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Postharvest application of chitosan and putrescine on maintaining the quality and extend shelf-life of cucumber (*Cucumis sativus* L.)

Samira Fakorizadeh[1], Mohammad Hossein Daneshvar[2], Mohammad Reza Zare-Bavani[3*]

1- Master's graduate. Department of Horticultural Science, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.
2- Professor, Department of Horticultural Science, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.
3- Assistant professor, Department of Food Science, Faculty of Animal and Food Science, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

ABSTRACT

Cucumber fruits have a short shelf life in the post-harvest stage due to high water content, high metabolic activity, and spoilage caused by the growth of microorganisms. In this research, the effects of putrescine (0, 0.5, 1, and 2 mM) and chitosan (0, 0.5, 1, and 1.5%) on some characteristics of cucumber fruit during storage at 12±1 degrees Celsius during storage (0, 10, and 20 days) and one day at 25 degrees Celsius was investigated. The results showed that chitosan and putrescine significantly prevent weight loss, maintain total soluble solids, titratable acidity, pH, firmness, total chlorophyll of skin, and ascorbic acid and improve the sensory quality of cucumber fruit during storage. The lowest weight loss and the highest firmness were obtained in the treatment of 1.5% chitosan and 2 mM putrescine at 10 and 20 days of storage. Also, the highest amount of soluble solids, titratable acidity, total chlorophyll, vitamin C, total antioxidant, total phenol, and the lowest amount of pH, and decrease in sensory quality in 10 and 20 storage times related to the treatments of 1.5% chitosan and 2 mM putrescine that showed a significant difference with the control treatment. 1% and 1.5% chitosan coating treatments showed no significant difference in other traits measured during storage, except for weight loss. It seems that pretreatment of putrescine (2 mM) and chitosan coating (1 to 1.5%) can maintain the quality of fruits for a more extended period while increasing the storage life of cucumber.

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*Corresponding Author E-Mail:
mzarebavani@asnrukh.ac.ir

1- Introduction

Cucumber (Cucumis sativus L.) with an annual production of about 91.3 million tons is one of the most important commercial vegetables cultivated in many regions of the world [1]. Nutritionally, cucumber has phenolic compounds, cucurbitacin, vitamins, antioxidant, anti-diabetic, anti-inflammatory, hyperglycemic and cancer compounds and has a lot of water and fiber and low calories [2, 3 and 4], which in this sense has an important place among It has consumers. However, this product is highly perishable, and during storage, transportation and post-harvest practices, it undergoes decay and its quality characteristics are easily lost [1, 4]. Loss of water and firmness of the texture, wrinkling, yellow or brown color change, rotting and freezing lead to reduction of the shelf life of cucumber to less than 14 days [5]. Increasing the shelf life of cucumber is very necessary for producers, traders, distributors and consumers, and different methods are used to reduce losses and maintain the quality of this product, including chemical methods (pesticides and fungicides), physical methods (packaged with modified and controlled atmosphere, cold storage and minimal processing). and new methods (edible coating) are used [1]. Among these approaches, chemical and physical methods are expensive, environmentally friendly, non-biodegradable and harmful to human health [6]. But edible coatings are a natural, environmentally friendly and cheap method [7]. In this process, the coating layers act as a barrier on the surface of the coated products and change the composition of the atmospheric gas in the product and slow down the loss of moisture and weight loss, as a result, they delay the aging process and prolong the shelf life of fresh fruits and vegetables. They make it longer [8 and 9]. Currently, edible coating for fruits and vegetables using different natural materials that have film-making properties such as polysaccharides (cellulose derivatives, starch, chitin and gums), proteins (soy protein, milk, gelatin, corn, zein and wheat gluten)) and lipids (oils, waxes) and resins are prepared [7]. Among edible coatings, chitosan is the most widely used in edible coatings due to its abundance, biocompatibility, biodegradability, low cost, superior film formation potential, and natural antimicrobial and antioxidant composition [1]. The beneficial effect of chitosan coatings on some cucumber cultivars has been reported, and its effectiveness has been affected by the storage temperature and the concentration used or the accompanying compounds [1, 10 and 11].

Polyamines are a group of compounds known in animals, plants and microorganisms that can help control fruit ripening due to their aliphatic nitrogen structure [12]. Polyamines are involved in a wide range of biological processes, such as plant growth, response to biotic and abiotic stresses, etc. The most known polyamines in plant cells are spermidine, spermine and putrescine [13]. Polyamines have been reported to be associated with antiaging properties [14]. But in most fruits and vegetables, the level of polyamines usually decreases during the ripening and aging process. Such a typical decrease in the levels of polyamines negatively affects the textural characteristics and shelf life of fruits and vegetables [13]. To solve this problem, the exogenous application of polyamines has been reported to extend the period of usability and maintain unchanged textural traits in several fruits such as strawberry [15], plum [16], apricot [17] and mango [18]. The use of putrescine treatment on cucumber fruit at 20 degrees Celsius showed that the treated fruits had better quality properties than untreated fruits during 10 days of storage [4].

The purpose of this study was to investigate the effects of postharvest application of different concentrations of putrescine and chitosan edible coating to maintain the quality and increase the shelf life of cucumber fruit during storage at 12±1 degrees Celsius.

2- materials and methods

1-2- Plant materials

Cucumber fruits (Cucumis sativus L.) variety Emerald were harvested in May 2018 from a greenhouse located in Shushtar city and immediately transferred to the Physiology Laboratory of Horticultural Science and Engineering Department of Khuzestan University of Agricultural Sciences and Natural Resources. Fresh fruits were selected manually for uniformity in size and shape (fruits about 20 ± 2 cm and smooth) and no external damage. First, the fruits were washed with chlorine water (0.05% sodium hypochlorite) and then with distilled water and allowed to dry in the air [9]. After drying in air, their initial physical and chemical properties were measured (day zero). The fruits were then pretreated with putrescine (0, 0.5, 1 and 2 mmol/l) and then coated with chitosan (0, 0.5, 1 and 1.5%). Before starting the treatment, chitosan (CAS 9012-76-4; Sigma-Aldrich) and putrescine (CAS 333-93-7; Sigma-Aldrich) solutions were prepared in 1% acetic acid and distilled water, respectively. After the treatment, the fruits were

weighed and stored in polyethylene baskets at a temperature of 12 ± 1 degrees Celsius and a relative humidity of 85 to 90% during a period of 20 days [19]. Measurements included weight loss, sensory quality, texture firmness, total soluble solids, titratable acidity, pH, total antioxidant capacity, content of total phenolic compounds, ascorbic acid and chlorophyll of the fruit peel. All measurements were performed on days 0, 10, and 20 after the start of the experiment, and three repetitions were used for all measured parameters. After leaving the warehouse, the fruits were placed in the laboratory environment temperature (25°C) for 24 hours and then the desired indicators were measured.

2-2- Preparation of chitosan and putrescine solution

For this purpose, 10 ml of acetic acid was added to each liter of distilled water to prepare a 1% acetic acid solution. One percent acetic acid solution was used to prepare chitosan concentrations of 0.5, 1, and 1.5 percent. To prepare the chitosan solution, 5, 10, and 15 grams of chitosan were slowly added to the acetic acid solution (1%) in one-liter beakers while stirring on a heating device equipped with an electric stirrer. After the complete dissolution of chitosan, the pH of the solution was brought to 5.2 by normal 0.1 soda [20]. The prepared solutions were kept overnight in the refrigerator and used for treatments the next day. Putrescine solutions in concentrations of 0, 0.5, 1 and 2 mM in distilled water were freshly prepared and used immediately.

2-3- Percentage of fruit weight loss

The measurement of the weight loss of the samples during the storage period in the cold storage using a digital scale with an accuracy of 0.01 grams was expressed as a percentage of the initial weight and was calculated using the following formula [20].

100x (initial weight / secondary weight - initial weight) = percentage of weight loss

4-2- Measurement of physicochemical characteristics

A digital refractometer (MA 871Milwaukee model, made in Romania) was used to determine the total dissolved solids of cucumber samples (°Brix) at 20 degrees Celsius. The titratable acidity of cucumber juice was determined by titration with sodium hydroxide (0.1 normal) until pH = 1.8 and based on malic acid (the dominant acid of unripe cucumber fruit), and the pH of homogenized cucumber samples (5 grams with 50 ml of distilled water) was measured with a pH meter (Ino Lab PH7110 made in Germany) [1].

5-2- tissue stiffness

The hardness of cucumbers was measured using a firmness meter (FT011) with a needle of 8 mm in diameter, and the hardness of cucumbers was presented in kilograms per square centimeter [1].

6-2- Determining sensory quality

Sensory quality of cucumber was evaluated on days 0, 10 and 20 of storage based on color and firmness characteristics. The sensory quality was expressed as a sensory index. For sensory evaluation, 10 students of Horticultural Science and Engineering Department of Khuzestan University of Agricultural Sciences and Natural Resources (3 men and 7 women) in the age range of 20 to 30 years were selected. For this purpose, the samples were placed in white plastic containers, which were randomly numbered with three-digit codes, and were given to the assessors, and they were asked to rate the samples based on sensory characteristics (color and freshness of the product, firmness). and smell) and give them a score from 0 to 5, so that the number 0 and 5 were considered as the least and the most amount of change in the color and freshness of the product, respectively. The evaluation on a scale of 0-5 was explained to the evaluators in this way. 0: excellent feature of cucumber fruit, green color, freshness, firmness, good smell; 1: a little loss of the green color of the skin, freshness, firmness, natural smell; 2: losing more greenness, having freshness and firmness; 3: light green skin, slightly cloudy, limited marketability; 4: bright yellow skin, a certain range of signs of decay and low quality; 5: Symptoms of severe decay, browning, moldiness, unpleasant smell, inedible [4].

2-7- Measuring the chlorophyll of the fruit skin

Chlorophyll of cucumber skin samples was extracted using 80% acetone and the light absorption of the extracts was read at two wavelengths of 645 and 663 nm and calculated according to the following formula [21]:

$$\text{chlorophyll} = [(20.2\times OD645)+(8.02\times OD663)]\times V/W$$

where OD645 is the number obtained at the wavelength of 645 and OD663 is the number obtained at the wavelength of 663, V is the volume of the extract in milliliters and W is the sample weight in grams.

2-8- Ascorbic acid

0.1 g of cucumber was extracted with 10 ml of metaphosphoric acid solution (1%) and then centrifuged. Then 1 ml of the resulting extract to 9 ml 2 and 6-dichloroindophenol (DIP) was added and then vortexed. In the last step, the absorbance of the samples was determined with a spectrophotometer (UV-2100Spectro photometr made in the USA) at a wavelength of 515 nm. The amount of ascorbic acid in the samples was calculated based on the standard chart of L-ascorbic acid [20].

9-2- total phenol

Total phenol content was measured using the Folin-Ciocalto method. 0.2 g of fresh fruit tissue was extracted in a Chinese mortar with 2 ml of methanol. The resulting extract was centrifuged for 15 minutes at a speed of 10,000 rpm and at a temperature of four degrees Celsius. 125 microliters of the resulting clarifier was mixed with 375 microliters of diluted distilled water and 2.5 mL Folin-Ciocalto reagent (10%), and then 2 mL sodium bicarbonate (7.5%) was added to it. The absorbance of the samples was recorded at 765 nm by a spectrophotometer. Gallic acid was used to prepare the standard chart. The amount of total phenol in the samples was expressed in terms of milligrams of gallic acid per 100 grams of fresh tissue [20].

2-10- Antioxidant capacity

The antioxidant capacity of cucumber fruits was measured using the free radical inhibition method of soluble 2 and 2 diphenyl 1-picrylhydrazyl (DPPH). One gram of fruit tissue was completely powdered by liquid nitrogen in a Chinese mortar and then extracted with 10 ml of pure cold methanol. 50 microliters of the obtained extract was poured into small falcon tubes and 950 microliters of methanolic DPPH solution (6.25×10 M5) was added to it and then vortexed. The resulting mixture was kept at room temperature in the dark. After 15 minutes, the absorbance of the samples was determined with a spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the whole fruits was expressed based on the reduction of absorption of the sample compared to the control in terms of percentage of DPPH inhibitory power according to the following formula [20]:

(Relation 4) % DPPHSC =(Acont – Asamp) Acont × 100

In this regard, %DPPHSC = inhibition percentage, Acont = control DPPH absorption rate, Asamp = sample absorption rate (sample + DPPH)

2-11- Statistical analysis

The experiment was carried out factorially and in the form of a completely randomized design with three replications. Each replicate included four fruits for each step of measurement (three fruits for sensory evaluation and one fruit for analytical measurements). In total, the test treatments included different concentrations of putrescine (0, 0.5, 1 and 2 mM) and chitosan (0, 0.5, 1 and 1.5 mM) and storage time (0, 10 and 20 days). In the desired time frame for the measurements, 4 fruits from each repetition in each treatment were taken out of the warehouse and kept at the temperature of the laboratory environment for 24 hours, and then the desired indicators were measured. The statistical analysis of the data of the present study was done by SAS V: 9.1 software, and the least significant difference (LSD) test was used to compare the average data.

3- Results and discussion

The results of variance analysis of the data of this research showed that the main effects of putrescine, chitosan and storage time and the double effects of putrescine × storage time on all studied traits were significant at the 1% probability level. The interaction effects of chitosan × putrescine and the effects of chitosan × putrescine × storage time were not statistically significant in any of the traits, but the double interaction effects of chitosan × time for titratable acidity ($p \leq 0.05$) and other traits ($p \leq 0.01$) were statistically significant.

1-3- Physiological weight loss

Comparing the average effects of putrescine, chitosan and storage time on the physiological weight loss of cucumber fruits showed that there is a significant difference ($p \leq 0.01$) between different levels of treatments. Generally, increasing the concentration of chitosan and putrescine in the treatments decreased and increasing the storage time increased the weight loss of cucumber fruits (Tables 1, 2 and 3). The mutual effects of putrescine × storage time showed that the highest amount of weight loss after 20 days of storage was related to the treatment of 0 mM putrescine (8.57%) and the

lowest weight loss in the same time was in the treatment of two mM putrescine (7.30%). , one millimolar putrescine (7.80%) and half a millimolar putrescine (8.09%). After 10 days of storage, the weight loss rate showed a significant decrease with the increase in putrescine concentration (Table 4). The results of comparing the average interaction between chitosan and storage time on weight loss showed that after 20 days of storage, the highest weight loss with an average of 9.44% was related to the 0% chitosan treatment, and the lowest weight loss with an average of 6.99% was from the treatment. 1.5 percent chitosan was obtained, which showed a significant difference with the treatment of 1 percent chitosan (6.52 percent) and half percent chitosan (8.60 percent) (Table 5). After 10 days of storage, with the increase in the concentration of chitosan coating, the rate of weight loss showed a significant decreasing trend, so that the lowest rate of weight loss was in the treatment of 1.5% chitosan (2.91%) and the highest rate of weight loss was in the treatment of 0% chitosan (0.5%). 5 percent) was observed (Table 5). Based on the obtained results, the highest percentage of weight loss was observed in the control samples. The treatments used had an effective role in maintaining fruit weight.

Weight loss of fruits during the storage period is a result of water evaporation from the surface of fruits, respiration and metabolic activities [17]. Weight loss is an important indicator of postharvest quality, which is associated with loss of vitamins, browning, tissue loss, and susceptibility to decay [22]. By binding to the carboxyl groups in the cell wall, putrescine increases the strength and integrity of the wall and reduces moisture transfer outside the product [23], also by binding to the cell membrane, putrescine stabilizes the membrane and removes epicuticular waxes, which play an important role in exchange Water plays through the skin, delays it and thus makes the process of losing weight slower [24].

The significant effects of putrescine treatment in preserving fruit juice and reducing the weight loss process of cucumber (temperature 20 degrees Celsius for 10 days) [4] and pepper [13] have already been reported. Chitosan coating with or without additives acts as a barrier against water evaporation and reduces transpiration [1]. The decrease in moisture content of fruits and vegetables during long-term storage can be attributed to evaporation and transpiration processes, which usually occur due to changes in temperature and relative humidity [6]. It has been reported that coating reduces moisture loss during cucumber storage [1, 6, 11, 19 and 25], which is consistent with the findings of this research.

2-3- Amount of soluble solids (TSS)

According to Tables 1, 2 and 3, increasing the concentration of chitosan and putrescine decreased and increasing the storage time increased TSS in cucumber fruits. The mutual effects of putrescine × storage time and chitosan × storage time showed that the highest amount of TSS after 20 days of storage and in the treatment of zero millimolar putrescine (6.04) (Table 4) and zero percent chitosan (6.08) (Table 5) And the lowest TSS was at the beginning of storage (Tables 4 and 5). After 10 and 20 days of storage, the amount of TSS measured was lower with increasing concentration of putrescine pretreatment or chitosan coating. The lowest TSS increase in putrescine treatment was observed after 10 or 20 days of storage in 2 mM treatment (Table 4). Regarding the chitosan coating, the lowest increase in TSS was recorded in the 1.5% chitosan treatment, which was not statistically significantly different from the 1% chitosan treatment at the same time (Table 5). Based on the obtained results, the highest increase in TSS was observed in the control samples (Tables 4 and 5).

The increase in fruit TSS can be due to various factors, including the breakdown of starch into sugar, hydrolysis of cell wall polysaccharides, and an increase in the percentage of dry matter due to water loss [12]. Chitosan as an excellent semi-permeable coating on the surface of the fruit changes the internal atmosphere of the fruit, reduces the amount of oxygen, increases the amount of carbon dioxide and suppresses the synthesis of ethylene [12 and 25]. As a result, due to reducing the rate of respiration and metabolic activities, it reduces the increase of TSS in fruit [1]. Putrescine prevents the increase of fruit TSS by reducing the respiration rate, ethylene production and delaying the sharp ripening process [24]. Similarly, previous reports showed that coated cucumber had lower TSS than uncoated samples [1, 25]. On the other hand, the use of polyamines has been reported to delay the increase of TSS during storage of cucumber [4] and also during ripening in mango [11], grape [26] and banana [12], which is in accordance with the findings of the present research. is.

Table 1 Mean comparison of puterscine treatments on cucumber fruits

Treats	Studied Indices									
	Weight loss (%)	Total soluble solid (ºBrix)	Titrable acidity (%)	pH	Firmness ($Kgcm^{-2}$)	Total Chlorophyll ($mg\ g^{-1}FW$)	Ascorbic acid ($mg100g^{-1}FW$)	Sensory index	Total phenols ($mgGA\ g^{-1}FW$)	Antioxidant capacity (%DPPHSC)
Putrescine (0 mM)	4.24±0.60 a	4.64±0.19 a	0.80±0.03 c	5.62±0.15 a	14.72±0.64 c	0.86±0.06 d	10.35±0.48 c	2.17±0.31 a	15.72±1.26 c	27.04±1.6 c
Putrescine (0.5 mM)	4.05±0.58 b	4.57±0.18 a	0.83±0.03 b	5.58±0.15 a	15.45±0.57 b	0.95±0.05 c	10.72±0.47 c	2.06±0.29 a	15.82±1.23 c	27.51±1.57 c
Putrescine (1 mM)	3.85±0.55 c	4.41±0.15 b	0.87±0.02 a	5.42±0.15 b	16.67±0.48 a	1.05±0.04 b	11.33±0.41 b	1.75±0.25 b	16.61±1.15 b	28.90±1.4 b
Putrescine (2 mM)	3.63±0.52 d	4.21±0.13 c	0.89±0.02 a	5.36±0.15 b	16.95±0.45 a	1.12±0.03 a	11.82±0.38 a	1.42±0.20 c	17.89±0.99 a	30.90±1.30 a

In each column, means that have at least one letter in common, not significant difference at 5% according to LSD test

Table 2 Mean comparison of chitosan treatments on cucumber fruits

Treats	Studied Indices									
	Weight loss (%)	Total soluble solid (ºBrix)	Titrable acidity (%)	pH	Firmness ($Kgcm^{-2}$)	Total Chlorophyll ($mg\ g^{-1}FW$)	Ascorbic acid ($mg100g^{-1}FW$)	Sensory index	Total phenols ($mgGA\ g^{-1}FW$)	Antioxidant capacity (%DPPHSC)
Chitosan (0%)	4.83±0.66 a	4.73±0.19 a	0.82±0.03 b	5.96±0.18 a	14.82±0.63 c	0.89±0.06 d	9.69±0.54 c	2.25±0.31 a	15.64±1.28 c	25.43±1.74 c
Chitosan (0.5%)	4.35±0.60 b	4.55±0.17 b	0.83±0.03 b	5.43±0.12 b	15.73±0.55 b	0.97±0.05 c	10.94±0.43 b	2.03±0.28 b	16.34±1.18 b	28.47±1.46 b
Chitosan (1%)	3.29±0.45 c	4.29±0.14 c	0.87±0.02 a	5.21±0.12 c	16.60±0.50 a	1.04±0.04 b	11.72±0.34 a	1.69±0.24 c	16.92±1.11 ab	30.12±1.34 a
Chitosan (1.5%)	3.30±0.49 c	4.27±0.14 c	0.88±0.02 a	5.38±0.13 b	16.63±0.50 a	1.07±0.04 a	11.87±0.36 a	1.42±0.21 d	17.14±1.10 a	30.32±1.30 a

In each column, means that have at least one letter in common, not significant difference at 5% according to LSD test

Table 3 Mean comparison of storage times on cucumber fruits

Time	Studied Indices									
	Weight loss (%)	Total soluble solid (ºBrix)	Titrable acidity (%)	pH	Firmness ($Kgcm^{-2}$)	Total Chlorophyll ($mg\ g^{-1}FW$)	Ascorbic acid ($mg100g^{-1}FW$)	Sensory index	Total phenols ($mgGA\ g^{-1}FW$)	Antioxidant capacity (%DPPHSC)
0	0.00±0.00 c	3.39±0.03 c	1.01±0.01 a	4.60±0.03 c	19.26±0.10 a	1.32±0.01 a	13.70±0.11 a	0.0±0.01 c	25.30±0.20 a	38.51±0.36 a
10	3.94±0.12 b	4.37±0.04 b	0.87±0.01 b	5.41±0.08 b	16.33±0.32 b	0.97±0.03 b	11.18±0.27 b	1.96±0.09 b	15.25±0.25 b	28.77±0.64 b
20	7.89±0.19 a	5.62±0.07 a	0.67±0.01 c	6.47±0.06 a	12.25±0.25 c	0.68±0.03 c	8.29±0.22 c	3.58±0.13 a	8.98±0.29 c	18.49±0.52 c

In each column, means that have at least one letter in common, not significant difference at 5% according to LSD test

Table 4 Mean comparison of puterscine treatments on cucumber fruits at different times

Treats	Time	Studied Indices									
		Weight loss (%)	Total soluble solid (ºBrix)	Titrable acidity (%)	pH	Firmness ($Kgcm^{-2}$)	Total Chlorophyll ($mg\ g^{-1}FW$)	Ascorbic acid ($mg100g^{-1}FW$)	Sensory index	Total phenols ($mgGA\ g^{-1}FW$)	Antioxidan capacity (%DPPHSC)
Putrescine (0 mM)	0	0.00±0.00 and	3.37±0.08 f	1.01±0.01 a	4.59±0.08 d	19.26±0.22 a	1.33±0.01 a	13.71±0.29 a	0.00±0.00 g	25.35±0.43 a	38.42±0.91 a
Putrescine (0 mM)	10	4.24±0.25 d	4.54±0.11 d	0.82±0.02 d	5.68±0.11 b	14.36±0.53 d	0.77±0.04 d	9.11±0.46 d	2.25±0.18 d	14.36±0.34 d	26.26±1.01 d
Putrescine (0 mM)	20	8.57±0.40 a	6.04±0.12 a	0.59±0.02 g	6.63±0.12 a	10.39±0.31 f	0.46±0.04 f	6.24±0.45 f	4.36±0.18 a	7.36±0.33 g	16.26±0.90 f
Putrescine (0.5 mM)	0	0.00±0.00 and	3.40±0.06 f	1.00±0.01 a	4.60±0.06 d	19.26±0.24 a	1.33±0.01 a	13.75±0.26 a	0.00±0.00 g	25.43±0.36 a	38.65±0.81 a
Putrescine (0.5 mM)	10	4.07±0.24 d	4.46±0.08 d	0.86±0.02 cd	5.59±0.13 b	15.56±0.44 c	0.90±0.03 c	10.96±0.49 c	2.17±0.14	13.97±0.26 d	26.76±0.98 d
Putrescine (0.5 mM)	20	8.09±0.39 ab	5.84±0.12 a	0.65±0.01 f	6.54±0.11 a	11.53±0.31 f	0.62±0.04 and	8.12±0.44 and	4.00±0.17 a	8.05±0.31 fg	17.11±0.79 f
Putrescine (1 mM)	0	0.00±0.00 and	3.41±0.05 f	1.01±0.01 a	4.62±0.06 d	19.27±0.16 a	1.32±0.01 a	13.71±0.18 a	0.00±0.00 g	25.25±0.50 a	38.43±0.62 a
Putrescine (1 mM)	10	3.86±0.25 d	4.31±0.08	0.89±0.02 bc	5.22±0.19 c	17.53±0.53 b	1.07±0.03 b	12.15±0.55 b	1.83±0.14 if	15.55±0.40 c	29.50±1.14 c
Putrescine (1 mM)	20	7.70±0.37 bc	5.52±0.10 b	0.71±0.02 and	6.42±0.12 a	13.21±0.33 and	0.76±0.04 d	9.30±0.37 d	3.42±0.17 b	9.02±0.41 f	18.77±0.82 f
Putrescine (2 mM)	0	0.00±0.00 and	3.38±0.05 f	1.02±0.01 a	4.60±0.06 d	19.27±0.20 a	1.32±0.01	13.62±0.17 a	0.00±0.00 g	25.17±0.39 a	38.53±0.59 a
Putrescine (2 mM)	10	3.58±0.21 d	4.17±0.07 and	0.92±0.01 b	5.15±0.19 c	17.87±0.50 b	1.15±0.03 b	12.51±0.42 b	1.58±0.16 f	17.11±0.47 b	32.54±1.18 b
Putrescine (2 mM)	20	7.30±0.36 c	5.10±0.08 c	0.74±0.02 and	6.34±0.13 a	13.71±0.39	0.89±0.04 c	9.49±0.35 d	2.67±0.18 c	11.38±0.54 and	21.63±1.05 and

In each column, means that have at least one letter in common, not significant difference at 5% according to LSD test

Table 5 Mean comparison of chitosan treatments on cucumber fruits at different times

Treats	time	Studied Indices									
		Weight loss (%)	Total soluble solid (ºBrix)	Titrable acidity (%)	pH	Firmness ($Kgcm^{-2}$)	Total Chlorophyll (mg g^{-1}FW)	Ascorbic acid (mg100g^{-1}FW)	Sensory index	Total phenols (mgGA g^{-1}FW)	Antioxidant capacity (%DPPHSC)
Chitosan (0%)	0	0.00±0.00 i	3.39±0.07 g	1.01±0.01 a	4.61±0.08 and	19.27±0.23 a	1.32±0.01 a	13.71±0.20 a	0.00±0.00 f	25.31±0.31 a	38.53±0.56 a
Chitosan (0%)	10	5.05±0.12 and	4.72±0.06 d	0.83±0.02 c	6.18±0.04 b	14.39±0.55 d	0.83±0.05 d	9.11±0.30 cd	2.50±0.15 cd	14.47±0.42 c	23.72±0.76 d
Chitosan (0%)	20	9.44±0.19 a	6.08±0.13 a	0.61±0.02 and	7.10±0.06 a	10.81±0.43 g	0.50±0.06 f	6.24±0.24 and	4.25±0.21 a	7.15±0.38 and	14.04±0.65 g
Chitosan (0.5%)	0	0.00±0.00 i	3.38±0.05 g	1.00±0.01 a	4.61±0.06 and	19.28±0.18 a	1.32±0.01 a	13.75±0.22 a	0.00±0.00 f	25.20±0.51 a	38.49±1.00 a
Chitosan (0.5%)	10	4.44±0.10 f	4.47±0.04 and	0.85±0.01 bc	5.36±0.09 c	15.98±0.51 c	0.94±0.05 c	12.29 ±0.45 b	2.17±0.11 d	15.05±0.55 bc	28.44±0.94 c
Chitosan (0.5%)	20	8.60±0.19 b	5.79±0.10 b	0.65±0.02 and	6.32±0.05 b	11.94±0.42 fg	0.65±0.06 and	8.62±0.32 d	3.92±0.21 a	8.76±0.40 d	18.49±0.56 f
Chitosan (1%)	0	0.00±0.00 i	3.38±0.06 g	1.01±0.01 a	4.61±0.06 and	19.28±0.20 a	1.33±0.01 a	13.71±0.21 a	0.00±0.00 f	25.30 ±0.37 a	38.52±0.64 a
Chitosan (1%)	10	3.34±0.10 g	4.16±0.05 f	0.89±0.01 b	4.88±0.13 d	17.48±0.56 b	1.04±0.05 bc	14.23±0.35 a	1.75±0.13 and	15.52±0.47 bc	31.39±1.01 b
Chitosan (1%)	20	6.52±0.17 d	5.33±0.10 c	0.71±0.02 d	6.14±0.06 b	13.04±0.45 if	0.77±0.05 d	9.38±0.21 c	3.33±0.21 b	9.93±0.65 d	20.46±0.74 if
Chitosan (1.5%)	0	0.00±0.00 i	3.41±0.05	1.01±0.01 a	4.59±0.06 and	19.23±0.22 a	1.32±0.01 a	13.62±0.28 a	0.00±0.00 f	25.39±0.47 a	38.48±0.71 a
Chitosan (1.5%)	10	2.91±0.11 h	4.13±0.06 f	0.90±0.02 b	5.22±0.11 c	17.46±0.52 b	1.08±0.04 b	13.76±0.43 a	1.42±0.10 and	15.95±0.54 b	31.52±0.92 b
Chitosan (1.5%)	20	6.99±0.17 c	5.26±0.10 c	0.72±0.03 d	6.33±0.04 b	13.20±0.43 and	0.82±0.05 d	8.99±0.19 cd	2.83±0.18 c	10.07±0.50 d	20.96±0.80 and

In each column, means that have at least one letter in common, not significant difference at 5% according to LSD test

3-3- Titratable acidity (TA)

The results of comparing the main effects of putrescine, chitosan and storage time on TA levels showed that increasing the concentration of putrescine and chitosan increased and storage time decreased TA (Tables 1, 2 and 3). The lowest and the highest amount of TA on the 20th day of storage were observed in the treatment of 0 mM (0.59%) and 2 mM putrescine (0.74%), respectively. On the 10th day of storage, the lowest and the highest amount of TA was related to the treatment of 0 mM (0.82%) and 2 mM putrescine (0.92%) (Table 4). In the case of chitosan treatment, after 20 days of storage, the lowest amount of TA was in the control treatments (0.61%), which was not statistically significant with the 0.5% chitosan treatment (0.65%), and the highest amount of TA was in the 1.5% treatment. Chitosan (0.72%) was observed, which was not significantly different from the treatment of 1% chitosan (0.71%). After 10 days of storage, a similar trend was recorded, so that the lowest amount of TA was in the treatment without chitosan (0.83%), which was not statistically significantly different from the treatment with 0.5% chitosan (0.85%), and the highest amount of TA was in treatment 5. 1% chitosan (0.90%) was observed, which was not significantly different from the 1% chitosan treatment (0.89%) (Table 5). In general, during storage, the acidity of cucumber samples decreased with the increase of storage time, and the lowest decrease trend was observed in the treated cucumber samples. The decrease in acidity during storage is probably due to the use of organic acids in respiration processes [13]. Since the loss of acidity of vegetables and fruits is related to the reduction of product quality during storage, maintaining acidity in treated cucumber samples is useful for maintaining quality and increasing product shelf life [6]. The external application of some compounds or coating reduces the rate of respiration and prevents the reduction of organic acids [13]. The findings of the present research are in accordance with the findings of other researchers regarding the effect of chitosan coating on titratable acidity of cucumber [25] and pepper [13] and the effect of putrescine on banana acidity [12].

4-3- pH

Increasing the concentration of putrescine and chitosan decreased and the storage time increased the pH of cucumber fruits (Tables 1, 2 and 3). After 20 days of storage, no statistically significant difference was observed between different concentrations of putrescine (Table 4). Also, no difference was recorded between the concentrations of 0.5, 1 and 1.5% chitosan, but these concentrations showed a significant difference with the control treatment (0% chitosan). (Table 6). After 10 days of storage, the lowest pH value was related to the 2 mM putrescine treatment (5.15), which was not different from the 1 mM putrescine treatment (5.22), but with the half-molar treatment (5.59) and the zero-molar putrescine treatment (5.68) showed a significant difference (Table 4). Regarding the chitosan treatment, after 10 days of storage, the lowest pH was in the 1% chitosan treatment (4.88), which is statistically different from the 1.5% chitosan treatment (5.22) and the 0.5% chitosan treatment (5.36). It was significant and the highest amount of TA was observed in the zero percent chitosan treatment (6.18), which was significantly different from other chitosan treatments (Table 5).

The results showed that putrescine and chitosan treatment reduced the increase in pH. During fruit storage, the pH of the fruit extract increases and the acidity decreases [27]. During the storage period, the pH of fruits increased, and according to researchers, in most fruits, the increase in pH is due to the reduction of organic acids during the storage period [28, 29, 30, 31]. These results are consistent with the results reported by other researchers who stated that the decrease in acidity during storage indicates fruit aging [29, 30]. The increase in pH may be due to the consumption of organic acids in the respiration process during the storage period [31]. The decrease in the process of changes in fruit pH may be due to the effect of treatments on the biochemical status of the fruit and the decrease in respiration rate and metabolic activity [32]. It has been suggested that chitosan forms a semipermeable coating on the surface of the fruit, which may alter the internal atmosphere, thus delaying ripening [10]. In this study, chitosan and putrescine treatments reduced pH and titratable acidity changes and effectively delayed fruit aging.

5-3- Fruit firmness

The results obtained in Tables 1, 2 and 3 show that increasing the concentration of putrescine and chitosan increases the firmness and storage time decreases the firmness of cucumber fruit. Comparison of the average interaction effects of putrescine × storage time and chitosan × storage time showed that increasing the concentration of putrescine and chitosan at the same times reduced the process of losing fruit firmness (Tables 4 and 5).

The firmness of the texture is one of the important qualitative characteristics in the consumer acceptance of fresh fruits and vegetables. Fruit firmness is a main characteristic that determines the post-harvest life and quality of the fruit [10]. The main cause of loss of firmness in fruits and vegetables is the breakdown of pectin polymers [22]. Cucumber is a fruit that quickly loses its firmness after harvesting, which makes it short-lived and susceptible to fungal contamination. Researchers have reported that covering cucumber fruits reduces respiration and this may be responsible for maintaining fruit firmness during storage [22]. The beneficial effect of increasing the concentration of chitosan on the firmness of cucumber fruit tissue [1, 10 and 25] has been previously reported, which is in accordance with the findings of the present research. The results showed that the loss of fruit firmness can be reduced with putrescine treatment. These results are in accordance with the findings of other researchers about cucumber [4]. Therefore, by using the anti-aging properties of putrescine, the aging and decay process can be delayed [4].

6-3- Sensory quality

Increasing the concentration of putrescine and chitosan decreased and the storage time increased the sensory quality of cucumber fruits (Tables 1, 2 and 3). After 20 days of storage, no statistically significant difference was observed between the control treatment and half-molar putrescine, but the 2-molar putrescine treatment showed the lowest reduction in sensory quality (Table 4). Also, the same trend was recorded in the case of chitosan treatments as with putrescine treatments (Table 5). . After 10 days of storage, the lowest sensory quality was related to 2 mM putrescine treatment (2.67) (Table 4). In the case of chitosan treatment, after 10 days of storage, the lowest sensory quality was observed in the 1.5% chitosan treatment (2.83) (Table 5). In the control group, the sensory index of cucumber fruit increased throughout the storage period. Sensory changes, including weight loss, color change, etc., were observed, but these symptoms were less recorded in fruits treated with chitosan and putrescine. It has been reported that fruits and vegetables are highly stressed after harvest due to the depletion of energy, nutrients, hormones, and water resources, leading to the rapid onset of senescence [33]. In cucumber fruits, the main symptoms Aging and quality reduction, yellowing and wrinkling are the result of water loss [9]. Putresin prevents the increase of the sensory quality of cucumber fruits by better preserving color, taste, firmness of texture, etc. [4]. Also, covering the fruit as a barrier reduces the loss of energy, nutrients, hormones and water, and therefore prevents weight loss and delays the onset of aging, as a result, it causes a better preservation of the sensory characteristics of the cucumber fruit [9], which is in accordance with the research findings. is present.

3-7- Chlorophyll

Increasing the concentration of chitosan and putrescine increased and the storage time decreased the amount of chlorophyll in the skin of cucumber fruits (Tables 1, 2 and 3). The interaction effects of putrescine × storage time showed that the lowest amount of chlorophyll after 20 days of storage and in the treatment of zero millimolar putrescine (0.46 mg g-1FW) and the highest amount of chlorophyll at the same time in the treatment of two millimolar putrescine (mg g- 1FW was 0.89). After 10 days of storage, the decrease in the amount of chlorophyll was slower with increasing concentration of putrescine (Table 4). The comparison results of the average chitosan × storage time interaction on the chlorophyll content of the fruit skin showed that after 20 days of storage, the highest chlorophyll content with an average of 0.82 mg g-1FW was related to the 1.5% chitosan treatment, and the lowest chlorophyll content was with an average of 50. 0 mg g-1FW was related to 0% chitosan treatment. During 10 days of storage, with the increase in chitosan concentration, there was a significant decrease in the amount of chlorophyll, so that the highest amount of chlorophyll in the treatment of 1.5% chitosan (80.1 mg g-1FW) and the lowest amount of chlorophyll in the treatment with zero percent chitosan (82 mg g-1FW) /0) was observed (Table 5). Based on the obtained results, the lowest amount of chlorophyll was observed in the control samples and the treatments used had an effective role in maintaining the amount of chlorophyll in the fruit.

According to consumers, fruit color is one of the most important indicators of fruit appearance. The right color leads to the creation of the desired appearance of the fruit, and therefore maintaining the color of the fruit is very important in its storage [27]. The green color of vegetables and fruits is due to the presence of pigments called chlorophyll [34]. Chemical changes during fruit storage usually decrease the amount of pigments [27]. The decomposition of chlorophyll can be attributed to three possible factors: pH changes, oxidative systems and chlorophyllase enzyme activity, all of

which are accelerated by respiration and the progress of the aging process [34]. It has been reported that polyamines can slow down the process of chlorophyll reduction in thylakoid membranes by stabilizing photosystem complexes during storage time [35]. The researchers stated that the coatings maintain the green color of the cucumber by creating a layer on the surface of the fruit and as a result prevent oxidative and enzymatic browning [1]. In accordance with our findings, previous reports have also shown that the reduction of cucumber fruit chlorophyll in edible chitosan coated samples compared to uncoated fruits during storage is associated with less reduction changes [1, 19]. In the case of putrescine treatment, the results were similar to chitosan treatment [4].

8-3- Ascorbic acid (AsA)

The results of comparing the main effects of putrescine, chitosan and storage time on the amount of AsA showed that increasing the concentration of putrescine and chitosan increased and storage time decreased AsA (Tables 1, 2 and 3). The lowest and highest amount of AsA after 20 days of storage was observed in the treatment of 0 mM (24.6 mg-100g FW) and 2 mM putrescine (9.49 mg-100g FW), respectively. After 10 days of storage, the lowest and highest amount of AsA was related to the treatment of 0 mM (11.9 mg-100g FW) and 2 mM putrescine (12.51 mg-100g FW) (Table 4). In the case of chitosan treatment, after 20 days of storage, the lowest amount of AsA was observed in the treatment without chitosan (6.24 mg-100g FW) and the highest amount of AsA was observed in the treatment with 1% chitosan (9.38 mg-100g FW), which was 1.5 mg in the treatment There was no significant difference in the percentage of chitosan (100g-1FW 8.99 mg). After 10 days of storage, a similar trend was recorded, so that the lowest amount of AsA was in the treatments without chitosan (11.9 mg-100g FW) and the highest amount of AsA was observed in the treatment with 1% chitosan (14.23 mg-100g FW), which with the treatment of 5 1.0% of chitosan (13.76 mg-100g FW) had no significant difference (Table 5).

During the storage time of cucumber fruit in the warehouse, the amount of AsA decreased, and the decrease of this parameter was observed in all samples. The reason for the reduction of AsA can be due to its oxidation in the environment. It is also due to the high respiration rate of the fruit [36 and 37]. In addition to oxidation, increasing pH due to enzymatic activity also destroys AsA [36]. The preservation of AsA indicates the preservation of the quality and nutritional value of the fruit [27]. By preventing the production of ethylene, reducing respiration and delaying the ripening of the fruit, putrescine prevents the decomposition of the cell wall and as a result reduces the production of free radicals and due to the low amount of free radicals, the need of the cell to consume AsA is reduced and as a result of this vitamin in The fruit is preserved, and polyamines, due to having positive charges, directly remove free radicals [38]. By creating a barrier on the surface of the fruit, the chitosan coating prevents the penetration of oxygen and reduces the fruit juice, and as a result, improves the preservation of AsA in cucumber [25] The results of this research are in agreement with the researches conducted by other researchers regarding the better preservation of AsA during storage using Putrescine and chitosan treatments correspond [25, 38].

3-9- Total phenol (TPC)

The results showed that increasing the concentration of putrescine and chitosan increased and storage time decreased the TPC of cucumber fruit (Tables 1, 2 and 3). Comparison of the average interaction effects of putrescine × storage time and chitosan × storage time showed that at the same times, increasing the concentration of putrescine and chitosan significantly reduced the process of fruit TPC loss (Tables 4 and 5). During storage, TPC of all cucumber samples decreased and reached the minimum values at the end of storage (20 days). TPC during storage was the lowest in cucumber samples without coating, while the treated samples had the highest level (Tables 5 and 6). The reduction of TPC during storage is most likely due to the use of these compounds as preservatives and antioxidant agents against microbial growth and oxidation processes in cucumber fruit [1]. Therefore, cucumber treatment with suitable coating and materials can significantly improve the storage of this perishable product. The decrease of phenolic compounds as an indicator of aging under the influence of increasing the activity of polyphenol oxidase enzyme can be an indicator of product aging [33]. The findings of this study are comparable with previous reports, which show that cucumber coated with chitosan has a higher total phenolic content than uncoated samples.

It decreases to a lesser extent during storage compared to uncoated samples [1]. Also, in another report on pepper, the use of putrescine and chitosan significantly slowed down the process of reducing phenolic compounds [33].

10-3- Antioxidant capacity

The results of comparing the main effects of putrescine, chitosan and storage time showed that increasing the concentration of putrescine and chitosan increased and storage time decreased the antioxidant capacity (Tables 1, 2 and 3). The lowest and highest antioxidant capacity after 20 days of storage were observed in the treatment of 0 mM (16.26%) and 2 mM putrescine (21.63%), respectively (Table 5). After 10 days of storage, the lowest and highest levels of antioxidant capacity were related to the treatment of 0 mM (26.26%) and 2 mM putrescine (32.54%), respectively (Table 4). Regarding the chitosan treatment, after 20 days of storage, the lowest antioxidant capacity was observed in the treatments without chitosan (14.04%), and the highest antioxidant capacity was observed in the 1.5% chitosan treatment (20.96%), which was observed with the 1% chitosan treatment (20.46%) had no significant difference (Table 6). After 10 days of storage, a similar trend was recorded, so that the lowest amount of antioxidant capacity was observed in the treatments without chitosan (23.72%) and the highest antioxidant capacity was observed in the treatment with 1.5% chitosan (31.52%) 1% chitosan (31.39%) had no significant difference (Table 5).

DPPH radical anti-radical activity of uncoated cucumber was lower than chitosan-coated and putrescine-treated samples (Tables 4 and 5). Putresin and chitosan treatments significantly maintained the antioxidant capacity of the fruit during storage. This effect of putrescine and chitosan treatment was probably due to the better maintenance of TPC and AsA content of the fruit during storage. It has been stated that there is a positive correlation between antioxidant activity and total phenolic and AsA of fruits [24]. The highest DPPH radical scavenging activity was observed in cucumber samples coated with 1.5% chitosan and 2 mM putrescine, which indicates that the treatments used improved the quality properties of cucumber samples, which indicates the antioxidant potential of these treatments in cucumber fruits and hence , can be used to increase food quality and health and increase the shelf life of cucumber [1]. In agreement with our findings, previous reports showed that coating treatment improves the antioxidant activity of fruits during storage [1, 4].

4- total resulting

Based on the results of the present research, it was found that putresin and chitosan treatments have beneficial effects in maintaining the quality indicators of cucumber fruits. The use of putrescine and chitosan provides favorable conditions for cucumber fruits and improves the storage quality of this product after harvesting. It was found that concentrations of 2 mM putrescine and 1-1.5% chitosan have the greatest effect on maintaining the quality indicators of cucumber fruit and the storage of cucumber fruits (at 12 ± 1 °C and 90% RH) can be increased up to 20 days. It has been reported that cucumber fruits are sensitive to storage conditions and its storage time is limited to less than 14 days. Achieving 20 days of acceptable quality storage for edibles is an important result for the future of postharvest science and technology. These results show that the combination of concentrations of 2 mM putrescine and 1 to 1.5% chitosan improves its efficiency and provides higher quality fresh products. Finally, in order to maintain the quality of the fruit, it is suggested that the fruits are pre-treated with putrescine and coated with chitosan so that cucumbers with higher shelf life and quality reach consumers.

5- Thank

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مقاله علمی_پژوهشی

کاربرد پس از برداشت کیتوزان و پوترسین بر حفظ کیفیت و افزایش ماندگاری خیار (*Cucumis sativus* L.)

سمیرا فکوری‌زاده [۱]، محمدحسین دانشور [۲]، محمدرضا زارع بوانی [۳*]،

۱- دانش‌آموخته کارشناسی ارشد، گروه علوم و مهندسی باغبانی، دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران،

۲- استاد، گروه علوم و مهندسی باغبانی، دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

۳- استادیار، گروه علوم و مهندسی باغبانی، دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

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* مسئول مکاتبات:

mzarebavani@asnrukh.ac.ir

چکیده

میوه‌های خیار در مرحله پس از برداشت، به دلیل رطوبت بالا، فعالیت متابولیکی زیاد و فساد ناشی از رشد میکروارگانیسم‌ها، عمر نگه‌داری کوتاهی دارند. در این پژوهش اثرات پوترسین (۰، ۰/۵، ۱ و ۲ میلی‌مولار) و کیتوزان (۰، ۰/۵، ۱ و ۱/۵ درصد) بر برخی ویژگی‌های میوه خیار در طی انبارمانی در دمای ۱±۱۲ درجه سلسیوس در زمان انبارمانی (۰، ۱۰ و ۲۰ روز) و یک روز در دمای ۲۵ درجه سلسیوس بررسی شد. نتایج نشان داد که کیتوزان و پوترسین تأثیر معنی‌داری بر جلوگیری از کاهش وزن، حفظ مواد جامد محلول، اسیدیته قابل تیتر، pH، سفتی بافت، کلروفیل پوست، اسیدآسکوربیک و بهبود کیفیت حسی میوه خیار در طی نگه‌داری دارند. کم‌ترین کاهش وزن و بیش‌ترین سفتی در تیمار ۱/۵٪ کیتوزان و ۲ میلی‌مولار پوترسین پس از ۱۰ و ۲۰ روز انبارمانی به دست آمد. هم‌چنین بیشترین میزان مواد جامد محلول، اسیدیته قابل تیتر، میزان کلروفیل، ویتامین ث، آنتی‌اکسیدان کل، فنل کل و کمترین میزان pH، پوسیدگی و کاهش کیفیت حسی در زمان‌های ۱۰ و ۲۰ انبارمانی مربوط به تیمارهای ۱/۵٪ کیتوزان و ۲ میلی‌مولار پوترسین بود که با تیمار شاهد تفاوت معنی‌داری نشان دادند. تیمارهای پوشش ۱٪ و ۱/۵ درصد کیتوزان به‌جز در کاهش وزن در دیگر صفات اندازه‌گیری شده در طی انبارمانی اختلاف معنی‌داری نشان ندادند. به نظر می‌رسد که پیش تیمار پوترسین (۲ میلی‌مولار) و پوشش کیتوزان (۱ تا ۱/۵٪) می‌تواند ضمن افزایش عمر انبارمانی خیار، کیفیت میوه‌ها را به مدت طولانی‌تری حفظ کند