

Initially, 1.5 mL of the clarified aromatized sunflower oil was transferred to a 10 mL falcon tube and diluted with 6 mL of cyclohexane, followed by shaking. LLE was conducted by adding 2 mL of 60% v/v ethanol, vigorously shaking for 3 min. The two phases were then separated by centrifugation at 4000 rpm for 10 min. The absorbance of the ethanolic phase was measured using a SP-UV500DB spectrophotometer (Spectrum, China) at



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wavelengths of 257 nm, 330 nm, and 440 nm for picrocrocin, safranal, and crocins, respectively. Standard curves were constructed within the concentration range of 5-25 μg $mL^{-1}$ for picrocrocin and 2-15 μg $mL^{-1}$ for safranal and crocins, using 60% v/v ethanol as the solvent.

## 2.4 Physicochemical characterization of flavored sunflower oil

### 2.4.1 Acid value (AV)

20 g (m) of oil was placed in a 250 mL Erlenmeyer flask, and approximately 100 mL of a neutralized ethanol-diethyl ether mixture (1:1) was added. Then, 3 to 4 drops of phenolphthalein reagent were added and mixed thoroughly. The resulting solution was titrated with 0.1 N standard solution of potassium hydroxide (N) until a persistent pale pink color appeared. Finally, AV was calculated in milligrams of potassium hydroxide per gram of fatty acid (mgKOH $g^{-1}$), (equation 1) with 'V' representing the volume of titrant consumed to reach the endpoint [20].

$$AV = \frac{V \times N \times 56.1}{m} \quad (1)$$

### 2.4.2 Peroxide value (PV)

5 g (m) of oil was placed in a 250 mL Erlenmeyer flask. A mixture of 50 mL glacial acetic acid:isooctane (3:2) was added to the sample and mixed thoroughly until complete dissolution of the oil was achieved. Subsequently, 0.5 mL of saturated potassium iodide solution (1.75 g $mL^{-1}$) was added to the mixture. The Erlenmeyer flask was sealed with its lid and stirred for 1 min. After removing the lid, 100 mL of freshly boiled distilled water was added promptly to the flask. The released iodine was titrated with 0.01 N sodium thiosulfate (N) in the presence of starch reagent until the blue color of the solution disappeared, indicating the endpoint. PV was expressed in milliequivalents of active oxygen per kilogram of oil ($meqO_2$ $kg^{-1}$), (equation 2) with 'V' representing the volume of titrant consumed to reach the endpoint [21].

$$PV = \frac{V \times N \times 1000}{m} \quad (2)$$

### 2.4.3 Determination of total phenolic content (TPC)

TPC in the sunflower oil was determined using the Folin-Ciocalteu assay [22, 23]. Initially, 100 μL of oil was mixed with 2 mL of methanol and stirred for 15 min, followed by centrifugation. Subsequently, 400 μL of 10% Folin-Ciocalteu reagent was added to the methanol fraction and left in darkness for 6 min. Then, 500 μL of 7.5% sodium carbonate was introduced, and the mixture was vortexed. The resulting solution was kept in darkness for 1 h, and the absorbance was measured at 765 nm. Gallic acid (10-85 μg $mL^{-1}$) in methanol was reacted with 10% Folin-Ciocalteu reagent to construct the calibration curve. The results were expressed as milligrams of gallic acid equivalents per gram of oil (mg GAE $g^{-1}$).

### 2.4.4 Total carotenoid content (TCC)

Initially, 7.5 g of oil was adjusted to volume in a 25 mL volumetric flask using cyclohexane. The TCC was measured using a spectrophotometer at a wavelength of 470 nm. The concentration of carotenoid pigment was determined in milligrams per kilogram (mg $Kg^{-1}$) using equation 3 [24].The density of the sunflower oil was experimentally determined to be 0.915 g $mL^{-1}$ at 25°C by measuring the weight of a known volume of oil.





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$$C_{carotenoid} = \frac{Abs_{470} \times 10^6}{2000 \times 100 \times density} \quad (3)$$

**2.4.5 Evaluation of the antioxidant activity**

Initially, 100 µL of oil was transferred to a falcon tube, covered with foil. Then, 2 mL of a DPPH solution (100 µM) was added to the tube and vortexed thoroughly. The sample was stored in the dark at room temperature for 30 min. Finally, the absorbance of both the control sample ($A_{control}$) and the flavored sunflower oil ($A_{sample}$) was determined using a spectrophotometer at 517 nm, with methanol as the blank. The results were expressed as the inhibition percentage according to equation 4 [25].

$$I\% = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (4)$$

**2.4.6 K232 and K268 extinction coefficients**

0.25 g and 0.05 g of oil were individually transferred to 25 mL volumetric flasks and diluted with isooctane for the determination of K268 and K232, respectively. The absorption ($E_\lambda$) at wavelengths of 232 and 268 nm was recorded with isooctane as the blank. Specific absorption ($K_\lambda$) was calculated using equation 5, where 'C' represented the concentration of the final solution (g $mL^{-1}$), and 'S' denoted the cell length (cm) [26].

$$K_\lambda = \frac{E_\lambda}{100 \times C \times S} \quad (5)$$

All measurements were conducted in triplicate. Statistical analysis, including calculation of mean, standard deviation, and ANOVA analysis, was performed using GraphPad prism software. Statistically significant differences were defined as those with p-values ≤ 0.05.

## 3 Results and discussion

### 3.1 UV-Vis measurment of the saffron stigma active compounds in oil samples

One of the significant methods for quantifying saffron compounds is the ISO 321 standard method, wherein picrocrocin, safranal, and crocins are assessed at wavelengths of 257, 330, and 440 nm, respectively [27]. In this study, instead of measuring E1%, as in Sena-Moreno's work [6], calibration curves were generated using standard solutions of all three compounds. Here, calibration curves for each of the mentioned compounds in 60% ethanol were plotted in the range of 5 to 25 µg/ml for picrocrocin and 2 to 15 µg/ml for safranal and crocins, as depicted in Figure 1a. Additionally, similar to the study by Suárez et al. [28], polar compounds extracted in oil were isolated using the LLE technique with an aqueous alcoholic solution in order to prevent sunflower oil interfering compounds adsorption, and the resultant aqueous-alcoholic extract (60% ethanol) was analyzed via UV-Vis spectroscopy. The concentrations of picrocrocin, safranal, and crocins were measured at their respective maximum wavelengths, as depicted in Figure 1b, which are 257, 330, and 440 nm, respectively. As depicted in Figure 1b, the absorption spectrum pattern of the LLE extract closely resembles that of the corresponding compounds in the standard solution, indicating the success of the extraction process.



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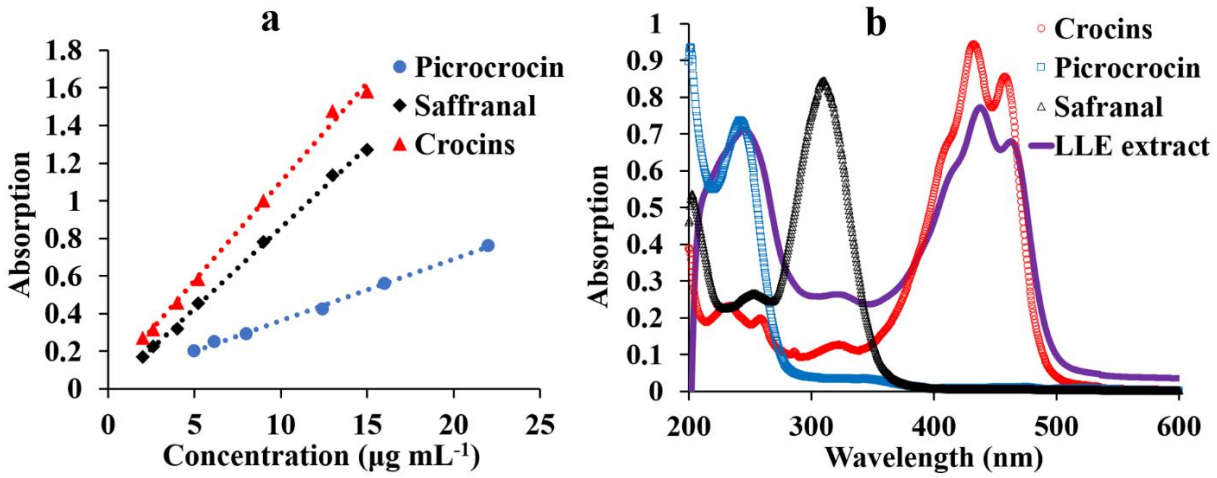


**Figure 1** Calibration curves (a) and Absorption spectra (b) of picrocrocin, safranal and crocins in standard or LLE extraction solution prepared in ethanol 60%. The calibration equations for picocrocin, safranal, and crocins are as follows: $y = 0.0328x + 0.0341$ ($R^2$=0.9973), $y = 0.0861x - 0.0031$ ($R^2$= 0.9989), $y = 0.1054x + 0.0474$ ($R^2$=0.9965).

### 3.2 Ultrasound-assisted extraction (UAE)

This study focused on aromatizing sunflower oil by blending it with saffron stigma using different solid-liquid extraction techniques. UAE was chosen for its notable efficiency, as highlighted in recent research [29]. The UAE involved both a probe device and a bath, and various parameters were explored and fine-tuned to optimize the process.

#### 3.2.1 Optimization of p-UAE process

The ultrasonic probe's power was adjusted between 50 to 200 W. Figure 2a shows that extraction levels of picrocrocin, safranal, and crocins increased as power increased. Purohit et al. found similar results with β-carotene extraction from carrot waste, noting a slower rate of increase with oil compared to other solvents [30]. Figure 2b demonstrates a linear increase in compound extraction within sunflower oil with longer ultrasonic probe time at 200 W. However, at 200 W and 45 min, extraction efficiency increased but with a noticeable burnt odor. Lara et al. also observed increased carotenoid extraction from papaya using sunflower and soybean oils with longer ultrasonic probe times from 10 to 60 min [31].

#### 3.2.2 Optimization b-UAE process

The ultrasonic bath, operating at lower intensities, offers milder extraction conditions, reducing sample damage [32]. Subsequently, in this study, the ultrasonic bath was also used for aromatization of sunflower oil with saffron stigma, with various parameters investigated. Results in Figure 2c show that the concentration of extracted compounds is lower in b-UAE compared to p-UAE even at 50 W power, showing lower efficiency of the bath method. However, at 70°C, b-UAE's extraction improved, yielding comparable results for picrocrocin and safranal to p-UAE (200 W, 10 min), and double the amount of crocins. Crocins exhibit greater stability in the milder conditions of the ultrasonic bath, explaining the difference. Similarly, Purohit et al. demonstrated enhanced beta-carotene





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extraction from carrot waste oil at higher temperatures with ultrasound [30]. In b-UAE, extraction remained relatively constant up to 1 h, increasing thereafter, with optimal extraction time considered as 30 min (Figure 2d). Goula et al. found carotenoid degradation during ultrasonic bath extraction, highlighting the importance of minimizing extraction time to less than 30 min to mitigate degradation [33].

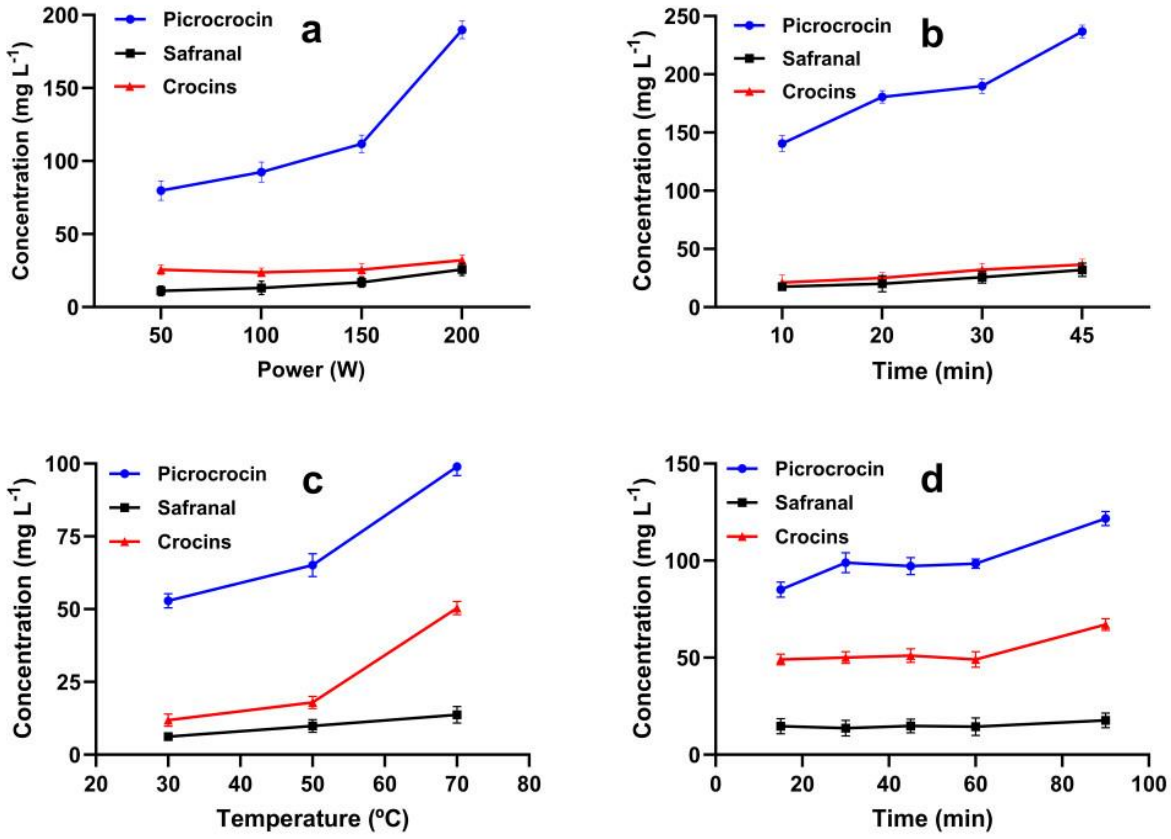


**Figure 2** Optimization of the p-UAE process: (a) ultrasonic power optimization (time: 30 min), (b) time optimization (power:200 W); optimization of the b-UAE process: (c) temperature optimization (time: 30 min), (d) time optimization (temperature: 70 °C) (saffron stigma: 0.7% w/v).

### 3.2.3 Multi-step extraction in b-UAE

Increasing saffron stigma powder in sunflower oil up to 17.8% w/v led to a 305% increase in picrocrocin content and a 141% increase in safranal content (Figure 3). However, adding more powder beyond this point did not significantly enhance these compounds further. In contrast, higher amounts of saffron stigma powder did not improve crocin extraction efficiency in the oil. The limited extraction of crocins is attributed to their higher water solubility (containing 24 hydrogen acceptors, and 14 hydrogen donors) [34], which makes its extraction in the oil phase more difficult. On the other hand, the instability of crocins against strong ultrasonic waves can also be an important factor in the degradation of the extracted amounts, which can be observed in





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comparison with the results of the SAM method.

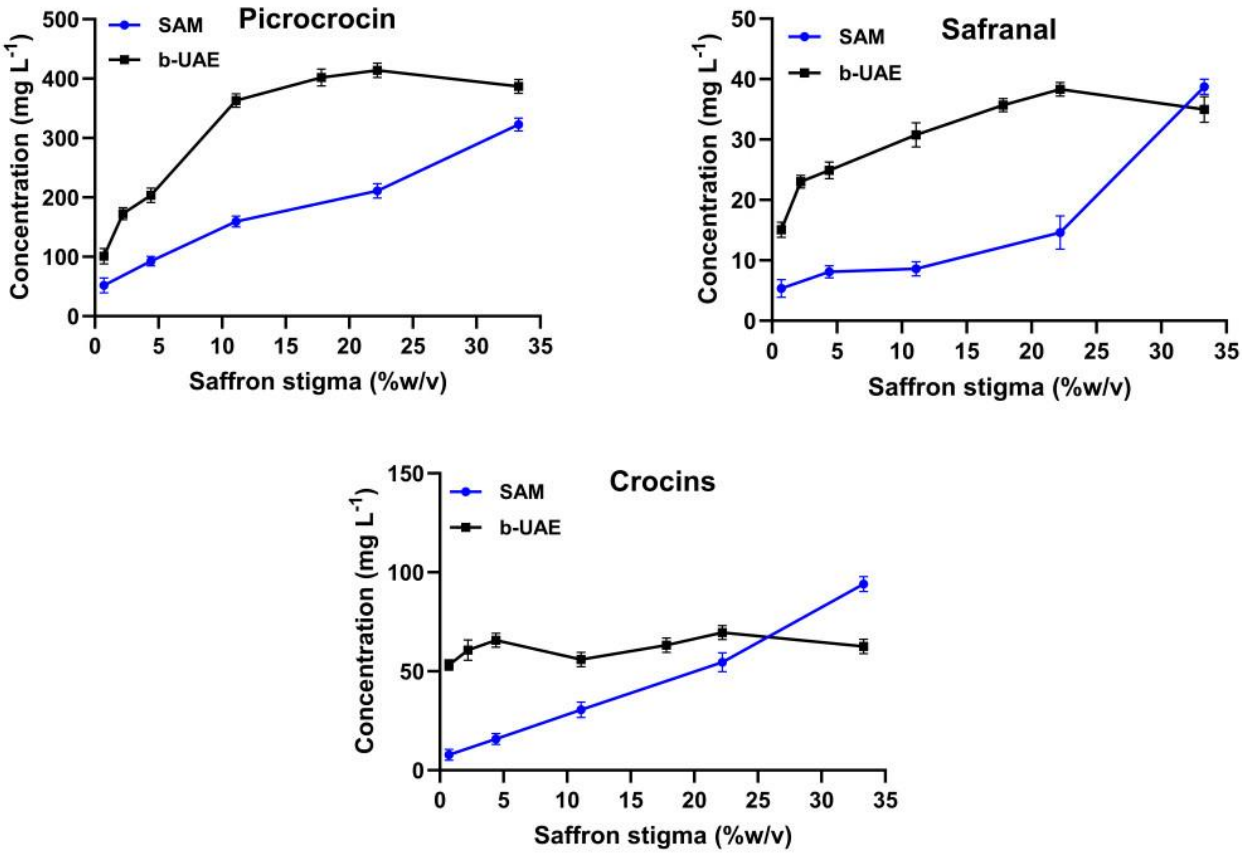


**Figure 3** The impact of wight of powder on the extraction efficiency in b-UAE (70 °C for 30 min) and SAM (30 °C for 60 min).

Considering that only a small percentage of saffron stigma bioactive compounds dissolved in sunflower oil during single-step bath ultrasonic-assisted extraction (b-UAE), a multi-step extraction approach was implemented to enhance efficiency. The remaining residue from the initial extraction stage underwent further extraction under similar conditions, repeated for up to 10 cycles. Results depicted in Figure 4 show increased cumulative extracted material with each cycle. Each b-UAE cycle comprised 10 min of ultrasonication followed by 5 min off. In some samples, stirring using a magnetic stirrer occurred during the off period. Overall efficiency was higher when ultrasonication was accompanied by stirring (b-UAE+SAM) compared to ultrasonication alone (b-UAE). Stirring aided in mass transfer of extracted compounds to the solvent bulk, facilitating contact between fresh solvent and settled saffron powder in subsequent ultrasonic cycles.





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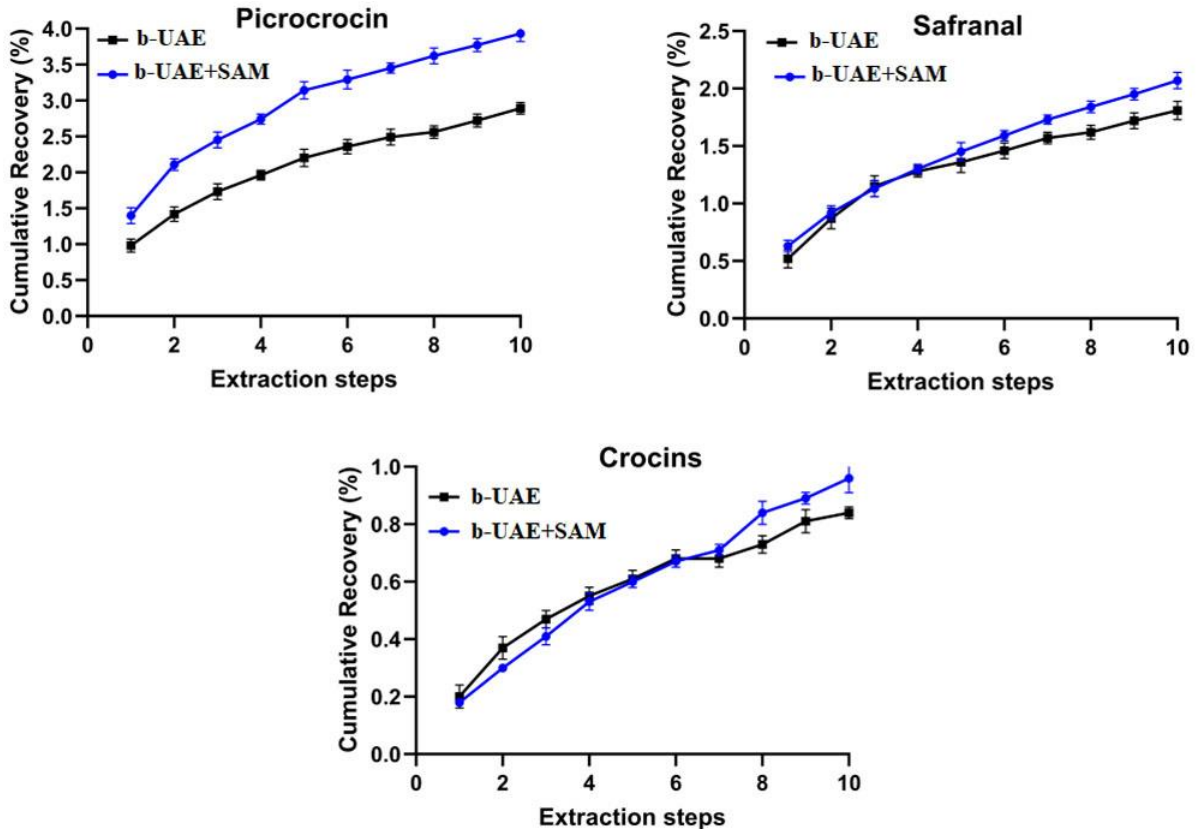


**Figure 4** The impact of the number of extraction cycles on the cumulative recovery of b-UAE (70 °C and 30 min).

In b-UAE at 70°C for 30 min with 10 extraction cycles and stirring, maximum extractable amounts of picrocrocin, safranal, and crocins from stigma were 3.85, 2.0, and 0.92%, respectively. The extraction efficiency of these compounds varies according to their polarity; the more polar the compound, the lower its extraction in oil. In first cycle, 36, 31, and 19% of picrocrocin, safranal, and crocins were obtained. About 81, 73, and 64% of total recovery for picrocrocin, safranal, and crocins occurred within the first 5 cycles. Reusing residual waste from the initial extraction stage does not achieve similar efficiency as fresh powder, as most compounds remain in the waste. With each cycle, extractable amounts decrease, and after 5 uses, residue no longer yields acceptable extraction efficiency. Since sunflower oil is a non-polar solvent, its capacity to extract polar compounds is limited, and its ability to permeate and penetrate the plant surface is also reduced. As the number of extraction cycles increases, the oil's penetration into plant cells appears to diminish, leading to decreased extraction efficiency. Therefore, it cannot be expected that oil will completely extract all saffron compounds; it can only incorporate a portion of the compounds, sufficient to enhance aroma and flavor. Furthermore, the extraction efficiency of compounds in the residual plant material decreases with increasing extraction cycles. The exact reason for this decline is not clearly understood, but one possible explanation is the degradation of these compounds in the plant due to the high energy of ultrasonic waves. Nonetheless, saffron is a highly expensive plant, and this solubility limitation can increase the final cost of the oil, restricting its applications to luxury uses.



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### 3.3 Stirring-assisted maceration (SAM)

The results indicate that UAE effectively extracts bioactive compounds from saffron stigmas into sunflower oil, albeit with a reduction in organoleptic properties. Conversely, the maceration method, being easily applicable in the industry and offering milder extraction conditions, avoids negative impacts on oil's organoleptic properties. Consequently, the study investigated sunflower oil aromatization with saffron stigmas using solvent-assisted maceration (SAM), optimizing certain influential parameters.

#### 3.3.1 Optimization of time in SAM

Figure 5 illustrates an increasing trend in bioactive compound extraction from saffron stigmas with longer maceration times at room temperature. Optimal levels for picrocrocin, safranal, and crocins were reached after 6, 4, and 5 days of maceration, respectively, with no significant changes beyond these durations. SAM extraction for one day yielded similar results to b-UAE, while after 4 days, SAM matched p-UAE. The key difference was the absence of burnt odor in SAM-produced oil. Moreover, SAM yielded 3- to 5-fold higher crocins content compared to b-UAE or p-UAE after 4 to 5 days, indicating less crocin degradation. Safranal and picrocrocin content in SAM oil was 3- and 7.1-fold higher than b-UAE and 2.5- and 3.1-fold higher than p-UAE, respectively. Despite the longer SAM duration, resulting concentration characteristics and organoleptic properties are noteworthy. Similarly, Jović et al. found optimal extraction times for aromatic plants in extra virgin olive oil using maceration to be around 2 weeks [35].

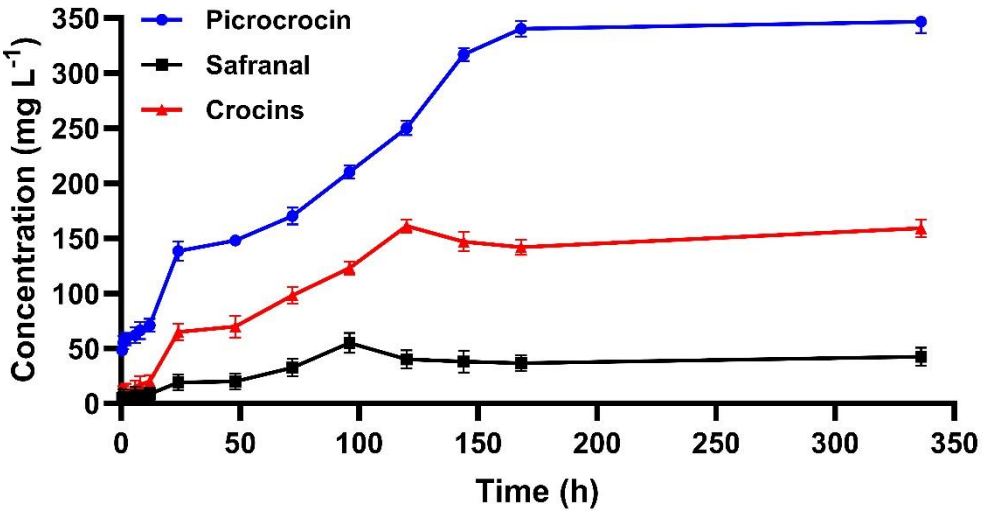


**Figure 5** The impact of time on the extraction efficiency of SAM method at room temperature (saffron stigma: 0.7% w/v).





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### 3.3.2 Optimization of power in MAE

To improve maceration extraction, microwave energy was incorporated alongside stirring. Following 5 min of stirring, the sample underwent 30 s of microwave treatment, continuing for a total of 60 min. Figure 6 shows that oil samples without microwave irradiation had higher bioactive compound content compared to microwaved samples. Microwave treatment at 30 W reduced the content of all compounds, even eliminating crocins. However, higher microwave power slightly increased extraction, with 100 W matching non-microwaved sample content. Doubling microwave time at 100 W marginally improved extraction (data not shown). This indicates microwave treatment enhances extraction but may also degrade compounds in oil, limiting its usefulness for saffron stigma bioactive compound extraction in sunflower oil.

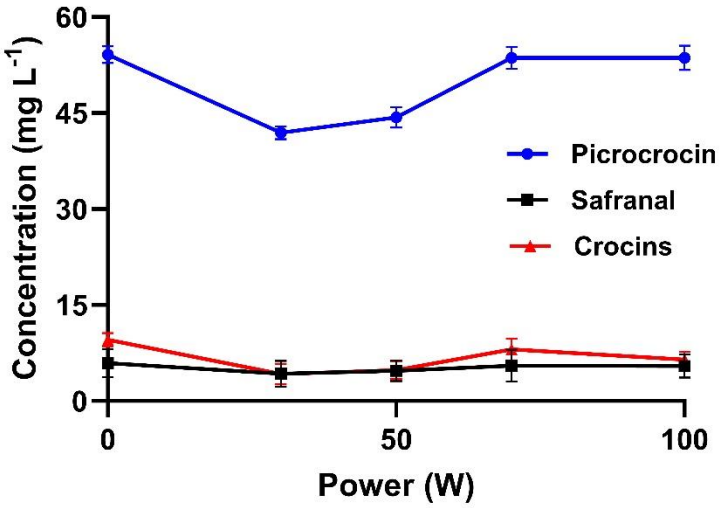


**Figure 6** The effect of microwave power on the extraction efficiency of MAE. (saffron stigma: 0.7% w/v).

### 3.3.3 Optimization of temperature in SAM

To improve the efficiency of extracting bioactive compounds from saffron stigmas in sunflower oil using the SAM method, various temperatures were explored. Figure 7 illustrates that SAM at 50°C resulted in higher dissolution of bioactive compounds from saffron stigmas in oil during the initial 12 hours compared to 30°C. However, over longer durations, compound content decreased due to temperature susceptibility. Therefore, for extended SAM processes, a more suitable ambient temperature is recommended.



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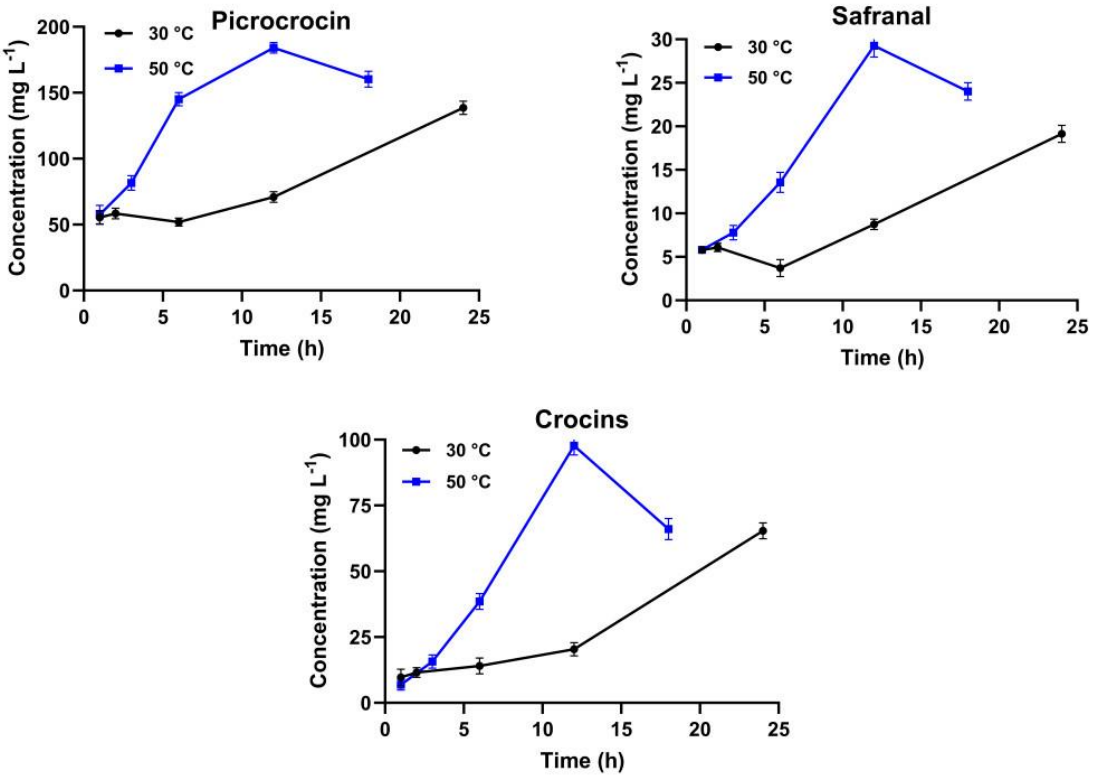


**Figure 7** The effect of temperature at different time intervals in SAM method (Saffron stigmas: 0.7 % w/v).

### 3.3.4 Optimization of weight/volume ratio in SAM

Increasing the weight-to-volume ratio of saffron stigma powder in the SAM method results in a nearly linear increase in extracted bioactive compound content in sunflower oil (Figure 3). At lower saffron powder weights, b-UAE extraction exceeds that of SAM. However, as saffron powder weight increases, b-UAE extraction becomes limited due to compound degradation. Conversely, SAM extraction increases linearly with saffron stigma mass without degradation. Both methods are constrained by the polar nature of saffron's bioactive compounds and the non-polar nature of sunflower oil. Similarly, Noor et al. demonstrated that carotenoid extraction from tomato waste in oil increases with plant weight [36].

### 3.4 The optimum concentration of bioactive compounds extracted via various aromatization methods

Table 1 presents the concentration of bioactive compounds from saffron stigmas in sunflower oil extracted using b-UAE, p-UAE, and SAM (one week) methods under optimal conditions Results show that picrocrocin content in sunflower oil is higher with the SAM method compared to UAE methods. The SAM method also yields the highest levels of safranal and crocins in sunflower oil. Figure 8 illustrates the color of oils obtained from these three methods, with the SAM method resulting in the most colorful sample.





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**Table 1** The concentration of bioactive compounds in sunflower oil obtained under optimal conditions using various methods. (Saffron stigmas: 0.7 % w/v)

| Method | Time | Temperature (˚C) | Picrocrocin (mg/L) | Safranal (mg/L) | Crocin (mg/L) |
|---|---|---|---|---|---|
| b-UAE | 30 min | 70 | 98.8 ± 1.11 | 14.7 ± 1.37 | 51.7 ± 1.23 |
| p-UAE | 45 min | RT | 231 ± 2.40 | 31.2 ± 1.68 | 36.4 ± 1.55 |
| SAM | 1 week | RT | 317.2 ± 0.91 | 55.8 ± 0.99 | 160.1 ± 1.36 |

RT= room temperature

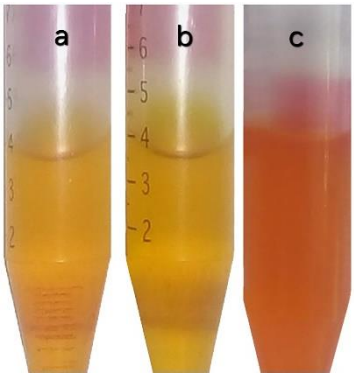


**Figure 8** Color of the aromatized oils using methods: a) b-UAE, b) p-UAE c) SAM.

### 3.5 Physicochemical properties of saffron stigma-aromatized sunflower oil

For the optimized oils, various physicochemical parameters were measured, including TPC, TCC, antioxidant capacity using the DPPH method, AV, PV, K232 and K268. The results in Table 2 show that TPC in aromatized sunflower oils is 1.4 to 1.8 times higher than the control, while TCC is 4 to 10 times higher. TPC and TCC from p-UAE are higher than b-UAE, likely due to the ultrasonic probe's higher extraction power. TPC from b-UAE with SAM is approximately equal. However, TCC using SAM is higher than UAE methods, suggesting slower, more stable extraction compared to UAE methods, which may degrade sensitive compounds like carotenoids. Hosseini et al. demonstrated the detrimental effect of ultrasonic probes on beta-carotene in sunflower oil, where increasing power over 5 min reduced beta-carotene levels [37].

The aromatized sunflower oils displayed greater DPPH free radical scavenging activity compared to the control, with oils from b-UAE and SAM methods exhibiting the highest antioxidant activity. This increase is likely attributed to higher phenolic and carotenoid contents, enhancing antioxidant capacity. Similarly, Bhimjiyani et al. found that flaxseed oil enriched with sea buckthorn had superior antioxidant capacity compared to the original oil [15]. Hamad et al. also demonstrated that adding spices like turmeric to palm oil using the maceration method improved antioxidant capacity [5].



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According to Gondkar et al., the acceptable AV range for sunflower oil is between 0.7 and 2.0 [38]. The AV of the control oil in this study fell within this range, consistent with typical properties of sunflower oil (1.17 ± 0.26). A slight increase in AV was observed during the aromatization process with saffron stigma, yielding values between 1.4-1.8. PV of the control oil was 9.7 ± 0.8, aligning with previous reports for sunflower oil [39]. Aromatizing the oil with saffron stigma resulted in a reduction in PV, indicating increased oil stability, likely due to decreased formation of primary oxidation compounds. As Table 2 shows, sunflower oil aromatized using the p-UAE method had higher TPC levels compared to oil obtained through b-UAE, resulting in a lower PV. Similarly, Moustakime et al. demonstrated that aromatizing olive oil with *Pimpinella anisum* using the maceration method increased AV and decreased PV [14]. Soares et al. showed that aromatizing extra virgin olive oil with rosemary and basil using b-UAE increased the PV [40], while Sousa et al. found that flavoring olive oil with garlic increased the AV and decreased the PV, with no significant effect observed for hot chili pepper aromatization [41]. Reports by Hosseini and Halim suggest that the ultrasonic process can increase PV of oil due to its destructive effect [37] [42]. However, saffron-aromatized sunflower oil in this study exhibited lower PV and higher stability compared to the control, attributed to its increased content of TPC and TCC introduced during the UAE process.

**Table 2** Physicochemical properties of saffron stigma-aromatized sunflower oil using different methods.

| Sample | TPC (mgGAE g oil$^{-1}$) | TCC (mg Kg$^{-1}$) | DPPH (%inhibition) | AV (mgKOH/g) | PV (meq$O_2$/kg) | K232 | K268 |
|---|---|---|---|---|---|---|---|
| Control** | 7.81 ± 1.11 | 0.22 ± 0.04 | 33.43 ± 0.65 | 1.17 ± 0.26 | 9.67 ± 0.85 | 11.6 ± 0.35 | 1.92 ± 0.25 |
| b-UAE | 10.69 ± 1.77 | 0.91 ± 0.05 | 53.09 ± 2.76 | 1.68 ± 0.30 | 5.05 ± 1.48 | 14.74 ± 0.40 | 1.75 ± 0.07 |
| p-UAE | 13.69 ± 2.01 | 1.71 ± 0.96 | 38.42 ± 1.28 | 1.45 ± 0.78 | 4.95 ± 1.91 | 16.13 ± 0.90 | 3.13 ± 0.01 |
| *SAM | 11.64 ± 0.76 | 2.24 ± 0.74 | 49.60 ± 1.63 | 1.79 ± 0.64 | 5.30 ± 1.98 | 11.09 ± 0.81 | 1.85 ± 0.07 |





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*Stirred for 1 week, **control= unflavored sunflower oil



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The extinction coefficients K232 and K268 are crucial indicators of oil stability and purity, with K232 reflecting primary oxidative degradation products and K268 indicating secondary products. Analysis of these coefficients in Table 2 reveals that both short-term and long-term stability indices of saffron stigma-aromatized sunflower oils obtained by the UAE method surpass the control, suggesting deteriorative conditions accompanied by a burnt odor. Due to the more rigorous conditions of p-UAE compared to b-UAE, the K232 and K268 indices for sunflower oil obtained through p-UAE exceed those obtained through b-UAE. Previous studies by Hosseini and Halim demonstrated that ultrasonic processes can elevate the K232 coefficient, reflecting ultrasound's destructive effect on sunflower oil [37, 42]. Conversely, these indices slightly decrease for saffron-aromatized sunflower oil obtained by the SAM method, attributed to the mild extraction conditions that preserve the oil's integrity during extraction, allowing saffron bioactive compounds to dissolve gently. Moustakime et al. also observed a slight reduction in K232 for aromatized oil compared to the control, with higher values noted for UAE compared to maceration [14]. Additionally, they reported an increase in K268 for aromatized oils, with a more pronounced increase observed for UAE compared to maceration. Similarly, Sousa et al. showed that K232 decreased slightly for aromatized oil with garlic and oregano compared to the control, while K268 remained relatively unchanged [41].

## 4 Conclusions

Various methods including ultrasonic waves, maceration, microwave, heat, and stirring were employed to enhance the dissolution efficiency of saffron stigma's active compounds in sunflower oil. While the ultrasonic method yielded good extraction efficiency in a shorter time, it also resulted in oil destruction, leading to undesirable properties in the oil. Substituting the ultrasonic probe with a bath reduced the destruction intensity but still couldn't prevent damage. Similarly, the microwave method only caused compounds degradation. On the other hand, the simpler and more cost-effective maceration method was also employed, which can be easily applied in the industry. Despite its slow process, this method achieved good extraction results comparable to the ultrasonic extraction method. It provided the highest concentrations of safranal, crocins, and picrocrocin without causing compound degradation. The resulting saffron-aromatized sunflower oil had superior physicochemical properties, including increased TPC, TCC, and antioxidant capacity, indicating enhanced oxidative stability. Moreover, the PV and extinction coefficients K232 and K268 decreased significantly, indicating less formation of degradation products. Therefore, the saffron-aromatized sunflower oil produced by the SAM method was deemed the most suitable for various applications, offering high concentration of active compounds, desirable color and aroma, and considerable oxidative stability. Moreover, the final product exhibited a desirable aroma and flavor, and no color changes were observed during the storage period.

## Acknowledgements





[ DOI: 10.22034/FSCT.22.160.142 ]

The research council and the graduate office of the University of Tehran are thanked for supporting this project.

## Conflict of Interest

The authors have declared no conflict of interest.

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[ DOI: 10.22034/FSCT.22.160.142 ]

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[ DOI: 10.22034/FSCT.22.160.142 ]

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# استخراج ترکیبات زیست فعال کلاله زعفران در روغن آفتابگردان: یک مطالعه مقایسه‌ای روش‌های مختلف استخراج جامد-مایع

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**اطلاعات مقاله**

**تاریخ های مقاله :**

**تاریخ دریافت: ۱۴۰۳/۲/۱۶**

**تاریخ پذیرش: ۱۴۰۳/۸/۶**

**کلمات کلیدی:**

استخراج به روش خیساندن،

استخراج به کمک ماکرویو،

معطرسازی روغن،

روغن آفتابگردان،

کلاله زعفران *Crocus sativus* L.،

استخراج به کمک فراصوت

**DOI: 10.22034/FSCT.22.160.142.**

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**چکیده**

معطر کردن روغن‌های خوراکی با هدف افزایش پایداری اکسیداتیو و ایجاد ویژگی‌های حسی مطلوب برای گسترش بازار آنها انجام می‌شود. هدف این مطالعه تولید روغن آفتابگردان معطر با طعم زعفران با استفاده از روش‌های مختلف استخراج جامد-مایع از جمله استخراج خیساندن به کمک همزدن (SAM)، استخراج به کمک فراصوت (UAE)، و استخراج به کمک مایکروویو (MAE) بود. این مطالعه با هدف بهینه‌سازی پارامترهای مؤثر در هر روش استخراج و شناسایی ویژگی‌های فیزیکوشیمیایی روغن‌های معطر حاصل انجام شده است. روش UAE به طور موثر ترکیبات زیست فعال کلاله زعفران را در زمان کوتاه‌تری استخراج کرد. با این حال، اعمال انرژی بالایی در طول فرآیند UAE منجر به تضعیف ویژگی‌های فیزیکوشیمیایی و حسی آن شد. به طور مشابه، در روش MAE، ترکیبات حساس و فرار زعفران تجزیه شده و از بین رفتند. به عنوان جایگزین، روش SAM با یک فرآیند ملایم و مداوم، موفق به انحلال غلظت بالای ترکیبات زعفران در روغن شد در حالی که ویژگی‌های فیزیکوشیمیایی و حسی آن را نیز حفظ کرد. غلظت بهینه‌ی پیکروکروسین، سافرانال، و کروسین به ترتیب ۳۱۷، ۵۶، و ۱۶۰ $mg\ L^{-1}$ در روغن آفتابگردان بدست آمد. روغن آفتابگردان معطر شده حاصل، یک محصول نوآورانه و پر امید برای کاربردهای متنوع غذایی و گسترش بازار می‌باشد.