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Synergistic effect of zinc and denak (*Oliveria decumbens*) essential oil to extend the storage life of pomegranate arils

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Abstract

Background Semi-processed pomegranates are increasingly being used around the world. Due to their perishability, however, arils currently have a limited supply and distribution. The treatment of arils with zinc and denak essential oil (DEO) can assist in reducing the growth of pathogens and can contribute to an increase in storage life. Since zinc is nutritionally valuable, the experiment involved immersing arils in 0.8% zinc sulfate ($ZnSO_4$) and packaging them in polypropylene (PP) containers. Then, labels were supercritically impregnated with DEO (25 and 50 µL L^{-1}) in the packages. Sampling was performed regularly at 10-day intervals for 60 days of storage.

Results Zinc and DEO had a synergistic impact on all indices. The treatment of arils with 0.8% $ZnSO_4$ and 50 µL L^{-1} DEO caused the maximum total soluble solids (TSS), firmness, titratable acidity (TA), total phenolic content (TPC), antioxidant activity, and anthocyanin content, as well as the lowest weight loss (WL) and polyphenol oxidase (PPO) activity.

Conclusions In general, the effect of 0.8% $ZnSO_4$ + 50 µL L^{-1} DEO was most efficient for the increase in storage life and maintained the qualitative characteristics of arils. Also, it caused the zinc content of arils to increase 36-fold. This may be an excellent strategy to meet the body's nutritional demand for zinc.

Keywords Essential oil, GC–mass, Postharvest, Storage life, Supercritical process, Zinc nutrition

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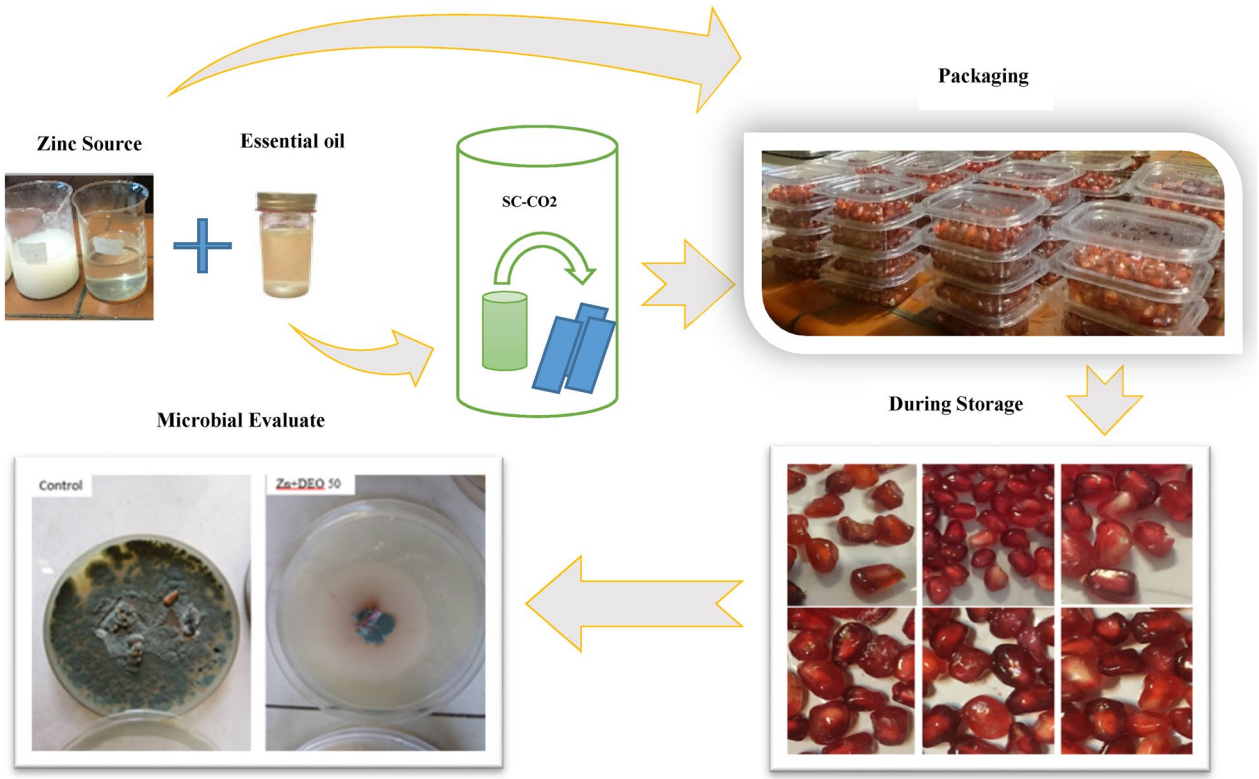
Graphical Abstract



Introduction

As a strategic, commercial fruit, pomegranate (*Punica granatum* L.) is a member of the *Lythraceae* family, capable of growing in tropical and subtropical climates [20]. Due to its high quality, the Iranian pomegranate is an unrivaled product in terms of export among agricultural products and is of great economic importance. Due to the high area under pomegranate cultivation in Iran and the increase in its production, it is highly important to maintain fruit quality and control effective factors that reduce the quality after harvest [5].

Hand peeling and separation of arils from pomegranates is a laborious task, which limits their usage. With technological and commercial improvements, there has been a growing trend toward using ready-to-eat fresh arils. Pomegranate arils, on the other hand, are perishable, because of an absence of protective layers against water loss and pathogen penetration, which would restrict their distribution as well as supply to markets [20].

One of the main factors that cause pomegranate storage waste is caused by the activity of pathogenic fungi. The use of chemical pesticides to reduce agricultural waste is declining due to its adverse effects on consumer health and also an increase in fungal resistance to chemical pesticides [28]. Therefore, it is important to maintain their quality to reduce the amount of waste after harvest and provide the consumer with better quality fruits. So far, many research attempts have been made to improve the shelf life of pomegranates [3, 16, 32]. The postharvest life of crops can be improved by using active packaging. Active packaging involves the process of combining additives in packaging systems to maintain product quality and increase its durability [11]. Several solutions have been suggested for active packagings, such as hydroalcoholic extracts or plant essential oils that play an important role as alternatives to chemical fungicides [11]. Essential oils are colorless natural compounds consisting of alcohol, aldehydes, and esters [40]. Denak (*Oliveria decumbens* Vent) belongs to the *Apiaceae* family and is native to Iran. This plant has antimicrobial properties against *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger*, *Staphylococcus aureus* and *Candida albicans*. The

antimicrobial activity of this essential oil is due to the presence of carvacrol and thymol [25].

Following iron, zinc is one of the main micronutrients needed by the body and is vital for humans, while having an essential role in numerous biological processes. For example, it acts as a kind of cofactor for superoxide dismutase (SOD) as an antioxidant enzyme [47]. Zinc is important in processes such as photosynthesis, sugar formation, fertility, protein synthesis, and growth, as well as disease resistance. With the occurrence of zinc deficiency, physiological activities are likely to be disrupted, and, thus, plant health and yield become severely influenced, reducing crop weight and quality [42]. Zinc deficiency could affect the biological membrane structure and increase membrane susceptibility to oxidative damage, thereby impairing their relevant functions [33]. Zinc concentrations are low in agricultural products, and zinc deficiency symptoms can be seen evidently in a vast majority of crops. Therefore, there is a need for some effective methods to supply zinc in fruits [49].

As the human body cannot store excess amounts of zinc, it should be used regularly but in small amounts in the diet. The maximum tolerable level of zinc is 40 mg per day, although individuals with zinc deficiencies require higher doses. The recommended daily intake (RDI) is 11 mg for adult males and 8 mg for adult females. An amount of 11 mg per day is recommended for pregnant women and 12 mg per day for lactating women [30].

Increasing pomegranate arils consumption in the world and enriching pomegranate arils with zinc could be regarded as an option for providing this essential micronutrient to the body. In addition to the properties mentioned for this element, zinc has antifungal properties such as *Penicillium expansum* and *Botrytis cinerea* [27]. An article also points to the effect of nano-zinc oxide and carboxy methyl cellulose on extending the shelf life of arils [32]. Zinc oxide has strong antimicrobial effects on a wide range of microorganisms while being a harmless compound for the human body [48]. Zinc is an environmentally friendly substance with low toxicity and is used in creams and ointments due to its antibacterial properties. In addition, it is widely used in dentistry and oral health products such as toothpaste and mouthwash, with no concerns over its toxicity or undesirable side effects if used in proper doses. In an experiment, the inhibitory effect of zinc showed significant growth even at low concentrations [2].

Supercritical carbon dioxide saturation is one of the most innovative methods used for active packaging. In this method, the supercritical fluid is the agent for transferring the active component (a variety of essential oils and non-harmful organic chemicals that prevent spoilage by slow and continuous release) from the reaction medium to the inner layer of multilayer packaging containers. At pressures above 7.38 MPa and temperatures over 31.1 °C, carbon dioxide gas is released from the gaseous state and acquires a supercritical fluid state. With changes in temperature and pressure in the supercritical range, the solubility of this fluid tends to change and much organic matter could be dissolved by supercritical carbon dioxide. Also, due to the low density and low surface tension, the supercritical fluid permeability is very high in most polymers. These properties allow the supercritical fluid to easily penetrate the desired active ingredients into the polymer layer of the package. Then, as the pressure decreases, the supercritical fluid will turn to gas and leave the polymer bed while the active component will be trapped inside the polymer bed [31]. Research in the field of active packaging has shown that using supercritical fluid can be used to add thymol to polyethylene labels. For this purpose, a reactor with a volume of 100 mL at pressures between 70 and 120 bar and a temperature of 313 K can be used [51]. In another study, eugenol was infused into a polyethylene film for 4 h by a supercritical fluid process at 45 °C and with pressures of 150, 120, and 100 bar. In the current research, the impact of pressure reduction rate was evaluated on the amount of essential oil remaining in the polyethylene film. The results showed that with these conditions, essential oil can be added to the polyethylene film [21].

Thus, an increase in public awareness about unnatural additives in products can double the need for extensive research on using active packaging materials with natural essential oils. Furthermore, there is a lack of comprehensive knowledge on the use of zinc as $ZnSO_4$ in particular for increasing the shelf life of horticultural crops. The present study aimed to find the best treatment to improve the shelf life and maintain the quality of pomegranate arils in storage. For this purpose, a new approach was used for zinc enrichment followed by active packaging along with DEO-impregnated labels produced by a supercritical fluid. Pomegranate arils were treated with $ZnSO_4$ and DEO. The evaluations were aimed at measuring the ability of zinc enrichment and the essential oil to prevent microbial growth and maintain aril quality in conditions of cold storage.

Materials and methods

Plant material, fruit treatment, and storage conditions

In this study, the pomegranate fruits (Rabab cultivar) were harvested from a pomegranate orchard in Farooq city (29° North and 53° East), Fars province, during the time of commercial harvest at the end of October. Pomegranates were harvested from mature trees when the fruits had a suitable ratio of total soluble solids/titratable acidity (TSS/TA = 24–27). Healthy pomegranates

were immediately sent to the postharvest laboratory of Shiraz University. Immersion of pomegranates in a 0.02% sodium hypochlorite (NaOCl) solution was done for 5 min before being washed with distilled water. The arils were hand-separated after peeling the fruits. Since a previous experiment [4] showed that 0.8% $ZnSO_4$ was the best treatment, it was used in this experiment. Arils were immersed in 0.8% $ZnSO_4$ (Merck, 99%) solution for one minute and drying was done at room temperature for 3–4 h. Then, they were divided into a set of three replications, with each weighing around 50 gr, and packaged in polypropylene packaging containers. In the next step, the labels that were soaked in DEO during the process of supercritical impregnation were placed in the packages (Fig. 1). All packages were stored at 5 °C and relative humidity of 95–90% for 60 days. Sampling was carried out on days 1, 10, 20, 30, 40, 50, and 60, and some physicochemical properties were measured. At the end of storage, trained panelists did the sensory evaluations. At the same time, pathogenic fungi were purified and identified.

Evaluation of physicochemical and quality factors

On days 1, 10, 20, 30, 40, 50, and 60 of cold storage, quantitative and qualitative data were evaluated. The arils were kept at room temperature for a 6-h duration at the very end of each storage period for the simulation of shelf life.

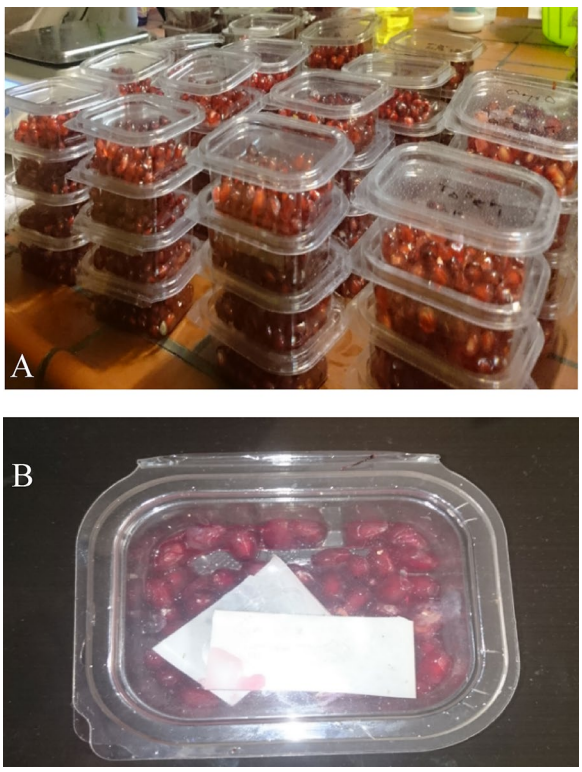


Fig. 1 Processes of packaging arils (**A**) and adding labels inside the packages (**B**)

Zinc content measurement

Aril juice samples were prepared with a volume of 1 mL and then entered into an atomic absorption spectrometer (Shimadzu Model 650AA, Japan) to determine the zinc content of all arils, expressed as mg kg-1 fresh weight (FW).

Extraction of DEO

The flowers of denak plants were dried at room temperature for one week after being collected from the natural habitats of the Khonj region, Fars province. The flowers were powdered and the essential oil extraction was performed by water distillation with a Clevenger apparatus. The total time of essential oil extraction was four hours. Dehydration of essential oil was done through the addition of a minimum amount of sodium sulfate. The anhydrous essential oil was subjected to storage in a dark container in the refrigerator at 4 °C [38].

Identification of essential oil compounds

DEO chemical composition determination was carried out using a gas chromatograph (Agilent 7890A, Santa Clara, CA, USA) coupled with mass spectrometer (Agilent 5975C, USA) and a mass fused silica capillary column (HP-5 MS, 30 m length × 0.25 mm internal diameter × 0.25 µm flm thickness, J & W Scientific, Folsom). The device was equipped with an HP-5 column with a length of 30 m, a diameter of 0.32 mm, and a stationary layer thickness of 0.25 microns. Column temperature programming was performed from 60 to 210 °C at a rate of 3 degrees per minute. The temperature of the injector and detector was 250 and 280 °C, respectively. Nitrogen was used as a carrier gas with an inlet pressure of 1 mL min^{-1} [14].

Impregnation of essential oil into labels by supercritical carbon dioxide

The impregnation of labels was done with the help of a supercritical carbon dioxide fluid reactor made of stainless steel with a volume of 200 mL (made in Iran). A strip of polyethylene terephthalate (PET) was used, having dimensions of 50 cm in length, 1 cm in width, and 0.1 mm in thickness. The strip was placed in the reactor with a large amount of DEO and saturated by DEO with the help of a supercritical fluid. The temperature and operating pressure of the reactor were 45 °C and 100 bar, respectively. After 1 h, the reactor pressure will decrease to ambient pressure within one minute [31]. The strip was weighed before and immediately after

the impregnation process. 0.1 g of the DEO in the reactor was added to the entire PET strip. To produce each label containing 0.006 g of DEO, the long strip was cut into 3 cm pieces and quickly glued into the polypropylene container of the arils. With this, the concentration of DEO in each container became 25 µl per liter. We also used two labels inside the container to have samples with a concentration of 50 µl per liter.

Storage life

The storage life of arils was estimated visually by investigating their apparent quality throughout the storage process. The storage life of arils was calculated from the packaging moment until the first signs of mold growth appeared.

Weight loss

The WL of arils during storage was estimated by measuring the difference between the initial weight of arils (W_1) and each sampling time (W_2). The following equation Eq. (1) was used to calculate WL [41]:

$$\text{Weight Loss (\%)} = (W_1 - W_2)/W_1 \times 100. \quad (1)$$

Juice pH, TSS, and TA

Measurement of the juice pH was done by using a pH meter (JENWAY 351, England) following calibration with pH buffers 4 and 7 [39]. TSS was determined at 20 °C by applying a kind of manual refractometer (ATAGO, Japan) and the obtained results were represented as percentages (%). TA was calculated by the titration of 3 mL of arils juice with 0.1 mol L^{-1} NaOH to an end point pH which was equal to 8.2. The result was represented as a citric acid percentage [32] Eq. (2):

$$\text{TA} = N \times V \times E/D \times 100. \quad (2)$$

TA is the amount of organic acid in the considered fruit extract, which was determined and then expressed in mg 100 mL^{-1}, while *N*, *V*, and *E* refer to the NaOH normality, the used NaOH volume, and the major organic acid gram valence (citric acid), respectively. D refers to the relevant volume of the sample in mL. The total amount of organic acids in the fruit extract was presented as g 100 mL^{-1}.

Determination of carbohydrates by HPLC

The chromatographic separation of sugar content determined the fractions of sucrose, fructose, and glucose, by applying an HPLC Rigole 3400 in an isocratic mode. The considered samples were subjected to analysis on a carbohydrate column (Dikma Company, Plastisil NH_2 25 cm. The RID temperatures were set at 35 °C. The mobile phase consisted of acetonitrile and water (75:25, *v*/*v*). Also, the rate of flow was 1.0 mL/min. The injection volume was 20 µL. Following the calibration of the column with a mobile phase, it was rinsed with water at the end of each experiment for a period above 30 min [53].

Total phenols content

Measurement of TPC was done per the Folin–Ciocalteu colorimetric method [36]. Briefly, 100 µL of the extract was combined with 100 µL of 2% sodium carbonate and left at room temperature for 3 min. The samples were then treated with 20 µL of 50% Folin and stored at room temperature for 30 min. The measurement of absorbance for a given mixture was done by applying a microplate spectrophotometer at 750 nm (Epoch Biotech, Germany). TPC was measured in mg of GAE L^{-1}.

Determination of phenolic compounds by HPLC

To separate, identify, and quantify phenolic components in arils, HPLC analysis was done on an Agilent 1200 series (USA), which was equipped with a Zorbax Eclipse (XDB)-C18 (150 × 4.6 mm I.D., 5 µm) film thickness, RP) and a photodiode array detector (PAD). Following an injection of 20 µL solution into the HPLC system, the rate of flow became 1.0 mL min^{-1}. Monitoring the elution was done at 280 and 320 nm. The selection of gradient elution was done to ensure maximum separation as well as sensitivity. Elution was realized by modifying the solvent A (formic acid 1% in deionized water) to solvent B proportion [methanol (*v*/*v*)] as represented here: formic acid 1%:methanol (90:10), (0 min); formic acid 1%:methanol (75:25), (10 min); formic acid 1%:methanol (40:60), (20 min) and, finally, formic acid 1%:methanol (30:70), (30 min). The total running time was 40 min. The column temperature was set to 30 °C. Identification of the compounds was done by comparison with analytical standards and getting the related calibration curves. The considered analytical standards were sinapic acid, catechin, gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, carvacrol, coumarin, vanillin, hesperidin, trans-ferulic acid, eugenol, ellagic acid, rosmarinic acid, hesperetin, and thymol. All were purchased from Sigma Co. The obtained results were presented in mg L^{-1}. Before injection in the analytical HPLC system, dissolution of all standards was done in methanol HPLC spectral grade. Before HPLC analysis was done, filtering of all solutions (sample and standards) was done through 0.2 µm nylon syringe filter (PTFE). Arils juices were subjected to centrifuging at 9000 g for 10 min at 25 °C to remove pulps and were subsequently stored at − 80 °C until the time of analysis [38].

Anthocyanin content

Anthocyanins were measured spectrophotometrically [36]. A system, composed of two buffers, was used for this purpose: sodium acetate buffer (0.4 M) for pH 4.5 and potassium chloride buffer (0.025 M) for pH 1.0 Eq. (3). The sample's dilution was carried out using the corresponding buffer (1:5). Absorbance measurement was implemented at 510 nm (A510) and 700 nm (A700) by applying a microplate spectrophotometer (Epoch Biotech, Germany). Total anthocyanin content was calculated based on (Eq. 3). The obtained findings were presented as cyanidin-3-glucoside equivalents (mg L^{-1}):

$$A = (\text{A510} - \text{A700})\ \text{pH}\ 1.0 - (\text{A510} - \text{A700})\ \text{pH}\ 4.5 \quad (3)$$

$$\text{Anthocyanin}\left(\text{mg}\,\text{L}^{-1}\right) = \text{A} \times \text{MW} \times \text{DF} \times 1000 / \varepsilon \times 1.$$

A represents the absorbance, MW refers to the molecular weight (449.2 g mol^{-1}), DF stands for the dilution factor, and ε indicates the molar absorptivity (26,900).

Antioxidant activity

Antioxidant activity for each extract was determined by applying the DPPH free radical scavenging method [12]. Briefly, it involved mixing 100 mL of the extract with 1 mL Tris buffer and 1 mL 0.1 mM DPPH (pH = 7.5). The resultant mixture was then kept at room temperature for 30 min, and the absorbance value was read at 517 nm by applying a microplate spectrophotometer (Epoch Biotech, Germany). Eventually, the formula (Eq. (4)) below was utilized to calculate the antioxidant activity:

$$\begin{aligned}&\text{Antioxidant activity (\%)}\\&= 1 - \text{A Sample (517 nm)}\\&\quad / \text{A Control (517 nm)} \times 100.\end{aligned} \quad (4)$$

Firmness

The firmness of arils per treatment was measured on the 20th day of storage (end of control storage life), when all treatments were available, by utilizing an Instron Universal Testing Machine (STM-20, Iran). The cell was half-filled with arils and a compression force was applied using a 35-mm cylindrical probe at a 100-mm min^{-1} rate. The maximum force needed to puncture the fruit peel was considered as the firmness of the fruit. The results were expressed as Newton (N) [48].

Polyphenol oxidase activity

Extraction and assay were performed using a method in the available literature [45], with minor modifications. To describe briefly, 10 g of arils were homogenized in 20 mL of phosphate buffer (pH 7.0, 0.05 M). The obtained mixture was centrifuged at 9000 g for 5 min at 4 °C, and applied as an enzymatic extract. Regarding the enzyme assay, 500 µL of catechol reagent was combined with 2 mL of phosphate buffer (pH 7.0) and 500 µL of enzyme extract. Reading the absorbance occurred at 420 nm (Spectronic, USA). PPO activity was expressed as the absorbance change at 420 nm considering 1 L of fresh weight per minute (U L^{-1}).

Microbiological evaluations

The microbial evaluation was continued until the appearance of mold growth. Three packages from each treatment were randomly chosen at each sampling time. The preparation of arils, with a serial tenfold dilution, was done in a 0.9% NaCl (*w*/*v*) sterile solution. The pour plate method on Plate Count Agar (PCA; Merck, Darmstadt, Germany) was used for counting the psychrotrophic bacteria and total aerobic microorganisms following incubation at 7 °C for 7 days or 30 °C for 72 h, respectively. Subsequently, Yeast Extract Glucose Chloramphenicol agar (YGC agar, Merck) and Violet Red Bile Lactose Agar (VRBLA; Merck) plates were used for determining the total yeast, mold, and coliform counts, following incubation at 25 °C for 5 days and at 37 °C for 24 h, respectively. The relevant tests were conducted twice, and the average colony counts were expressed as log10 cfu g-1 [9].

Reproduction, purification, and DNA sequencing

Amplification of the ITS1-5.8SrDNA-ITS2 region using lead starter (ITS-1-F) and backward starter (ITS-4-R) was done (Table 1). Polymerase chain reactions (PCR) in 25 µL were considered for each tube, containing 2.5 µL of buffer, 10X PCR + $MgSO_4$, 1 µL of each primer (with a concentration of 10 pico mol), 0.5 µL of dNTPs (0.2 mM), Taq polymerase DNA, and 1 µl of fungal DNA (100 ng) were prepared. All reactions were provided by Sina Gen Company. Amplification was examined in a thermocycler (Techne- TC 512, England) according to conditions as follows: initial denaturation at 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s and the last extension at 72 °C for 7 min. PCR products were electrophoresed on 1.2% agarose gel which was stained with

Table 1 Initial sequences for region propagation ITS

Initial sequence	Primer
ITS-1-F	5′-TCCGTA GGTGAACCT GCGG-3′
ITS-4-R	5′-GCATCG ATGAAG AAGAAC GCAGC-3′

ethidium bromide (2 µg/mL) for 15 min. PCR bands were envisioned with a UV trans illuminator (BIORAD, UK) [1]. The PCR reaction product was recycled and purified using a PCR product purification kit (Kiagen Company). Then, the product was recycled by Sanger sequencing (Genetic codon Co.) Local alignments (BLAST) in Gen Bank databases were registered.

Sensory evaluation

Aril sensory properties, such as sweetness, sourness, aroma, color, luminosity, and sliminess, were specified by applying a panel test including 10 individuals, consisting of men and women with an age range of 20 to 50 years. On day 20 of storage, at 5 °C, the storage period ended for the arils of the control group. According to these sensory attributes, the panelists' overall acceptance of the samples was assessed based on a score ranging from 1 to 10. A score of 1 represented 'worst quality', whereas a score of 10 represented 'excellent quality'. The evaluation was based on mean scores [16].

Experimental design and statistical analysis

The distribution of the treatments was done according to a factorial experiment by applying a randomized completely block design (RCBD) involving three replications. Physicochemical data were analyzed. The comparison of mean values was done by applying Duncan's multiple range test ($P \leq 0.01$). All analyses were implemented by using SAS software (SAS Institute Inc., USA), version 9.1. Pearson's correlation coefficients were measured among the relevant parameters.

Results and discussion

Zinc content

In this research, the amount of zinc in the pomegranate arils of the control was 0.52 mg Kg^{-1}, which increased by about 36 times in those treated with zinc. Arils treated with $ZnSO_4$ displayed the highest concentration of zinc in comparison with the other treated arils. Arils of the control group had the least amount of zinc, which was significantly different from the other treatment groups (Fig. 2). According to experts, zinc is required daily for human health [48] and can be incorporated into food packaging or food additives [13]. The high tolerable level of zinc is 40 mg per day, which can be within the limit of daily allowance (LDA), and, therefore, can be thoroughly safe. The recommended daily allowance (RDA) for zinc is 11 and 8 mg per day for men and women, respectively (Institute of Medicine (US) Panel on Micronutrients, 2001). According to Fig. 2, the maximum amount of zinc in arils (14.88 mg kg^{-1}) was observed in response to 0.8% $ZnSO_4$, which is in accordance with the standards and did not exceed the RDA. In fact, the amount of zinc in all treated arils was within the range of daily allowance (LDA), suggesting that the treatments were safe. Accordingly, the zinc RDA for the human body can be met by fortified pomegranate arils.

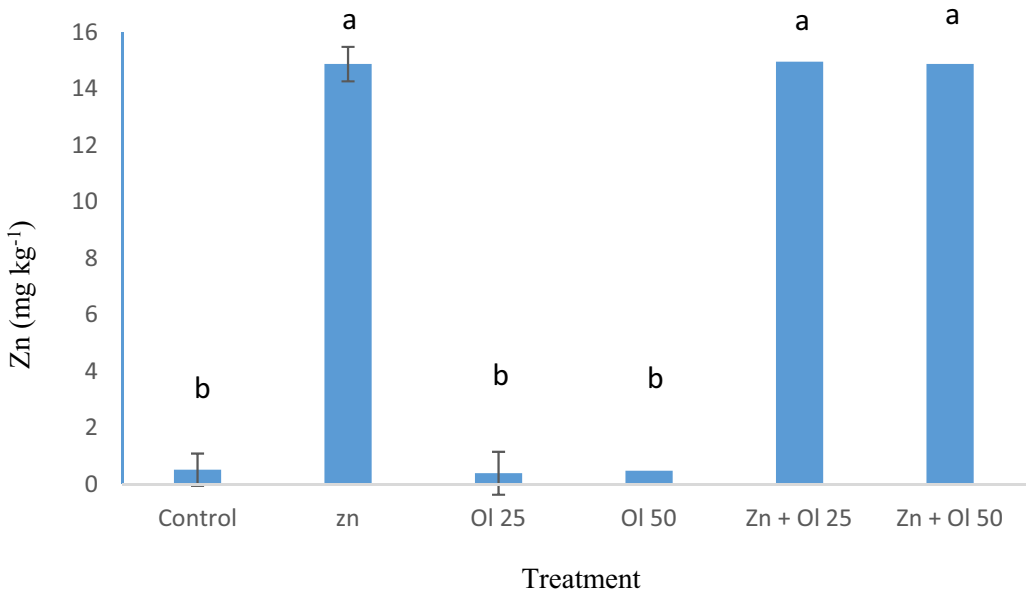


Fig. 2 Zinc content in arils treated with zinc and without zinc at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

Table 2 Pearson's correlation coefficients in arils treated with different concentrations of DEO and zinc sulfate ($ZnSO_4$) at the end of the storage life at 5 °C

	WL	pH	TSS	TA	TPC	Anthocyanin	Antioxidant	PPO	T aerobic count	Psychotropic	T yeast and mold	Zn	Firmness	Shelf life
WL	1													
pH	0.92[b]	1												
TSS	− 0.85[b]	− 0.90[b]	1											
TA	− 0.90[b]	− 0.93[b]	0.84[b]	1										
TPC	0.91[b]	0.78[b]	− 0.85[b]	− 0.77[b]	1									
Anthocyanin	− 0.77[b]	− 0.77[b]	0.84[b]	0.83[b]	− 0.67[b]	1								
Antioxidant	− 0.70[b]	− 0.51[b]	0.51[b]	0.47[b]	− 0.66[b]	0.89[b]	1							
PPO	0.89[b]	0.83[b]	− 0.78[b]	− 0.74[b]	0.88[b]	− 0.86[b]	− 0.62[b]	1						
T aerobic count	0.98[b]	0.92[b]	− 0.84[b]	− 0.89[b]	0.88[b]	− 0.83[b]	− 0.71[b]	0.89[b]	1					
Psychotropic	0.97[b]	0.91[b]	− 0.82[b]	− 0.89[b]	0.87[b]	− 0.84[b]	− 0.72[b]	0.89[b]	0.99[b]	1				
T yeast, mold	0.96[b]	0.88[b]	− 0.83[b]	− 0.88[b]	0.88[b]	− 0.86[b]	− 0.71[b]	0.89[b]	0.97[b]	0.97[b]	1			
Zn	0.78[b]	0.70[b]	0.77[b]	0.69[b]	0.71[b]	0.61[b]	0.61[b]	− 0.57[b]	− 0.71[b]	− 0.78[b]	− 0.75[b]	1		
Firmness	− 0.54[b]	0.62[b]	0.71[b]	0.61[a]	0.59[b]	0.62[b]	0.65[b]	0.59[b]	− 0.84[b]	− 0.83[b]	− 0.84[b]	0.74[b]	1	
Shelf life	0.22[a]	0.20[a]	0.1 ns	0.1 ns	0.13 ns	0.1 ns	0.25[a]	− 0.1 ns	− 0.71[b]	− 0.71[b]	− 0.70[b]	0.71[b]	0.87[b]	1

[a] Significant at p = 0.05; [b]significant at p = 0.01; *ns* non-significant

Chemical composition of DEO

The results of the GC–MS analysis identified many chemical compounds in the DEO. Eight of these compounds were dominant, including γ-terpinene (28.18%), thymol (19.45%), carvacrol (13.97%), α-terpinene (13.31%), myristicin (8.04), p-cymene (7.32%), D-limonene (2.81), β-pinene (1.68) (Table 2). These results were in line with previous reports [25], but with slight differences in the concentration of the components.

Storage life

Arils of the control group decayed after 20 days of storage, whereas arils treated with 0.8% $ZnSO_4$ and 25 µL L^{-1} DEO were degraded after 30 days of storage. Arils treated with DEO 50 decayed after 40 days of storage. The treatment of arils with Zn + DEO 25 extended the storage life up to 50 days. At most, arils treated with Zn + DEO 25 remained healthy until day 60 of storage (Fig. 3), while maintaining the physicochemical qualities of the arils up to 60 days of storage. This tripled their storage life compared to the control. Such findings indicated that zinc and essential oil have a synergistic impact on improving storage life. Using DEO and eucalyptus essential oil (50 µL L^{-1}) on two strawberry fruit cultivars reportedly caused improvements in the storage life of strawberries [18]. Thakur et al. [50] stated that zinc increases the shelf life of crops, thereby confirming the findings of this experiment. There was a correlation coefficient ($r=71$) between storage life and zinc content. Also, zinc limited the growth of microorganisms considerably (Table 3).

Weight loss

WL was observed in all treatment groups throughout the storage period ($P \leq 0.01$) (Fig. 4A). The maximum WL belonged to the arils of the control. Arils treated with Zn + DEO 50 continued to be healthy until day 60 and showed a lower level of WL in comparison with the other treatment groups. WL usually occurs throughout the storage period because of water exchange between the inside and outside of a given product, followed by respiration and tissue degradation [4]. According to previous studies, postharvest zinc treatments reduced WL in kiwifruit [35], pomegranate [32], and strawberry [3, 48]. The ameliorative effect of zinc treatment on WL ($r=0.78$) could be attributed to its role in decreasing the microbial load ($r=0.84$) and maintaining membrane integrity. Tissue softening and an increase in WL during storage are usually concurrent with fruit rot. Due to its oily structure, the essential oil covers the surface of the fruit in a thin layer. With its antimicrobial properties, it replaces the damaged cuticle and increases the cuticle function, thereby affecting the exchange of carbon dioxide, oxygen, and water vapor. Thus, it delays the process of weight loss and softens the fruit tissue [26].

Juice pH, TSS, TA

The pH of aril juice increased continuously throughout the storage period. Greater changes were observed in the arils of the control group (Fig. 4B). After 20 days of storage, the pH of the control group was higher than in the other treatment groups. On the last day of storage (day 60), the minimum pH was observed in arils treated with Zn + DEO 50. The decrease in TA correlated with the increase in the pH of aril juice ($r=-0.91$). The results showed that zinc and essential oil prevented the reduction of TA and TSS, thereby preventing the rise of pH. This also showed a considerable correlation between pH and TA ($r=-0.93$) (Table 3). The highest levels of TSS (data not shown) and TA were found at harvest time but then gradually declined throughout the storage period. Nevertheless, the rate of reduction in the control group was greater when compared to that of the zinc and essential oil treatment groups (Fig. 5A). Throughout the storage, the treated arils had a higher level of TSS and TA, when compared to the control arils. The highest TA and TSS occurred in response to the Zn + DEO 50 treatment, which was significantly different from the other treatment groups. The lowest levels of TA and TSS belonged to the control group. The organic acids content in fruits decreased over time, owing to respiration and energy consumption, fermentation, and organic acid oxidation [39]. The TA content correlated with the concentration of the organic acid. The decline in acidity at the end of the storage period could be ascribed to the consumption of organic acids in the process of respiration [15]. Zinc indirectly preserved intracellular acids by reducing the microbial load ($r=0.69$). Guilin et al. [23] reported that essential oil can reduce the consumption of organic acids in the product by decreasing the rate of oxidative processes such as respiration. Amal et al. [3] indicated that essential oils reduced the rate of respiration and retained carbohydrates by inhibiting the activity of sucrose phosphate synthase (a key enzyme in the conversion of acids to sucrose). Any factor that reduces respiration and aging can reduce the consumption of acids in the cell [22]. The results of analyzing individual sugar components, with HPLC on the 20th day, showed insignificant amounts of sucrose. Meanwhile, the amount of fructose was higher than that of glucose (Table 4).

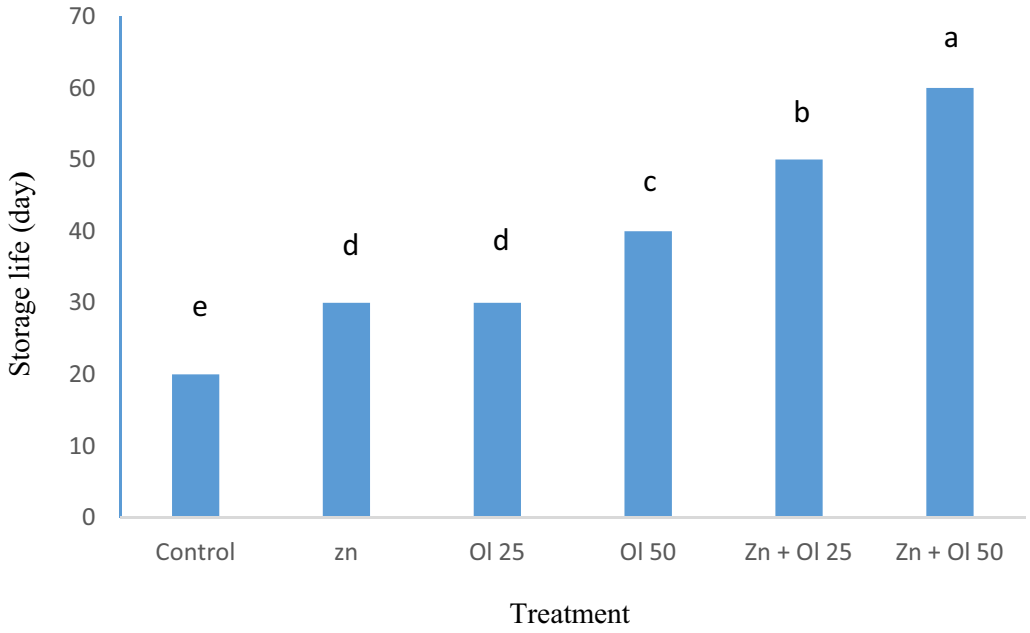


Fig. 3 Storage life of control and treated pomegranate arils at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

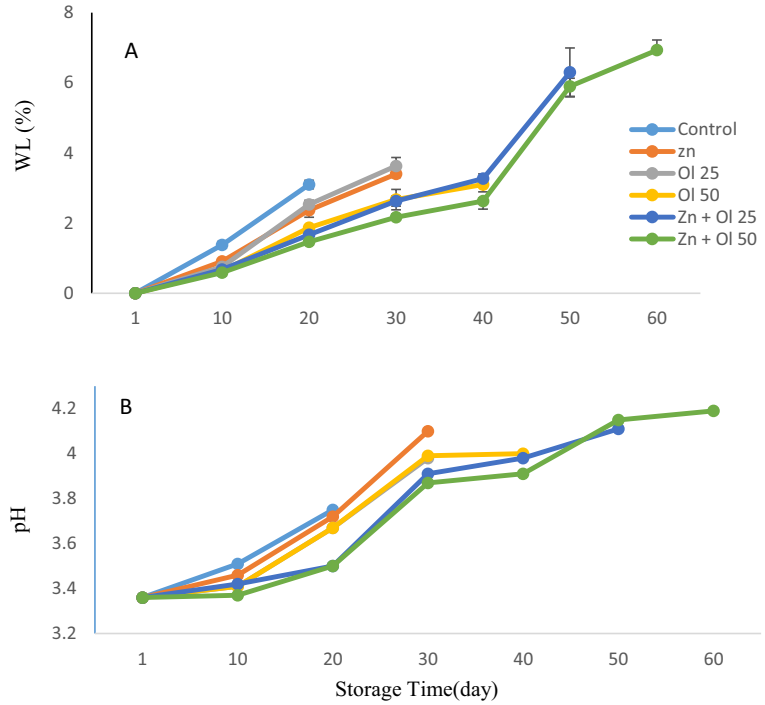
Total phenolic content

In the current experiment, TPC increased in the first 30 days of storage, thereafter decreased until the 40th day, and increased again until the end of storage (Fig. 5B). The TPC in the arils of the control group was lower than that of other treatment groups. At all sampling times, the maximum TPC occurred in response to Zn + DEO 50, whereas the lowest was observed in the control group. The increment of TPC during storage may be related to the stimulation of the activity of some enzymes involved in phenolic biosynthesis due to cold storage [24], or aging as a result of the breakdown of the cell structure [41]. The decrease of the TPC in pomegranate arils may be due to the decomposition of TPC as a result of enzymatic activity during storage; it could also be owing to the oxidation or polymerization of TPC [37] or the breakdown of the cell structure throughout product aging [19]. One of the reasons for the observed decline in phenolic compounds in the postharvest period is polyphenol oxidase (PPO) activity, which causes the oxidation of phenolic compounds that form brown polymers and lead to the browning of the fruit [46]. Due to their oily structure [26], essential oils reduce oxygen contact with the fruit surface. Also, zinc reduces PPO activity, respiration, and microbial load [48], thereby maintaining TPC. Essential oils usually contain phenolic compounds, which can be a robust scavenger of free radicals and can aid in maintaining plant health. Phenolic compounds are known for their high antifungal activity that damages microbial cell walls, cell membranes, and cell organelles. Phenolic compounds can be regarded as a hydrophobic fraction in essential oils and are subjected to dissolution in the cytoplasmic membrane, especially the hydrophobic domain between acyl lipid chains, thereby disintegrating the outer membrane. In turn, this can enhance cytoplasmic membrane permeability to adenosine triphosphate (ATP) in pathogenic cells and cause cell death. In essential oils, thymol and carvacrol disrupt the integrity of the bacterial cell membrane and affect the pH homeostasis and mineral ion balance in the pathogen. Since thymol has high antifungal activity, it can be assumed that essential oils are characterized by antifungal activity because of their

Table 3 Soluble sugars (fructose, glucose and sucrose) of pomegranate arils extract determined by HPLC after 20 days of storage at 5 °C

Sample	Concentration (g L^{-1})		
	Fructose	Glucose	Sucrose
Control (20 day)	45.5e	44e	≤ 0.5
Zn (20 day)	51d	50.5d	≤ 0.5
L25 (20 day)	59.5c	59c	≤ 0.5
L50 (20 day)	59c	58.5c	≤ 0.5
L25 + Zn (20 day)	68.5b	67.5b	≤ 0.5
L50 + Zn (20 day)	74.5a	73.5a	≤ 0.5

(Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})



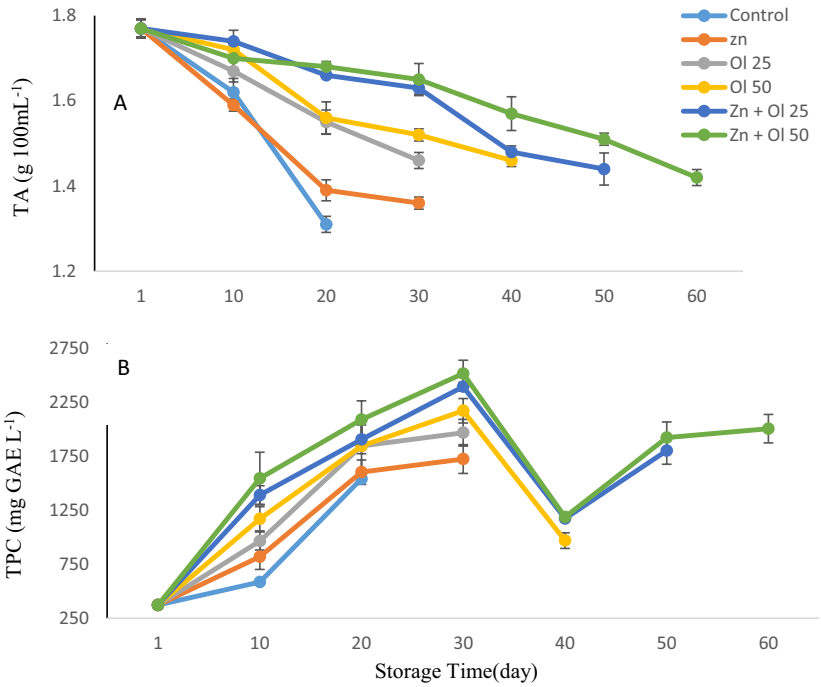
A of V GLM	DF	Mean Sq WL	Mean Sq pH	significance WL	significance pH
Block	2	0.97	0.17	$P \leq 0.01$	$p \leq 0.05$
treatment (T)	5	1.44	0.031	$P \leq 0.01$	$p \leq 0.01$
Storage Time (ST)	6	46	1.87	$P \leq 0.01$	$p \leq 0.01$
T * ST	17	0.28	0.001	$P \leq 0.01$	$p \leq 0.05$
Error	56	0.69	0.004		

Fig. 4 Changes of WL (**A**) and pH (**B**) in pomegranate arils during storage at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

high phenolic composition, which facilitates the binding of these compounds to proteins, especially the cell membrane of pathogens. This facilitates membrane disintegration and causes the loss of cellular content [26]. The results also indicated a considerable correlation between TPC and PPO ($r = -0.88$). Arils that were treated with zinc and essential oil had more TPC than the control group, due to a reduced microbial load and the protection from cellular destructions of fungal attack [26, 32]. The results of this experiment, thus, indicated a considerable correlation between zinc and TPC ($r = 0.71$) (Table 3). During the measurement with HPLC on the 20th day of storage (when all treatments were available), 17 phenolic compounds were measured, of which only 7 compounds were present in pomegranate. These compounds were, namely, gallic acid, catechin, caffeic acid, chlorogenic acid, vanillin, hesperidin, and ellagic acid. Three of these compounds, i.e., gallic acid, catechin, and ellagic acid, had the highest levels (Table 5).

Anthocyanin content

Pomegranate color is related to the anthocyanin content of arils, consisting of cyanidin, delphinidin, and pelargonidin. Anthocyanins are unstable compounds in plants and have strong antioxidant properties that are beneficial to human health [52]. In this study, the anthocyanin content decreased throughout storage. However, the decrease was less prominent in zinc- and essential



A of V GLM	DF	Mean Sq TA	Mean Sq TPC	significance TA	significance TAC
Block	2	0.004	173041	ns	$p \leq 0.01$
treatment (T)	5	0.071	524034	$P \leq 0.01$	$p \leq 0.01$
Storage Time (ST)	6	0.225	5418712	$P \leq 0.01$	$p \leq 0.01$
T * ST	17	0.014	75564	$P \leq 0.01$	$p \leq 0.05$
Error	56	0.002	33250		

ns = non-significant

Fig. 5 Changes of TA (**A**) and TPC (**B**) in arils treated during storage at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

oil-treated arils, compared to the control (Fig. 6A). The lowest amount of anthocyanins was recorded in the arils of the control group, whereas the highest amount occurred in arils treated with Zn + DEO 50. The decrease in anthocyanins throughout storage could be ascribed to TPC oxidation via PPO catalysis, as well as its degradation and conversion to other types of anthocyanins in pomegranate [48]. This experiment also confirmed a considerable correlation between anthocyanins and PPO ($r = -0.86$). A decrease in anthocyanins during storage may be due to various factors such as pH, storage temperature, oxygen, metal ions, sugars, and their products which influence the rate of anthocyanin degradation [17]. Essential oils have strong antioxidant properties that prevented anthocyanin oxidation during storage. When treated with thymol, strawberries reportedly had higher levels of anthocyanins compared to the corresponding control [3], which was in agreement with the findings of the current research. Zinc was also effective in maintaining the anthocyanin content of arils. The zinc and anthocyanin content of fruits correlated significantly with each other ($r = 0.61$) (Table 3).

Antioxidant activity

Antioxidant compounds are molecules that scavenge free radicals in the body and inhibit oxidative processes, thereby benefiting human health. During fruit ripening, reactive free radicals and oxygen species (ROS) increased due to an increase in oxidative metabolism. To prevent the effects of free radicals, plant cells use antioxidant systems. Usually, antioxidant activity decreases during storage, which could be associated with aging as well as decay [48]. In this research, the rate of reduction was found to be less in zinc and essential oil-treated arils than in arils of the control group (Fig. 6B). The lowest level of antioxidant activity was recorded in arils of the control group, following 20 days of storage. At such a stage, the highest antioxidant activity was observed in arils treated with Zn + DEO 50. Pomegranate is known to have many compounds that exhibit antioxidant activity. While anthocyanins are one such compound, the results revealed a considerable correlation between antioxidant activity and anthocyanin content ($r = 0.89$) (Table 3). Some essential oils reportedly increased the antioxidant capacity of harvested products by increasing their antioxidant enzyme activity and synthesizing antioxidant compounds [22, 29]. The results showed that zinc and DEO treatments partially prevented the decrease in antioxidant activity by reducing microbial growth and delaying the process of cellular aging. A correlation between antioxidant activity and zinc content ($r = 0.61$) was recorded. In this regard, the findings of this research are similar to those reported in the available literature [48].

Firmness

At harvest, the firmness of the arils was 0.91 Newton (N). The firmness of the arils declined over time and during storage, but arils treated with zinc and essential oil showed greater firmness than the control. Untreated arils decayed after 20 days of storage and showed a firmness value of 0.16 N. Arils that were treated with Zn + DEO 50 remained apparently healthy until 60 days in storage, and their ultimate firmness value was 0.21 N (Fig. 7A). There was a high negative correlation between firmness and microbial contamination ($r = 0.84$) (Table 3), which suggests that zinc and essential oils increased the firmness of arils indirectly due to their antimicrobial effects on the microbial load [35]. Subsequently, an increase in firmness was associated with a longer storage life ($r = 0.87$) (Table 3). Also, essential oils formed a thin layer on arils, which acted as a barrier against water loss, and, thus, maintained firmness [26, 44].

Table 5 Chemical composition of *Oliveria decumbens* essential oil

No.	Compound	Relative peak area (%)
1	γ-Terpinene	28.18
2	Thymol	19.453
3	Carvacrol	13.975
4	α-Terpinene	13.312
5	Myristicin	8.042
6	p-Cymene	7.317
7	D-Limonene	2.813
8	β-Pinene	1.677

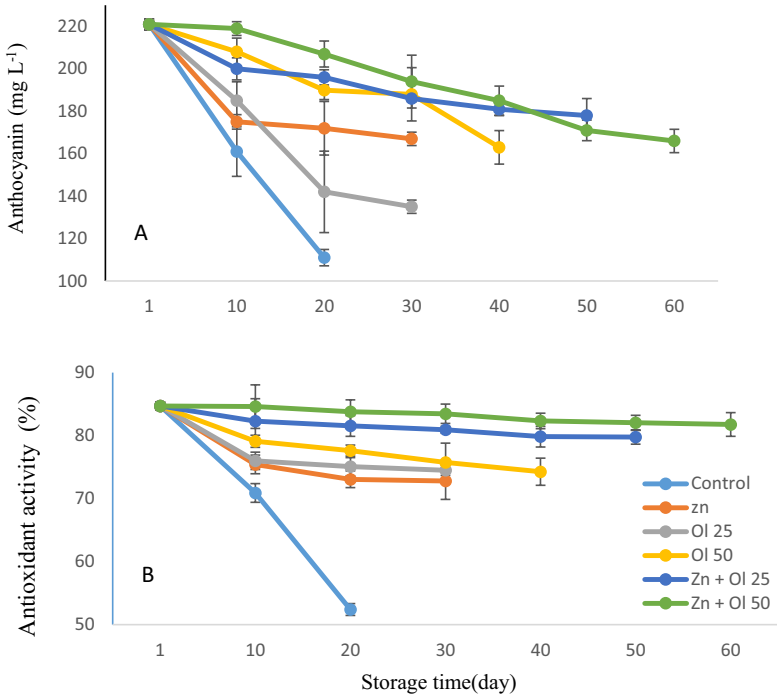
Polyphenol oxidase activity

PPO activity increases with aging and longer storage periods. It causes the dissolution of pectin materials in the cell wall, causes microbial contamination, disruption of membranes, and oxygen penetration [26]. PPO activity was more significant in the arils of the control group (Fig. 7B). The lowest level of activity was observed in arils treated with Zn + DEO 50. A gradual rise in PPO activity could explain the early, rapid browning of the arils. The PPO enzyme penetrates the thylakoid membrane, acts on the adjacent cytoplasm layer (sub), and oxidizes phenolic compounds, thereby

Table 4 Phenolic compounds of pomegranate arils extract determined by HPLC after 20 days of storage at 5 °C

Sample	Concentration (mg L^{-1})						
	Gallic acid	Catechin	Caffeic acid	Chlorogenic acid	Vanillin	Hesperidin	Ellagic acid
Control (20 day)	263e	35.5f	3.9f	4.37d	4.29f	1.02f	11.32d
Zn (20 day)	300.5d	38.37e	7.2e	7.17c	6.45e	1.61e	11.81d
L25 (20 day)	313.5c	41.99d	9.26d	7.3c	8.9d	2.24d	11.78d
L50 (20 day)	319.5c	60.5c	9.38c	8.37b	12.97c	2.91c	14.01c
L25 + Zn (20 day)	351.5b	63.71b	10.95b	8.41b	16.1b	5.06b	15.31b
L50 + Zn (20 day)	387a	78.86a	11.8a	9.25a	27.89a	9.65a	16.15a

(Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})



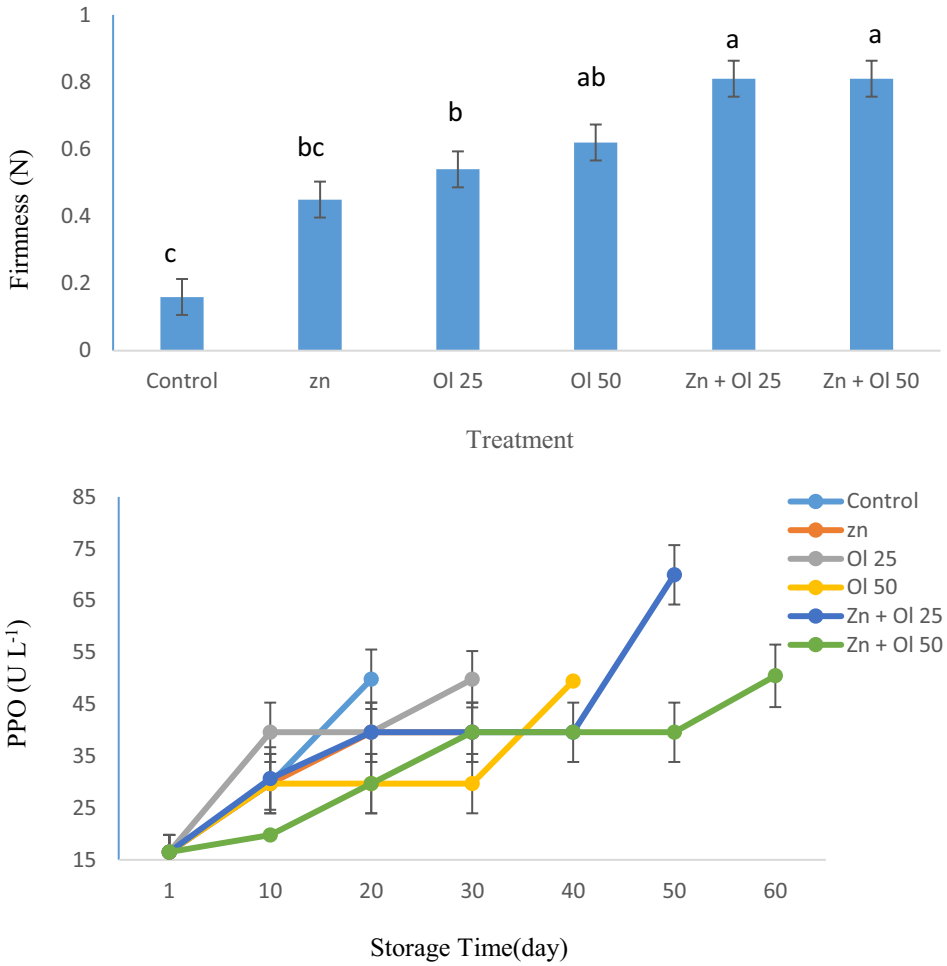
A of V GLM	DF	Mean Sq Anthocyanin	Mean Sq Antioxidant	significance Anthocyanin	significance Antioxidant
Block	2	465	47.35	$P \leq 0.05$	$p \leq 0.01$
treatment (T)	5	4363	68.16	$P \leq 0.01$	$p \leq 0.01$
Storage Time (ST)	6	7227	512	$P \leq 0.01$	$p \leq 0.01$
T * ST	17	780	24.59	$P \leq 0.01$	$p \leq 0.01$
Error	56	146	7.12		

Fig. 6 Changes of anthocyanin (**A**) and antioxidant activity (**B**) in control and treated arils during storage at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

browning the arils [45]. In the present research, the pH value of the brown arils increased simultaneously ($r = 0.83$) and their moisture content was lower than that of their healthy counterparts. These changes brought an imbalance between oxidative and reducing processes, which decreased membrane integrity and facilitated enzymatic oxidation, leading to the production of brown pigments in the arils [46]. Sulfites can be regarded as the main inhibitors of PPO activity in fungi and plants because they affect the active sites of enzymes directly while reducing reactive products [34]. Due to its oily structure, essential oils are usually placed as a thin layer on the product and affect the exchange of oxygen gas, thereby reducing PPO activity [26, 44]. In addition to zinc, essential oils reduce microbial contamination and inhibit PPO activity.

Microbial evaluation

The microbiological properties related to untreated and treated pomegranate arils throughout the storage

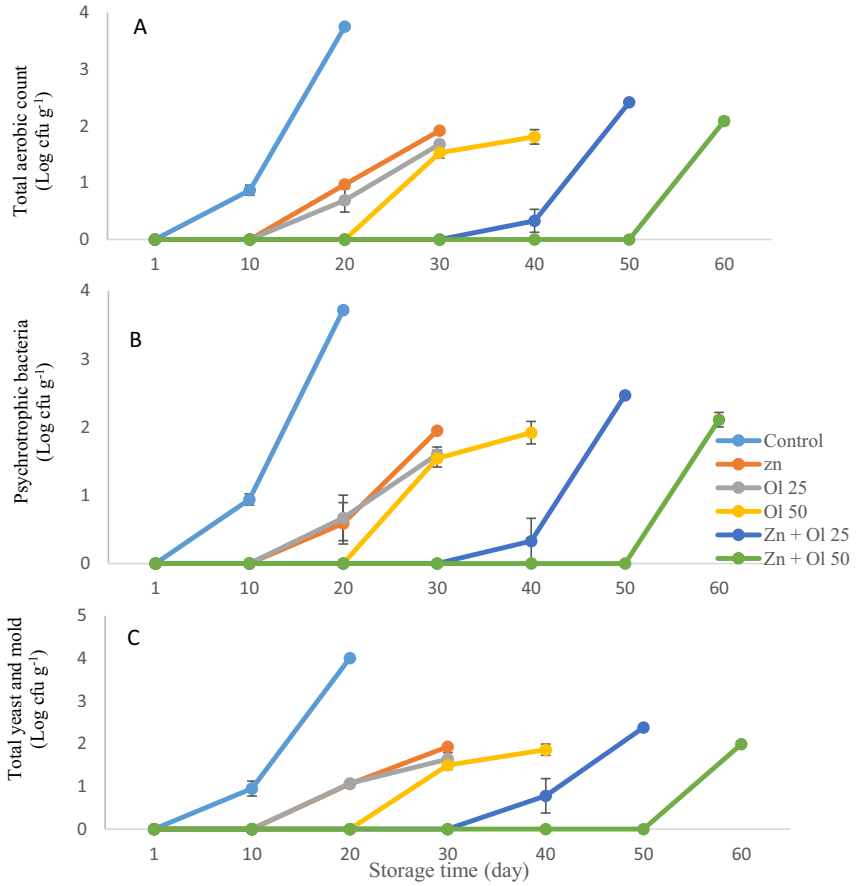


A of V GLM	DF	Mean Sq PPO	significance PPO
Block	2	69.1	ns
treatment (T)	5	288	$P \leq 0.01$
Storage Time (ST)	6	1887	$P \leq 0.01$
T * ST	17	129	$P \leq 0.05$
Error	56	64.5	

ns = non-significant

Fig. 7 Changes of firmness after 20 days of storage (**A**) and PPO during storage (**B**) in pomegranate arils at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

period are presented in Fig. 8. Regardless of treatment type, the microbial count increased significantly during storage ($P \leq 0.01$). Coliforms could not be seen in the treated arils. The highest acceptable limits for total yeast, mold and total aerobic bacteria in fresh fruits are 5–7 log CFU g-1 [9]. The total aerobic count in the arils of the control group increased to 4 log CFU g-1 on day 20. In the other treatment groups, however, it continued to remain below the upper acceptable limit throughout the study period. On day 20, the control group was more contaminated than the other treatment groups. Accordingly, it was revealed that Zn and DEO treatments maintained aril quality by reducing microbial contamination. The efficiency of this treatment could be improved by



A of V GLM	DF	Mean Sq Total aerobic count	Mean Sq Psychrotrophic bacteria	Mean Sq Total yeast and mold	significance
Block	2	0.047	0.07	0.61	ns
treatment (T)	5	2.86	3.19	3.28	$p \leq 0.01$
Storage Time (ST)	6	5.54	5.71	5.62	$p \leq 0.01$
T * ST	17	1.18	1.30	1.21	$p \leq 0.01$
Error	56	0.012	0.39	0.025	

ns = non-significant

Fig. 8 Total aerobic count (**A**), psychrotrophic bacteria (**B**), and total yeast and mold (**C**) in pomegranate arils during storage at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL, OL 50 = *Oliveria decumbens* 50 µL

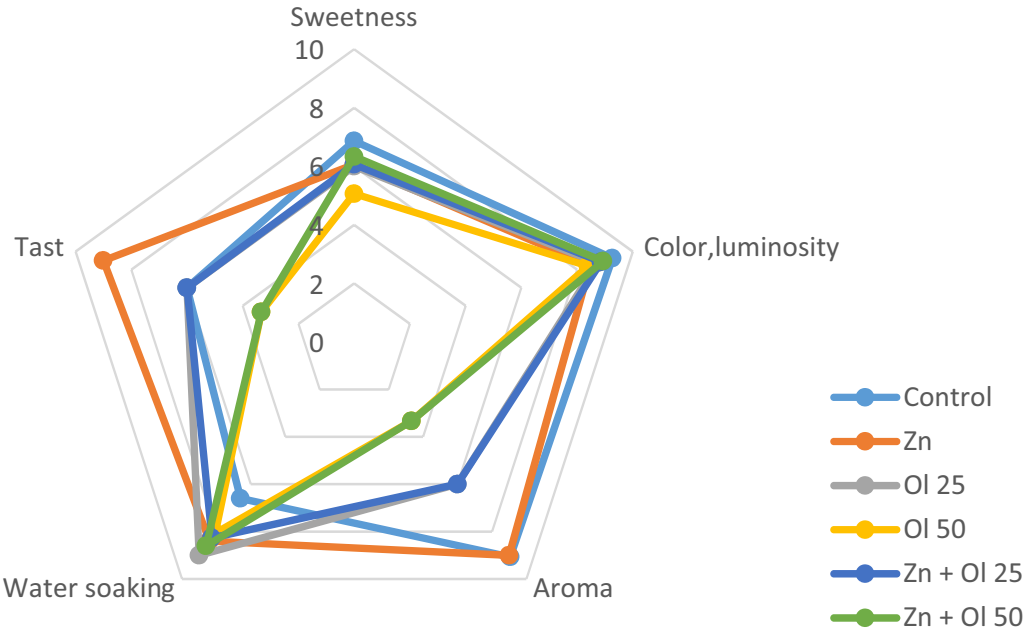


Fig. 9 The average sensory scores in pomegranate arils after 20 days of storage at 5 °C. (Zn = $ZnSO_4$, OL 25 = *Oliveria decumbens* 25 µL L^{-1}, OL 50 = *Oliveria decumbens* 50 µL L^{-1})

increasing the essential oil concentrations. Therefore, the combination of zinc and essential oil treatments was much more effective than each of the treatments alone, regarding the microbial count and the visual quality of fruits during storage. The results, thus, were in agreement with those of the previous studies on pomegranates [32] and strawberries [3]. Zinc treatment reduced microbial growth, thereby delaying the process of cellular aging and decay [43]. Furthermore, a correlation existed between microbial contamination and the zinc content of arils ($r = -0.75$). Zinc treatment usually suppresses fungal mycelium growth [7]. Zinc acetate reportedly decreased microbial contamination. Similarly, $ZnSO_4$ can release zinc after dissolution in water. In the $ZnSO_4$ treatment, following dissolution in water (solubility of $ZnSO_4$ = 57.7 g 100–1 mL), zinc ions are released and bind to the microorganism membrane, thereby delaying cell division and the growth cycle [6]. The antimicrobial and antifungal activity of essential oils emanates from the qualities of terpenes/terpenoids, which could be ascribed to their highly lipophilic nature and low molecular weight. They can impair cell membrane permeability, induce cell death, inhibit spores, and delay food spoilage. Essential oils damage the cellular membrane of pathogens, weaken the cell wall, impair membrane integrity, and disturb membrane permeability, causing leakage and release of vital intracellular substances such as proteins and K^+. This can damage or kill the microbes and allow the entry of antibiotics. The antimicrobial activity of DEO may be ascribed to its main components, including terpinene, thymol, and carvacrol which belong to the category of monoterpenes [10, 38]. In another study, two essential oils of cumin and DEO were used at concentrations of 1, 3, 5, 10, and 15%. The antimicrobial properties of these emulsions were evaluated, revealing that both emulsions had an inhibitory effect on two bacteria (*Escherichia coli* and *Staphylococcus aureus)*, while DEO was effective against *Staphylococcus aureus* [8]. In the current study, the visual quality of arils in storage is depicted in Fig. 9.

Sensory evaluation

The panelists evaluated the sweetness, color, luminosity, and water soaking, all of which gained better scores in the treated arils, compared to the arils of the control group. The zinc treatment had no adverse impact on the aroma, color, and luminosity of the arils. Treating the arils with two concentrations of DEO did not change the color or luminosity, but the aroma of the arils changed. Nonetheless, arils treated with DEO 25 had a more acceptable aroma, compared to those of the DEO 50 treatment. Among the treatments, Zn + DEO 25 had more desirable sensory properties and overall acceptability, compared to the other treatments on day 20 of storage. Furthermore, the findings revealed that zinc and essential oil treatments reduced browning by decreasing phenolic oxidation. Zinc and essential oil treatments also decreased the microbial

Fig. 10 Visual quality of arils during storage **A**: control at the end of the storage life (20th day), **B**: zinc sulfate 0.8% ($ZnSO_4$ 0.8%) at the end of the storage life (30th day), **C**: *Oliveria decumbens* essential oil 25 µL (ODEO25 µL) at the end of the storage life (30th day), **D**: *Oliveria decumbens* essential oil 50 µL (ODEO50 µL) at the end of the storage life (40th day), **E**: zinc sulfate 0.8% + *Oliveria decumbens* essential oil 25 µL ($ZnSO_4$ 0.8% + ODEO25 µL) at the end of storage life (50th day), **F**: zinc sulfate 0.8% + *Oliveria decumbens* essential oil 50 µL ($ZnSO_4$ 0.8% + ODEO50 µL) at the end of storage life (60th day)

growth during storage, which led to a positive impact on the sweetness, aroma, and water soaking of the arils (Fig. 10). The current results were consistent with previous reports on pomegranates [32] and a fermented dairy drink, 'doogh' [54].

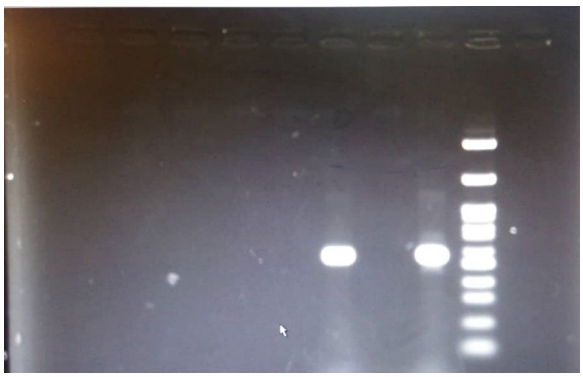


Fig. 11 PCR product from ITS region amplification for 11 isolates studied from left to right, respectively, with access number from HM629973 to HM629958

Reproduction, purification, and DNA sequencing

After purification and identification, the results confirmed the presence of *Penicillium glabrum*. The Zn + DEO 50 treatment significantly prevented the growth of this fungus, compared to the condition of the control group. Also, in Fig. 11, the existence of one type of fungus was confirmed. Accordingly, Fig. 12 clearly shows the effect of Zn + DEO 50 treatment on the prevention of fungal growth. The results of fungal identification were available from the database of NCBI (GenBank: MT159429.1 and MT159430.1).

Conclusion

This study revealed that zinc and DEO treatments increased the postharvest life of arils, compared to the control group. The combination of these two treatments, however, had a synergistic effect. Zinc and DEO treatments reduced PPO and WL. They improved the TSS, TA, anthocyanin content, and antioxidant activity. Regarding the RDA, the zinc treatment retained the nutritional features of the arils, compared to the control

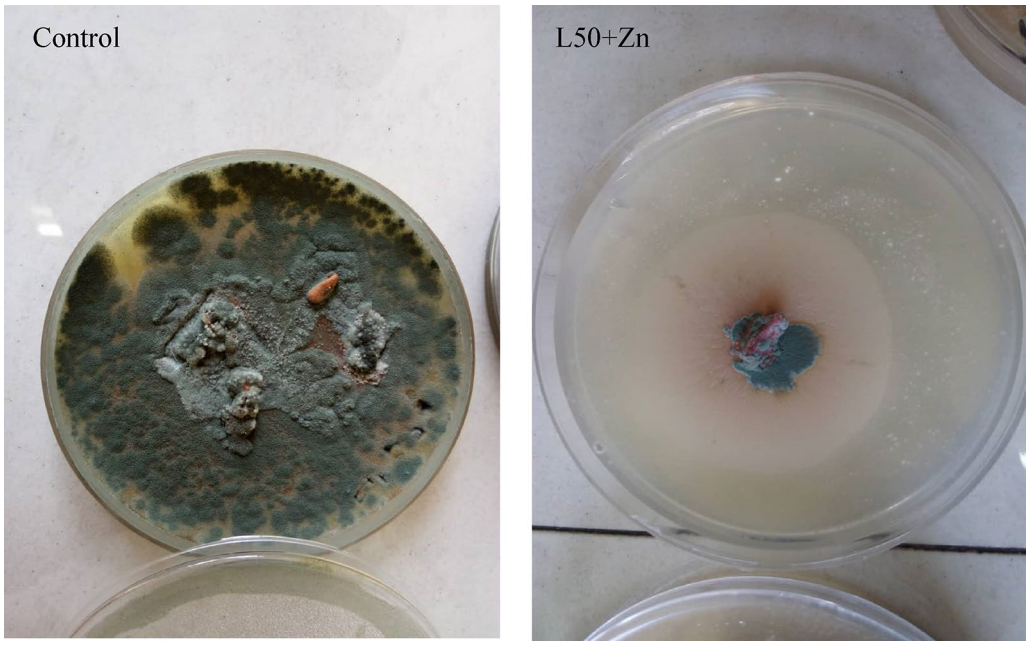


Fig. 12 The effect of L50 + Zn (0.8% $ZnSO_4$ + *Oliveria decumbens* 50 µL) treatment on preventing the in vitro growth of *Penicillium glabrum*

samples. Among all treatment groups, Zn + DEO 50 performed most optimally by maintaining the quality of pomegranate arils, although it was not acceptable by the panelists because of its adverse effects on the aroma of arils. Overall, the outcome of Zn + DEO 25 was more acceptable. Fungal decay appeared after 20 days in the arils of the control group. However, fungal decay was delayed up to 60 days in arils treated with zinc and essential oil. The zinc and DEO treatments can be regarded as a straightforward, effective, and fast method to lengthen the storage period of arils and meet the daily nutritional requirement for zinc. The combined use of zinc and DEO can be recommended as an optimal treatment for improving the storage life and maintaining the qualitative characteristics of pomegranate arils while lowering their microbial contamination. Also, the use of CO_2 supercritical fluid for transferring the essential oil to labels can be recommended as a fast, efficient, and cost-effective way to pack agricultural products.

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Author contributions
AR: data curation, formal analysis, writing original draft; AR: supervision, lab equipment's, editing paper. All authors read and approved the final manuscript.

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Availability of data and materials
All data are available upon request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors have given their personal consent for publication.

Competing interests
The authors declare that they have no competing interests.

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