Effect of salt and organic acid on storage quality of minimally processed Indian spinach

Raju Ahmmed1, Farjana Akter Sukhi2, Fardana Binte Zaman3, Shimul Das3, Md. Fakhruл Hasan3, Md. Junaed Khan1

1 Department of Food Engineering, Dhaka University of Engineering and Technology, Gazipur, Bangladesh.
2 Department of Food Technology and Engineering, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh.
3 Department of Horticulture, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh

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Abstract
Postharvest processing of horticultural products not only adds value to the products, but also makes the products more convenient to the consumers. The objective of this study was to investigate the storage quality of minimally processed Indian spinach as influenced by organic acid and salt. The vegetables were washed and immersed in cold (7°C) water with 100 mg L\(^{-1}\) free chlorine for sanitation. The samples were treated with NaCl, KMS and citric acid in 9 treatments viz. T\(_1\): 1% NaCl, T\(_2\): 1.5% NaCl, T\(_3\): 2% NaCl, T\(_4\): 1.5% citric acid, T\(_5\): 1% citric acid, T\(_6\): 0.5% citric acid, T\(_7\): 0.5% KMS, T\(_8\): 1% KMS and T\(_9\): 1.5% KMS. The treated samples were stored for 0 to 6 days. Among the treatments, changes in quality traits decreased significantly (p = 0.05) with the increasing rate of organic acid (T\(_6\)) compared to the control and other treatments during the storage period. Treatment T\(_1\) was better than other treatments as the treated spinach had higher titratable acidity (33.47, 22.60 & 18.83), lower TSS (0.30, 0.30 & 0.40), lower pH (6.99, 3.43 & 4.12), higher vitamin C (17.03, 16.33 & 11.40), lower weight loss (10, 9.90 & 9.40) and higher firmness (15.45, 13.29 & 10.67) at 2, 4 and 6 days. The storage ability was good for all treatments, but keeping quality was not satisfactory for all treatments. There was significant variation among all treatments, and 1.5% citric acid was accepted for keeping the good storage quality of minimally processed Indian spinach.

Introduction
In Bangladesh, there are still significant losses of agricultural products due to inadequate use of technology during cultivation, handling, storage, and preservation. An alternative technology to minimize post-harvest losses is the minimal processing of vegetables (Chitarra, 1999). The demand for minimally processed vegetables has promoted an increase in the nutritional quality and safety of a variety of food products. In Bangladesh, total area of Indian spinach cultivation is about 10217 hectares with the production of 78665 metric tons in 2015-2016 (BBS, 2016). Minimal processing is an integrated approach, where raw materials handling, processing, packaging, and distribution must all be properly considered to make shelf-life extension possible and offer consumer with high nutrition. Being living materials, fresh-cut produce can modify the atmosphere in their packages as a result of respiratory \(O_3\) consumption and \(CO_2\) evolution (Pirovani et al., 1998). Major problems associated with fresh-cut vegetables are the development of strong off-odors and decay, discoloration, and tissue softening (Zagory and Kader, 1988). The physical damage accrued during minimal processing, especially with wounding, causes disruption of the cellular membrane, putting enzymes and their substrates in direct contact, which accelerates the loss of quality and storage life. Unit operations applied in the commercial production of minimally processed vegetables usually include cleaning, peeling, cutting, washing, and packaging. To increase the shelf life of vegetables, different types of synthetic chemicals are used commercially that are harmful for human beings. But organic substances and safe salts can be the alternative of hazardous synthetic chemicals. To overcome the undesirable changes, cut produce are immersed in different treatment solutions at the final stage of handling operations. Compounds such as ascorbic and citric acids, sodium meta bisulphite and sodium chloride have been utilized in many parts of the world as suitable preservatives for maintaining quality of minimally processed produce such as potato, spinach and apple (Ahvenainen, 1996; Cortez-Vega et al., 2008).

At present, Indian spinach is selected to minimally process due to its rich nutritional contents and higher use in our daily life for preparing a variety of foods. Moreover, nutrients like iron, calcium and vitamin K are present in higher concentrations due to which it becomes more beneficial against some serious health problems like anaemia, haemorrhage and clotting dysfunction and to maintain electrolytes balance in our body (Raheem et al., 2019).
Storage quality of minimally processed spinach

al., 2015). Now a day’s, people, especially the office going ladies, are becoming busy for different activities and they have no enough time to process vegetables by washing, cutting, peeling for ready to eat. Again, sometimes the quality of those vegetables is not so satisfactory for health; it may not be fresh, mature or other problems as well. Due to those reasons, people lose interest to take vegetables. This kind of problems can be overcome by providing minimally processed vegetables. Therefore, the present study was conducted to evaluate the physical and chemical properties of minimally processed Indian spinach, to evaluate the effects of NaCl, KMS and Citric acid on minimally processed Indian spinach, and to study the hygienic quality of minimally processed Indian spinach.

Materials and Methods

Sample collection
Indian spinach was collected directly from the farmers' fields of Dumki, Patuakhali at the optimum stage of maturity. Sample of matured and freshly harvested Indian spinach was carried at the Horticultural Laboratory, Patuakhali Science and Technology University (PSTU).

Sample processing
The sanitation of the process was ensured by personal hygiene and the use of aprons and gloves. All the utensils used were previously sanitized in 200 mgL⁻¹ free chlorine. The sample was washed in fresh water, cut with sharp stainless-steel knives and immersed in cold water (7ºC) for 15 min. Indian spinach was cut into 3-mm thick slices having round shape using a knife. These were then immersed in cold water (7ºC) with 100 mgL⁻¹ free chlorine at pH 7.0 for 15 min for sanitation. After completing the cleaning process, all the samples were kept in air-tight polyethylene bags as per treatments of the study and then stored in refrigerator under low temperature. Data were recorded at 0, 2, 4 and 6 days after storage as per treatments.

Physicochemical analysis
Firmness: Firmness was measured by using the Magnness-Taylorpressure testers (Model GY-802). The firmness testing machine was equipped with a 4-mm diameter cylindrical probe that was penetrated in the normal direction. The reading was taken for each sample at collar, middle and end points according to Hassan (2006).

Weight loss: Weights of packages with sample slices were measured using an electronic balance (Sartorius BP 6100, R & M marketing, UK). The difference in the weight was expressed as gram of weight loss of the samples (Anthony et al., 2003).

Titratable acidity: Titratable acidity of the filtered sample was measured with sodium hydroxide at 0.01N (the results for the Indian spinach was expressed in % of citric acids) (AOAC, 1995).

pH: pH was determined in the triturated sample by a digital electronic pH meter. Before measurement, the pH meter was standardized with a buffer solution as described by (Ranganna, 1994).

TSS: Ten (10) g sample of Indian spinach was blended with distilled water (40 ml) in a homogenizer (Black & Decker, BX 250, Hunt Valley, USA) for 2 min. The homogenate was filtered through a muslin cloth, and few drops of the filtrate were used to measure TSS using a hand-held Refractometer (BOE-32195).

Vitamin C: The content of vitamin C was determined with Tillman’s reagent (2,6 dichlorophenolindophenol) titration until a slightly pink coloration was stabilized for 15 s. The results were expressed in mg of ascorbic acid per 10 g of sample (Pregnolatto and Pregnolatto, 1985).

Data Analysis
Statistical analyses were done using Mixed-model analyses of variance (ANOVAs). Mean values of the samples were compared using Fisher’s Least Significant Difference (LSD) test among the treatments.
Results and Discussion

Effect of treatments on weight-loss
Weight-loss values for minimally processed Indian spinach showed differences among treatments. The weight-loss values at different days decreased with the increasing storage period (Fig. 1). There was significant difference among all treatments. At 2, 4, and 6 days T₄ attained the highest score (10 g, 9.90 g and 9.40 g, respectively); the difference was significant (p = 0.05). At 2 and 4 days, T₀ got the lowest score (9.10 g, 8.21 g) and at 6 days T₉ provided the lowest score (7.57 g). Water loss results in weight loss, wilting and shrivelling. At the time of harvest, the water content of vegetables is very high and has a fresh appearance and crisp texture. Harvesting removes the plant part from its water supply and the product begins to lose weight. This loss of water has an immediate economic effect in that it reduces saleable weight. Maximum permissible water loss for a number of Indian spinach is 3% (Kays and Paull, 2004). Any type of package slowed water loss when compared to using no package. In this study, water loss rate was low in T₄. Although we used polythene bag, the slight changes occurred due to small pores in the bags. So, T₄ treatment is considered more acceptable than other treatments for long storage period and better quality of minimally processed Indian spinach.

![Graph showing weight loss over days for different treatments](image_url)

**Fig. 1.** Variation of weight-loss on minimally processed Indian spinach with storage time under different treatments

Effect of treatments on firmness
In the minimally processed Indian spinach, the average values found for firmness ranged from 7.77 to 15.45 kg cm⁻² during different storage periods and among treatments (Tables 1 and 2). The firmness at different days of the minimally processed Indian spinach decreased with increasing storage period. At 2 days, T₄ attained the highest score (15.45), but treatment T₀ got the lowest score (12.01). At 4 days, treatment T₄ attained the highest score (13.29) and treatment T₆ got the lowest score (10.33). The highest score at 6 days was gained by the treatment T₄ (10.67) and treatment T₉ gained the lowest score (7.77). According to LSD values at 5% significant level, the difference was significant among all treatments (Tables 1 and 2).

Firmness is an important attribute that determines the acceptance or rejection of minimally processed produce by consumers. According to Ekanayake (2014), minimally processed immature jack fruit pre-treated with citric acid and ascorbic acid showed a slight decrease in firmness after 7 days of storage at 5-7°C. Textures of vegetables lose as moisture decreases. According to Ben-Yehoshua et al. (2005) if leafy vegetables lose more than 3% of the original fresh weight, they become unsalable. The texture of vegetables’ vegetative tissue is dependent on the cell wall. The decrease in firmness with storage may be due to the destruction of parenchymal cells, leading to softening of the plant material. In our study, a slight decrease in firmness was observed in treatment T₄; this is desirable for better quality of minimally processed Indian spinach than other treatment including the control (Table 1).

Effect of treatments on Total Soluble Solids (TSS)
In the minimally processed Indian spinach, the average values found for TSS ranged from 0.3⁰ Brix to 0.7⁰ Brix during different storage periods and among treatments (Tables 1 and 2). The total soluble solids at different days increased with increasing storage period. At 2 days, T₄ attained the highest score (0.60), but treatments T₃ and T₄ got the lowest score (0.30). At 4 days, treatment T₇ attained the highest score (0.70) and treatment T₄ got the lowest score (0.30). The highest score at 6 days was gained by the treatment T₈ (0.70) and treatment T₄ gained the lowest score (0.40) (Table 2). According LSD values at p=0.05, there was no significant difference among all treatments (Table 1 and 2). Vegetables contain many compounds, which are soluble in water
such as sugars, acids, vitamin C and amino acids. These soluble compounds form the soluble solid content of the vegetables. Increase in TSS content reflects hydrolysis of starch into sugars and makes vegetable sweeter. But, the treatments impede the process and keep accurate quality of vegetables and make them acceptable to the consumer. The lower value of TSS means lower content of sugar and better quality of vegetables. So, treatment T4 was more acceptable than the other treatments for TSS.

**Effect of treatments on Titratable Acidity (TA)**

The contents of titratable acidity, TA, affected by the storage period and presented statistical differences among treatments for Indian spinach. The average values found for TA ranged from 12.33% to 33.47% during different storage periods and among treatments (Tables 1 and 2). Titratable acidity at different days decreased with increasing storage period. At 2 days, T4 attained the highest score (33.47) but treatment T0 got the lowest score (14.30). The highest score at 6 days was gained by the treatment T3 (18.83) and treatment T0 gained the lowest score (13.83) (Table 2). According to LSD values at p= 0.05, the difference was significant among all treatments (Tables 1 and 2). At 2 days, T4 attained the highest score (17.03) but treatment T0 got the lowest score (11.00) (Table 1). At 4 days, treatment T4 attained the highest score (22.60) and treatment T0 got the lowest score (16.07) (Table 1). At 6 days, treatment T4 attained the highest score (21.80) and treatment T0 got the lowest score (16.04) (Table 1). At 8 days, treatment T4 attained the highest score (21.80) and treatment T0 got the lowest score (16.04) (Table 1). Each data point represents the mean of three replicates ± standard error.

Vitamin C levels oscillated with a slight decrease during the period of storage in all treatments carried out (Table 1). Similar results were found by (Benedetti et al., 2002) in sliced green peppers packed in expanded polystyrene trays and wrapped in PVC (polyvinyl chloride) film stored at 5ºC for 10 days. Vitamin C was lost during minimal processing through cutting during storage. Chemical treatments retained the nutritional quality as well as vitamin C from spoilage. In our research, 1.5% citric acid treatment (T4) showed low decrease level of Vitamin C during storage period. So, treatment T4 was more acceptable than other treatment at 5% level of significance.

**Effect of treatments on Vitamin C**

The vitamin C contents of the samples of minimally processed Indian spinach presented differences among treatments (Tables 1 and 2). Vitamin C at different days decreased with increasing storage periods. According to LSD values at p= 0.05, the difference was significant among all treatments (Tables 1 and 2). At 2 days, T4 attained the highest score (17.03) but treatment T0 got the lowest score (11.00) (Table 1). At 4 days, treatment T4 attained the highest score (16.33) and treatment T0 got the lowest score (10.70). The highest score at 6 days was gained by the treatment T4 (11.40) and treatment T0 gained the lowest score (3.60) (Table 2).

Girardi et al., (2005) showed that decline of titratable acidity during storage is inevitable, but citric acid treatment can help to better retain titratable acidity. In our research, 1.5% citric acid treatment (T4) hold high content of TA. According to Economos and Clay (1999), citrus vegetables are a rich source of vitamins, minerals and dietary fiber that are essential for normal growth and development and overall nutritional well-being. So, T4 was considered more acceptable for long storage period and good quality of Indian spinach, but it slightly reduces flavour and affects tastes.

**Table 1. The physicochemical properties of minimally processed Indian spinach on day 0 and day 2 due to the effects of different treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Firmness (kg cm(^{-2}))</td>
<td>TSS (°Brix)</td>
</tr>
<tr>
<td>(T_0)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_1)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_2)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_3)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_4)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_6)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_7)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_8)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>(T_9)</td>
<td>16.04±0.00</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(T1: 1% NaCl, T2: 1.5% NaCl, T3: 2% NaCl, T4: 1.5% Citric acid, T0: 1% citric acid, T6: 0.5% citric acid, T7: 0.5% KMS, T8: 1% KMS, T9: 1.5% KMS treatment). Each data point represents the mean of three replicates ± standard error.
Table 2. The physicochemical properties of minimally processed Indian spinach on day 4 and day 6 due to the effects of different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Firmness (kg cm⁻²)</td>
<td>TSS (°Brix)</td>
</tr>
<tr>
<td>T₀</td>
<td>12.02±0.87</td>
<td>0.50±0</td>
</tr>
<tr>
<td>T₁</td>
<td>12.06±0.87</td>
<td>0.40±0</td>
</tr>
<tr>
<td>T₂</td>
<td>11.77±0.87</td>
<td>0.60±0</td>
</tr>
<tr>
<td>T₃</td>
<td>11.29±0.87</td>
<td>0.40±0</td>
</tr>
<tr>
<td>T₄</td>
<td>13.29±0.87</td>
<td>0.30±0</td>
</tr>
<tr>
<td>T₅</td>
<td>13.07±0.87</td>
<td>0.40±0</td>
</tr>
<tr>
<td>T₆</td>
<td>10.33±0.87</td>
<td>0.50±0</td>
</tr>
<tr>
<td>T₇</td>
<td>11.01±0.87</td>
<td>0.70±0</td>
</tr>
<tr>
<td>T₉</td>
<td>10.52±0.87</td>
<td>0.50±0</td>
</tr>
<tr>
<td>T₀</td>
<td>12.44±0.87</td>
<td>0.60±0</td>
</tr>
<tr>
<td>LSD₀.₀5</td>
<td>1.589</td>
<td>0.1523</td>
</tr>
</tbody>
</table>

(T₀: 1% NaCl, T₁: 1.5% NaCl, T₂: 2% NaCl, T₃: 1% citric acid, T₄: 0.5% citric acid, T₅: 0.5% KMS, T₆: 1% KMS, T₇: 1.5% KMS treatment). Each data point represents the mean of three replicates ± standard error.

**Effect of treatments on pH**

In the minimally processed Indian spinach, the average pH ranged from 7.1 to 3.9 during different storage periods (Tables 1 and 2); pH increased with increasing storage period. There was significant difference in pH at p= 0.05 among all treatments due to the effect of citric acid. NaCl and KMS treatments. At 0 days, T₄ attained the highest score (6.95) but treatment T₀ got the lowest score (5.02) (Table 1). At 2 days, T₄ attained the highest score (6.99) but treatment T₀ got the lowest score (3.43) (Table 1). At 4 days, treatment T₄ attained the highest score (7.02) and treatment T₀ got the lowest score (3.93). At 6 days, treatments T₁ and T₉ got the highest score (7.27) and treatment T₀ got the lowest score (4.12) (Table 2).

The pH depends on the total quantity of acids as well as the strength of the acids present (Schmidl and Labuza, 2000). According to Daranagama et al., (2012), minimally processed banana pre-treated with ascorbic acid and citric acid stored at 5-7 °C showed slight increase of pH (lower acidity) after 7 days of storage. High content of pH diminishes the quality of vegetables. In our research, pH content was low in T₄ since Indian spinach is alkaline when raw. So, T₄ is considered more acceptable treatment for better storage of minimally processed Indian spinach.

**Overall effect of treatments on Vitamin C, TA and pH**

According to Hartwig and McDaniel (1995), pH is inversely related with organic acid and Vitamin C. At low pH, titratable acidity and Vitamin C content will be high and vice versa. For minimally processed Indian spinach, low pH holds up good storage and nutrient quality. The results show significant variation for Vitamin C, titratable acidity and pH with the treatments (T₁: 1% NaCl, T₂: 1.5% NaCl, T₃: 2% NaCl, T₄: 1.5% Citric acid, T₅: 1% Citric acid, T₆: 0.5% Citric acid, T₇: 0.5% KMS, T₈: 1% KMS, T₉: 1.5% KMS). Among all treatments, T₄ shows the best result for keeping quality of storage life. In T₄, pH level was 6.99, 3.43 and 4.12 at 2, 4 and 6 days respectively. As pH is inversely related with Titrable acidity and Vitamin C, treatment T₄ contained high ranges of Titrable acidity (33.47, 22.60 and 18.83%) and Vitamin C (17.03, 16.33 and 11.40 mg/10g). Total Soluble Solids (TSS) or sugar contents remain low in vegetables. In T₄ treatment, TSS level (0.30, 0.30 and 0.40 °Brix) was also low. The physical changes (weight loss and firmness) were also at acceptable levels in T₄ treatment. It is thus revealed that T₄ (1.5% Citric acid) treatment is more acceptable for long storage periods and keeping good quality of minimally processed Indian spinach.

**Conclusion**

This study revealed a significant effect of treatments on physical and chemical properties of minimally processed Indian spinach. Indian spinach, treated with 1.5% citric acid, showed a longer shelf life. KMS treatment also showed good result in processed Indian spinach. But from the storage point of view, T₄ (1.5% citric acid) may be accepted as it contains higher amount of Vitamin C, low pH, low TSS and higher TA than other treatments. Further studies may include detailed analysis of physicochemical properties of minimally processed Indian spinach.

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