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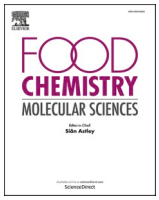

What do microRNA concentrations tell us about the mechanical damage and storage period of strawberry fruits?

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ABSTRACT

Although much research has been performed to investigate the effects of storage conditions, such as mechanical damage and storage period, on the morphological and physiological properties of strawberry fruits, almost all of them have considered severe stress conditions. Finding fruit characteristics that exert significant changes even toward mild and moderate stress conditions can help provide valuable information about the fruit quality during storage. This study aims to investigate various characteristics of strawberry fruits during storage to determine which type of fruit characteristics exert such significant changes toward stress conditions. Identical strawberry samples were subjected to mechanical loading at three levels (1, 2, and 3 N) and then stored at 6 °C for 13 days. Morphological and physiological features, as well as the concentration of several microRNAs involved in strawberry storage, were measured at three-day intervals. The effects of mechanical loading on morphological and physiological characteristics were not significant, while their effects were significant on miR-164, miR-167, and miR-399a. Moreover, while low correlation coefficients were observed between the fruit morphophysiological traits (< 0.6) toward storage conditions, high correlations were obtained between the concentrations of microRNAs. Instead of measuring the morphological and physiological characteristics of fruits, whose behavior is not generally specific toward the stresses, the results show that microRNA concentrations, which can be measured by an electrochemical biosensor, provide us with noteworthy information about fruit quality during storage. These small non-coding molecules exhibited remarkable responses even in mild and moderate stress conditions, making them reliable markers of fruit quality assessment.

1. Introduction

Various characteristics of agricultural products change during the transportation process and the storage period. The measurement of such characteristics might be a suitable indicator of mechanical damage and storage quality during the storage period. The senescence and unfavorable storage conditions of many fruits such as strawberries are among the genetically regulated processes characterized by natural fruit degradation (Zhang & Zhang, 2018). This degradation occurs mainly in the form of a change in texture, flavor loss, changes in lycopene-type carotenoids, and sugar accumulation (Wang et al., 2017; Wang et al., 2020). However, such fruit behaviors are not specific to the storage conditions. For example, various factors, such as storage at high temperatures, mechanical damages during transportation, etc. can result in similar changes in fruit texture (Asefpour Vakilian, 2019; Hashemi Shabankareh et al., 2023). Finding fruit characteristics that can act with high specificity toward the stressors during storage can be beneficial for those who want to know in what conditions fruits have been stored.

In the last decade, the investigation of post-storage quality factors of products has been carried out by investigating the concentration of microRNAs in products such as tomatoes, bananas, pears, and peaches (Ma et al., 2020). microRNAs are small non-coding RNA molecules in plants, animals, and some viruses that consist of about 17–23 nucleotides and have a significant effect on gene expression (Asefpour Vakilian, 2020). The gene and microRNA expression networks help fruit production and quality formation. Research has shown that miR-156/157 as an essential microRNA is involved in almost all stages of fruit development (Wang et al., 2016). The target gene pathway of miR-156 maintains the meristem status of fruit ovary tissues and thus regulates the early stages of development and determines the number of fleshy fruits. On the other hand, miR-160, which targets auxin response factors, regulates long-term embryo development and is essential for fruit set (Lin et al., 2015). Also, miR-397 regulates Arabidopsis seed number and lignin biosynthesis by targeting laccase (Wang et al., 2014).

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Growth of plants such as Arabidopsis is inhibited by reducing miR-172 activity through target gene overexpression or expression of miR-172-resistant AP2, resulting in smaller fruit (José Ripoll et al., 2015). Although miR-172 accumulation increases fruit size in Arabidopsis, the miR-172-AP2 module exhibits a different function in apples. Apple fruit development is negatively regulated by the overexpression of miR-172, leading to a significant reduction in fruit size (Yao et al., 2015). This is because the fruits of different plants grow from different tissues. In contrast to Arabidopsis and tomato fruits, both of which arise from ovaries (Yao et al., 2016), apple fruits are derived primarily from the hypanthium, which is assumed to consist of the fused bases of sepals, petals, and stamens, while the lower ovary turns into a nucleus. These studies show that the effect of specific microRNAs on fruit growth may be specific to fruit type and plant species. In tomatoes, research has revealed a novel role for miR-396 in regulating sepals and fruit size by targeting SlGRF, providing a new way to improve tomato fruit yield (Cao et al., 2016).

In addition to the size and shape of the fruits, other characteristics such as taste and color contribute to the market value of the fruits. Fruit flavor increases with the accumulation of primary metabolites (such as sugars and acids) and secondary metabolites (such as flavonoids and phenolics) (Wang et al., 2022). In strawberries, the presence of miR-399 increases phosphate uptake (Bari et al., 2006), which increases fructose, glucose, and soluble solid content in ripening fruit (Wang et al., 2017). In persimmon, miR-395p-3p and miR-858b regulate bHLH and MYB, respectively, which simultaneously regulate structural genes of tannin biosynthesis (Luo et al., 2015). Fruit color during ripening can be obtained by determining chlorophyll or anthocyanin (Li et al., 2020). Texture is another main quality of fruit that is of interest to consumers (Kamal-Eldin et al., 2020). Studies show that miR-397a inhibits laccase (LAC) gene expression, resulting in reduced fruit quality, as the laccase enzymes are involved in the synthesis of lignin in the cell walls of fruits (Xue et al., 2019). Fruit softening also affects fruit tissue, where cell wall degradation occurs (Pose et al., 2019). microRNAs such as miR-479, miR-399 g, miR-397a, miR-3627-5p, and miR-2950, are involved in the softening of berries and grapes through regulating fruit softening-related target genes. Although thousands of short non-coding RNAs are identified in various organs of strawberry plants, some of them have been recognized as the most effective ones in regulating the fruit response toward senescence and quality loss during cold storage: miR-164, miR-167, miR-172, and miR-399a (Ma et al., 2020).

There are various methods for extracting microRNAs, including polymerase chain reaction (PCR), northern blotting, and biosensing-based spectroscopic and electrochemical techniques. The limitations in previous microRNA extraction methods have led to the expansion of research on the use of biosensors (Turner, 2013). Biosensors have significant advantages such as high specificity, fast response, reproducibility, and cost-effectiveness. According to these advantages, biosensors can replace conventional methods (Bazin et al., 2017). If it can be shown that mechanical loading and storage conditions have a significant effect on microRNA concentrations, then portable biosensors can be used to determine strawberry quality during storage without the need to transfer the samples to the laboratory and measure time-consuming morphological and physiological characteristics. Therefore, the research objective was to determine the significance of the effects of the treatments on the morphological, physiological, and biochemical characteristics of strawberry fruits to investigate if microRNAs can tell us something more than morphophysiological traits.

2. Materials and methods

2.1. Plant material and experimental design

Identical and full-red fresh strawberries (*Fragaria ananassa* L. var. Albion) variety were obtained from a research greenhouse near Gorgan City, Golestan province, Iran (36.84° N, 54.43° E) in Spring 2022, and transferred to the laboratory with full caution. For subjecting the strawberry samples to quasi-static loads, a universal testing machine (STM5, Santam, Tehran, Iran) equipped with compression platens was utilized. The compression platens allowed for the application of forces in two perpendicular directions on the lateral sides of the samples. Forces of 0 (control), 1, 2, and 3 N were applied at a speed of 0.015 m s^{-1}. The purpose of this treatment was to gain a better understanding of the changes in qualitative properties, color parameters, and concentration of microRNAs due to the application of mechanical force during the transportation and storage of strawberries. In the following, the samples were stored at a constant temperature of 6 °C for 13 days and the sampling to measure morphological, physiological, and microRNA characteristics was done with three-day intervals, i.e., at five storage periods (day 1, 4, 7, 10, and 13). All experiments were performed with 20 replications. Considering the fact that four levels of mechanical loading were applied to the fruits and five storage periods were investigated in this work, while the study was replicated with 20 replications, a total of $4 \times 5 \times 20 = 400$ strawberry fruits were used in this study.

2.2. Measuring morphological traits

The images of strawberry samples were captured by a 12-megapixel mobile phone camera (iPhone 13 Pro Max, Apple, Cupertino, USA) from two sides in the ambient lighting conditions and a distance of 30 cm from the sample. After image acquisition, a sub-image with dimensions of approximately 250 × 150 pixels containing the red part of the strawberries was cut from each image, and the new images were processed by a program written in the MATLAB R2020b software environment (MathWorks, Natick, USA) and color information based on scale-invariant features was measured in each image.

The morphological characteristics of the samples included color characteristics in three models RGB, HSV, and L*a*b* (Table 1) as well as image texture including energy, entropy, and local homogeneity of the image. The gray-level co-occurrence matrix (GLCM) was used to calculate the textural characteristics of the images. As the elements of GLCM, $P(k,l)$ is obtained by counting the number of pairs of pixels (x_1, y_1) and (x_2,y_2) that have gray values k and l. According to previous studies, three characteristics of image texture are more important than other characteristics (Asefpour Vakilian & Massah, 2017), which are: entropy, which shows the irregularity of the gray level of the image (Eq.

Table 1
Color models and features used in this work.

Color model	Feature	Equation
RGB	R	$R = R/(R+G+B)$
	G	$G = G/(R+G+B)$
	B	$B = B/(R+G+B)$
HSV	H	$H = \begin{cases} 0^\circ & \text{if } M-m=0 \\ 60^\circ \times \left(\frac{G-B}{M-m} mod\, 6\right) & \text{if } M=R \\ 60^\circ \times \left(\frac{B-R}{M-m}+2\right) & \text{if } M=G \\ 60^\circ \times \left(\frac{R-G}{M-m}+4\right) & \text{if } M=B \end{cases}$ where $M = max(R,G,B)$ and $m = min(R,G,B)$
	S	$S = \begin{cases} 0 & \text{if } M=0 \\ \frac{M-m}{M} & \text{if } M \neq 0 \end{cases}$
	V	$V = M$
L*a*b*	L*	$L^* = 116 \times f(Y/Y_n) - 16$ * where $f(t) = \begin{cases} \sqrt[3]{t} & \text{if } t > 0.0089 \\ 7.787 \times t + 0.1379 & \text{otherwise} \end{cases}$
	a*	$a^* = 500 \times (f(X/X_n) - f(Y/Y_n))$
	b*	$b^* = 200 \times (f(Y/Y_n) - f(Z/Z_n))$

* X, Y, and Z in the L*a*b* color model are the color stimulus considered, while X_n, Y_n, and Z_n describe a specified white achromatic reference illuminant.

1)

$$\text{Entropy} = -\sum_{k}\sum_{l} P(k,l)log(P(k,l)) \quad (1)$$

energy, which shows the darkness or lightness of the colors in the image (Eq. 2)

$$\text{Energy} = \sum_{k}\sum_{l} P^2(k,l) \quad (2)$$

and local homogeneity, which shows the uniformity of adjacent colors in the image (Eq. 3).

$$\text{Local Homogeneity} = \sum_{k}\sum_{l} \frac{P(k,l)}{1+(k-l)^2} \quad (3)$$

2.3. Measuring physiological traits

Physiological properties of strawberry samples, including firmness and total soluble solids, were measured according to existing laboratory protocols. The firmness of the samples was measured using a penetrometer (FT327, QA Supplies, USA) in kg cm^{-1}. Total soluble solids were measured using a refractometer (MT-032ATC, Zebra Skimmers, USA).

2.4. Apparatus and chemicals for microRNA analysis

Electrochemical measurements were performed using a potentiostat introduced by Asefpour Vakilian and Massah (2018). The electrochemical experiments were carried out at room temperature in a conventional three-electrode system including a modified gold as a working electrode, a platinum wire as a counter electrode, and an Ag/AgCl (3 M KCl solution) as a reference electrode, all with a diameter of 3 mm purchased from Metrohm (Herisau, Switzerland). The HPLC-purified sequences of oligonucleotides complementary to target microRNAs were purchased as thiolated probes from Pars Farzanegan Chemical Company (Tehran, Iran). Polyethylenimine (PEI) (MW ~ 25 kDa), silver nitrate, and phosphate-buffered saline (PBS) were of analytical grade (> 99 %) purchased from Merck & Co. (Rahway, USA).

2.5. Measuring the concentration of microRNAs

The microRNAs measured in this study are listed in Table 2. The method presented by Hakimian and Ghourchian (2020) was used for microRNA concentration measurements at femtomolar (fM) levels. According to their method, when the microRNAs available in the samples are hybridized with complementary oligonucleotides immobilized onto the electrode surface, the negative charge near the electrode surface increases. This allows for the absorption of positively charged polyethyleneimine-silver (PEI—Ag) nanoparticles as electroactive labels onto the microRNA/oligonucleotide hybrid. This increases the electric peak current proportional to the concentration of target microRNA hybridized onto the electrode surface (Fig. 1). To prepare PEI-Ag nanoparticles, 100 mL of silver nitrate with a concentration of 10 mM and 1 mL of 2 % (*w*/w) PEI were heated to boiling temperature while stirring. After boiling for 15 min, the color of the solution changed from colorless to green. After centrifuging the solution at 8000 ×*g* for 5 min, its sediment was washed with deionized water by an ultrasonic homogenizer for 1 min (Kim et al., 2012). The thiolated probes were immobilized on the working electrode due to the presence of the thiolated group. For this purpose, 8 μL of the probe (1 μM) and 8 μL of PBS were mixed and poured onto the working electrode of the biosensor. Then, the electrode was dried under infrared light for 30 min and washed three times with deionized water.

Table 2
List of microRNAs measured in this work.

microRNA compound	microRNA sequence
miR-164	5′-UGGAGAAGCAGGGCACGUGCA-3′
miR-167	5′-UGAAGCUGCCAGCAUGAUCU-3′
miR-172	5′-AGAAUCUUGAUGAUGCUGCAU-3′
miR-399a	5′-UGCCAAAGGAGAGUUGCCCUG-3′

In the next step, the total RNA content from strawberry juice was extracted using the Trizol protocol (Davis et al., 2006). The electrode was immersed in a solution consisting of PBS with a concentration of 0.1 M at a temperature of 60 °C for 30 s, and immediately after that, 20 μL of the extracted total RNA was mixed with 20 μL of PBS and poured on the surface of the electrode to perform hybridization of the probe and target microRNA. Then, the electrode was dried under the infrared lamp and washed with deionized water. Finally, positively charged PEI-Ag nanoparticles (1.8 μg in 6 μL of deionized water) were poured onto the surface of the electrode to be absorbed into the hybridized probe-target microRNA that had a negative charge. After drying and washing, a cyclic voltammetry curve was obtained in 2 mL of PBS (0.1 M) with sweeping the electric potential in the range of 600 to −600 mV and a scan rate of 150 mV s^{-1}. The higher the concentration of target microRNA in the sample was, the sharper the peak current observed during the cyclic voltammetry (Fig. 1).

2.6. Statistical analysis

After data collection, the analysis of variance was performed on all traits. Statistical operations including mean comparisons and measuring correlation coefficients between the traits were done using MATLAB R2020b software (MathWorks, Natick, USA) using Duncan's method at a significance level of 1 %.

3. Results and discussion

3.1. Fruit morphological traits

Tables S1 to S4 in the Supplementary Information file show the results of the analysis of variance obtained for image processing characteristics of strawberry samples. As shown in the tables, the interaction effects of the storage period and the mechanical loading applied to the samples were not significant. However, the individual effect of storage time on the average of the red band, the average of the blue band, and the energy of the image of the samples was significant ($P < 0.01$). Fig. 2 shows the average effect of the storage period on these traits. Over time, the mean of the red band has decreased and the mean of the blue band has increased. Also, a decrease in image energy was observed over time, which indicated the darkening of the samples over time. A decrease in image energy with plant growth has been shown in previous research (Asefpour Vakilian & Massah, 2017).

Based on the obtained results, the mechanical loading applied to the strawberry samples did not cause a specific response of the color characteristics in the RGB, HSV, and L*a*b* bands, as well as the image texture, indicating that the mechanical forces applied to the fruits cannot be investigated with the help of image processing characteristics during the studied storage period. Gao et al. (2018) used the finite element method combined with image processing to detect the mechanical load applied to potatoes and reported that this method can determine the mechanical load on the samples when falling from a certain height. They used image processing to analyze finite element data and did not extract color features from the images. Afsharnia et al. (2017) used image processing to detect wear in blackberries due to mechanical load. They showed that the L*a*b* characteristics of the images decreased with increasing storage period. Most of the studies performed on specific detection of mechanical load or storage period are based on hyperspectral image processing. This type of processing has been successfully applied to fruits such as tomato (Sun et al., 2021), and peach (Zhang et al., 2022).

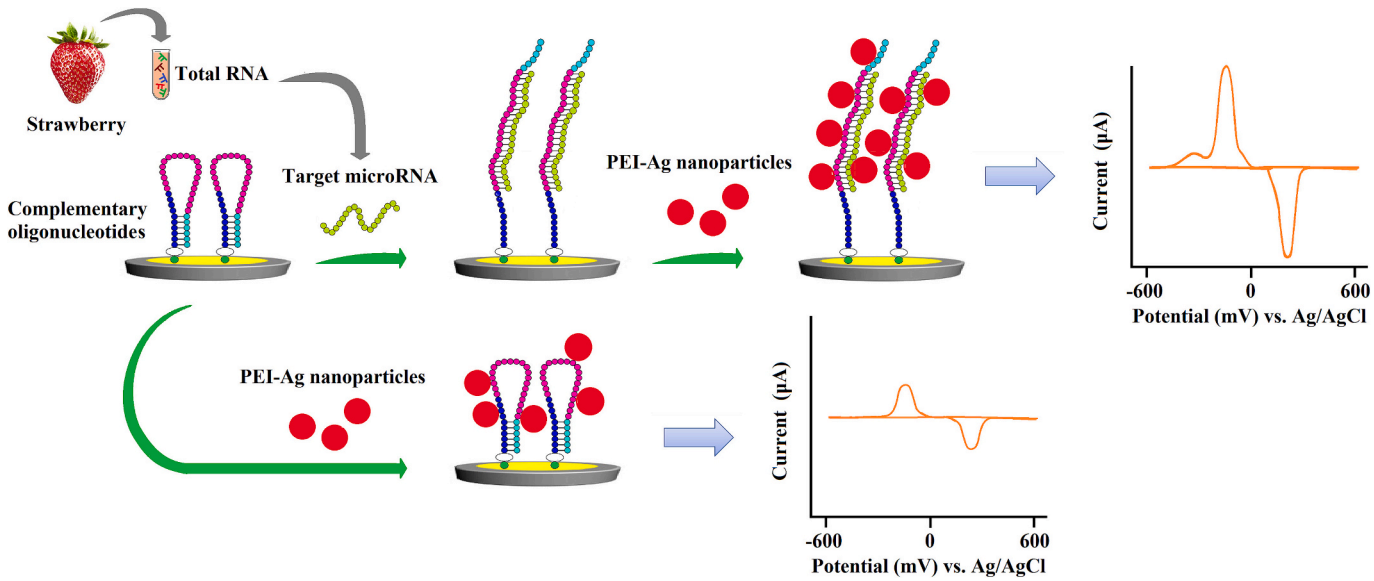

Fig. 1. The biosensing approach utilized for the microRNA concentration measurement.

3.2. Fruit firmness

Table S5 in the Supplementary Information file shows the results of the analysis of variance obtained for strawberry firmness values. As shown in the table, the changes in tissue firmness due to the individual factor of storage period were significant ($P < 0.01$). However, mechanical loading did not exert a significant effect. Also, the table shows that the interaction effect of mechanical loading and storage period had a significant effect on the firmness of strawberry fruits.

As shown in Fig. 3, the highest value of fruit firmness (1.54 kg cm^{-1}) was observed on the first day of storage after applying a loading force of 1 N, while its lowest value (0.64 kg cm^{-1}) was observed on the last day of storage period (13th day) when applying a mechanical load of 3 N. Many researchers have also shown that the texture of agricultural products becomes softer during storage and loses its original quality (Hashemi Shabankareh et al., 2023). Although the current research showed that mechanical loading did not have a significant effect ($P < 0.05$) on the firmness of the samples, previous studies reported a significant decrease in the firmness (Wang et al., 2023). The reason might be due to the differences in the intensity of the mechanical force applied to the fruits. In various studies, the level of mechanical loading is generally considered in such a way that the damage to the fruit is visible, but the levels studied in this work were not so much that during the storage period, it could cause visible effects in the fruits. This highlights the need for some markers that can detect the behavior of the fruits toward mild to moderate stress conditions that, although seeming harmless to fruits at first, eventually reduce the fruit quality after a storage period.

3.3. Fruit total soluble solids

Table S6 in the Supplementary Information file shows the results of the analysis of variance obtained for the amount of total soluble solids. According to the table, changes in the amount of total soluble solids due to the storage period were significant ($P < 0.01$). The mechanical loading did not exert significant effects. However, the interaction of storage period and mechanical loading was also significant ($P < 0.01$). Fig. 4 shows the effects of mechanical loading and storage period. The highest total soluble solids (12.8 %) was observed after a storage period of 13 days. With the evaporation of moisture in agricultural products over time, total soluble solids in the samples increase. According to the figure, this increase is evident over time during the experiment.

3.4. Fruit miR-164 concentration

Table S7 in the Supplementary Information file shows the results of the analysis of variance obtained for the strawberry miR-164 concentration. As shown in the table, the changes in miR-164 concentration due to both individual factors of storage period and mechanical loading were significant ($P < 0.01$). Also, their interaction had a significant effect at the 1 % level on miR-164 concentration. The significant effects of the treatments on the concentration of this microRNA indicate that contrary to the morphological and physiological characteristics that were discussed above, the miR-164 concentration has the ability to identify the post-harvest treatments examined in this research. Fig. 5 shows the effects of mechanical loading and storage time on miR-164 concentration. This microRNA plays a role in determining the post-storage quality of strawberry fruit. The highest concentration (371 fM) was reported in the loading force of 3 N and the first day of storage. The lowest concentration of this microRNA (118 fM) was recorded in the loading force of 1 N and on the 10th day of storage. The concentration of miR-164 decreased significantly with increasing storage time.

The miR-164 sequence is a conserved family in plants. According to the miRBase database, this family includes three members in Arabidopsis, including miR-164a, miR-164b, and miR-164c. Also, it should be noted that miR-164a and miR164b have the same mature sequence. Increasing the concentration (expression) of miR-164a or miR-164b to a certain extent leads to disruption of the plant embryo pattern (Laufs et al., 2004). The miR-164c molecule is critical for petal development, as its mutant causes extra petals to form in premature flowers (Baker et al., 2005). Also, in wild strawberry plants, three target genes for miR-164 molecules have been identified by bioinformatics methods (Xia et al., 2015). However, their biological signs and symptoms as well as their target genes in strawberries have not yet been fully identified (Zheng et al., 2019). In Arabidopsis, miR-164 can bind to six NAC target genes, some of which, such as CUC1 and CUC2, are responsible for controlling the proper number of plant organs (Baker et al., 2005). The balance between mi-R164a and CUC2 determines the number of plant leaves (Nikovics et al., 2006). Also, miR-164 constructs promote lateral root development in response to auxin (Guo et al., 2005). In the research conducted by Li et al. (2017), it was found that miR-164 has an essential role in strawberry fruit aging in response to storage at different temperatures by negatively affecting the expression of NAC domain transcription factor genes.

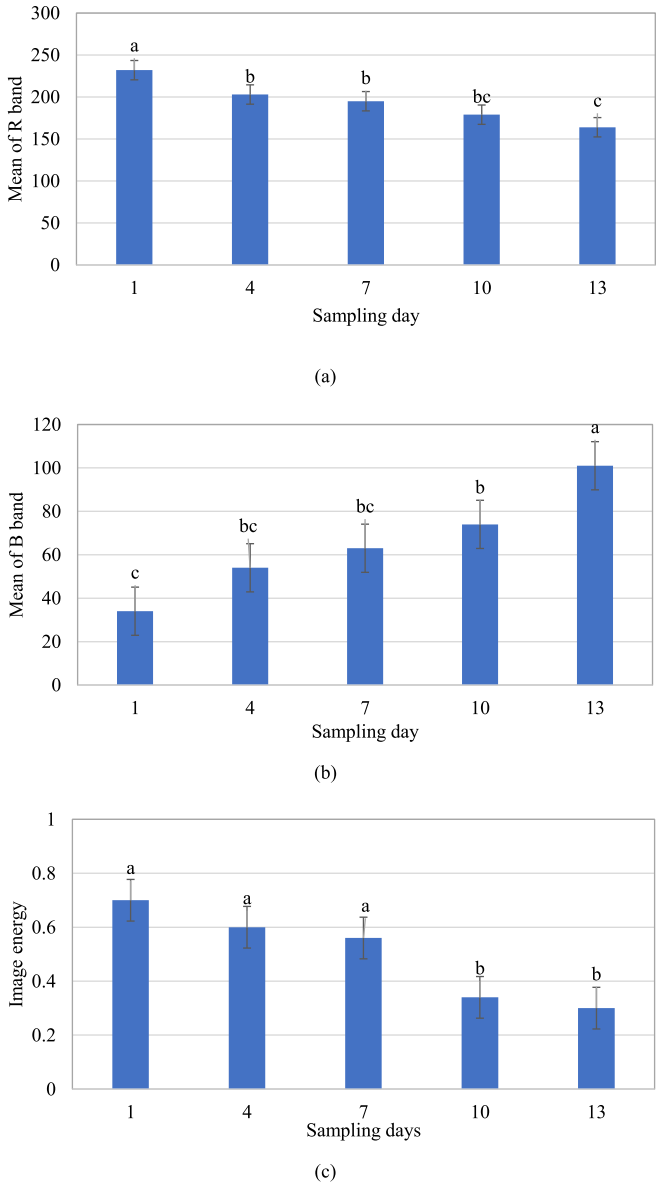

Fig. 2. The effects of storage period on the (a) mean of the red band, (b) mean of the blue band, and (c) the image energy of the strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Fruit miR-167 concentration

Table S8 in the Supplementary Information file shows the results of the analysis of variance obtained for the miR-167 concentration. According to the table, the changes in miR-167 concentration due to all individual factors and their interaction were significant ($P < 0.01$). In addition, the storage period had a greater effect on the miR-167 concentration than the mechanical loading. Fig. 6 shows the effects of mechanical loading during the storage period on miR-167 concentration. This figure shows that during the storage time, the concentration of this microRNA has decreased. The highest concentration (525 fM) was reported in the loading force of 0 N and the first day of storage. The lowest concentration of this microRNA (54 fM) was recorded in the loading force of 2 N and on the 13th day of storage. Research has shown that overexpression of miR-167 in tomato fruit effectively reduced electrolyte leakage, hydrogen peroxide content, malondialdehyde content, and chilling index during the low-temperature storage period, and also caused more accumulation of ascorbic acid, proline, lycopene, and phenolics and preserved the activity of antioxidant enzymes (Li et al., 2023), which indicates the direct effect of increasing this microRNA on increasing product quality. Also, in another research, it was reported that increasing the expression level of miR-167 in strawberry fruit increases its quality during cold storage (Xu et al., 2015). Similar to these results, in the present study, when the quality of the fruits decreased due to the increase in storage period, the concentration of this microRNA decreased remarkably.

3.6. Fruit miR-172 concentration

Table S9 in the Supplementary Information file shows the results of the analysis of variance obtained for miR-172. As shown, the changes in the concentration of miR-172 due to the individual factor of storage time were significant ($P < 0.01$). The individual factor of mechanical loading did not have a significant effect. Also, the interaction of loading force and storage period did not have a significant effect on the miR-172 concentration. This indicates that the storage time had a greater effect on miR-172 concentration than the loading force. Fig. 7 shows the effects of storage time on miR-172 concentration. In general, miR-172 with the target gene crtISO plays a role in determining the post-harvest quality of strawberries. The highest concentration of miR-172 (275 fM) was observed on the first day of storage. Although the lowest concentration of this microRNA (139 fM) was observed on the 10th day of storage, the values on the 10th and 13th days of the experiment were not different significantly. Therefore, it can be concluded that in general, the concentration of this microRNA decreased with increasing storage time. These results agree with the results of Gao et al. (2015) regarding the effects of ethylene treatment during the storage of tomatoes on miR-172 concentration.

Plant growth is inhibited by reducing miR-172 activity through target gene overexpression or miR-172-resistant AP2 expression, resulting in smaller fruit (José Ripoll et al., 2015). Although miR-172 accumulation increases Arabidopsis fruit size, apple fruit development is negatively regulated by the overexpression of miR-172, leading to a dramatic reduction in fruit size (Yao et al., 2015). This is because the fruits of different plants grow from different tissues. These studies show that the effect of microRNAs on fruit growth is specific to fruit type and plant species. Table S9 shows that, unlike the two previously studied microRNAs, the changes in miR-172 concentration due to the treatments were not significant and did not show a specific response. This indicates that the specific response of microRNAs that are involved in the post-storage of fruits should be evaluated under different treatments to gain more knowledge about the role of microRNAs in the post-storage period.

3.7. Fruit miR-399a concentration

Table S10 in the Supplementary Information file shows the results of the analysis of variance obtained for the miR-399a concentration. According to the table, the changes in the concentration of miR-399a due to all individual factors and their interaction were significant ($P < 0.01$). Fig. 8 shows the effects of mechanical loading during the storage period on miR-399a concentration. The highest concentration of miR-399a (1654 fM) was observed on day 13 of storage and a loading force of 3 N. Its lowest concentration (213 fM) was observed on the first day of storage and a loading force of 0 N. The concentration of miR-399a increased during the storage period. In strawberries, the presence of miR-399 increases phosphate uptake (Bari et al., 2006), which increases fructose, glucose, and soluble solid content in ripening fruits (Wang et al., 2017). On the other hand, this microRNA is involved in fruit softening during the storage period.

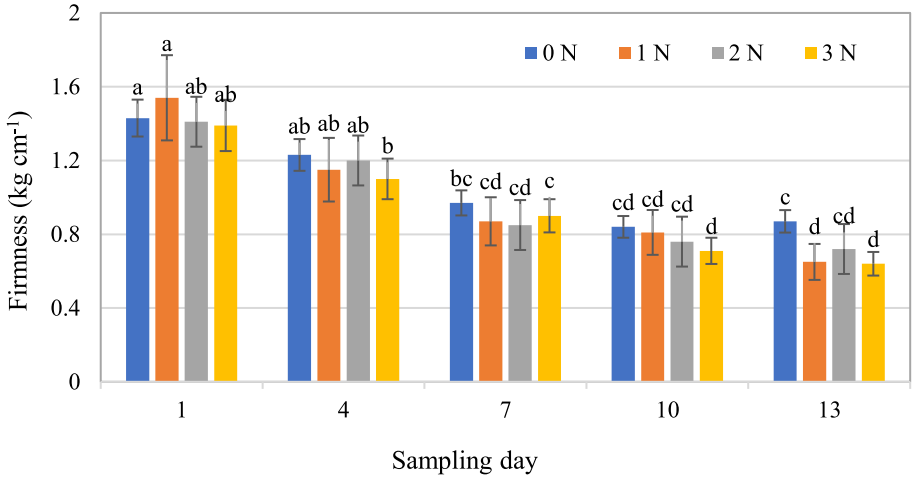

Fig. 3. The effects of mechanical loading and storage period on the firmness of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

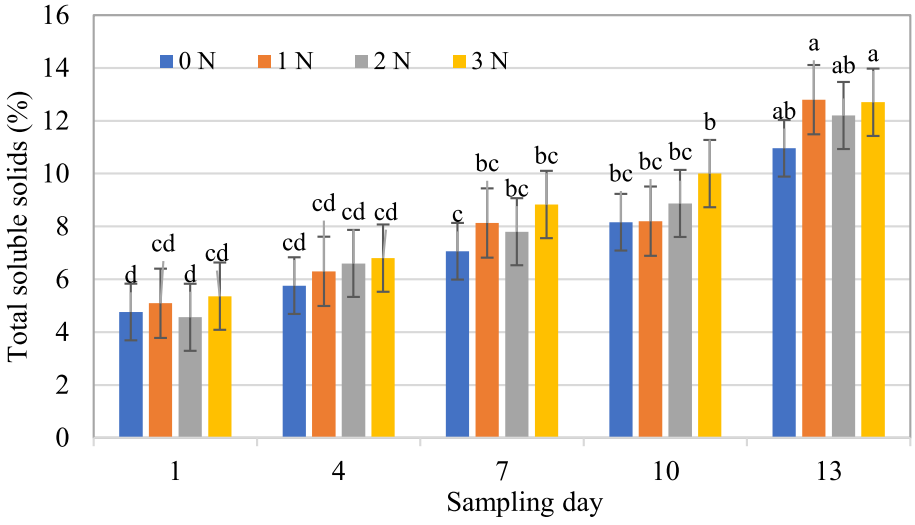

Fig. 4. The effects of mechanical loading and storage period on the total soluble solids of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

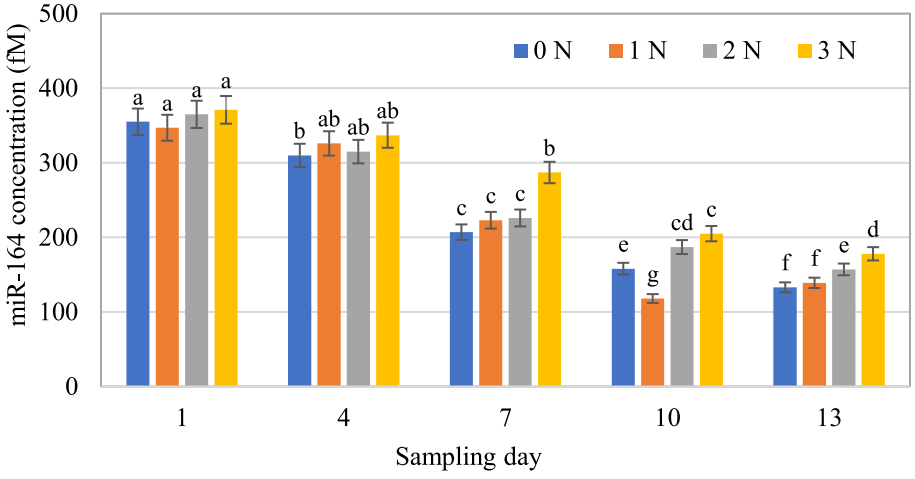

Fig. 5. The effects of mechanical loading and storage period on the miR-164 concentration of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

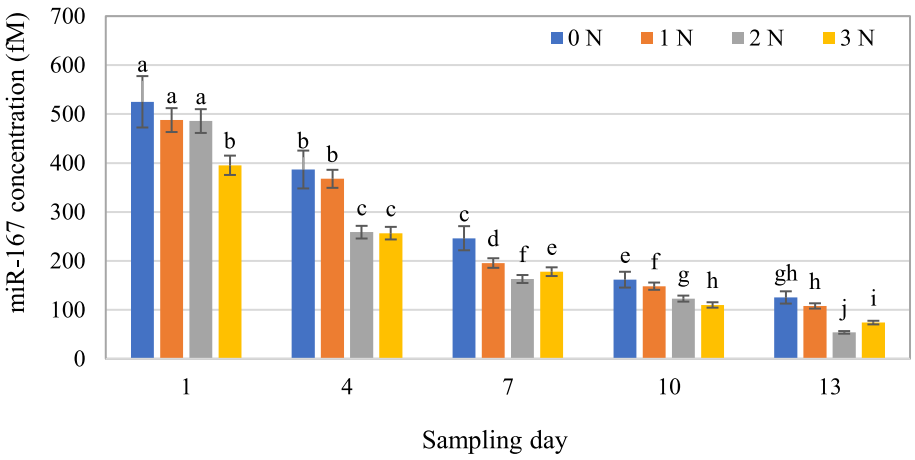

Fig. 6. The effects of mechanical loading and storage period on the miR-167 concentration of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

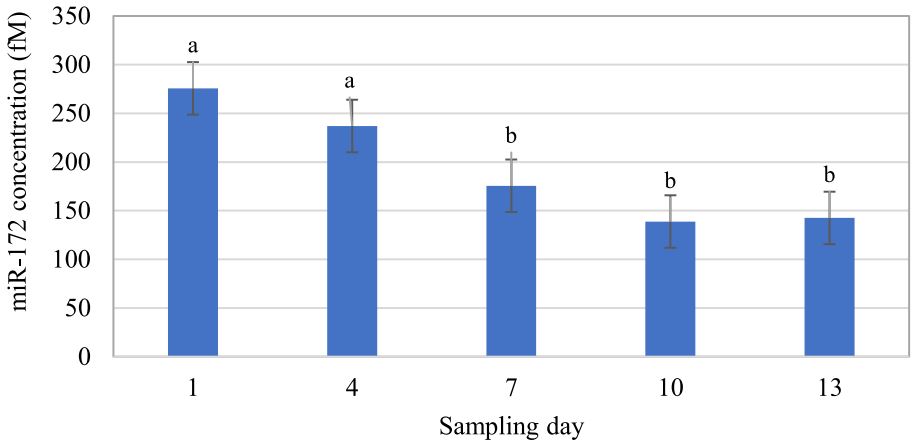

Fig. 7. The effects of mechanical loading and storage period on the miR-172 concentration of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

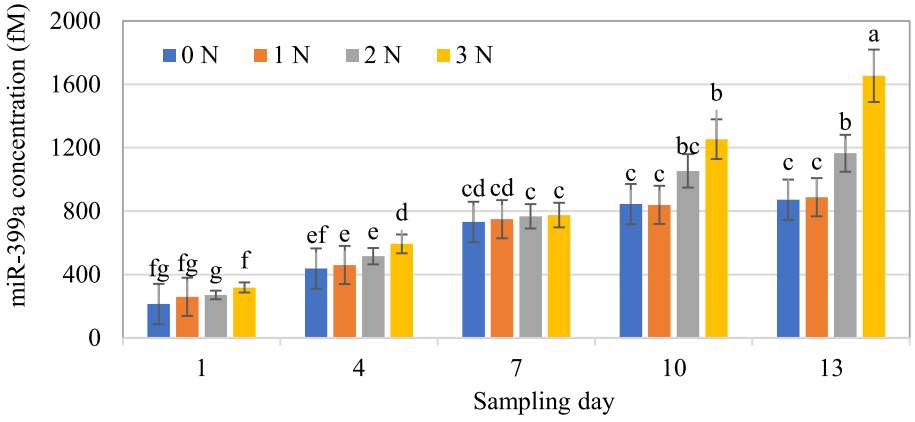

Fig. 8. The effects of mechanical loading and storage period on the miR-399a concentration of strawberry samples. Columns with the same letters do not have a significant difference ($P < 0.01$).

3.8. Correlation coefficients

Table 3 shows the absolute values of the correlation coefficients between the characteristics investigated in this work. It can be seen that there is no remarkable correlation in most of the traits, especially the morphological characteristics obtained from fruit image processing, where the correlation coefficient does not exceed 0.6. However, among microRNA concentrations measured in this study, the values of the correlation coefficient range from 0.6 to 0.9. The highest correlation coefficient (0.9) was observed between the concentrations of miR-164 and miR-167. On the other hand, the correlation of microRNA concentrations and the morphological and physiological traits is small. During

Table 3
The correlation coefficient between the traits measured in this study.

Traits	R	G	B	H	S	V	L	a	b	Energy	Entropy	Local homogeneity	Firmness	Total soluble solids	miR-164	miR-167	miR-172	miR-399a
R	1																	
G	0.2	1																
B	0.6	0.4	1															
H	0.3	0.2	0.4	1														
S	0.1	0.1	0.2	0.5	1													
V	0.4	0.5	0.1	0.1	0.4	1												
L	0.1	0.2	0.3	0.1	0.2	0.5	1											
A	0.2	0.4	0.5	0.1	0.1	0.2	0.1	1										
B	0.1	0.3	0.1	0.3	0.4	0.4	0.3	0.3	1									
Energy	0.3	0.5	0.2	0.3	0.1	0.1	0.5	0.5	0.5	1								
Entropy	0.4	0.1	0.4	0.1	0.3	0.1	0.1	0.1	0.1	0.1	1							
Local homogeneity	0.2	0.1	0.1	0.4	0.5	0.1	0.1	0.2	0.2	0.3	0.2	1						
Firmness	0.3	0.2	0.2	0.2	0.6	0.3	0.3	0.2	0.4	0.5	0.1	0.2	1					
Total soluble solids	0.3	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.4	0.1	0.3	0.1	0.2	1				
miR-164	0.4	0.4	0.3	0.5	0.2	0.1	0.5	0.4	0.1	0.1	0.5	0.5	0.1	0.6	1			
miR-167	0.4	0.1	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.5	0.1	0.3	0.4	0.9	1		
miR-172	0.2	0.2	0.5	0.5	0.4	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.3	0.6	0.7	1	
miR-399a	**0.1**	**0.3**	**0.1**	**0.1**	**0.2**	**0.1**	**0.1**	**0.6**	**0.4**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.8**	**0.9**	**0.8**	**1**

the experiments, samples treated by similar levels of treatments exerted rather large standard deviations in their morphological and physiological traits, while this standard deviation was remarkably lower for their microRNA concentrations. This can be the reason for low correlation coefficients between microRNA concentrations and other traits investigated in this work. The high correlation of the microRNAs with each other might be due to the specificity of the changes in concentrations of microRNAs that were studied as biochemical characteristics. However, many factors are effective in the changes of the morphological and physiological characteristics of the fruits and it seems that microRNA concentrations can provide stakeholders (e.g., exporters/importers, wholesale buyers, and warehouse managers) with more information than other characteristics to determine/identify the treatments applied to the fruits during the storage.

4. Conclusion

The fruit characteristics that show a more specific behavior than other characteristics toward storage treatments can be widely and even commercially used for the quality assessment of agricultural products. This is because in this situation, by measuring such specific characteristics, it is possible to increase the knowledge of suppliers and wholesale buyers of agricultural products regarding product quality. The *F* values obtained from the analysis of variance for the concentrations of miR-164, miR-167, and miR-399a compared to the values obtained for the morphological and physiological characteristics indicated a stronger effect of the treatments investigated in this work on changes in biochemical traits. This is an essential result of this research since instead of measuring conventional morphological and physiological characteristics whose changes might not be completely affected by stressors, it is possible to use the measurement of microRNAs as a measure of product quality. The electrochemical biosensor used in this work to measure the microRNA concentrations is a portable device and can be used by suppliers, or even wholesale buyers of agricultural products to determine the storage condition of the fruits without the need to transfer the fruit samples to the laboratory.

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CRediT authorship contribution statement

Mahdieh Sharbati: Writing – original draft, Methodology, Data curation. **Keyvan Asefpour Vakilian:** Writing – review & editing, Supervision. **Mohsen Azadbakht:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2025.100250.

Data availability

Data will be made available on request.

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