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Effects of washing with boric acid solutions and tap water on some quality characteristics of fresh-cut spinach

Borik asit çözeltisi ve musluk suyu ile yıkamanın taze kesilmiş ıspanakta bazı kalite özelliklerine etkisi

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Abstract

Fresh-cut spinach samples were washed with different concentrations of boric acid solutions (0.1%, 0.5%, and 1%) for different times (2, 5, and 10 min), stored at +4°C for 15 days, and the effects on the quality and shelf life of spinach were investigated. The results were compared with unwashed spinach samples that were washed with tap water for the same periods. Accordingly, the highest decrease in total mesophilic aerobic bacteria counts measured on day zero was 4.75 logarithmic units in spinach samples washed with 1% boric acid solution for 10 min. At the same time, this value was 6.40 log value in unwashed samples (1.65 log decrease). The antioxidant activity values of the samples washed with boric acid solution and tap water were lower than those of the unwashed samples. In addition, the increase in the concentration of boric acid solution and washing time caused a decrease in the antioxidant activity values of the samples. After the washing process, there was a decrease in the puncture force and puncture distance values of the samples, but it was determined that this decrease was not at a sensory observable level. The high L value measured in spinach was directly affected by the concentration of the boric acid solution and the increase in washing, and this value was measured as 40.94 in samples washed with 1% boric acid solution for 2 min, while this value was 43.02 in samples washed for 10 min. As a result, it was determined that the 10 min washing process with 1% boric acid solution could extend the product's shelf life up to 10 days without causing a significant decrease in quality.

fboricKeywords: Boric acid, Fresh-cut spinach, Washing, Shelf life

Öz

Taze kesilmiş ıspanak örnekleri farklı konsantrasyonlardaki (% 0.1, %0.5 ve %1) borik asit çözeltileri ile farklı sürelerde (2, 5 ve 10 dk) yıkanmış, 15 gün boyunca 4°C'de depolanmış, ıspanağın kalitesine ve raf ömrüne etkisi incelenmiştir. Elde edilen sonuçlar aynı sürelerde musluk suyu ile yıkanan ve yıkanmamış ıspanak örnekleri ile kıyaslanmıştır. Buna göre toplam mezofilik aerobik bakteri sayısındaki en yüksek azalış sıfırıncı günde ölçülen %1'lik borik asit çözeltisi ile 10 dk süreyle yıkanan ıspanak örneklerinde 4.75 logaritmik birim olurken yıkanmamış örneklerde ise bu değer 6.40 log bulunmuştur (1.65 log azalma). Borik asit çözeltisi ve musluk suyu ile yıkanan örneklerin antioksidan aktivite değerleri yıkanmamış örneklere göre daha düşük seviyede ölçülmüştür. Ayrıca borik asit çözeltisinin konsantrasyonunun ve yıkama süresinin artışı örneklerin antioksidan aktivite değerlerinde azalmaya neden olmuştur. Yıkama işlemi sonrası örnekleri delme kuvveti ve delme mesafesi değerlerinde azalma olmuş fakat bu azalışın duyusal olarak gözlemlenebilir düzeyde olmadığı belirlenmiştir. Ispanaklarda ölçülen yüksek L değeri, borik asit çözeltisi konsantrasyonunun ve yıkama artışı ile doğrudan etkili olup % 1'lik borik asit çözeltisi ile 2 dk süreyle yıkanan örneklerde bu değer 40.94 olarak ölçülürken 10 dk süreyle yıkanan örneklerde bu değer 43.02 bulunmuştur. Sonuç olarak, %1 'lik borik asit çözeltisiyle yapılan 10 dk yıkama işlemi ürünün raf ömrünü kalitede önemli bir azalmaya neden olmaksızın 10 güne kadar uzatabileceği belirlenmiştir.

Anahtar kelimeler: Borik asit, Taze kesilmiş ıspanak, Yıkama, Raf ömrü

1 Introduction

In the context of a healthy diet, vegetables play an indispensable role. Vegetables are a rich source of nutrients, such as various vitamins and fibre [1]. Green leafy vegetables are usually consumed raw or minimally processed. The primary examples are spinach, chard, arugula and some types of lettuce [2]. Green leafy vegetables, such as spinach, contain many beneficial nutrients for our bodies. These include manganese, magnesium, vitamins A, C, E, and B, potassium and calcium [3]. 100 g of spinach contains 28.1 mg of vitamin C, 2.03 mg of vitamin E, 194 µg folic acid, 99 mg of calcium, 2.71 mg of iron and 2.2 mg of dietary fibre [4]. Recently, consumers prefer fresh-cut or fresh leafy vegetables. The reasons behind this preference are advantages such as ease of consumption and freshness of the products [5].

Minimally processed vegetables are products cut, disinfected, and not subjected to heat treatment. Consumers often prefer minimally processed spinach, but it has a limited shelf life. The short shelf life is due to tissue damage caused by cutting, increased respiration, and perspiration [6].

Thoroughly washing minimally processed vegetables is crucial for preventing rotting, reducing the presence of bacteria, and prolonging the shelf life of the veggies. Disinfectant washing significantly decreases the microorganisms present in fresh-cut vegetables [7]. Disinfectants like chlorine, chlorine dioxide, ozone, electrolyzed water, and acids are used to remove contaminants from fresh-cut vegetables [8]. Chlorine, while frequently employed as a disinfectant in the food industry, has the potential to react with organic chemicals and produce harmful substances like trihalomethanes. Consequently, additional research is required to cleanse fresh-cut vegetables of contaminants [9]. Boric acid, on the other hand, is generally

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recognized as environmentally friendly. Therefore, boric acid is a good disinfectant alternative [10].

Boron does not naturally occur in its elemental form but is found in compounds such as borax, boric acid, colemanite, kernite, ulexite, and other borates [11], [12]. Boron oxide (B_2O_3) and boric acid (H_3BO_3) are the simplest boron compounds [13]. Today, boron compounds, especially boric acid and borax, are used in many products [11]. Specifically, boric acid and sodium salts of boron, such as borax and disodium tetraborate, are employed as antiseptics and bactericides [14]. Boron is one of the most essential sources in Türkiye [15]. Türkiye possesses abundant soils containing the element boron, with around 73% of the global reserves inside its borders [14]. Boron is applied as a food additive in the food industry to extend the shelf life of food goods by suppressing the growth of bacteria that cause rotting [15]. Boric acid is a preservative in meat and dairy products, an antifungal agent in citrus fruits, and a mold inhibitor in fruits [18]. Boron is also widely used in disinfectants, mouthwashes, and antiseptics for its antimicrobial properties [19].

This study investigated the effect of boric acid, a type of boron element, on fresh-cut spinach's quality characteristics and shelf life. It also evaluated the effects of different boric acid concentrations and washing times.

2 Material and method

2.1 Material

The spinach samples used in the study were obtained from a local market in Ankara. The green leafy parts of the spinach were used for the analysis. The spinach samples were washed with a boric acid (Merck, D-6100 Darmstadt, Germany) solution prepared at various concentrations and tap water, and the excess water was removed in a centrifugal vegetable dryer (Bager Plastic, Istanbul).

2.2 Preparation of Spinach Samples and Experimental Plan

The boric acid concentration and washing times used in the study are given in Table 1.

Table 1. Boric acid concentration and washing times (Temperature 20°C)

Washing treatments	Boric acid concentration (%)	Washing time (min)
Boric acid solution	0.1	2
		5
		10
	0.5	2
		5
		10
	1.0	2
		5
		10
Tap water	-	2
		5
		10
Unwashed (control)	-	

In the study, solutions of boric acid concentrations indicated in Table 1 were prepared as 100 mL each, and these solutions were brought to 20°C in a water bath. Spinach leaves were cut into 10 g pieces of 2x2 cm size using a stainless-steel knife. The prepared boric acid solutions were added to the spinach samples and placed in a beaker. Each sample was mixed at 250 rpm in a shaking mixer for the times indicated in Table 1. Before analysis, a centrifugal dryer was used to remove excess water from the washed spinach samples. Spinach samples were washed with tap water, and unwashed spinach samples were used to compare. The times indicated in Table 1 were also applied to the samples washed with tap water. The washed spinach and unwashed samples were placed in plastic containers, sealed airtight with plastic wrap, and then stored in the refrigerator at +4°C for 15 days. The prescribed analyses were performed on spinach samples every 5 days, and the first analysis day was set as day zero.

2.3 Total Mesophilic Aerobic Bacteria Count

The total count of mesophilic aerobic bacteria was examined using the methodology outlined by Halkman [20]. According to this method, the washed and unwashed spinach samples were taken from the container with a sterile clamp, placed in 90 mL of sterile FTS (physiological salt solution, 0.85%), and mixed. 1 mL of each sample was transferred into 9 mL tubes containing FTS, which were sterilized in an autoclave. After dilution, the samples were prepared in the following dilutions: 10^{-2}, 10^{-3}, and 10^{-4}. These dilutions were duplicated on the PCA medium using the spread plate method. At the end of inoculation, the Petri dishes were incubated at 28°C for 48 h, colonies were counted, and the results were expressed as log CFU/g. The same procedure was repeated every 5 days during storage.

2.4 pH Measurement

The samples' pH values were determined using a pre-calibrated pH meter (inoLab pH 720, WTW 82362, Weinheim, Germany).

2.5 Color Determination

The color of the samples was measured using a colorimeter (Konica Minolta, CR-400, Japan), which was calibrated before measurement. Color values were established as L* (0=black, 100=white), a* (+ red, - green) and b* (+ yellow, - blue).

2.6 Texture Measurement

The texture measurement of spinach samples was carried out on a TAXT Plus texturing device (Stable Micro Systems, TAXT Plus, Surrey, UK), which used an HDP/FSR Film Support Rig (with HDP/90+ P/5S) probe. In this analysis, the puncture strength of the samples and the displacement of the probe at the moment when the spinach leaves break were measured. The highest force was expressed as the puncture force, and the displacement was expressed as the puncture distance, which indicates the spinach leaf's flexibility.

2.7 Antioxidant Activity

To assess antioxidant activity, the DPPH technique utilized in leafy vegetables by Brand-Williams et al. [21] was used. In this method, 0.0025 g of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100 mL of methanol, blended with 10 g of spinach sample, and transferred to a centrifuge tube. A mixture of methanol and water (80:20, v/v) was added to a final volume of 25 mL. The samples were subsequently agitated using a shaking mixer (Biosan, OS-10, Lithuania) at 220 rpm for 1 hour and then centrifuged at 27,123 g for 10 min (Hettich Zentrifugen-Universal 320R, Germany) to obtain the extract. A volume of 60 µL of the extract was combined with 1940 µL of DPPH solution in tubes, mixed via vortex, and left to incubate in the dark for 1 hour. The absorbance at 515 nm was measured using a spectrophotometer (Shimadzu, UV-1601, Japan). The samples' inhibition levels (%) were computed using Equation (1).

$$\% \, inhibition = \frac{[A_{control} - A_{sample}]}{A_{control}} \times 100 \qquad (1)$$

$A_{control}$: absorbance of control sample
A_{sample}: absorbance of sample

2.8 Statistical Analysis

The data received were examined using the variance analysis (ANOVA) method in factorial order. Duncan's multiple comparison test prevented mean differences. All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and differences between samples were judged significant at $p<0.05$.

3 Results and discussion

3.1 Effects of Different Washing Treatments on Total Mesophilic Aerobic Bacteria Count in Spinach

Tables 2, 3, and 4 show the effects of boric acid solutions used at specific concentrations and different durations of washing with tap water on the total mesophilic aerobic bacteria level during storage.

Microbial reduction was observed in spinach washed with different concentrations of boric acid solution and tap water at different times. The changes in the total number of mesophilic aerobic bacteria in the samples treated for 2 min, 5 min and 10 min were statistically significant ($p<0.05$). The highest reduction in total mesophilic aerobic bacteria count was observed in samples washed with 1% boric acid solution for 10 min compared to unwashed day zero samples (1.65 log).

Table 2. The effect of various washing treatments for 2 min on the total count of mesophilic aerobic bacteria during storage (log CFU /g)

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	5.35±0.15^{bA}	5.35±0.15^{bA}	5.45±0.15^{bcA}	5.50±0.10^{bA}
0.5% boric acid	5.15±0.15^{bcA}	5.15±0.15^{bcA}	5.25±0.15^{cdA}	5.30±0.10^{cA}
1% boric acid	5.00±0.20^{cA}	5.00±0.10^{cA}	5.05±0.15^{dA}	5.15±0.15^{cA}
Tap water	5.40±0.00^{bA}	5.40±0.00^{bA}	5.50±0.00^{bA}	5.50±0.00^{bA}
Unwashed	6.40±0.10^{aA}	6.45±0.05^{aA}	6.50±0.10^{aA}	6.55±0.05^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 3. The effect of various washing treatments for 5 min on the total count of mesophilic aerobic bacteria during storage (log CFU /g)

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	5.25±0.15^{bA}	5.30±0.20^{bA}	5.35±0.15^{bA}	5.40±0.10^{bA}
0.5% boric acid	5.10±0.20^{bcA}	5.10±0.20b^{cA}	5.20±0.20^{bcA}	5.25±0.15^{bcA}
1% boric acid	4.90±0.20^{cA}	4.93±0.15^{cA}	5.05±0.15^{cA}	5.10±0.10^{cA}
Tap water	5.30±0.00^{bA}	5.30±0.00^{bA}	5.40±0.00^{bA}	5.40±0.00^{bA}
Unwashed	6.40±0.10^{aA}	6.45±0.05^{aA}	6.50±0.10^{aA}	6.55±0.05^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 4. The effect of various washing treatments for 10 min on the total count of mesophilic aerobic bacteria during storage (log CFU /g)

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	5.20±0.20^{bA}	5.20±0.20^{bA}	5.30±0.20^{bA}	5.35±0.10^{bA}
0.5% boric acid	5.00±0.20^{bcA}	5.05±0.20^{bA}	5.15±0.20^{bA}	5.15±0.15^{cA}
1% boric acid	4.75±0.15^{cB}	4.80±0.10^{cAB}	4.90±0.10^{cAB}	5.00±0.10^{cA}
Tap water	5.20±0.00^{bD}	5.25±0.00^{bC}	5.30±0.00^{bB}	5.40±0.00^{bA}
Unwashed	6.40±0.10^{aA}	6.45±0.05^{aA}	6.50±0.10^{aA}	6.55±0.05^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Boric acid primarily targets microbial membranes, aiming to slow down the metabolic processes of microorganisms. The antimicrobial effect of boric acid varies depending on factors such as the target microorganism, exposure time, and concentration. These parameters are critical determinants for achieving either a bactericidal or bacteriostatic effect on microorganisms [10]. In their study, Kara et al. [22] stated that treating pomegranates with boric acid at different concentrations created an antifungal effect and that increasing the concentration strengthened the inhibitory effect. Boric acid and borate salts have been used in medicine since the 1860s as bactericidal, fungicidal, and antiseptic agents. These compounds are available in wettable powder, liquid (spray or aerosol), emulsions, concentrates, granules, or powders. Boric acid is safer than highly volatile synthetic chemical pesticides [23]. Therefore, as the boric acid concentration and treatment time increased, the total number of mesophilic aerobic bacteria reduction increased. Therefore, as the boric acid concentration and treatment time increased, the total number of mesophilic aerobic bacteria reduction increased.

In order to better express the data obtained in the discussion section of this study, the results of storage on the 10th day with

1% boric acid solution were compared since the best results were obtained in washing with 1% boric acid solution and the quality decreased to an unacceptable level from the 10th day of storage.

Spinach samples washed with varied boric acid concentrations (0.1%, 0.5% and 1%) and washing durations (2, 5 and 10 min) exhibited a decrease in the total number of mesophilic aerobic bacteria compared to unwashed samples. When the tables offered are studied, it is evident that the reduction in the number of bacteria is higher in the samples washed with boric acid solution than in the samples washed with tap water. Our study observed that the samples washed with boric acid solutions of different concentrations and durations had the best results compared to the unwashed samples in the samples washed with 1% boric acid solution for 10 min. Liu et al. [24] washed fresh-cut cabbages using aqueous ozone for different times, such as 1, 5 and 10 min. They stored the samples at +4°C for 12 days and monitored the total mesophilic aerobic bacteria count change during this period. They determined that the total number of aerobic bacteria increased in all samples during storage (0, 4, 8 and 12 days). They observed that the samples washed with ozonated water on the 12th day of storage showed a more significant decrease in the total aerobic bacteria than the control (distilled water) samples. The study reported that the samples washed with aqueous ozone for 1, 5 and 10 min reduced 1.2, 1.5 and 1.6 log, respectively, compared to the samples washed with distilled water for 5 min. The researchers discovered that the washing duration was significant ($p<0.05$) on the total quantity of aerobic bacteria, and 10 min of washing time produced the maximum inactivation. Our work is similar to this literature in that the longer washing time resulted in a more substantial drop in the overall number of mesophilic aerobic bacteria. Chung et al. [25] examined the bactericidal effect of chlorine dioxide and sodium hypochlorite solution concentrations on 6 different fresh-cut vegetables and fruits (cucumber, lettuce, carrot, apple, tomato and guava). For this study, they employed 50, 100, and 200 ppm chlorine dioxide and sodium hypochlorite solutions. Before washing, the researchers observed that the total bacterial counts for fresh-cut cucumber, lettuce, carrot, apple, tomato and guava were 6.41, 6.58, 6.48, 6.97 and 6.30 log, respectively. Samples treated with 50 ppm chlorine dioxide showed a decrease in the total number of bacteria and were found to be 4.15, 3.30, 4.48, 4.56 and 5.18 log, respectively. When the concentration of chlorine dioxide used in the washing process was increased to 100 ppm, the decrease in the total number of bacteria in the samples was found to be higher. These results were obtained as 3.89, 3.08, 3, 4, 3.04 and 4.78 log for fresh-cut cucumber, lettuce, carrot, apple, tomato and guava, respectively. The study found that increasing the disinfectant concentration had a more significant effect on reducing bacterial counts in samples washed with sodium hypochlorite. When the tables in our study (Tables 2, 3 and 4) are examined, it is seen that increasing the concentration in the samples washed with boric acid solution applied at different concentrations provides a more significant reduction in the total number of mesophilic aerobic bacteria.

3.2 Effects of Different Washing Treatments on the pH Value of Spinach

Tables 5, 6, and 7 show the effects of different duration treatments of washing processes with boric acid solution and tap water used at specific concentrations on pH change during storage, respectively.

Statistically significant differences were found between the samples in pH during the storage period at 2, 5 and 10-min washing times of the washing treatments with different concentrations of boric acid solution and tap water ($p<0.05$).

Table 5. The effect of various washing treatments for 2 min on the pH during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	6.46 ± 0.10^{bC}	6.50 ± 0.11^{bC}	6.73 ± 0.05^{bcB}	7.21 ± 0.01^{cA}
0.5% boric acid	6.44 ± 0.13^{bC}	6.48 ± 0.12^{bBC}	6.67 ± 0.11^{cB}	7.15 ± 0.00^{dA}
1% boric acid	6.37 ± 0.01^{bC}	6.46 ± 0.08^{bC}	6.61 ± 0.09^{cB}	7.06 ± 0.05^{eA}
Tap water	6.48 ± 0.04^{bD}	6.64 ± 0.09^{abC}	6.86 ± 0.07^{abB}	7.30 ± 0.02^{bA}
Unwashed	6.63 ± 0.05^{aC}	6.72 ± 0.08^{aC}	6.92 ± 0.03^{aB}	7.42 ± 0.02^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 6. The effect of various washing treatments for 5 min on the pH during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	6.45 ± 0.07^{bC}	6.50 ± 0.10^{bC}	6.71 ± 0.05^{bcB}	7.18 ± 0.02^{cA}
0.5% boric acid	6.45 ± 0.08^{bC}	6.52 ± 0.12^{bBC}	6.68 ± 0.10^{cB}	7.14 ± 0.01^{cA}
1% boric acid	6.34 ± 0.03^{bC}	6.47 ± 0.05^{bB}	6.58 ± 0.08^{cB}	6.99 ± 0.09^{dA}
Tap water	6.45 ± 0.05^{bD}	6.61 ± 0.07^{abC}	6.83 ± 0.03^{abB}	7.29 ± 0.02^{bA}
Unwashed	6.63 ± 0.05^{aC}	6.72 ± 0.08^{aC}	6.92 ± 0.03^{aB}	7.42 ± 0.02^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 7. The effect of various washing treatments for 10 min on the pH during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	6.46 ± 0.05^{bC}	6.50 ± 0.08^{bcC}	6.70 ± 0.01^{bcB}	7.18 ± 0.01^{bA}
0.5% boric acid	6.45 ± 0.04^{bC}	6.49 ± 0.04^{bcC}	6.63 ± 0.11^{cdB}	7.11 ± 0.03^{bA}
1% boric acid	6.34 ± 0.01^{cB}	6.41 ± 0.04^{cB}	6.50 ± 0.09^{cB}	6.87 ± 0.17^{cA}
Tap water	6.47 ± 0.05^{bD}	6.61 ± 0.04^{bC}	6.77 ± 0.05^{bB}	7.25 ± 0.03^{bA}
Unwashed	6.63 ± 0.05^{aC}	6.72 ± 0.08^{aC}	6.92 ± 0.03^{aB}	7.42 ± 0.02^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Lee and Baek [26] packed spinach treated with chlorine dioxide, sodium hypochlorite, water in vacuum, nitrogen, air, and CO_2 gas and stored at 7°C for 7 days. They measured the pH for each washed sample on days 0, 3 and 7 of storage. It was reported that the pH level was higher in spinach washed with water than in samples washed with chlorine dioxide and sodium hypochlorite. The researchers observed increased pH levels in each washed and packaged sample during storage. In our study, the pH levels of the samples washed with tap water were higher than those washed with boric acid solution. In our study, the pH levels of all samples increased during storage. Kenny and O'Beirne [27] washed fresh-cut iceberg lettuce with tap, distilled, and chlorinated water and stored these samples at 4°C for 8 days. The researchers examined the pH levels of minimally processed iceberg lettuce on preset storage days (1, 2, 4 and 8). They wanted to investigate the effects of different washing procedures on shelf life and quality in terms of pH. On the first day of the study, the pH levels of the samples washed with tap water, distilled water and chlorinated water were measured as 6.12, 6.07 and 6.11, respectively. On the 8th day, the last day of the storage period, the pH values increased and were found to be 6.41, 6.44 and 6.53, respectively. The researchers found an increase in pH during storage and interpreted the increase as a mix of bacterial growth and metabolic activity. In our investigation, the increase in total mesophilic aerobic bacteria count, and pH level recorded in the samples during storage is similar to that observed in this study.

3.3 Effects of Different Washing Treatments on Color Characteristics of Spinach

In our study, since there is a visible loss of quality in color values from the 10th storage day, Figure 1 shows the results of that day.

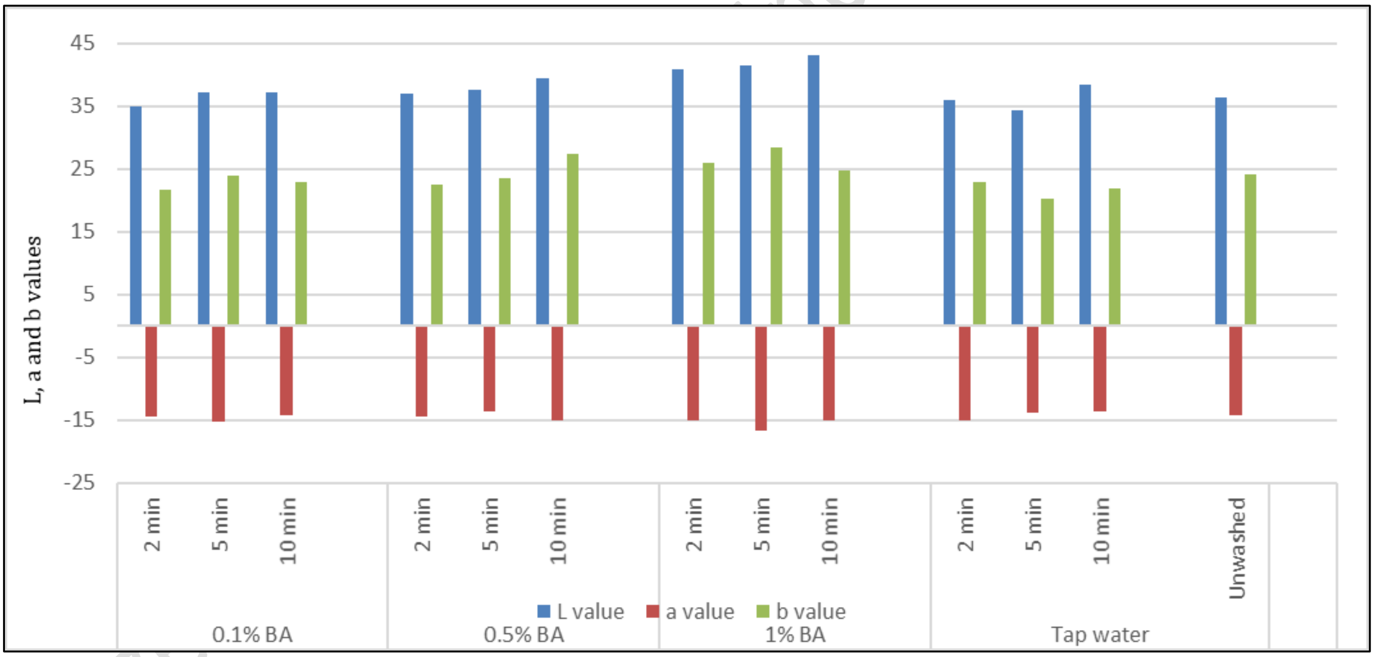

Figure 1 Effects of different washing treatments on color coordinates of spinach on the 10th day of storage period (BA: boric acid)

Color changes resulting from chlorophyll degradation are a natural process throughout the shelf life of green leafy vegetables [10]. In Figure 1, the increase in washing times generally negatively affected color. According to the statistical results, washing treatments affected the color values of the spinach samples. Changes in the green color during storage can also be associated with chlorophyll loss. Figure 1 shows that the highest L value on the 10th day was 43.02 in spinach samples washed with a 1% boric acid solution for 10 min, while the lowest L value was 36.40 in unwashed spinach samples. In other words, the comparisons were made based on the values of the samples washed for 10 min and stored for 10 days. Qi et al. [28] measured the L values of unwashed spinach leaves washed with electrolyzed oxidized water (EO) with different chlorine concentrations for different times [120 mg/l, 15 min (EO_1), 120 mg/l, 8 min (EO_2) and 70 mg/l 15 min (EO_3)] and spinach washed with deionized water. According to the results obtained in the study, EO_1= 29.54, EO_2=30.32, EO_3=29.86, 30.95 in samples washed with deionized water and 29.49 in unwashed samples. According to the results obtained by the researchers, the samples washed with deionized water had the highest L value, while the unwashed samples had the lowest L value. Similarly, in our study, samples washed with different washing treatments had higher L values than unwashed samples. Bermudez-Aguirre and Barbosa-Canovas [29] cleaned lettuce using varied concentrations of citric acid solution (0.5%, 1% and 1.5%). In the study, the L, a and b values of lettuce samples washed with 1% concentration citric acid solution were 47.07, -7.77 and 15.90, respectively, while these values were 55.00, -9.11 and 23.87 for the samples washed with 1.5% concentration citric acid solution. According to these results, citric acid concentration affects the sample's L, a and b values. In our study, the concentration of the boric acid solution used in the washing process also affected the color values of the samples, which is consistent with this study.

3.4 Effects of Different Washing Treatments on Textural Properties

In our study, the probe's puncture strength and displacement at the moment of spinach leaf breakage were measured for spinach samples with and without different washing methods. The puncture force values are given in Tables 8, 9 and 10, and the puncture distance values are given in Tables 11, 12 and 13. Leaf texture is becoming an increasingly important criterion in spinach quality control. Senescence in vegetables is a process in which cell walls degrade, leading to cell death; during this process, water and solids are released into the intercellular space, causing tissue loss. The firmness level is generally associated with ripeness, freshness, high quality, and marketability, as firmness gradually decreases during the ripening process and subsequent shelf life [30]. In our study, the textural improvement observed in spinach samples was better when washed with a boric acid solution during storage than when washed with tap water. It can be stated that washing with boric acid has a more protective effect on spinach than washing with tap water. The statistical differences between the washing groups were significant ($p<0.05$). The washing treatments significantly influenced the texture properties of the spinach samples. Since boric acid is an acidic solution, it was noted that the chemical content was better preserved in treated products, which may have played a role in maintaining the texture [10]. Nguyen et al. [31] washed fresh-cut baby spinach using different methods, such as chlorine dioxide, neutral electrolyzed water and peroxyacetic acid. The washed spinach samples were stored at 4°C for 13 days. In the study, the puncture force value measured on day zero of the samples washed with chlorine dioxide was 2.02 N, while this value was found to be 1.54 N on the 10th storage day. The researchers determined that the maximum load force applied to spinach decreased during storage. In our study, a similar situation was observed in the spinach sample washed with 0.1% boric acid solution for 5 min, and the puncture force value of the spinach sample was measured as 1.11 N on day zero. In contrast, this value was 1.02 N on the 15th storage day. Prabawa et al. [32] claim that this scenario is affected by the storage duration of the tissues of fruits and vegetables after harvest and that the tissue loss is related to the decrease in turgor pressure in the cell wall.

3.5 Effects of Different Washing Treatments on Antioxidant Activity

Tables 14, 15, and 16 show how spinach samples' antioxidant activity (% inhibition) changes during storage when different washing methods are applied based on time and concentration. Significant differences ($p<0.05$) were observed in the antioxidant activity levels of samples washed for 2, 5, and 10 min with various concentrations of boric acid solution and tap water during storage.

This study assessed the antioxidant activity of fresh-cut spinach samples washed under different conditions and compared them with unwashed controls using the DPPH assay. The antioxidant activity levels of the samples washed with varying concentrations of the boric acid solution dropped with increasing concentration and washing period. Antioxidant activity values of all samples decreased during storage. The antioxidant activity levels of the samples washed with varying concentrations of the boric acid solution dropped with increasing concentration and washing period. Antioxidant activity values of all samples decreased during storage. This result can be explained by the effect of water-soluble antioxidant compounds that can transfer into the washing water. During prolonged washing, a significant portion of these compounds may dissolve into the washing water, leading to a loss in the product's antioxidant activity [10].

Table 8. The effect of various washing treatments for 2 min on the puncture force (N) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	1.02±0.31^{aA}	1.27±0.22^{aA}	1.58±0.46^{aA}	1.29±0.45^{aA}
0.5% boric acid	0.99±0.26^{aA}	1.15±0.01^{aA}	1.42±0.39^{aA}	1.12±0.28^{aA}
1% boric acid	0.91±0.24^{aA}	1.16±0.20^{aA}	1.21±0.17^{cA}	1.14±0.20^{aA}
Tap water	1.02±0.02^{aB}	1.04±0.04^{aB}	1.07±0.06^{aAB}	1.14±0.03^{aA}
Unwashed	1.18±0.16^{aB}	1.27±0.12^{aB}	1.58±0.02^{aB}	1.60±0.04^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 9. The effect of various washing treatments for 5 min on the puncture force (N) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	1.11±0.15^{aAB}	1.21±0.17^{aAB}	1.33±0.16^{abA}	1.02±0.11^{bB}
0.5% boric acid	0.97±0.21^{aA}	1.30±0.24^{aA}	1.36±0.18^{abA}	1.12±0.27^{aA}
1%boric acid	0.93±0.08^{aA}	1.27±0.34^{aA}	1.31±0.32^{abA}	1.15±0.15^{bA}
Tap water	1.04±0.04^{aB}	1.17±0.02^{aA}	1.20±0.07^{bA}	1.01±0.10^{bB}
Unwashed	1.18±0.16^{aB}	1.27±0.12^{aB}	1.58±0.02^{aB}	1.60±0.04^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 10. The effect of various washing treatments for 10 min on the puncture force (N) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	0.95±0.14^{aA}	1.05±0.01^{bAB}	1.27±0.15^{bA}	1.14±0.22^{bAB}
0.5% boric acid	1.01±0.14^{aA}	1.04±0.24^{bA}	1.21±0.24^{bA}	1.19±0.14^{bA}
1% boric acid	0.93±0.08^{aA}	1.03±0.00^{bA}	1.10±0.13^{bA}	1.09±0.07^{bA}
Tap water	0.91±0.22^{aA}	1.12±0.04^{abA}	1.16±0.12^{bA}	1.11±0.16^{bA}
Unwashed	1.18±0.16^{aB}	1.27±0.12^{aB}	1.58±0.02^{aB}	1.60±0.04^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 11. The effect of various washing treatments for 2 min on the puncture distance (mm) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	3.278±0.28^{aA}	4.203±0.61^{aA}	4.105±0.71^{aA}	4.130±0.74^{aA}
0.5% boric acid	3.131±0.40^{aA}	4.183±0.67^{aA}	4.135±0.74^{aA}	4.141±0.78^{aA}
1% boric acid	3.519±0.20^{aA}	4.226±0.55^{aA}	4.206±0.65^{aA}	4.156±0.70^{aA}
Tap water	3.025±0.46^{aA}	4.208±0.63^{aA}	4.198±0.67^{aA}	4.182±0.71^{aA}
Unwashed	2.991±0.51^{aA}	4.148±0.62^{aA}	4.068±0.67^{aA}	4.140±0.70^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 12. The effect of various washing treatments for 5 min on the puncture distance (mm) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	3.504±0.02^{aA}	4.184±0.60^{aA}	4.146±0.72^{aA}	4.170±0.76^{aA}
0.5% boric acid	3.535±0.03^{aA}	4.166±0.65^{aA}	4.178±0.75^{aA}	4.067±0.69^{aA}
1% boric acid	3.452±0.05^{aAB}	4.167±0.64^{aA}	4.137±0.71^{aA}	4.164±0.69^{aA}
Tap water	3.239±0.26^{aAB}	4.197±0.66^{aA}	4.112±0.59^{aA}	4.176±0.72^{aA}
Unwashed	2.991±0.51^{aB}	4.148±0.62^{aA}	4.068±0.67^{aA}	4.140±0.70^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 13. The effect of various washing treatments for 10 min on the puncture distance (mm) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	3.298±0.20^{aAB}	4.182±0.68^{aA}	4.176±0.72^{aA}	4.150±0.76^{aA}
0.5% boric acid	3.878±0.38^{aA}	4.176±0.66^{aA}	4.212±0.75^{aA}	4.009±0.73^{aA}
1% boric acid	3.432±0.07^{aAB}	4.195±0.66^{aA}	4.179±0.67^{aA}	4.191±0.68^{aA}
Tap water	3.936±0.35^{aA}	4.198±0.66^{aA}	4.206±0.59^{aA}	4.142±0.69^{aA}
Unwashed	2.991±0.51^{aA}	4.148±0.62^{aA}	4.068±0.67^{aA}	4.140±0.70^{aA}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

According to Karaca [9], this decrease is because ascorbic acid is a water-soluble vitamin, significantly contributing to the antioxidant activity of the vegetables studied. Even with water washing, a certain amount of ascorbic acid loss in the product is likely. In preserving the nutritional components of fresh-cut products, the disinfectant dosage used and the destructiveness of the applied processing method (washing, cutting, etc.) are important factors. The smaller the product is cut into pieces, the more cell fluid will leak from the broken plant tissues, and the greater the loss of the product's nutritional components will be. Based on our findings, the reductions observed in the washed samples can be explained by the water-soluble nature of ascorbic acid, which influences antioxidant activity and can dissolve into the washing water. Therefore, during prolonged washing processes, a significant portion of antioxidant compounds may remain in the washing water, which could account for the decreased antioxidant activity observed in the washed spinach samples [9]. Bottino et al. [33] observed a decrease in the antioxidant activity of spinach samples after washing during storage.

Karaca and Velioglu [34] examined the effects of distilled water, ozonated water and chlorinated water treatments on antioxidant activity values in leafy vegetables such as lettuce, spinach and parsley. The samples were treated using several washing methods and washed at 5°C for 15 min. The researchers found higher antioxidant values in lettuce and spinach washed with ozonated water than in unwashed samples, but this value did not change in parsley. While the antioxidant content decreased in parsley and lettuce samples washed with distilled water, this value increased in spinach samples. All samples washed with chlorinated water had lower antioxidant values than unwashed samples. Based on these results, the researchers concluded that ascorbic acid, which contributes the most to the antioxidant capacity of leafy vegetables, is a water-soluble vitamin, and the loss of ascorbic acid in samples washed with distilled water lowers antioxidant activity. In our study, the antioxidant activity values of the samples washed with different washing treatments were lower than those of the unwashed samples, similar to those in these studies.

Table 14. The effect of various washing treatments for 2 min on the antioxidant activity (inhibition%) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	8.628±1.38^{bA}	7.110±1.09^{bAB}	6.329±1.26^{bB}	5.317±0.56^{bB}
0.5% boric acid	7.953±0.41^{bA}	5.952±084^{bB}	5.120±0.82^{bcBC}	4.269±0.40^{cC}
1%boric acid	6.635±0.84^{bA}	5.106±0.90^{bB}	4.153±0.47^{cBC}	3.118±0.13^{dC}
Tap water	8.563±1.90^{bA}	6.817±1.55^{bAB}	5.592±0.68^{bcB}	5.141±0.74^{bcB}
Unwashed	11.875±2.02^{aA}	10.110±0.94^{aAB}	8.531±0.54^{aBC}	7.110±0.60^{aC}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 15. The effect of various washing treatments for 5 min on the antioxidant activity (inhibition%) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	8.180±1.08^{bA}	6.565±0.70^{bB}	5.840±0.92^{bB}	5.026±0.45^{bB}
0.5% boric acid	7.296±0.77^{bA}	5.798±0.83^{bB}	4.796±0.65^{bcBC}	3.876±0.18^{cC}
1% boric acid	6.268±0.90^{bA}	4.807±1.04^{bB}	3.834±0.45^{cBC}	2.638±0.00^{dC}
Tap water	8.193±1.67^{bA}	6.509±1.55^{bAB}	5.197±0.74^{bB}	4.559±0.51^{bcB}
Unwashed	11.875±2.02^{aA}	10.110±0.94^{aAB}	8.531±0.54^{aBC}	7.110±0.60^{aC}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

Table 16. The effect of various washing treatments for 10 min on the antioxidant activity (inhibition%) during storage

Washing treatments	Storage duration (day)			
	0	5	10	15
0.1% boric acid	7.962±1.01^{bA}	6.216±0.80^{bB}	5.439±0.83^{bBC}	4.546±0.32^{bC}
0.5% boric acid	6.854±0.77^{bA}	5.490±0.83^{bB}	4.471±0.48^{bcBC}	3.498±0.15^{cC}
1% boric acid	5.973±0.90^{bA}	4.493±1.04^{bB}	3.510±0.45^{cB}	1.982±0.05^{dC}
Tap water	7.826±1.74^{bA}	6.125±1.61^{bAB}	4.713±0.57^{bB}	3.903±0.56^{bcB}
Unwashed	11.875±2.02^{aA}	10.110±0.94^{aAB}	8.531±0.54^{aBC}	7.110±0.60^{aC}

^{aA} Different letters within the same column (a) and row (A) denotes statistically significant differences ($p<0.05$)

4 Conclusion

What is needed for the decontamination of fresh-cut vegetables is to preserve parameters such as microbiological stability, color values and texture strength. Today, many disinfectants are used for this purpose. The most widely used of these is chlorine. However, chlorine can produce harmful by-products when it reacts with organic matter. Therefore, there is a need for environmentally friendly and safe disinfection methods to replace chlorine. While chlorine poses a risk of forming harmful substances, boric acid offers a safe and environmentally friendly alternative as a disinfectant. Boron does not occur in its elemental form in nature but is found in compounds such as borax and boric acid. Boric acid is one of the simplest boron compounds. The effect of boric acid on health depends on the concentration used and the duration of exposure. At low concentrations and with controlled use, boric acid can reduce the microbial load on vegetables and extend their shelf life due to its antimicrobial properties. The antimicrobial effect of boric acid has been proven through various studies. This effect has been highlighted in several works, demonstrating its use in medical fields such as eye treatments and cancer therapies.

Our study evaluated the effects of boric acid, which is used as an alternative to chlorine in disinfecting fresh-cut spinach. Spinach was minimally processed, i.e. only cut and subjected to different washing treatments. In the samples washed with different concentrations of the boric acid solution, a decrease in the total number of mesophilic aerobic bacteria, a decrease in pH level, an increase in L value and a decrease in antioxidant activity level was observed. When the analysis was evaluated, the increase in boric acid concentration and washing time positively affected the samples. For example, the reduction in the total number of mesophilic aerobic bacteria in samples treated with 1% boric acid solution for 10 min on the first day of storage produced the most significant reduction compared to unwashed samples (1.65 log). Considering the other findings, washing with 1% boric acid solution for 10 min increased the shelf life of fresh-cut spinach up to 10 days and kept its quality. In this context, washing with boric acid, a by-product of boron, can be a potential alternative. This is particularly significant because our country ranks first globally with a 73% share of global boron reserves. Due to its availability, boric acid is used as a disinfectant in various products for daily use. Washing with boric acid is a good alternative because it improves food quality and is cost-effective. Furthermore, studies such as residue analysis of boric acid should also be conducted to ensure long-term sustainability.

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6 Author contribution statement

In this study, Author 1 was responsible for conceptualization, data analysis, and the literature review; Author 2 handled evaluating the results, material procurement, and further data analysis; Author 3 contributed to the manuscript's review and editing to ensure accuracy and consistency.

7 Ethics committee approval and conflict of interest statement

Ethical approval was not required to prepare this article. The authors declare no conflict of interest with any individuals or institutions involved.

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