Effect of *Eucalyptus globulus* and *Mentha spicata* essential oils on post harvest Qualitative attributes of pomegranate ariels

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Abstract

Pomegranate fruit is one of the most important and popular horticultural products, which is used in different parts of the world fresh processed and processed in many parts of the world. Since preparing this fruit for consumption is a little difficult and time consuming. The researchers have recommended a new-cut or ready-to-eat method. In this way, in order to maintain the quality of the product, preservatives should be used which, given the awareness of consumers and their desire to consume healthy, natural and low-risk foods for the environment, use of natural preservatives for conventional fresh-cut products. These preservatives are of animal and plant origin. Some of these herbs are essential oils. In this research, the effect of two herbal essential oils, Eucalyptus globulus and Mentha spicata on qualitative traits of pomegranate Robab neyriz cultivar was studied as independent tests. The experiments were designed as a factorial in a completely randomized design. For each treatment, three levels of essential oil were measured at 300, 600 and 900 µl / l in three replicates. For each essential oil, one control sample was considered in three replicates. After applying the treatment for 5 minutes, 300 g of pomegranate seeds were stored in zipper bags and stored for 4.5 days at 4.5 to 5 ° C with relative humidity of 85-90%. The length of the experiment period for the sampling was divided into four sections: 0, 5, 10 and 15 days, and the data obtained were compared with each other. Regarding the essential oil of Tasmanian bluegum (Eucalyptus globulus), the results showed that with the treatment of this essential oil at a level of 900 µl / l and a little at 600 µl / l, in the time interval of 5 to 10 days, the best quality indices compared to the control and 300 µl / l have been But it should be noted that the treatment of this essential oil at the level had no positive effect on the amount of soluble solids (TSS) and the level of PH and vitamin C content. But in the rest of the indicators, it has had a positive and significant effect on the flavor index (TSS / TA), taste index, microbial load, color, phenol content, total acidity (TA), and total anthocyanin levels. However, regarding the essential oil of Spearmint (Mentha spicata), it can be stated that by treating this essential oil at a level of 600 µl / l and a little at a level of 900 µl /l, it would be best to maintain the qualitative properties during 5 to 10 days of storage compared to the sample Control and 300 µl / l levels. However, this essential oil treatment did not have a beneficial effect on soluble solids (TSS) and PH content. But in other parameters, such as flavor index (TSS / TA), taste index, microbial load, color rate, phenol content, total acidity (TA), total anthocyanin levels and vitamin C levels, is effective.

Keywords: Pomegranate, Warehouse, Herbal Essences, Tasmanian bluegum, Spearmint, Qualitative Characteristics

1. INTRODUCTION

In recent years, the use of fresh- cut products, with minimal processing, has grown with regard to human living conditions, which is why consumers are looking for the most healthy product in terms of quantity and quality. The use of edible coatings from natural ingredients in fresh- cut products has recently been a common way to meet this consumer's need. Since the fresh- cut products are more vulnerable to injuries due to processing, and the rate of respiration, loss of environmental pathogens and browning are the main factors in reducing their quality.

in recent years there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. On the other hand, the interest in the possible use of natural alternatives to food additives to prevent bacterial and fungal growth has notably increased. plant products can represent a source of natural alternatives to improve the shelf-life and the safety of food. In fact, they are characterised by a wide range of volatile compounds. [28]. Essential oils in plants are generally produced to protect plants from disease. Many of them are part of the plants' preinfection or postinfection defense systems against infectious or parasitic agents [21]. For example, vegetables such as horseradish, mustard, and wasabi from the *Brassicaceae* family contain glucosinolates that are inactive. When the plant tissue is damaged, the enzyme myrosinase is released and converts glucosinolates to thiocyanates including AIT, which is a potent antimicrobial [7][16].

this study examines the effect of two types of essential oils Eucalyptus and mint as a natural and organic coating in different concentrations, have been used to maintain the quality of the post-harvesting properties of pomegranate ariels during storage. plant volatiles have been widely used as food flavouring agents and most of them are generally recognized as safe (GRAS). [18]. Application of essential oils is considered a safe treatment for the control of postharvest decay of fresh produce, and therefore, should not have any regulatory issues [25]. Due to with out skin in the environment, pomegranate ariels have short-lived post-harvest life, the main reason is being loss of water, drying and shrinkage. All the aforementioned problems will destroy the fruit's properties, including decreasing the antioxidant capacity, reducing vitamin C, reducing the amount of sugar in the crop, and ultimately reducing the quality of the food. The pomegranate" *Punica granatum* L". (Punicaceae) is one of the oldest fruit and medicinal fruits and is one of the most important commercially valuable fruits of the Central Asian region of Iran and Turkmenistan to the north of India. And is native to these areas [20]. The pomegranate's edible part is its seeds, along with ariel or juicy pulp, which are separated by a thin white coating [13]. Aril, peanut and pomegranate texture contain antioxidants such as soluble polyphenols, tannins and anthocyanins, and have been reported against a variety of diseases such as arthritis, prostate cancer, and cardiovascular disease [2].

The use of essential oils in the food industry is a novel way to maintain the quality of the maintenance of food products and agricultural products. Essences are complex compounds of runaways and form secondary metabolites and have an important protective role in nature as countermeasures. Bacterial, antifungal, antiviral and insecticidal products in plants [3].

Essential oils can be utilised as a natural alternative in food preservation and their usage complies with

consumers' expectations for natural food with marginal chemical treatments [1][9]. It has also been shown that essential oils have a significant potential in controlling fungal damage during the post-harvest period and can replace the use of chemical fungicides. Emission of bioactive vapour/volatile compounds inside packaged food product offers the advantage of preventing surface growth of microbes without having to come into direct contact with the food product. [15]. On the other hand, due to its high antifungal properties in recent years, and due to the biodegradability of essential oils, the use of these essential oils and natural compounds has increased as natural fungicides. In this research, the aim of this study is to investigating the effect of Eucalyptus and Mint essential oils on preserving the quality of pomegranate arils.

2. Material and methods

At first, pomegranates " *Robab Neyriz*" cultivar, used in this experiment, were purchased from Tehran's central fruit and vegetable sales center, regardless of size and randomly. After washing and disinfecting the selected pomegranate with sodium hypochlorite solution, 200 µl /liter was used to peel off and remove the arils by a sharp knife. The essential oils used in the experiment as main treatments were prepared as follows: Also, Eucalyptus essential oil from the cultivar *(Eucalyptus globulus)* and Spearmint essential oil (*Mentha spicata*) from the company of the production of essential oils of (Apak Ararat based in Tehran-Iran). The essential oils used were as follows: Eucalyptus essential oil with the highest active ingredient (cineole) with a purity of 78% and spearmint essential oil with the highest active ingredient (carvone) with a purity of 80%. After extraction of pomegranate ariels and the mentioned treatments, packing was done. Made from lightweight polyethylene (LFO 200) 15 x 15 cm zipped bags.Then, in each zipped bag, 300 gr of pomegranate ariels were placed in three replicates and the bags were kept in the refrigerator at a temperature of 4.5 to 5 ° C for 15 days. for analysis at day 0, 5, 10 and 15. At every step during the whole process of preparation of pomegranate arils, polyethylene hand gloves were used to avoid any contamination. Also, all sampling devices, such as spoons, dishes, test tubes, etc., were washed with distilled water after disinfection with 89% ethanol.

Solutions made from the essential oils were prepared at levels and in the following manner:
From each essential oil of mint and eucalyptus, three levels of 300 µl / liter, 600 µl / liter and 900 µl / liter were prepared by first dissolving the essential oils in 2 cc of alcohol (ethanol 89%) Then we mixed in a liter of distilled water. After preparation of the treatment solutions and their uniformity after mixing with the magnet, for 3 minutes, the pomegranate ariels were poured into the solutions and remained immersed for 5 minutes, after which time The ariels are said to have been withdrawn from the solution for 30 seconds in the vicinity of the air to reduce the amount of surface moisture from 100% to 90%.

At the end, after packing in the manner described, for each treatment, one control was carried out, which was kept without any additive and treatment during the 15-day period.

2.1.Methods

Pomegranate arils were crushed to extract juice for determination of total soluble solids (TSS) content.manual simple refractometer (working with ambient light) was used to measure the TSS of the juice extracted and the values were expressed as Degree Brix (°B) Refractometer was calibrated by distilled water

prior to measuring TSS of samples. The pH was determined by digital pH meter (Microprocessor based design-ATC bench top meter-SAT-401/AUTO CAL- Canada) and results represented as unit of pH. It should also be noted that the measurement was carried out at 20 ° C. Titratable acidity(TA) was determined by the[8] method. Titration with sodium hydroxide was 0.1 normal and the phenol-phethalene reagent 1% was used up to pH 1.8. First, 5 ml of each extract was poured into the test dish, then three drops of phenol-phethalene were added. Following this, titration was performed with sodium hydroxide solution of 0.1 normal to appear purple and remain stable. After that, the amount of titration solution consumed was noted and, by placing it in the formula, the total acidity was calculated: (1)

$$Acidity\ Titration = \frac{(Normality\ of\ SH * The\ dominant\ acidity\ factor * 100 * \text{SH consumed})}{5}$$

SH: Sodium Hydroxide

Results were expressed as percentage with respect to citric acid. The acid is the dominant fruit of pomegranate citric acid, whose coefficient is 0.06404. 100 in the form of a fraction due to the use of acidity as a percentage, and the number 5 in the denominator indicates the use of 5 milliliters of the extract.
Fruit ripening index was determined according to [17] as ratio between TSS and TA.

The amount of total phenol was measured by the proposed method of Reading optical absorption spectrophotometer(Perkinelmer UV/VIS – Lambda 25) By the Folin-Ciocalteu Reagent [24]. First, combined 1 milliliter of the centrifuge extract with 1 milliliter of Folin-Ciocalteu solution from the German company Merck, diluted with distilled water at a ratio of 1 to 10. After 5 minutes, we added 1 ml of 20% sodium carbonate solution (20 g Na2CO3 + 100 ml distilled water) and finally, 7 ml of distilled water added to the total solution and after 1 hour The absorption was read at 760 nm. For blanking of the spectrophotometer, a mixture of 7 milliliters of distilled water, 1 mL of folin solution and 1 mL of sodium carbonate 20% were added without adding 1 milliliter of the extract. The standard 100 ppm tannic acid graph for obtaining the comparison formula was prepared as follows: 100 mg of tannic acid was dissolved in 1 liter of water and the concentrations of 0, 20, 40, 60, 80 and 100% Distilled water was prepared. Then, the absorbance of the concentrations was read at 760 nm, respectively, and finally, its standard graph along with the slope of the line was presented to determine the total phenol content.

The color rate was measured by the proposed method [17] with same spectrophotometer apparatus at 520 nm. The total anthocyanin level was measured using the differential PH method described by [17] PH differential method was used to check this index. At first, 1 milliliter of the extract with 2 ml of potassium chloride solution was 0.025 molar with PH 1 and 1 milliliter of extract with 2 milliliters of 0.4 M sodium acetate with pH 4.5. After centrifugation at 4000 centrifuges, it lasted for 5 minutes at 15 minutes. Then, the absorbance of each solution was read by the spectrophotometer at 510 and 700 nm wavelengths and used for blanching of ethanol. Finally, the total anthocyanin content with the dominant anthocyanin of pomegranate cyanidine 8-glucoside was compared by the following formula: (2)

$$(TAC) = \frac{A * MW * DF}{MA * L}$$

$$A = (A510 - A700)PH1.0 - (A510 - A700)PH4.5$$

where A=Absorbance values at 510 nm and 700 nm

MW= The molecular weight of the dominant anthocyanin (cyanidine 8-glucoside = 449.2 g/ mole).

DF= Dilution factor(3).

MA= Molar absorption coefficient of cyanidine 8-glucoside (26900 g/ mole).

L= cell path length(1cm).

Measurement of vitamin C was done by the proposed method [5]. The absorption rate was then read by the spectrophotometer device at 515 nm. According to the standard solution of ascorbic acid (0.01 to 0.1), its final rate was measured.

For sensory analysis, in each period of the experiment, some items such as taste, color and perfume were evaluated. For this experiment, 15 individuals were randomly selected At the end, each testor declares the results from a score of 1 to 5. That score 1 low quality and score 5 showed the highest quality.

Microbial analysis was carried out by the proposed method of [11]. The study of microbial load (change in microbial population) was evaluated by counting the total microbial population during storage, which was observed on days 0, 5, 10 and 15 days The number of colonies formed from microbes was done.

At first, 10 g of each sample was mixed with 90 ml of distilled water. Serially, concentrations of 1 to 10 were prepared. The prepared concentrations were grown on agar medium and then kept for 48 hours in incubator at 37 ° C for growth, which was then placed at 25 ° C for 24 hours. . Grown colonies were counted on the culture medium and their number was evaluated based on the unit (log CFU / g), which is the number of units formed in the colony of microbes per gram

For Statistical analysis all appointments were made three times, and the data were expressed as the average of three repetitions ± standard deviation. Experimental data was used using the SAS software and outcomes of the software using the Microsoft Excel program to draw charts and tables. In general, the importance of different treatments, storage and intercourse was considered at $p < 0.05$.

3. Results and discussion

All the conclusions in the text are based on the analysis of the graphs in each section.

3.1. Effect of different concentrations of Eucalyptus globulus essential oil and storage time on quality characteristics of pomegranate seeds:

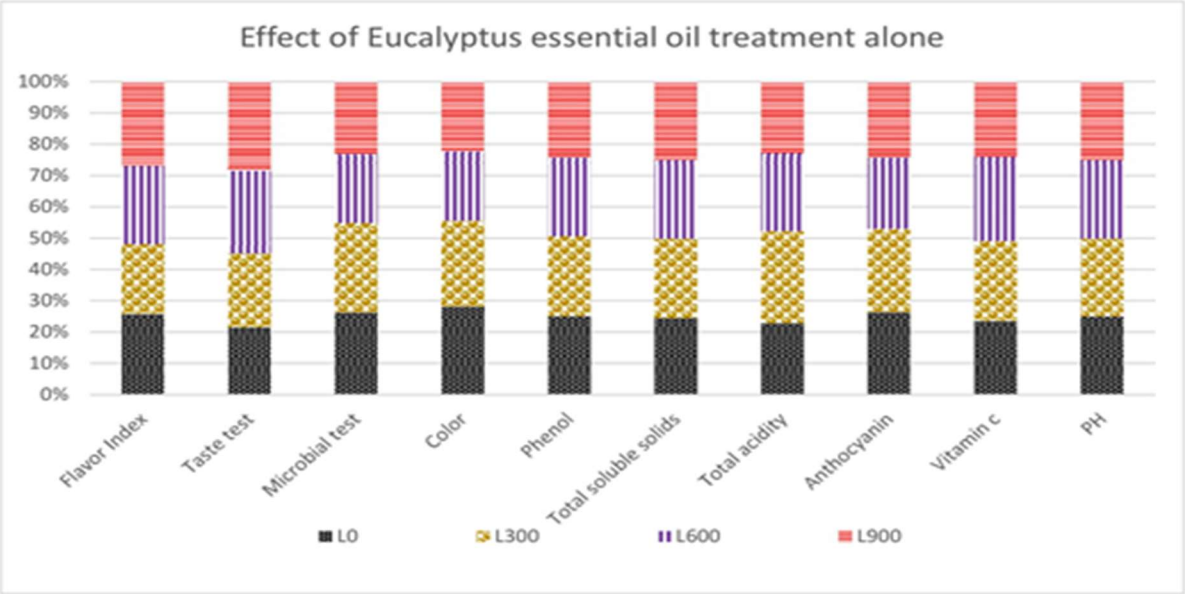

Fig. 1. Effect of eucalyptus essential oil treatment (Eucalyptus globulus) alone.

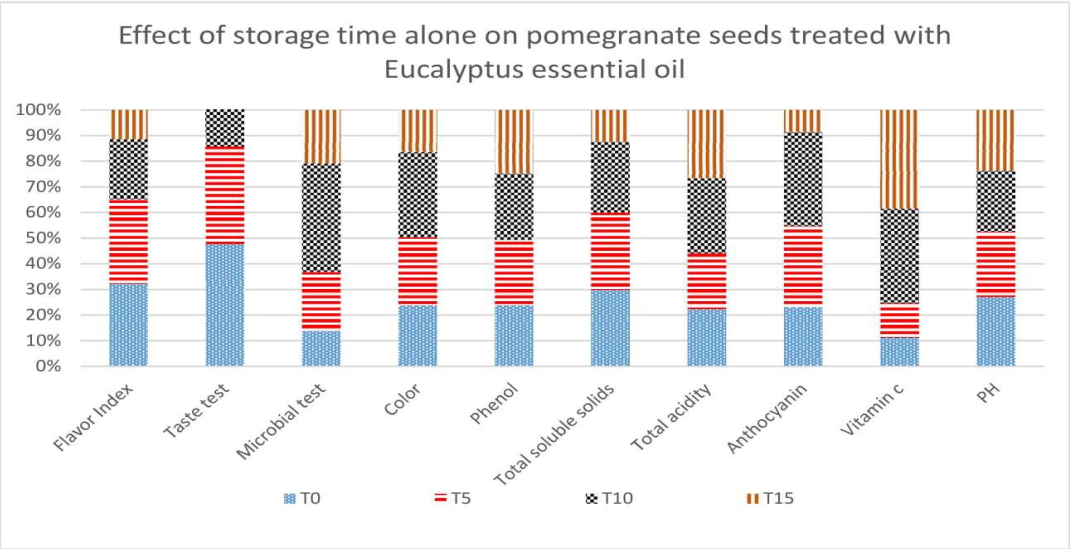

Fig. 2. Effect of storage time alone on pomegranate seeds treated with eucalyptus essential oil (Eucalyptus globulus).

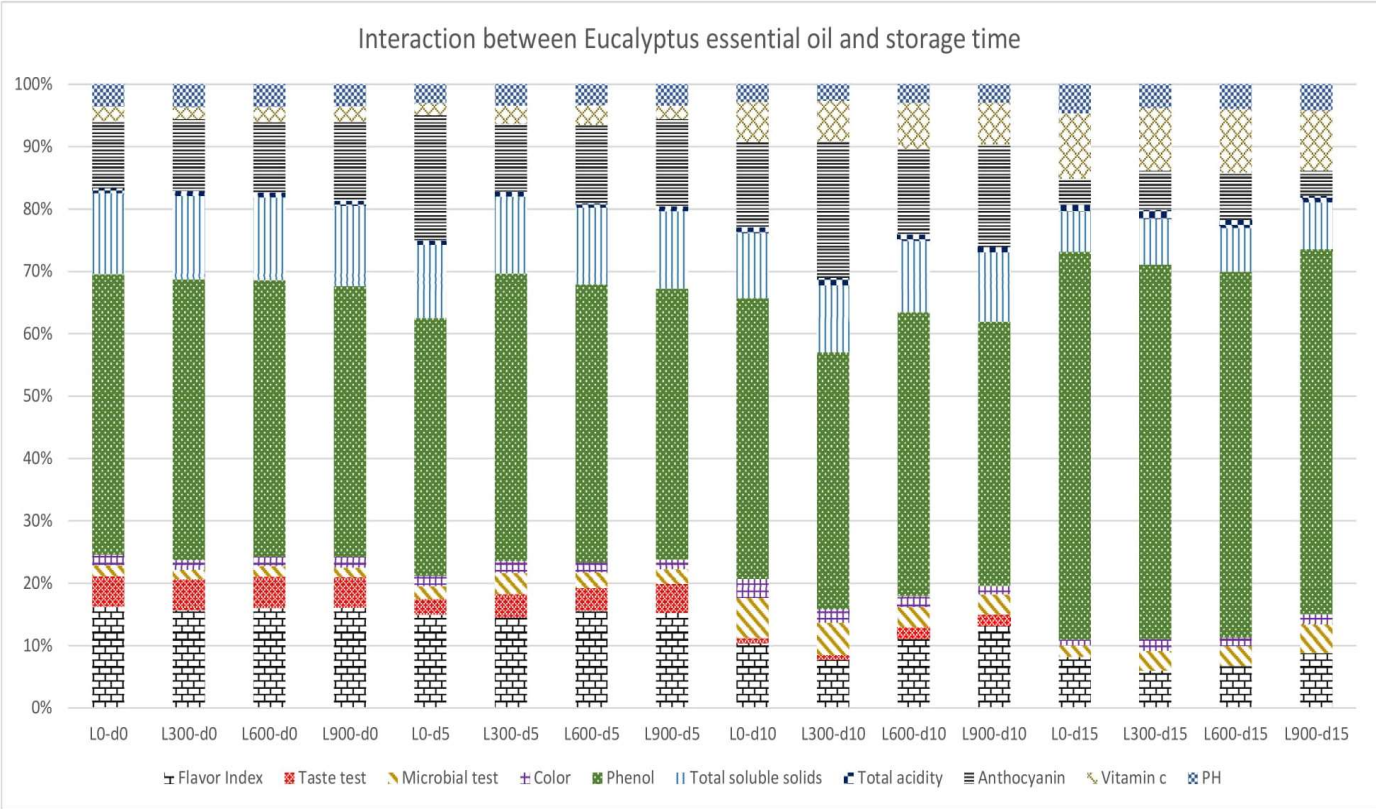

Fig. 3. Interaction between eucalyptus essential oil (Eucalyptus globulus) and storage time.

Regarding the taste index and according to fig. 3, it can be stated that the use of 600 and 900 µl / l of essential oil of Eucalyptus Nile has the ability to maintain a flavor index higher than the concentration of 300 µl / l. According to the results of most studies, the flavor index, which results from the division of total soluble solids (TSS) into titrable acidity (TA), decreases during storage [29]. As shown in this experiment, the highest flavor index of that control sample was on the fifth day of the whole period. But in general, concentrations of 600 and 900 µl / l have been shown to have a good effect on maintaining the flavor index.

In terms of taste scoring and the level of affection and according to fig. 3, it can be said that the best use time was from zero to ten days, with the highest concentration of 900 µl / l.

According to the results of microbial contamination and growth and proliferation of pathogens and according to fig. 3, it can be stated that during the fifth to tenth day the essential oil function was appropriate for preventing the activity of pathogens at a concentration of 900 µl / l. So that it has a good effect on the control and other concentrations. But during the 10th to the 15th day, it has the lowest performance, which triggers intense microbial activity and ultimately increased contamination. This is probably due to the reduced resistance of the essential oils to the increased population of pathogens. The use of herbal essential oils in edible coatings has been effective in reducing the growth of pathogens in fresh-cut products during storage. For example, the use of lemon grass essential oil in edible coating containing alginate on apple and fresh-cut pineapple It has reduced the growth of microbial agents, which is similar to other experiments [4][23].

According to the data obtained from the test and according to fig. 3, the amount of transparency and color opacity can be stated that the use of concentrations of 600 and 900 µl / l preserves the quality and clarity of color, but the storage time has had a significant effect on this quality of preservation And the long period from the fifth to the fifteenth day has prevented the proper functioning of the essential oil in this regard. Changes in color in fresh-cut products due to the destructive reaction of the polyphenol oxidase enzyme and the oxidation of phenolic compounds in the presence of oxygen and copper. In most studies, the use of edible coatings with essential oils prevents and reduces color changes in fresh-cut products [26][19]. This is also close to the results of this experiment.

About the changes in the phenol content in this experiment and according to fig. 3, it can be noted that the concentration of 900 µl / l of the Eucalyptus Indigenous essential oil played a significant role in keeping the phenol levels low until the 15th day of the experiment. According to similar research results, it can be stated that most of the antimicrobial activity of the essential oils is directly related to the prevention of the production of phenolic compounds, which makes it possible to use plant essential oils to reduce phenolic compounds and reduced the effect of free oxygen [27]. As a result, reducing the amount of phenol will prevent destructive effects and reduce color and lower quality.

According to the results of the experiment on the TSS and according to fig. 3, we can say that the results have the highest yield during the fifth to the fifteenth day of storage at a concentration of 300 µl / l. The incremental amount of total soluble solids in this experiment was up to fifth day and then declined. According to the results of other investigations [14], With regard to this and meaningless The effect of essential oils on total soluble solids can be deduced that the properties of indigenous eucalyptus essential oil have a negative effect on this and reduce the total soluble solids during storage.

Regarding the study of the data on the amount of TA and according to fig. 3, it can be stated that during storage, the highest acidity was at the concentration of 600 µl / l from the tenth to the fifteenth day and, in contrast, the concentration of 900 µl / l, Has the lowest total acidity. According to sources, during the storage period, the acidity of the whole fruit of the pomegranate, the dominant acid of which is citric acid, increases [12]. Therefore, according to the data, the concentration of 900 µl / l has been able to maintain the total acidity level at the low and even constant level until the end of the maintenance period. The results of this experiment are similar to those of [29], which have come to the conclusion.

Regarding the results of the experiment and according to fig. 3, the total anthocyanin level, it should be said, that by applying the treatment, we increased the total anthocyanin from the fifth to the tenth day. Which has an intermittent and rational increase of anthocyanin at concentrations of 600 and 900 µl / l. This is due to other research, similar to the results of [19].

Regarding the results of the test and according to fig. 3, it is possible to state that vitamin C has the highest yield of 300 and 600 µl / l in vitamin C increase. The amount of vitamin C usually decreases during storage, but the use of edible coatings maintains vitamin C during storage [30]. Therefore, it can be concluded that the use of essential oil of Eucalyptus indigenum in the mentioned concentrations preserves vitamin C.

According to the results of the experiment and according to fig. 3, the amount of PH changes can be concluded that different levels of treatment during the storage period had no effect on PH changes. The PH range from day zero to fifth day was about the same as the day of the tenth and fifteenth day of storage.

However, in the fifth, tenth, and fifteenth days, the difference in pH was between 600 and 900 µl / l in comparison with control samples and a concentration of 300 µl / l.The result of this test is similar to that of other tests. According to the results of other experiments, it is not possible to establish a direct relationship between PH and total acidity due to the change in the buffering capacity of organic acids, and it has been shown that in post-harvest respiration, the use of organic acids in the reaction The enzymatic levels of respiration are lower [6]. Therefore, the constant pH is normal and the essential oil does not have a positive effect on it.

3.2. Effect of different concentrations of Mentha spicata essential oil and storage time on quality characteristics of pomegranate seeds:

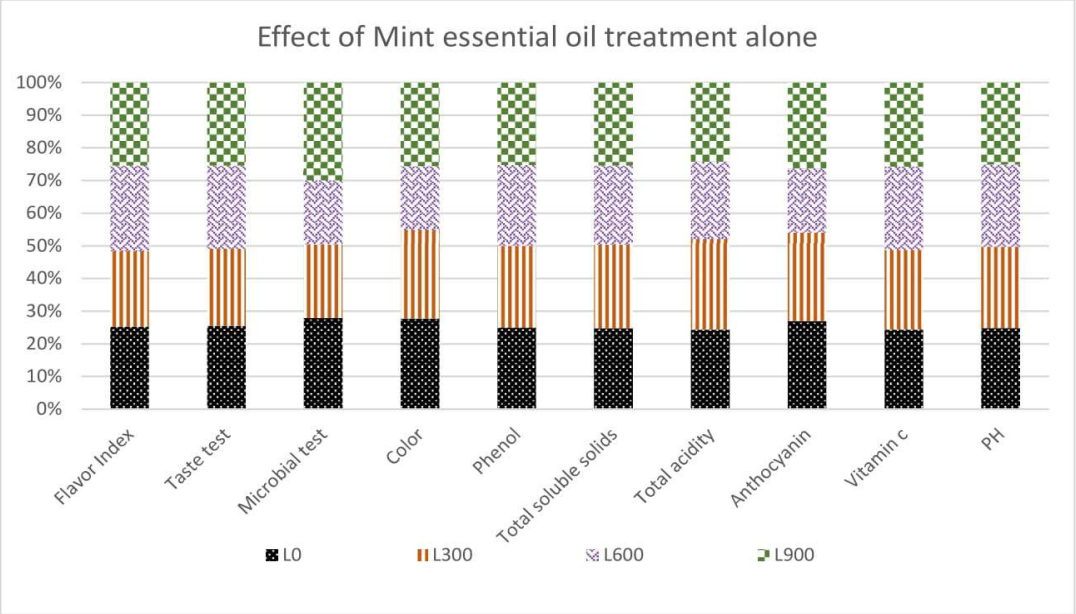

Fig. 4. Effect of mint essential oil (Mentha spicata) treatment alone.

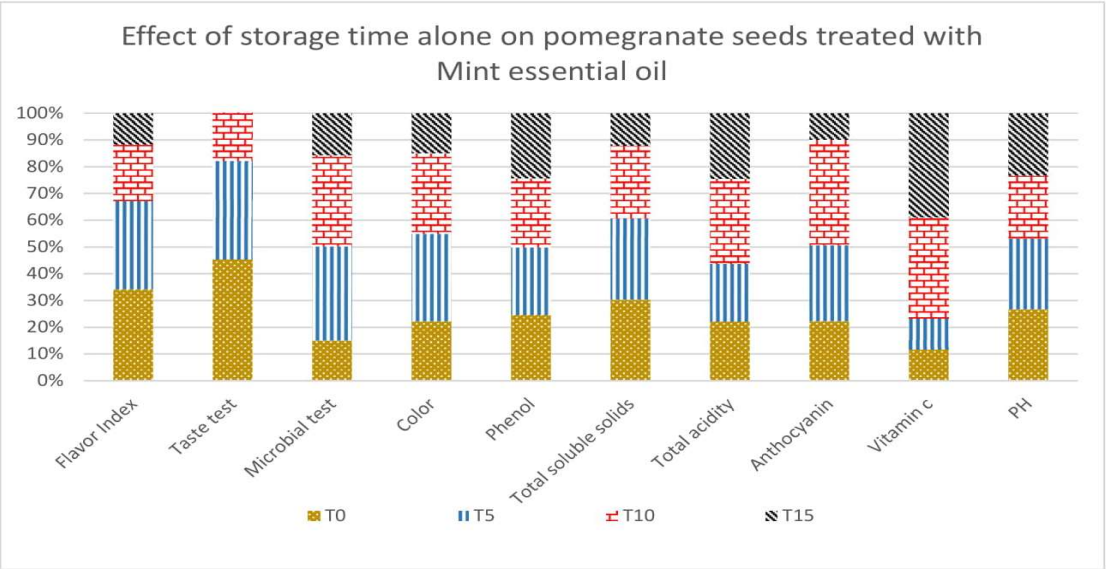

Fig. 5.effect of storage time alone on pomegranate seeds treated with mint essential oil (Mentha spicata).

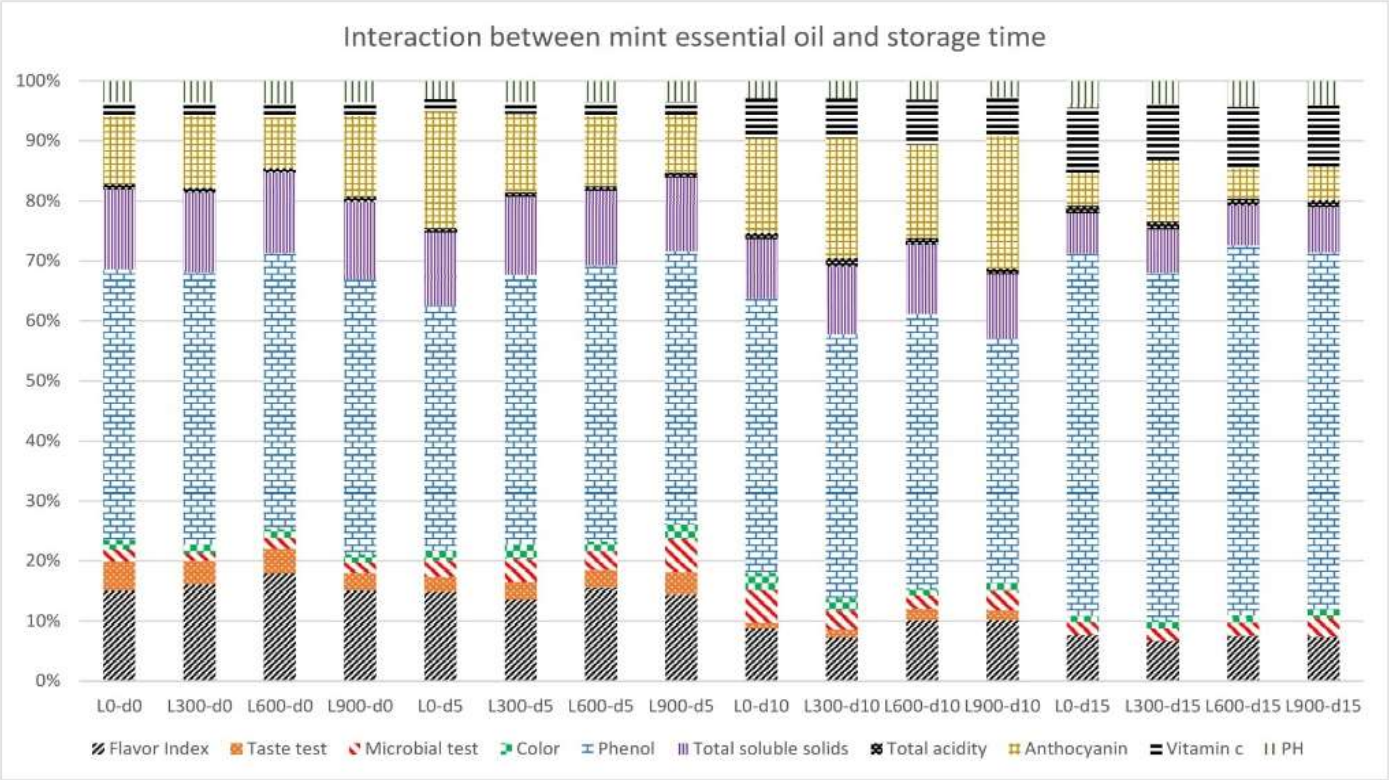

Fig. 6. Interaction between mint essential oil (Mentha spicata) and storage time.

Concentration of 900 µl / l of Spearmint had the highest performance in maintaining a higher flavor index than others. According to the results of most studies, the flavor index, which results from the division of soluble solids (TSS) into titratable acidity (TA), decreases during storage [22]. As noted in this study, high concentrations of essential oil have the appropriate effects to maintain a flavor index. Regarding the level of likelihood and score of the audience to the taste of the samples, it can be stated that the best use time was from zero to fifth day, with the concentration of 900 µl / l also most enjoyable.

According to the results of the study and according to fig.6, pathogen infections and growth, it can be stated that during the fifth to the 10th day the essential oil function was suitable for preventing the activity of pathogens at a concentration of 600 µl / l. So that it has been able to perform well in comparison with control and other concentrations. The use of herbal essential oils in edible coatings has been shown to be effective in reducing the growth of pathogens in fresh-cut products during the storage period, for example. The use of essential oils of lemon grass in the food coating containing alginate on apples and fresh-cut pineapple has reduced the growth of microbial agents [4][23]. The total result of this experiment was similar to other experiments with a concentration of 600 µl / l. It has been reported in the study that spearmint Essence has been shown to be beneficial in inhibiting the growth of bacteria and fungi [10].

From the results of testing the changes in the degree of transparency and color opacity and according to fig. 6, it can be concluded that using a concentration of 600 µl / l retained the quality and clarity of color, but

the storage time had a significant effect on this quality preservation, and a long period Between five and fifteen days the essential oil has been blocked.

Changes in color in fresh-cut products due to the destructive reaction of the polyphenol oxidase enzyme and the oxidation of phenolic compounds in the presence of oxygen and copper. In most studies, the use of edible coatings combined with vegetable oils prevents and reduces color changes in fresh-cut products [26][19]. This is also observed at a concentration of 600 µl / l of mint peppermint and is close to the results of this experiment.

In order to interpret the results of the experiment and according to fig. 6, the amount of phenol changes can be said that the concentration of 600 µl / l of spear mint in keeping the amount of phenol up to the 15th day of the experiment played a significant role. According to similar research results, it is possible that most of the antimicrobial activity of the essential oils is directly related to the prevention of the production of phenolic compounds, which makes it possible to use plant essential oils to reduce harmful effects Phenolic compounds were used in fresh-cut products and reduced the effect of free oxygen [27].

As a result, decreasing the amount of phenol will prevent destructive effects, and reduce color and lower quality. Therefore, it can be stated that by applying a concentration of 600 µl / l of mint peppermint, the amount of total phenol can be kept low to prevent its destructive effects at high levels.

According to the results of testing of changes in total soluble solids and according to fig. 6, we did not perform well during the fifth to the fifteenth day of storage considering the treatments. The incremental amount of total soluble solids in this experiment was up to fifth day and then followed a downward trend. Regarding this issue, and without mentioning the effect of essential oils on total soluble solids, it can be concluded that the properties of spearmint essential oil have a negative effect on this and reduce the soluble solids during the storage period. This is similar to the results of [22][19].

Regarding this study and according to fig. 6, it can be stated that during storage, the highest acidity was observed at 300 µl / l concentration from tenth to fifteenth day and in contrast, the concentration of 600 µl / l had the lowest total acidity had. According to sources, during the storage period, the acidity of the whole fruit of the pomegranate, the dominant acid of which is citric acid, increases [12]. Therefore, according to the data in the tables, the concentration of 600 µl / l has been able to maintain the total acidity level, at a low and even constant level, until the end of the maintenance period. The results of this experiment are similar to the experiment [29].

From the results of the experiment and according to fig. 6, changes in the total anthocyanin level can be said that by applying the treatment, we increased the total anthocyanin from the fifth to the tenth day. Which has an intermittent and natural increase of anthocyanin at a concentration of 900 µl / l. This result, according to recent research, is similar to those of [19].

Considering the results of the test, and according to fig. 6 the evaluation of vitamin C changes can be made that the highest performance in increasing the amount of vitamin C has a concentration of 900 µl / l and then 600 µl / l. The amount of vitamin C usually decreases during storage, but the use of edible coatings maintains vitamin C during storage [30]. Therefore, it can be concluded that the use of spear mint Essence in the mentioned concentrations preserves vitamin C.

Regarding pH changes and according to fig. 6, it can be stated that there is a significant difference in PH data at zero days but the difference between the numbers is very close and does not matter. In general, it has a low level than the others at a concentration of 600 µl / l. On the fifth day, the highest concentration of PH is about 900 µl / l, at which point numbers other than the highest concentration were also close, and the difference was 0.01. However, a 10-day review of the more severe reduction trend indicates that the highest concentration is at 900 µl / l and the lowest concentration is 300 µl / l. In the review of the fifteenth day, the downward trend continues, but not extreme, and it can be said that it remains constant. Which is the highest for 900 µl / l and the lowest for the control sample. Therefore, it can be concluded that different levels of treatment during the storage period had no effect on PH changes. The pH range from zero to fifth day, with very little difference, was higher than the tenth and fifteenth day of storage. According to the table data, no surface of spear mint Essence has been able to maintain and maintain the pH.

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