RESPONSE POSTHARVEST QUALITY OF POMEGRANATES TO THERMAL TREATMENTS, IMMERSING IN BLACK SEED AND FLAXSEED OILS

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ABSTRACT
This study was carried out to investigate thermal treatments, immersing in black seed and flaxseed oils on postharvest quality pomegranate fruits cv. Salakhan. Fruits were randomly divided into two experiments: First, thermal treatment by which fruits are immersed for four minutes at 25, 40 and 50°C in hot water (HW), and fruits are exposed to hot air (HA) for one hour at 40 and 50°C plus control treatment. Second, fruits are immersed for 4 minutes in 1 and 2% for the black seed and flaxseed oil, plus control treatment. Then, fruits are placed in the storage at 5±1 ºC and 85-95% of relative humidity for four months. Both experiments performed by using Complete Randomize Design. The results indicated that soaking fruits in HW for four minutes at 40°C significantly decreased the weight loss and physiological disorder, whilst significantly increased most of studied attributes compared to control. However, HA treatments exposure for 1 hour at 40 and 50°C, with increasing in the HA levels, decreased the weight loss and physiological disorder, but increased some of studied attributes compared to control. Moreover, the immersing fruits in 1% black seed or 1% flaxseed oil significantly reduced weight loss and physiological disorder. In addition, the immersing fruits in 1% flaxseed oil lead significantly increased most of studied attributes compared to control.

Keywords: anthocyanin; oil extract; flavonoids; phenols; salakhan pomegranate.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family. Pomegranate is one of the archaic the familiar edible fruits (1). With increasing its production, this fruit needs to be properly stored and marketed to meet the request at the local and foreign markets (12). Over the last few years, the physical treatment has been utilized to prolong the storage life of pomegranate fruits by research studies on the pomegranate postharvest storage and maintenance of the main quality of the freshly harvested fruits (12, 30). To expand the ability of the storage and marketing of various fruit crops, significant results were observed with thermal applications, such as inundation in hot water, hot water dips and vapor and forced hot air. These applications can stimulate the resistance of fruits to lower temperature, decreased weight loss, decay, and chilling injury through storage and rising shelf life of much of crops (14, 29). Moderate heat treatment, especially hot water, has the potential to inactivate enzymes and minimize microorganisms (25), but it is not permanently appropriate with fresh plant foods because it may lead to a decrease to consumer acceptability because of unwanted changes of flavour, colour, nutritional quality and texture (28). Furthermore, (19) showed that the black seed oil application significantly affected the protection of fruit weight and quality. (15) noted that the black seed oil contains thymoquinone, which has good antimicrobial vigor. They also reported that the black seed oil had a high antibacterial activity opposed to all the strains of *L. monocytogenes*. Much bioactivity characteristic have been to black seeds (*Nigella sativa*), fixed or essential oil, involving antioxidant activities (6). According to the results of (5) study, using *Nigella sativa* essential oil coating would keep the Manfalouty pomegranate fruit and maintain their quality for longest storage period. Due to a lack of research study on its effects on postharvest life in fruits and vegetables, the flaxseed oil was used in the current study. In Iraqi Kurdistan Region, the Halabja governorate is considered a good source for the production of pomegranate, especially cv. Salakhani due to its suitable climate for this cultivar. The fruits are characterized as a medium to enlarge fruits in size, and the peel is thick reddish-yellow in color, impregnated with pink color. Further, arils are juicy with a red to pink color, with a good flavor and sour sweet taste, and this cultivar is also the most important for exporting to other countries (2). Since this cultivar is desirable for exporting, storing and prolonging its freshness and quality at the markets, this study was carried out to knowledge of the impact of two types of thermal treatments, black seed and flaxseed oils on postharvest quality of “Salakhani” pomegranate fruits.

MATERIALS AND METHODS

This study was conducted during the year 2019 on Salakhani Pomegranate fruits were obtained from the private Orchard, at Halabja governorate. The fruits were harvested during the last week of October at uniform stage of maturity, and the size, free of insects, diseases and damage were selected randomly. The harvested fruits were used immediately. This study was conducted based on two experiments:

**Experiment 1:** The effect of thermal treatments on postharvest quality of pomegranate cv. Salakhani.

The fruits were randomly divided into six equal groups (treatments). Each treatment was replicated three times, 15 uniform fruits were chosen randomly for each replicate, after being dried and placed in carton boxes. Then, the fruits were placed in a storage at 5±1 ºC and 85-95% RH for four months. The treatments were conducted as follows:

1. The fruits were dipped in hot water at 25ºC for 4 minutes, symbolized by (HW 25ºC) as the control treatment for hot water treatments.
2. The fruits were dipped in hot water at 40ºC for 4 minutes, symbolized by (HW 40ºC).
3. The fruits were dipped in hot water at 50ºC for 4 minutes, symbolized by (HW 50ºC).
4. The fruits were not treated thermally; symbolized by (Con. Air), as the control treatment for hot air treatments.
5. The fruits were heat treated at 40ºC for one hour, symbolized by (HA 40ºC).
6. The fruits were heat treated at 50ºC for one hour, symbolized by (HA 50ºC).

**Experiment 2:** The effect of the black seed and flaxseed oils on postharvest quality of pomegranate cv. Salakhani.
The fruits were randomly divided into six equal groups (treatments). Each treatment was replicated three times, 15 uniform fruits were chosen randomly for each replicate, after being dried placed in carton boxes. Then, the fruits were placed in a storage at 5±1 °C and 85-95% RH for four months. Black seed and Flaxseed oils were obtained by cold pressing *Nigella sativa* seeds and *Linum usitatissimum* seeds respectively. The treatments were conducted as follows:

- a- The fruits of the control treatment were immersed in distilled water for 4 minutes.
- b- The fruits were immersed in (1%) black seed oil for 4 minutes, (1% BS).
- c- The fruits were immersed in (2%) black seed oil for 4 minutes, (2% BS).
- d- The fruits were immersed in (1%) flaxseed oil for 4 minutes, (1% FS).
- e- The fruits were immersed in (2%) of flaxseed oil for 4 minutes, (2% FS).

After four months of the cold storage period for each experiment, the following parameters were measured:

**Physical parameters**

Weight loss as a percentage from initial weight was calculated. Fruit juice %, fruit arils % and fruit peel % were measured from total weight of fruit for each sample. Fruit physiological disorder %, the number of damaged fruits (skin and arils browning) was measured as a percentage from the total number of each replicate. It was calculated after the cold storage period and seven days of shelf life at room temperature.

**Chemical juice parameters**

A total soluble solid (TSS) was measured by Hand Refractometer. For Titratable Acidity (TA) % samples (fruit juice) were titrated with NaOH using phenolphthalein indicator and the acidity was determined as citric acid content followed by the method of (16). According to the method of Horwitz (16), total soluble solids to TA were measured by dividing TSS on TA. Ascorbic acid in juice was estimated by titration against 2,6 dichlorophenol indophenol blue dye. The pH value was estimated by using a pH meter instrument. According to (18), total sugars were determined by using 1ml of the sample in the test tube followed by adding 1ml of phenol 5%, shake well and then adding 5 ml H2SO4 (97%). Afterwards, the solution was put in water bath for thirty minutes at 60 °C. Before it was centrifuged at 3000 rpm, for 15 minutes, finally, total sugars were estimated by spectrophotometer at 490 nm. Anthocyanin (mg.100 ml⁻¹ juice) and total phenol (mg.100 ml⁻¹ juice) were estimated according to (32) by adding 5 ml of juice and 5 ml of the solution (95% ethanol and 1.5 N hydrochloric acid (HCl) at 85:15 each respectively) and the mixture was mixed well. Then, it was stored overnight in a refrigerator at 4°C, after that it was placed in the centrifuge for 15 minutes at 3000 rpm. Anthocyanin was measured by using a spectrophotometer at the wavelength 535 nm., and the phenol was determined by the same method as it was only read at the wavelength 280 nm. The proportion of flavonoids in the peel and juice was estimated according to (8) by extracting 10 g of the sample with 100 ml of methanol (80%) at room temperature followed by solution filtration through the filter paper (Whatman No.42). The residue was evaporated until drying on a water bath and the residual weight of the precipitate was calculated based on a percentage.

**Statistical analysis**

The Complete Randomize Design was used for these experiments, and the collected data was submitted to the analysis of variance (ANOVA) by using SAS 9.1 software. Mean comparisons was carried out by using Duncan multiple range test at p≤ 0.05.

**RESULTS AND DISCUSSION**

**Effect of some thermal applications on postharvest quality of pomegranate cv. Salakhani**

**Weight loss:** Following four months of cold storage, in both types of the thermal treating (hot water and hot air), it was observed that the lowest weight loss was recorded in fruits which was treated with 40°C, while the highest value was recorded in fruits which was treated with 50°C as shown in Table 1. This result was in agreement with others (13). The thermal treatments were characterized by their ability to reduce the weight loss percent, and this may be due to the action of heat in causing some physiological changes in the fruits peel, which are similar to the changes that occur when conducting the operations of...
conditioning or Curing, which leads to the increase of polyamines that increase the stability of cell membranes and accompany nucleic acids and in the structure of membranes, which increases the thickness and hardness of the outer peel and reduces water loss (22, 35).

**Physiological disorder:** Table 1 shows that in both types of the thermal treating of pomegranate fruits at 40 and 50ºC significantly reduced the physiological disorder compared to other treatments. The results of the current study confirm these previous results with different fruits (20, 35). They found that thermal treatments have the potential to reduce chilling injury in fruit during the cold storage. In the pomegranate skin, 10 fatty acids, five saturated, three poly-unsaturated, and two mono-unsaturated

**Table 1. Effect of Thermal Treatments on Weight Loss, Physiological Disorder, Arils, Peels, and Juice of Pomegranate Fruits cv. Salakhan**

| Treatments  | Weight Loss (%) | Phys. Disorder (%) | Arils (%) | Peel (%) | Juice (%) |
|------------|-----------------|-------------------|-----------|----------|-----------|
| HW 25 ºC  | 7.322           | 26.667            | 70.014    | 29.986   | 56.226    |
| HW 40 ºC  | 6.337           | 20.000            | 74.845    | 25.155   | 60.411    |
| HW 50 ºC  | 8.941           | 20.000            | 71.981    | 28.019   | 57.461    |
| Con. Air  | 5.588           | 33.333            | 74.276    | 25.724   | 58.683    |
| HA 40 ºC  | 5.386           | 20.000            | 70.089    | 29.911   | 56.267    |
| HA 50 ºC  | 5.895           | 20.000            | 67.316    | 32.684   | 52.670    |

Values in the same column with different letters are statistically different ($p < 0.05$)

were identified and quantified. Among the saturated, the prevailing acid was palmitic fatty acid ($≈33\%$), whilst, among the unsaturated fatty acid, the prevailing acid was linolenic acid ($≈25\%$). However, pending the storage of all fatty acids concentricities in pomegranates, which were thermally treated, remained significantly more than those in the control pomegranates, (24). The increment in the concentrations of membrane unsaturation lipids was utilized as a mechanism of increasing the resistance to low temperatures (10), and this leads to the protection of membrane liquidity during storage at these temperatures and might be in charge of the skin browning and lower electrolyte leakage. Thus, the findings of this study indicate that the control treatment caused an increase in the chilling injury (CI), which might be due to the inability of the treatment to improve this mechanism of acclimation. Therefore, CI considerably appeared in and boosts by the essential relationship found between the reduction in unsaturated fatty acids percentage to saturated fatty acids and the increment in electrolyte leakage through storage. However, thermal applications may be stimulate this response by preservation of unsaturated fatty acids through storage, and in turn, decreased the severity of CI symptoms (23). Thus, it may be responsible for reducing CI found in the thermally treated pomegranates.

**The arils percentage:** The highest percentage was recorded in fruits that were immersed in HW at 40ºC compared to control treatment, which obtained the lowest percentage. Whereas, according to the Table 1, the arils percentage significantly decreased with increasing the hot air levels than that of the control treatment.

**The peel percentage:** Fruits treated with HW at 40ºC and 50ºC significantly reduced the peel percentage compared to the control treatment (25ºC). Whilst, the hot air peel percentage of fruits was significantly increased with an increases in the level of hot air treatments. The reason for these increases or decreases in the percentage of arils in the fruit could be return to the increases or decreases of the peels in the fruit, because this ratio was measured as a percentage and reversely.

**The juice percentage:** The percentage of juice in fruits was significantly increased when fruits were dipped in HW at 40ºC than those of the control pomegranates. Concerning the effects of hot air on juice percentage in fruits, the increases in the hot air levels treatments caused a decreases in the juice percentage of fruits. This reduction of fruits juice might be attributed to the effect of thermal applications to the arils percentage.

**Total Soluble Solid:** Data in Table 2 shows that immersion of fruits in the hot water for 4 minutes at 40ºC recorded the maximum TSS.
content at the end of storage duration, at the same time the lowest value was obtained in fruits that were treated with the hot air for 1 hour at 50°C than those of the control fruits.

**Titratable acidity:** Fruits which were immersed in HW at 50°C boosted the titratable acidity in fruits juice, while the lowest value was observed in fruits immersed in the HW at 40°C. Furthermore, the hot air treatments revealed that with rising the temperature of the hot air, a significant reduction was obtained in the titratable acidity in fruits juice (Table 2). The cause of the acidity of the pomegranate fruit juice is mainly due to the increases of citric acid concentration which is produced in mitochondria by the citrate synthase enzyme, which works to combine the Oxaloacetate with the Acety-CoA to form the citric acid. While, the reduction in the titratable acidity in fruits juice might be due to metabolic changes and a rapid conversion of organic acids to sugars at the end of the storage duration (Table 2). Furthermore, organic acids would be exposed to the oxidizing during the respiration process and producing energy, as some organic acids enter directly into the Krebs cycle, and the others are converted to another form of organic acid that is one of the intermediate acids in the Krebs cycle and is consumed in the respiration process (25).

**TSS/TA ratio:** Results in Table 2, show the results indicate that immersing fruits in HW at 40°C significantly increased TSS/TA ratio compared to the other hot water treatments. While, fruits which were exposed to hot air for 1 hour at 50°C, were significantly superior on the other hot air treatments. This increase might be owing to the solubilization of carbohydrates compounds or loss of water in the fruit. In addition, it is known that during storage, there is antagonistic relation between TSS and acidity. The reduction of acidity could be because of the process of respiration in fruits which consume acidity for respiration. Thereby, the total soluble solid became higher than acidity. Thus TSS/acid ratio was increased.

**Ascorbic acid content:** In fruits were treated with HW at 40°C significantly increased than those of the other treatments in this parameter. At the same time, according to Table 2, the hot air treatment with the highest level of ascorbic acid was observed in fruits which were treated with 50°C hot air. The ascorbic acid (vitamin C) concentration in fruits is one of the indexes of good storage, but its sensitivity to storage conditions is the important point, as it is oxidized when storing fruits at temperatures that are higher than what is recommended for a certain type, or when storing fruits at lower temperatures than recommended for a certain type this leads to chilling injury, or because of water loss in fruits, or due to oxidation by Oxidative enzymes such as the ascorbase, the phenolase or the oxidase enzyme. This reaction happens fast, once the fruit juice is exposed to the air containing oxygen (3). However, thermally treated strawberries raise its level of vitamin C because of prompting preservative enzymes against oxidative molecules that is reported by others (27).

**Table 2. Effect of Thermal Treatments on TSS, TA, TSS/TA, Vitamin C, pH and Total Sugar of Pomegranate Fruits cv. Salakhani**

| Treatments | TSS (%) | TA (%) | TSS/TA | Vit. C (mg/100 ml juice) | pH | T. sugar (%) |
|------------|---------|--------|--------|--------------------------|----|-------------|
| HW 25 °C  | 15.000  | 1.579  | 9.499  | 52.000                   | 3.110 | 2.625       |
| HW 40 °C  | 15.767  | 1.365  | 11.548 | 55.778                   | 3.160 | 3.752       |
| HW 50 °C  | 15.200  | 1.621  | 9.379  | 40.556                   | 3.017 | 3.3128      |
| Con. Air  | 15.600  | 1.427  | 11.206 | 40.667                   | 3.015 | 2.289       |
| HA 40 °C  | 15.067  | 1.259  | 11.981 | 40.556                   | 3.177 | 3.176       |
| HA 50 °C  | 14.733  | 1.003  | 14.714 | 46.556                   | 3.380 | 3.176       |

Values in the same column with different letters are statistically different (p < 0.05)

**pH:** as demonstrate in Table 2, the maximum pH was observed in juice of fruits which were treated with HW at 40°C, whereas the minimum pH recorded with HW at 50°C. However, hot air treatments significantly increased pH with increasing the levels of hot air, whereas the lowest value was observed in non-thermally treated.
**The total sugar:** Total sugar content in fruits juice increased significantly in both thermal treatments at 40°C or 50°C compared with that of the control treatment. The impact of thermal treatments on raising the total sugars could be owing to the raise in arabinin, galactosidase, and glucosidase activities, which produce sugars from the polyomers presented in the cell wall, as suggested by (7) in kiwi fruit after thermal applications. Furthermore, the greater organic acids and sugar content after thermal treatments would preserve the organoleptic quality of the pomegranate arils, due to these components in accordance with the taste and flavor (30). While these greater concentrations might be causing reduce respiration process, because during the respiration using these compounds as substrates and thermally applications were advised to prevent or reduce the respiration process in plums (33). Thus, more research studies are required to determine the reasons of these changes in pomegranates.

**Total phenol:** Total phenol in fruits juice was non-significantly influenced by both thermal treatments.

**Anthocyanin content:** At the end of storage period, the highest value was recorded in fruits which were immersed in hot water at 25°C, with non-significantly difference at 50°C. While the lowest value was recorded in fruits treated with hot water for 4 minutes at 40°C. This result was in consistent with that reported by (25). Anthocyanin is unsteady and sensitive to degradation, leading to a brownish color during storage. Many factors have effects on the anthocyanin stability, including the temperature of storage, the anthocyanin chemical nature (glucosylation or acylation),

**Effect of black seed and flaxseed oils on postharvest quality of pomegranate cv. Salakhan**

**Weight loss:** As presente in Table 4, all black seeds and flaxseed oils levels were significantly decreased in terms of the weight loss in fruits compared to the control. Weight loss is an important quality that demonstrates the storage quality of fruits. It minimizes the attractions of pomegranate fruits by changing the shape, browning the peel and hardening fruits peel (36). This result is in agreement with the result of a study conducted by others (19). The positive effect of oils on reducing fruit weight loss might be due to making a thin film of oil around the fruit peel and a modification of microclimatic of fruits. Oil coating methods act as permeable barrier against moisture, carbon dioxide and oxygen, and in turn, decreasing oxidation reaction rates, water loss and respiration (5, 30).

**Physiological disorders:** The fruits that were immersed in 1% BS or 1% FS significantly

### Table 3. Effect of Thermal Treatments on Anthocyanin, Total Phenol, Flavonoids in Juice and Flavonoids in Peel of Pomegranate Fruits cv. Salakhan

| Treatments | Anthocyanin (mg.100 ml⁻¹) | Total Phenol (mg.100 ml⁻¹) | Flavonoids in Juice (%) | Flavonoids in peel (%) |
|------------|--------------------------|---------------------------|------------------------|-----------------------|
| HW 25 °C   | 257.420 a                | 340.020 a                 | 0.143 a                | 0.140 a               |
| HW 40 °C   | 235.377 b               | 335.031 a                 | 0.140 b                | 0.183 b               |
| HW 50 °C   | 251.202 a               | 339.450 a                 | 0.140 a                | 0.197 a               |
| Con. Air   | 231.813 bc              | 341.303 a                 | 0.145 c                | 0.150 c               |
| HA 40 °C   | 212.853 d               | 337.312 a                 | 0.143 d                | 0.147 od              |
| HA 50 °C   | 222.598 cd              | 339.308 a                 | 0.137 e                 | 0.150 f               |

Values in the same column with different letters are statistically different (p < 0.05)

sugars, pH, H₂O₂, ascobic acid, light and metals (31). It is more likely that hot water resinate peroxidases (POD) activity and stopped phenyl alanine ammonia-lyase (PAL) activity at the same time, and this cause a rapid anthocyanin reduction in arils. Similarly, hot air, both hot air levels caused reduce the anthocyanin content in fruits juice compared with that of the control treatment. This reduction in the anthocyanin and total phenol in the treated fruits might be attributable to the metabolic changes that led to the analysis the anthocyanin to its initial compounds like sugars, which caused an increase in the total sugar (Table 2).

**Flavonoid:** At the end of cold storage period, the statistical analysis shows non-significant differences of flavonoid amount in fruits juice by both thermal treatments. Furthermore, flavonoid in fruits peel was significantly increased by both hot water treatment (40 and 50°C). However, non-significant effects were recorded among hot air treatments.

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reduced physiological disorders than those of the control pomegranates. This result is similar to the finding reported by (19). This reduction in the percentage of physiological disorders might be attributable to the role of these applied oils which increase bright, attractive and flexibility of

Table 4. Effect of Black seed and Flaxseed essential Oils on Weight loss, Arils, Peels, Juice, and Physiological Disorder of Pomegranate Fruits cv. Salakhani

| Treatments | Wt. Loss (% | Phys. Disorder (%) | Arils (%) | Peel (%) | Juice (%) |
|------------|--------------|--------------------|-----------|----------|-----------|
| Control    | 5.144 a      | 10.000 b           | 70.395 ab | 29.605 b | 54.793 ab |
| 1% BS      | 2.830 c      | 3.333 d            | 68.121 b  | 31.879 b  | 49.838 b  |
| 2% BS      | 3.632 b      | 10.000 b           | 64.342 c  | 35.658 a  | 50.495 b  |
| 1% FS      | 3.684 b      | 6.667 c            | 73.705 a  | 26.295 c  | 58.474 a  |
| 2% FS      | 3.968 b      | 15.833 e           | 69.445 b  | 30.555 b  | 53.454 ab |

Values in the same column with different letters are statistically different (p < 0.05)

Table 5. Effect of Black Seed and Flaxseed Essential Oils on TSS, TA, TSS/TA, Total Sugar, pH, and Vitamin C of Pomegranate Fruits cv. Salakhani

| Treatments | TSS (%) | TA (%) | TSS/TA | pH | Vit. C (mg.100 ml⁻¹) | T. sugar (%) |
|------------|---------|--------|--------|----|----------------------|--------------|
| Control    | 14.633 b | 1.259 a | 11.686 a | 3.183 a | 41.889 b              | 2.986 e      |
| 1% BS      | 15.000 ab | 1.493 a | 10.443 a | 3.037 a | 33.777 d              | 2.272 e      |
| 2% BS      | 15.667 a  | 1.131 a | 15.458 a | 3.203 a | 44.445 c              | 3.649 e      |
| 1% FS      | 15.400 ab | 1.344 a | 11.866 a | 3.100 a | 42.225 b              | 5.059 e      |
| 2% FS      | 14.700 b  | 1.109 a | 13.596 a | 3.190 a | 38.667 c              | 4.050 b      |

Values in the same column with different letters are statistically different (p < 0.05)
ascorbic acid (20), or may be due to the reasons that were mentioned in the first experiment.

**Total sugar:** All levels of black seeds and flaxseed oils were significantly superior to the control treatment in total sugar of fruits juice except fruits that were treated with 1% BS, which recorded the minimum percentage. The positive effect of oils on reducing fruit weight loss may be due to making a thin film of oil all around the fruit peel and a modification of microclimatic of fruits. Oil coating methods act as permeable barrier against moisture, carbon dioxide and oxygen, thereby decreasing oxidation reaction rates, water loss and respiration (35, 36).

**CONCLUSIONS**

In general, the thermal application (hot water and hot air) significantly maintained the pomegranate weight and quality. The results indicate that dipping fruits in hot water for 4 minutes at 40°C significantly decreased weight loss and physiological disorder that is directly associated with the fruit quality after harvest, also maintain the high quality of the fruit compared to control. In the case of hot air levels exposure (40 and 50°C for 1 hour), with increases in the hot air levels, caused a decrease in weight loss, physiological disorder, whereas preserving the high quality of the fruit than the control. Additionally, the current study shows that immersing fruits in 1% black seed oil significantly reduced weight loss and physiological disorder than the control pomegranates. However, the fruits treated with 1% flaxseed oil significantly reduced weight loss and physiological disorder but significantly increased the arils%, juice%, total sugars, anthocyanin and total phenols compared to the control treatment. According to the results, we can recommend using thermal treatments and 1% of black seed and flaxseed oil to minimize weight loss and physiological disorder of pomegranate. As well as, conduct other researches by using other levels of these seeds oil on pomegranate or others to limit the best level of them, to maintain high fruits quality and prolong the storage period.

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