The ethanol dip and vapor delays chilling injury of Cucumber (*Cucumis sativus* L. cv. Ceylan),

during storage.

Ethanol treatments for decreasing chilling injury of cucumber

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Abstract

In this study, the effects of ethanol which is applied in different forms as liquid and vapor after harvest on chilling injury, color and overall quality of cucumber (*Cucumis sativus* L. cv. Ceylan) during storage were examined. For the liquid ethanol treatments, cucumber fruits were immersed in water comprising different concentration of ethanol for 5 minutes. In order to apply ethanol in the form of steam, a pad was firstly impregnated with ethanol at different doses. This pad was placed in a polystyrene foam dishes, and after the cucumber fruit is placed on it, the dishes wrapped with stretch film. The doses for used the both ethanol treatment as: $\text{Control}_{\text{dip}}$(CD), $\text{Control}_{\text{vapor}}$ (CV), 200 µL/L, 400 µL/L , 800 µL/L and 1600 µL/L. It was concluded that the liquid ethanol treatment at the dose of 1600 µL/L was effectively delayed of chilling injury, softening and decay rate.

Key Words: *Cucumis sativus* L., ethanol, chilling injury, firmness, decay.

Subject Classification: Postharvest Technology of Vegetables, Horticulture

Type (Method/Approach): Research Article

Supporting Agencies: Kocaeli University

Date of Publication: 30.05.2018

DOI: 10.24297/jaa.v8i1.7399

ISSN: 2349-0837

Volume: 8 Issue:1

Journal: Journal of Advances in Agriculture

Website: https://cirworld.com

Introduction

According to the data of 2016, about 1.075 billion tons of vegetables are produced in the world. Turkey is taken place 4th rank after China, India and the USA in vegetable production of the world [1]. Approximately 28 million tonnes of vegetables is produced by Turkey, and 85% portion of these comprised fruity vegetables [2].One of the major vegetable kinds into the fruity vegetables is cucumber, both in the Turkey and the World. Cucumber production in our country is about 1.8 million tons [2]. Whereas cucumber is widely freshly consumed, pickle production is the second important use area. Due to the high water content (96.7%) [3] and rapid growth, the quality loss of the cucumber is rapidly increased after harvest [4]. The most important technique used to reduce post-harvest losses is rapidly cooling of the crop [5]. However, in many types of vegetables, including cucumbers, even decreasing the temperature to just above freezing point causes the formation of a physiological disease called chilling injury [6].

Chilling injury (CI) begin under the temperature of 6-7^{o}C in cucumber fruits. Symptoms are surface pitting, regional yellowing, shrinking that begin and spreading near the stalk, and the formation of gel on the surface. In the later stages of the disease, an infection occurs and the fruit rot [4]. CI is prevented by storage in controlled conditions; however, low temperatures during production, harvest, transportation, marketing and storage on uncontrolled conditions cause CI. For this reason, various applications are being made to prevent the CI that occurs in uncontrolled temperatures.

Ethanol is not only a reliable antiseptic but also an agent that can be effective in delaying aging in tissues [7, 8]. Forexample, it was determined that ethanol treatments are effective to delaying ripening in tomatoes [9]; to increase the vase life in carnations, [10]; to controlling the superficial scald of the apples [11]; preventing of wilting in Jeruselam artichoke [12] and extending the shelf life of fresh-cut apple slices [13].

Decay caused by pathological reasons in many fresh fruit and vegetable causes considerable crop losses during postharvest process. It is possible to control diseases that cause rot during postharvest process with ethanol applications different kind of fruits; such as cherries [14], grapes [15], peach-nectarine [16] and mango [17].

Ethanol delays the breakdown of chlorophyll that causes aging in green colored crops. Ethanol has only been studied in broccoli about the effect of preventing yellowing, but no such study has been found in any other species [18, 19, 20, 21]. Moreover, no studies have been conducted on the effects of ethanol on CI.

In this study, we aimed to investigate the effects of two different forms of ethanol on chilling injury, color loss and overall quality of cucumber during postharvest period.

Material and Methods

Plant Material

The cucumber fruits belonging to variety of the Ceylan F1 used in the research was taken from a producer in Kocaeli. Cucumber fruits, which were carried in the laboratory immediately, were examined according to deformation, injured fruit or infection criteria and those not suitable for the experiment were separated.

Ethanol Treatments

Ethanol have been treated to Cucumber fruits in two different ways as vapor and liquid. For vapor treatment, 10 mL of ethanol at different doses was sucked into the pads, and then the pads were placed in a polystyrene (PS) foam dish. The cucumber fruids were placed in PS foam dishes prepared, and wrapped in stretch film. The ethanol was applied by release from ped during storage. The doses of ethanol used are as follows: [200 µL/L (V200), 400 µL/L (V400), 800 µL/L (V800), 1600 µL/L (V1600)]. Cucumber fruits treated with only water steam without ethanol were used as controls (CV).The application of ethanol as a liquid carried out by dipping the cucumber fruits into the water containing ethanol in different concentrations for 5 minutes. The doses of ethanol used for this purpose are: [200 µL/L (D200), 400 µL/L (D400), 800 µL/L (D800), 1600 µL/L (D1600)]. Cucumber fruits dipped only water (CD) were used as controls. Cucumber fruits were dried on drying paper at room temperature, after dipping treatment, and then packaged with PS foam dishes explained as above.

Storage Conditions

Packaged cucumber fruits were stored in cold room containing 4 ± 1°C temperature and 90-95% relative humidity

Texture Analysis

Shimadzu LX Tekture analyzer was used for this purpose. Texture measurements were made from three different points of the cucumber fruit using 3 mm piercing probes and expressed as Newton (N).

Color Measurement

The skin color of the fruit was measured using the Minolta CR 400 (Minolta Co., Osaka, Japan) Chroma portable colorimeter with a D65 lamp. The color of the fruit is determined by the coordinates of the *L*, a*, b** color space (CIELAB). Color meter calibrated with white standard calibration plate [22, 23]. Furthermore, with using of (L *, a *, b *) values, Hue angle values were calculated by the following equations [24]. Measurements were made at three different points of each fruit, and at least five fruit for every replicate.

$$H = 180 + \arctan\left({b*}/{a*}\right)$$

Electrolyte Leakage

For electrolyte leakage measurement, 5 mm thick discs were cut from cucumber fruits, and the cut samples were washed twice with 50 mL of distilled water. 50 mL of water was added to the same samples for the last time, then allowed to stand at room temperature for two hours and after that the electrical conductivity was measured. In the samples frozen and dissolved, the electrical conductivity value was measured again when sample temperature reached 18 ° C, and calculated as% [25].

Total Soluble Solids (TSS)

In fruit juice, the amount of water soluble total dry matter was measured as % using Atago DR-A1digital refractometer (Atago Co. Ltd., Japan).

Visual Quality Scores

For this purpose, scoring by considering the hardness, appearance, color, brightness and decay of the fruits; It was made by 9 panelists according to the scale of 5 points. According to this; 5-Excellent, 4-Good (very small flaws), 3-Medium quality (medium size flaws), 2-Bad quality (extremely flawed), 1-Very poor quality (unusable).

Decay rate (%)

It was calculated as the ratio of the number of infected fruits to the total number of fruits, and referred (%).

Chilling Injury (CI) Scores

Based on the criteria such as pit formation on the surface, the wrinkle of the end part and yellowing; it was evaluated over 4 points by 9 panelists. According to this; the scores shows that 0: no CI symptoms, 1: 25% symptom of CI, 2: 50% symptom of CI, 3: 75% symptom of CI, 4: 100% symptom of CI.

Weight Loss (%)

The same samples were weighed both at the beginning of the study and each analysis period, weight loss was evaluated as% relative to initial.

Statistical Analysis

The experiment was set up in 3 replications according to the complete randomized design. Variance analysis was performed using SPSS 16.0 statistical program and Duncan test was applied for comparison of the averages.

Results and Discussion

Chilling Injury Scores

The symptoms of CI on cucumber fruit started to be seen from the 15th day of the storage and the severity of the symptoms increased on the 20th day (Fig. 1). In this respect, differences among the treatments average was found significant statistically. In addition, it was observed that in the research the CI scores and the visual quality scores were changed in parallel. The lowest CI was detected in the D1600 application, whereas the highest CI score was found in the D400 treatment. Again, it has been found that the application of ethanol-dip is more effective than vapor treatments for delaying chilling injury. In a previous study, it was determined that vapor ethanol treatment alleviated CI to some extent, prevented the rise in respiration and ethylene [26]. In the present study, no CI symptoms seen until 10th day of storage, but at the day 15, CI symptoms started in all treatments, however, the cucumbers in D800 and D1600 treatments have less CI scores than the other treatments. Electrolyte leakage can be used to determine changes in membrane permeability caused by environmental stress factors such as CI [27, 28]. It is therefore a useful method is measurement the K ion leakage for determining chilling damage, and it has been reported that in cucumbers, an increase in ion leakage is associated with cell damage caused by CI [29, 30, 31]). As a matter of fact, in this study, the low electrolyte leakage of the cucumber in the D800 and D1600 applications shows that these applications reduce the CI damage.

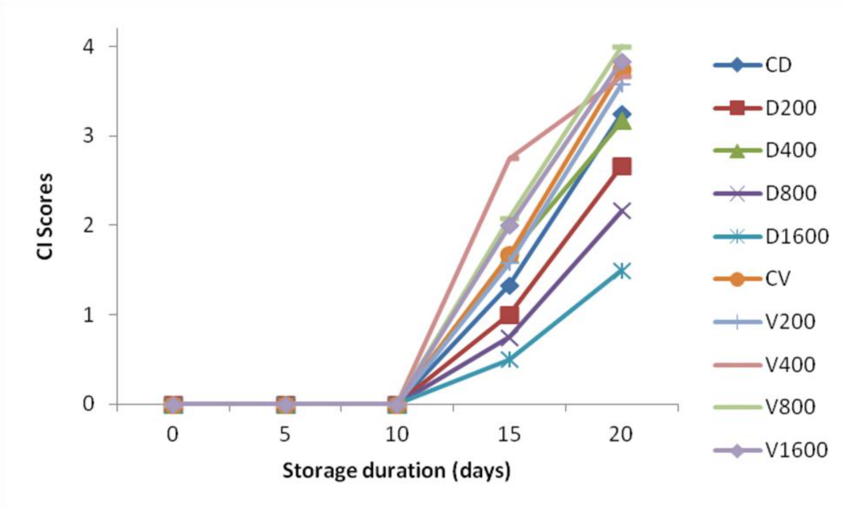

Figure 1. The changes at CI scores of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Decay rate

In the study, the decay rate increased in all treatments from Day 15 of the experiment. (Fig. 2). However, the lowest decay rate was determined in D1600 with 37.50% and the highest rate was obtained in V800 application at 100%, at the end of the storage. In this respect, the difference between D1600 application and other treatments is statistically significant. It has also been found that ethanol-dip applications give better results than vapor application in the control of decay. It is believed that the chilling damage in the fruit of cucumbers, caused incerasing the decay rate of the fruit by reducing the defensive mechanism of the fruit. Because it was previously reported that with prevention of CI by ethanol treatments was delayed decay rate, and also antimicrobial effect of ethanol is important in this phenomenon [16, 15, 14, 17].

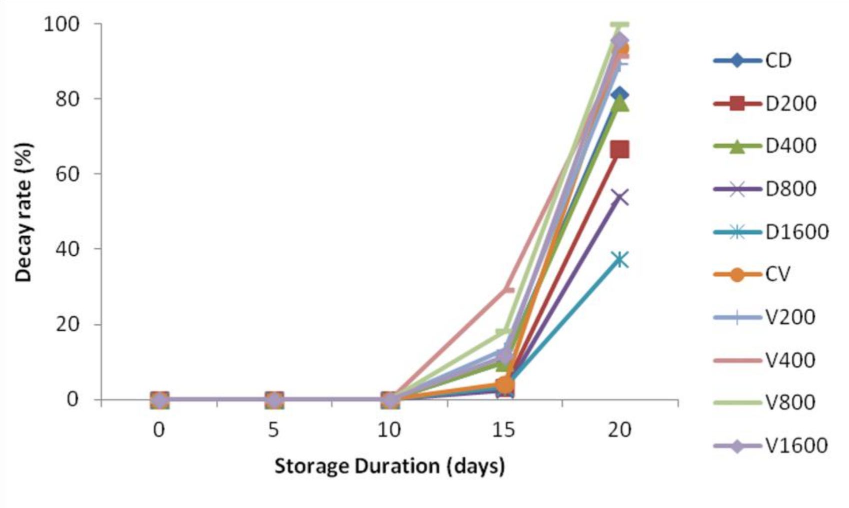

Figure 2. The changes at decay rate of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Fruit firmness (N)

While the firmness value of cucumber fruits was 12.53 N at the beginning of the experiment, it getting increase up to 10th day of storage and was change 14.89-16.03 N in all applications (Fig. 3). After this period, although the value of hardness decreased in all applications, it increased (15.57 N) only in the samples in D1600 application. In general, it can be said that ethanol vapor applications cause more rapid softening in cucumber fruits and immersion applications are more effective in firmness protection. Especially while the D1600 application has been more effective in protecting the hardness, it was followed by D800 and CD. Jin et al. [32] applied vapor ethanol at doses of 0.5 mL/kg and 3 mL/kg to melons and found that both treatments delayed the softening. But in the present study, the vapor ethanol treatments caused softening, whereas the ethanol-dip treatments maintain firmness of cucumber. This result may be related to crops, because we used cucumber in this experiment, and this fruit may be get better absorbed ethanol as liquid whereas, maybe melons could effectively be use vapor ethanol. So we suggests the ethanol-dip treatment could be use for delaying softening for cucumber during storage.

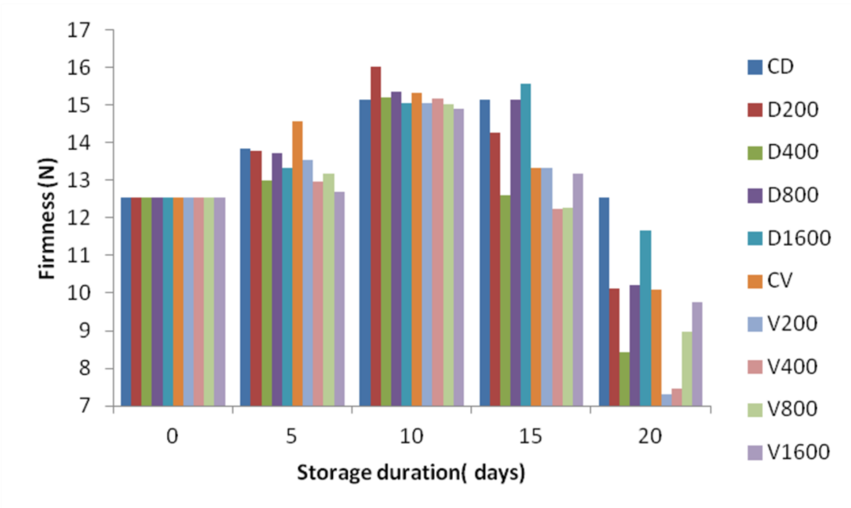

Figure 3. The changes at firmness of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Electrolyte Leakage (%)

The electrolyte leakage changes of cucumbers in all treatments were given as Fig. 4. In cucumber applied with ethanol by both vapor and dipping method, no significant change in electrolyte leakage was observed until the 5th day of storage. However, it has started to increase after the 10th day and reached an average value of 66.76% on the 20th day. There was no statistically significant difference ($P<0.05$) in electrolyte leakage between applications, but it was higher in fruits where ethanol was applied as a vapor. In a previous study it was found that in fresh cut eggplants, the vapor ethanol treatment decreased electrolyte leakage through maintain cell membran integrity [8]. In the present study, the ethanol treatments both dip and vapor decreased electrolyte leakage first fifth days but after that it increased in all treatments during the storage periods. The elecktrolyte leakage of samples treated with ethanol-dip lower than that of vapor treatments. So, it can be said that ethanol-dip treatment more succesfull for maintain cell integrity.

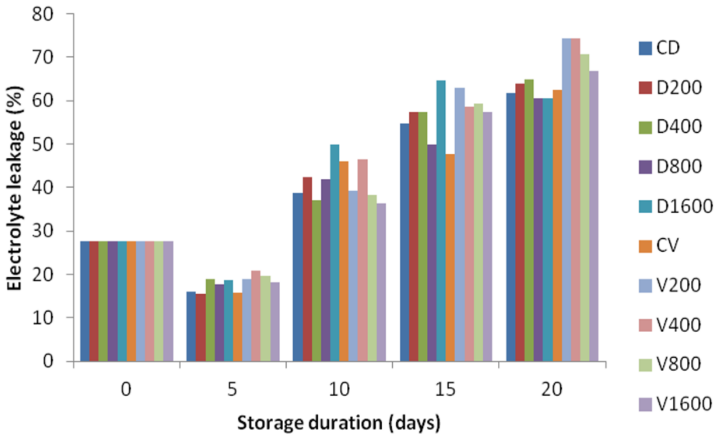

Figure 4. The changes at electrolyte leakage of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Weight Loss

Different ethanol treatments have had different effects on weight loss on cucumber during storage and especially the application of ethanol as a vapor has reduced weight loss (Fig 5). Generally, as storage time increases, weight loss was increased. While the lowest weight loss was detected in the V1600 treatment with an average 2.25%, the highest weight loss occurred in the application of D400 with 3.16% during the storage, and the difference between these two applications was statistically significant at the level $P<0.05$. Ethanol vapor treatment was maintain weight of tomato (at 6 mL kg-1, [33] and also in eggplant [8]. Similary in the present study it was determined that the weight losses of cucumber were delayed with vapor ethanol treatments. Therefore, it was concluded that the vapor ethanol treatment was more effective in regard to delaying weight loss than immersion treatments for cucumber.

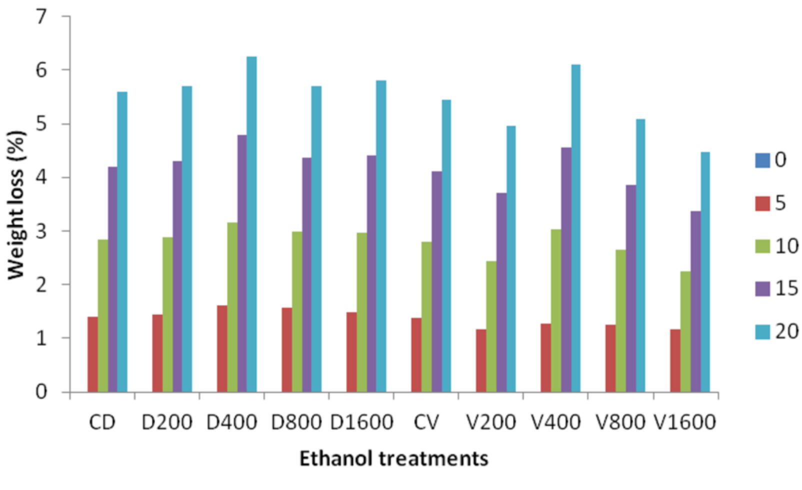

Figure 5. The changes at weight loss of cucumber fruits treated with ethanol both vapor (V) and dip (D). The different colored bares represent storage duration.

Total Soluble Solids (TSS)

It is observed that the amount of TSS of cucumber fruits in all applications decreased during storage (Fig. 6). While the TSS value of samples were 4.25% at the begining of storage, it decreased during storage, and changed between 3.10 to 3.45% at the end of storage, and also the effect of storage time on the amount of TSS was also found statistically significant. When the ethanol applications were examined, the most decrease in the amount of TSS was obtained in CD and D800 treatments with 3.61% and 3.65%, while the highest TSS contents were found in V400 (3.80%), V800 (3.80%) and D400 (3.81%) applications, respectively. Furthermore, while the difference between immersion treatments and control (CD) is found to be statistically significant, the difference between vapor applications and control (CV) was insignificant. It was found by Atta-Ally et al. [34] that the 10% ethanol vapor treatment was significantly reduced total soluble solids content of tomato and also 15% ethanol treatment was caused reduction on TSS, too. But, in this study both immersion (D400;400 µL/L) and vapor (V400 and V800; 400 and 800 µL/L) ethanol treatments provided less decreasing of total soluble solids content of cucumber fruits.

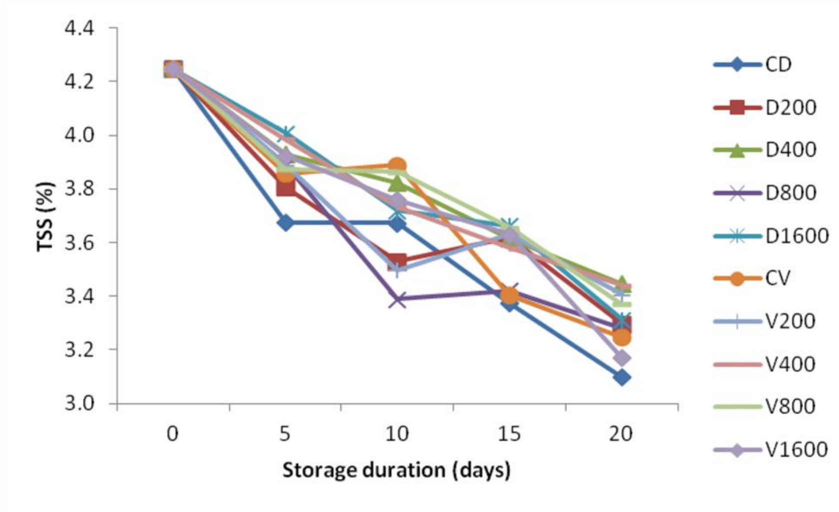

Figure 6. The changes at TSS of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Color b and hue angle values*

An increase in b * is an indication that the products have been yellowed [35]. It has been determined that ethanol applications, both vapor and dipping, control the yellowing. (Fig. 7). While the b* values of samples treated with ethanol-vapor the range 19.89 to 20.04 and it change from 20.32 to 20.59 in the ethanol-dip treatments, b* values of the samples in the control group of both application were found to be higher (CD: 20.89 and CV: 20.99). However, it has also been found that the dipping applications are more effective than the vapor treatments in the control of the yellowing. The lowest b * value in the study was obtained from D200 application with 19.89. The effects of different ethanol applications on hue angle values of cucumber fruits are given in Fig. Accordingly, at the beginning of the experiment, the hue angle value was 123.16, and decreased to the range 120.45-122.35 at the end of the storage period, so that the hue angle values reduce in all applications during storage. Also, the differences among the storage times was found to be statistically significant at the level of $p<0.05$. The hue angle values of cucumber fruits were best preserved in D200 and D1600 treatments. On the other hand, the application of vapor ethanol resulted in decrease of hue angle values, and the difference between the applications was also found statistically significant. Roy et al. [33] found that both 0.5 mL/kg and 3 mL/kg ethanol doses significantly delayed skin color changes of melons. Similary we determined that the both vapor and liquid ethanol treatments delayed yellowing of cucumber fruits, but the immersion treatments effectively than vapor applications. Yanuriati et al. [36] was reported that 2 mL/kg ethanol treatments on tomatoes in two maturity stages (breaker and mature green) significantly slowed down the color changes and the effect of ethanol application is greater when tomatoes stored at 5 °C temperature. Also, in this study cucumber fruit was stored at 4 ± 1°C temperature, and ethanol dip treatment at 200 mL/L effective to control yellowing of cucumber.

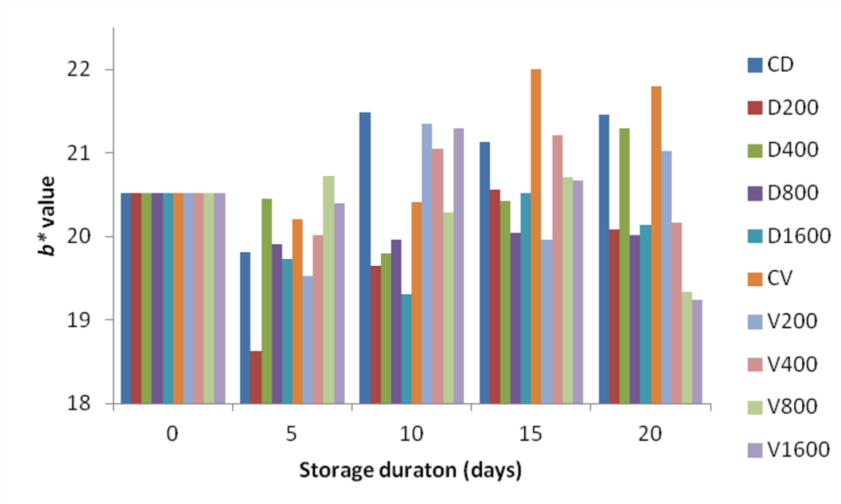

Figure 7. The changes at color *b** values of cucumber fruits treated with ethanol both vapor (V) and dip (D).

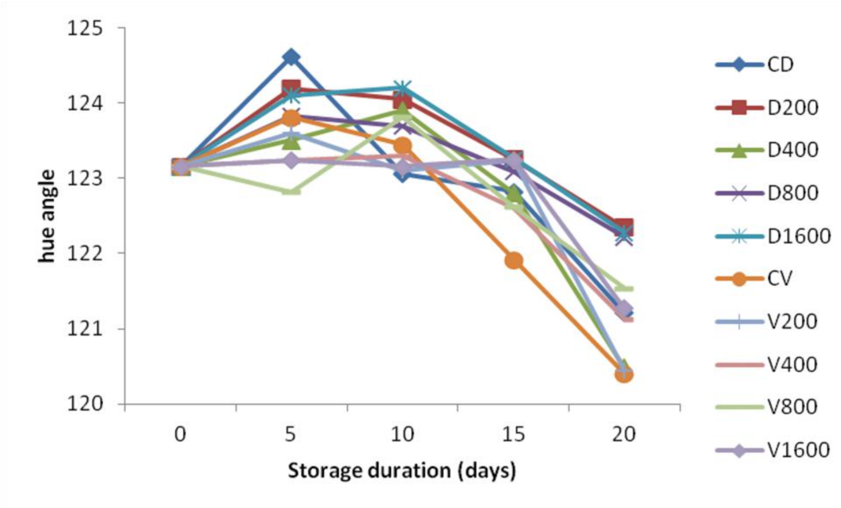

Figure 8. The changes at hue angle color values of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Visual Quality Scores

Visual quality scores decreased during storage in all applications (Fig. 9). While the maximum decrease was obtained in CV (2.86) application, the least decrease was in the applications of D200 (4.01) and D1600 (4.25), and the difference between the applications was statistically significant. It has also been found that ethanol-dip applications are more effective in protecting visual quality than vapor treatments. In the present study liquid ethanol treatment delayed softening of fruit, decreased electrolyte leakage, maintained TSS content and color, so that the improving these characteristics caused increasing visual quality of cucumbers. Furthermore, vapor treatment at the doses of 400 and 800 µL/L contributed improving appearance of fruit through maintaining TSS and decreasing weight losses of fruit.

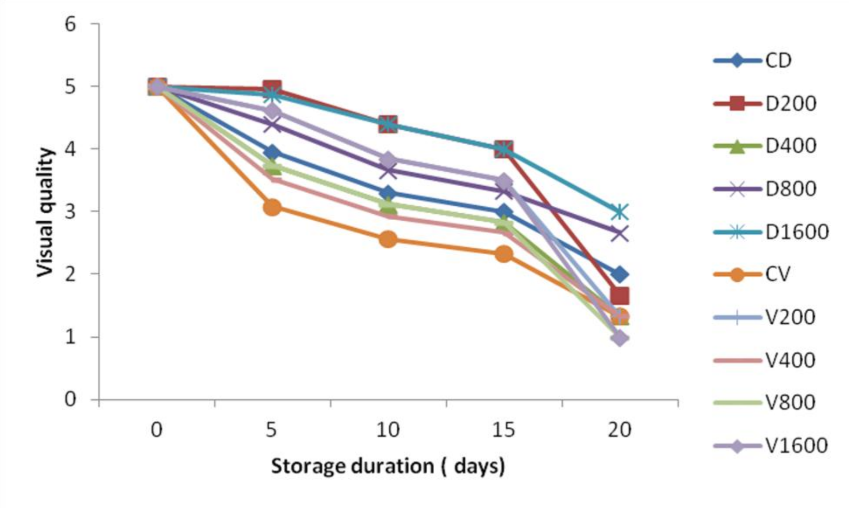

Figure 9. The changes at visual quality scores of cucumber fruits treated with ethanol both vapor (V) and dip (D).

Conclusion

Ethanol has potent antimicrobial activity and the treatment of ethanol both dip and vapor were reported to control postharvest diseases different kind of vegetables or fruits. The purpose of this study, therefore, investigate to the effect of ethanol treatments on chilling injury, decay rate, color, total soluble solids, weight loss and elektrolyte leakage of cucumber during storage. For this aim, the ethanol both dip and vapour treated to cucumber at the doses of 200, 400, 800 and 1600 µL/L. According to the results of this study, vapor treatment of ethanol accelerated softening of cucumber fruit, whereas dip treatments maintain firmness. The weight losses of cucumber in vapor treatment, however, lower than the ethanol dip treatments. But, the ethanol-dip treatments decreased yellowing of fruit, and especially W1600 treatment was effectively improved visual quality, decreased CI and decay rate of cucumber. So, it was concluded that the ethanol-dip treatments were maintained quality significantly compared to vapor ethanol treatments.

Conflicts of Interest

The authors declare that this manuscript is not, any conflict of interest.

Funding Statement

"This reseach has been funded by Kocaeli University Project Number: 2014/075

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