

Research Article

Antibacterial Effect of Noni Juice In Vitro and Applied to Fresh-Cut Papaya to Control *Escherichia coli* O157:H7

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This study aimed to evaluate the effect of noni (*Morinda citrifolia* L.) juice (NJ) *in vitro* and applied it to fresh-cut papaya to control *Escherichia coli* O157:H7. Furthermore, the NJ effect on the physicochemical characteristics of fresh-cut papaya was evaluated. We determined the minimal inhibitory concentration (MIC) in a microplate reader by the microdilution method using TSB using an initial concentration of 1×10^7 cells of *E. coli*/mL. Cubes of fresh-cut papaya fruit were immersed in an *E. coli* suspension (1×10^5 cells/mL) and after that in NJ three times (0, 2.5, and 5 min) and then stored at $4 \pm 1^\circ\text{C}$ for 18 days. The presence of *E. coli* and total coliforms, as well as pH, TSS, and titratable acidity, were evaluated every three days. The results of *in vitro* assays showed that NJ at 20% inhibited the microbial growth of *E. coli*, finding a maximum growth rate (μ_{max}) of -0.0066 h^{-1} . Immersion in NJ for 5 min presented a reduction of *E. coli* of $3.72 \pm 1.43 \log_{10} \text{ CFU/g}$ of fresh-cut papaya fruit treated with this bacteria on day 9 of storage regarding control. Likewise, fresh-cut papaya fruit immersed in NJ for 5 min maintained the total coliforms between 1 and $-1 \log_{10}$ coliforms CFU/g for 18 days. However, the immersion treatment in NJ modified some physicochemical parameters of the fresh-cut papaya fruit, such as acidity and pH ($P < 0.05$). The application of NJ to fresh-cut papaya fruit showed *in vitro* and *in vivo* inhibition of *E. coli* and total coliforms, evidencing it as a possible bacterial control agent in the precut fruit industry for up to 18 days.

1. Introduction

The demand of consumers for safer, fresh, higher-quality foods with long shelf life has increased [1]. This has led to the development of preservation technologies through minimal processing techniques such as applying natural additives (such as polyphenols, organic acids, essential oils, and plant extracts) and physical methods (ozone-based treatment, ultraviolet radiation, pulsed light, cold plasma, ultrasound,

and novel packing practices) [1–3]. Consequently, this kind of processing has become a subject of special care due to the presence of certain pathogens such as *Escherichia coli* O157:H7, which is considered by the WHO [4] as one of the 14 pathogens causing severe gastrointestinal diseases. Nonetheless, there is little information about the behavior of these pathogens in precut fruits such as papaya [5].

Papaya is mostly consumed due to its sensory and functional properties [6]; nevertheless, this tropical

climacteric fruit presents a short shelf life due to a rapid pulp softening and microbial contamination (less than eight days of storage) [7, 8]. The food processing industry uses conservatives of synthetic origin to assure the quality and innocuousness of this fruit and others, which become a danger to the health of consumers and the development of resistance of microorganisms [9]. Therefore, the search for natural conservatives with effective antibacterial properties that preserve the quality of a fresh-cut product and do not cause deterioration in the product's sensorial attributes has emerged [10]. In this regard, noni juice (NJ) has demonstrated an efficient activity for preserving sensory proprieties and microbiological control over fresh-cut fruits such as mango. The soaking of mango cubes in NJ had an antimicrobial effect for 15 days, reducing the count of aerobic mesophilic, fungi, and yeast; besides, it maintained the color (CIE $L^* a^* b^*$, Chroma, and Hue angle) during 12 days of storage [11].

Morinda citrifolia L., known as “noni,” is a small tree used traditionally as a folk medicine for the treatment of many diseases [12]. More than 200 phytochemical compounds have been identified in this plant such as phenolic compounds, anthraquinones, flavonoids, phytosterols, polysaccharides, iridoids, fatty acid esters, sugars, triterpenoids, lactones, carotenoids, and volatile compounds [13–16]. NJ is made from the fruits of this plant and is widely consumed due to its properties. As mentioned before, NJ can inhibit the growth of certain bacteria such as *Staphylococcus aureus* (2.5 and 5 mg/mL), *Pseudomonas aeruginosa* (2.5 mg/mL), *Proteus morgani* (2.5 and 5 mg/mL), *Bacillus subtilis* (5 mg/mL), *Escherichia coli* (2.5 and 5 mg/mL), *Helicobacter pylori* (5 mg/mL), *Salmonella* (>20 mg/mL), *Shigella* (5 mg/mL), and *Candida albicans* (40–60 mg/mL) [12, 16, 17]. Therefore, this study aimed to evaluate the effect of NJ *in vitro* and applied it to fresh-cut papaya to control *Escherichia coli* O157:H7. Furthermore, the NJ effect on the physicochemical characteristics of fresh-cut papaya was evaluated.

2. Materials and Methods

2.1. Noni Juice Preparation. Noni fruits (*Morinda citrifolia* L.) in the maturity of consumption were harvested in San Blas, Nayarit, Mexico. Fruits were carried to the laboratory of the Unidad de Tecnología de Alimentos of the Universidad Autónoma de Nayarit, in Tepic, Nayarit, Mexico. Fruits were washed with tap water; then, the fruit was placed in sealed plastic jars and left in natural fermentation into the dark for 60 days at $28 \pm 3^\circ\text{C}$ [11, 18]. Fermented juice was filtered and centrifuged for 15 min at 3,050 xg (Centrifuge 5804 R, Eppendorf AG, Germany). The supernatant was decanted and stored in dark jars at 25°C until its use. The NJ yield was 84% *w/w* (juice/original weight of fruit). The physicochemical characteristics of the NJ were pH 3.46 ± 0.01 , titratable acidity of $0.1 \pm 0.011\%$ of citric acid, and 9.9 ± 0.2 °Brix.

2.2. Papaya Preparation. Fruits of papaya (*Carica papaya* L.) var. Maradol at consumption maturity (edible condition) were acquired in a local market. Fruits were disinfected by

immersion in a sodium hypochlorite solution at 200 ppm for 1 min [19]. After that, papaya fruits were rinsed with water, peeled, and then cut into cubes of 8 cm^3 .

2.3. Inoculum Preparation. *Escherichia coli* O157:H7 (isolated and studied before by Castro-Rosas [20], donated by the Universidad Autónoma del Estado de Hidalgo) was cultured in tryptic soy broth (TSB) added with rifampicin at 100 mg/L and incubated at 37°C for 24 h [21]. Cell suspensions were adjusted to 10^4 , 10^5 , 10^6 , and 10^7 cells of *E. coli*/mL using a saline solution (NaCl 0.85%).

2.4. Inhibitory Evaluation of NJ *In Vitro*. The inhibitory effect of NJ was determined in liquid culture and measured with a microplate reader (Multiskan GO model 1510, Thermo Fisher Scientific Oy, Vantaa, Finland) using 96 well plates. Each well (sample) contained 150 μL of TSB at concentrations of 10^4 , 10^5 , and 10^6 cells/mL of *E. coli* and 50 μL of NJ using a TSB with strain as a control. Plates were incubated for 24 h at 37°C . The cell density was determined by measuring the absorbance at 600 nm [22]. The pH of the medium after NJ addition was 5.2 ± 0.1 . The treatments were performed six times; each experimental unit was a well. The experiment was repeated three times.

The minimum inhibitory concentration (MIC) was considered the lowest concentration of NJ that inhibits the growth of *E. coli* [23]. Each well of the microplate contained 95 μL of nutrient broth, 5 μL of the inoculum (*E. coli* at 1×10^7 cells/mL), and 100 μL of NJ at concentrations of 50, 40, 30, 20, 10, 5, and 2.5%. Subsequently, the plates were incubated for 24 h at 37°C , shaking for 20 s each *h*. The bacterial growth was determined by measuring absorbance at 600 nm using a microplate reader (Multiskan GO model 1510, Thermo Fisher Scientific Oy, Vantaa, Finland). The treatments were performed six times, and each experimental unit was a well. The experiment was repeated three times.

2.5. Antibacterial Evaluation of Noni Juice *In Vivo*. The experiment was divided into two stages. In the first stage, 200 g of fresh-cut papaya fruit were immersed in an *E. coli* suspension (1×10^5 CFU/mL) for one minute. Then, the remaining suspension was drained out. Then, fresh-cut papaya fruits were immersed in pure NJ, evaluating three treatments (immersion in NJ for 0, 2.5, and 5 min) and a control (immersion in sterile distilled water). In the second stage, the NJ effect was evaluated on total coliforms natives of the papaya using the same treatments of immersion as previously described but with no immersion in pathogen suspension.

The treatments were stored in polyethylene domes at 4°C and monitored for 18 days, with a sampling every third day to evaluate the presence of total coliforms. Fresh-cut papaya cubes were homogenized in a stomacher (Stomacher 400, AbaTec, México). Then, serial dilutions were prepared. The counting plate method was used to determine the number of CFU of either *E. coli* or total coliforms (CFU/g) using VRBA. *E. coli* was determined in papaya cubes that were inoculated,

and total coliforms were determined in papaya that were not inoculated [24].

2.6. Physicochemical Analysis of Papaya. For 18 days, physicochemical parameters (pH, titratable acidity, and total soluble solids (TSS)) were evaluated according to the Official Methods of Analysis of AOAC [25]. The pH was measured in a Denver pH-meter model 250 (Denver Instrument Company, Denver, CO, USA); TSS was measured by refractometer Auto Abbe model 10500 (Reichert-Jung, Leica Inc., Buffalo, NY, USA). Titratable acidity was determined by NaOH 0.1 N neutralization. The results were expressed in % of citric acid (AOAC, 2000.939.05).

2.7. Statistical Analysis. Data were analyzed by analysis of variance (ANOVA) with a statistical significance of 5%. The Tukey test ($P \leq 0.05$) was used when ANOVA found statistical differences. Statistical analyses were performed using STATISTICA 10 software.

3. Results and Discussion

3.1. Microbiological Analysis

3.1.1. In Vitro Treatments. The NJ inhibition effect against *E. coli* O157:H7 at concentrations of 10^4 , 10^5 , and 10^6 cells/mL is shown in Figure 1. As can be seen, all the concentrations treated with NJ maintained a static growth behavior compared to control. The control treatments stop growing between 14 and 17 h of incubation because, during this period, they reach the stationary phase. Besides, the maximum growth was different for each inoculum concentration. These results may be due to bacteria in the stationary phase accumulating toxic catabolic products in the environment in a short time, resulting in a decrease in the number of viable cells, also known as the death phase [26].

The MIC was determined using seven NJ concentrations starting from an *E. coli* concentration of 1×10^7 cells/mL. Figure 2 represents the growth behavior of the bacteria. The lowest NJ concentrations (10, 5, and 2.5%) showed no inhibitory effect on *E. coli*, in contrast with higher concentrations (50, 40, 30, and 20%). This result suggests a static effect, which was corroborated with the maximum rate value (μ_{max}). Table 1 shows significant differences among the (μ_{max}) of the treatments ($P < 0.05$). The lowest concentration able to decrease the microbial growth was NJ 20% ($\mu_{max} -0.0066 \text{ h}^{-1}$), while bacteria control (*E. coli* suspension of 1×10^7 CFU/mL) had a $\mu_{max} 0.36175 \text{ h}^{-1}$. Also, Table 1 shows the values of phase lag, although there was not a relationship between the concentration of NJ and the time of the lag phase.

The antibacterial activity of noni fruit and noni juice has been related to the diversity of bioactive compounds they contain. Deng et al. [26] identified iridoids (deacetylasperulosidic and asperulosidic acids) from noni fruit with antibacterial activity against *E. coli*, *Candida albicans*, and *Staphylococcus aureus*. Anugweje [27] reported secondary

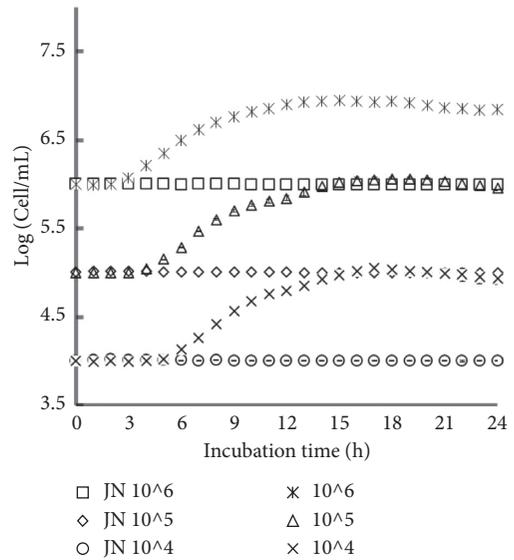


FIGURE 1: Growth kinetics of *Escherichia coli* O157:H7 at initial concentrations of 10^4 , 10^5 , and 10^6 cells/mL in the presence or absence of NJ, monitored for 24 h at 37°C.

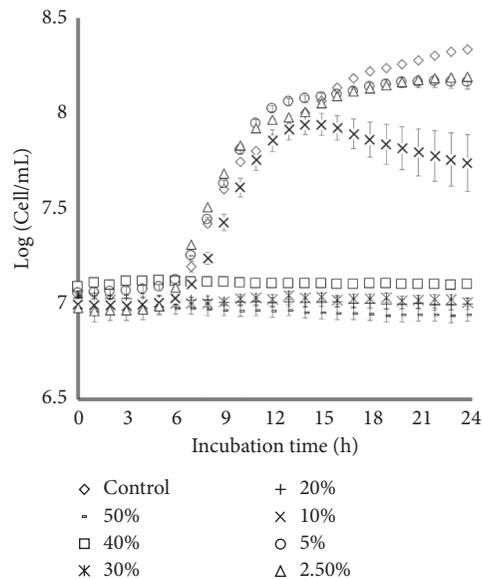


FIGURE 2: Effect of NJ on the growth of *E. coli* in vitro at different concentrations.

TABLE 1: Kinetic parameters of *E. coli* in vitro treated with NJ at different concentrations.

	Time lag (h)	Mmax
Control	5.00 ± 0.16 b *	0.1939 ± 0.0219 a
2.5%	4.60 ± 0.52 b	0.1669 ± 0.0031 b
5%	6.00 ± 0.00 c	0.1671 ± 0.0157 b
10%	5.80 ± 0.41 c	0.1423 ± 0.0273 bc
20%	4.30 ± 1.97 bc	-0.0025 ± 0.0022 d
30%	7.60 ± 3.44 bc	-0.0014 ± 0.0014 d
40%	4.00 ± 1.26 bc	-0.0016 ± 0.0010 d
50%	0.00 ± 0.27 a	-0.0036 ± 0.0056 d

* Values are expressed as an average ± standard deviation. The values followed by different letters in the same column differ statistically ($P < 0.05$).

metabolites like phenols, steroids, terpenoids, alkaloids, tannins, flavonoids, saponins, glycosides, reducer sugars, and acid compounds. Wall et al. [28] found organic acids in NJ such as acetic, ascorbic, butyric, citric, dehydroascorbic, galacturonic, malonic, succinic, shikimic, and tartaric acids. These organic acids are responsible for NJ's pH, which was 3.16 ± 0.05 [11]. Negi [10] summed up some natural antimicrobial action mechanisms, describing that terpenoids and phenols can cause cell membrane disruption. Besides, phenols and flavonoids can chelate metals. Also, the authors mentioned that coumarins and alkaloids could affect the genetic material producing microbial inhibition. NJ could be considered as one of the fermented juices with higher antimicrobial activity. Sripraya and Trachoo [29] found that fermented juices of lime, orange, kaffir lime, pineapple, avocado, star fruit, and sugar cane had antimicrobial activity. These fermented juices showed pH values between 3.1 and 3.4; however, unlike NJ, these juices' antimicrobial activity was related to acidity.

On the other hand, some authors found differences in the MIC to control *E. coli*. Natheer et al. [30] reported inhibition of *E. coli* due to the application of NJ at 25 mg/mL. Tintino et al. [31] elucidated the inhibitory capacity from ethanolic extract of noni fruit at 50–100 mg of dry extract/mL, testing *in vitro* against *Pseudomonas aeruginosa* and *Escherichia coli*. These variations in MIC may be related to the tolerance capacity of the bacteria to noni juice. In this study, the MIC of NJ to the bacteria was 20% (200 mg/mL), a high concentration compared to those reported by Natheer et al. [30] and Tintino et al. [31]. A possible explanation is that the bacteria studied in this work could present similar resistance mechanisms to those observed with the antibiotic rifampicin [20,21].

3.2. Antibacterial Evaluation of NJ In Vivo. In the first stage of treatments applied to fresh-cut papaya fruit (Figure 3(a)), i.e. samples were immersed for one min. In *E. coli* suspension, the bacterial population was constant on papaya control (without NJ immersion). There were no significant statistical differences throughout the storage days ($P > 0.05$), keeping a concentration between 2 and 3 \log_{10} CFU/g of fresh-cut papaya fruit. Singh and Shalini [32] mentioned that pH, water activity, and temperature act as barriers against the growth of microorganisms. However, Abadias et al. [33] and O'Beirne et al. [34] reported similar behavior to those presented in this work. These authors, as well as Strawn and Danyluk [5], mentioned that at 4 ± 1 °C *E. coli* O157:H7 could survive due to papaya's intrinsic factors such as pH, which could have influenced the *E. coli* population to remain stable. As seen in the later section, the papaya cubes treated with NJ and *E. coli* maintained a pH of between 4.88 and 5.72 throughout the experiment.

Concerning monitoring the papaya treated with noni juice, in treatment 3 (immersion in NJ for 5 min), logarithmic decreases of *E. coli* were observed. On day 0, a reduction of 3.7 log units regarding the initial inoculum

was observed; however, an increase of 0.3 log units concerning control was found on day zero. On the other hand, a reduction of 1.6 \log_{10} UFC/g of fresh-cut papaya fruit was found on the sixth day of storage regarding control. Furthermore, there was a reduction of 3.4 \log_{10} UFC/g of fresh-cut papaya fruit on the 12th day of storage regarding control (Figure 3(a)). Likewise, in treatment 2 (immersion in NJ for 2.5 min), there was a decrease of 2.96 \log_{10} CFU/g of the population of *E. coli* on the 9th day of sampling relative to control. In treatment 1 (immersion for 0 min), we found a difference of about 1 \log_{10} CFU/g relative to the control during the experiment, which shows an antibacterial effect even when the exposure time to NJ is minimal.

Although the NJ had a bacteriostatic effect on *E. coli* in the *in vitro* treatments, it had a bactericidal effect *in vivo* treatments. This may be due to differences in the composition of the culture medium used *in vitro* treatment and the composition of papaya. Zampieri et al. [35] mention that differences between carbon sources can affect the resistance to antimicrobials due to changes in energy metabolism and intracellular and extracellular putative metabolite profiles.

The bacteriostatic effect *in vitro* and the bactericide effect *in vivo* of the NJ could be related to its metabolites. The presence of compounds such as coumarins, flavonoids, phenolic acids, and iridoids has been reported in NJ [36]. Kumar et al. [17] mention that the antibacterial effect could be due to iridoids or other antimicrobial compounds in noni fruit. Atkinson [37] reported that the antimicrobial effect of noni fruit is due to the presence of phenolic compounds such as L-asperuloside, acubin, scopoletin, alizarin, and other anthraquinones. Duncan et al. [38] showed that scopoletin inhibits *E. coli*. Likewise, Sina et al. [16] reported the phytochemical composition of *M. citrifolia* fruit juice, finding chemical compounds such as alkaloids, flavonoids, tannins, leuco-anthocyanins, saponosids, reducing compounds, and O-heterosids in fresh and fermented juice. Kang and Song [39] mention that the hydroalcoholic extract of noni fruit causes cell damage and increases the cell membrane permeability of *Listeria monocytogenes*, which could cause the death of the cell. Nagalingam et al. [40] conducted extractions with ethanol and methanol of noni fruit, identifying the presence of steroids, flavonoids, tannins, and saponins, which are also attributed to antimicrobial properties.

In the second stage (Figure 3(b)), papaya control (not treated with *E. coli*), the total coliforms population was kept at 3 \log_{10} CFU/g approximately. On the other hand, in fresh-cut papaya fruit immersed between 0 and 2.5 min in NJ, the total coliform population was kept between 0 and 1 \log_{10} CFU/g for nine days. In contrast, for the fresh-cut papaya fruit immersed for 5 min in NJ, total coliforms were maintained between -1 and 1 \log_{10} CFU/g until the 18th day. Differences between samples treated and non-treated with *E. coli* can be due to the initial inoculum concentration and that the total coliforms could be more sensitive than *E. coli*. The strain of *E. coli* used in this study is resistant to the antibiotic rifampicin [20]. Martinez [41]

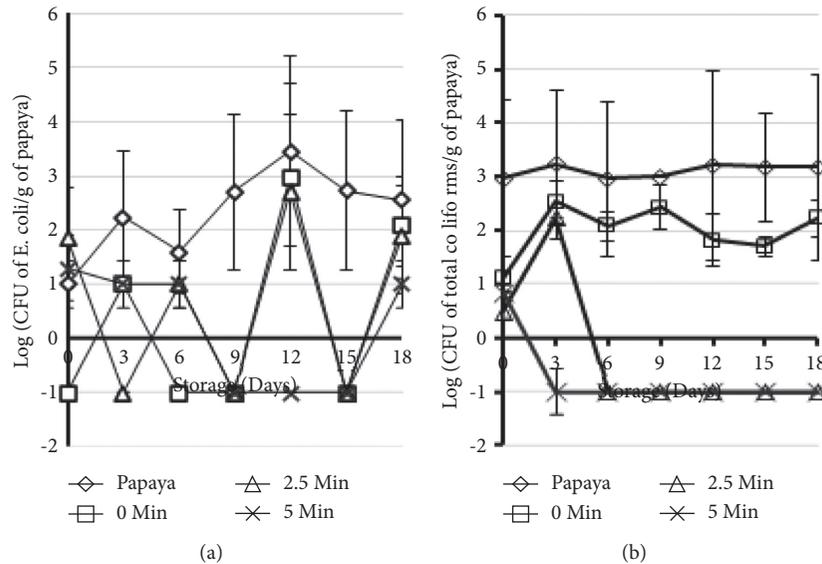


FIGURE 3: The microbial population on fresh-cut papaya fruit stored at 4 °C for 18 days. (a) Papaya inoculated with *E. coli*. (b) Papaya not inoculated.

mentions that bacteria can present diverse antibiotic resistance mechanisms, which could help *E. coli* resist the effects of the noni juice metabolites. This resistance of *E. coli* to NJ is not very efficient *in vivo* treatments. It could be due to NJ having several metabolites with different action mechanisms [36], the carbon sources available on the papaya cubes [35], and the intrinsic factors of the papaya such as pH and acidity [5]. However, the immersion time could be an important factor in the inhibition of *E. coli* and total coliforms. Alam et al. [42] mention that the immersion time in osmotic processes is related to solute gain. In this sense, with the longer immersion time, the metabolites present in NJ could migrate into the papaya cubes, making the inhibitory effect of *E. coli* and total coliforms more effective.

3.3. Physicochemical Analysis

3.3.1. Total Soluble Solids. The TSS results of fresh-cut papaya fruit during storage at $4 \pm 1^\circ\text{C}$, control, and treated with *E. coli* by the effect of the immersion time in NJ are shown in Figure 4(a) and Figure 4(b), respectively. On day 18, the samples, either without inoculum and treated by NJ immersion or with inoculum and treated by NJ immersion, had a significant decrease in TSS content ($P > 0.05$). However, the magnitude of treatments varied from 3.16% to control and 14.9% to the treatment of 0 min of immersion. This is similar to the report by Riviera-López et al. [43] on papaya Maradol stored in refrigeration. They reported that the product stored at 5°C had lower respiration rates and showed a small decrease in TSS content. This indicates that NJ and *E. coli* did not significantly affect the TSS of the fresh-cut papaya fruit ($P > 0.05$).

3.3.2. pH Values. The behavior of pH in fresh-cut papaya during their storage at $4 \pm 1^\circ\text{C}$, not inoculated, and inoculated with *E. coli*, for the effect of the immersion time in NJ is shown in Figures 5(a) and 5(b), respectively. The fresh-cut papaya treated with NJ has a lower pH than those treated with water (control); this may be due to the organic acids present in the NJ.

The treatment control, either in the presence or absence of *E. coli*, showed a significant decrease in the pH values ($P < 0.05$). These changes in the pH values during the storage period may be associated with the growth of microorganisms and the subsequent production of organic acids [44]. In general, the papaya fruit immersed in NJ kept the pH values constant, with a slight increase at the end of the storage period.

Fajar-Falah et al. [45] found that organic acid content will decrease during the ripening process, but TSS and pH will increase; however, acidity increases (a pH decrease) at the end of the shelf life of the fresh produce as an indicator of fresh product for the consumer. This means that the fresh-cut product is not acceptable to eat. The abovementioned indicates that the papaya cubes untreated with NJ lose their quality quickly, and they are not acceptable for consumption on day 3.

3.3.3. Titratable Acidity. The papaya acidity tended to increase in both treatments (immersion in *E. coli* and not immersion in *E. coli* treatment) at the end of the experiment. Besides, NJ treatment for 0, 2.5, and 5.0 min (Figure 6) showed that the longer the immersion time in NJ, the acidity value is higher.

In the treatments without the addition of *E. coli*, a decrease in the acidity is shown during the first 12 days of storage, to then increase significantly at day 15 ($P < 0.05$) and

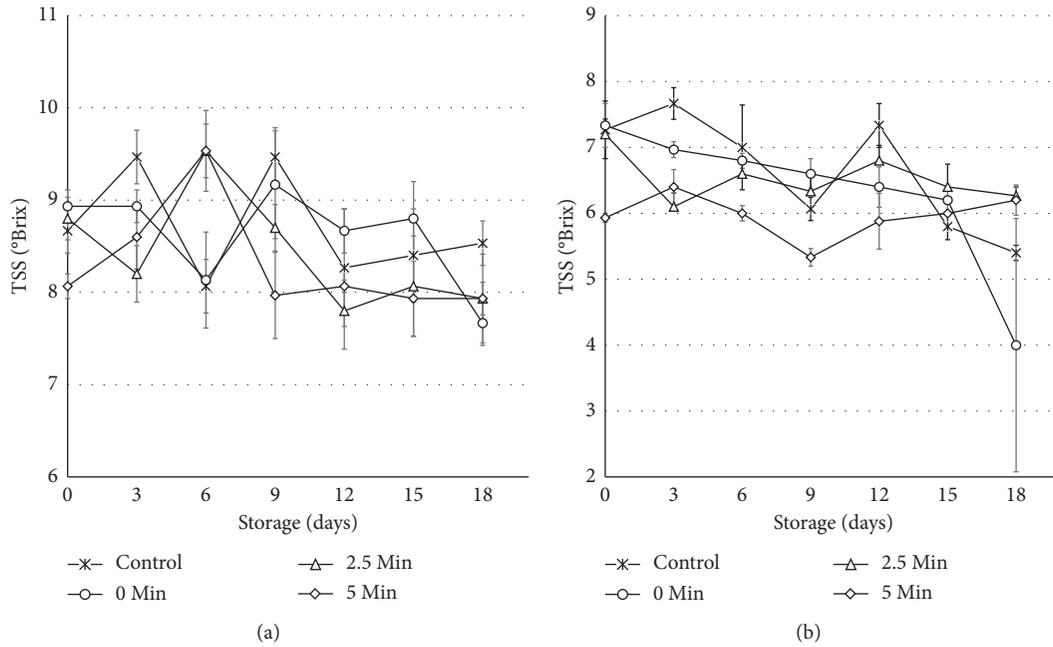


FIGURE 4: Effect of the immersion time of fresh-cut papaya fruit in NJ on the content of TSS during their storage at 4°C. Control (a) and treated with *E. coli* (b).

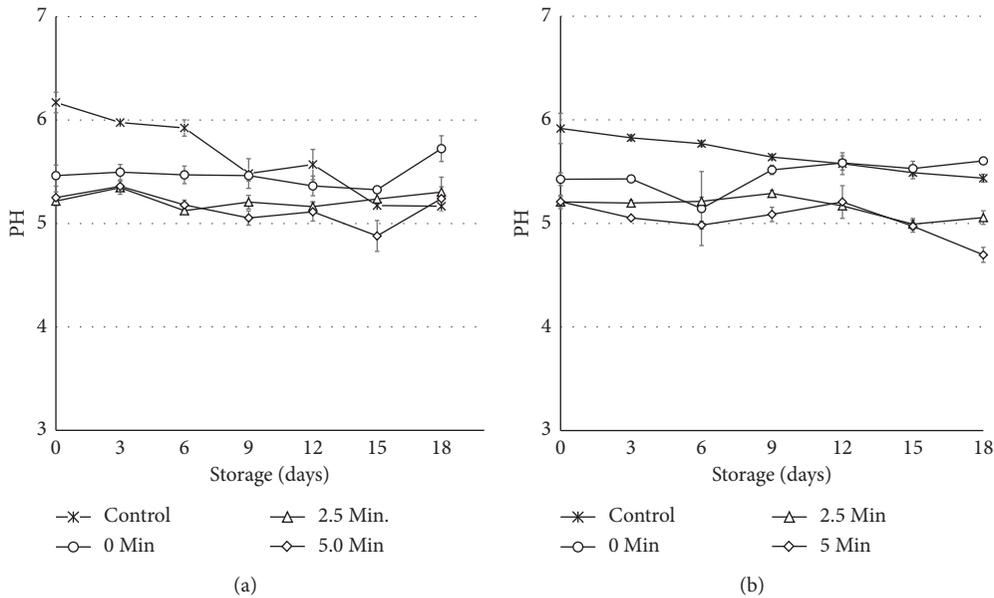


FIGURE 5: Immersion time effect on pH values during storage of papaya cubes treated. Control (a) and *E. coli* treated cubes (b).

end with a decrease in acidity, that is, normal behavior in pre-cut fruits [45].

However, the fresh-cut papaya was immersed in a suspension of *E. coli* and (Figure 6(b)) showed a constant

increase in acidity throughout the storage period. This behavior has been observed in papaya cubes by edible coating and stored at 5°C for 12 days [46]. NJ could contain polysaccharides [13–16], which could form an edible coating [47].

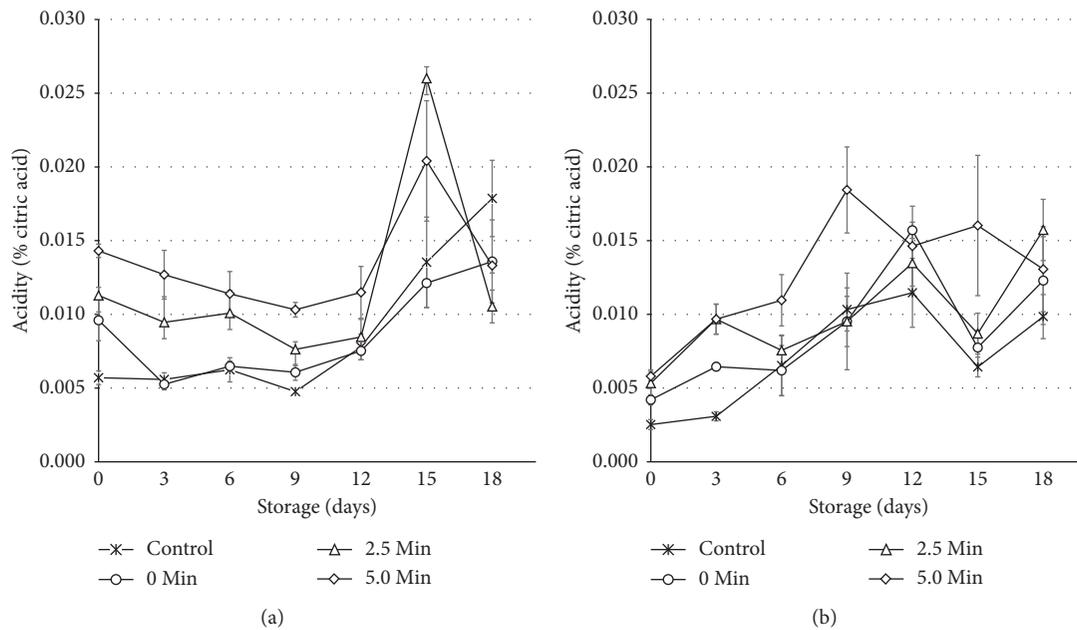


FIGURE 6: Immersion time effect on acidity during storage of papaya cubes. Control (a) and *E. coli* treated cubes (b).

4. Conclusions

Based on the results obtained in this study, NJ showed a bacteriostatic effect on *E. coli* *in vitro*. Besides, the application of NJ to fresh-cut papaya fruit showed bacterial inhibition *in vivo*, evidencing it as a possible bacterial control agent in the pre-cut fruit industry. The immersion of fresh-cut papaya on NJ for 0 min is enough to control *Escherichia coli* O157:H7; however, an immersion for at least 2.5 min is necessary to control others coliforms bacteria. The pre-cut fruit immersion time in NJ is a critical factor in bacteria survival. However, further studies are needed regarding the conservation of the physicochemical and sensory properties of the fruit when applying the NJ.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of Interest.

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