

**COMBINATION OF ULTRASOUND AND OXALIC ACID TO CONTROL CHILLING INJURY IN
CAPIA PEPPER (*Capsicum annuum* L. cv Yalova Yaglık)****Mehmet U. Kasım, Rezzan Kasım, Kübra Yaşar**

Kocaeli University, Vocational School of Arslanbey, Kartepe-Kocaeli, Turkey

Corresponding author: rkasim@kocaeli.edu.tr

Abstract

The chilling injury is caused important produce losses in peppers, after harvest. These losses are emerged both quantity and quality. The purpose of this study is to determine the effect of ultrasound (52 dB) and oxalic acid treatments on chilling injury (CI) and color preservation of Capia peppers at 4°C temperature. The peppers harvested at the green maturation stage divided into four group and the following treatments were done; 1. Control (C, dip to water at 25°C for 15 min), 2. Oxalic acid (OA, dip to the 3 µMol oxalic acid solution at 25°C for 15 min), 3. Ultrasound (US, dip to water bath in a 52 dB and at 25°C for 15 min), 4. US+OA (dip to the 3 µMol oxalic acid solution in a 52 dB water bath for 15 min). After treatments, the peppers were dried, placed in polystyrene foam dishes as three pepper per dish, and wrapped polyethylene stretch film. Then, the packaged peppers were stored in a cold room at 4±1°C temperature and 85-90% RH. The study was carried out in three replicates, and total soluble solids (TSS), color (L*, a*, b*), weight loss, electrolyte leakage, visual quality scores and CI scores were determined during storage (at the beginning, 14th days, 14th+72h, 28th days, 28th+72h). According to the results, CI scores of samples in three treatments (OA, US, US+OA) were lower compared to control group. But, OA treatment was suppressed CI symptoms than the other treatments during all storage period. The color preservation of samples in OA, US, US+OA treatments were lower than control. In conclusion, it was concluded that, while the effect of US treatment on prevent CI symptoms is limited, the OA treatment is successful.

Keywords: Pepper, ultrasound, oxalic acid, chilling injury.**Introduction**

Pepper has an important place among the fruit-consumed vegetable species. Peppers have different uses such as tomato paste, spices, dried, frozen, canned and sauce as much as they can be consumed freshly. For this reason, peppers are produced in significant amounts both in the world and in Turkey. 32 324 345 tonnes (FAO, 2014) in the world and 2 457 822 tonnes (TUIK, 2016) fresh peppers are produced in Turkey.

There are different types of peppers, including pointed, stuffed, spicy and chili peppers. Capia pepper is a type of pepper used for produce pepper-paste and it constitutes 40% of pepper production of our country. Since flesh of the pepper is thick and the amount of fat and dry matter is high, it can be used as either processed or freshly in both green and red mature period. The storage time of the pepper varies between 2-4 weeks depending on the varieties and can reach up to 4 weeks under optimum conditions. This time can be extended with some postharvest application such as low temperature, controlled atmosphere, storage in modified atmosphere (Halloran et al. 1995). On the other hand, the cold storage technique, which is required to reduce postharvest losses in all fresh fruits and vegetables, is also important for the pepper casing. However, chilling injury that occurring due to low temperature during long term storage in the case of subtropical and tropical species, limits the storage of the pepper at low temperature, so the storage temperature is partially kept high. Because peppers are among the susceptible species. Generally, chilling injury (CI) occurs at temperatures below 10°C. The symptoms of CI are speckle-shaped depressions on the surface of the fruit, decay in the later stages, discoloration of the stem and drying, and also seed browning. CI cause fruit to lose market value altogether (Erdoğan et al. 2015). Treatments such as UV-C

applications (Vicente et al. 2005), methyl jasmonate (Meir et al. 1996), hot water applications (González-Aguilar et al. 2000) are being used for preventing or delaying of CI. Recently, ultrasound applications have been used in addition to these applications (Yang et al. 2012).

Studies on post-harvest ultrasound applications in fresh fruit and vegetables are noteworthy. Studies have shown that ultrasound application reduces the softening of fruit flesh in plums (Chen and Zhu 2011; Bal, 2016) and peaches (Wang et al. 2006); it is effective in the preservation of some biochemical compounds in litchi (Chen et al. 2012) and tomato fruit (Pinheiro et al. 2016); it has been found that reduce of rotting in strawberries (Cao et al. 2010). In this study we aimed to determine the effects of ultrasound and oxalic acid applications on chilling injury and other quality parameters during low temperature storage of pepper harvested in green period.

Material and methods

Plant Material: The capia pepper (*Capsicum annum* var. *conoides* cv. Yalova Yaglık) using in this study has been cultivated in a research and application greenhouse of Arslanbey Vocational School in Kocaeli University. Peppers were harvested during the green period, when they received their full size, but the redness did not begin. The harvested peppers were quickly moved to the laboratory and those that were not suitable for the experiment were removed.

Ultrasound ve oxalic acid treatment: Peppers were divided into 4 groups one group as control and the following applications being made.

1. Control (C, dip to water at 25°C for 15 min)
2. Oxalic acid (Oxalic acid (OA, dip to the 3 µMol oxalic acid solution at 25°C for 15 min),
3. Ultrasound (US, dip to water bath in a 52 dB and at 25°C for 15 min),
4. US+OA (dip to the 3 µMol oxalic acid solution in a 52 dB water bath for 15 min).

Following the applications, peppers are dried on drying papers and packed.

Packaging and storage conditions: After applications, the peppers are placed in foam dishes in sized 210 x 120 mm including three fruits in each, and covered with stretch film. The packaged products were stored for 28 days in a cold room containing 4±1°C temperature and 85-90% relative humidity. Measurements and observations were made at the beginning of storage and every 14 days during storage, as well as in peppers kept in room conditions (18-22°C) for 72 hours to determine the shelf life.

Weight Loss: Initially three pack of each treatment were separated for weight loss measurements and weighed at each analysis period. No weight loss measurements were made in +72 hours samples. The weight loss is calculated as a percentage according to initial value according to the following formula:

$$W.L. (\%) = (initial\ weight - weight\ at\ the\ time\ of\ analysis) \times 100 / initial\ weight$$

Total soluble solids: The juice obtained from the pepper fruits using micro-pressing was measured at 20°C using the Atago DR-A1 digital refractometer (Atago Co. Ltd., Japan) and was determined as (%).

Color measurements: For the color measurements, three sample packages were initially separated and measurements were made in the cold room. Measurements were made from stretch film, from three points of each fruit and as totally nine reading. No color measurements were made in +72 hours samples. For this purpose, L*, a* and b* values were measured with CR-400 colorimeter (Konica Minolta, Inc. Osaka, Japan) consisted with D65 lamp. Before measurements were made, the instrument was calibrated with a calibration plate. Accordingly, L* (0 = black, 100 = white), a* (+ red, - green), and b* (+ yellow, - blue) indicate color. Using the measurements, hue angle values (h°) (Kasim and Kasim 2015) and the total redness index (TCI) (Obande, 2010) were calculated by the following equations.:

$$h^{\circ} = 180 + \tan^{-1}(b/a)$$

$$TCI = \frac{2000 a}{\sqrt{L(a^2 + b^2)}}$$

The raw values obtained were compared with the initial color values, and given as the change ratio (%).

Electrolyte Leakage: Five 5 mm wide discs were taken from the pepper for electrolyte ion leakage measurements, washed twice with 50 mL distilled water and kept in 50 mL distilled water for 2 hours. After two hours, the electrical conductivity (EC) value in the solution was measured and the samples were frozen. Frozen samples were thawed and EC values were determined when the solution temperature reached 18°C; total electrolyte leakage was calculated as the ratio of these obtained values to the initial EC values and expressed as (%) (Kasim and Kasim 2014).

Chilling injury (CI): During each analysis period, the fruits were examined for CI symptoms such as shallow pitting, fruit stem shrinkage, end shrinkage and were evaluated by a 9-person jury on the scale of 0-4 (0: no sign of CI, 1: mild, 2: moderate, 3: severe 4: very severe). The assessments were made on fruit basis and averages were taken.

Visual quality: During each analysis period; The fruits were evaluated by a 9-person jury in terms of visual quality criteria such as brightness, firmness and color and evaluated according to the 5-1 scale (5: very good, 4: good, 3: moderate, 2: poor, 1: very poor). The assessments were made on fruit basis and averages were taken.

Statistical analysis: The experiment was conducted as a completely randomized design with factorial (applications x maintenance time) arrangement, and a minimum of-three replications. Variance analysis was performed using SPSS 16.0 statistical program and Duncan test was applied for comparison of the averages.

Results and Discussion

Weight Loss

As a result of the measurements, no statistically significant difference in weight loss between the applications was found. As a result of 28 days of storage, the maximum weight loss was found to be 11.16% in US treatment and the lowest weight loss was found in OA applications with 9.9% (Fig. 1). Plant tissues are in equilibrium with the environment, in the condition that at the same temperature conditions and with 99-99.5% relative humidity environment, there is no water loss (Burton, 1982). The protective layers such as waxy layer, cuticle layer, fur etc. into the skin and on surface of the skin of fruit and vegetables, serve to reduce water loss (Kasim and Kasim 2016). In the case of a loss that occurs in these layers, water loss, and therefore weight loss, increases. It is observed that the applications do not damage the skin and surface texture and therefore there is no significant difference between the applications in weight loss. It is also seen that ultrasound applications of peach are not effective on weight loss (Bal, 2013). However, US and US+OA treatments seem to have more weight loss than control and OA treatments. This result suggests that the application of ultrasound causes a slight change in the skin.

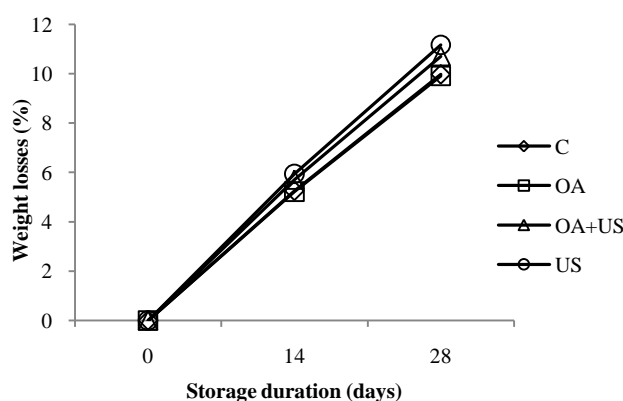


Figure 1. Effects of oxalic acid and ultrasound application on weight loss during pepper storage during storage

Total soluble solids (TSS)

At the beginning, 4.7% TSS values show slight fluctuations during storage, but linear regression analysis shows a slight increase tendency except OA application (Fig 2). However, statistically significant changes in TSS values were not found. In the studies conducted by Cao et al. (2010) on strawberry and Yang et al. (2012) on peach, it is seen that the results of TSS are not significantly affected by ultrasound. However, it is noteworthy that the TSS in the OA application remains more stable. There was a slight increase in the amount of TSS in the samples kept at room temperature for 72 hours following storage. This is thought to be due to higher water loss at high temperatures.

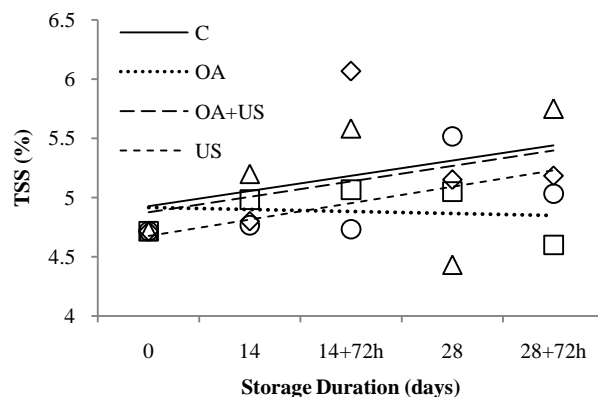


Figure 2. The effects of oxalic acid and ultrasound applications during storage on TSS of peppers

Visual quality

As a result of the panel evaluation, the visual quality scores of control application was slightly lower, but the difference with other applications was not significant statistically. In all applications it did not fall below the three points that is marketable limit value at the end of the 28 days storage period (Fig 3).

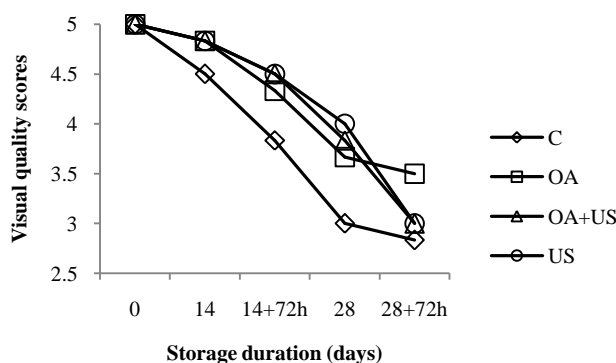


Figure 3. The effects of oxalic acid and ultrasound application on visual quality in pepper during storage

Chilling injury and electrolyte leakage

It was determined that pitting on the surface was more severe in both US and OA + US applications. Therefore, it was seen that US treatments increased the chilling injury (Fig 4). It has been determined that only OA treatment reduces the severity of CI compared with both control and other treatments. The differences between the treatments are also found to be statistically significant.

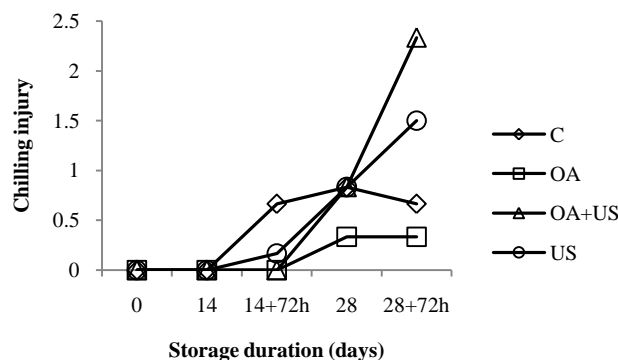


Figure 4. The effects of oxalic acid and ultrasound treatments on the severity of chilling injury of peppers during storage

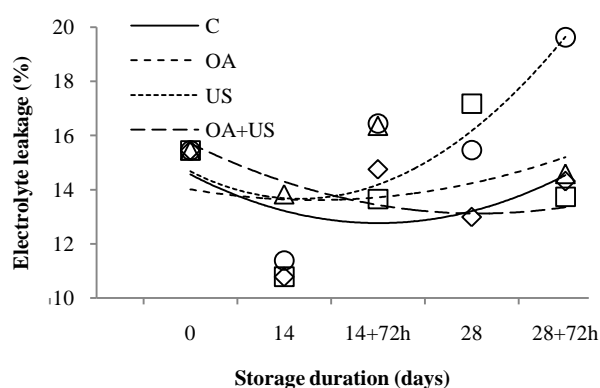


Figure 5. The effects of oxalic acid and ultrasound on electrolyte leakage of peppers during storage (symbols represent mean values, lines represent polynomial slope).

Similar results were obtained with measurements of electrolyte leakage that using to determine chilling injury symptoms on a cellular basis (Fig 5). As can be seen in Fig.5, it was found that the greatest increase was found in US treatment. It is not seen that a significant increasing of electrolyte leakage of samples in the other treatments.

The symptoms of CI were more evident of samples kept at room temperature for 72 hours than that of samples taken just after storage. CI scores of samples which is treated with OA+US and stored 28 days and after that hold in room temperature during 72 h (28+72h) were found to be the highest (2.33); is followed by US application with 1.5 points, and the CI scores of peppers treated with OA has the lowest. According to these results, US application has weakened skin texture, and US application together with OA seems to increased the damage even more. It is also noteworthy that the OA alone is delaying the chilling injury. Bal (2013) determined that the ultrasound treatment to peach is delayed of chilling injury but Yang et al. (2012) concluded that ultrasound application alone did not affect the chilling injury of peach.

Changes of color

As a result of examinations made at a * value, it was found that there is a rapid change in control application (Fig 6). A change of 14.6% was occurred especially on the 14th day. This change means a decrease in green color. Even on the 28th day of storage in other applications, it has not been observed the changes like this. The differences between control and other applications is found to be significant, statistically.

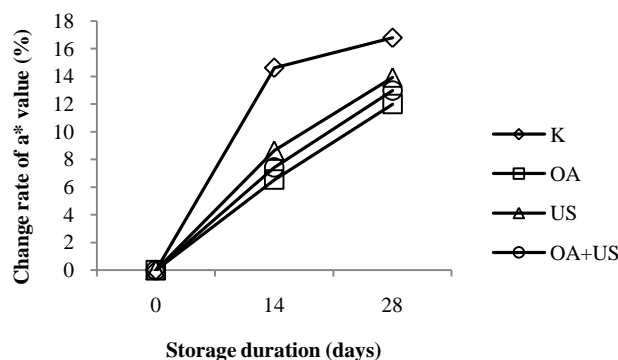


Figure 6. Effects of oxalic acid and ultrasound application on a* value in pepper during storage

It has been seen that the changes of b* values is also parallel to the a* values (Fig 7). The color changes in control treatment was significantly higher than in other applications. This indicates that the green color of the control samples is rapidly disappearing and that the color of samples are started to yellow. This increase may be due to accelerated metabolism because of increased chilling injury in control samples. In addition, there is no significant change in the red color in the control values in terms of angle values and redness index data (Fig 8 ve 9). Therefore, it is thought that a rapid aging occurs in the samples due to the chilling injury. In addition, the application of ultrasound alone or in combination with oxalic acid improves both chilling and coloration in peppers (Fig 4 ve 9). On the other hand, the application of OA on its own seems to increase the quality of storage by delaying both coloring and chilling injury.

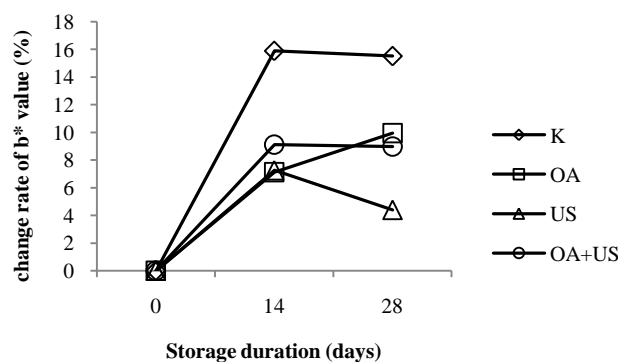


Figure 7. Effects of oxalic acid and ultrasound application on b* value of pepper during storage

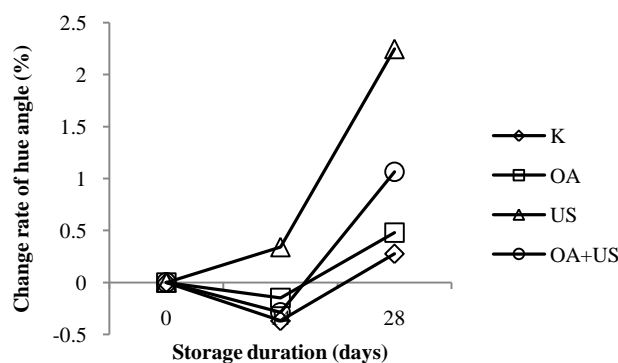


Figure 8. The effect of oxalic acid and ultrasound treatments on hue angle values of pepper during storage

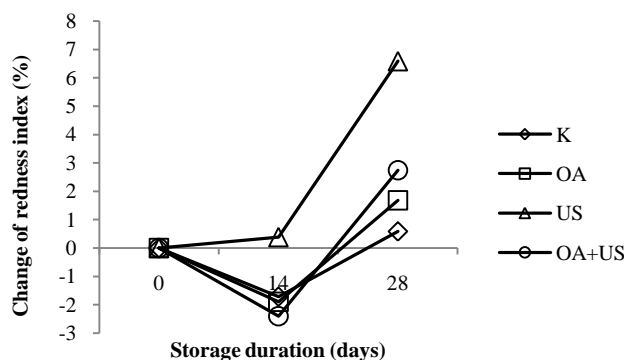


Figure 9. The effect of oxalic acid and ultrasound treatments on redness index of peppers during storage

Conclusions

In this study, it was evaluated that the effect of ultrasound (52 dB) and oxalic acid treatments on chilling injury (CI) and color preservation of Capia peppers at 4°C temperature. For this purpose, oxalic acid alone, ultrasound alone and oxalic acid and ultrasound together treated to Capia pepper, and after application peppers were stored in a cold room at 4 C and 85-90% RH. As results of research, it was found that, when ultrasound alone or ultrasound and oxalic acid together applied to peppers, they accelerated senescence of peppers. Also chilling injury of samples in both US and US+OA is high due to damaging of tissue. However, oxalic acid treatment both delayed color formation and CI. Therefore it could be said that oxalic acid treatment a promising application for delaying CI symptoms of peppers at the lower temperature.

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