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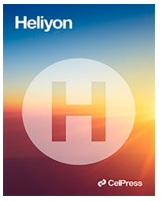

Research article

Ultrasound treatment on saffron *(Crocus sativus* L.*)* corm: Impact on textural, morphological, and microbial properties and stigma-derived metabolite compositions

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ABSTRACT

This research aimed to assess the influence of ultrasound at a frequency of 37 kHz on various parameters of saffron (*Crocus sativus* L.) plants, including flowering efficiency, morphological, and textural properties, corm surface microbial population, and the concentrations of key secondary metabolites in saffron stigmas. Ultrasound was applied to saffron corms during two distinct growth stages, namely flower induction and flower initiation, utilizing varying sonication durations (0, 15, 30, and 60 min). Notably, flowering efficiency experienced a significant decrease ($P \leq 0.05$) after 60 min of sonication compared to the control at both stages. In contrast, an increase in sonication time from 0 (control) to 15 and 30 min resulted in a noteworthy augmentation in the number of flowers and flowering efficiency. The structural morphology of corms did not exhibit significant alterations under ultrasound at different durations within each stage. Extended exposure time showed efficacy in diminishing microbial population on corm surfaces and reinforcing corm resistance against compressive forces. High-performance liquid chromatography (HPLC) analysis of saffron stigmas revealed that a significant increase in crocin content compared to the control at both stages, a trend that persisted with increasing sonication time up to 60 min. However, picrocrocin and safranal contents exhibited a decrease in both stages with prolonged sonication. These findings imply that ultrasound not only impacts the flowering efficiency of saffron corms but also exerts an influence the concentrations of secondary metabolites in the resultant stigma.

1. Introduction

Ultrasound refers to sound with a frequency higher than 20 kHz which is greater than the human audible range (0.25–16 kHz). A number of studies have reported the effect of sound waves with a wide range of frequencies on plants and their growth [1–4]. Maleki Farahani et al. [5] investigated the effect of ultrasound with two frequencies (40 and 59 kHz) on the seed germination of *Cuminum cyminum* at different exposure times (0, 2, 4, 6, 8 and 10 min). They concluded that application of ultrasonic wave had a positive effect on *C. cyminum* seeds germination. Omidi and Dashab [6] reported the effect of ultrasound at different powers (60, 80, and 100 W), time

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durations (2, 4, 6, 8, and 20 min) and frequencies (40 and 59 kHz) on seed germination of rapeseed cv. Modena. They observed an improvement in seed germination with pre-treatment of seeds at both frequencies of 40 and 59 kHz with the power of 100 W. Some studies have been carried out on the effect of ultrasound on the secondary metabolites production of the plants [4,7–10]. Li et al. [11] applied sound on different organs of *Dendrobium candidum* such as leaves, stems and roots, for 6 days. These treatments were caused to observe some changes in the antioxidant enzyme activity. Moreover, Tovar-Pérez et al. [12] studied the effect of ultrasound on the quality of tomato fruits. They measured the contents of polyphenols, lycopene and rutin after harvesting. It was found that the contents of polyphenol, lycopene and rutin significantly increased in both pulp and tomatoes skin. On the other hand, the effect of ultrasound was also studied on fruits and vegetables in different trials such as surface microbial contaminations [13–16], mechanical characteristics [17], and their morphology [18,19].

Saffron (*Crocus sativus* L.) is one of the most world expensive medicinal plant which is known due to the constituents of its dry stigma such as apocarotenoids, flavonoids and anthocyanins [20]. The main secondary metabolites from the dry stigma are including, crocin [crocetin di-(β-gentiobiosyl) ester], picrocrocin [4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde] and safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1- carboxaldehyde) [21] which are responsible for stigma color, taste and aroma, respectively. These compounds can be analyzed with various methods of chromatography [22–26].

In the context of saffron cultivation, phonological studies have traditionally been conducted with the primary objective of practical application in agriculture. These studies aim to establish the most suitable timing for implementing crucial practices such as fertilization, the application of crop protection agents (such as herbicides, fungicides, and insecticides), utilization of plant growth regulators, strategic pruning, effective pollination techniques, acquiring propagation material, and informed decision-making regarding the optimal harvest period [27]. Phonological cycle of saffron was illustrated in Fig. 1. A fully developed corm has a long dormancy during May to early September (Fig. 1A). In this phase, apparently, there is no physiological activity. However, at the end of this period the bud flower induction happens, and subsequently flower initiation will be carried out in early October. This is succeeded by flower formation through emerging cataphylls containing flowers (Fig. 1B). Finally, the flowers will appear based on the environmental conditions in the middle of October to Mid-November.

Despite numerous reports on the effect of ultrasound on plant production performance, physico-chemical properties, and the

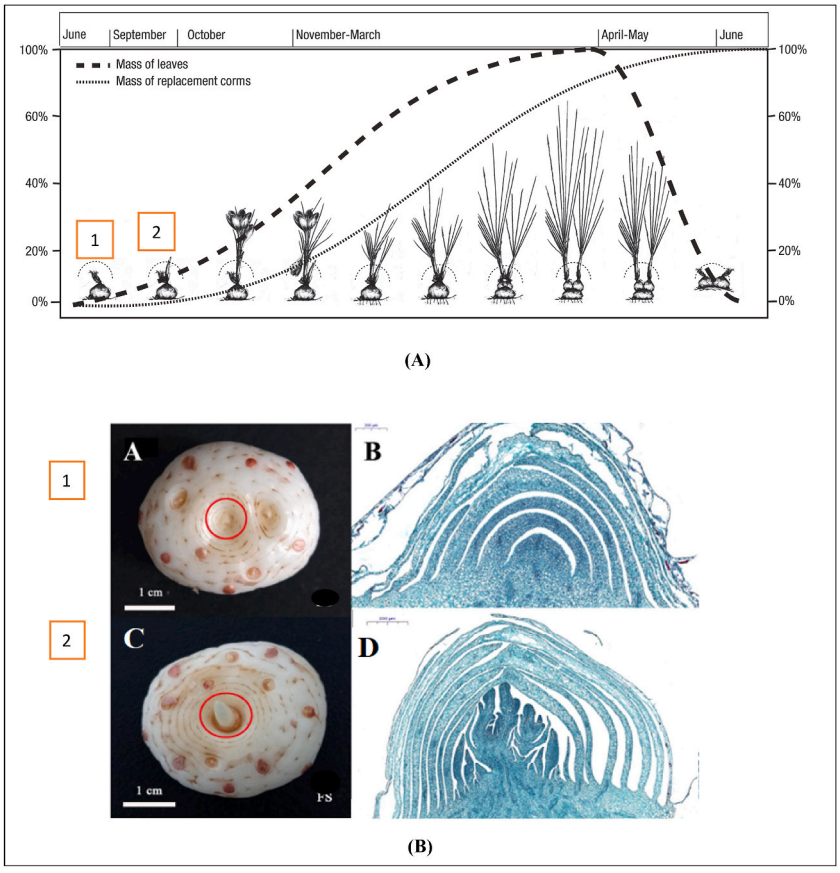

Fig. 1. A) Phonological stages of saffron plant according to the BBCH Scale; (1) flower induction and (2) flower initiation [27]. B) Morphological and developmental changes during saffron apical bud outgrowth (1, a & b) Apical bud; an undifferentiated tissue inside the corm when the corm is in dormant period, early August. (2, c & d) Apical bud; a differentiated tissue when the corm starts the physiological activities and forming the flowering organs [48].

formation of active compounds (primary or secondary metabolites) [10,28,29], a gap in the literature is evident concerning the application of ultrasound on saffron corms growth and the extraction of secondary metabolites from stigmas. Consequently, this study was conducted to comprehend the effect of ultrasound on flowering efficiency and the stigmas content of main secondary metabolites (i.e. crocin, picrocrocin and safranal) derived from sonicated corms under aeroponic greenhouse cultivation. Additionally, given the need to remove outer shells in greenhouse cultivation due to the high relatively humidity (75–85 %), the potential of corm surface contamination was also assessed. Furthermore, to establish the relationship between flowering yield and the textural and morphological properties of sound-treated corms, texture and scanning electron microscope (SEM) analysis of the corms were also addressed.

2. Materials and methods

2.1. Materials

This study was performed for two consecutive years during August to October in 2021–2022. Saffron corms were collected from Zaveh region (Torbat Heydarieh, Razavi Khorasan provience, IRAN) with coordinates of 59°, 27′, 57.390″ (Longititude); 35°, 16′, 19.553″ (Lattitude); and 1350 m (altitude). Plate count agar (PCA) as medium was provided from Ibresco (Iran). Methanol and acetonitrile (HPLC grade), natrium coloride (NaCl), Standards for crocin PHL80391: ≥95 %) and safranal (W338907: ≥90 %) were purchased from Merck Company (Germany). The standard of picrocrocin (sc-479341) was purchased from Santa-Cruz Biotechnology (Germany). For all tests, HPLC grade distilled water was used.

2.2. Ultrasound treatment

2.2.1. Preparation of saffron corms

Saffron cormwith the range of 10–20 g were selected for this study (total number of corms was 414). After removing the outer shells, the corms were placed in the baskets (dimensions of 20 cm × 30 cm) on nursery of coco peat. Each treatment had three baskets as replication. The equal numbers of 46 corms were arranged inside each basket whose weight distributions in each basket were considered the same as well. The baskets were put up on the shelves of the growth chamber after coding.

2.2.2. Sonication

Sonication on saffron corms was carried out in two separate steps, flower induction early in August and the flower initiation, in the middle of October, according to Fig. 1A. For ultrasound treating, 46 saffron corms (each basket) were packed in a double zipped plastic bag with the dimension of 25 cm × 30 cm. The bags were then completely sealed and placed in an ultrasonic bath (Elma, Elmasonic P, Germany). The sonication was applied in a frequency of 37 kHz (as installed on the device) at the amplitude of 100 % in different time durations (10, 30, and 60 min per day) during a period of 7 days for each step (flower induction and flower initiation). Sonication was conducted in a bath water at the temperature of 25 °C ± 2 °C. For the control samples, the similar procedure was followed without sonication.

2.2.3. Growth chamber conditions

All the baskets were incubated in the growth chamber for flower induction and initiation under aeroponic (soilless) cultivation as in Table 1.

Lightning to the saffron corms during flower initiation stage was provided by light-emitting diode (LED) solar lens (Samsung led, SMD 5730 RGB LED chip, Korea) which placed on the top of the baskets with a distance of 50 cm [30].

2.2.4. Collecting saffron flowers

After corms flowering, all the harvested flowers were collected, counted, and substantially weighed and recorded based on flower weight per corm and basket. Then, stigmas were separated and weighed before and after drying at room temperature.

For better understanding of the effect of ultrasound on corm physiological properties, the flowering efficiency (FE) was also evaluated based on equation (1), where N_c and N_t denoted as the number of flowers in the control and sonicated corms, respectively:

$$\text{Flowering Efficiency } \% = (N_t - N_c)/ N_c \times 100 \quad (1)$$

Table 1
The environmental conditions in growth chamber during two stages of flower induction and initiation.

Conditions	Stage	
	Flower induction	Flower initiation
Date of ultrasounic treating	Aguste 1-7	September 24–30
Ultrasounic duration (day)	7	7
Temprature (°C)	26 ± 1	15 ± 1
Relative humidity (%)	75 ± 5	80 ± 5
Photoperiod (h)	Dark (Completely darkn)	8 h light (and 16 h dark)

2.3. Characterization

2.3.1. Texural and morphological analyses

Texture analyzing was carried out for the treated corms after sonication in each stage. The corms without shells were cut into small pieces with a dimension of 1 cm^3. Compression test was done by a texture analyzer device (TA.XT plus, Stable Micro Systems, UK). A cylindrical probe (20 mm diameter and 0.83 mms^{-1} speed) was used and the loading force was adjusted to 50 % of the sample height while the pick positive force was defined as firmness (N(of the corms. The sample orientation was kept constant for all samples. Each test was performed in three replications [31].

The microstructure of the dried (in liquid N_2) slices of saffron corms was studied by scanning electron microscope (SEM) (HITACHI, Model S4160, Japan) at 10 kV voltages. For this study, corm slices were prepared in 3 mm thickness [32].

2.3.2. Microbial test (total plate count)

For the evaluation of the corms microbial population, a sterile swab was impregnated with sterile physiological serum (NaCl solution, 8.5 g L^{-1}), drawn on the surface of the corm (2 cm × 2 cm) in different directions. It was then put transferred into a falcon tube containing 9 ml of sterile physiological serum. The rest of the steps were followed from Ref. [33]. Pour plate culture method (PCA) was used for total counting of the microbial flora. The prepared plates were placed in an incubator (Memmert, Unb400, Germany) at 37 °C for 48 h. At the end of the incubation period, microbial colonies were counted and reported (Log CFU cm^{-2}). Limit of detection was 1 LOG CFU cm^{-2}.

2.3.3. HPLC analyses

Quantification of crocin, picrocrocin, and safranal content of stigma extracts were performed by HPLC/UV–Vis [34–36]. For this purpose, saffron stigmas were collected at the end of flowering step, dried and powdered with a mortar and pestle. The extraction was carried out based on Razavizadeh & Arabshahi Delooei [37] protocol. Accordingly, in this method, (0.1 g) flower powder was added into the (10 mL) Methanol (80 %), and shaked for 1 h in the darkness. It was then centrifuged (Ortoalresa, digicen 21, Spain) at 4000 rpm for 15 min (Minisart, RC25). Subsequently, the supernatant was filtered by a cellulose acetate siring filter (diameter 0.45 μm). 20 μL of stigma extract was injected to the HPLC (Waters, Model: 1525 Binary HPLC Pump, USA) equipped with a GLScience C_{18} reverse phase column (specifications: 150 mm × 4.6 mm, and particle size 5.0 μm) (GLScience, InertSustain C_{18} Analytical Column, Japan) and UV–Vis detector. Detection of metabolites was made at the wavelengths of 254, 440 and 330 for picrocrocin, crocin, and safranal respectively [34,35].

The crocin standard solution was prepared by dissolving 10 mg of standard powder in 5 ml of 50 % methanol to make a final concentration of 2 gL^{-1}. The analysis of crocin was carried out at 30 °C with the eluents of water and methanol as solvent A and B, respectively. The gradient flow program was started as A to B (80:20 %, v/v), with a linear change to 20:80 %, (v/v) at 1 mL min^{-1} in 60 min. All HPLC measurements were performed in 3 replicates [37].

For HPLC analysis of picrocrocin, a standard solution (1 mg picrocrocin per L methanol as a solvent) was used. Picrocrocin analysis was done at 30 °C with the eluents of water (solvent A) and acetonitrile (solvent B) in a gradient program: 80 % A, 0–5 min; 80 to 20 % A, 5–15 min; and 20 % A, 15–20 min with a flow rate of 0.8 mL min^{-1} [35,36].

Safranal standard solution (10 mg mL^{-1} in methanol as the solvent) was also prepared. It was then used for preparing different dilutions (10, 20, 30, 40, and 50 μg L^{-1}). Safranal was then analyzed by HPLC as explained for picrocrocin [38].

2.4. Statistical analysis

Statistical analysis of the recorded data was performed based on multilevel factorial experiments under completely randomized design. The input parameters were included of two factors; flowering steps (Factor A) at two levels (flowering induction and initiation) and sonication time (Factor B) at four levels (0, 15, 30, and 60 min). The output variables were included of flowering efficiency, textural and morphological characteristics, corm microbial populations, and the quantification of secondary metabolites in stigmas derived from sonicated corms.

All experimental treatments were replicated three times. Analysis of variance (ANOVA) was conducted using Minitab software

Table 2
Statistical analysis repoert for the effect of ultrasound on saffron corms in two steps of flower induction and initiation in different exposure times (Mean ± SD).

Stage	Time (min)	Corms Weight (g)	Flowers No.	Flowers Weight (g)	Fresh stigma weight (g)
Flower induction	0	568.6 ± 0.7^{a}*	41.0 ± 1.0 ^{d}	8.929 ± 0.497 ^{ab}	0.914 ± 0.051 ^{b}
	15	567.0 ± 0.4^{a}	43.5 ± 1.5 ^{cd}	8.246 ± 0.046 ^{b}	0.866 ± 0.003 ^{b}
	30	568.0 ± 2.5^{a}	53.5 ± 1.5^{a}	10.062 ± 0.802^{a}	1.039 ± 0.128 ^{b}
	60	566.3 ± 0.7^{a}	35.0 ± 0.5^{e}	5.764 ± 0.790^{c}	0.602 ± 0.066^{c}
Flower initiation	0	568.6 ± 0.7^{a}	41.0 ± 1.0 ^{d}	8.929 ± 0.497 ^{ab}	0.914 ± 0.051 ^{b}
	15	568.2 ± 2.7^{a}	47.0 ± 0.0 ^{b}	9.748 ± 0.474 ^{ab}	0.979 ± 0.050 ^{b}
	30	567.2 ± 1.0^{a}	46.0 ± 1.0 ^{bc}	9.223 ± 0.658 ^{ab}	1.477 ± 0.068^{a}
	60	566.6 ± 0.0^{a}	17.0 ± 1.0^{f}	3.359 ± 0.406 ^{d}	0.280 ± 0.042 ^{d}

*Different letters in each column indicate a significant difference based on the Tukey test at the 5 % level ($P \leq 0.05$).

version 4.2.19, and mean comparisons were executed through the Tukey test with a confidence interval of 95.0 %. This rigorous statistical methodology provided a comprehensive understanding on the effect of flowering steps and sonication time on various saffron cultivation parameters.

3. Results and discussion

3.1. Ultrasound effect on flowering efficiency

The results of ultrasound application on saffron corms in two stages of flowering step-induction and initiation-were shown in Table 2. The highest and the lowest significant number of flower was obtained in the flower induction stage for the exposing time of 30 min (with an average value of 53.5 ± 1.5) the flower initiation at 60 min (with average value of 17.0 ± 1.0), respectively. The statistical results showed that the number of flowers in the both stages was increased with compared to their respective controls when the increasing the time of sounding to 30 min. However, this trend was non-significant for the treatment of 15 min in flower induction stage ($p > 0.05$). Increasing the exposing time to 60 min in both stages caused a significant decrease in the number of flowers compared to the control ($p \leq 0.05$).

According to Table 2, the average weight of saffron flowers in the both stages for the exposure times of 15 and 30 min was not significantly different from the control ($P > 0.05$). In contrast, the flower weight at the time of 60 min showed a significant decrease compared to the control ($p \leq 0.05$). Moreover, increasing the exposure time to 30 min did not show any significant effect on the stigma fresh weight in the flower induction stage ($p > 0.05$). However, extending the exposure time to 60 min caused a significant decrease in stigma fresh weight compared to the other periods in this stage ($p \leq 0.05$). Similar results were observed in flower induction stage, except the time of 30 min, which showed the highest significant stigma fresh weight (1.47 ± 0.068 g) compared to the other treatments ($p \leq 0.05$).

The flowering efficiency of saffron corms was plotted in Fig. 2. The results of flowering efficiency show that the times of 15 and 30 min in both stages, was significantly higher than the control ($p \leq 0.05$). In the flower induction stage, the highest flowering efficiency was obtained in 30 min with the average of 30.49 % while, in the flower initiation stage; the flowering efficiency in 15 min was found to be 14.63 %, which was not significantly different from the treatment of 30 min (12.20 %) ($p > 0.05$). However, the flowering efficiency in 60 min in the both stages significantly decreased to the average of −58.54 % and −13.41 %, respectively ($p \leq 0.05$). These results indicated that the ultrasound exposure time of 60 min negatively influenced on flowering efficiency of saffron corms especially in the flower initiation stage, which was much more severe.

The sound waves are characterized by some parameters such as the frequency, sound pressure level and sound exposure time. Inaddition to these parameters, the physiological stage of the plant can also inffluence the response, when a plant is exposed to the sound waves [22,39–42]. Several studies demonstrated the effect of ultrasound waves on the growth of seed plants, including germination, seedling and water uptake [3,43,44]. Honarmand et al. [45] exposed shoot apex explants to ultrasonic waves (with a constant frequency of 37 kHz) at different times (0–300 s). They concluded that the percentage of shooting was significantly reduced while, the number of shoots per explant and the percentage of callusing significantly increased due to ultrasound. Lo Porto et al. [44] found that treating soybeans with ultrasound improved seed germination and of sprout formation by increasing the plant water uptake. Afkhami Hoor et al. [46] also evaluated the effect of treating saffron corms with ultrasound of on saffron callus induction and growth. They found that ultrasound stimulated all these parameters. Moreover, the effects of ultrasonic waves on seed germination of some medicinal plants including, *Atriplex lentiformis, Cuminum cyminum,* and *Zygophyllum eurypterum* were also studied by Sharififar et al. [47]. The experiments were performed bsed on different sound exposure time (1, 3, 5, 7, and 9 min). The highest seed germination was found at 5 and 7 min, while a negative effect was observed for 9 min of exposure. They concluded that these improvements could be attributed to effect ofultrasonic waves on the seed cellular walls. Their results reveald that longer exposure time (9 min), might cause a destructive effect on the cell wall. López-Ribera & Vicient [43] also studied the germination of *Arabidopsis thaliana* seeds at different sonication exposure times (30 s–64 min), at the frequency of 45 Hz and a temperature of 24 °C. The optimum germination of the seeds was obtained at 30 s.

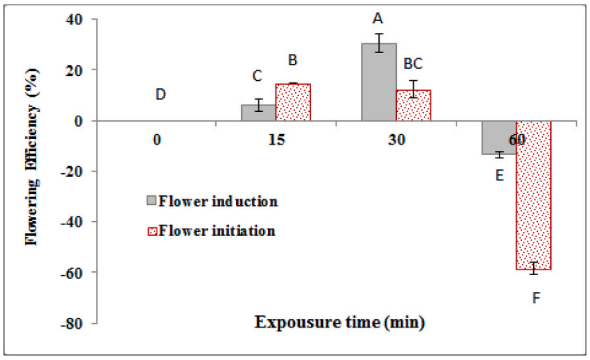

Fig. 2. Flowering Efficiency of the saffron corms in two growth stages of flower induction and flower initiation at different exposure times.

According to the results in the present study, exposing saffron corms to the ultrasound waves (37 kHz) for up to 30 min could improve the flowering efficiency, especially in the stage of flower induction, while increasing the duration to 60 min had a negative effect on the flowering. Comparing the reported results regarding the application of ultrasound on different plant organs a common point emerges: ultrasound at certain level has a positive effect on some plant traits, but in higher levels some undesirable effects might happen. The impact of ultrasound, in addition to the physical characteristics of the sound is influenced by several physiological parameters of the exposed plant organs. For instance, in the present research and study by Sharififar et al. [47], in addition to the applying different frequncy of waves (37 KHz in this study and 43 KHz by Sharififar et al.), both studies exposed saffron corms to ultrasound at different times but with unsimilar purpose. In this study, undifferentiated tissue (induction stage, Fig. 1A) and in the other one a differentiated tissue was subjeted for sounding with aiming induction differentiated organ, flower, and induction of undifferentiated tissue, callus, respectively. Morever, Sharififar et al. [47] simustaneously evaluated the effect of plant growth regulators on callus induction. Due to these different conditions, it is revealed that the level and exposure time of ultrasound can vary for different plant organs.

3.2. Textural and morphological studies

Texture analysis was performed with compression probe on the corms and their results were shown in Fig. 3. Results revealed that in both stages, the applied ultrasound on corms at different exposure times of 15 and 30 min significantly increased the corm firmness compared to the control ($p \leq 0.05$). While, for the exposure time of 60 min, the differences to the control and other duration times were not significant ($p > 0.05$). It seems that increasing the sonication to 30 min resulted in rising the firmness of the corm against the compressive force.

Fig. 4 shows the micrograph images of the cut (vertical) slices of saffron corms at different exposure times during the flower induction stage. The saffron corm exhibits almost regular honeycomb structures of the cell walls. However, some of the cell walls appear torn or damaged, with small particles visible inside the cells. A comparison of the micrograph images of the treatments with the control (Fig. 4A–C) reveals no significant differences among the control and treated corms (15 and 30 min) in the size and structure of corm cell wall holes. However, the 60 min exposure time (Fig. 4D) shows damage to the cell walls.

Micrograph images of the saffron corms in the stage of flower initiation at different exposure time are presented in Fig. 5(A–D). It shows that the cell walls were completely destroyed, with all the cell contents including starch particles of the corms, visible. In addition to the intracellular contents, fibers related to the cell walls are also observable (Fig. 5A–D). The control treatment (Fig. 5A) shows similarly structure to the other treatments in the flower initiation stage. Overall, the differences in textural characterization between Figs. 4 and 5 can be attributed to the physiological conditions of the corms. Fig. 4 presents the corm textural conditions during the dormancy period, indicating that corms tend to become firmer and more turgid as they conserve water and nutrients. This firmness results from the corms' reduced metabolic activity and water loss prevention mechanisms. In contrast, Fig. 5 represents the tissue at the end of dormancy, when corms prepare for sprouting; at this stage, they may become less firm due to the mobilization of stored nutrients and increased metabolic activity. Fig. 1B also confirms these changes, showing that during the flowering induction stage, the tissue is undifferentiated, whereas in the flowering initiation stage, differentiated tissue appears [48].

End of Dormancy: As dormancy ends and the corms prepare for sprouting, they may become less firm due to the mobilization of stored nutrients and increased metabolic activity.

The application of ultrasound may affect the morphology and texture of plant organs, depending on the processing conditions and the type of tissues [17]. Saeidirad et al. [49] studied on the effect packaging for saffron corms concluded that the application of compressive force is influenced by the moisture content of saffron corms and concluded that the application of compressive force is influenced by the moisture content of saffron corms. They found a positive relationship between corm crispness and corm moisture content, noting that increased moisture makes the corms softer and reduces their resistance to compressive forces. On the other hand,

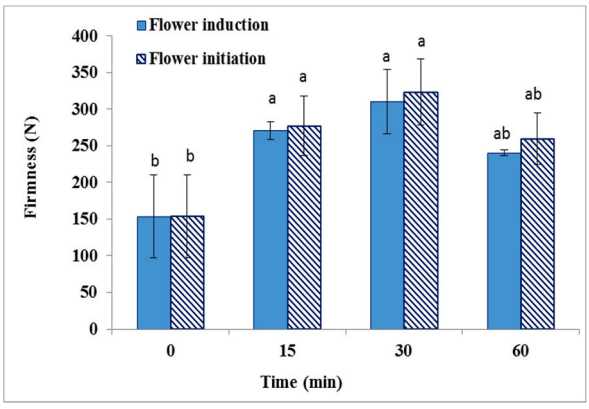

Fig. 3. The effect of ultrasound on firmness of the saffron corms at different sonication exposure times for two growth stages of flower induction and flower initiation (Different letters in each column indicate a significant difference based on the Tukey test at the 5 % level ($P \leq 0.05$)).

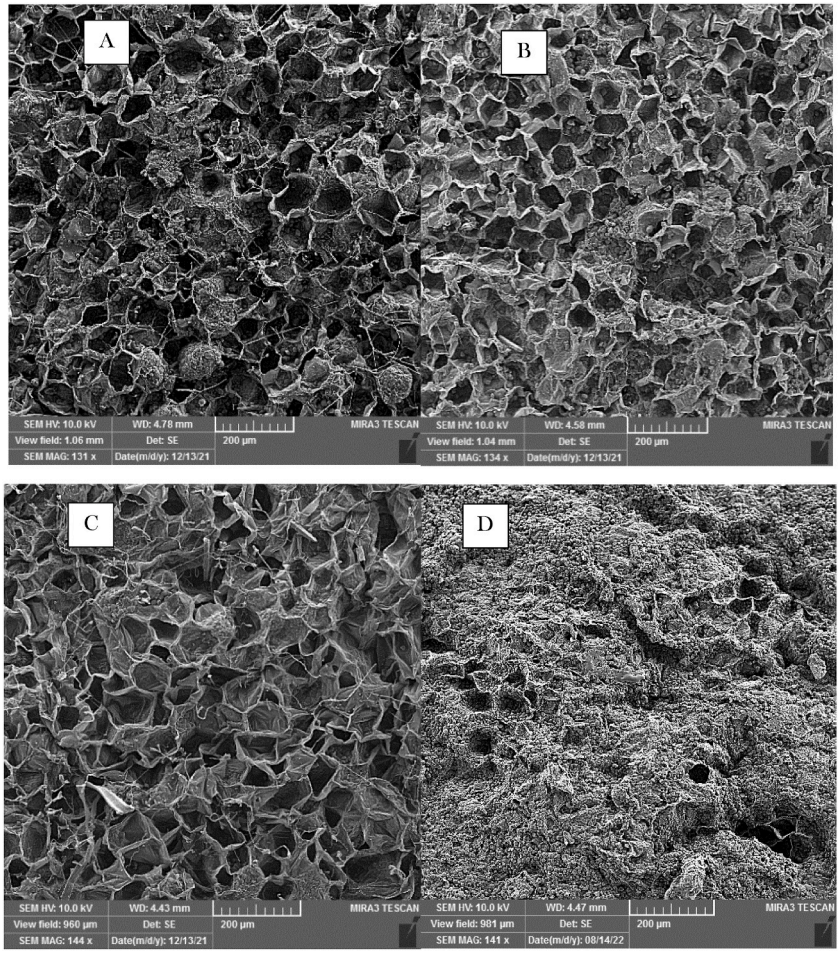

Fig. 4. SEM micrographs of dried corm slices at the flower induction stage and different exposure sonication times: A) 0 min (control), B) 15 min, C) 30 min, D) 60 min,.

Mitra et al. [32] found a non-significant increase in product firmness in their study on vacuum-dried onions, attributing this result to the damage of the porous structure of onions due to disintegration of the cell walls. They attributed this result to the damaging of porous structure of onions due to disintegration of the cell walls. It is important to note that the structural integrity and texture of plant products are primarily determined by factors such as primary cell wall solidity and cell-to-cell adhesion [17]. Thus, ultrasound treatment, ultrasound treatment, along with other biological elicitors, may strengthen plant cell walls through peroxidase-catalyzed oxidation of structural proteins and polyphenols, leading to the formation of cross-links and increased firmness in plant organs.

Moreover, reports on the pretreatment of plants (such as potatoes, carrots, and pears) with low-intensity ultrasound revealed the improvement in structure and texture [50,51]. It has been suggested that these results are due to stress induction, activation of the peroxidase catalyzed oxidation of cell wall structure proteins and phenolic compounds, pectin methylesterase catalyzed de-esterification of pectin, or a combination of these mechanisms. However, the specific mechanism has not yet fully understood [17].

Our results suggest that changes in the cellular structures of the corms could be due to the effect of growing stag during which the cell walls disintegrate and the starch content changes as germination progresses, as proposed by Ref. [52].

3.3. Microbial population

The effect of exposure time on the surface microbial contamination on of saffron corms were shown in Fig. 6. These results indicated that the effect of ultrasound on surface microbial contamination depends on the stage at which it is applied. As shown in Fig. 6, increasing the ultrasound exposure time during the flower induction stage significantly decreased the microbial load compared to the control ($p \leq 0.05$). However, no differences were found among exposure times (15, 30, 60 min). In the flowering initiation stage, no significant reduction in the microbial population was observed except for the 60 min exposure, which was significantly lower than others ($p \leq 0.05$).

Ding et al. [14] investigated the effect of ultrasound combined with slightly acidic electrolyzed water on microbial contamination and the quality of cherry tomatoes and strawberries. They found that this method reduced total aerobic bacteria, yeasts and molds on

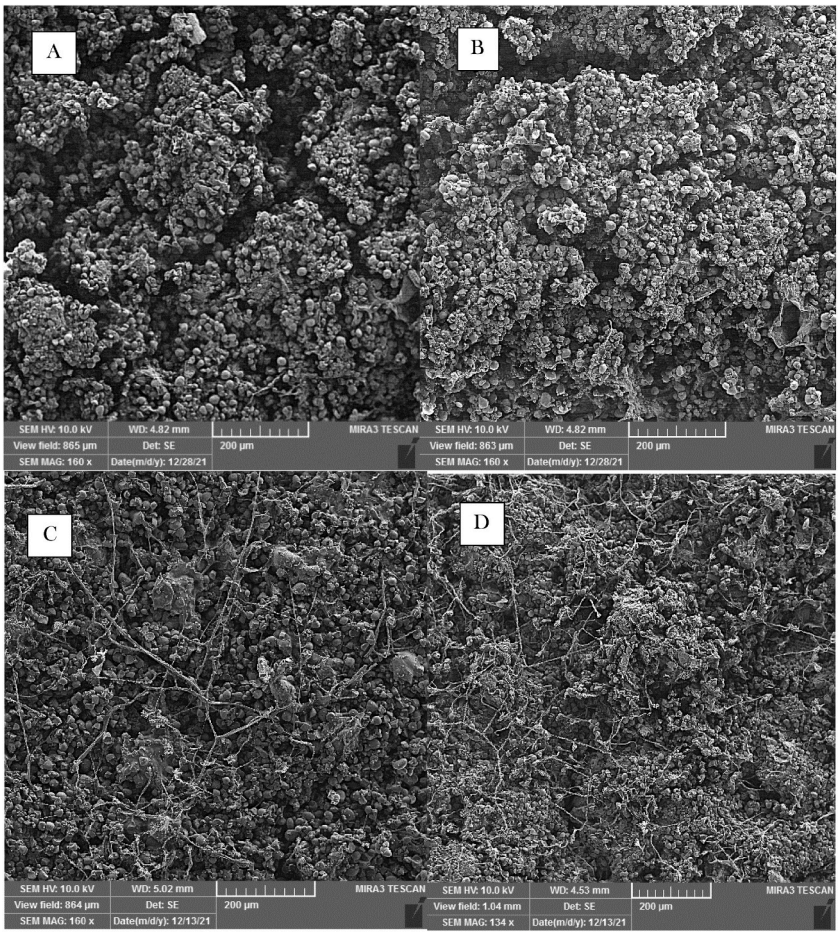

Fig. 5. SEM micrographs of dried corm slices at the flower initiation stage and different exposure sonication times: A) 0 min (control), .B) 15 min, C) 30 min, and D) 60 min.

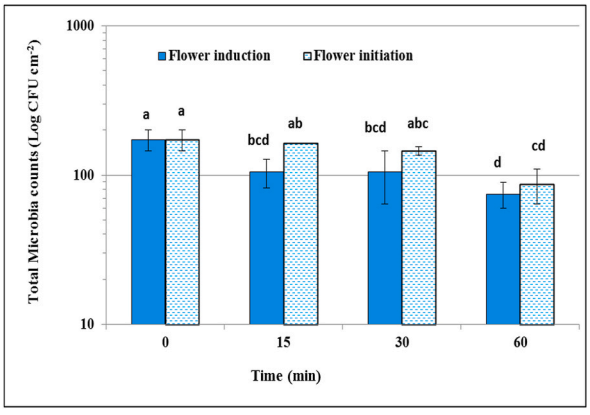

Fig. 6. Microbial papulation of the saffron corms surface at different sonication exposure times for the stages of flower induction and flower initiation (Different letters in each column indicate a significant difference based on the Tukey test at the 5 % level ($P \leq 0.05$)).

the fruits. Fan et al. [15], in their review, proposed that application of ultrasound on fruit and vegetables can inactivate spoilage and pathogenic microorganisms on the surface of these products. Similarly, the present findings show that ultrasound significantly reduced microbial contamination on the corm surfaces, especially during the flower induction stage.

3.4. Secondray methabolites quantification

The main secondary metabolites extracted from sonicated corm-derived stigmas were quantified by HPLC. As shown in Fig. 7A, the crocin content in ultrasonic-treated corms samples significantly increased over time ($p \leq 0.05$). The highest crocin levels were obtained at 60 min in both the flower induction and initiation stages with values of 27.06 % and 25.86 %, respectively ($p \leq 0.05$). Although an increase in crocin content was observed at sonication times of 15 and 30 min compared to the controls in both flower induction and initiation stages, the difference in crocin content between these two exposure times was not significant ($p > 0.05$).

Fig. 7B shows the quantification of picrocrocin content was extracted from the sonicated corm-derived stigmas. With increasing sonication time, the picrocrocin levels in the flower induction stage did not significantly chang compared to the control, ($p > 0.05$). However, in the flower initiation stage, the amount of picrocrocin significantly decreased ($p \leq 0.05$) compared to the control, particularly at 30 and 60 min. At 15 min, the difference from the control was not significant ($p > 0.05$).

The quantification of extracted safranal from the sonicated corm-derived stigmas is presented in Fig. 7C. According to this figure, safranal content significantly decreased with increasing sonication time compared to the control in both stages ($p \leq 0.05$), except for the time of 15 min, where the decrement was not significant ($p > 0.05$).

Several studies have investigated the effect of ultrasound on the contents of chemical componentes and metabolites in different plants [7,10,12]. Lin et al. [8] investigated the effects of ultrasound on the growth and secondary metabolite biosynthesis of *Panax ginseng* cell cultures at a frequency of 38.5 kHz and power of 810 W with sonication times ranging from 1 to 4 min in an ultrasound

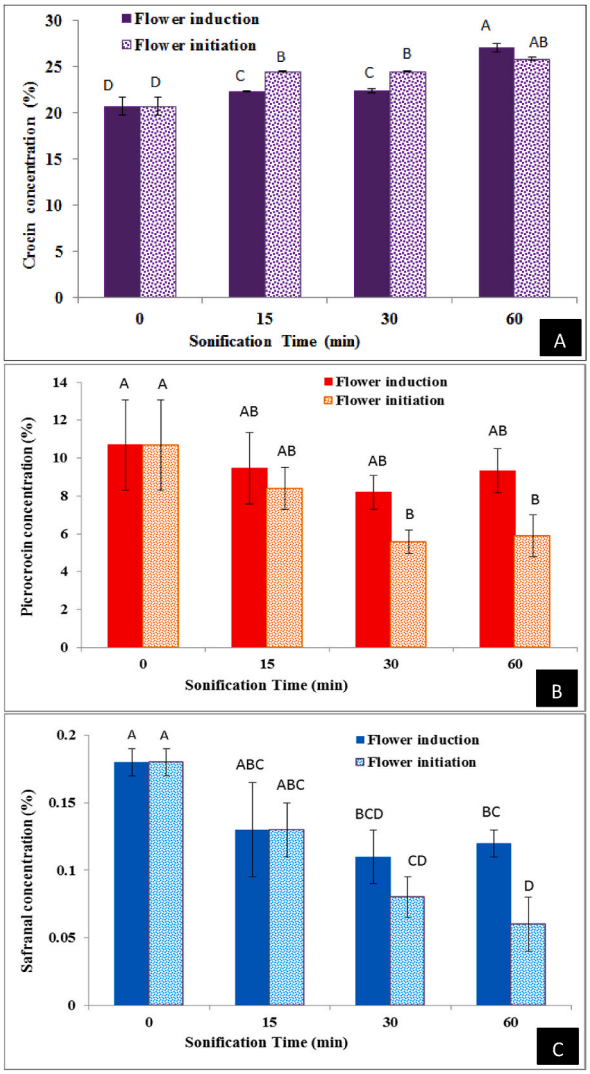

Fig. 7. The metabolites contents in the resulted stigmas from the corms vs. different times of sonication at flower induction and flower initiation stages: (A) crocins, (B) picrocrocin, and (C) safranal (Different letters in each column indicate a significant difference based on the Tukey test at the 5 % level ($P \leq 0.05$)).

bath. They found that ultrasound treatment stimulated the biosynthesis of secondary metabolites and increased the total saponin content of the cells. Russowski et al. [53] used ultrasonic waves (40 kHz for 2.5–5 min) on the liquid culture of *Valeriana glechomifolia* plants and concluded that the content of secondary metabolites including valepotriate was increased. Lu et al. [54] quantified the contents of various secondary metabolites (such as total phenolic, lycopene, carotenoids and ascorbic acid) and antioxidant capacity from ultrasonic treated tomato fruits (25 kHz) in a water bath for 1–4 min. The results showed that ultrasound treatment could increase the contents of secondary metabolites and improve the beneficial health effects of tomatoes.

Several studies have focused on changes in the content of secondary and primary metabolites in saffron under different conditions, such as environmental factors, types of growth regulators, and drying methods. For example, Chrungoo and Farooq [52] found that during the flower induction stage, the carbohydrates content and amylase activity in the saffron corms increased, whereas the starch content decreased. Bagri et al. [55] studied the changes in metabolites contents in saffron corm in different stages of the growth. They revealed that the contents of sugars, amino acids and fatty acids increased as the growth stage shifted from dormancy to flower induction. Fan et al. [15] concluded in their review on ultrasound effects on fruits and vegetables that while ultrasound can decrease mass and cause color change, it also enhances enzyme activity, inhibits spoilage, and preserves nutritional components. Additionally, several reports concluded that ultrasound treatment of a plant organs combined with other biological and physical stressors, may increase the synthesis of secondary metabolites such as phenolic compounds [51,56]. In another study on the effect of acoustic sound waves (in the range of 0.5–16 kHz, audible to human) on the saffron corms during flower induction and initiation stages in the greenhouse, it was found that the contents of secondary metabolites in the stigmas depended on the stage of corm growth, sound wave frequency, and the duration of sounding [37,42]. Although ultrasound may have a different mechanism from acoustic wave, acoustic cavitation has more power and can stimulate the plant cell defense system, causing physiological changes in plant cells and stimulating the production of secondary metabolites [7].

4. Conclusions

Plant treating with ultrasound is considered as a physical stress, so the plant response depends on the organ and its physiological conditions. Although several studies have been conducted on saffron corms, there are limited reports on the effect of the ultrasound saffron characteristics. The findings of the present study indicated that ultrasound treatment on the corms at different stages of growth (flower induction and flower initiation stages) can significantly influence the flowering efficiency and main stigma-derived secondary metabolites such as crocin, picrocrocin and safranal. The results of this research can be applied to saffron production in greenhouse with the aim of increasing flower efficiency and consequently the main and valuable secondary metabolites for industrial applications, including pharmaceuticals, perfumery, and food industry. However, due to the impact of many parameters influencing these phenomena, which have not yet fully understood, more research is need in future to address the remained questions.

CRediT authorship contribution statement

Bibi Marzieh Razavizadeh: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Seyed Mahdi Ziaratnia:** Writing – review & editing, Validation, Investigation, Data curation, Conceptualization.

Availability of data and material (data transparency)

Not applicable.

Ethics approval

Not Applicable.

Consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Code availability (software application or custom code)

Not applicable.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The author declares there is no competing financial interest or personal relationship in this paper.

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