Exposure to Volatile Essential Oils of Myrtle (*Myrtus communis* L.) Leaves for Improving the Postharvest Storability of Fresh Loquat Fruits

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1.Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical plant which was originated from south-central China. It belongs to the Rosaceae family [1] and widely adapted to many regions of the world, including South Africa, the United States of America, Asia, and Mediterranean countries [2]. Loquat fruits are nutritious and have high amount of diverse phytochemicals [3]. Loquat fruits have also been reported as delicious fruits, which are highly appreciated by consumers and have been used for many purposes, including fresh consumption, syrups, jams, juices, and wines [2]. Loquat fruits also have a long history in traditional Chinese medicine [4]. One of the main disadvantages of loquat fruits for marketing is the short storability of the fruits due to enzymatic browning, weight loss, and pathogenic decay [5]. Weight loss and pathogenic decay were reported to be controlled by storing the fruits at low temperatures (5 to 7°C). However, low temperatures are known to adversely affect fruit quality due to chilling injury [6]. The main symptoms of chilling injury at the loquat fruits are the internal and external browning together with increased fruit firmness and reduced juice content. Heat treatment [7] and application of edible coatings [8–10] have been reported to reduce fruit browning.

Postharvest pathogens are the other important factors reducing the marketability of loquat fruits. Over the last 70 years, fungicides and other agrochemicals have been the
main methods for controlling postharvest pathogens. However, the acceptability of agrochemicals has been decreasing since the beginning of the 20th century, due to the confirmation of their possible adverse effects on human health and the environment [11]. Therefore, alternatives to the agrochemicals have been the subject of current studies, which mainly focus on the use of biomaterials [12]. The use of plant-derived biomaterials in postharvest handling of fruits has had great interest since the 20th century, mainly because of their high acceptability by the consumers due to their extended threshold for toxicity [13]. The plant extracts [14–18], essential oils [19–21], and edible coatings and/or films [22–24] have been reported to have high potential for improving the postharvest storability of fresh fruits and vegetables and controlling postharvest pathogens.

The mode of action of the plant-derived biomaterials is mainly a result of the semipermeable barrier for water vapor, oxygen, and carbon dioxide which help to reduce transpiration and respiration. Biomaterials also include diverse phytochemicals which are known to activate some enzymes (ascorbate peroxidase-APX, catalase-CAT, and superoxide dismutase-SOD) and/or deactivate some others (polyphenoloxidase-PPO and peroxidase-POD), which help for preserving postharvest fruit quality and/or controlling fruit pathogens [17, 25]. Essential oils (EOs), with their diverse phytochemical compounds and biologically active characteristics, have high potential for preserving postharvest fruit quality [26–28]. Another advantage of the EOs is their ability to be applied as vapors [29]. Myrtle (Myrtus communis L.) plants grow wildly in the Mediterranean basin and are an important element in the maquis [30]. The essential oils of myrtle leaves are reported to be used in the perfume and flavor industry and have high antimicrobial activity [31]. Several studies have been performed about the chemical composition of the essential oils of myrtle leaves [31–34]; however, the postharvest studies with the EOs of myrtle leaves are limited. Therefore, this study was conducted to investigate the effectiveness of volatile essential oils of myrtle (M. communis L.) leaves for preserving the postharvest storability of loquat fruits. Parallel to the postharvest studies, the essential oils of the myrtle leaves of the present study were also determined.

2. Materials and Methods

2.1. Materials. Loquat fruits Eriobotrya japonica cv. “Morphitiki” of the present study were hand-collected at horticultural maturity (27 April 2020) based on skin color (fully yellow-orange) from a commercial plantation located in Yayla village in Northern Cyprus. The SSC: TA was also used to determine horticultural maturity. The SSC was between 11.00% and 12.00%, whereas the TA was between 0.95g to 1.05g [10]. Fruits were then immediately transferred to the laboratory and were selected to ensure homogeneous size and color and to eliminate any damaged fruits. At the same time, leaves of myrtle (Myrtus communis L.) were collected from the same village, about 400 m away from the loquat plantation. In the experiments, the young leaves of myrtle are used because their balsamic time (June) is very near and the old ones are eliminated. The use of the old leaves can be quite dangerous because normally they contain some specific pathogens which may aggravate the decay of the treated fruits.

2.2. Experimental Methodologies. The experimental studies were planned to continue for 42 days and quality parameters to be measured within a 7-day interval (7, 14, 21, 28, 35, and 42). For each measurement point, 3 replications with 10 fruits in each were assigned. Therefore, totally 180 fruits (6 fruits in each group) were randomly divided into 5 groups (# of treatments) of 180 fruits in each group. These groups were then treated with one of the following treatments: (i) water vapor (2 min), (ii) myrtle leaves (3% w/w of total weight of fruits), (iii) myrtle leaf vapor (2 min), (iv) myrtle leaf vapor (10 min), and (v) untreated control. Fruits were all dipped in distilled water for 1 min duration and air dried for 30 min before exposing to the treatments explained below. Detailed explanation for the treatments is given in Table 1.

After exposing to the abovementioned treatments and packaging, all fruit trays were transferred to the storage conditions of 4 ± 1°C and 95% relative humidity. Studies were continued for 42 days, and a total of 3 trays from each group were taken out from cold rooms with 7-d intervals (7, 14, 21, 28, 35, and 42) to measure the quality characteristics. Study duration and measurement points were determined according to the several previous studies which used 5/7-day intervals for 35–40 d storage durations [7, 35].

2.3. Data Collection. Weight loss, decay incidence (DI), browning index (BI), fruit firmness, soluble solids concentration (SSC), and titratable acidity (TA) were all measured according to the following methods. Weight loss (%) was determined by subtracting the final weight from the initial weight and dividing the solution to the initial weight and multiplying with 100. Initial and final weights of the fruits were all measured with a digital scale (±0.01 g). DI of the fruits was assessed by using the four-point scale formula [36]. To do so, all fruits were individually graded from 0 to 4 where 0 means no decay, 1 represents slight decay (≤25%), 2 equals moderate decay (25% < 50%), 3 means severe decay (>50%). The following formula was then used to calculate the DI.

\[
DI = \frac{[(1 \times N_1) + (2 \times N_2) + (3 \times N_3)] \times 100}{(3 \times N)}
\]

Here, \(N\) equals to the total number of fruits where \(N_x\) represents the number of fruits with \(x\) score of decay severity.

The BI was used to measure the chilling severity of the fruits. The 0–4 scale of Rui et al. [37] was used to determine BI. In this scale, 0 equals no browning, 1 represents slight browning (less than <5%), 2 means moderate browning (5–25%), 3 represents moderately severe browning (25–50%), and 4 equals to severe browning (>50%). Scoring of the fruits was performed after 1 additional day at 20°C. Next, the following formula was used to calculate BI.

\[
BI = \frac{\sum[(BI \times scale) \times (number of fruit at that BI)]}{(4 \times total number of fruit in each treatment)}
\]
acceptability (marketability) of the fruits was defined as a BI index of 0.4 according to Ghasemnezhad et al. [8]. A hand penetrometer with a 5 mm-diameter probe was used at a speed of 1 mm s⁻¹ to measure the fruit firmness (kg cm⁻²). Then, SSC of each loquat fruits was assessed by using a hand refractometer. Weight loss, fruit firmness, decay incidence, browning index, and SSC were measured for 30 individual fruits for each treatment. Finally, TA of loquat fruits was assessed according to the method of AOAC [38] as g/100 g of malic acid. For TA determination, a mixture of the juice of 10 fruits belonging to the same replication of each treatment was used. Thus, a total of 3 replications were used for each treatment.

2.4. Isolation and Analyses of Essential Oil. The steam distillation method (by using Clevenger-type apparatus (European Pharmacopoeia)) was used to separate volatile essential oils from the plant leaves, and the oil was kept at amber vials at +4°C until analysis. Essential oil components were analysed with a GC-MS (Gas Chromatography-Mass Spectrometry) device, Thermo Scientific ISQ Single Quadrupole. 5 μl of essential oil was diluted in 2 ml cyclohexane. The column model was TG-Wax MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter * 60 m length, and 0.25 μm film thickness). The ionization energy was calibrated as 70 eV, and the mass interval was m/z 1.2–1200 amu. The scan mode was used as the screening more in data collection. The MS transfer line temperature was 250°C, MS ionization temperature was 220°C, and column temperature was 50°C at the beginning; then, it was increased up to 220°C with 3°C min⁻¹ rate of temperature increase. The structure of each component was defined by using mass spectrums (Wiley 9) with Xcalibur software. Retention indices were determined using retention times of n-alkanes (C₈–C₄₀) that were injected after the M. communis essential oil under the same chromatic conditions.

2.5. Data Analysis. Raw data of the experiments were subjected to the analysis of variance (ANOVA) by using SPSS 22.0 software. Comparison of the means of different treatments was assessed by Tukey’s HSD test at P = 0.05. Microsoft excel was then used to prepare figures from the means and standard deviations.

3. Results

3.1. Chemical Composition of the Volatile Essential Oils. Volatile essential oil of myrtle leaves was determined to consist of 30 different compounds. The three most abundant compounds were determined as eucalyptol (39.38%), α-pinene (24.98%), and linalool (8.18%). The full list of the chemical compounds is given in Table 2. Eucalyptol is a colorless organic liquid compound, a cyclic ether, and a monoterpene. α-Pinene is a colorless terpenoid and liquid plant metabolite, which is substituted by methyl groups at 3 positions. Linalool is also a monoterpene, which is substituted by methyl groups at 2 positions and a hydroxy group at 1 position. Moreover, α-terpineol and limonene was found to have 6.94% and 6.16% in the essential oil of myrtle leaves.

3.2. Weight Loss and Fruit Firmness. Results of the present study showed that, as expected, the weight loss of the loquat fruits increased during the storage (Table 3.). Two (myrtle leaf (3% w/w) and myrtle leaf vapor (2 min)) of the four test applications were found to be highly effective in preventing the weight loss during storage (Figure 1(a)). Water vapor, which was tested as the second control of the present study was found to have slight influence on the weight loss prevention, but it was not statistically significant. Besides that, myrtle leaf vapor (10 min) application was also ineffective in preventing the weight loss, as compared with the myrtle leaf vapor (2 min). These results suggest that the increase in the exposing duration to the myrtle leaf vapor causes a reduction in the effectiveness of the application. At the end of the 42 days of storage, the minimum weight loss was noted from the myrtle leaf (3% w/w) treatment as 17.60% and was followed by the myrtle leaf vapor (2 min) treatment with 18.05%. No significant difference was noted between these two treatments. Moreover, the highest weight loss was observed from the control treatment with 23.20%.
parameters (Table 3.). In the first weeks of storage, a slight tendency during storage as compared with the other quality of storage.

Table 3: Effects of cold storage on the different quality attributes (average of five different treatments) of loquat fruits during 42 days of storage.

| Storage duration | WL (%) | DI (%) | BI | Firmness | SSC | TA |
|------------------|--------|--------|----|----------|-----|----|
| At harvest       | 0.00 g | 0.00 c | 0.00 d | 0.42 e | 11.52 c | 0.98 |
| 7 days           | 1.60 f | 0.00 c | 0.03 d | 0.39 f | 10.39 e | 0.76 e |
| 14 days          | 4.50 e | 2.89 c | 0.19 c | 0.43 e | 9.82 f | 0.66 f |
| 21 days          | 8.66 d | 6.22 c | 0.28 bc | 0.47 d | 10.99 e | 0.59 f |
| 28 days          | 11.88 c | 10.22 c | 0.31 bc | 0.51 c | 11.27 c | 0.89 cd |
| 35 days          | 14.96 b | 48.67 b | 0.39 ab | 0.57 b | 12.08 b | 1.12 b |
| 42 days          | 20.60 a | 83.56 a | 0.45 a | 0.61 a | 12.73 a | 1.42 a |

Means of quality attributes in the same column with the same letters showed no significant differences according to Tukey’s HSD test at P ≤ 0.05.

Firmness of the loquat fruits showed a slightly different tendency during storage as compared with the other quality parameters (Table 3.). In the first weeks of storage, a slight decrease was noted in the fruit firmness, and then, it increased continuously corresponding to the decline of fruit weight and increase in BI. Fruit firmness was significantly lower at the fruits treated with myrtle leaf vapor (2 min) and myrtle leaf vapor (10 min) as compared with the untreated control fruits (Figure 1(b)). However, this was higher than the initial fruit firmness. The initial firmness of loquat fruits in present study was 0.42 kg cm⁻² and was found to increase until 0.72 kg cm⁻² at the untreated control fruits after 42 days of storage. During this period of cold storage, the application of myrtle leaf vapor (2 min) and myrtle leaf vapor (10 min) was observed to keep fruit firmness at lower degrees, 0.52 kg cm⁻² and 0.55 kg cm⁻², respectively.

3.3. Decay Incidence and Browning Index. As expected, DI (%) and BI were observed to have an increasing trend during cold storage. However, the decay incidence was not significant from the harvest date until the 28th day of storage (Table 3). Similar with the results of weight loss, the myrtle leaf (3% w/w) and myrtle leaf vapor (2 min) treatments were found to be effective in preventing the DI (Figure 2(a)). At the end of the storage (42 days of storage), the minimum DI was observed from the myrtle leaf (3% w/w) treatment with 50%. Although it was effective in reducing the DI, this was not acceptable for marketing. Therefore, it can be concluded from the results that the loquat fruits can be stored for 35 days in cold rooms, with the application of either myrtle leaf (3% w/w) or myrtle leaf vapor (2 min).

The results about BI were slightly similar with the effects on the weight loss and DI. However, it was noted that the myrtle leaf vapor (2 min) application has higher influence than the myrtle leaf (3% w/w) (Figure 2(b)). In addition to that, myrtle leaf vapor (10 min) application was also successful in preventing the BI. At the end of the storage period (42 days), the lowest browning index was noted from the myrtle leaf vapor (2 min) application with a score of 0.225 and was followed by the myrtle leaf (3% w/w) and myrtle leaf vapor (10 min), with BI scores of 0.392 and 0.408, respectively. At that time, the BI was observed as 0.708 at the untreated control fruits.

3.4. Soluble Solids Concentration and Titratable Acidity. In present study, the fruit SSC and TA were observed to have a decreasing trend during the first 21 days of cold storage, and then, the trend was changed to reverse (Table 3). The initial SSC was 11.52% and was noted to increase up to 12.91% at the untreated control fruits in 42 days of storage. Results suggested that all the applications have a significant influence on the SSC, and the lowest SSC (12.52%) was measured from the fruits treated with myrtle leaf vapor (2 min) (Figure 3(a)). A similar trend was observed for the TA. The initial TA content was 0.98 g 100 ml⁻¹ malic acid. The TA of the untreated control fruits decreased to 0.55 g 100 ml⁻¹ malic acid in 21 days of storage, and then, it showed an increasing trend. The final TA content of the untreated control fruits was 1.51 g 100 ml⁻¹ malic acid. Results suggested that all treatments have significant effect on TA. Thus,
the minimum TA content (1.32 g 100 ml−1 malic acid) was measured from the myrtle leaf vapor (2 min) (Figure 3(b)).

4. Discussion

Novel results of the current work suggested that exposing loquat fruits to the volatile essential oils of myrtle leaves, either by packing together in a concentration of 3% w/w or exposing to the myrtle leaf vapor for 2 min, improves the postharvest storability of fruits. Overall results about the quality parameters of untreated control fruits are in accordance with the findings of Song et al. [9]. The dominant chemical compounds of the volatile essential oils were found to be eucalyptol, α-pinene, and linalool for the myrtle leaves in the present study. Similarly, eucalyptol and α-pinene compounds were noted to be the two most dominant
compounds of the essential oils extracted from *M. communis* L. leaves [33, 34]. Myszka et al. [34] reported 24 different chemical compounds from the essential oils of myrtle leaves, where 18 of them were similar to those of the current work. There is not much study about the effects of myrtle essential oils on the fruit weight, but similar results were noted for different essential oils including similar compounds in their chemical structures [15, 17, 19, 21, 24].

These terpenes were previously noted to have high bioactivity against several food spoilage microorganisms and pathogens [34, 39, 40]. The mechanism was reported be mainly disruption of the membrane functions by damaging...
The cell wall and membrane structures [39]. The two most important functions were noted to be electron transfer or enzyme activity. Therefore, the high influence of the myrtle leaf and myrtle leaf vapor on the prevention of the decay incidence in the present study can be attributed to the high eucalyptol and α-pinene contents in the volatile essential oils of myrtle leaves. Similar effects were previously noted for the essential oils of myrtle leaves on the *Salmonella typhimurium* [41], Gram-positive and Gram-negative bacteria [42], and *Cryptococcus neoformans* (yeast) [43]. In the study of Fadil et al. [41], it was noted that a mixture of *Thymus vulgaris* L. (55%) and *M. communis* L. (45%) essential oils provides high

![Figure 3: Effects of volatile essential oils of myrtle leaves on the (a) soluble solids concentration and (b) titratable acidity of loquat fruits during 42 days of storage. Different lowercase letters denote significant differences among different treatments for the same sampling time, and different capital letters denote significant differences among different sampling times for the same treatment, according to Tukey’s HSD test at *P* < 0.05.](image-url)
antibacterial activity against *Salmonella typhimurium*. In another study carried out in Algeria, it was found that the *M. communis* L. essential oil has strong antifungal activity against *Cryptococcus neoformans* (yeast) and *Epidermophyton floccosum, Microsporum canis*, and *Trichophyton rubrum* (dermatophytes) [43]. Current results of this study also reveal the traditional use of the Myrtle plants as a disinfectant in Greece [44]. In a most similar study, the essential oil vapors of *M. communis* L. were tested against *Penicillium digitatum* in *in vitro* conditions, and the results showed that it elapsed time between the fungal inoculum and vapor contact [45]. However, in the mentioned study, the essential oils of *M. communis* L. leaves were noted to have lower efficacy than those of *Rosmarinus officinalis* L. The volatile essential oil of myrtle leaves was also tested *in vitro* against two stored-product insects, *Tribolium confusum* and *Callosobruchus maculatus*, and the results of this research showed that the volatile essential oils of myrtle leaves might be used as a potential biocontrol agent these insects [46].

Fruit browning, in conjunction with chilling injury, is among the most important postharvest problems of loquat fruits [47]. Current work demonstrated that exposing fruits to the volatile essential oils of myrtle leaves either by packing together or exposing to leaf vapor is an effective method for reducing the fruit browning. Several studies suggested that the application of different hormones or chemicals, i.e., methyl jasmonate, salicylic acid, and 1-methylcyclopropene, was effective in reducing the browning index of loquat fruit [5, 48].

In agreement with the weight loss results, the results of the present study showed that the volatile essential oils of myrtle leaves are effective in preventing the changes in the fruit firmness. According to Song et al. [35], firmness of loquat fruits increases during storage, together with the lignin contents, and those are the typical symptoms of fruit browning. The main cause of the increase in fruit firmness was also attributed to the cell expansion and secondary lignification in cell wall [48]. Thus, the increase in lignin content causes a rigidity characteristic at the cell wall, which then causes an increase in fruit firmness [49]. Therefore, contrary to many of the fruits, increase in fruit firmness is not a desirable characteristic for the loquat fruits which has less extractable juice [50]. No measurement has been performed in the current work about the lignin content of the loquat fruits, but results showed that the essential oils of myrtle leaf provide favorable conditions for preventing the increase in fruit firmness. Furthermore, results of the present study showed that the volatiles of myrtle leaf have a slight influence on the SSC and TA contents of loquat fruits during storage. In a similar work, myrtle leaf oil application was noted to display similar effects on the SSC of strawberry fruits during storage [51].

5. Conclusions

To sum up, results of the current work suggested that the diverse composition of the volatile essential oils of myrtle leaf makes it a suitable agent for postharvest handling. Among the described 30 chemical compounds, eucalyptol, α-pinene, and linalool were found to be the most abundant compounds. Results also demonstrated that packaging of loquat fruits with myrtle leaves (3% w/w) or exposing them to the myrtle leaf vapor (2 min) provide higher efficacy for the reduction of weight loss, prevention of decay incidence, and reducing the fruit browning. Overall, results suggested that the fresh loquat fruits can be stored for 35 days at 4 ± 1°C and 95% relative humidity by exposing to myrtle leaves (3% w/w) and myrtle leaf vapor (2 min). Thus, the current study would be a preliminary study to investigate the effects of volatile essential oils of myrtle leaf on fruit quality and pathogenic mechanisms, and the results would guide further studies with different fruits.

Data Availability

All data used to support the findings of this study are included within the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

“Conceptualization was carried out by N.P.B., I.K., and C.W.; I.K. and C.W. were responsible for the methodology; investigation was conducted by N.P.B. and I.K.; data curation was performed by I.K.; writing of the original draft was carried out by N.P.B. and I.K.; C.W. was involved in writing the review and editing; visualization was performed by I.K. All authors have read and agreed to the published version of the manuscript.”

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