Postharvest Biology and Technology 155 (2019) 120–129

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

# Quality and safety attributes on shredded carrots by using *Origanum majorana* and ascorbic acid

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ARTICLE INFO

*Keywords:*
Ascorbic acid
*Daucus carota*
Fresh produce
Essential oil
Hydrosol
Minimally processed products

ABSTRACT

The increased need for consumption of raw vegetables has led to the production of minimally processed products like shredded carrots. There is a current trend towards the use of natural agents for the preservation of fresh produce quality, as alternatives to synthetic compounds such as chlorine. The purpose of this study was to evaluate the effects of different washing treatments with aqueous solutions of marjoram (*Origanum majorana*) essential oil (EO) (1:1500 v/v), marjoram hydrosol (Hyd) (1:15 v/v), ascorbic acid (AA) (1%) and their respective combinations on the quality of shredded carrot under storage at 4 °C for 9 d. The EO-treated carrots had acceptable aroma and AA-treated carrots obtained acceptable carrot-like aroma. The carrots' orange color preserved with the AA application compared to the untreated control but marjoram Hyd application resulted to a final product with darker color and increased respiration after 6 and 9 d of storage at 4 °C and 90% RH. Furthermore, the application of AA increased total phenolic content and antioxidant activity of shredded carrots, while the combination of AA with EO and Hyd increased carotenoid content. Total soluble solids were increased following the application of marjoram Hyd. The application of AA increased total acidity and lowered pH values of shredded carrots at the 9th day of storage. Decay incidents, as observed by the total viable counts and yeast and filamentous fungi, were decreased by single or combined treatment during storage. Ascorbic acid alone or in combination with Hyd or EO maintained quality and preservation of processed carrots and in that way can be proposed as alternative sanitizers.

## 1. Introduction

According to dietary guidelines, a balanced and healthy diet should include daily intake of fruit and vegetables (HHS and USDA, 2015). Phytochemicals are abundant in fresh produce and may promote human health, reduce the risk of heart diseases and prevent diseases such as cancer (Food and Agriculture Organization of the United Nations (FAO, 2015; Formica-Oliveira et al., 2017). Carrots (*Daucus carota* L.), as all vegetables, are an important source of nutrients, rich in carotenoids, vitamins A and E as well as antioxidants (Formica-Oliveira et al., 2017). Regular consumption of dietary antioxidants may decrease sensitiveness on several diseases (Altunkaya and Gökmen, 2009).

Fresh-cut vegetables are widely consumed as a healthy, nutritional and convenient option for the increase of daily vegetable intake (Klaiber et al., 2005; Vandekinderen et al., 2008; Olaimat and Holley, 2012). One of the most commonly consumed salad vegetable is the minimally processed carrots (Fai et al., 2016). Minimal processing includes washing and preparation of fresh produce for consumption such as shredding, slicing, peeling and trimming (Alegria et al., 2010).

Minimal processing should be able to provide the market with fresh-like products with high nutritional value and extended shelf life that can satisfy consumer's needs for fresh and safe products (Klaiber et al., 2005; Alegria et al., 2010). However, fresh-cut vegetables are perishable products with short shelf life and this can be attributed mainly to mechanical damage, physiological disorders and availability of nutrients on the cut surfaces that can favour biochemical changes (i.e. enzymatic browning) and microbial growth (spoilage and pathogen microorganisms) (Alegria et al., 2010; Lucera et al., 2010; Fai et al., 2016). Wounding is the primary stress subjected to the minimally process products which results in adjustments on plant metabolism, activating defence-related mechanisms, including higher production of polyphenols to overcome or to prevent further damage (Han et al.,

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https://doi.org/10.1016/j.postharvbio.2019.05.015
Received 8 March 2019; Received in revised form 18 May 2019; Accepted 19 May 2019

2017). For instance, during processing of shredded carrots loss of the bright orange color might be noticed due to dehydration and lignification that will lead to surface whiteness of the product (Fai et al., 2016). Storage conditions, including temperature and preservative means, have additional role on shredded carrot safety and quality indices (Lavelli et al., 2006; Xylia et al., 2018).

Washing of fresh produce is one of the most common steps during minimal processing of vegetables and chlorine is the most common agent used by the food industry in the washing water (mainly as sodium hypochlorite). However, the use of chlorine can lead to the production of undesirable by-products such as trichalomethanes with the incomplete oxidation of organic matter (Tzortzakis et al., 2007), while its use is already prohibited in some countries in Europe, Netherlands, Sweden, Germany to name a few (Gil et al., 2009). Trichalomethanes are harmful, carcinogenic compounds that can negatively affect human health and the environment (Parish et al., 2003). Thus, there is need for alternative, environmentally friendly and safe agents that can be used instead of chlorine and many researchers have been studying naturally occurring substances with antimicrobial and antioxidant properties. Essential oils from aromatic plants (Singh et al., 2002; Scollard et al., 2013; Stavropoulou et al., 2014; Tzortzakis et al., 2016; Xylia et al., 2018) and ascorbic acid (vitamin C) (Akbas and Ölmez, 2007) have been considered as potential agents for the disinfection and preservation of vegetable quality due to their antimicrobial and antioxidant properties. On top of the well documented antimicrobial activity of the EOs (Stavropoulou et al., 2014; Xylia et al., 2018), sensorial impacts of the processed produce is also of high attention to researchers and industry. Siroli et al. (2015) reported that oregano and thyme EO-treated lettuce obtained the same sensorial analysis and product self-life similar to that obtained with chlorine. In contrast, lettuce washed with oregano and thyme EO solution were rejected of overall appreciation after 7 d, but carrots treated with EO were acceptable by a sensory panel throughout storage (Gutierrez et al., 2009). However, the different preservation means, the huge variation and variety of the EOs, doses, application time, combination among preservatives and fresh produce can alter the effectiveness of a simple preservative (Alegria et al., 2009; Tzortzakis, 2009; Stavropoulou et al., 2014).

During distillation (usually hydro- or steam-distillation) for the production of essential oils, the hydrosol (colloidal suspensions of essential oil and water-soluble components) that is produced, appears to have various biocidal properties. For example, the antimicrobial properties and effectiveness of mint hydrosol on shredded carrots have been previously reported (Xylia et al., 2018). Moreover, marjoram EO has been applied for fresh produce preservation (Hyun et al., 2015a). The aim of this study was to examine the effects of marjoram essential oil (EO), marjoram hydrosol (Hyd), ascorbic acid (AA) and their combinations in the preservation of quality attributes of refrigerated fresh shredded carrots.

## 2. Materials and methods

### 2.1. Plant material and EO extraction

Fresh carrots (*Daucus carota* L.) were obtained from a local market in Limassol, Cyprus. Carrots purchased from Cypriot local market do not receive any sanitation treatment, rather than washing with water for soil residues removal. Then they were selected for uniformity in appearance and the absence of physical defects or injury and stored at 4 °C and 90% RH until use (within 24 h). Prior to processing, carrots were rinsed with running tap water and then were paper dried.

Marjoram plants (*Origanum majorana* L.) were obtained from the experimental farm of Cyprus University of Technology, where they were cultivated in soil. Marjoram plant tissue was harvested, air-dried (in oven at 42 °C), chopped and approx. 1 kg of dried tissue were hydrodistilled for 3 h, using Clevenger apparatus (Chrysargyris et al., 2016). The obtained EO was stored in amber glass bottles at −20 °C until use. Following EO hydrodistillation, the water solution (hydrosol) was collected and filtered to remove any plant residues. Freshly prepared hydrosol (Hyd) was used for all the studies. The composition of marjoram EO was determined as described previously (Chrysargyris et al., 2016), and the main components were terpinen-4-ol, γ-terpinene, *trans*-sabinene hydrate, and α-terpinene (Table 1).

**Table 1**
Chemical composition of the essential oils (% of peak area) of marjoram. Values in rows are means of three replications with standard error (SE).

| Peak no. | Compound* | RI[a] | Mean % | SE |
|---|---|---|---|---|
| | α-thujene | 926 | 1.09 | 0.003 |
| 1 | α-pinene | 933 | 0.69 | 0.001 |
| 2 | Sabinene | 973 | 4.80 | 0.031 |
| 3 | β-pinene | 977 | 0.38 | 0.001 |
| 4 | β-myrcene | 989 | 1.67 | 0.002 |
| 5 | α-phellandrene | 1004 | 0.39 | 0.003 |
| 6 | α-terpinene | 1017 | 10.71 | 0.055 |
| 7 | p-cymene | 1022 | 1.77 | 0.008 |
| 8 | Sylvestrene | 1026 | 3.86 | 0.017 |
| 9 | Eucalyptol | 1031 | 0.27 | 0.000 |
| 10 | γ-terpinene | 1058 | 17.20 | 0.101 |
| 11 | *cis* Sabinenehydrate | 1067 | 3.31 | 0.030 |
| 12 | Terpinolene | 1089 | 3.37 | 0.018 |
| 13 | *Trans* Sabinenehydrate | 1100 | 13.05 | 0.005 |
| 14 | *cis* p Menth-2-en-1-ol | 1120 | 1.37 | 0.031 |
| 15 | iso-3-Thujanol | 1137 | 0.69 | 0.027 |
| 16 | Terpinen-4-ol | 1178 | 24.82 | 0.029 |
| 17 | α-terpineol | 1197 | 4.41 | 0.003 |
| 18 | *trans* Sabinene hydrate acetate | 1254 | 0.21 | 0.008 |
| 19 | Linalool acetate | 1255 | 2.16 | 0.021 |
| 20 | Bornyl acetate | 1283 | 0.15 | 0.009 |
| 21 | Terpinene-4-ol acetate | 1297 | 0.72 | 0.018 |
| 22 | Caryophyllene *E* | 1411 | 1.38 | 0.011 |
| 23 | Bicyclogermacrene | 1512 | 1.23 | 0.002 |
| | Monoterpenes hydrocarbons | | 45.94 | 0.188 |
| | Oxygenated monoterpenes | | 47.92 | 0.119 |
| | Sesquiterpenes hydrocarbons | | 1.42 | 0.001 |
| | Oxygenated sesquiterpenes | | 0.00 | 0.000 |
| | Others | | 3.24 | 0.056 |
| | Total Identified | | 98.53 | 0.014 |

[a] Retention index relative to *n*-alkanes on the ZB-5 column.
* Only compounds representing more than 0.05% of the total chromatogram area are presented.

### 2.2. Preliminary screening

Fresh carrots were washed with chlorinated water (0.1 g $L^{-1}$) and rinsed with distilled water, hand peeled and shredded (2 mm x 3 mm x 40–60 mm). Then, 100 g of shredded carrots were dipped for 10 min (according to preliminary tests) into 0.5 L of treatment solution. The following thirteen dipping solutions were studied: 1) distilled water, 2) marjoram EO (1:500, 1:1000, 1:1500 and 1:2000 v/v), 3) marjoram hydrosol (1:5, 1:10, 1:15 and 1:20 v/v) and 4) ascorbic acid (0.25%, 0.5%, 1%, and 2% w/v). Afterwards, carrots were drained, and 25 g were placed into 90 mm Petri dishes and stored at 4 °C and 90% RH until the day of sampling (Days 1, 3, 5 and 7). Four biological replicates per treatment/concentration were sampled and the appropriate amount of tissue was stored at −20 °C until analysis. For preliminary screening, aroma and marketability assessed by 7 untrained panelists, while the weight loss and the content of total polyphenols were measured and used for choosing the adequate dipping solutions.

Carrots weight loss was monitored every second day, as the weight of each Petri dish was registered before and during storage at 4 °C for 7 d (Days 1, 3, 5 and 7) and results were presented as percentage of weight loss. Overall, aroma and chroma (visual quality) were evaluated after storage for 7 d. Aroma evaluation was assessed with a 5-point hedonic scale (0.5 interval) where 5 = not acceptable; 3 = not carrot but acceptable; 1 = carrot. Visual quality was assessed with a 5-point

hedonic scale (0.5 interval) where 5 = brown; 3 = orange; 1 = white. The content of total phenolics was determined from methanolic extracts as previously described by Tzortzakis (2007) and results were expressed as mg of gallic acid equivalents per g of fresh weight (mg $g^{-1}$ GAE).

### 2.3. Main study for the determination of quality and antioxidant activity

Following preliminary screening, carrots were selected, prepared and shredded as above. Then, 150 g of shredded carrots were dipped for 10 min into 0.5 L of treatment solution selected from the preliminary screening. The following six dipping solutions/combinations were further studied: 1) distilled water, 2) marjoram EO (1:1500 v/v), 3) marjoram hydrosol (1:15 v/v), 4) Ascorbic acid (1% w/v), 5) marjoram EO (1:1500) + Ascorbic acid (1%) and 6) marjoram hydrosol (1:15) + Ascorbic acid (1%). Afterwards, carrots were drained, and 25 g were placed into a 90 mm Petri dish at 4 °C and 90% RH until the day of sampling (Days 0, 3, 6 and 9). Four biological replicates per treatment/day were sampled and the appropriate amount of tissue was stored at −20 °C until analysis.

#### 2.3.1. Weight loss and color

Carrots weight loss was determined as mentioned above. Shredded carrot's color was evaluated with a colorimeter (Chroma meter CR400 Konica Minolta, Japan) where the $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) value were recorded on Days 0, 3, 6 and 9 (four measurements per treatment). Chroma value (C), hue (h) and whiteness index (WI) were calculated by the following equations $C = (a^{*2} + b^{*2})^{1/2}$, $h = b^*/a^*$ and $WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Bolin and Huxsoll, 1991; McGuire, 1992).

#### 2.3.2. Respiration rate

Respiration of the processed product was estimated by measuring the $CO_2$ concentration of the packages using a dual gas analyzer (International Control Analyser Ltd, Kent, UK). Briefly, one Petri dish with open lid was enclosed in a polypropylene plastic container (1 L) at room temperature for 1 h and afterwards container's air was withdrawing for 40 s through a hole on the lid whilst recording the % $CO_2$. Respiration rate was computed according to the volume and weight of the shredded carrot.

#### 2.3.3. Total soluble solids, total acidity, pH, ascorbic acid, total carotenoid content

Carrot tissue (four biological replicates/treatment/day) was grinded/pressed to extract the juice with a domestic blender. The pH of the carrot juice was measured with a pH-meter (HANNA HI2211, Cluj-Napoca, Romania). Total soluble solids (TSS) content was determined using a digital portable refractometer (Atago, Tokyo, Japan) and results were expressed in percentage of TSS. Titratable acidity (TA) was determined by titration with 0.1 N NaOH as described by Rocha et al. (2007) and results were expressed as percentage of citric acid (% TA). The ratio of TSS/TA was used to evaluate the sweetness of carrots (Picouet et al., 2015).

AA content was quantified by titration with 2,6-dichlorophenol-indophenol (AOAC International, 2007) and results were expressed as g of AA per kg of fresh weight. Total carotenoid content was determined as described by Rocha et al. (2007); absorbance was measured at 480 nm, and results were expressed as g of carotenoids per kg of fresh weight (g $kg^{-1}$ carotenoids).

#### 2.3.4. Total phenolic content and antioxidant activity

Total phenolics were determined as mentioned above. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activities were determined according to the procedures described before by Wojdyło et al. (2007). Results expressed as g of trolox per kg of fresh weight (g $kg^{-1}$ trolox).

#### 2.3.5. Hydrogen peroxide content and lipid peroxidation

The hydrogen peroxide ($H_2O_2$) concentration was determined according to the method described previously by Loreto and Velikova (2001). Results were expressed as μmol of $H_2O_2$ per kg of fresh weight (μmol $kg^{-1}$ $H_2O_2$).

Lipid peroxidation of shredded carrots was estimated according to the 2-thiobarbituric acid reactive substances (TBARS) method as previously described by de Azevedo Neto et al. (2006). The absorbance was measured at 532 and 600 nm and results were expressed as μmol of malondialdeyde (MDA) per kg of fresh weight (μmol $kg^{-1}$ MDA).

#### 2.3.6. Activity of antioxidant enzymes

Fresh carrot tissue was grinded with liquid nitrogen and then 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVPP), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.05% triton X-100, was used for the enzyme extraction. Catalase (CAT; EC 1.11.1.6) and superoxide dismutase (SOD; EC 1.15.1.1) activity were assayed as described by Chrysargyris et al. (2018). For the peroxidase (POD; EC 1.11.1.7) activity the method described by Tarchoune et al. (2012) was adapted, using pyrogallol and following the increase in absorbance at 430 nm. Results were expressed as units of enzyme per mg of protein. The Bradford method was used for the quantification of protein content in the carrot extracts.

#### 2.3.7. Microbiological analysis

Total viable count (TVC) as well as yeast and filamentous fungi were determined as described by Alegria et al. (2010) using Plate count agar (PCA, Merck, Darmstadt, Germany) and Rose Bengal Chloramphenicol Agar (Liofilchem s.r.l, Italy), respectively. Results were expressed as log CFU per g of fresh weight (log CFU $g^{-1}$).

### 2.4. Statistical analysis

Statistical analysis was performed comparing data means with one way-analysis of variance (ANOVA) using IBM SPSS version 22 and Duncan's multiple range test was performed for P = 0.05. Four (n = 4) biological replicates were used and values refereed to mean ± standard error (SE). Microbiological analysis was done with duplicate plates for each of the three replicates. Results are expressed on a fresh weight basis.

## 3. Results

### 3.1. Effectiveness of screening preservation means

The screening of the effects of marjoram EO, Hyd and AA on shredded carrots during storage is presented in Figs. 1 and S1. The content of total phenolics increased at treatments with 1–2 % AA, even at the 1st day of storage, and this effect was maintained up to 7 d of storage. The applications of Hyd (from 1:5 to 1:20), AA (from 0.25% to 2%) and EO at 1:500 and at 1:2000 reduced weight loss when compared to the control treatment (Fig. S1). The preservative applications affected the carrot aroma after 7 d storage at 4 °C and 90% RH, whereas the EO marked with 3 out of 5 scale, which reflected an acceptable but not a carrot-like aroma. The application of AA marked as acceptable carrot-like aroma, whereas Hyd acceptability was marked between AA and EO acceptability (Fig. 1). Carrot marketability changes were also observed in terms of orange color appearance. It was found that both AA and EO maintained the orange carrot color whereas the application of Hyd decreased the marketability as the processed carrots had browner chroma (scored 4 out of 5 units), and this was evident even at the first day of storage (Fig. S2). Control treatment also revealed brown color on carrots after the 5th day (Fig. 1).

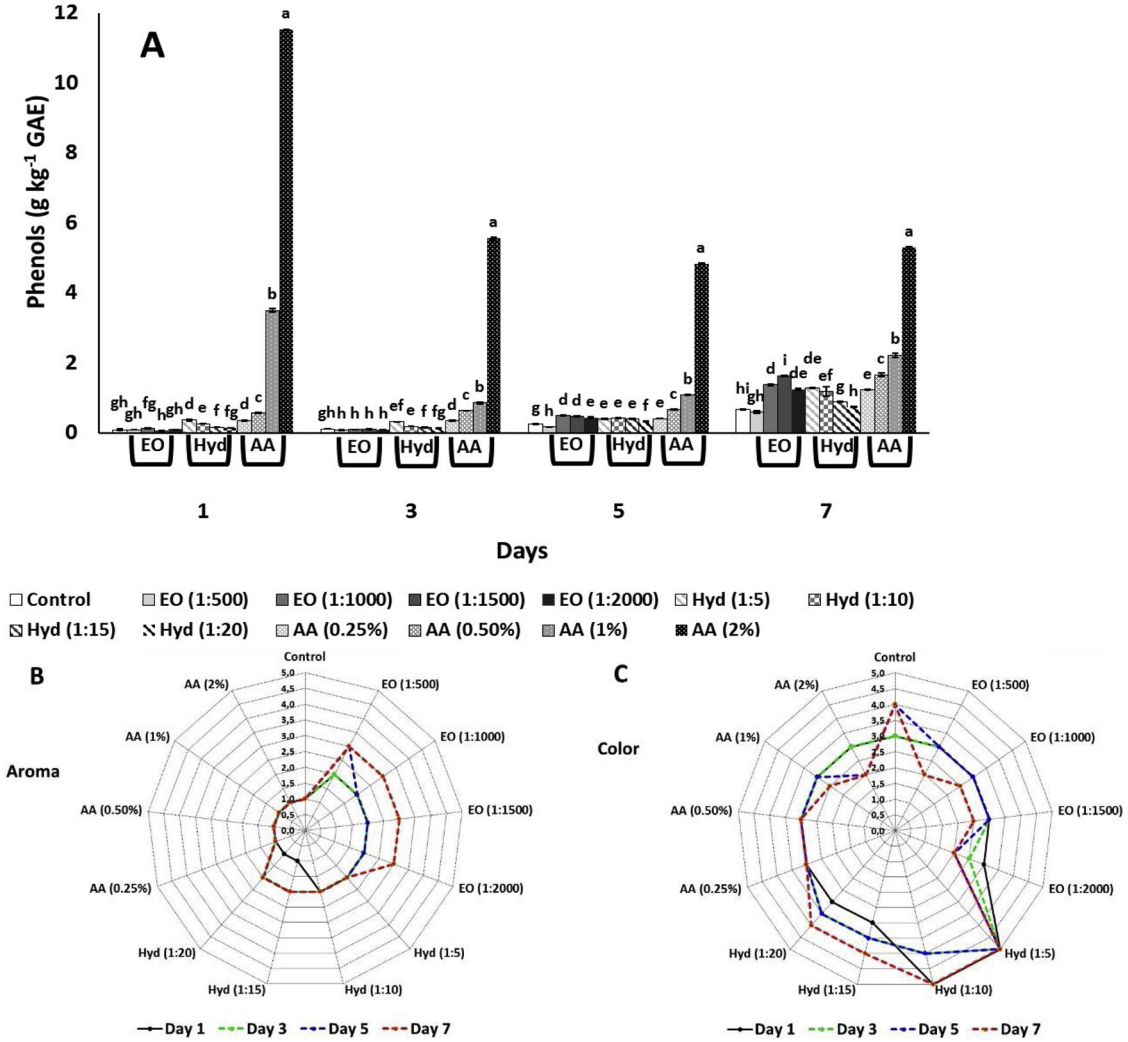


**Fig. 1.** Screening of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots (A) total phenols (g $kg^{-1}$ GAE), (B) aroma (scale range from 1-carrot like to 5-not acceptable) and (C) color/marketability (bottom right, scale: 1-white; 3-orange; 5-brown) after 7 d of storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters for different days. Values of phenolics represent means ( ± SE) of measurements on four biological replicates per treatment. Aroma and color evaluation were assessed by 7 untrained panelists.

### 3.2. Impacts on quality and antioxidant activity

#### 3.2.1. Effects on weight loss and respiration rates

The application of marjoram EO increased (up to 2.25%) weight loss on shredded carrots stored up to 9 d, while both Hyd and AA maintained weight losses to similar levels with the ones observed at the control treatment (Fig. 2). Indeed, the combinations of EO + AA and Hyd + AA resulted in higher weight losses compared to the individual effects of the AA or Hyd.

Respiration was increased (3.2-fold) with the combination of

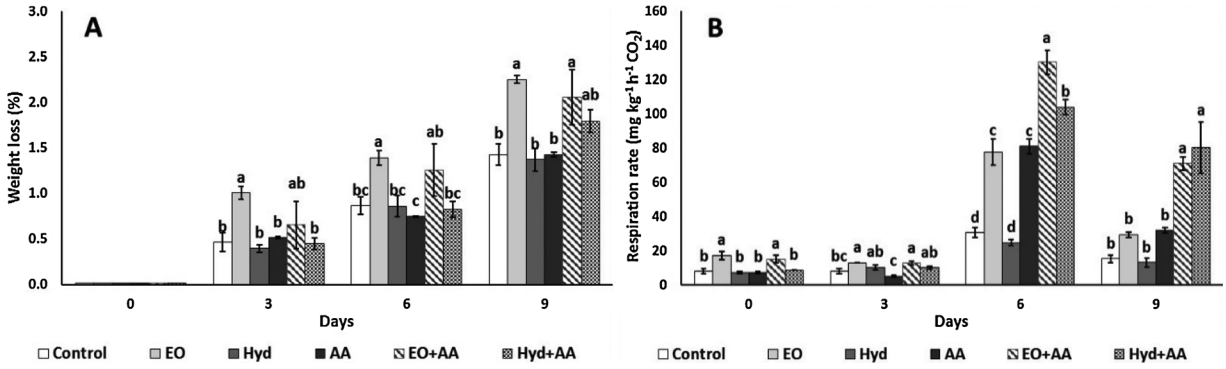


**Fig. 2.** Effect of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots (A) weight loss (%) and (B) respiration rate (mg $kg^{-1}$ $h^{-1}$ $CO_2$) after treatment and 9 d of storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters for different days. Values represent means ( ± SE) of measurements made on four biological replicates per treatment.

EO + AA on the 6th day, whereas Hyd maintained respiration rate to similar levels as the one observed at the control treatment, on the same day (24.61 mg $kg^{-1}$ $h^{-1}$ $CO_2$) (Fig. 2). Furthermore, the combinations of EO + AA and Hyd + AA increased (3.6-fold and 4.2-fold, respectively) respiration rate on the 9th day, while no differences were observed by the single application of EO, Hyd and AA. Oxygen level was slightly decreased and averaged at 18% (data not presented) and remained almost constant throughout the storage period.

*3.2.2. Effects on color*

The application of marjoram Hyd decreased carrots lightness ($L^*$ values) and redness ($a^*$ values) up to the 9th day of storage, while yellow color ($b^*$ value) was increased with the application of marjoram EO and the combination of EO + AA (46.38 and 47.12, respectively) (Figs. S3A, S3B, S3C). The application of AA and Hyd + AA did not affect the lightness and the red color of the carrots. The combination of EO + AA increased (up to 8.7%) Chroma value compared to the control shredded carrots at the 9th day. The application of AA (alone or with the combination of EO or Hyd) decreased (up to 7.1%) Hue value while EO + AA application decreased (up to 7.3%) the Whiteness index when compared to control after 6 d of storage (Figs. S3D, S3E, S3F).

*3.2.3. Effects on total soluble solids, acidity, sweetness and pH*

Total soluble solids (TSS) and sweetness (TSS/TA) of shredded carrots were decreased (up to 56.4% and 60.3%, respectively) with the application of marjoram Hyd compared to the control treatment at the 9th day, whereas an increase of TSS was observed with the combination of EO + AA and Hyd + AA (up to 30.2% and 63.0%, respectively) up to the 9th day of storage (Fig. 3A, 3C). An increase in total acidity value was observed with the application of AA on the day of the application and after six and nine days (up to 49.2%, 58.4% and 2.6-fold, respectively) when compared to the control (Fig. 3B). The application of the combined treatments of EO + AA and Hyd + AA decreased (up to 13.9% and 15.1%, respectively) the pH values of the shredded carrots during the 9th day of storage at 4 °C and 90% RH (Fig. 3D). Noticeably, AA acidified further the processed carrots and led to decreased pH values up to 24.3% compared to the control.

*3.2.4. Effects on total phenolics, antioxidants, ascorbic acid and total carotenoids content*

Total phenolic content was increased with the application of AA, and its combination with marjoram EO and Hyd as illustrated in Fig. 4A. The application of AA and the combination of Hyd + AA increased (up to 2.8-fold and 2.6-fold as assayed by DPPH and ABTS) antioxidant activity of shredded carrots on the day of the application whereas the effects were almost exhausted following 3 d of storage (Fig. 4B, 4D). Ascorbic acid content of shredded carrots was increased with the application of AA and the combination of Hyd + AA on the day of the application (up to 3.9-fold and 12-fold, respectively), after six (up to 1.5-fold and 1.2-fold, respectively) and nine days (up to 2.5-fold and 2.3-fold, respectively) (Fig. 4C). The combination of Hyd + AA maintained carotenoid content (0.111 g $kg^{-1}$ carotenoids) to similar levels to the control treatment, while a decrease of carotenoids was observed with the application of marjoram EO, marjoram Hyd and AA (up to 24.1%, 25.7% and 26.6%, respectively) on the 9th day at 4 °C and 90% RH (Fig. 4E).

*3.2.5. Effects on hydrogen peroxide, lipid peroxidation and antioxidant enzymes*

The impacts of marjoram EO, marjoram Hyd and AA on hydrogen peroxide, lipid peroxidation and antioxidant enzymes are presented in Fig. 5. On the first day (after treatments), the AA application increased reactive oxygen species (ROS) production. The application of the combination of EO + AA followed by the EO treatment increased (up to 55.3% and 32.6%, respectively) $H_2O_2$ while the application of AA decreased (up to 39.6%) $H_2O_2$ compared to the control, on the 9th day of storage (Fig. 5A). All the AA treatments increased MDA especially with the combination of Hyd (Hyd + AA; up to 33.4%) or EO (EO + AA; up to 63.7%) during the 9 d of storage (Fig. 5B). The enzymatic activity of SOD was increased (up to 54.8%) with the application of AA, while a

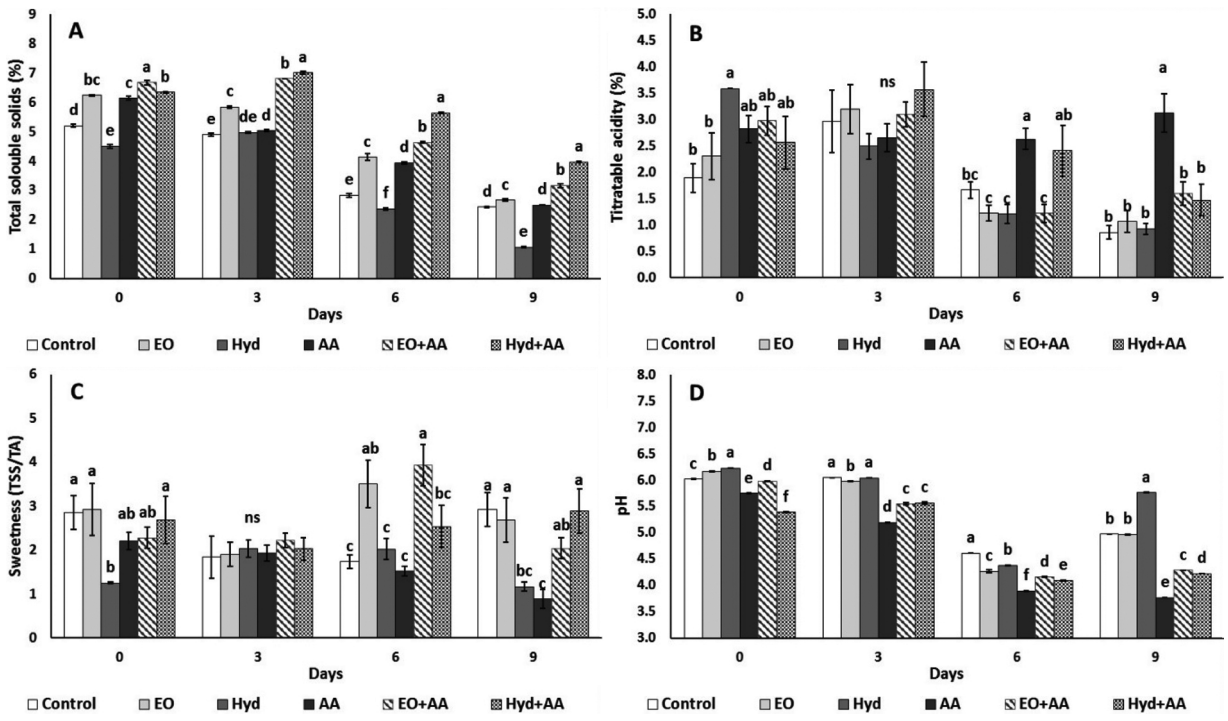


**Fig. 3.** Effect of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots (A) total soluble solids (TSS; %), (B) total acidity (TA; %), (C) sweetness (TSS/TA), and (D) pH after treatment and 9 d storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters; ns indicates no significant differences for different days. Values represent means ( ± SE) of measurements made on four biological replicates per treatment.

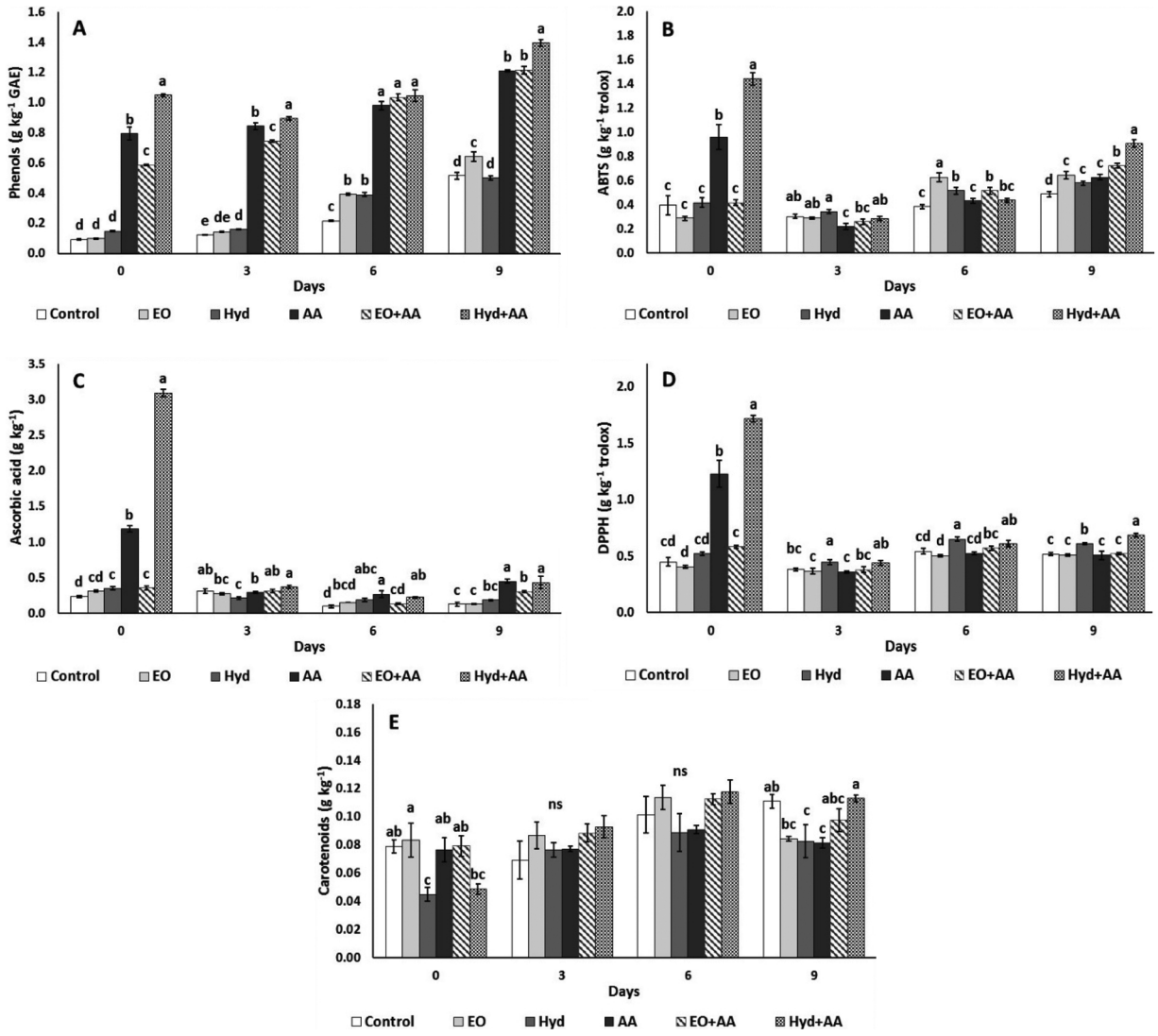


**Fig. 4.** Effect of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots (A) total phenolics (g $kg^{-1}$ GAE), (B) antioxidant activity (DPPH; g $kg^{-1}$ trolox), (C) ascorbic acid (g $kg^{-1}$), (D) antioxidant activity (ABTS; g $kg^{-1}$ trolox), and (E) carotenoids (g $kg^{-1}$) content after treatment and 9 d of storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters; ns indicates no significant differences for different days. Values represent means ( ± SE) of measurements made on four biological replicates per treatment.

decrease was observed with the application of marjoram EO (up to 19.4%) and Hyd + AA (up to 17.4%) on the 3rd day, compared to the control (Fig. 5C). The application of marjoram EO increased CAT on the 3rd day, whereas the application of AA, EO + AA and Hyd + AA decreased the CAT activity on the 3rd and 6th day (Fig. 5D). Moreover, the application of marjoram Hyd increased (up to 62.7%) POD on the day of the application, while the application of AA and its combinations with marjoram EO and Hyd decreased POD (73.9%, 77.6% and 56.5%, respectively). Interestingly, the combined treatments seem to cause decreases in POD activity, in general (Fig. 5E).

### 3.2.6. Microbiological analysis

Microbial quality of shredded carrot subjected to single or combine treatments are illustrated in Fig. 6. A significant decrease in total viable counts (TVC) was detected on the 6th day in all the examined treatments and on the 9th day with the application of AA and the combination of EO + AA and Hyd + AA (up to 20.1%, 16.5% and 24.2%, respectively), compared to the control (Fig. 6A). No differences were found the first 3 d for the TVC. Yeast and filamentous fungi were decreased after 3 d for all the treatments (with exception the Hyd + AA) and the effects were more pronounced with the application of the combined treatment on the 9th day. Therefore, Hyd + AA revealed the greatest decrease (up to 49.1%) of yeasts and moulds at the 9th day of application compared to the control (Fig. 6B).

## 4. Discussion

Minimally processed vegetables are quite popular nowadays as a source of phytochemicals, however they are perishable products as they tend to have short shelf life. The use of natural products such as essential oils and naturally occurring substances as an ecofriendly and healthier preservative method is rapidly growing, attracting scientific interest and consumer's acceptance (Song et al., 2017).

The antioxidant and antimicrobial activity of EOs from aromatic and medicinal plants have been previously mentioned (Sellami et al., 2009; Tzortzakis, 2009; Xylia et al., 2017). It has been reported that marjoram EO possess high antioxidant and antimicrobial activity and its main components include terpinen-4-ol, terpinolene, 1,8-cineole (Vera and Chane-Ming, 1999), and this is in line with the main components found in the examined marjoram EO. Marjoram EO has been applied to fresh cabbage, lettuce and radish sprouts with encouraging results (Hyun et al., 2015a, b). The use of AA as an anti-browning agent

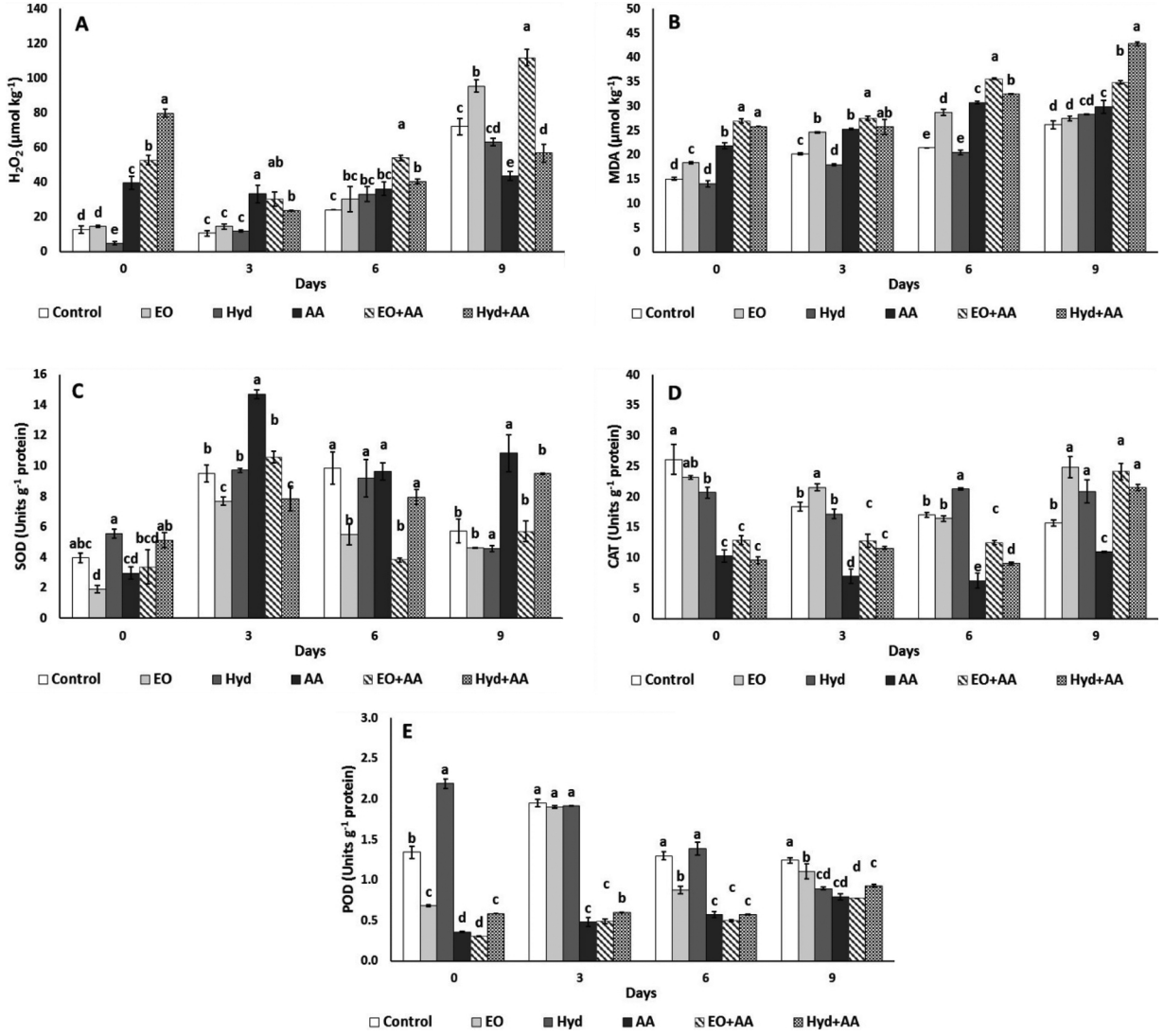


**Fig. 5.** Effect of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots on (A) $H_2O_2$ production (μmol kg$^{-1}$), (B) lipid peroxidation (μmol kg$^{-1}$) and antioxidant enzymes activity of (C) superoxide dismutase (SOD; units g$^{-1}$ protein), (D) catalase (CAT; units g$^{-1}$ protein) and (E) peroxidase (POD; units g$^{-1}$ protein) after treatment and 9 d of storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters for different days. Values represent means ( ± SE) of measurements made on four biological replicates per treatment.

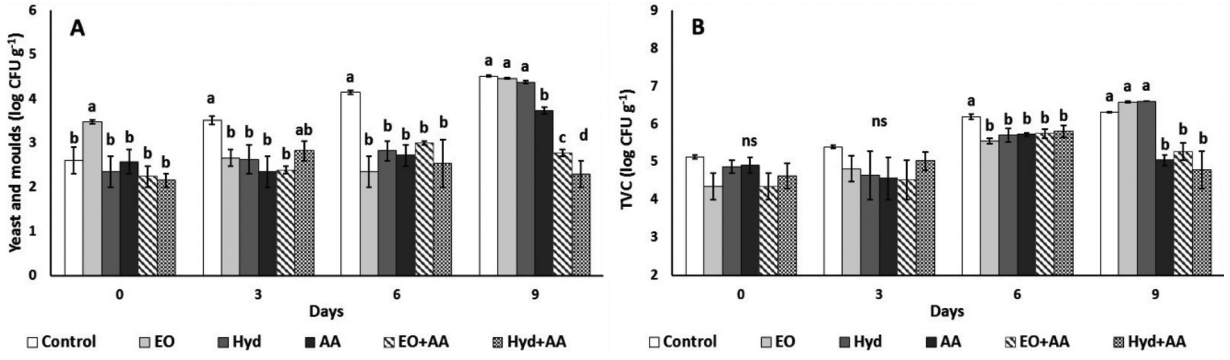


**Fig. 6.** Effect of marjoram essential oil (EO), marjoram hydrosol (Hyd) and ascorbic acid (AA) on shredded carrots on (A) yeast and filamentous fungi (log CFU g$^{-1}$) and (B) total viable count (TVC; log CFU g$^{-1}$) after 9 d of storage at 4 °C and 90% RH. On the columns, significant differences ($P < 0.05$) among treatments are indicated by different Latin letters; ns indicates no significant differences for different days. Values represent means ( ± SE) of measurements made on four biological replicates per treatment.

in fresh produce has been studied due to its high antioxidant activity (Akbas and Ölmez, 2007; Altunkaya and Gökmen, 2009; Ölmez and Temur, 2010; Park et al., 2011).

In this study no considerable weight losses (< 2.3%) were determined. The higher weight losses were observed after the application of marjoram EO, and the treatments of EO + AA and Hyd + AA. The increased surface exposure to air of the processed products can lead to moisture loss and furthermore surface whitening but also to decay presence (Li and Barth, 1998). It is noteworthy that Rocha et al. (2007) have reported up to 9% weight loss after storage at 2 °C for 10 d.

Increased respiration was observed on the sixth day with the application of EO + AA whereas marjoram Hyd lead to a decrease of the respiration rate. Heat treatment efficiently maintained shredded carrot quality by decreasing respiration rates (Algeria et al., 2010). As it has been previously mentioned by Rocha et al. (2007), cutting increases the respiration rate of fresh produce and causes protein, lipid and carbohydrates degradation and off-flavors and off-odors development.

The bright orange color of carrots is one of the most important attributes that can affect consumers buying decision. The application of EO + AA increased Chroma value resulting in a lighter orange color throughout storage. In a previous study the application of mint hydrosol (1:10, v/v) on shredded carrots resulted in a less orange product on the sixth day of storage with lower Chroma value compared to the untreated samples (51.64 and 53.20, respectively) (Xylia et al., 2018). Rocha et al. (2007) reported that surface whitening might be attributed to increased moisture loss. Barry-Ryan and O'Beirne (1998) also pointed out that surface whitening and lignification of fresh cut carrots may be the results of moisture loss. The application of EO + AA decreased Whiteness index of shredded carrot exhibiting less surface whitening. Whitening index (averaged in 31.2 value) in the control treatment in the present study was at similar levels with the one found in untreated and chlorine-treated carrots (Klaiber et al., 2005). Decreased Chroma and lightness ($L^*$ values) reflect in a less intense orange color and at the same time Whiteness index increases suggesting quality deterioration (Mastrocola and Lerici, 1991; Lavelli et al., 2006). Lavelli et al. (2006) correlated the visual appearance of whitening and the perception of off-odor on minimally processed carrots and concluded that WI can be regarded as the most sensible indicator of sensory quality. The increases in WI that were observed during storage are related to the reversible dehydration of outer tissue layers which can be related to the formation of lignin and concomitant whitening of the shreds (Cisneros-Zevallos et al., 1995).

Total soluble solids were decreased by the marjoram Hyd treatment and increased by the EO + AA and Hyd + AA applications. In the present study, TSS values ranged between 1.07–6.80 % which were lower to the values observed in a relevant study with the application of mint EO and hydrosol in shredded carrots (TSS range: 8.26–9.33 %), indicating the variation of EO effectiveness from different plant species, marjoram versus mint (Xylia et al., 2018). Other studies have reported higher TSS values, ranging between 8.3 and 9.6% (Koca and Karadeniz, 2008) or even up to 11.8% (Alegria et al., 2010). Total acidity was increased (up to 3.12% on the 9$^{th}$ day of storage) with the AA application while Rodrigo et al. (2003) observed TA values up to 0.6% in carrot juice.

Decreased pH values were observed with the application of AA (up to 5.75), EO + AA and Hyd + AA (up to 4.09), and these findings are in accordance with previous reported studies (Alegria et al., 2009, 2010; Xylia et al., 2018). It is noteworthy that Rocha et al. (2007) mentioned that decreased pH values (pH 5.4–6.2) may be the result of $CO_2$ production that can react with water of the plant tissue and release $H^+$. In this sense, the increased respiration rates found in EO + AA and Hyd + AA could contribute to the $H^+$ release and acidified the processed carrots leading to pH decreases.

EOs can induce plant defense mechanisms, both directly and indirectly, by the production of phenols and antioxidants (Amorati et al., 2013). Total phenolic content was increased with the application of AA, EO + AA and Hyd + AA (0.98, 1.03 and 1.04 g kg$^{-1}$ GAE, respectively) compared to control (0.21 g kg$^{-1}$ GAE) after 6 d of storage. On the other hand, the application of mint EO on shredded carrots decreased total phenols (1.65 g kg$^{-1}$ GAE), whereas mint Hyd increased total phenols (5.22 g kg$^{-1}$ GAE) after six days of storage (Xylia et al., 2018). Han et al. (2017) mentioned shredded carrots phenolic content ranging between 0.23 and 0.44 g kg$^{-1}$ GAE after storage at 4 °C, and these levels are in accordance with the present findings for the control treatment. Increased antioxidant activity was observed with the application of marjoram Hyd and Hyd + AA whereas similar results were observed with the application of mint Hyd on shredded carrots in a previous study (Xylia et al., 2018).

The application of AA and Hyd + AA increased carrots ascorbic acid content. Hyd application caused a light increase in ascorbic acid content, being in accordance with previous studies with mint Hyd on shredded carrots (Xylia et al., 2018). Carotenoid content varies between carrot cultivars (0.004-0.026 g kg$^{-1}$) (Nicolle et al., 2004). The application of Hyd + AA maintained carotenoid content whereas the application of marjoram EO, Hyd and AA decreased carotenoids compared to the control during 9 d of storage. On the other hand, the application of mint EO and Hyd did not significantly affect carotenoids of shredded carrots (Xylia et al., 2018), indicating a variation of the EO and Hyd effectiveness among different plant species (marjoram versus mint). According to Rocha et al. (2007) carotenoids reduction is related to the duration of the storage period and therefore, nutritional value of the produce is decreased during storage. Carotenoids are concentrated mainly to the phloem of the carrot tissue. The loss of carotenoids seems to be attributed to the oxidation as minimally processing (peeling and shredding) exposes phloem to light, air and enzymes (Gross, 1991). On the other hand, carotenoids are relatively stable in their natural environment, but postharvest treatments or processing operations may enhance the pigments' degradation.

Processing induces wounds in plant tissue and as a consequently, this is causing a stress response of the wounded tissue. Indicators of damage index often presented by the increased lipid peroxidation (MDA) content and hydrogen peroxide production (Chrysargyris et al., 2018). Han et al. (2017) reported levels of $H_2O_2$ ranging between 8.56 and 9.33 μmol kg$^{-1}$ in shredded carrot stored at 4 °C which were affected by cutting style and storage temperature (Han et al., 2017). In our study EO + AA increased $H_2O_2$ levels and lipid peroxidation in terms of MDA production. This indicates an increased oxidative stress, by increasing ROS production, related to the applied treatment. Increased MDA was also found as a result of Hyd + AA treatment after 9 d of storage. The increased AA content in processed carrots seems to be not enough to detoxify the tissue, as MDA remained at high levels. Therefore, regarding the examined non-enzymatic antioxidants (ascorbic acid, polyphenols), the protection against oxidative stress was mainly related to the increased polyphenols. In the presence of increased free radicals and in a way to fight against oxidative stress, plant tissues have developed a series of mechanisms to scavenge these reactive compounds, including phenols and antioxidant production, as well as the activation of antioxidant enzymes such as SOD, POD and CAT (Han et al., 2017; Chrysargyris et al., 2018). The application of AA increased SOD and at the same time decreased POD and CAT activity. Moreover, the application of EO decreased SOD and increased CAT activity, whereas POD activity was decreased with the application of EO + AA and Hyd + AA. Alegria et al. (2010) observed a correlation of POD activity and quality attributes of carrots (color, flavor, texture and nutritional value) when treated with hot water at 100 °C for 45 s. For example, the inhibition of POD activity was correlated with preservation of the orange color of carrots (Alegria et al., 2010). These findings are in accordance and support our findings.

The application of AA, marjoram EO + AA and marjoram Hyd + AA decreased TVC values of shredded carrot to similar levels of a commercial sanitizer (chlorinated tap water) as reported by Klaiber et al. (2005), being approximately 20% lower than the untreated sample on

the ninth day (6.31 log CFU $g^{-1}$). In another study the application of ozonated-water, hot water and ultrasonication resulted in a TVC load ranging between 0.4 and 1.7 log CFU $g^{-1}$ reduction depending on treatment (Alegria et al., 2009). Yeast and filamentous fungi have been decreased with the application of marjoram Hyd + AA and are in similar levels with a study in which their count was around 3.8 log CFU $g^{-1}$ after the application of ozonated-water, hot water and ultrasonication (Alegria et al., 2009). In both studies, yeast and filamentous fungi did not exceed 5 log CFU $g^{-1}$ that could be characterized as the acceptable maximum limit for these microorganisms (Jacxsens et al., 2002). However, Lavelli et al. (2006) reported that the maximum limitation of microorganism can be obtained in higher storage temperature, for example 10 °C when compared to 4 °C that was the temperature in the present study. The antimicrobial action of EOs targets the cell membrane of the microorganisms, where they cause a disruption, resulting to cell death. This action is ascribed to the EO bioactive aromatic components and their hydrophobicity, such as terpenes and phenolics (Patrignani et al., 2015). Ascorbic acid antimicrobial action is mainly related to the antioxidant capacity and the decrease of the pH which is unfavorable for the majority of microorganisms (Akbas and Ölmez, 2007).

Most naturally occurring substances and EOs are classified as generally recognized as safe (GRAS) and can be used for food preservation. However, they should be used with caution as high concentrations may be required in order to ensure food safety and quality and these high concentrations may cause non-acceptable changes in the organoleptic characteristics of each product (Tzortzakis, 2007; Gündüz et al., 2010). The findings of this study are promising and further studies are needed for the investigation of the conditions (time, concentrations) of the application of these and similar natural products on fresh produce. Additionally, deeper knowledge of the physicochemical properties of these natural substances should be examined. In terms of stability, single components or the synergistic effect of EOs and/or their components with the desirable antimicrobial/antioxidant properties needs to be investigated.

## 5. Conclusions

Shredded carrots are considered high perishable fresh produce while successful and eco-friendly preservation means are still under investigations. The results of the present study provide enquiring information for the use of EOs and AA in food sector. Marjoram EO and hydrosol alone were not as promising as AA application for the preservation and quality of processed carrots, while their combination with AA (Hyd + AA and EO + AA) provided increased quality attributes such as higher carotenoids content. Additionally, AA increased polyphenols and antioxidant status and decreased pH of the shredded produce, providing valuable role in quality maintenance of the produce. During screening test, AA-treated carrots were marked as having an acceptable carrot-like aroma, while EO-treated carrots were marked with an acceptable aroma but not carrot-like. Hyd treated carrots were marked as being between AA and EO, in terms of product acceptability. The antimicrobial and antioxidant role of natural products is continually studied, and the combined/optimized applications of different components and/or EOs are of great interest for postharvest preservation and quality maintenance of fresh and minimally processed products.

## Acknowledgements

The authors are grateful to the project AgroLabs that has been developed under the Programme Interreg V-B Balkan - Mediterranean 2014-2020, co-funded by the European Union and National Funds of the participating countries.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2019.05.015.

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