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Development of Basil Essential Oil (BEO) as a Novel Alternative to Prolong the Storage of Tomato (*Lycopersicum esculentum* L.)

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Abstract: Antimicrobial compounds used as post-harvest treatment of fruit and vegetables can extend their shelf life by reducing the rate of microbial growth. Essential oils extracted from herbs or spices can also enhance shelf life due to their antimicrobial nature. Tomatoes harvested at consumption maturity were treated by spraying with aqueous solutions of basil essential oil (BEO) and glycerol in different concentrations (50, 100, 150, 200, 250 and 300 ppm) and stored by refrigeration at 8 °C and 85% relative humidity. The BEO used was obtained by extraction from indigenous crops of *Ocimum basilicum* and was analyzed by GC-MS for discerning of the constituents present in it. The main components identified in BEO were: eucalyptol, linalool, estragol, eugenol methyl-cinamate, trans-α-bergamotene, germacrene D, γ-cadinene and T-cadinol. During storage, in order to highlight the effect of the applied treatment, the following were determined: dry matter (DM), total soluble content (TSS), total phenols content (TP), antioxidant activity (AOA), color, weight loss and total number of aerobic mesophilic bacteria (AMB) during storage. It has been shown that spraying the fruit with solutions of different concentrations of BEO has significant effects on weight loss, DM, TSS, TP, AOA, color and TAMB, during storage. The lowest TP value was found in the control and the variant treated only with 2.5% aqueous glycerol solution (52.18 mg/100 g fw GAE) and the highest value in the variants treated with concentrations of 200, 250 and 300 ppm BEO (54.37, 55.00 and 57.81 mg GAE/100 g fw). The highest AOA values were found in the 300 ppm BEO-treated variant (119.23 µmol TE/100 g fw). Spraying tomatoes with aqueous solutions of glycerol 2.5% and BEO at a dose of 250 ppm prolongs their storage while maintaining their quality for fresh consumption.

Keywords: total phenolic content; antioxidant activity; color; total number of mesophilic bacteria; storage

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1. Introduction

Fruit and vegetables are perishable products that require protection against spoilage in order to extend their shelf life. Only storing them at low temperatures cannot ensure that the quality of fruit and vegetables is maintained, so they must be protected with antimicrobials [1].

Antimicrobials are used to avoid contamination of products with microorganisms, thus preventing their early decline and death.

The use of some antimicrobials such as benzoic acid or sorbic acid is currently restricted and others are completely banned due to their toxic effect on human health [2].

Nowadays, consumers have become aware of the harmful effect of synthetic chemicals used in post-harvest treatments of fruit and vegetables, which has prompted researchers to look for natural broad-spectrum antimicrobials.

Essential oils from plants have been shown to have antimicrobial potential [1]. Essential oils, which are synthesized by the secondary metabolism of plants, can be used to extend the shelf life of fresh fruits and vegetables due to their antioxidant and antimicrobial

properties [3]. Preparation of edible coatings containing essential oils extracted from various aromatic plants has been reported in recent literature [4–6]. Basil *(Ocimum basilicum* L.) is an aromatic plant belonging to the *Lamiaceae* family cultivated for the special aroma of its leaves. Essential oils extracted from basil leaves of various species (*O. basilicum*, *O. sanctum var. shyama*, *O. basilicum var. thyrsiflorum*, *O. citriodorum*, *O. gratissimum*) contain volatile organic substances with biological activities [7]. Basil essential oil (BEO) is used as a flavoring in food or as a fungicide and insecticide in pharmaceutical and industrial products [8] and contains terpenes, esters, alcohols, aldehydes, ethers and ketones; the main compounds being estragole, linalool, eugenol, methyl-eugenol and eucalyptol [9,10]. BEO has a high volatility and hydrophobicity, which makes it difficult to use in pathogen control treatments on the surface of fruit and vegetables, various studies having been carried out on its possible attachment to the surface of products [11–13].

Tomato (*Lycopersicum esculentum* L.) is a species of major importance for vegetable farming, being appreciated both for fresh consumption and for various processed products. Tomatoes are climacteric fruits with a short shelf life and low firmness, sensitive to attack by microbial agents [14]. The degree of ripeness of tomatoes can be judged, among internal characteristics, by their color, as they are eaten at the consumption maturity when they are red and firm enough to be transported and handled [15]. Consumer acceptance of tomato fruits depends on their physical and chemical properties, i.e., color, total soluble solids, dry matter, total polyphenol content, acidity, firmness and polyphenol content [16,17]. Tomatoes, one of the most produced and consumed vegetables [17], are a source of nutrients and secondary metabolites such as flavonoids, β-carotene and lycopene, of particular importance for health [18,19], the characteristic color of tomato being given by the interaction between the chlorophyll, β-carotene and lycopene content [20]. Tomato varieties have different contents in terms of types and concentrations of carotenoids and other phenolic compounds [21–23], lycopene representing more than 80% of the total carotenoids in tomatoes at full maturity [24]. One of the biggest problems with tomatoes is extending their shelf life. Due to their intense metabolism with the release of ethylene [25], this affects the fresh storage and quality of tomatoes [26], which does not ensure a continuous supply to the market and the canning factories. Various packaging methods and treatments have been applied to tomatoes in order to extend their shelf life [27–33].

For the first time, the post-harvest spraying of tomatoes with aqueous solutions of BEO and glycerol of different concentrations on their physicochemical properties was studied. Post-harvest treatments were carried out with the aim of extending the storage duration of tomatoes. Knowing the antimicrobial potential of plant essential oils [1], the effect of spraying tomatoes with BEO on microorganisms on tomatoes was also studied. Unlike other studies using essential oils in the preparation of edible coatings [4–6], in this paper, for the first time, an aqueous solution of basil oil and glycerol was prepared and used. Since essential oils are not soluble in water but soluble in glycerol, in order to ensure the solubility of the oil in the aqueous solution and the uniformity of the spray, we used glycerol as a solvent for it.

2. Materials and Methods

2.1. Chemicals

The following reagents were used: sodium carbonate and methanol from Merck (Darmstadt, Germany; Folin-Ciocalteu reagent, Gallic acid, sodium acetate, DPPH (2, 2 -diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) from Sigma-Aldrich (Germany), the other chemicals being of analytical grade. Solvents (n-hexane, water—LiChrosolv ®® grade) were all purchased from Merck (Burlington, MA, USA).

2.2. Plant Material

Tomatoes belonging to the variety Rozalina Rossa F1, grown in protected cropping (plastic tunnels), in Dabuleni (43°48′04″ N 24°05′31″ E), Dolj county, Romania were used in this study in order to extend their storage. Pink Rozalina Rossa F1 tomatoes are intended

for early crops both in the field and in protected areas. The fruits of the hybrid are a deep pink color and are extremely firm, without a green cap. The Rozalina Rossa F1 tomatoes have a shelf life of more than 10 days and, resistance to *Verticillium*, *Fusarium* and Tobacco Mosaic Virus (TMV) [34].

Planting in plastic tunnels was carried out with 60 days old seedlings on 15–20 March. Prior to planting, the irrigation system was installed. Planting distance was 80/35 cm (about 3600 plants/1000 m^2). Basic fertilization was carried out with 20 t/ha manure. During the cultivation, all phases of the protected area cultivation technology have been completed (gap filling, staking, copulation, removal of side shoots from the main stem, budding, pruning, flower stimulation, phase fertilization, aeration and phytosanitary treatments). Starter fertilization was carried out with Yara Mila Cropcare NPK 11-11-21 at a rate of 60 g/m^2. Weed control was performed by hand pruning. Phase fertilization was carried out in irrigation water using Agroxilato-K, weekly in doses of 1000–1500 mL/1000 m^2. Harvesting was carried out in mid-June, selecting fruit at the light red stage.

Dried and ground basil (*Ocimum basilicum* L.) leaves (100 g) grown in the same area were mixed 1000 mL water and used to extract the essential oil by hydro-distillation using a Clevenger apparatus for 180 min at 102 °C. The quantity of the oil collected was 1.2 ÷ 1.4 mL/100 g dry matter, and kept at a temperature of 4 °C until use.

2.3. Sampling

Tomatoes, harvested at consumption maturity with calyx, were sorted (with uniform color and size) and treated by spraying with aqueous solutions of glycerol (2.5%), in which BEO of different concentrations was incorporated. After treatment, the tomatoes were air-dried at a temperature of 20 °C, packed in 7 kg wooden boxes, and stored at a temperature of 8 ± 1 °C and 85% relative humidity. 42 kg of tomatoes (6 wooden crates) were used for each variant of treatment.

Depending on the concentration of BEO used, several experimental variants were set up (Table 1).

Table 1. Basil oil concentrations used in tomato spraying.

Variant	The Treatment Used
Control	untreated
V1	aqueous glycerol solution 2,5%
V2	50 ppm BEO in 2.5% aqueous glycerol solution
V3	100 ppm BEO in 2.5% aqueous glycerol solution
V4	150 ppm BEO in 2.5% aqueous glycerol solution
V5	200 ppm BEO in 2.5% aqueous glycerol solution
V6	250 ppm BEO in 2.5% aqueous glycerol solution
V7	300 ppm BEO in 2.5% aqueous glycerol solution

The following analyses and determinations were performed: dry matter (DM), total soluble content (TSS %), total phenols content (TP mg/100 g fw), antioxidant activity (AOA mM Trolox/100 g), color and weight loss during storage. The color index (CI) and a*/b* ratio was also calculated. Analyses were carried out before spraying, every 7 days of storage and at removal from storage (21 days). In order to highlight the action of BEO on the microbiological load, samples were taken, and the total number of aerobic mesophilic bacteria was determined (TAMB log CFU/g) and BEO obtained by hydro-distillation was analyzed to identify the components.

2.4. Analytical Methods

Extraction of BEO was carried out using an assembly of a heating mantle (NAHITA, Spain), directly connected to a Clevenger type continuous distiller (Glasco USA) described previously [35]. Before injection into the GC-MS the BEO had been diluted 1000 times.

GC analysis was performed for discerning of the constituents present in the essential oil of *Ocinum basilicum* by perceptible elucidation comparing their mass spectra with reference spectra (NIST Library) using a Thermo Scientific Focus GC coupled with a DSQ II mass detector equipped with TraceGOLD, TG-5SilMS column (60 m × 0.25 mm × 0.25 µm). The volume of the injection at the flow of 1.5 mL/min and a split ratio of 1:10 was 2 µL using helium as carrier gas. The initial oven temperature raised to 110 °C was maintained for 2 min, increased to 120 °C at the rate of 3 °C/min and maintained for 3 min; the temperature was further increased to 140 °C at the rate of 3 °C/min and maintained for 3 min and finally increased to 230 °C at the rate of 3 °C/min and kept for 7 min. MS transfer line temperature of 250 °C, ion source temperature of 230 °C electron impact ionization (EI) 70 eV were the other required conditions for GC-MS analysis. Spectra were scrutinized in the full scan mode over the range of 35 to 350 mass range and the retention times (RI) of all constituents were registered.

Weight loss was determined by weighing tomatoes with a digital scale (Sartorius CP124S, UK, accuracy = 0.01 g) both at the beginning of the experiment and every 7 days of storage, results being expressed as percentage of weight loss to initial weight [36].

Dry matter (DM %) was determined gravimetrically by drying 5 g finely divided fresh tomato in a laboratory oven (Memmert, Germany) set at 105 °C until constant weight was reached. The results were expressed as percentage of dry matter [37,38].

Total soluble solids content (TSS %) was measured with a digital refractometer (Hanna Instruments, Woonsocket, RI, USA) from a sample obtained by blending tomatoes in an electrical blender. The total and the results were expressed as percentage of soluble solids [36].

2.5. Extraction

Three grams of tomato homogenate were extracted with 10 mL of methanol for 60 min using an ultrasonic bath at room temperature. They were then centrifuged at 6000 rpm for 15 min and the supernatants were collected and stored at −40 °C. Furthermore, the extracts were used for the determination of total phenolic content and DPPH free radical scavenging activity.

2.6. Total Phenolic Content Evaluation

Total phenolic content was evaluated with the Folin–Ciocalteu procedure [39,40]. A total of 100 µL of extract was mixed with 5 mL of distilled water and 500 µL of Folin–Ciocalteu reagent. Up to 1.5 mL of sodium carbonate solution (20% *w*/*v*) was added, and the mixture was made up to 10 mL with distilled water. The mixture was shaken vigorously and incubated in the dark at 40 °C for 30 min. Absorbance was measured at 765 nm on a Varian Cary 50 UV spectrophotometer (Varian Co., Palo Alto, CA, USA). Prior, a calibration curve was made using standard solutions of Gallic acid. The results were expressed in milligrams of Gallic acid equivalent (GAE) per 100 fresh weight (fw).

2.7. Antioxidant Activity Evaluation

Antioxidant activity (AOA) was evaluated by the procedure (free radical scavenging activity of the extracts against DPPH free radical) described by Oliveira et al. [41]. With some modifications [38], results being expressed as µmol Trolox equivalents (TE) per 100 g fresh weight (fw). A total of 50 µL of tomato extract was mixed with 3 mL of 0.004% DPPH methanolic solution. The mixture, vigorously shaken, was kept 30 min in darkness. Absorbance was measured at 517 nm on a Varian Cary 50 UV-VIS spectrophotometer. A blank sample was made by mixing methanol with DPPH solution instead of extract. The results were calculated according to the formula:

DPPH scavenging activity (%) = [1 − absorbance of sample/absorbance of blank] × 100.

2.8. Color Evaluation

The color of tomato was evaluated by measuring the lightness L*, redness a* yellowness b*, chroma and hue angle values of the CIELab system using a Thermo Scientific Evolution 600 UV/VIS spectrophotometer, with quartz cuvettes of 1 mm. According with Kumar et al. [42], L is the lightness ranging from 0 (black) to 100 (white); a is a scale ranging from −100 (green) to +100 (red); and b is a scale ranging from −100 (blue) to +100 (yellow). The hue angle denotes the hue of the color, with the following values: red-violet: 0, yellow: 90, blue-green: 180, and blue: 270. Chroma expresses the saturation of the color and with it the degree of difference of a hue compared to a grey color of the same brightness is determined [43].

In addition, the a*/b* ratio and color index (CI) were calculated by the following formula [43]: CI = 2000 $\frac{a}{L\bullet\sqrt{a^{2}+b^{2}}}$ The data reported were the mean set of three determinations on different fruits, each one consisting of three measurements on opposite points of the tomato.

2.9. Bacteriological Analysis

Determination of the number of mesophilic aerobic bacteria was conducted according to the method described by [44]. The 10 g samples of tomato from each variant were sterile collected and placed in sterile BagLight 400 bags homogenized with 90 mL sterile peptone water. The homogenization of the samples was performed in a stomacher type BagMixer, Interscience for 2 min at maximum speed. Decimal dilutions were made from this sample, up to 10^{-5}, using two successive dilutions in the inoculation. The inoculation was carried out with 1 mL of the dilutions considered optimal using a culture medium (GranuCult Merk nutrient agar) which allowed the growth of aerobic mesophilic bacteria (TAMB). Sterile 90 mm Petri dishes were used. Incubation was carried out at 30 °C for 48 h. All colonies developed on the surface of the medium were counted and the result was converted to log CFU/g. From the colonies that exhibited the same colonial morphology characters, stained Gram smears were performed in order to determine what kind of bacteria were present in each sample. Tests were conducted in triplicate, mean values per sample being used for statistical analysis.

2.10. Sensory Analysis

The sensory evaluation of the tomato at the end of the storage was performed with voluntary students of the Horticultural Faculty of the University of Craiova. Each student individually completed an organoleptic tasting sheet. The evaluation was carried out using a 5-point hedonic scale, with 1 meaning "dislike totally" and 5 meaning "like totally". The attributes considered were: taste, color and general acceptance. The students who carried out the tasting were not given any information about the samples assessed, as they were coded with numbers. People were also asked to rinse their mouths with distilled water before each tasted sample.

2.11. Statistical Analysis

All the tests and analysis were conducted in triplicate, mean values per sample being used for statistical analysis. The results were statistically analyzed using Statgraphics Centurion XVIII (Statgraphics Technologies, Inc., The Plains, VA, USA).

In order to summarize the variability in the datasets, standard deviation was used, utilising Microsoft 365 Excel and data are presented as means ± SD. To see if there were significant differences between the means of the treatments used and the three storage periods, we used one-way ANOVA, using $p < 0.05$ to test the null hypothesis, followed by a least significant difference (LSD) test for establish the statistical difference at the 0.05 significance level.

3. Results

3.1. Components of Basil Essential Oil

The BEO was analyzed in order to identify the main components. From the chromatogram (Figure 1), the following main components were identified: eucalyptol, linalool, estragol, eugenol methyl-cinamate, trans-α-bergamotene, germacrene D, γ-cadinene and T-cadinol (Table 2), which is according with data from the literature [9,10,45].

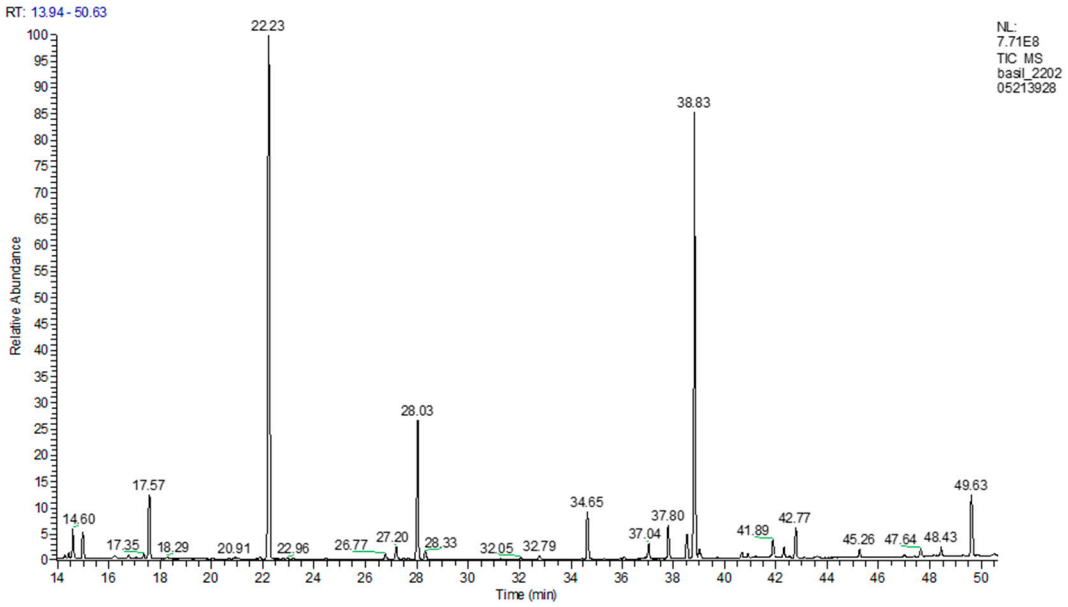

Figure 1. Chromatogram of the components identified in BEO.

Table 2. Main components of BEO.

Rt	Name	RI	Match
17.57	Eucalyptol	1032	940
22.23	Linalool	1099	936
28.03	Estragole	1196	952
34.65	Methyl-cinnamate	1302	923
37.80	Eugenol	1358	932
38.52	Trans-α-Bergamotene	1435	890
38.83	Caryophyllene	1327	938
41.89	Germacrene D	1481	908
42.77	γ-Cadinene	1513	931
49.63	T-Cadinol	1640	927

3.2. Weight Loss

Weight loss of tomatoes increased steadily ($p < 0.05$) during storage (Figure 2). In the control, the greatest weight loss was observed in the last 14 days of storage. During this period, the weight loss increased by 9.32%. In the BEO aqueous solution-treated variants, the weight losses were much lower compared to the control and glycerol-treated variants. The values recorded at the end of storage (21 days) were close to those recorded for the control and glycerol-treated variant only after 14 days of storage. As regards the differences between the variants, the highest value of the weight loss was found in the control (12.80%), followed by V1 (9.67%), and the lowest value in the V7 variant (3.95%). It is observed that the weight loss in the control was significantly ($p < 0.05$) higher than in the BEO-treated variants. It

is also observed that at small differences in the concentration of BEO used, the differences between the variants were insignificant in terms of weight loss (7.27% in the variant treated with 100 ppm BEO, respectively, 6.74% in the variant treated with 150 ppm BEO).

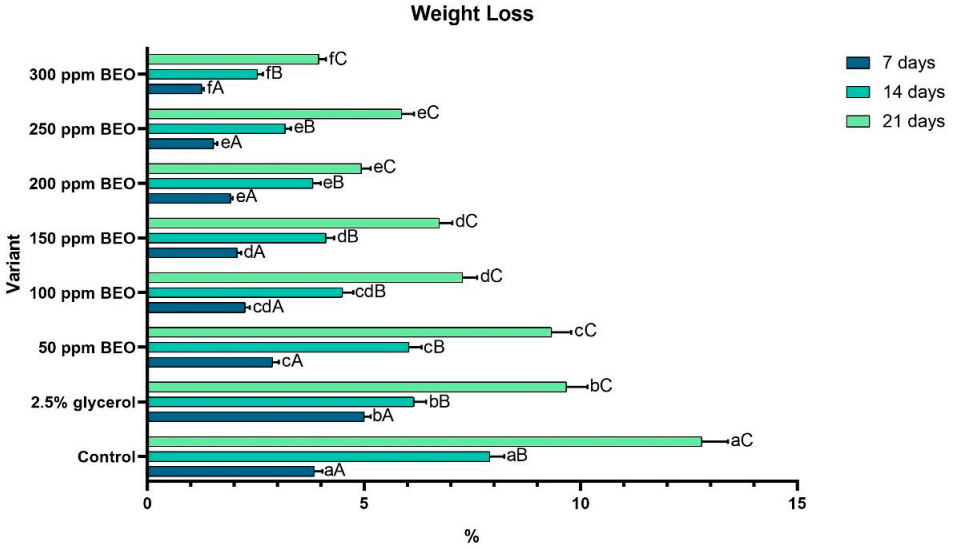

Figure 2. Effects of applied treatments on weight loss (%) during storage for 21 days at 8 ± 1 °C.

Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$); Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

3.3. Dry Matter (DM %)

The tomato DM content is low, this being affected as well by growing conditions and growing season (Figure 3).

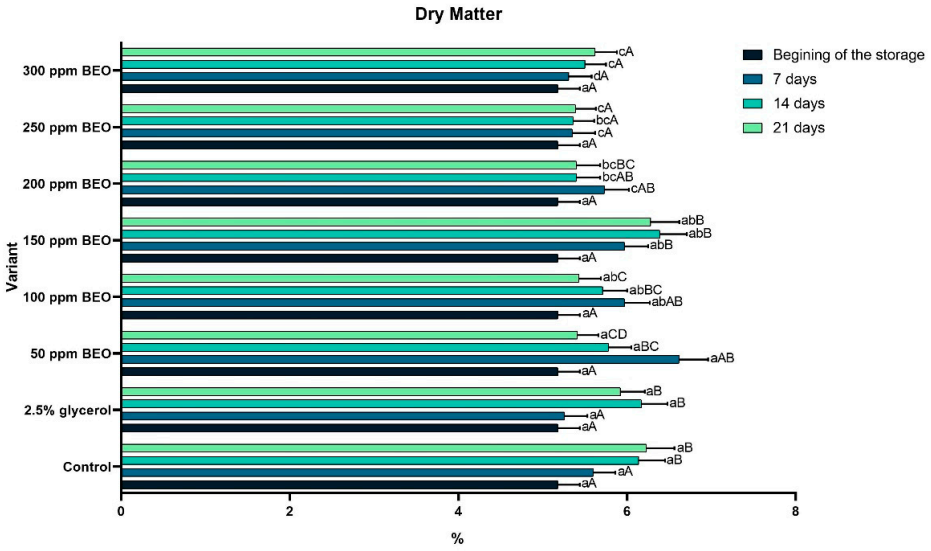

Figure 3. Effects of applied treatments on dry matter (%) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

In the beginning of the experiment, DM content of tomato was 5.18%. During storage, the dry matter content increased significantly ($p < 0.05$) with the highest values found in the control (6.23%) and glycerol-treated variants (V1 = 5.92%). The same increasing trend was observed in the BEO-treated variants, with significant differences ($p < 0.05$) between variants. At the end of storage, the lowest dry matter content was found in the 200 ppm (5.40 ± 0.28 %) and 250 ppm (5.39 ± 0.2%) BEO-treated variants. It is thus observed that BEO spray treatments had a positive effect on the dry matter content of tomatoes during storage. The treatments applied kept water in the tomato cells, maintaining almost constant values.

3.4. Total Soluble Solids Content (TSS %)

TSS ranges from 4.8% (beginning of the experiment) to 5.3% (Figure 4). At the control and V1, TSS increased slowly up to 21 days of storage.

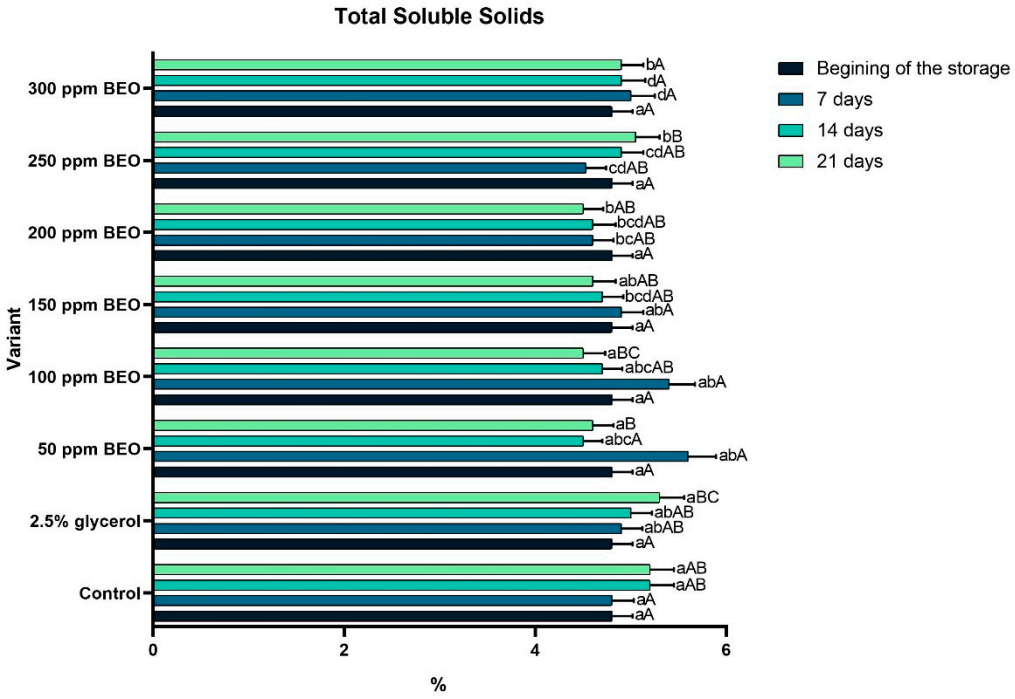

Figure 4. Effects of applied treatments on total soluble solids (%) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

Significant differences ($p < 0.05$) were found between the variants at the end of storage, both in terms of the influence of the storage period and the treatment applied. However, the variants treated with lower concentrations of BEO (50, 100, 150 and 200 ppm) had close values of TSS content at the end of storage (V2 = 4.6%; V3, V5 = 4.5%; V4 = 4.6%). The lowest TSS content at the end of storage was observed in the variant treated with 300 ppm BEO (4.9%). It is also observed in this variant that in the last 14 days of storage, the content in the TSS remained the same, unlike the other variants.

3.5. Total Phenolic Content (TP, mg GAE/100 g fw)

In the beginning of the experiment, TP was 30.62 mg/100 g fw GAE which is a low value compared with other fruits, vegetables and tomatoes grown in the field; Lutz, Hernández and Henríquez [46] reporting contents of 2.3 mg/g GAE (Figure 5). The lowest TP value was found in the control and the variant treated only with 2.5% aqueous glycerol

solution (52.18 mg/100 g fw GAE) and the highest value in the variants treated with concentrations of 200, 250 and 300 ppm BEO (55.0, 54.37 and 57.81 mg GAE/100 g fw).

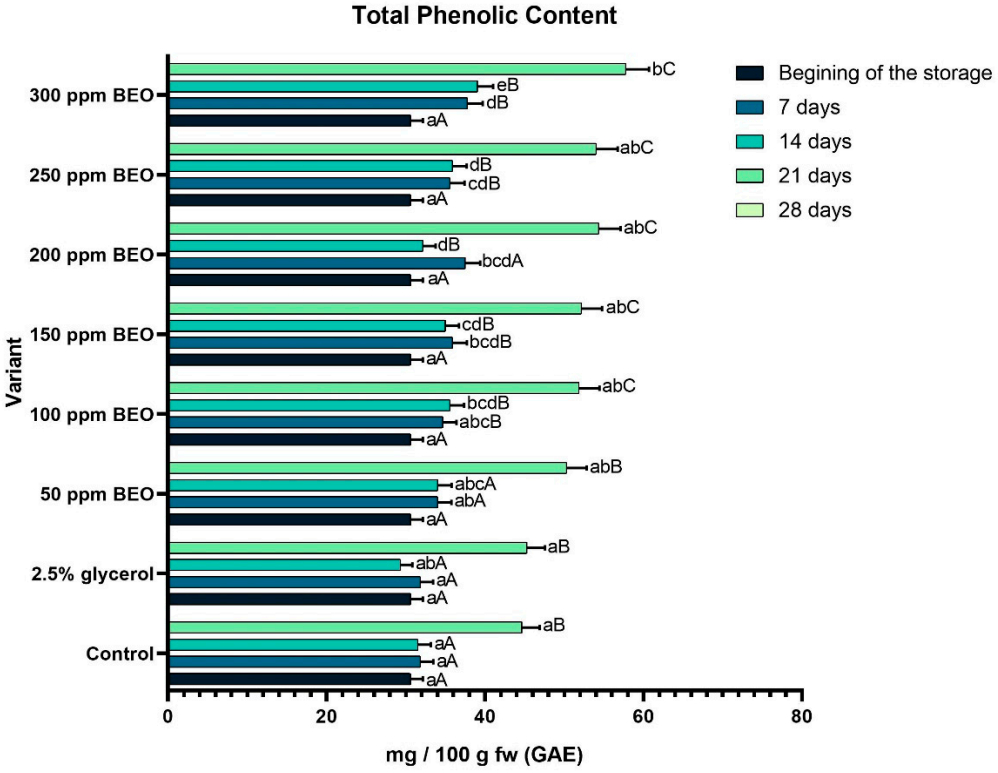

Figure 5. Effects of applied treatments on the total phenolic content (mg GAE /100 g fw) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

3.6. Antioxidant Activity (AOA, µmol TE/100 g fw)

Tomatoes belonging to the variety Rosalina Rossa F1, grown in protected cropping (plastic tunnels), showed relatively low AOA values (54.10 µmol TE/ 100 g fw). Analyzing the AOA of the tomatoes after 21 days of storage, it can be seen that the highest values were found in V5 (88.90 µmol TE/100 g fw), V6 (94.3823 µmol TE/100 g fw) and V7 (119.23 µmol TE/100 g fw). The variants treated with 200 (V5), 250 (V6) and 300 (V7) ppm solutions of BEO proved to be the most effective after 21 days of storage (Figure 6).

3.7. Color Variation

During storage, CIE a* value ranged from 24.39 to 27.93, which shows that the tomatoes changed color from light pink to dark pink to red. The b* value ranged from 15.89 to 26.38 and lightness value varied from 36. 84 at the beginning of the storage to 33.77 at the end of the storage (Figure 7). It was found that the color index (CI) (Figure 8) increased with the storage period (from 45.48 to 47.01 at the control after 21 days of storage). Chroma (Figure 9) and hue angle (Figure 10) ranges at the beginning and the end of storage were 28.61–38.64 and 31.66–43.46, respectively. There were significant ($p < 0.05$) differences between variants during storage and at the end of it, for all color parameters. Treatments with BEO solutions slowed down the color modification of tomatoes, the lowest color index being found in V4 (150 ppm BEO solution). The a*/b* ratio (Figure 11) values ranged from 1.53 to 1.06 at the control.

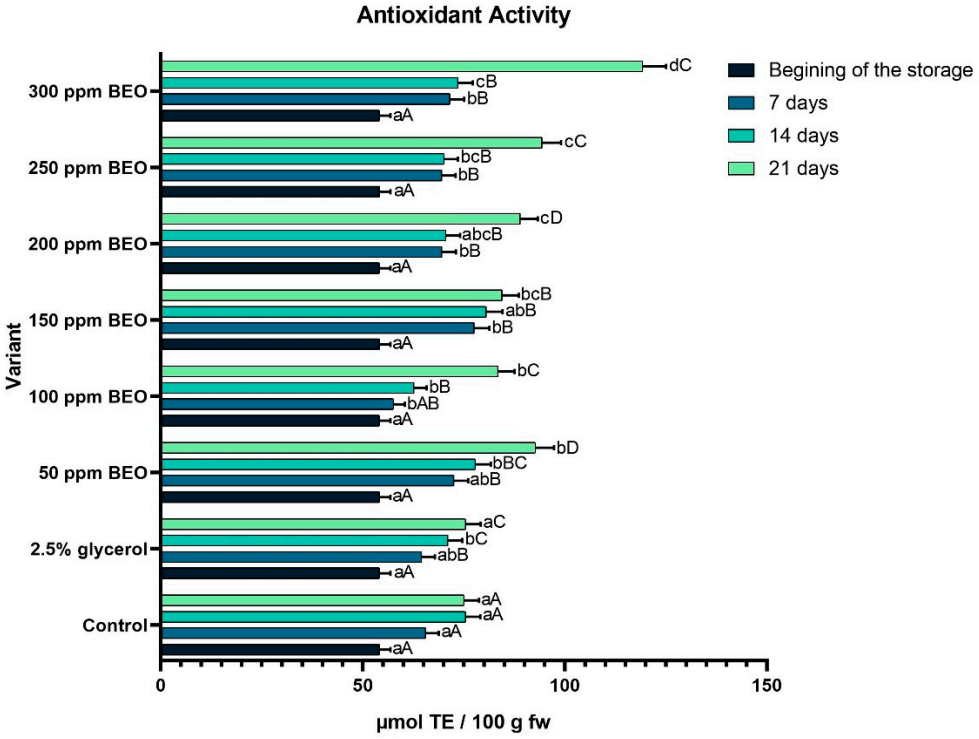

Figure 6. Effects of applied treatments on the antioxidant activity (µmol TE/100 g fw) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

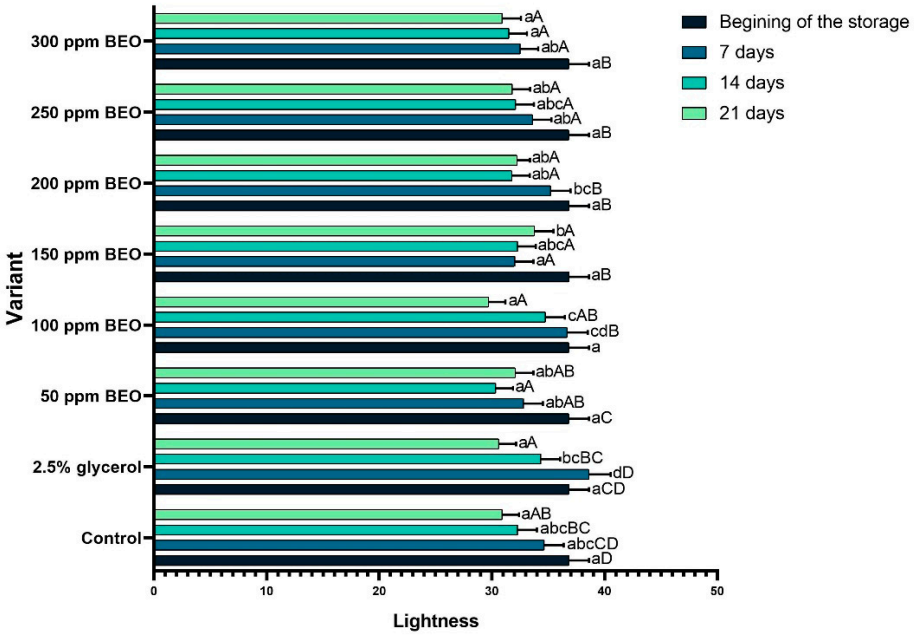

Figure 7. Effects of applied treatments on the Lightness (fruit color parameter) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

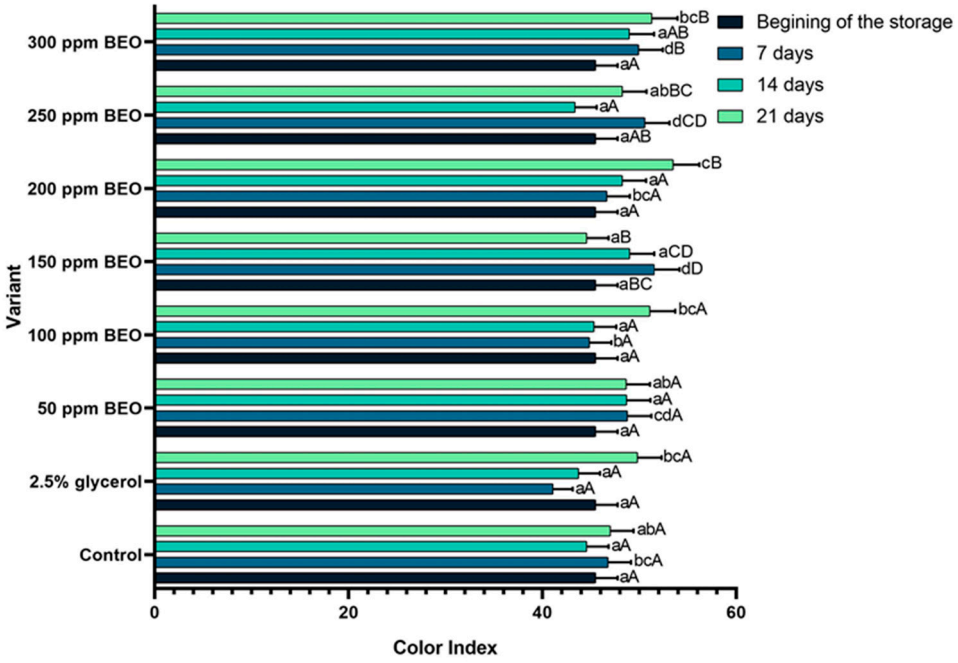

Figure 8. Effects of applied treatments on the Color index (fruit color parameter) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

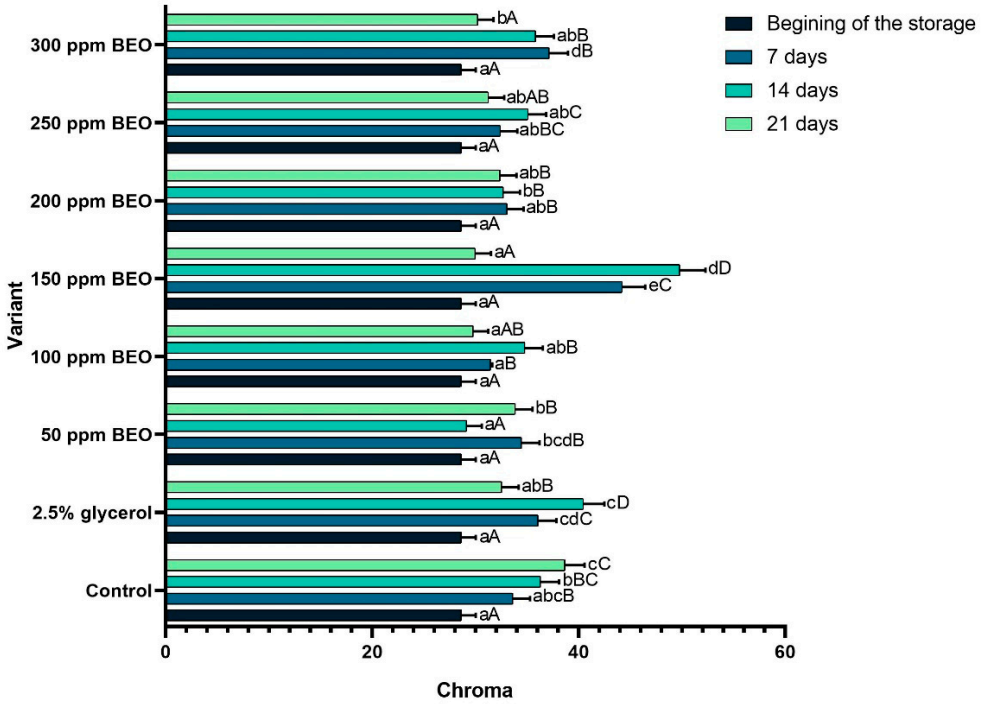

Figure 9. Effects of applied treatments on the Chroma (fruit color parameter) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

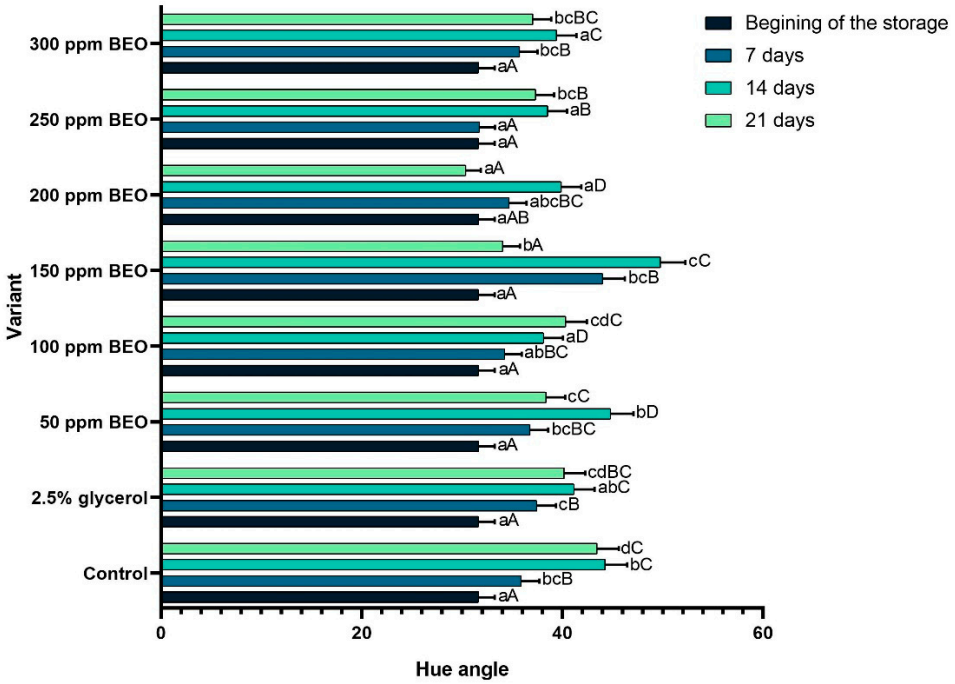

Figure 10. Effects of applied treatments on the Hue angle (fruit color parameter) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

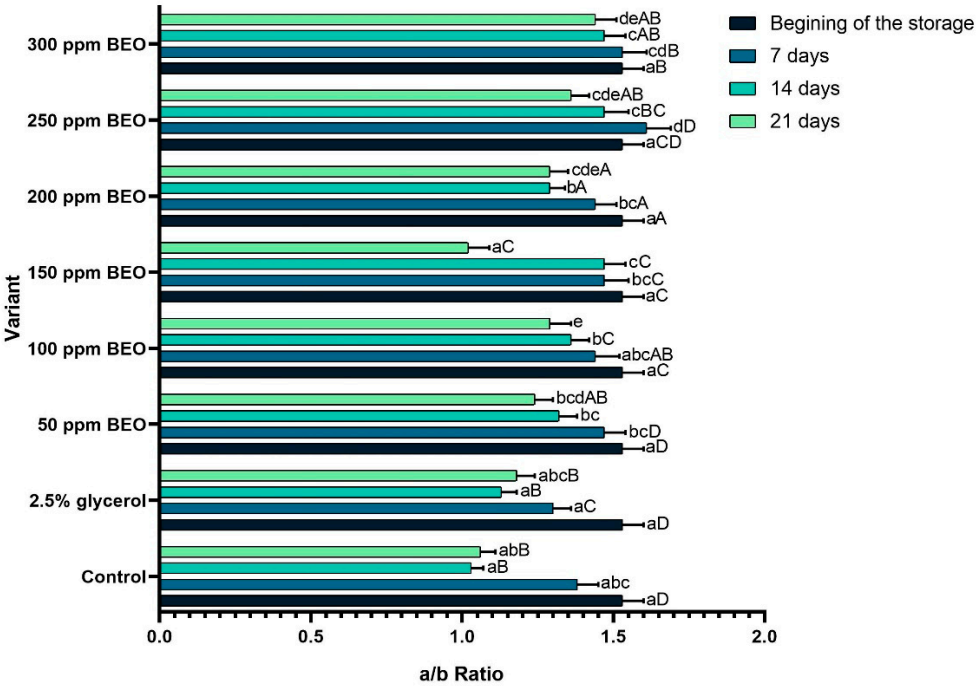

Figure 11. Effects of applied treatments on the a*/b* ratio (fruit color parameter) during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

3.8. Bacteriological Analysis

Previous studies have highlighted the antimicrobial activity of basil essential oil with a pronounced effect on numerous strains of gram (+) bacteria (*Staphylococcus aureus*, *Streptococcus*), gram (-) bacteria (*Escherichia coli* and *Salmonella* spp.) and the fungus (*Candida albicans*) [47]. Before BEO treatment, the total number of mesophilic aerobic bacteria (TAMB) in the tomato was 5.82 (log CFU/g). Compared to the control, a decrease of 4.05 log CFU/g was detected (Figure 12). It is observed that bacteria are inhibited at values higher than 100 ppm BEO.

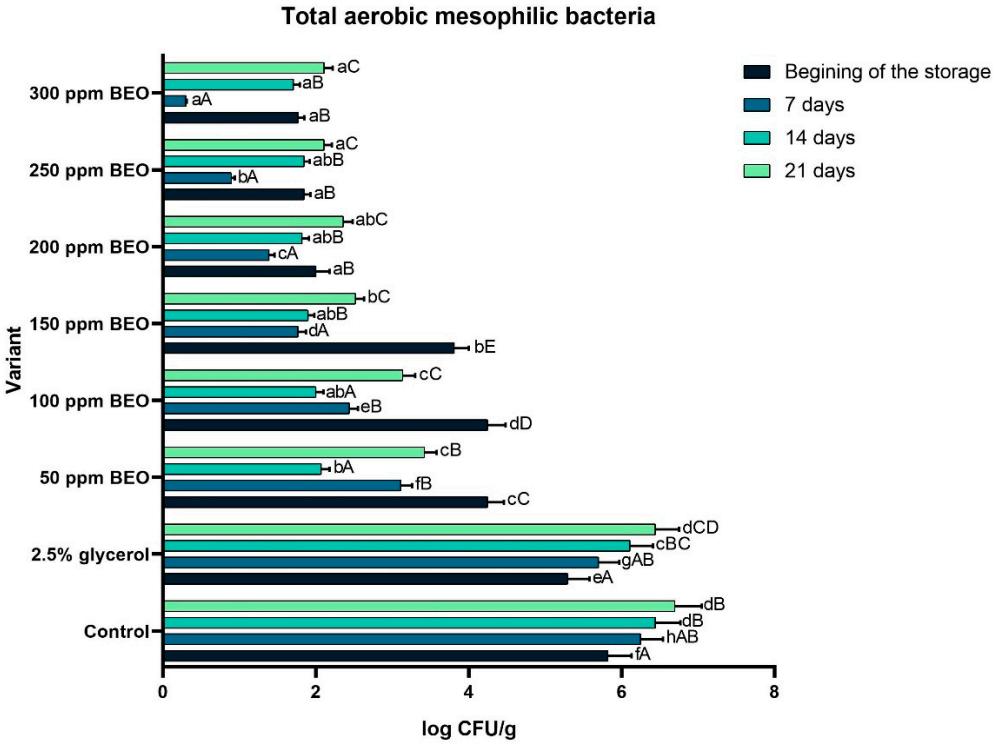

Figure 12. Effects of applied treatments on the total number of aerobic mesophilic bacteria during storage for 21 days at 8 ± 1 °C. Bars for the same sampling time followed by different lowercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$). Bars for the same treatment during storage time followed by different uppercase letters are significantly different based on the least significant difference (LSD) test ($p < 0.05$).

3.9. Sensory Analysis of Tomato at the End of Storage

The results of the sensory analysis of the tomatoes at the end of storage are shown in Figure 13. Treatments with BEO aqueous solution on tomatoes resulted in significant effects on their taste, color and general acceptance.

In terms of taste, the control variant received the lowest score (2.01), while the variant treated with 250 ppm BEO received the highest score (4.41). Regarding the color, the control variant and that treated only with 2.5% glycerol received the lowest score (2.25 and 2.62, respectively) while the variants treated with 250 and 300 ppm BEO received the highest score (4.85 and 4.50, respectively).

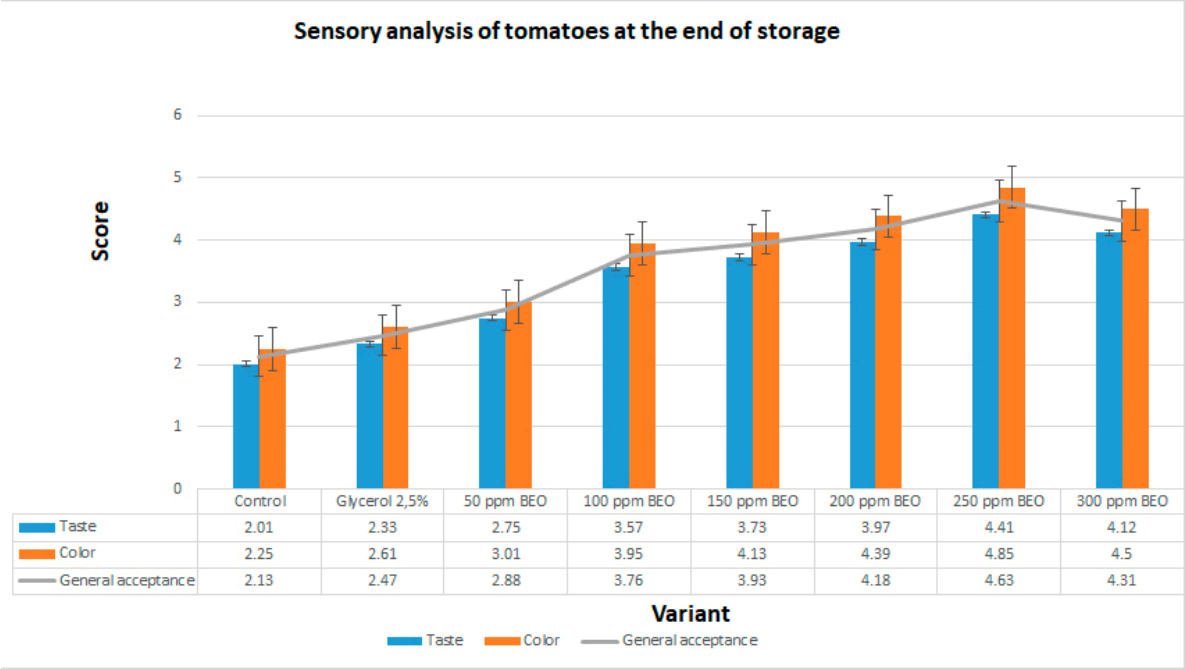

Figure 13. Sensory analysis of the tomato at the end of storage.

4. Discussion

The main identified components in BEO were eucalyptol, linalool, estragole and eugenol. The high amount of linalool and estragole are characteristics of the European chemotype, just as Lawrence et al. [48] classified basil based on essential oil composition [49]. Linalool, eucalyptol and caryophyllene were responsable for the exhibition of antimicrobial activity [50,51].

Tomato weight loss increased steadily during storage with significant differences between untreated and spray-treated variants. During storage, the biggest weight loss was recorded in the last 2 weeks for all the variants studied, significant increase of weight loss being observed with storage time. Similar results were observed by Soto-Zamora et al. [52] and Pinheiro et al. [53]. In fact, according to the data obtained, the control and V1 variants, after 3 weeks of storage, no longer showed the quality parameters required for fresh consumption. It was shown that spraying the fruit with solutions of different concentrations of BEO have significant effects on weight loss during storage. Additionally, the oil concentration of the solution used influences the weight loss of tomatoes during fresh storage.

DM content is affected by storage time and by the applied treatments. It is found an increase of DM content is related to water loss through evapotranspiration. Similar values of DM were reported by Alenazi et al. [54] and Tilahun et al. [17].

The major components of the soluble solids are sugars and acids which affect the taste and flavor of tomatoes (Tilahun et al. [17]). TSS is affected by the storage time. After 14 days of storage the TSS decreased, remaining almost constant for the next 7 days. It was thus found that the variants treated with a solution with concentrations of 250 and 300 ppm of BEO had a positive effect in maintaining water and TSS in the tomato cells during storage.

Phenols are molecules with antioxidant properties in fruits and vegetables [17] and the importance of TP as a component of AOA has been previously documented [16,18,21,23].

During storage, TP increases steadily with a peak at 21 days of storage. The same trend was observed in all experimental variants. These data are in correlation with those found by Raffo et al. [55], who mentioned that TP increase occurs in the late stages of tomato ripening. In terms of the effect of the spray treatments, it is observed that the TP increases proportionally to the concentration of the BEO used. Significant differences were

found between the control, V1 and the variants treated with 250 and 300 ppm BEO (V6 and V7). Moreover, it is found that in the late stages of ripening, the variants treated with BEO at concentrations of 250 and 300 ppm recorded minor decreases in TP, which remained almost constant.

AOA varies in tomatoes depending on genotype, phenophasis, maturity and fresh-storage period [21,23]. AOA analysis represents the most common analytical approach in food science [56]. During storage, AOA increased in the 21 days of storage (Figure 6). The same trend was observed in both the control and the BEO-treated variants, which is consistent with the statements of Hanson et al. [23]. This increase may be due to the accumulation of lycopene during fruit ripening. At full maturity AOA also falls, due to the decrease in phenolic content, the correlation between them being known [21,23]. Differences between AOA were also found due to the treatments applied, with BEO contributing to the maintenance of higher AOA values.

The color of tomato fruit is an important quality factor valued by consumers [57]. The analysis of tomato color during storage is very important to identify the stage of ripening [58]. During storage, tomatoes change color, which is associated with ripening and ethylene production [59]. Treatments with BEO solutions slowed down the color variation of tomatoes. According to Ciptaningtyas et al. [59], the alteration of the color of tomatoes occurs due to the accumulation of some pigments such as anthocyanins, flavonoids and betaines. The a*/b* ratio of the components of the CIE L*a*b* color space, which is associated with the green-red color, had the highest variation during storage. It was found that the a*/b* ratio slowly decreased the 21 days of storage. It was found that in the variants treated with BEO solutions, the a*/b* ratio had a less dramatic decrease. The variants treated with solutions in concentrations of 200 (V5), 250 (V6) and 300 (V7) ppm BEO showed the highest a*/b* ratio values. The increase of the chroma value at the end of storage showed a higher color intensity of the samples perceived by the naked eye. These values demonstrate the maturation of tomatoes during storage. However, the results obtained show that BEO treatments slow down the maturation processes during tomato storage. Thus, the chroma value in the 250 and 300 ppm BEO-treated variety was significantly lower than in the control and glycerol-treated variants. It shows that treatment with BEO solutions delays the ripening of tomatoes during storage while maintaining the color of the fruit.

Immediately after spraying the tomatoes with BEO and weaning them, it was found that the TAMB decreased considerably, the best results being recorded in the variant treated with 300 ppm BEO in 2.5% aqueous glycerol solution. During storage by refrigeration, the decrease in TAMB occurred only during the first 7 days, after which a proliferation of psychrophilic bacteria occurs. After 14 days of storage, the TAMB values are simi-lar to those determined immediately after the application of the treatment, for variants treated with 200, 250 and 300 ppm BEO in 2.5% aqueous glycerol solution. For variants treated with 50, 100 and 150 ppm BEO in 2.5% aqueous glycerol solution, the TAMB continues to decrease, after which a proliferation of bacteria appears. The same decreasing trend in bacterial number within the first hours after the application of high concentration essential oil treatments was also reported by Rowland et al. [60]. In the untreated BEO variants (control and V1) the bacteria grew continuously for the entire storage period. Microscopic examination in Gram-stained slides, showed in all BEO-treated variants the presence of sporulated bacteria of the genus *Bacillus* spp. Some unsporulated gram (−) *Bacillus* and gram (+) *Bacillus* belonging to *Staphylocccus aureus* species were also found in the untreated BEO variants (control and V1). Similar results were reported by Bello et al. [61].

As a result of the sensory evaluation, it was determined that people scored close to the maximum for the 250 ppm BEO-treated variant. The tasters also mentioned that the 300 ppm BEO-treated variant had a slight unidentified taste and therefore gave a lower score than the 250 ppm BEO-treated variant.

5. Conclusions

Spray treatments of tomatoes with aqueous solutions of glycerol (2.5%) and different concentrations of BEO extend the storage of tomatoes by retaining water in the cells (from 93.77% in control to 94.61 at the variant treated with 250 ppm BEO) and slowing down the ripening process. These treatments also slow down the degradation of phenolic substances at full maturity (from 52.18 mg GAE/100 g fw in control to 57.81 mg GAE /100 g fw in the variant treated with 300 ppm BEO), thus maintaining the antioxidant activity of the tomatoes.

Spray treatments with BEO on tomatoes can extend their storage up to 21 days at 8 °C and 85% relative humidity. The most effective concentrations of aqueous BEO solution were 200, 250 and 300 ppm. However, the 300 ppm BEO-treated variant received a lower score in the sensory analysis than the 250 ppm BEO-treated variant due to the slightly changed taste of the tomatoes as a result of the treatment.

BEO concentrations higher than 200 ppm decrease in TAMB during the first 7 days of storage (from 5.82 log CFU/g before treatment to 1.39 in variant treated with 200 ppm BEO to 0.3 log CFU/g in variant treated with 300 ppm BEO), followed by a proliferation during the next 14 days of storage, but are still less in value than before treatment (2.55 log CFU/g). The untreated BEO variants (control and V1) showed constant increases in TAMB. At the end of the storage period, the TAMB had 2–3 times higher values compared to the BEO-treated variants, which considerably increases the risk of spoilage. The most resistant bacteria to BEO treatment were those of the Bacillus spp.

Spraying tomatoes with aqueous solutions of glycerol (2.5%) and different concentrations of BEO leads to a considerable reduction in the number of microorganisms responsible for fruit decay immediately after treatment. As a result of this reduction in the number of microorganisms, microbiological spoilage and early fruit decline are delayed.

The use of post-harvest treatments by spraying tomatoes with aqueous solutions of BEO and glycerol extends the shelf life of tomatoes by about 7 days. The advantage of these treatments is to reduce the microorganisms in the fruit and delay the appearance of microbiological spoilage. It also has the advantage of using natural products as opposed to using chemicals that remain in the product.

The concentrations of BEO used are relatively low (ppm) and do not affect the taste and smell and aroma of the fruit except at doses of 300 ppm.

Spraying tomatoes with such solutions can be practically applicable because it does not require major interventions in the storage technology flow. These treatments can be carried out in the conditioning room using shower washing facilities. However, the oils used must have a known origin and be natural, from organic crops.

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