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Effect of Some Citrus Essential Oils on Post-Harvest Shelf Life and Physicochemical Quality of Strawberries during Cold Storage

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Abstract: Utilization of essential oils alone or incorporation with edible films is an appropriate technique to conserve the quality attributes and reduce post-harvest deterioration in fresh vegetables and fruits. Strawberries, being perishable fruits have a short shelf life, and using essential oils is considered one of the most suitable methods to prolong their shelf life during storage. The current study assessed the impact of different essential oils, including lemon oil (L), orange oil (O) and mandarin oil (M) on the physicochemical and microbial load of strawberries (*Fragaria* × *ananassa* cv. Festival) stored at 2 ± 1 °C and 95% relative humidity (RH) for 18 days. The differences in the physicochemical and microbial properties of strawberries were assessed by determining the following parameter changes: weight loss, decay percentage, firmness, soluble solids content, titratable acidity, color, anthocyanins, vitamin C, total phenol, total antioxidant, catalase activity, polyphenol oxidase activity, sensory evaluation, microbial content, total coliforms, molds, and yeasts. The results of this study indicated that the fruits treated with all essential oils treatments (L, O and M) had higher total antioxidant content and physicochemical properties than untreated fruits, due to protection against the microbial growth of molds, and yeasts. At the end of the storage period, the treated fruits showed a greater acceptance and sensory attributes than the untreated fruits. Furthermore, the correlation study showed a significant and negative relationship between the total antioxidant of treated fruits and following quality attributes including, weight loss, decay percentage, respiration rate soluble solids content, polyphenol oxidase activity, molds, and yeasts. It is noteworthy that all the essential oil treatments extended the shelf-life of strawberries and delayed their deterioration up to 18 days.

Keywords: essential oils; *Fragaria* × *ananassa*; antioxidant content; antimicrobial effect; shelf-life; lemon oil; mandarin oil; orange oil; fruit quality

1. Introduction

Strawberries (*Fragaria* × *ananassa*) are in high demand because of their richness in minerals, vitamins, anthocyanins, flavonoids, and phenolic compounds that play a vital role in the human diet and health [1]. Usually, the fruits of strawberries are consumed fresh or processed in juices. Strawberries are also considered a perishable fruit and extremely sensitive to microbial contamination that can affect their quality traits and diminish nutritional value. Furthermore, the possible existence of pathogens (such as *Escherichia coli*) and their toxins can even pose a threat to consumer safety and contribute to foodborne illness. Although synthetic fungicides application, mainly benzimidazole, dimethyl

inhibitors, and dicarboximide, were shown to be extremely effective against a wide range of pathogens in fruit and vegetables [2], their potential negative impact on the environment and human health has encouraged scientific researchers to find alternative natural agents to inhibit undesired microbial growth, prevent food spoilage and preserve fruit quality during storage. Likewise, the continued and repeated addition of fungicides has led to the development of resistant strains from fungi [2,3]. Therefore, the application of appropriate technologies is important to diminish losses and to extend the post-harvest shelf life during the storage of fruits [4]. Natural and organic compounds have gained significant importance for their applications in foods, as they are beneficial to health with little or no side effects, cost-effective, and environmentally friendly compared to nonorganic synthetic compounds. Therefore, plant-derived natural antimycotics have become ideal alternatives to commercial synthetic chemical preservatives for improving food quality and safety [5]. In this regard, among the plant essential oils, citrus essential oils (CEOs) have drawn more attention because of their broad-spectrum insecticidal, antibacterial, and antifungal properties along with their high yields, aromas, and flavors [6].

CEOs enhance the sensory attributes of fruits and prevent microbial growth at low concentration. Furthermore, CEOs are non-toxic, hypoallergenic, and safe for consumption [7]. Essential oils (EOs) are natural substances extracted from medicinal and aromatic plants, commonly by distillation processes. These compounds have an important role in food preservation contributing to safety and shelf-life extension of food products. The improvement in food safety is due to the inhibition of pathogenic microbial growth. The extension of food products' shelf-life results from enzymatic reduction, mainly due to their antioxidant activity. The EOs effectiveness is attributed to the presence of phenolic natural compounds and they are an important and healthy alternative to synthetic preservatives and chemical additives [8]. The waste of citrus processing, which is the peel, can be extracted as an essential oil which has been listed in the Code of Federal Regulations as Generally Recognized as Safe (GRAS) for food consumption [9]. CEOs consist of some major biologically active compounds like α-/β-pinene, sabinene, β-myrcene, d-limonene, linalool, α-humulene, and α-terpineol belonging to the monoterpenes, monoterpene aldehyde/alcohol, and sesquiterpenes group, respectively. These compounds possess several health beneficial properties like antioxidant, anti-inflammatory, anticancer properties, etc., in addition to antimicrobial properties that can inhibit or reduce the growth of many pathogens like *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *P. aeruginosa*, *B. licheniformis*, *B. altitudinis*, *Candida albicans* and *Aspergillus flavus*, which have immense potential for food applications [9].

In fact, due to their importance as selective barriers to moisture and gas, edible films can effectively decrease the growth of undesirable microorganisms in fruits by reducing the diffusion rate of antimicrobial compounds from film materials into the food, consequently sustaining a high concentration of antimicrobial compounds on the surface of the fruit for a long period [4,5,10].

A variety of natural products, including essential oils (EOs) have been examined for their antimicrobial potential against pathogens and spoilage fungi in various fruits and vegetables products, especially strawberries. Essential oils (EOs) have recently been used in edible films to improve physicochemical, antioxidant, and antimicrobial properties [2,11]. Some studies reported varying antimicrobial activity at different concentrations and an overall effect of improving the shelf life and maintaining the quality of strawberry fruits of citrus essential oils (lemon, sweet orange, lime peel essential) [12]. The performance of edible films was enhanced by the combination of different bioactive compounds, mainly essential oils or extracts of aromatic plants that can not only improve antimicrobial properties but also decrease biochemical deteriorations produced by processing, such as texture collapse, enzymatic browning, and off-flavors development [13].

The antimicrobial activity of essential oils was correlated to their chemical compounds, mainly phenolic compounds, terpenes, ketones, aliphatic alcohols, acids, and aldehydes, and these compounds are capable of inhibiting the development of undesirable microorganisms and preventing fruit spoilage. Generally, the EOs possessing the strongest antimicrobial properties against foodborne pathogens contain a high percentage of phenolic compounds such as carvacrol, eugenol and thymol. Phenolic

compounds possesses great structural variations and are one of the most diverse group of secondary metabolites. The hydroxyl (-OH) groups in phenolic compounds are thought to cause inhibitory action as these groups can interact with the cell membrane of bacteria to disrupt membrane structures and cause leakage of cellular components [14]. In fact, the use of EOs for reducing the development of plant and human pathogenic microorganisms in food, particular fruits and vegetables, requires exhaustive knowledge of their antimicrobial activity and their interactions with product components and with other antimicrobial compounds [14]. Therefore, this research work aimed to study the effectiveness of three different citrus essential oils as emulsions on the post-harvest shelf life, and nutritional quality of strawberries that could be greatly promising. This study could introduce a more effective treatment to prolong the post-harvest shelf-life of strawberries, which would benefit consumers, sectors of the agri-food industry, and the wider producers.

2. Materials and Methods

2.1. Fruit Samples

Fresh strawberry fruits (*Fragaria* × *ananassa*) of the variety Festival were harvested at commercial stage (75% of fruits surface with red color) from a private farm located in Cairo, Egypt (latitude 30.0131° N, longitude 31.2089° E, 694 m). Fruits were investigated visually for fungi contamination and physical damages. Uniform berries in appearance (color and shape) were selected for the experiment.

2.2. Essential Oils

Essential oils of the lemon, orange and mandarin were acquired from El-Marwa food industries (6th of October City, Egypt) (Juhayna group). The cultivars of citrus used to extract the essential oils were Balady for orange, Adalia for lemon and Description for mandarin. Chemical composition of the essential oils were assessed by gas chromatography-mass spectrometry (GC-MS) at National Research Center, Egypt.

2.3. Estimated Antimicrobial Activity of Lemon, Orange and Mandarin Oils

The antimicrobial activity of essential oils was determined using the disk diffusion technique against different types of microorganism such as (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 33221, *Salmonella typhimurium* ATCC 20231, *Aspergillus niger* NRRL 2035, *Aspergillus flavus* NRRL 3357, *Saccharomyces cerevisiae* NRRL 2034 and *Candida lypolitica* NRRL 1095).They were obtained from Northern Regional Laboratories, Peoria, IL, USA (NRRL strains), American Type Culture Collection, Manassa, VA, USA (ATCC strains). The strains were activated and obtained the growth in 2.5×10^5 cfu (colony-forming units) mL^{-1} number of cells. The disks of 5 mm in diameter were placed on the nutrient agar surface (Sigma chemical company, St. Louis, MO, USA) and separately fecundated with 5 µL of each essential oil (El-Marwa food industries, Egypt) using four concentrations (50, 100, 150 and 250 $\mu L \cdot m^{-1}$). The plates of mold and yeast were incubated at 28 °C for 48 h, while, the plates of bacteria were incubated for 35 °C for 48 h. Antimicrobial activity was defined as absenteeism of microbial growth in the area around the disk. The diameter of the inhibition zones was determined with a ruler [15].

2.4. Antiradical Scavenging Activity of Essential Oils

Radical scavenging procedure DPPH (radical 2,2′-diphenyl-1-picrylhydrazyl) (Sigma chemical company, St. Louis, MO, USA) was used to test the antioxidant capacity and determined the IC50 of essential oils extracted from differ citrus fruit peels (orange, mandarin and lemon) Different concentrations of essential oils were used to assess the antioxidant activity compared with ascorbic acid (IC50 (30.32 ± 0.16) $\mu g \cdot ml^{-1}$) according to Kizil et al. [16]. Different concentrations were prepared of each essential oils (50, 100, 150, 200 and 250 $\mu L \cdot ml^{-1}$ methanol), then 50 µL of each concentration was diluted with 1 mL of methanol (Qualikems, Vadodara, India) and added to 5 mL of a DPPH

methanol solution (0.004 percent) the recent mixtures were incubated for 30 min at room temperature in the dark. The absorbance of sample and control was measured at 517 nm using spectrophotometer (model UV-2401 PC, Shimadzu, Milano, Italy). Inhibition free antioxidant (%) was calculated as follows:

$$I\% = (A \text{ control} - A \text{ sample}/A \text{ control}) \times 100 \quad (1)$$

where, *A* control is the absorbance of the DPPH radical solution (0.004%) + methanol, and *A* sample is the absorbance of the test oils antioxidant reaction.

Inhibition % against concentration was plotted after 30 min, and compared to standard/commercial antioxidants (vitamin C), the inhibitory concentration (IC50) of the essential oil required to inhibit 50% of the DPPH radicals obtained from the standard curve. A lower value of IC50 indicates greater antioxidant activity. Tests were performed in triplicate.

2.5. Preparation of Emulsions for Coating

The coating forming solutions were prepared according to the method of Rojas-Graü et al. [17]. The 2% (*v*/*v*) of lemon, orange, or mandarin essential oils and Tween 80 as an emulsifier (10% of the volume of the essential oil) were dissolved into distilled water. Emulsion was stirred at 13,000 rpm for 2 min using High-performance disperser (IKA T25-Digital Ultra Turrax, Staufen, Germany) at room temperature.

2.6. Application of Emulsions to Strawberry

Sorted strawberries were submerged in the respective solutions of treatments for 4 min, corresponding to L = lemon essential oil emulsion (2%), O = orange essential oil emulsion (2%), M = mandarin essential oil emulsion (2%) and C = control (Untreated fruits) then air-dried for two hours in a sterile room. After the coating process was performed, the coated fruits of each treatment were equally packed into polyethylene terephthalate packages (dimensions of 178 × 127 × 89 mm) and stored at 2 ± 1 °C for 18 days. A total of 144 boxes were used to perform all the experiments (36 boxes/treatment). For determination of the changes in microbial communities and physiochemical properties of treated fruits, six boxes from each treatment were randomly taken after 0, 4, 7, 10, 14, and 18 days of storage and never returned back. Zero day is after 12 h after application of treatments. The whole set of experiment was carried out two times. The experimental design was completely randomized design with six replications.

2.7. Physical Parameters of Strawberry

2.7.1. Weight Loss

At the beginning of the experiment, the strawberry fruits of each treatment and replicates were separately labeled and weighed prior to storage and also every sampling date during the storage period (4, 7, 10, 14, and 18 days) for determining the weight loss. The weight loss percentage was calculated using the following Equation (2).

$$\text{Weight loss percentage (WL\%)} = \frac{FW - IW}{IW} \times 100 \quad (2)$$

where, *FW* is the final weight and *IW* is the initial weight.

2.7.2. Decay Percentage

The methodology used to determine the decay was a modification as described by [18]. Strawberries were visually evaluated on days 4, 7, 10, 14, and 18 days, and then the weight of

decayed strawberries was recorded. Mycelia growth, visible lesion, brown spots and softening on fruits were considered as decayed. The decay percentage was calculated using the following Equation (3).

$$D\% = \frac{WIF}{WAF} \times 100 \quad (3)$$

In which, D% is the decay percentage and *WIF* and *WAF* are the weight of decayed fruits and total weight of strawberry fruits at sampling date, respectively.

2.7.3. Surface Color

The surface color of the strawberries was determined using a hand-held tristimulus reflectance colorimeter (model CR-400, Minolta Corp., Newburgh, NY, USA). Calibration was performed using a white plate. The measurements were performed on fruit surfaces before storage (after coating process) and also every sampling date during the storage period (0, 4, 7, 10, 14, and 18 days). To avoid the effects of heterogeneity on the fruit surface, measurements were taken in the same previously labeled most expanded shoulder of each fruit. CIE-*L** (Lightness), *a** (-greenness to + redness), and coordinates were recorded [19]. *a** value is the main index for fruit senescence.

2.7.4. Fruit Firmness

The firmness of the strawberries of each treatment during the storage periods was measured using a digital force gauge (model M4-200, MARK, Copiague, NY, USA) equipped with 8 mm diameter cylindrical probe [20]. Three different locations were used to measure firmness on the most expanded region of the fruit, especially around the equatorial area. The firmness was expressed as gram per centimeter square of force (g cm^{-2}). The firmness was determined after 0, 4, 7, 10, 14, and 18 days.

2.7.5. Soluble Solid Content (SSC)

The sample was crushed in a mortar and squeezed by hand to acquire juice. A digital refractometer (model PR101, Co. Ltd., Atago, Tokyo, Japan) was used to measure total soluble content (SSC) in fruit juices of different treatments and the SSC value was expressed as a percentage on the Brix scale. Soluble solid content was determined after 0, 4, 7, 10, 14, and 18 days.

2.7.6. Sensory Assessment

Sensory evaluation and general appearance was obtained by submitting samples to a trained panelist from the vegetables department, Faculty of Agriculture, Cairo University, Giza, Egypt to make evaluations. Samples were identified in descending trend with 5 being the best and 1 being the worst. Each sample was rated using score system as follows: 5 = excellent, 4 = very good, 3 = good, 2 = fair and 1 = very poor). This scale describes fresh appearance, fresh calyx, change of color and decay. Data was collected on color, texture, flavor, appearance and overall acceptability on all the sampling days. A general score of 3 was considered as the limit for consumption ability. Sensory evaluation was determined according to [21].

2.7.7. Respiration Rate

Respiration rate was expressed as a concentration of carbon dioxide and oxygen inside the gas jar containing the fruits. Oxygen and carbon dioxide analyzer (Model 902P, Quantek Instruments, Grafton, MA, USA) equipped with oxygen/carbon dioxide sensor PS-2110 was used to measure the respiration rate. This was done in a 175 mL hermetically sealed glass jar using two fruits. After two hours, the respiratory rate of the fruits was recorded on days 0, 4, 7, 10, 14, and 18 for each treatment, using six replications per treatment. To calculate the rate of respiration in a static system, we need to know the volume of container, weight of tissue, initial CO_2 concentration, Length of time and final CO_2 concentration.

2.8. Chemical Parameters of Strawberry

2.8.1. Titratable Acidity and Ascorbic Acid

Titratable acidity (TA) was determined by using 10 g aliquots of strawberry fruits mixed in 90 mL of distilled water, then homogenized, and filtered; 10 mL of the filtrate was titrated with 0.1N NaOH (El-Nasr Company, Giza Governorate, Egypt) to an end-point (pH 8.0–8.4). TA was expressed as a percentage of citric acid and was calculated using the method reported by Association of Official Analytical Chemists (A.O.A.C.) [22]. The ascorbic acid content was assessed using a 2, 6-dichlorophenol indophenol titration method as described in A.O.A.C. [21]. 10 g of the fruit tissue was homogenized with 90 mls of oxalic acid (3%) (Nice chemical LTD, Kerala, India), the sample was then filtered using a filter paper (Whatman paper); 25 mls of the filtrate was titrated by 2, 6-dichlorophenol indophenol (Qualikems, Gujarat, India).

2.8.2. Total Phenolic Compounds (TPC)

The Folin–Ciocalteu method, with minor modification, was used to measure total phenolic compounds (TPC) [23], which based on the colorimetric reaction of phenols. Briefly, 100 g of strawberry fruit were homogenized and 5 g was mixed with 50 mL methanol (50%). The mixture was shaken (for 30 min at 200 ppm) and filtered through filter paper (Whatman paper). The filtrate was collected, then 0.2 mL was taken and mixed with 1 mL Folin reagent (1:10 Folin: water)(Loba Cheme, India), 0.8 mL of sodium carbonate (7.5%) (El-Nasr Company, Egypt) was added then kept in the dark for 1 h at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Model UV-2401 PC, Shimadzu, Milano, Italy). TPC was calculated as gallic acid equivalents (GAE) in mg· 100 g^{-1} fresh fruit.

2.8.3. Total Anthocyanin

For this assay, 100 g of strawberry fruit were homogenized, 4 g of the strawberry puree was dissolved in 40 mL of extraction solvent (ethanol:0.1 M HCl—85:15%, *v*/*v*) (Merk, Darmstadt, Germany) and sonicated for 10 min. After filtration the supernatant was collected and used for total anthocyanin determination by the pH differential spectroscopic method as described by [24]. Extractions were done in triplicate and the results were calculated according to following Equations (4) and (5).

$$Asp = (A510 - A700)\ pH1.0 - (A510 - A700)\ pH\ 4.5 \quad (4)$$

$$TA = (Asp \times M \times DF \times 1000)/(\xi \times \lambda \times m) \quad (5)$$

where, TA: total anthocyanin mg·kg^{-1} fresh weight fruit; M: molecular weight value was 445; DF: the dilution factor; ε: the molar absorptivity coefficient values 26,900 M^{-1} cm^{-1}; λ: the cuvette optical path length (1 cm) and m: the weight of the sample (g).

2.8.4. Antioxidant Activity

Antioxidant activity was measured by using of the free-radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH, from Sigma Company, St. Louis, MO, USA) test, with minor modification, as described by [25]. For this assay, 0.2 mL of previous extract aliquot were dissolved in 3.8 mL of a methanol DPPH solution (0.0024 g·100 mL^{-1}). The mixture was shaken and kept in the dark at room temperature for 30 min. The absorbance was measured at 510 nm. The antioxidant activity was calculated as % of inhibition according to Equation (6):

$$\text{Inhibition } (\%) = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100 \quad (6)$$

In which, *A* control and *A* sample are the absorbance of the control and sample, respectively.

2.8.5. Enzymes Activity

Aliquots (10 g) of the homogenized strawberry samples were dissolved in100 mL of phosphate buffer (pH 7.0, 0.1 M) and 0.5% (*w*/*v*) of polyethylene glycol (Qualikems, Gujarat 391340, India). After that, the homogenate was filtered and was centrifuged (Centurion Scientific Model C2041, West Sussex, UK) at 10,000 rpm for 10 min under cooling to obtain crude enzyme extracts.

Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) was determined by spectrophotometer (Unico, UV2000, Rochester, NY, USA) using Catechol (0.2 mm L^{-1}) (Qualikems, Gujarat 391340, India) as a substrate and the activity was defined as unit $mg^{-1}{\cdot}min^{-1}$. One unit equal the change in absorbance at 420 nm of 0.001 per minute at pH 7 and the reaction mixture at 25 °C [26].

Catalase

Catalase (CAT) was determined according to the method by Aebi [27] by recording the quinoneimine dye absorbance at 510 nm of a reaction mixture containing 0.50 mL sodium phosphate buffer (pH 7.0, 0.1 M), 0.10 mL diluted H_2O_2 (10 µL H_2O_2:10 mL distilled water) and 0.05 mL of sample (enzyme extract) after one min at 25 °C add 0.20 mL chromogen- inhibitor and 0.50 mL peroxidase then incubated 10 min. at 37 °C. The blank was determined using the same above steps without sample. The catalase kits were purchased from Biodiagnostic Company, Egypt. Enzyme activity was expressed as U g^{-1}. Unit of CAT activity is calculated using the following equation:

$$\text{U g}^{-1} = A \text{ standard} - A \text{ sample}/A \text{ standard} \times 1/\text{weight of sample (g)} \quad (7)$$

2.8.6. Microbiological Analysis

These analyzes were performed in duplicate, for the count of coliform group bacteria, bacteria, molds and yeast on days 0, 4, 7, 10, 14 and 18 on the treatments. Briefly, 50 g of strawberries from each treatment were mixed with sterilized saline solution (450 mL, 0.85% NaCl), and then homogenized using an electric blender in aseptic conditions. Serial 10-fold dilutions were prepared in each treatment. Then 1 mL of each sample was transferred to dishes containing nutrient agar for total count bacteria, potato dextrose agar (PDA) for mold and yeast and eosine methylene blue agar (EMBA) for total coliform group determination. Thereafter, plates for total count bacteria and mold and yeast were incubated at 28 °C for 48 h, while plates for coliform bacteria (coliform group) were incubated at 35 °C for 48 h. The experiment was performed using two replicate counts, and results were presented in Log cfu g^{-1} [28]. (All the medium of agar were purchased from the Sigma chemical company, St. Louis, MO, USA)

2.9. Statistical Analysis

One way analysis of variance (ANOVA) and the least significant difference method (LSD) for mean separation, with a confidence level of 95%, were used in this study to assess the impact of the different treatments at each storage period (0, 4, 7, 10, 14 and 18) on the studied variables of strawberries. The results were represented as means ± standard deviations and ANOVA was performed by using the M.Stat program (Version 2.1, Michigan state university, East Lansing, MI, USA). Pearson correlation was performed by using SPSS program (Inc., Chicago, IL, USA, version, 14) to understand the relationships between the total antioxidant content of tread fruit with essential oils and different physicochemical additionally microbiological properties of fruits.

3. Results and Discussion

3.1. Chemical and Antimicrobial Characterization of Essential Oils

3.1.1. Antiradical Scavenging Activity of Essential Oils and their Components

Figure 1 demonstrates the radical scavenging activity of methanol extracts of different citrus fruit peels as the percent inhibition of DPPH. Methanolic extracts were shown to have antioxidant activity, and essential oils had radical scavenging effects at all concentrations. However this effect was not as prominent as the standard ascorbic acid's radical scavenging effect. The IC50 values for the different extracts were 229.2, 208.9 and 200 $\mu L \cdot ml^{-1}$, compared to ascorbic acid IC50 (30.61 $\mu L \cdot ml^{-1}$) for the orange, lemon and mandarin oils, respectively. IC50, the median inhibitory concentration, was determined from the graph, which was calculated by plotting inhibition percentages (50%) against concentrations (200 $\mu L \cdot ml^{-1}$) of mandarin oil as an example. The essential oils studied included orange oil which possessed the weakest radical scavenging activity. Meanwhile, at all concentrations the mandarin essential oil was assayed with the strongest radical-scavenging effect. Citrus essential oils in terms of antioxidant may cause free radical scavenger limonene activity [29]. The major constituents of the essential oils were as follows: mandarin: D-limonene (93–96%); lemon: limonene (59–80%) and orange: limonene (85–96%) were presented in Table 1. Our findings were in line with [30–32]. Nonetheless, essential oils' antioxidant activities can differ depending on the variations in chemical composition. An essential oil's antioxidant activity is mainly attributed to its major components, although consideration must be given to the synergistic or antagonistic effect of one compound in a minor percentage of the mixture [33].

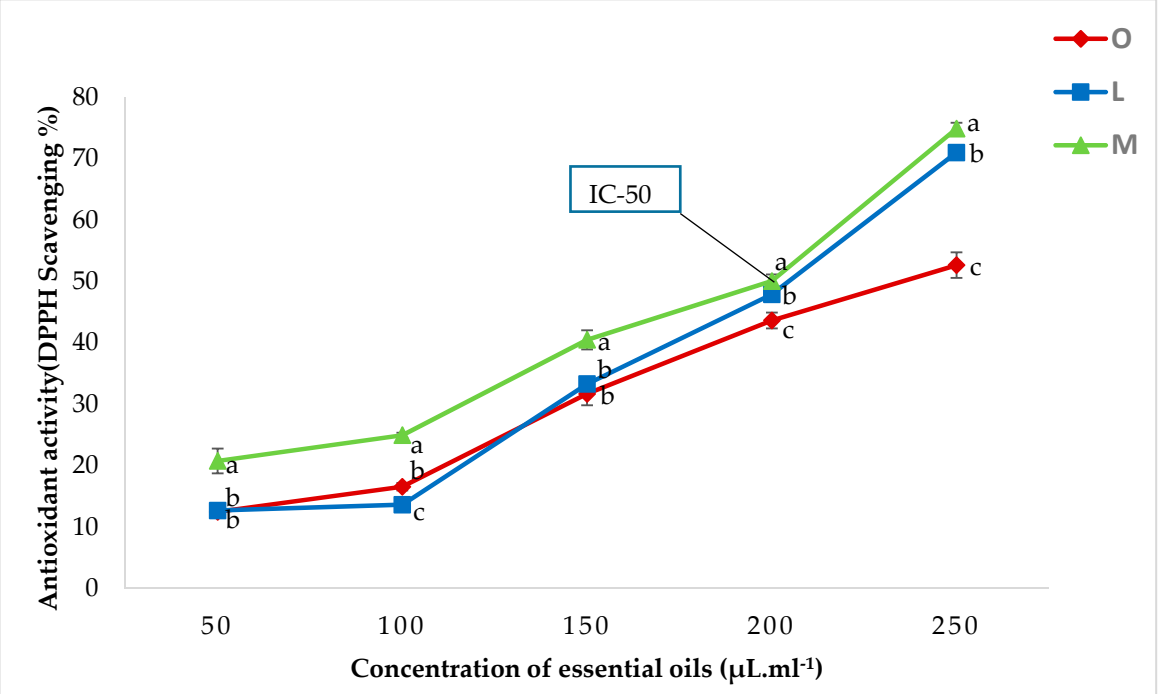

Figure 1. Percentage free radical scavenging activity and IC50 of citrus peel essential oils (L = lemon, O = orange, and M = mandarin essential oil). Vertical bars indicate standard deviation (n = 3). Means and different letters indicate significant difference between essential oils at each concentration (Duncan's of 95%).

Table 1. Chemical composition of citrus essential oils.

Items	Mandarin	Lemon	Orange
Refractive index at 20 °C	1.470–1.476	1.473–1.479	1.474–1.478
Specific gravity at 20 °C	0.842–0.850	0.845–0.858	0.840–0.855
D-limonene	93–96%	59–80%	85–96%
Total aldehyde	1.1–3.0% (Decanal)	1.99–3.66% (Citral)	0.66%

3.1.2. Antimicrobial Activity of Essential Oils

The results of estimating the antimicrobial activity of the essential oils of lemon, orange and mandarin were studied on the growth of different microbial groups (bacteria-fungi and yeast) using different concentrations (50-100-150 and 200 µL) of the aforementioned essential oils (Table 2). The higher antimicrobial activity observed for lemon with the different concentrations was used, where it showed a positive effect on all tested strains, except for the *Bacillus subtilis* ATCC 33221 strain, followed by lower antimicrobial activity for orange and mandarin EOs. Orange oil was showed an effect on all strains, including *Bacillus subtilis* ATCC 33221, but the inhibition zone area was less than the lemon oil. Finally, the mandarin oil showed the least effect on the tested strains.

Table 2. The antimicrobial capacity of the essential oils on the different microbial strains.

		Inhibition Zone (mm) ±SD in Different Microorganism Strains							
Eos Type	**Eos ppm**	**S.A.**	**P.A.**	**B.S.**	**S.T.**	**A.N.**	**A.F.**	**L.**	**K.**
Lemon	50	15 ± 0.000	0	0	9 ± 0.031	0	5 ± 0.014	5 ± 0.004	6 ± 0.002
	100	16 ± 0.010	10 ± 0.023	0	9 ± 0.000	0	8 ± 0.020	7 ± 0.002	7 ± 0.001
	150	17 ± 0.000	12 ± 0.001	0	10 ± 0.002	5 ± 0.000	8 ± 0.016	7 ± 0.000	9 ± 0.005
	200	17 ± 0.002	14 ± 0.000	0	15 ± 0.005	7 ± 0.002	9 ± 0.001	10 ± 0.005	10 ± 0.001
Orange	50	10 ± 0.030	5 ± 0.000	5 ± 0.004	0	0	0	0	7 ± 0.001
	100	15 ± 0.050	5 ± 0.021	7 ± 0.025	0	0	0	0	7 ± 0.042
	150	15 ± 0.001	6 ± 0.004	9 ± 0.001	0	0	0	6 ± 0.000	9 ± 0.002
	200	16 ± 0.003	7 ± 0.001	9 ± 0.007	0	0	0	7 ± 0.003	9 ± 0.010
Mandarin	50	5 ± 0.005	0	0	0	0	0	5 ± 0.000	5 ± 0.005
	100	9 ± 0.001	0	0	0	0	0	5 ± 0.010	5 ± 0.021
	150	9 ± 0.002	5 ± 0.015	0	0	0	0	5 ± 0.041	5 ± 0.033
	200	14 ± 0.000	5 ± 0.024	5 ± 0.001	0	0	0	5 ± 0.003	5 ± 0.002

Essential Oils (Eos), S.A.—*Staphylococcus aureus* ATCC 25923, P.A.—*Pseudomonas aeruginosa* ATCC 9027, B.S.—*Bacillus subtilis* ATCC 33221, S.T.—*Salmonella typhiomrium* ATCC 20231, A.N.—*Aspergillus niger*, A.F.—*Aspergillus flavus*, L.—*Saccharomyces cerevisiae* NRRLY2034, K.—*Candida lypolitica* NRRLY 1095.

3.2. Physicochemical and Antimicrobial Properties of Strawberry Fruits

3.2.1. Weight Loss

The weight loss of strawberries is mainly correlated to the respiration rate and evaporation of moisture through the fruit skins. Furthermore, the rapid loss of water from the fruit skin is considered one of the important factors that participate in the perishability of strawberries [34]. This drives the dryness of fruits and eventually shrinkage and deterioration. As shown in Figure 2, insignificant differences were observed among fruit samples covered with essential oil coatings (lemon, orange, and mandarin). However, a highly significant difference was found between treated and untreated strawberry samples at the end of storage periods ($p = 0.008$ ***). This reduction in weight loss rate in coated fruits, during storages, was due to barrier property and antioxidant activity of essential oils coatings [2,4,34]. This type of coatings might be able to decrease the gas and water exchange between fruit surfaces and the surrounding environment resulting in delaying the respiration rate, water loss and oxidation of metabolic compounds [34–36]. Similar findings were reported by Sánchez-González et al. [37] who found that a significant reduction of weight loss was achieved when essential oils were added into edible coatings than using the edible coatings alone.

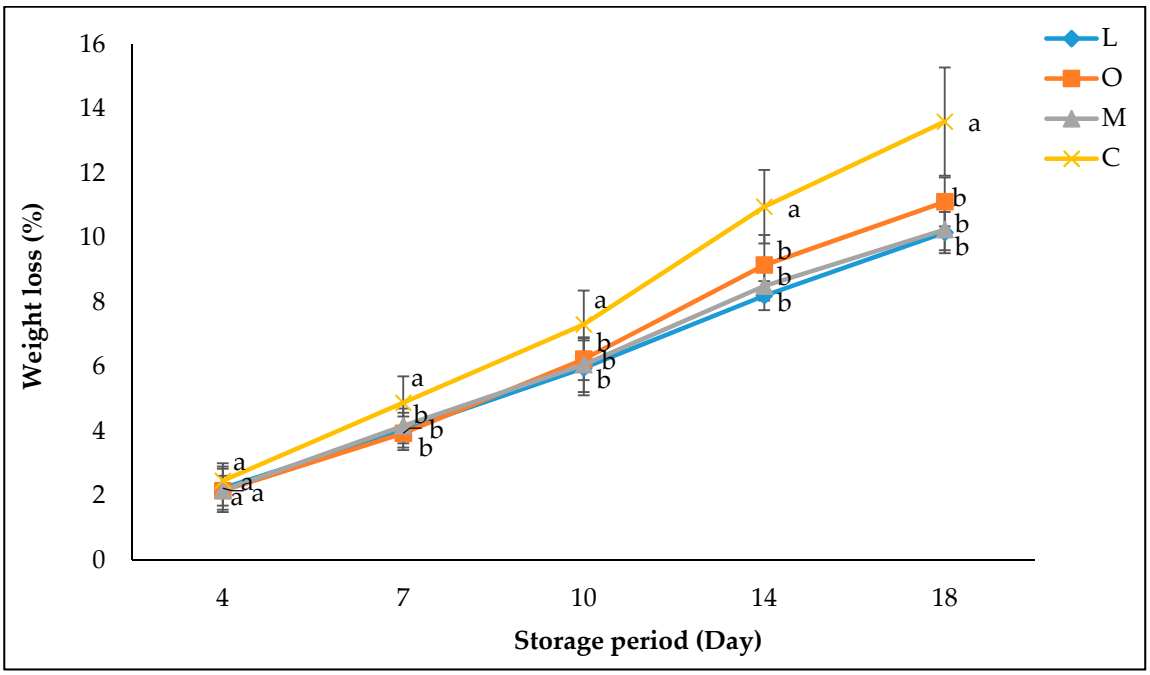

Figure 2. Effect of edible emulsion incorporated with essential oils (L = lemon, O = orange and M = mandarin essential oil) on weight loss percentage of strawberry fruits at 2 °C. Vertical bars indicate standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.2. Decay Percentage

The decay percentage of strawberry samples during the storage period is presented in Figure 3. The signs of fungal decay were observed in untreated fruits at day 7 of the cold storage and increased significantly with increasing the period of storage and recorded the highest value at the end of the storage period (at day18). Signs of fungal decay were observed in treated fruits with orange and lemon oils at day 10 of cold storage and in treated fruit with mandarin oil at day 14 of cold storage. All treated fruits with essential oils, at the end of storage periods showed lower decay percentage compared with untreated strawberry (p = 0.034 **). There was not a significant difference in decay percentage among treated fruits with different essential oils. This finding indicated that the treatment with essential oils was effective to reduce fruit spoilage by inhibition of the fungal growth on the fruit surfaces. This might be attributed to antimicrobial compounds found in applied essential oils and caused damage for cell membranes of fungi [4,15,16]. In addition to their effectiveness in reducing the respiration rate and water loss, which kept the strawberries under high carbon dioxide concentration (CO_2) and low-moisture content, thus not supporting the growth of fungi. This result was in accordance with several scientists who reported that the treated fruit samples with limonene, cinnamon, oregano, bitter orange, and mandarin essential oils were lower prone to spoil and deteriorate than untreated fruits [34,38–40].

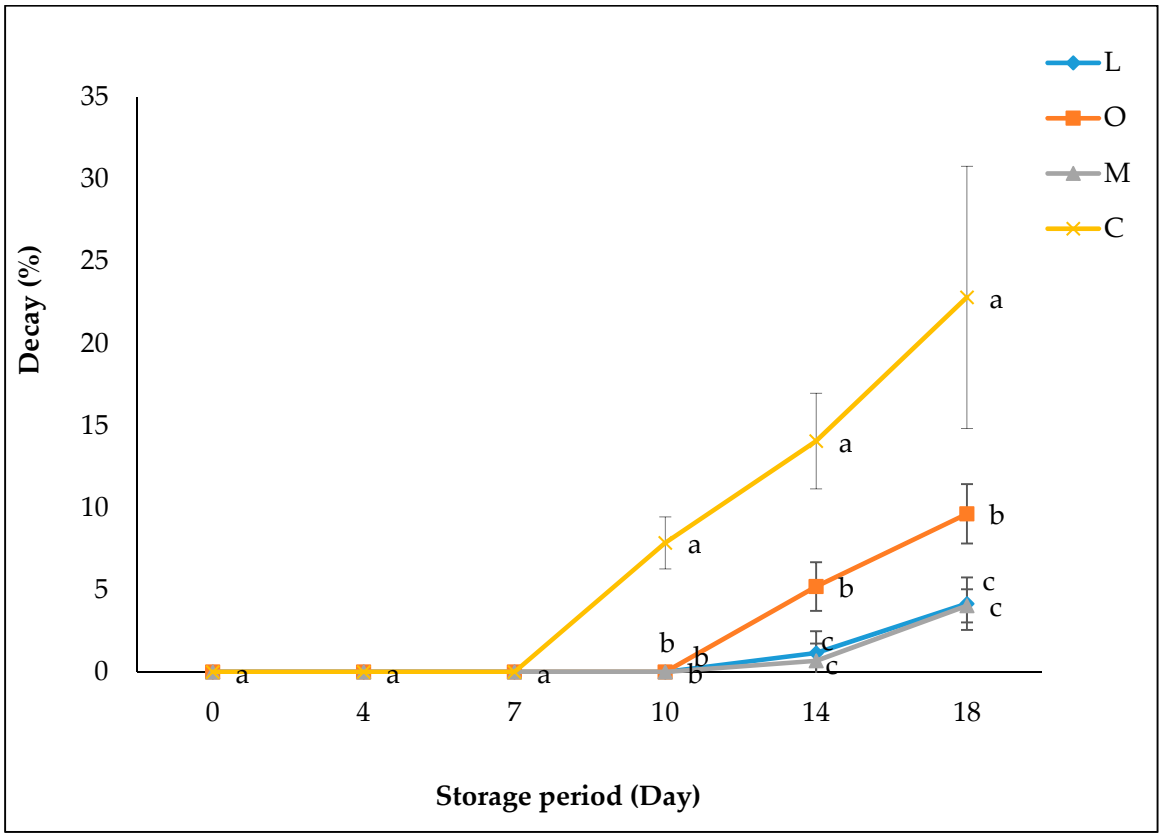

Figure 3. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on decay of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.3. Firmness

Fruit firmness is one of the most important quality attributes for strawberries. The change in the firmness of strawberry fruits treated with essential oils is shown in Figure 4. During the storage period, the firmness values of all stored samples decreased with increasing storage periods. At the end of storage periods, after 18 days, the lowest value of firmness was recorded in the untreated strawberry fruits than treated fruits with mandarin essential oil ($p = 0.192$). Several authors reported that the tissue of untreated strawberry fruits becomes soft due to loss of cell wall structure through increasing the enzyme activity, which results in diminishing the hardiness of fruits during the preservation period [4]. On the other hand, the maximum values of firmness were recorded in treated fruits with mandarin oil followed by lemon and orange oils compared to untreated fruits. Similar results were observed by Severino et al. [41] who reported that the firmness and the other quality attributes of treated fruits with mandarin and lemongrass oils were maintained for 15 days of cold storage. This probably was due to selective permeability of coatings material to gas and water transmission, thus reducing respiration rates, enzyme activities, and most of the metabolic changes, and thereby delaying ripening and over softening of strawberry fruits [42].

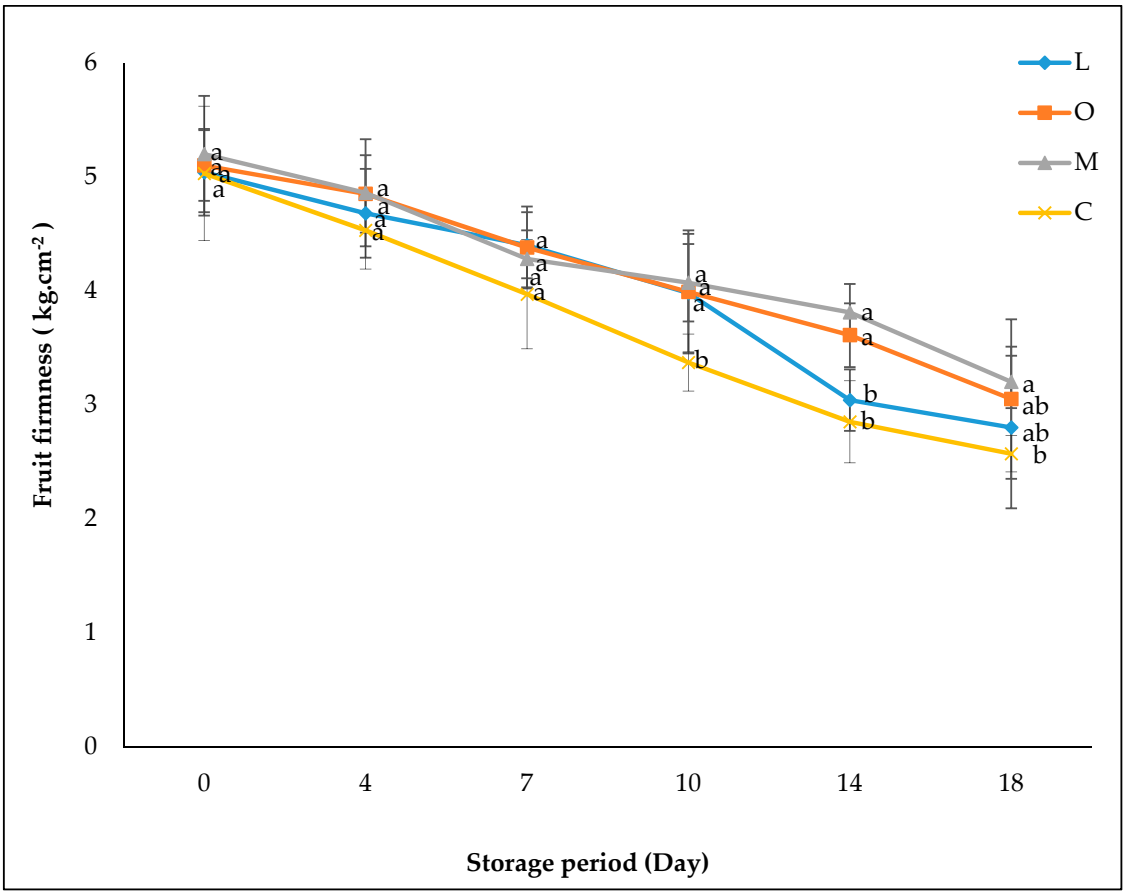

Figure 4. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on firmness of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.4. Soluble Solid Content (SSC)

The change in soluble solids content (SSC) of strawberry during the storage period is shown in Figure 5. Generally, the SSC values of all strawberry samples gradually decreased with increasing storage period. No significant changes were observed in SSC content among all coating treatments. After 18 days of storage, the maximum values of SSC were observed in uncoated fruits while the lowest values were recorded in treated fruits (p = 0.0054 **). The increase in the SSC value of untreated fruits stored at 2 °C might be due to rapid water loss from the fruit surface [26]. Conversely, the reduction in SCC values of coated fruits was probably associated with slow water loss from fruit surface, this was due to the ability of coating material to reduce the migration of water from the fruit surface to the surrounding environment [4].

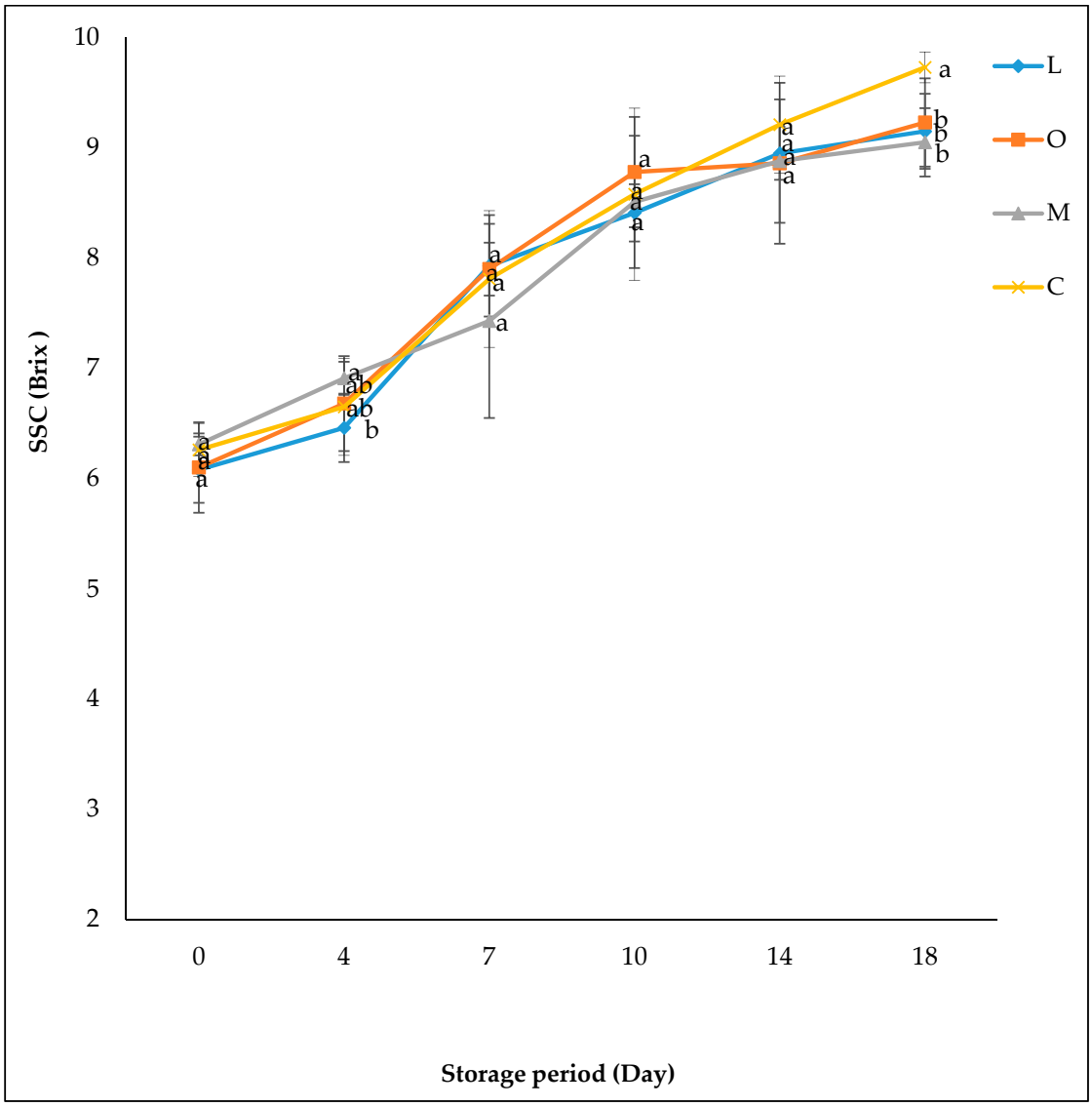

Figure 5. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on soluble solid content (SSC) of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.5. Surface Color

Color is considered one of the most important quality parameters for strawberry fruits. The red color in strawberry fruits is more associated with the accumulation of anthocyanin in the ripe fruits [34]. This pigment also is largely affected by preservation conditions, in particular, temperature and the respiration rate (the CO_2 concentration), as reported by Jin et al. [43]. The surface color of strawberries was determined by recording the lightness value (L^*) and redness value (a^*), as shown in Figure 6. The value of lightness (L^*) and redness (a^*) of all strawberry fruits was affected by increasing the storage period. During the storage periods, the strawberry fruit treated with essential oils resulted in a lighter color (high L^* values) while untreated fruit had dark color (lower L^* values), as shown in Figure 6A. By contrast, the redness values (a^*) of all fruits were increased with increasing storage periods for all treatments, but the treated fruits had less redness (Low a^* value) than untreated fruits (high a^* value) without any significant treatment between then until 14 days, as illustrated in Figure 6B. In general, at the end of the storage (18 days), the colors of the treated fruits were slightly light red and

of untreated fruits dark red, thus indicating the effectiveness of essential oil coatings in decreasing the anthocyanin accumulation and delaying the senescence of fruits (p = 0.046 * for L^* value and 0.0919 for a^* value). This could be related to the capability of coatings formulated from essential oils to increase the internal CO_2 concentration surrounding fruits which able to reduce the respiration rate (Figure 7) and delay the accumulation of the anthocyanin pigments in treated strawberries (Figure 8). These findings were in agreement with those reported by Jin et al. [43], who found that treatment with CO_2 significantly reduced the accumulation of anthocyanin and changed significantly the color of stored fruit.

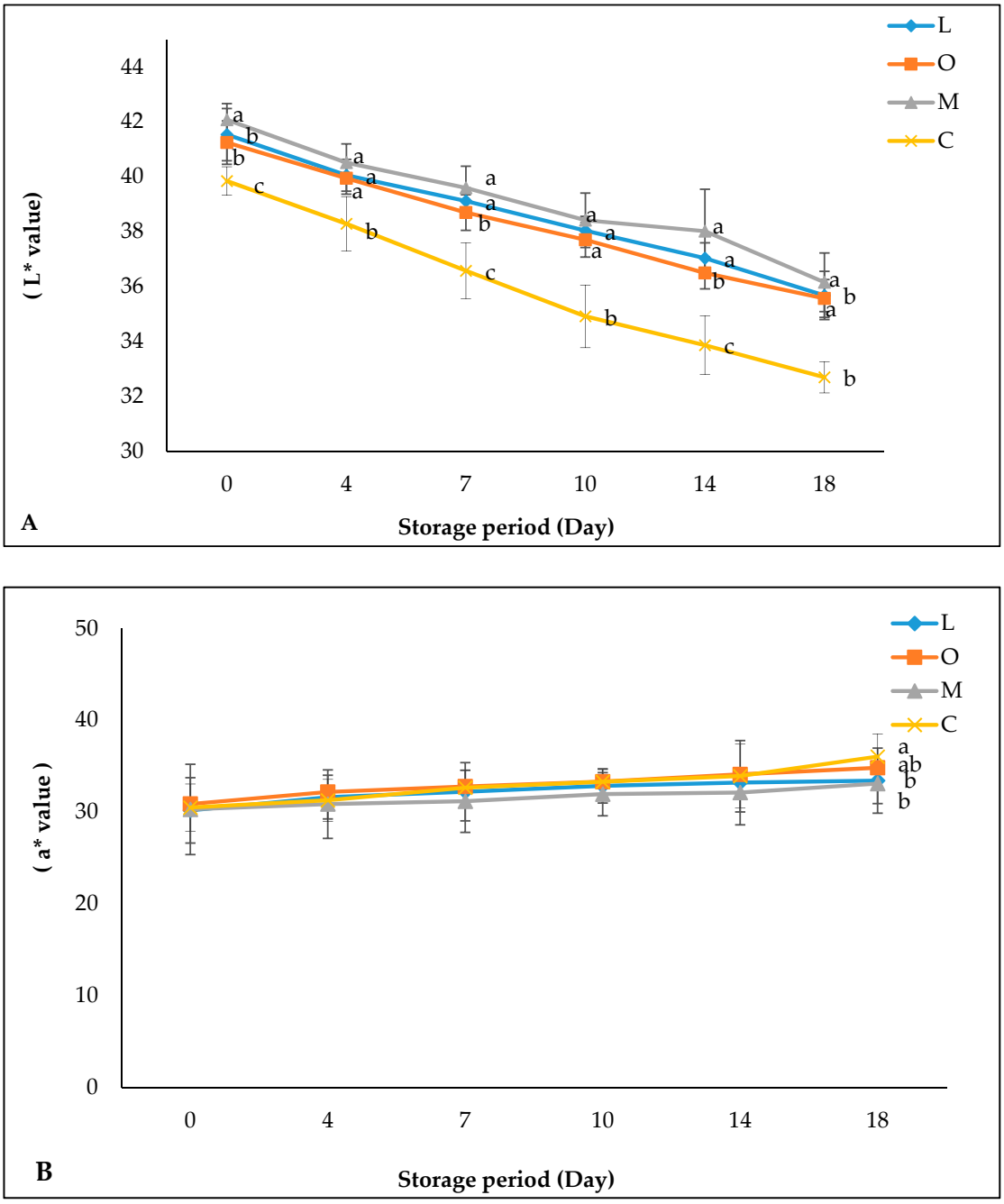

Figure 6. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on color surface of strawberry fruits stored at 2 °C for 18 days. (**A**) = lightness (L^* value) and (**B**) = redness (a^* value). Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

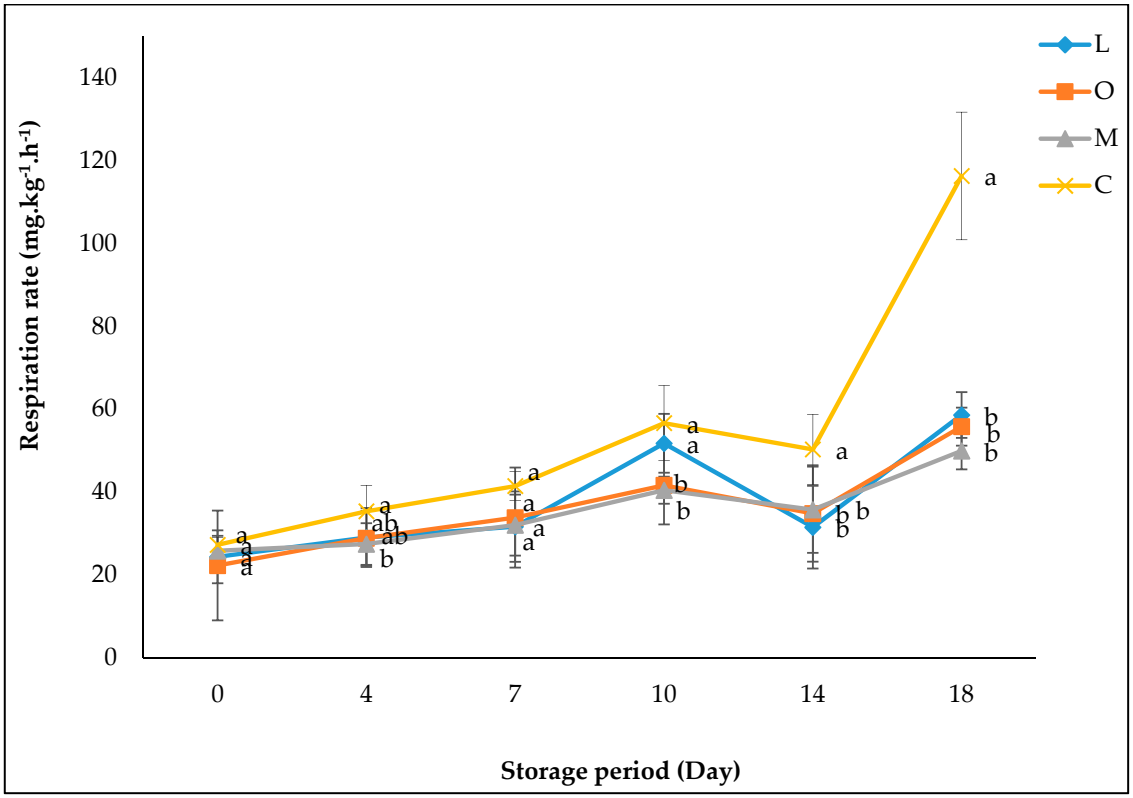

Figure 7. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on respiration of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

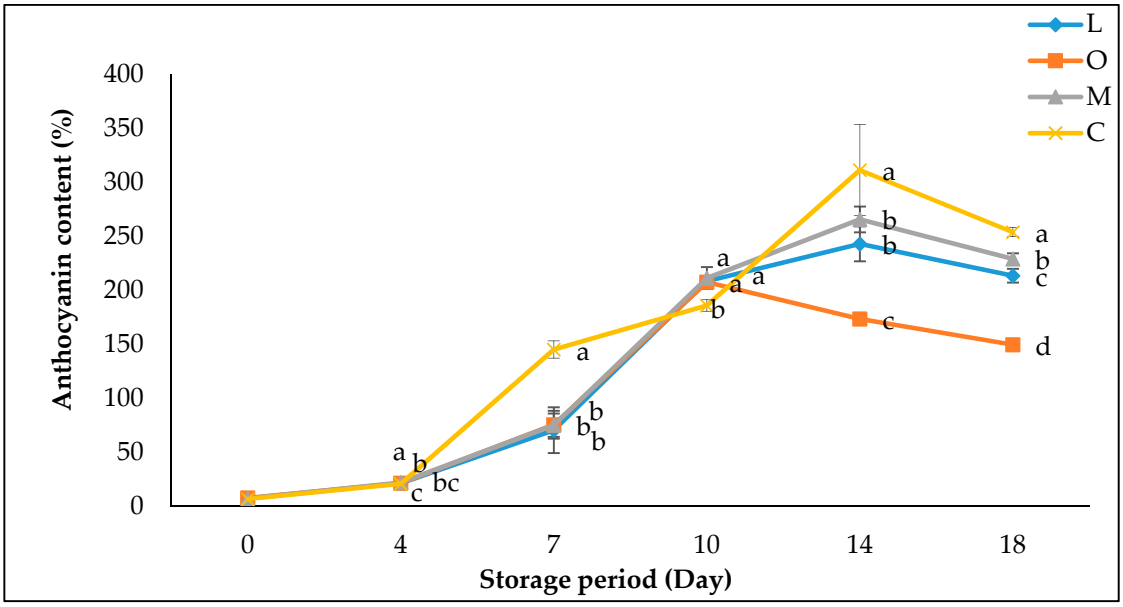

Figure 8. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on anthocyanin content of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.6. Respiration Rate

Perishable fruits, such as strawberries, are considered as having a high respiration rate and are severely affected by time and temperature of storage [2,36]. Figure 7 shows the level of the respiration rate, expressed as mg of CO_2 $kg^{-1}{\cdot}h^{-1}$, on days 0, 4, 7, 10, 14, and 18. The respiration rate of all samples was increased by increasing the storage period. At the end of the storage period, the lowest rates of the respiration rate were recorded in the treated fruits with different essential oils while the highest rates were observed in the untreated fruits ($p = 0.006$ **). No significant differences were observed in the respiration rates among the fruits treated with different essential oils (lemon, orange and mandarin). These results indicate that the treatment with different essential oils caused a significant reduction in the respiration rate. The findings suggest that application of essential oils coatings significantly reduced the respiration rate of fruits in a similar method to storage under a controlled atmosphere. This was due to an antioxidant activity and the gas permeability of used essential oils covered the fruit surface that could have changed the endogenous content of CO_2 and O_2 gas, thus reducing the respiration rate of fruits [4]. Similar results were reported by Marín et al. [44], who found that the use of lemon essential oil with chitosan significantly decreased the respiration rate compared to the use of chitosan coating alone after 12 days of storage.

Natural antimicrobial edible coatings are widely used in fresh and minimally processed fruits and vegetable, to decrease respiration, reduce loss of flavor compounds, and extend the self-life of fruits and retard the maturation process [4]. Nevertheless, the change in the internal gas composition due to the application of coating film can produce alcoholic flavors as a result of anaerobic fermentation linked to the accumulation of carbon dioxide and reduction of oxygen content [45]. For that reason, it is required to control the alcoholic flavors in sensorial panels. In our study, there were no off-odors and off-tastes produced in the coated fruits, during the storage periods. The efficiency of edible films in conserving the quality of fresh vegetables is strongly associated with the use of a proper coating material that is able to introduce a desirable internal gas composition to reduce the transpiration rates of fruits without production of the fermentative compounds that contribute to the development of off-odors and off-tastes [4,44].

3.2.7. Total Anthocyanin Content

Anthocyanins belongs to the polyphenol groups and it is responsible for the red color in the ripe strawberry fruits. A regulatory enzyme, the phenylalanine ammonia-lyase is accountable for the biosynthesis of anthocyanin in fruits and vegetables [46]. Generally, the concentrations of total phenols and total anthocyanins in the fruits depend on varieties and the maturity stage of strawberries at harvest in the open field or during storage. The results pointed out that the untreated fruit showed the maximum value of anthocyanin concentration (253.97 $mg{\cdot}kg^{-1}$) at the end of the storage, compared to 6.82 $mg{\cdot}kg^{-1}$ as the storage began (Figure 8). The significant increase, especially in control, could be explained as a natural process during fruit ripening, in addition to the effect of the high weight loss (Figure 2) that might have contributed to the concentration of pigments. However, the treated fruits with essential oils showed a lower concentration of anthocyanin than the untreated ones. Furthermore, significant differences were found in total anthocyanin among coated fruits with essential oil types. The lowest concentration of total anthocyanin content was recorded in treated fruits with mandarin oil followed by lemon and orange oils compared with untreated fruits ($p = 0.0005$ ***). This result confirmed what was previously shown in Figure 6, that the color of treated fruits with essential oils were lower darker and redder than untreated fruits. This was due to delaying the synthesis of anthocyanin and the other pigments contributing to the red color in strawberries [47]. Similar results have been observed in different fruits treated with coatings based on chitosan [26]. Moreover, the better findings were achieved when essential oils were added with those edible coatings [48]. The reduction in anthocyanin concentration in treated fruit could be linked to the antioxidant property of the essential oils that reduced diffusion of oxygen and increased the CO_2 accumulation around the strawberry surfaces, and thus consequently reduced the delayed activity of enzymes and biochemical

reactions responsible for anthocyanin biosynthesis [49]. This result was observed by Pelayo et al. [50], who found that the level of anthocyanin in the stored strawberries was inhibited due to increasing the concentration of CO_2. Moreover, the treatment with essential oils significantly reduced the respiration rate (Figure 7) which consequently reduced the water loss from the fruit surface and this explained the reduction of anthocyanin concentration in coated fruits when compared with uncoated fruits. Therefore, treatments with essential oils can effectively delay the fruit ripening and extend the post-harvest self-life of strawberries.

3.2.8. Total Phenolic Compounds (TPC)

Total phenols are quite important compounds for the quality of strawberry fruits, not only due to their involvement with anthocyanins in the flavor and the color of most fruits and vegetables [51] but also their involvement in the preservation of fruit quality during storage. Polyphenol compounds possess antimicrobial, anti-aging and antioxidant properties, making these compounds an important indicator to evaluate the fruit and vegetable conservation effect [52]. Changes in the total phenolic content of all the stored strawberries are presented in Figure 9. The trends of total phenols content in all samples increased gradually after storage began and peaked on Day 10, then the trend slowed down gradually and peaked on Day 18 (p = 0.0035 **). The fast accumulation of total phenols content in all samples in the first ten days could be linked to an increase in the accumulation of other phenolic compounds [53].

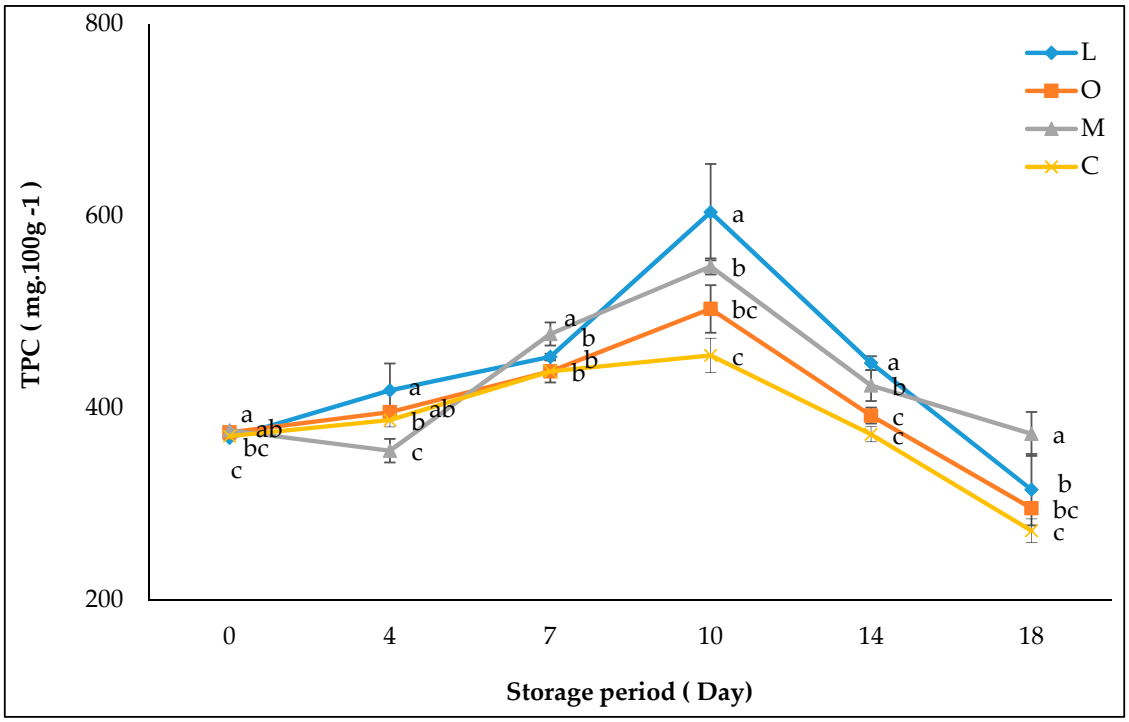

Figure 9. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on total phenol content (TPC) of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

Shin et al. [54] stated that differences in the total phenolic content of strawberry fruits during storage were affected by the maturity of fruits at the harvest and storage period. At the end of storage periods, the highest content of total phenols content was recorded in coated fruits treated with mandarin essential oil followed by lemon oil and orange oil compared with uncoated fruits. This might be associated with the antioxidant properties of applied essential oils that reduced the oxidation of

phenolic compounds [55]. These results were in agreement with those obtained by Gol et al. [49] who found that the content of phenolic compounds was significantly increased in coated fruit treated with chitosan incorporated with essential oils than uncoated fruits. On the other hand, the decline of phenolic content, at the end of the storage period, in uncoated strawberries could be due to continuous respiration rate and the breakdown of cell structure [36].

3.2.9. Titratable Acidity

The amount of titratable acidity (TA) in the strawberry is directly correlated to the content of organic acids existing in the fruits. The changes in the amount of TA of strawberry during storage are shown in Figure 10. The amount of TA of strawberries during storage decreased gradually with the extension of the storage periods. At the end of the storage periods, the amount of TA in the coated fruits was greater than that of the uncoated fruits, indicating that edible coatings with essential oils are favorable to maintain the level of TA in the fruits during preservation (p = 0.0466 *). Similar findings were observed by Dhital et al. [34] and Gol et al. [49]. They found that the edible coatings incorporated with essential oils reduced the water loss, respiration rate, and microbial growth which consequently decreased the consumption of organic acids (i.e., titratable acid) in the respiratory metabolic activities of strawberry fruits. This efficiently reduces the loss of titratable acid, extending the post-harvest shelf life of stored fruits.

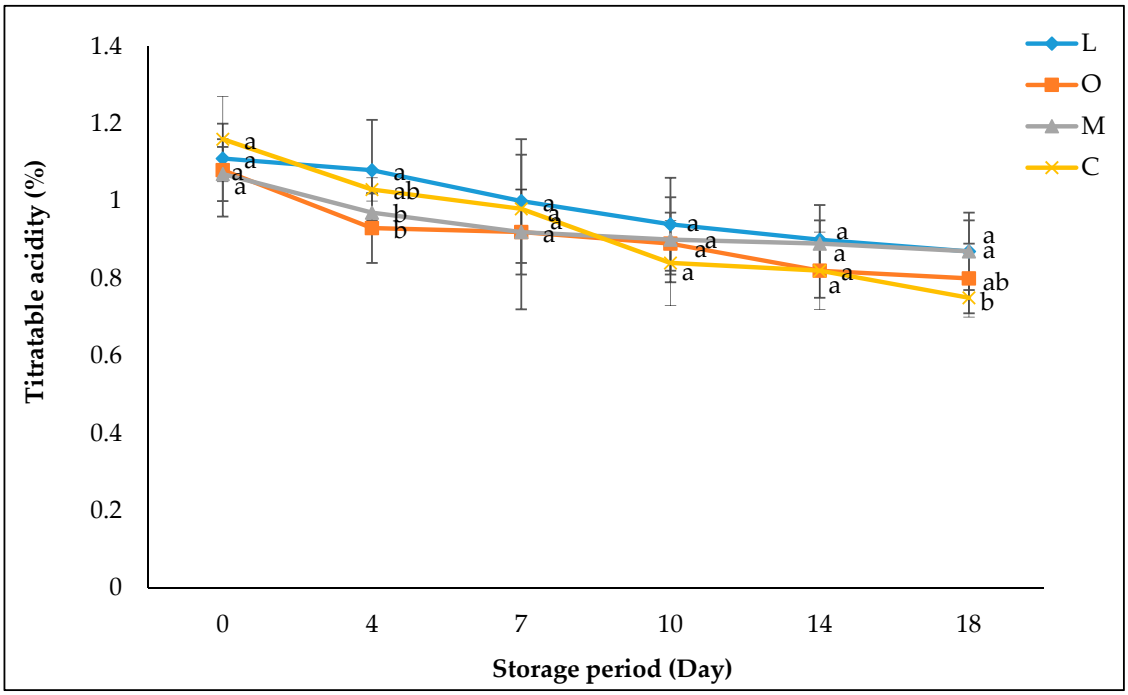

Figure 10. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on titratable acidity of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.10. Ascorbic Acid

Ascorbic acid plays an important role in fruit preservation during storage [36]. Change in ascorbic acid concentration of strawberries during storage is presented in Figure 11. The total ascorbic acids of all coated and uncoated samples of the strawberry fruits significantly decreased with increases in the storage periods. This reduction could be related to its oxidation through superoxide and hydroxyl radicals in the strawberry fruits [2,36]. At the end of the storage period, the concentrations of ascorbic acid were higher in coated fruits with different essential oils than the uncoated fruits (p = 0.057).

Significant differences in ascorbic acids were found among the treated fruits with different essential oils. The maximum values of vitamin C were observed in the strawberry fruits treated with mandarin essential oil followed by lemon and orange essential oils compared with untreated fruits (control). The result of this study suggested that the treatment with essential oils can effectively reduce the loss of vitamin C (ascorbic acid) in coated strawberry fruits during storage. This might be attributed to the antioxidant properties of essential oil coatings (Figure 1) which reduced the diffusion of oxygen, decreased the rate of respiration, as shown above in Figure 7, and consequently reduced the ascorbic acid oxidation [4,44].

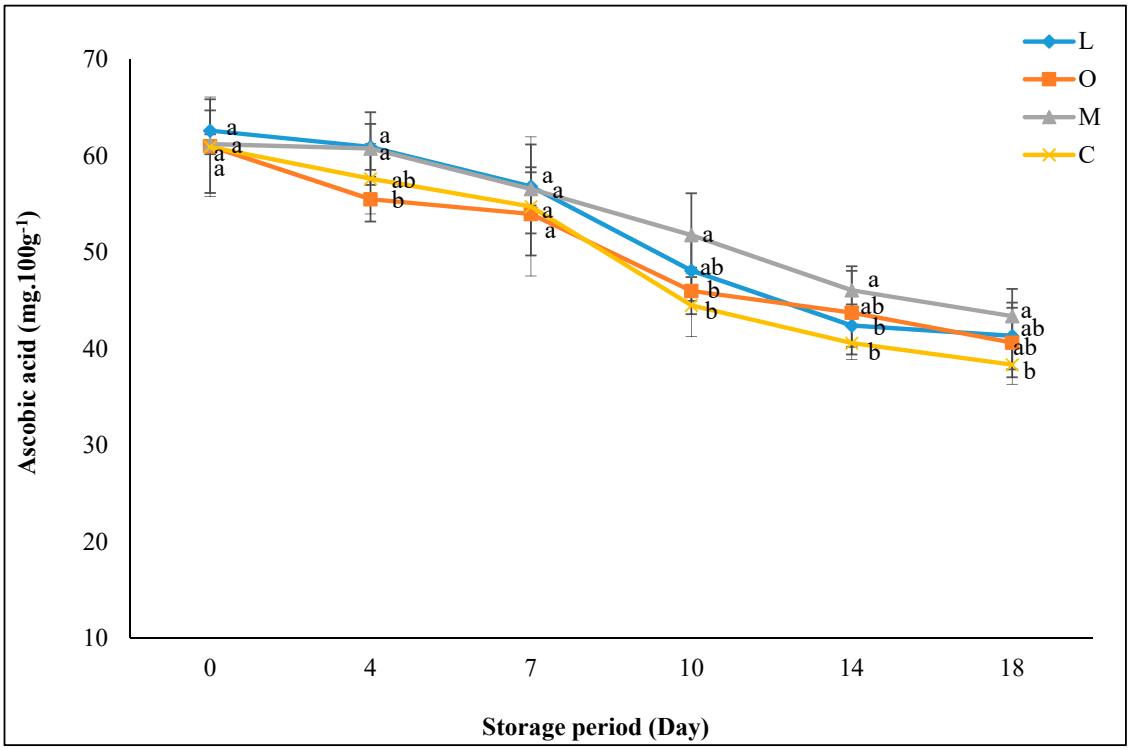

Figure 11. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on ascorbic acid of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.11. Antioxidant Capacity

Antioxidant capacity is a very important factor in assessing fruits and vegetables. Several techniques are used to evaluate the total antioxidant ability, including the oxygen radical absorbance capacity (ORAC) technique, the 1-diphenyl-2-picrylhydrazyl (DPPH) technique, the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, ABTS) technique, the ferric reduction antioxidant (FRAP) technique and so on. The DPPH technique has been extensively used to determine the antioxidant capacity of foods, especially fruits and vegetables, and their byproducts due to their sensitivity, speediness, reliability, and simple technique [56].

The change in the antioxidant ratio of strawberry samples treated with essential oils during the storage periods is shown in Figure 12. The antioxidant content of the treated fruits with different essential oils significantly increased with an increase in the storage periods until 14 days of storage. After that, the total antioxidant content of the treated and untreated strawberries was decreased. However, the reduction in the antioxidant content in treated fruit was lower than the untreated fruit. The maximum values of antioxidants were recorded in fruits treated with mandarin oil followed by lemon and orange oils compared with untreated fruits (p = 0.0006 ***). The increase of the total

antioxidant capacity of treated fruits with essential oil might be associated with the relative amounts of antioxidant compounds added to strawberries after coating with essential oils, especially polyphenol compounds [2,57]. Moreover, these types of essential oils have the ability to retard the loss of other antioxidant compounds of the coated fruits such as total phenols, ascorbic acid, and anthocyanin contents, as previously mentioned (Figures 8, 9 and 11). Meanwhile, the reduction of antioxidant capacity in untreated fruits at the end of the storage period might be due to senescence [58]. The findings of this study were consistent with the results of Martínez et al. [2] who found that edible chitosan coatings incorporated with *Thymus capitatus* essential oil significantly improved the total antioxidant capacity of coated strawberries over uncoated fruits.

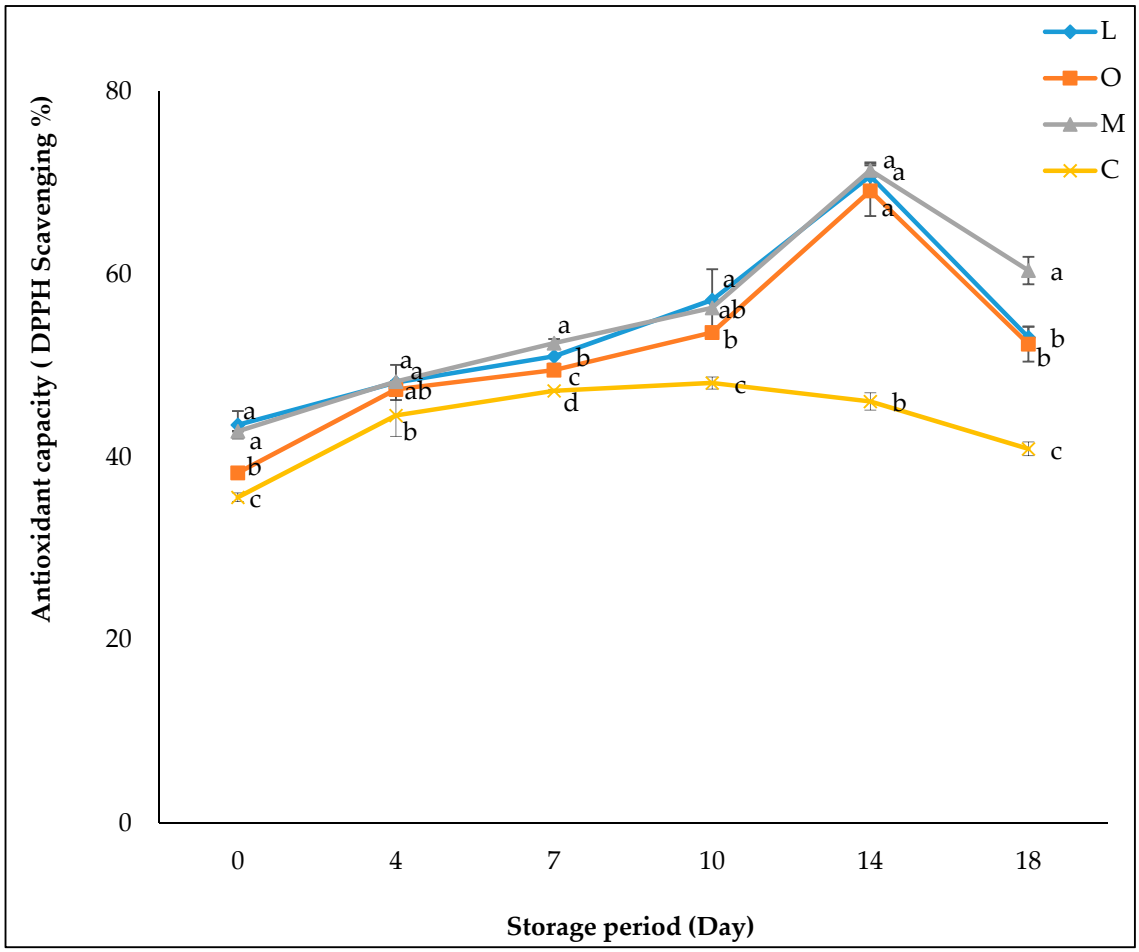

Figure 12. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on antioxidant capacity of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

3.2.12. Antioxidant Enzymes (Catalase and Polyphenol Oxidase)

Catalase (CAT) is considered one of the most important antioxidant enzymes in protecting the plant cell from oxidative damage by reactive oxygen species [59]. The activity of this enzyme usually increases during fruit maturation [60]. As shown in Figure 13, the activity of the CAT enzyme in all fruit samples rapidly decreased with increasing storage periods, but the reduction in the coated fruits with different essential oils was lower than uncoated fruits, at the end of the storage period (p = 0.0001 ***).

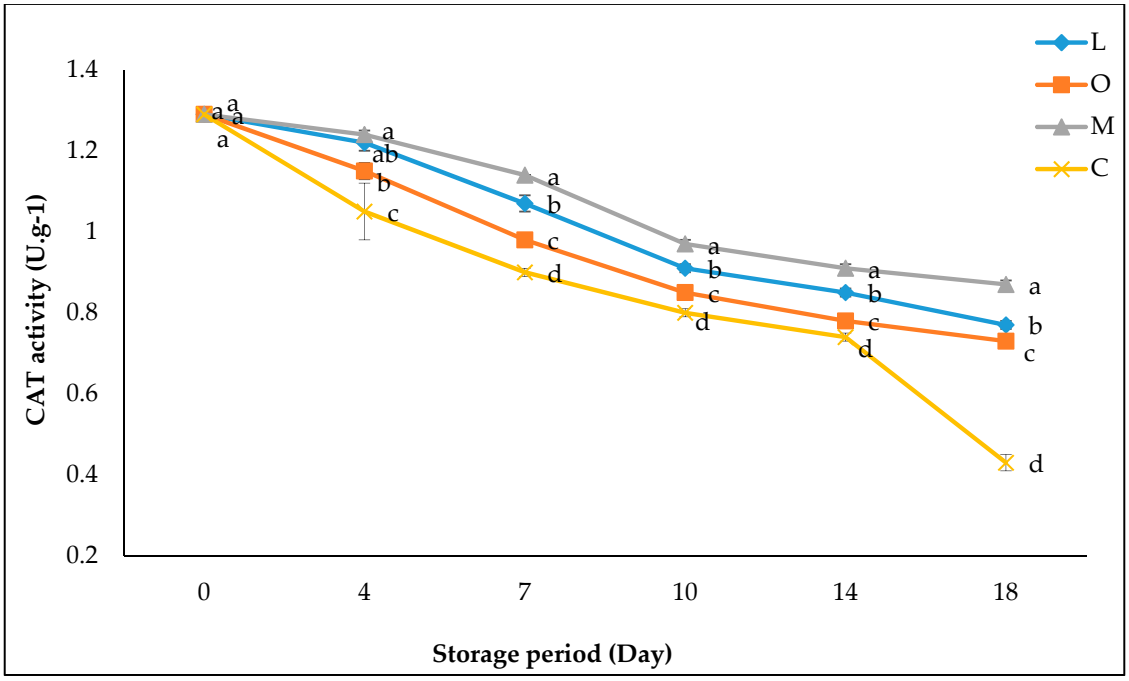

Figure 13. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on catalase activity (CAT) of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

The highest CAT activity was observed in fruits treated with mandarin essential oil followed by lemon and orange than uncoated fruits. The polyphenol oxidase (PPO) enzyme is responsible for browning reaction, mainly occurs from the oxidation of phenolic substances, and leads to quality loss [61]. PPO activity in all fruits increased with the extension of storage periods, and PPO activity in coated fruits with different essential oils showed a lower level compared to uncoated fruits ($p = 0.0004$ ***). At the end of storage, the lowest PPO activity was in strawberries treated with mandarin oils followed by lemon and orange oils (Figure 14). Similar findings were reported by Badawy et al. [26] who observed that the use of essential oils containing thymol (0.02%) or geraniol (0.04%) increased CAT activity and reduced PPO activity. These results suggested that the use of essential oils improves the levels of oxyradical detoxification enzymes including CAT and declines the PPO activity that prevents the oxidation damages, thus promoting prolongation of the shelf-life and preserving the quality of strawberries during the storage.

3.2.13. Sensory Assessment

The sensory assessment of the treated strawberries is necessary since it shows the acceptability on the part of the final consumer of the fruits treated with the essential oils [62]. The findings of the hedonic evaluations are presented in Table 3 during the 18 days of storage. Results indicated that the strawberries treated with different essential oils (L, O, and M) had higher acceptance regarding color, texture, flavor, and appearance attributes than untreated strawberries. Furthermore, significant differences were found between treated fruits with different essential oils in sensory attributes, especially in color, appearance, and general acceptance at the end of the experiment.

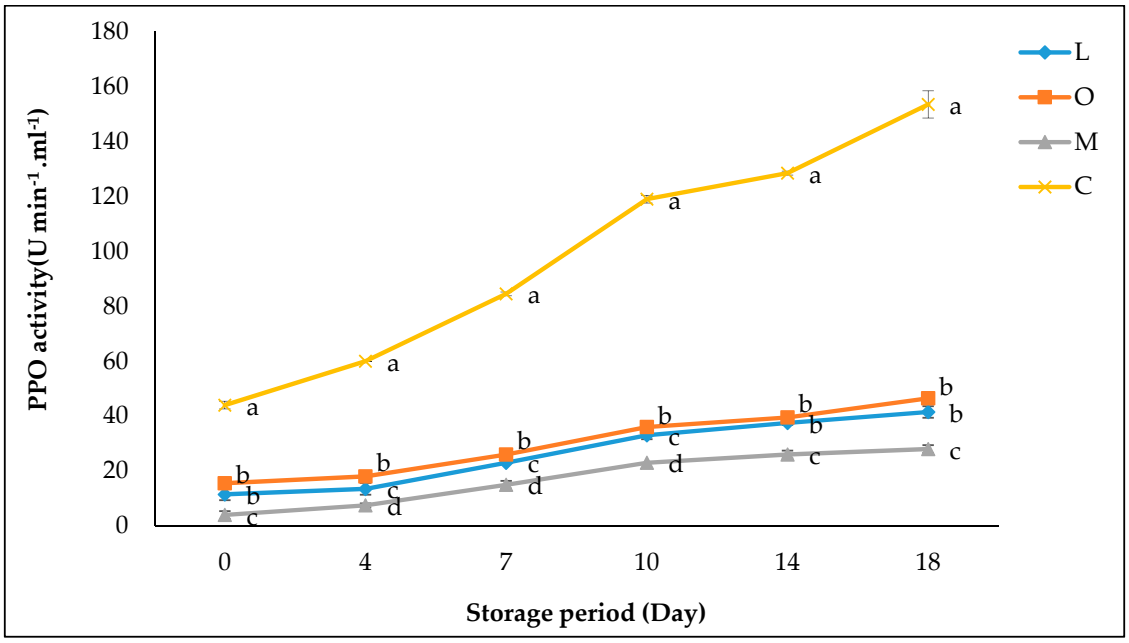

Figure 14. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on polyphenol oxidase activity (PPO) of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicates significant difference between treated and control fruits at each storage period (Duncan's of 95%).

Table 3. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on color, texture, appearance of strawberry fruits stored at 2 °C for 18 days. Different letters indicates significant difference between treated and control fruits at each storage period (Duncan's of 95%).

Treatments		Storage Duration (Days)					
		0	4	7	10	14	18
Color	L	5.00a	5.00a	4.80 ± 0.45a	4.10 ± 0.89a	4.40 ± 0.55a	3.90 ± 0.22ab
	O	5.00a	5.00a	5.00a	4.40 ± 0.55a	4.10 ± 0.55a	3.70 ± 0.27b
	M	5.00a	5.00a	4.90 ± 0.22a	4.50 ± 0.71a	4.80 ± 0.45a	4.10 ± 0.22a
	C	5.00a	5.00a	4.00b	4.00 ± 0.61a	3.70 ± 0.27b	2.70 ± 0.27c
Texture	L	5.00a	5.00a	4.90 ± 0.22a	4.40 ± 0.55a	3.90 ± 0.22ab	3.30 ± 0.45a
	O	5.00a	5.00a	4.80 ± 0.45a	4.60 ± 0.55a	4.10 ± 0.55a	3.20 ± 0.27a
	M	5.00a	5.00a	5.00a	4.80 ± 0.45a	4.40 ± 0.55a	3.40 ± 0.22a
	C	5.00a	5.00a	4.00b	3.70 ± 0.27b	3.50b	2.20 ± 0.45b
Flavor	L	5.00a	5.00a	5.00a	5.00a	4.40 ± 0.22a	3.90 ± 0.22a
	O	5.00a	5.00a	5.00a	5.00a	4.40 ± 0.22a	3.90 ± 0.22a
	M	5.00a	5.00a	5.00a	5.00a	4.30 ± 0.27a	3.90 ± 0.22a
	C	5.00a	5.00a	5.00a	4.60 ± 0.42b	3.40 ± 0.65b	2.40 ± 0.42b
Appearance	L	5.00a	5.00a	4.60 ± 0.55a	4.80 ± 0.45a	3.90 ± 0.22a	3.90 ± 0.22a
	O	5.00a	5.00a	4.80 ± 0.45a	4.40 ± 0.55a	4.00a	3.40 ± 0.55ab
	M	5.00a	5.00a	4.80 ± 0.45a	4.50 ± 0.50a	4.00a	3.50 ± 0.61ab
	C	5.00a	5.00a	4.20 ± 0.45a	4.30 ± 0.67a	3.10 ± 0.22b	3.00 ± 0.50b
General acceptance	L	5.00a	5.00a	4.30 ± 0.67a	4.10 ± 0.55a	3.80 ± 0.27a	3.40 ± 0.22ab
	O	5.00a	5.00a	4.50 ± 0.50a	4.50 ± 0.50a	3.50a	3.10 ± 0.42b
	M	5.00a	5.00a	4.60 ± 0.55a	4.60 ± 0.42a	3.90 ± 0.22a	3.70 ± 0.27a
	C	5.00a	5.00a	3.90 ± 0.65a	3.00a	2.30 ± 0.45b	1.80 ± 0.67c

At the end of storage, the analysis of color was higher in strawberry fruits treated with mandarin than those treated with lemon and orange oils. The appearance of fruits treated with lemon essential oil was slightly higher than the fruits treated with mandarin and orange essential oils. There were no significant changes among the treated fruits in texture and flavor. Regarding analysis of general

acceptance, all the treated fruits with different essential oils showed a higher acceptability than untreated fruits at the end of storage. This behavior indicates that the treatments with essential oils improve the organoleptic characteristics and physicochemical attributes of the strawberries [4,63]. The fruits treated with orange essential oil (O) had a lower acceptance of the sensory attributes than mandarin and lemon essential oils, but these fruits had higher acceptability of sensory properties than untreated fruits. This result might be due to rapid changes in metabolic compounds of untreated fruits (C) and then treated fruits with essential oil of orange (O) that finally affect organoleptic characteristics of strawberry fruits. Therefore, future studies should be conducted to correct the concentration of lemon essential oil can facilitate its acceptance without affecting negatively on organoleptic properties of strawberry fruits.

3.2.14. Microbial Study

The changes in contamination with molds, total count and coliform group on coated strawberry samples are presented in Figures 15–17. The results of the samples treated with essential oil showed a strong effect on the total count of microbes, which was reflected in the preservation period. The treatments of lemon and mandarin recorded numbers less than log 1 until the end of the storage period, while treatment of orange recorded log 3.38 at the end of storage (p = 0.002 **). The control sample reached the number of log 6.86 at the end of storage.

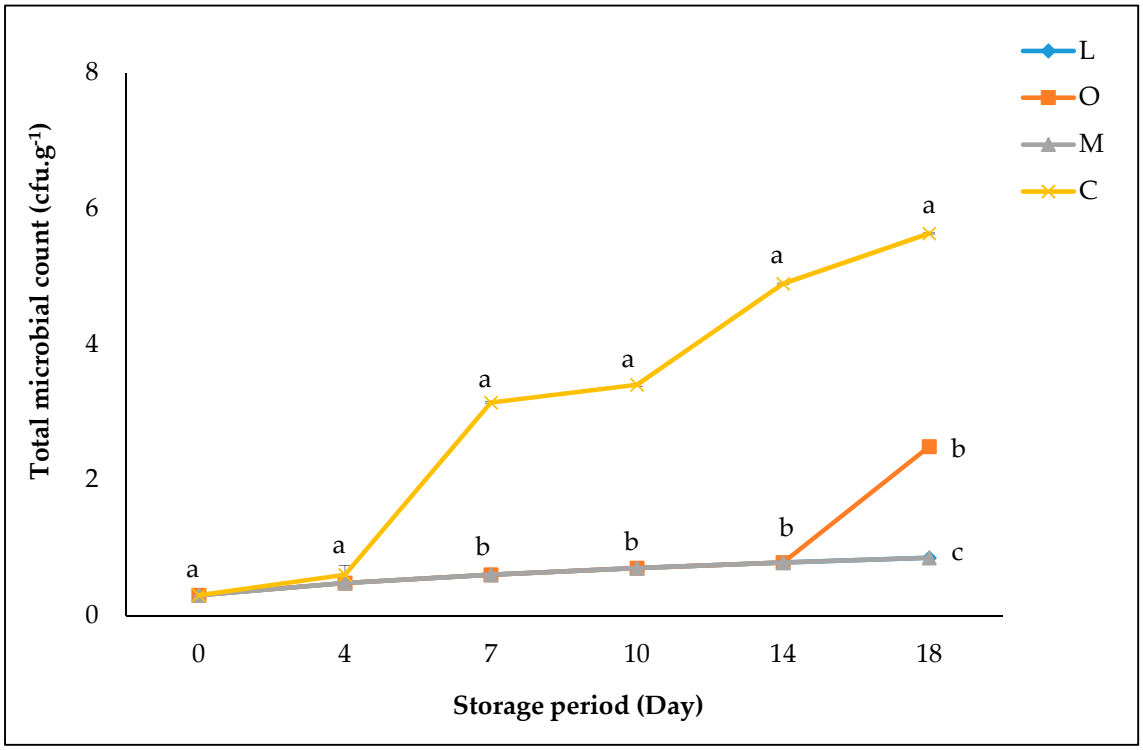

Figure 15. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on total microbial content of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

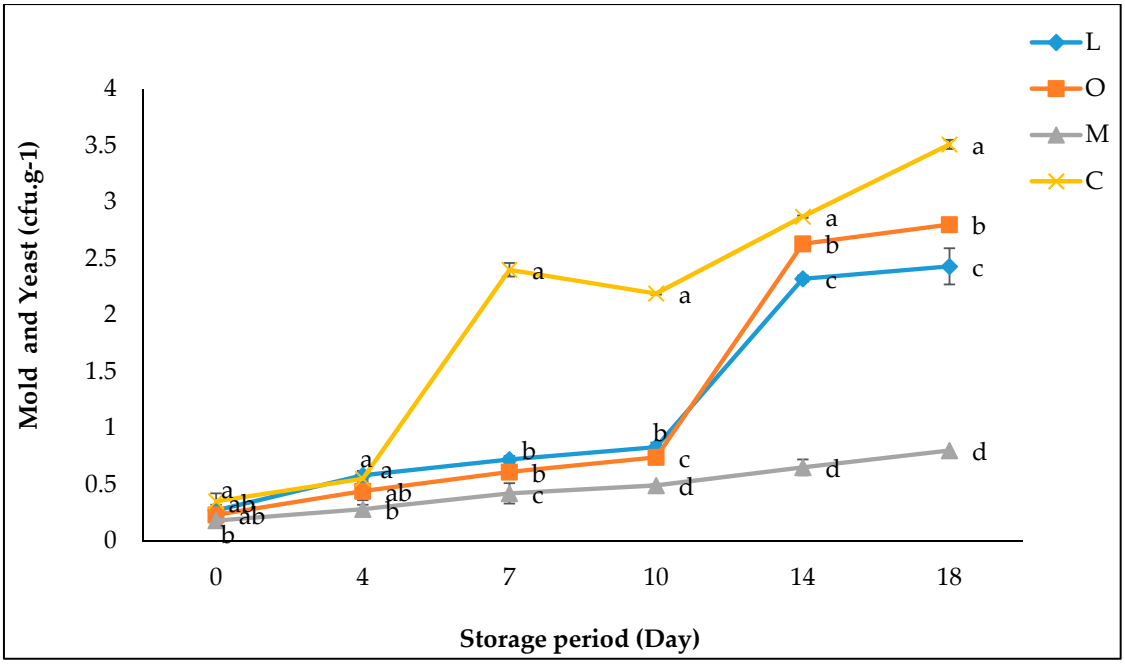

Figure 16. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on mold and yeast of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

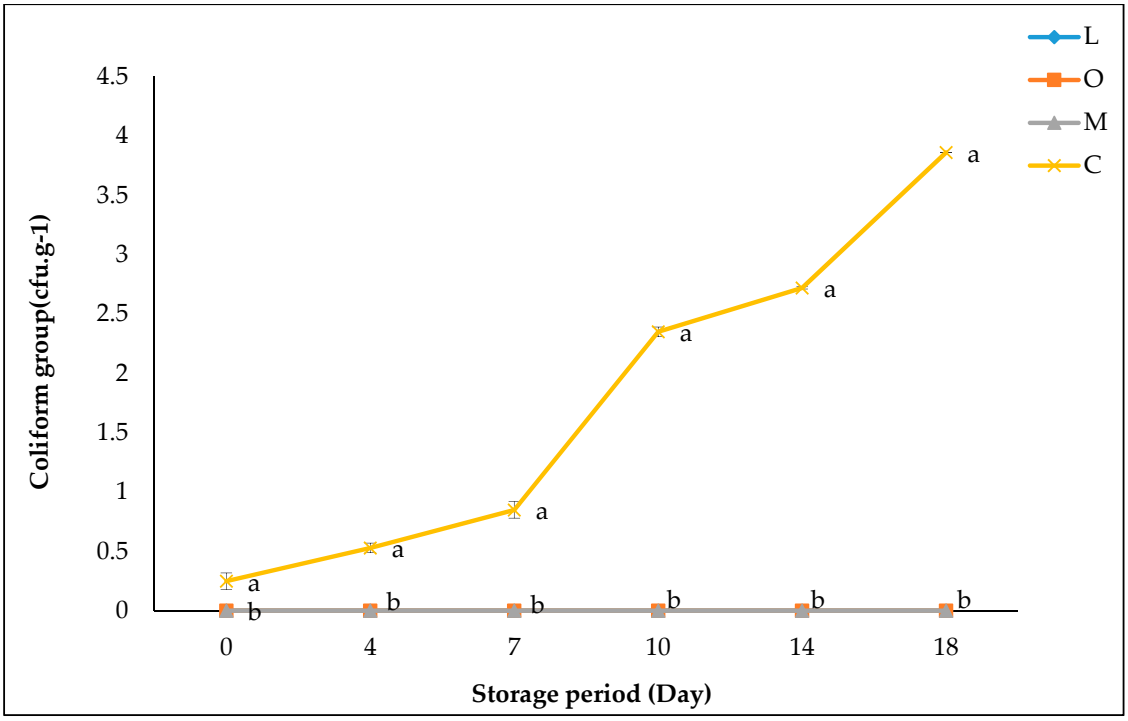

Figure 17. Effect of different essential oil emulsions (L = lemon, O = orange, and M = mandarin essential oil) on total coliform group of strawberry fruits stored at 2 °C for 18 days. Vertical bars indicate the standard deviation (n = 6). Means and different letters indicate significant difference between treated and control fruits at each storage period (Duncan's of 95%).

In the case of estimating the numbers of mold and yeast, the results showed that treatment with mandarin essential oil is the best, as no more than one logarithm was recorded during storage,

except for the last sample at storage end, log 2.22, while treatments of lemon or orange essential oil were recorded by the end of storage following log 3.62 and log 3.96, respectively (p = 0.004 **). However, the positive control sample recorded log 4.19 at the end of storage.

When estimating the coliform group, all treatments of essential oils did not record any numbers, which shows the positive effect of all treatments compared to the control treatment which recorded log 4.1 at the end of the storage period (p = 0.007 **). These results might be due to the anti-microbial effect that characterizes essential oils because they contain active compounds such as carvacrol, thymol, limonene, and cinnamaldehyde [64–66].

3.3. Correlation Study

Pearson analysis in Table 4 shows a significant and positive correlation between total antioxidant content of treated strawberries and vitamin C, TPC, and CAT while a significantly negative relation was observed for weight loss and SSC content. The total antioxidant content of treated fruits significantly correlated negatively with decay percentage, respiration rate, and PPO. A similar relationship was noted for total microbial account and total coliform as well as mold and yeast. This correlation suggested that increasing the level of the antioxidant content in fruits plays an important role in reducing the changes in physicochemical properties and extending the shelf life of strawberries' fruits [4]. Conversely, the lack of a relationship was noted with fruit firmness, thus indicating that the fruit firmness could be correlated strongly with the water loss and fruit respiration, as mentioned by Badawy et al. [26].

Table 4. Correlation study between total antioxidant activity of treated fruits and physiochemical and microbial properties of strawberry fruits.

Positive correlation								
Antioxidant activity of strawberry	Firmness	Vit. C	Acidity	TPC	Catalase			
	0.41 *	0.50 *	0.533 **	0.97 **	0.98 **			
Negative correlation								
Antioxidant activity of strawberry	Weight Loss	Decay	SSC	Respiration	Polyphenol	Total Count	Mold& Yeast	Coliform
	−0.76 **	−0.83 **	−0.62 **	−0.88 **	−0.94 **	−0.92 **	−0.90 **	−0.88 **

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

4. Conclusions

Compared with untreated fruits, utilization of essential oils emulsion for orange, lemon and mandarin could effectively reduce the weight loss, respiration rate, human and plant pathogenic microorganisms, and activity of antioxidant enzymes (PPO and CAT) of strawberry fruits. In addition, our tested essential oil delayed the deterioration of fruit firmness, soluble solid content, and titratable acidity. Our treatments maintained ascorbic acid, anthocyanins and total phenols at the end of the storage period. The strawberry fruits had a higher antioxidant capacity at the end of storage, thus extending the storage ability and delaying fruit senescence. Therefore, emulsions of citrus essential oils had better preservation effects, and emulsion of mandarin essential oil was slightly better than orange and lemon essential oils.

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