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ORIGINAL RESEARCH PAPER

THE EFFECT OF NOVEL PACKAGING CONTAINING PHASE CHANGE MATERIAL ON BROCCOLI QUALITY DURING STORAGE

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Abstract: Farmers and distributors find it challenging to maintain broccoli as a fresh vegetable during shelf life. Novel packaging can be used to extend the shelf life and preserve the nutritional value of the plant. Innovative aspects of this package include the use of high latent heat phase change materials, such as short chain paraffins in the polymer matrix. The weight loss, DPPH radical scavenging activity, glucosinolates, total phenol and moisture content, texture, color and microbiological test, have been investigated for 15 days by 5 days' interval of storage under refrigerator temperature using PE packages containing phase change material and PVC packaging with ozone pretreatment. According to the results, the sample was packaged in PE containing phase change material during 15 days of storage had the lowest microbial count and the least amount of weight loss, the highest total phenol content and the highest content of glucosinolates. During 15 days of storage, the samples treated with 1.6 ppm ozone and the novel packaging showed more freshness and crispness. In comparison to the control sample, the L, a, and b indicators changed the least in the sample packed in novel packaging. Therefore, broccoli's shelf life can be successfully extended by using novel packaging with better preservation of its durability, sensory properties, and nutritional value and freshness of the agricultural product.

Keywords: *glucosinolates, ozone, paraffin, polymer, post-harvest, total microbial count*

INTRODUCTION

Broccoli and other cruciferous vegetables have high detoxification activity because of glucosinolates. Broccoli contains glucoside compounds that containing sulfur which is produced from the breakdown of glucosinolates, isothiocyanates, and indoles. Glucosinolates levels vary based on the growing season, growing conditions, and species. The sulforaphane in broccoli plants inhibits the cell cycle in cancer cells and is found in broccoli sprouts, which are an important source of sulforaphane [1]. Oxidative stress results from an unbalanced reaction between free radicals and antioxidants, which is the root cause of most chronic diseases [2]. Phenolic compounds, beta-carotene, ascorbic acid and vitamin E are health-promoting and antioxidant agents that should be stored properly during storage [1]. A relative humidity of 90 to 95 % and a temperature of 10 °C are recommended for keeping agricultural products fresh and reducing crop respiration. Between 10 and 20 °C, crop respiration and deterioration are intensified [3].
A lack of proper packaging can result in food waste and loss. From farm to fork, appropriate packaging is essential to maintain quality and freshness. The post-harvest packaging and cover provides some sustainability during post-harvest, especially in developing countries with high waste rates [4].
Phase change materials (PCM) with high latent heat are the thermal energy storage materials that can improve the performance and reliability of packaging [5]. By putting PCMs into the structure of packaging materials, this well-known function can be performed better for food products by buffering or minimizing temperature fluctuations caused by heat transfer between the product and the surrounding medium [6]. A PCM absorbs energy and releases it into the environment during the heating process and reverse cooling process, respectively [7]. Phase-change materials with a melting point of 10 °C can be used in agricultural products to reduce the effects of temperature changes. Low molecular weight paraffin is used in passive cooling. Paraffin-based PCMs are typically encapsulated or in shape-stable matrixes and this new form is in a flexible polymer matrix [7, 8].
Immersing broccoli in ozone-containing water minimizes chemical and microbial contamination. The use of ozone appears to extend the shelf life of fresh and uncut crops like broccoli [9]. One of the best benefits of ozone is that excess ozone decomposes quickly on its own, producing oxygen without leaving any residue in food [10]. Ozone and ozone-derived free radicals cause bacterial cell lysis by attacking proteins, fats, cell walls, and enzymes in spores and lipids [11, 12]. In this study, ozone pretreatment and packaging materials containing PCMs were investigated in combination to extend shelf-life of broccoli.

MATERIALS AND METHODS

The broccoli used in this study was a hybrid seed with liberty cultivar prepared from a greenhouse in Mehrshahr, Karaj. Samples were taken from the greenhouse 6 hours after harvesting.
Packaging material that was used: transparent stretch film containing S65 grade PVC granules with additives such as soybean oil and anti-phage (WVTR (Water vapor transmission rate) = 0.07 %, OTR (Oxygen transmission rate) = 0.15 %, tensile strength

= 30 N, shrinkage percentage = 67 to 70 %) from Almas Film Azerbaijan Iran. A three-layer polyethylene film with a thickness of 85 microns, which in the middle layer contains short-chain paraffin as a phase-change material (WVTR=2.90 $g \cdot m^{-2} \cdot day^{-1}$, OTR=26.9 $mL \cdot m^{-2} \cdot day^{-1}$, tensile strength =26 N) was used [13].

In this study, the ozone water treatment was performed by an Ozonizer machine 5 $g \cdot h^{-1}$ that was produced by Noavaran Company, Pasargad Ozone Makers. The incoming water volume was 20 liters. With 99 % purity, the inlet flow rate was one liter per minute. Treatment with ozone water was performed at 0.7 and 1.6 ppm, and broccoli was exposed for 15 minutes each. A detector tablet was used to measure the amount of ozone in the water and residual ozone. We measured the amount of ozone by removing it from the container and placing it on a sterile tube with a detector dissolved in it.

The broccoli, including the florets and stems, was freeze-dried. Because crushing releases the enzyme Myrosinase, which converts glucosinolates to glucose, the amount of glucosinolates can be estimated by determining the amount of glucose. In accordance with the reference method, two preparation methods were used [14].

Each sample was subjected to a texture analysis. The sample was positioned longitudinally in the center of the texture gauge machine. The Varner cutting blade probe was performed at a speed of 120 $mm \cdot min^{-1}$. Three replications of each sample were performed.

The Folin–Ciocalteu reagent was used to measure phenolic content in samples containing flower and stem tissue, which were dried in the medium. An ultrasonic homogenizer and 70 % ethanol were used to prepare the extract [15]. The absorbance was measured at 765 nm.

To determine the level of free radical scavenging activity, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used. DPPH radical scavenging activities of broccoli were determined by measuring the absorbance of the solution at 517 nm [16].

Statistical analyses were performed by one-way analysis of variance (ANOVA) using SPSS software ver. 22.0.

For comparing the means, a Duncan's multiple range test at 95 % confidence level was used.

RESULT AND DISCUSSION

Ozone is an unstable gas. After ozone injection in water and washing broccoli for 15 minutes, the results showed there was no trace of ozone in the water and the photometer showed no number, indicating that there was no ozone in the solution. A few minutes of ozone lasts in clean water and only seconds in dirty water [10].

According to microbiological tests, the lowest microbial population is associated with the sample treated with 1.6 ppm ozone and the film containing phase change material, while the highest is associated with the control sample unpacked and unwashed (Table 1). Rinmarn *et al.* [17] demonstrated that washing with tap water removed a small number of germs, whereas ozone treatment reduced the number of microorganisms by way of a logarithmic cycle. Additionally, sugar beets are reduced in spoilage when using PE containing PCM [18].

Table 1. *Total microbial count of different broccoli samples during refrigerated storage*

Treatment	Day0	Day 5	Day10	Day15
Control Sample	31800±311 ^{Da}	527000±7671 ^{Cc}	785000±8071 ^{Bb}	995000±43698 ^{Aa}
SO0	31800±311 ^{Da}	535000±7071 ^{Cc}	725000±7071 ^{Bb}	969000±29698 ^{Ab}
SO0.7	31800±311 ^{Ba}	13350±1202 ^{De}	15000±4242 ^{Ce}	88500±777/81 ^{Ad}
SO1.6	31800±311 ^{Da}	475000±1414 ^{Cb}	520000±14142 ^{Bd}	674500±50204 ^{Ad}
PCMO0	31800±311 ^{Da}	110000±14142 ^{Aa}	90500±707 ^{Ca}	92500 ± 1060^{B}
PCMO0.7	31800±311 ^{Da}	67900±1555 ^{Ab}	57500±3535 ^{Bc}	43500±3535 ^{Ce}
PCMO1.6	31800±311 ^{Aa}	18750±353 ^{Bd}	17400±565 ^{Ce}	17000±2828 ^{Df}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment)).

Water content of broccoli samples showed that the packaging with PE containing phase change material experienced an increase in humidity on day 15 ($p < 0.05$). Based on the results in Table 2, the samples packaged in PE containing PCM materials were associated with higher moisture content due to the reduction of OTR of this type of packaging and reduced respiration. Storing agricultural products at a lower temperature than the melting point of paraffin reduces permeability and reduces respiration, which can lead to loss of water content and weight. The main causes of weight loss in fruits and vegetables during storage and after harvest are respiration and sweating [13].

Table 2. *Moisture content of different broccoli samples during refrigerated storage*

Treatment	Day0	Day 5	Day10	Day15
Control Sample	85.02±0.014^{Ca}	86.00±0.070^{Ac}	85.92±0.070^{Bb}	82.83±0.070^{Dd}
SO0	85.02±0.014^{Aa}	84±0.070^{Bd}	84.00±0.070^{Bb}	84.00±0.070^{Bb}
SO0.7	85.02±0.014^{Ca}	89.00±0.014^{Aa}	86.00±0.014^{Ba}	83.01±0.063^{Dc}
SO1.6	85.02±0.014^{Aa}	82.04±0.014^{De}	82.485±0.007^{Cc}	84.925±0.035^{Bb}
PCMO0	85.02±0.014^{Ba}	84.00±0.035^{Cd}	84.775±0.021^{Cb}	88.1±0.01^{Aa}
PCMO0.7	85.02±0.014^{Ca}	87.24±0.014^{Ab}	86.07±0.014^{Ba}	87.3±0.014^{Aa}
PCMO1.6	85.02±0.014^{Da}	87.735±0.070^{Ab}	87.020±0.070^{Ba}	86.520±0.070^{Cb}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment)).

The difference in water vapor pressure between the texture of the product and the atmosphere around the product causes sweating. Accordingly, it can be reported that vegetable weight loss can be affected by packaging type. Results of changes in weight loss in PVC showed the greatest weight loss after 15 days of storage (Table 3).

Table 3. *Weight loss of different samples of broccoli during storage at refrigerator temperature*

Treatment	Day 5	Day10	Day15
Control sample	1.00 ± 0.014^{Ab}	4.00 ± 0.014^{Bb}	12.00 ± 0.021^{Cc}
SO0	1.00 ± 0.014^{Ab}	3.00 ± 0.014^{Ba}	9.00 ± 0.021^{Cb}
SO0.7	8.07 ± 0.00^{Ac}	10.00 ± 0.424^{Bc}	14.00 ± 0^{Cd}
SO1.6	0.00 ± 0.014^{Aa}	4.00 ± 0.014^{Bb}	9.00 ± 0.070^{Cb}
PCMO0	1.42 ± 0.00^{Ab}	4.00 ± 00^{Bb}	6.07 ± 00^{Ca}
PCMO0.7	1.23 ± 0.00^{Ab}	11.95 ± 00^{Bc}	14.11 ± 00^{Cd}
PCMO1.6	1.00 ± 0.00^{Ab}	3.25 ± 00^{Ba}	6.90 ± 00^{Ca}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment)).

Comparing the packaging, it was found that the packaging containing PCM had significantly less weight loss ($p < 0.05$), which can be attributed to its low permeability. The color experiments revealed that ozone treatment and packaging containing PCM did not adversely affect color but washing PVC-packaged samples with ordinary water reduced transparency and created unfavorable effects (Table 4).

Table 4. *Changes in color parameters (L *, a and b) of different samples of broccoli during storage at refrigerator temperature*

Treatment	Day0	Day5	Day10	Day15
L*				
Control sample	40.00 ± 0.00^{Aa}	34.00 ± 0.007^{Cf}	32.15 ± 0.007^{De}	36.00 ± 0.007^{Be}
SO0	40.00 ± 0.00^{Aa}	37.00 ± 0.007^{Bc}	36.15 ± 0.007^{Cd}	36.00 ± 0.007^{Ce}
SO0.7	40.00 ± 0.00^{Aa}	37.00 ± 0.007^{CC}	38.00 ± 0.007^{Bb}	40.00 ± 0.00^{Ac}
SO1.6	40.00 ± 0.00^{Ba}	35.00 ± 0.007^{De}	37.00 ± 0.007^{Cc}	41.00 ± 0.007^{Ab}
PCMO0	40.00 ± 0.00^{Da}	41.00 ± 0.007^{Ca}	42.00 ± 0.007^{Ba}	43.00 ± 0.007^{Aa}
PCMO0.7	40.00 ± 0.00^{Aa}	36.00 ± 0.007^{Cd}	37.00 ± 0.007^{Bc}	37.00 ± 0.00^{Bc}
PCMO1.6	40.00 ± 0.00^{Aa}	39.00 ± 0.007^{Bb}	39.00 ± 0.007^{Bb}	39.00 ± 0.007^{Bd}
A				
Control sample	-3.00 ± 0.707^{Ca}	-4.00 ± 0.00^{Da}	-2.00 ± 0.007^{Ba}	-1.00 ± 0.007^{Aa}
SO0	-3.00 ± 0.707^{Ba}	-5.00 ± 0.00^{Cb}	-3.00 ± 0.007^{Bb}	-2.00 ± 0.007^{Ab}
SO0.7	-3.00 ± 0.707^{Aa}	-4.00 ± 0.00^{Ba}	-4.00 ± 0.007^{Bc}	-4.00 ± 0.007^{Bc}
SO1.6	-3.00 ± 0.707^{Aa}	-6.00 ± 0.007^{Cc}	-5.00 ± 0.007^{Bd}	-6.01 ± 0.007^{Cd}
PCMO0	-3.00 ± 0.707^{Aa}	-6.00 ± 0.007^{Dc}	-5.00 ± 0.007^{Cd}	-4.00 ± 0.007^{Bc}
PCMO0.7	-3.00 ± 0.707^{Aa}	-4.00 ± 0.007^{Ba}	-4.00 ± 0.007^{Bc}	-4.00 ± 0.00^{Bc}
PCMO1.6	-3.00 ± 0.707^{Aa}	-4.00 ± 0.00^{Ba}	-4.00 ± 0.007^{Cc}	-5.15 ± 0.007^{Dd}
B				
Control sample	8.00 ± 0.00^{Da}	11.00 ± 0.00^{Be}	17.00 ± 0.007^{Ac}	10.00 ± 0.007^{Ce}
SO0	8.00 ± 0.00^{Da}	12.00 ± 0.00^{Cd}	19.00 ± 0.007^{Bb}	22.00 ± 0.007^{Ab}
SO0.7	8.00 ± 0.00^{Da}	19.00 ± 0.007^{Aa}	10.00 ± 0.007^{Cf}	12.00 ± 0.00^{Be}
SO1.6	8.00 ± 0.00^{Da}	15.00 ± 0.014^{Cc}	16.00 ± 0.007^{Bd}	17.00 ± 0.00^{Ac}
PCMO0	8.00 ± 0.00^{Da}	22.00 ± 0.007^{Cb}	23.00 ± 0.007^{Ba}	25.00 ± 0.007^{Aa}
PCMO0.7	8.00 ± 0.00^{Da}	15.04 ± 0.007^{Cc}	15.00 ± 0.007^{Be}	16.00 ± 0.00^{Ad}
PCMO1.6	8.00 ± 0.00^{Da}	8.00 ± 0.007^{Bf}	9.05 ± 0.007^{Af}	9.00 ± 0.00^{Af}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment)).

The broccoli sample wrapped in polyethylene containing PCM and ozone treated with 1.6 ppm had the least amount of jaundice in the florets. According to the control sample, the highest yellowing was observed after washing the broccoli with ordinary water. This means that the novel packaging controls respiration and reduces color and quality loss. Changes in color are not noticeable at the level of ozone concentration at which it is normally used; it can in some cases improve fruit color [19].

Based on glucosinolates content, high correlations between glucosinolates (R^2 = 0.94) were observed at a 95 % confidence level. The highest content is related to the sample packed with novel packaging and ozone treated with 1.6 ppm broccoli, and the lowest content is related to the sample packed with PVC compared with PE containing PCM broccoli (Figure 1). Sugar content and therefore glucosinolates are not affected by ozone treatment [19], however, the novel packaging reduces respiration. Accordingly, glucosinolates levels did not decrease.

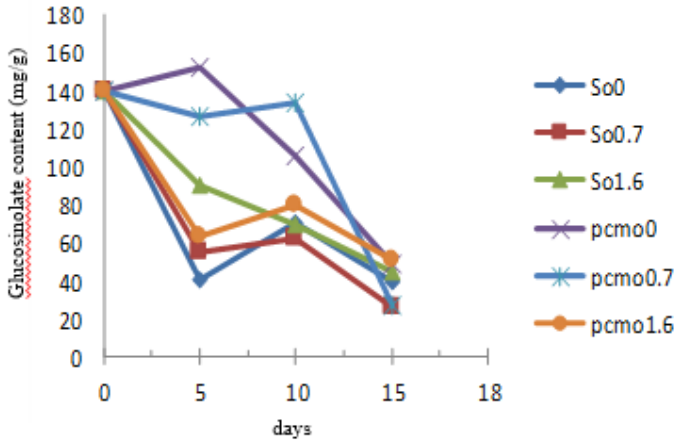

Figure 1. *Diagram of glucosinolates levels in broccoli with different treatments (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment))*

According to Table 5, in the results of the texture analysis, the control sample had a significant difference with the sample treated with ozone at 1.6 ppm and packaged with phase change materials. A sample wrapped in polyethylene containing PCM and washed with ozone after 15 days of storage had a more favorable texture. This result corresponds to the water content and respiration rate in packaging containing PCM.

Table 5. *Changes in Texture Stiffness (Newton) of Different Broccoli Samples during Storage at Refrigerator Temperature*

Treatment	Day0	Day 5	Day10	Day15
F(max)				
Control sample	222 ± 0^{Aa}	166 ± 0^{Be}	155 ± 0^{Ce}	143 ± 0^{De}
SO0	222 ± 0^{Aa}	187 ± 0^{Dd}	195 ± 0^{Cc}	201 ± 0^{Bd}
SO0.7	222 ± 0^{Da}	282 ± 0^{Aa}	229 ± 0^{Cb}	238 ± 0^{Bc}
SO1.6	222 ± 0^{Ca}	202 ± 0^{Dc}	226 ± 0^{Bb}	255 ± 0^{Ab}
PCMO0	222 ± 0^{Ca}	240 ± 0^{Ab}	230 ± 0^{Bb}	222 ± 0^{Cd}
PCMO0.7	222 ± 0^{Aa}	145 ± 0^{Cf}	180 ± 0^{Bd}	120 ± 0^{Df}
PCMO1.6	222 ± 0^{Da}	291 ± 0^{Aa}	287 ± 0^{Ba}	272 ± 0^{Ca}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment)).

High correlations between total phenols ($R^2 = 0.98$) were observed at a 95 % confidence level.). The results of total phenol in Table 6 indicate the highest concentrations of phenolic compounds were related to the sample that was treated with 0.7 ppm ozone and packaged with PCM, while the highest reduction in phenolic compounds was observed in the control sample without packaging and washing. In previous research, packaging material was found to have a direct impact on phenolic compounds. Some authors confirm that low storage temperatures can cause to accumulate polyphenols [20]. Packaging that contains PCM has a synergistic effect on storage temperature of phenolic compounds.

Table 6. *Total phenol content of different samples of broccoli during refrigerated storage*

Treatment	Day0	Day 5	Day10	Day15
Control Sample	191.00 ± 0.028^{Aa}	121.02 ± 0.070^{Bd}	113.00 ± 0.014^{Ce}	101.00 ± 0.014^{Df}
SO0	191.00 ± 0.028^{Aa}	174.00 ± 0.070^{Cb}	183.00 ± 0.070^{Ba}	166.00 ± 0.014^{De}
SO0.7	191.00 ± 0.014^{Aa}	180.42 ± 00.01^{Ca}	146.25 ± 1.173^{Dd}	182.08 ± 00.01^{Bd}
SO1.6	191.00 ± 0.014^{Ba}	137.08 ± 00.01^{Dc}	189.58 ± 00.01^{Cf}	207.92 ± 00.00^{Ab}
PCMO0	191.25 ± 00.01^{Aa}	110.42 ± 00.01^{De}	129.58 ± 00.01^{Be}	112.91 ± 1.180^{Cf}
PCMO0.7	191.00 ± 0.014^{Ba}	121.25 ± 0.014^{Df}	153.75 ± 0.042^{Cc}	227.08 ± 0.014^{Aa}
PCMO1.6	191.25 ± 00.01^{Ba}	175.00 ± 0.059^{Cb}	172.92 ± 00.01^{Db}	194.58 ± 0.014^{Ac}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment).

In Table 7, the results of DPPH radical scavenging activity showed that packaging with PE containing PCM prevented the reduction of antioxidant content of broccoli significantly. Thus, novel packaging and cold storage increase polyphenols in a synergistic way. Researchers have found that storage conditions and time can affect antioxidant levels, and that the antioxidant levels of broccoli decrease during storage. Lettuce that was treated with ozone-containing water was not observed to exhibit appearance or antioxidant properties. The results showed that ozone treatment reduced lettuce ascorbic acid [21].

Table 7. *Antioxidant content of different broccoli samples during refrigerated storage*

Treatment	Day0	Day 5	Day10	Day15
Control Sample	69.46±1.39^{Da}	80.00±0.01^{Be}	82.00±1.00^{Ad}	78.00±1.00^{Cd}
SO0	69.46±1.39^{Ca}	84.00±0.07^{Bc}	84.00±0.01^{Bc}	86.00±2.00^{Ab}
SO0.7	69.46±1.39^{Da}	88.30±0.07^{Aa}	84.76±0.01^{Cc}	85.54±0.01^{Bc}
SO1.6	69.46±1.39^{Ca}	82.04±0.06^{Bd}	82.90±0.48^{Bd}	85.12±0.63^{Ac}
PCMO0	69.46±1.39^{Ba}	88.80±0.76^{Aa}	88.44±0.81^{Aa}	87.75±2.53^{Aa}
PCMO0.7	69.46±1.39^{Da}	87.78±2.01^{Bb}	86.66±0.00^{Cb}	87.88±0.03^{Aa}
PCMO1.6	69.46±1.39^{Ca}	87.35±0.01^{Ab}	86.76±0.01^{Bb}	86.54±3.23^{Bb}

* Capital letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in the row and lower-case letters indicate a significant difference in 95 % confidence level ($p < 0.05$) in each column. (S (stretch film), PCM (PE containing phase change material, O0 (without ozone), O0.7 (0.7 ppm ozone) and O1.6 (1.6 ppm ozone treatment).

CONCLUSION

A novel packaging system could be able to decrease weight and moisture losses when it comes to vegetables and fruits due to a high percentage of waste globally. Additionally, ozone washing reduces the microbial population of agricultural products, which prevents them from spoiling. Since broccoli has a high nutritional value, which is beneficial for human health, it is important to use these methods to reduce waste and provide it to consumers at a reasonable cost. Studies have shown that storing broccoli at refrigeration temperature, along with packaging that contains phase-change material, can reduce waste and reduce yellowing of broccoli florets and deterioration. As a result, broccoli packed in a polyethylene bag containing phase change materials showed the least changes during storage. Additionally, it was found that adding ozone to water had positive effects on microbial populations and color, as well as providing synergistic packaging protection and improving broccoli texture during storage.

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