Effect of Banana Leaf Extract, Pumpkin Seed Extract and Bee Honey Treatment on the Inhibition of Browning of Fresh Cut Guava

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ABSTRACT

Purpose: Enzymatic browning is one of the most common phenomena happenings in guava slices as well as in all fresh-cut produces. This research was aimed at studying the potentiality of bee honey (BH), banana leaf extract (BLE), and pumpkin seed extract (PSE) for inhibition of enzymatic browning of fresh-cut guava slices.

Research Method: Guava slices were dipped in solution (slice: solution = 1:1) containing either BH or BLE or PSE for 5 min and the changes in physicochemical properties such as browning intensity, color, vitamin C, acidity and microbial attributes were evaluated during their storage at 4°C up to 6 days.

Findings: All treatments had the potentiality to inhibit enzymatic browning reactions. Treatment with BH had the best browning inhibition capacity (75.68%) followed by BLE (59.93%) and PSE (36.67%). Moreover, all three treatments worked against increasing of microbial load, degradation of acidity, ascorbic acid and color. All treated samples had higher lightness (L*) values upon storage than control (without treatment). The reaction rate constants for degradation of vitamin C based on 1st order kinetic model were as 0.0153, 0.0117, 0.0047 and 0.0037 per day for control, PSE, BLE and BH treatments, respectively.

Originality: Findings of this study indicate that the BH, BLE and PSE had potency against quality deterioration and browning of fresh cut guava slices during storage.

Keywords: Banana leaf extract, Browning intensity, Guava slice, Bee honey, Pumpkin seed extract

INTRODUCTION

Fresh cut food products are also defined as minimally processed foods that have been trimmed and/or peeled and/or sliced into complete useable foods. Fresh-cut fruit products have enjoyed a growing presence on the market due to customer demand, both for retail and food service applications (Gross et al., 2016). However, the integrity of the fresh-cut produces has been changed due to this minimal processing and consequently affects negatively on product quality such as formation of brown color, degradation of texture, development of off-flavor, changes of nutritional and microbial attributes etc. Customers typically evaluate the fresh-cut fruits’ quality based on their physical appearance and freshness at purchase time (Rojas-Graü et al., 2009). Thus, retardation of such detrimental consequences is of great concern to all stakeholders involved in fresh-cut produce production and distribution.

Guava (Psidium guajava) is a highly perishable climacteric fruit (>80% moisture content) and due to which its post-harvest loss is considerably

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high up to 4.0-15.5% (Madan and Ullasa, 1993). All fresh-cut products are susceptible to enzymatic browning which is consequently responsible for pest and microbial attacks (El-Anany and Hassan, 2013). During post-harvest handling and processing, enzymatic browning affects the nutritional value of fresh cut fruits and vegetables. As a result, fresh produce industry faces severe economic losses (He and Luo, 2007). Enzymatic browning is induced by the activity of polyphenol oxidase (PPO) on its phenolic substrates in the presence of oxygen. The oxidative products PPO undergo further reactions to very complex polymers, which are responsible for browning (McEvily et al., 1992). Perishable and highly perishable fruits and vegetables namely, guava, potatoes, mushrooms, banana, peaches and apples are more prone to this oxidative/enzymatic browning (McEvily et al., 1992).

Several approaches, such as the use of dipping treatments, based on individual anti-browning or firming agents or mixtures of them such as calcium chloride with ascorbic acid (McEvily et al., 1992), ascorbic acid and cysteine (Raheem et al., 2013), honey and/or citric acid (Eman et al., 2015) are explored to inhibit the browning of fresh-cut guava due to enzyme action. Fruit slices show faster rate of enzymatic browning and softening due to wounding (Deepthi et al., 2016; Kaur and Kapoor, 2000). On the other hand, Deepthi et al. (2016) also remarked that the lack of post-harvest treatments results in decrease of weight, firmness, acidity, ascorbic acid and sensory scores. Industrially, ascorbic acid and its derivatives, sulphur dioxide and sulphites are used to inhibit polyphenol oxidase (PPO)-induced browning reactions for a wide range of fresh cut fruits including guava (McEvily et al., 1992). Eman et al. (2015) studied on the post-harvest treatments of guava whole fruit and slices by with 15% bee honey with or without 2% citric acid compared without any treatments (control) at 5±1°C and 85-90% relative humidity and they mentioned that treatments attributed positively against quality loss of the guava slices. Additionally, they obtained that a combination of bee honey (15%) and citric acid (2%) significantly reduced the post-harvest loss and respiration rate of guava whole fruits and slices. Treatment with banana leaf extract was also effective in restricting the enzymatic browning of mushroom, apple and potato slices due to its natural preservative actions (Kaur and Kapoor, 2000). Moreover, they added that supplementation of banana leaf extract with ascorbic acid and 4-hexylresorcinol significantly inhibited the enzymatic browning during storage of apple pieces, trimmed mushroom and potato slices at 4°C.

Bee honey is an acidic food (pH ranges from 3.4 to 6.1) which is used to inhibit the growth of some pathogenic micro-organisms and also acts as an antioxidant. Research has confirmed the potentiality of honey as an anti-browning agent in raisins (Wessels et al., 2014), juices (McLellan et al., 1995; Lee, 1996), fresh cut apple (Gacche et al., 2009; Oszmianski and Lee, 1990; Son et al., 2001; Jeon and Zhao, 2005) and fresh-cut persimmon (Ergun and Ergun, 2010). However, the effect of honey, banana leaf extracts and pumpkin seed extracts are yet to be studied against browning and quality changes of fresh-cut guava. Based on the above stand points, this study was undertaken to evaluate the effect of BLE, PSE and BH on the quality of guava slices in preventing the post-harvest oxidative and enzymatic browning of fresh cut guava slices.

MATERIALS AND METHODS

Minimal processing of guava

Matured guava fruits were purchased from a local fruit market (Mymensingh, Bangladesh), graded, sorted and cleaned with distilled water and soaked in 100 ppm NaOCl (sodium hypochlorite) for 3 min. Cleaned guava fruits were then dried by natural convection process at room temperature (25-30°C) (Nasution et al., 2015) and sliced into uniform sizes (approximately, 2 cm × 2 cm × 2 cm) with a sharp knife. Bee honey was bought from the local market.

Preparation of pumpkin seed extracts

Pumpkin seeds (1 kg) were collected from the regional market and washed thoroughly in running water. The seeds were then dried in a
vacuum dryer at 55°C for 6 hr and grinded by a grinder (Yeasmen and Islam, 2015). Fifty (50) g of pumpkin seed powder was mixed with 500 ml of 96% ethanol followed by magnetic stirring at 900 rpm 30 min and sonicated at 45°C for 55 min to increase the efficiency of extraction (Bisht et al., 2016; Rahman et al., 2019). Three (3) stage extractions were used for the extraction of PSE i.e. 50 g pumpkin seed powder was mixed with 500 ml of ethanol for 3 times. The extract was centrifuged at 7500 g for 15 min maintaining the temperature -15°C followed by evaporation of the supernatant using a rotary evaporator. Alcoholic flavor was removed from the PSE passing through nitrogen gas. Extracted PSE was kept at 4°C for further processing (Bisht et al., 2016; Rahman et al., 2019).

**Preparation of banana leaf extracts**

Banana leaf (200 g) was taken and cut into 0.01 m long pieces and properly washed through potable water. It was then soaked in water (60°C) for 6 hr and filtered through Whatman No. 01 filter paper (Rahman et al., 2019). The brown color filtered was decolorized through activated charcoal and used as banana leaf extracts (BLE) (Kaur and Kapoor, 2000).

**Treatments of BH, BLE and PSE for inhibiting enzymatic browning from fresh cut guava slices**

In this study, total four (4) samples were prepared and three (3) treatments were performed by individually dipping 100 g prepared guava slices (2 cm × 2 cm × 2 cm) in 100 ml of 35% (v/v) bee honey (21% moisture content), 100 ml of banana leaf extract solution and 100 ml of pumpkin seed extract solution for 5 min. Besides, a control sample was also prepared in which no treatment was used. Table 01 represents the experimental plan for this study. These four treated or untreated guava slices were stored at 4°C for 6 days after packing in double layer polyethene. Browning intensity, superficial colors, total color difference, vitamin-C, titratable acidity, and microbial analysis were determined for both treated and control samples. Apart from vitamin-C, triplicate determinations were performed for each analysis.

**Determination of browning intensity**

For the determination of browning intensity, 5 g of treated guava slice was extracted in 30 ml of 65% (v/v) ethanol and stirred for 60 min at room temperature. The suspension was filtered using filter glass and measured the absorbance at 420 nm using a UV spectrophotometer (Shimadzu, model 1800) (Mazumder and Ranganathan, 2020; Sapers and Douglas, 1987). Browning intensity was expressed as $\Delta OD_{420}$/100g fresh weight (FW).

**Color measurement**

Color parameters ($L^*$, $a^*$ and $b^*$) were measured by using a Konica Minolta Colorimeter (Chromameter CR-400, Japan). Total color difference ($\Delta E^*$) of the treated guava slices were calculated using the formula in equation (i) (Monira, 2016):

$$\Delta E^* = \sqrt{(\Delta L^* \,^2 + \Delta a^* \,^2 + \Delta b^* \,^2)} \quad \text{..................... (i)}$$

**Table 01: Experimental Plan**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>Storage condition</th>
<th>Analysis Frequency</th>
<th>Parameters observed</th>
</tr>
</thead>
</table>
| Guava slice (2 cm × 2 cm × 2 cm) | Control (without treatment), banana leaf extract (BLE), pumpkin seed extract (PSE) and Bee honey (BH) | Refrigerated storage (4°C): 6 days | 0 (initial) , 1st, 3rd and 6th day | 1. Browning Intensity  
2. Color  
3. Vitamin-C  
4. Titratable acidity  
5. Total viable count |
Evaluation of the inhibition of browning

Sapers and Douglas (1987) suggested that the difference between final and initial L values should be calculated to minimize the variability in the natural pigmentation of food products. Tristimulus colorimetry methodology was used to evaluate the browning of guava slices. The degree of inhibition of enzymatic browning was expressed as the percent difference between control and treatment after specific storage time t:

\[
\% \text{ inhibition value} = \frac{\Delta L \text{ control} - \Delta L \text{ treatment}}{\Delta L \text{ Control}} \tag{ii}
\]

Where, \(\Delta L\) is the difference between final and initial L value.

Determination of Vitamin C (ascorbic acid) of fresh cut guava slices

Ascorbic acid (vitamin C) content was determined by using 2, 6-Dichlorophenolindophenol dye method (Ranganna, 2005). Five (5) g of treated guava slice was blended with 3% metaphosphoric acid (HPO\(_3\)) solution and volume made up 100 ml by 3% metaphosphoric acid solution. The solution was filtered through Whatman No. 01 filter paper and filtrate was titrated against the dye taken in the burette, till the pale pink (permanent) color was obtained. The amount of ascorbic acid in the given solution was calculated by the following formula:

\[
\text{mg of ascorbic acid per 100 g sample (iii)} = \frac{T \times D \times V_1 \times E}{W \times V_2 \times 100} \times 100
\]

Where, \(T\) = Titer value, \(D\) = dye factor, \(V_1\) = volume made up, \(V_2\) = volume of sample taken for estimation, \(W\) = weight of sample.

1st order kinetic model used to express the vitamin-C changes during storage of guava slices, as this model fitted well for degradation of vitamin-C (Zhang et al., 2016; Olivera et al., 2013). The model is given in equation (iv):

\[
\ln C = \ln C_0 - k_1 t \text{ or } C = C_0 \exp^{-k_1 t}
\]

Where, \(C_0\) is the initial concentration of the Vit-C while \(C\) is the concentration of Vit-C at time (t). Plotting of \(C\) vs time (t) on semi log coordinate gives a straight line (slope), which is the first order reaction rate constant, \(k_1\).

Determination of titratable acidity of fresh cut guava slices

Five (5) g of treated guava slice was blended with distilled water and volume made up 100 ml by distilled water. The solution was mixed properly and filtered through Whatman filter paper 04. Five (5) ml of sample was pipette out into a clean conical flask. Three (3) drops of phenolphthalein indicator was added to the solution and titrated against standard sodium hydroxide (0.1 N) until first permanent pink color. The titratable acidity was calculated using the following formula (Ranganna, 2005):

\[
\% \text{ titratable acidity} = \frac{T \times N \times V_1 \times E}{W \times V_2 \times 100} \times 100 \tag{v}
\]

Where, \(T\) = Titer value; \(N\) = Normality of NaOH; \(E\) = Equivalent weight of acid; \(V_1\) = volume made up, \(V_2\) = volume of sample taken for estimation, \(W\) = weight of sample.

Microbial analysis of fresh cut guava slices

0.1 ml sample solution of each 10-fold serial dilution was transferred onto agar plate using micropipette. The inoculated samples were spread on the surface of the plate using glass spreader and incubated at 37°C for 24-48 hr and 30-300 colonies were counted. The total viable count was calculated using modified method of ISO (1995) and expressed as log Colony Forming Unit (CFU) per gram. The total viable count was determined by multiplying the average number of colonies by dilution.

Statistical analysis

The obtained results of this study were analyzed by the Statistical Analysis Method (SAS, 1985) for analysis of variance (ANOVA) to know whether there were any significant differences among samples’ quality during storage period. Fisher’s least significant difference (LSD) test procedure was applied to find the significant differences if any at 5% significance level (p<0.05) using StatView (Abacus Concepts Inc., 2005).
RESULTS AND DISCUSSION

Effect of BH, BLE and PSE on color parameters of fresh cut guava slices

Figure 01 illustrates the changes in lightness (L*) of four samples (control, BH, BLE and PSE) during 6 days of storage. L* values of the guava slices was decreased continuously with storage period which was concomitant and consequently increased the darkness. The result indicates that browning was increasing during storage of guava slices. Dea et al. (2010) suggested that a decrease in L* value is an indicator of flesh browning. At first (1st) day of storage, L* value slightly increased (not significant with initial L* value) in all three treatments except in the control sample (significantly differed with control). The L* values of control and PSE treated guava slices differed significantly (p<0.05) from BH treated sample on 3rd day storage. L* value of BLE treatment was equally acceptable to both BH and PSE treated guava slices. On the 6th day, the highest L* value (72.03±1.12) was observed for guava slices treated with 35% BH and the lowest L* value (56.55±1.11) was observed at control slices. The L* values for BLE and PSE were 69.50±2.33 and 65.70±1.69, respectively. Significant differences were noticed among all four samples for L* values on the 6th day of storage. The result suggested that all three treatments had the ability to keep the lightness of the guava compared to control and inhibit browning. At 6th day of storage, L* value followed such order: BH>BLE>PSE>control. However, BH treated guava slices were the brightest which indicated lower browning and acceptable physical appearance of the guava slices which was followed by BLE, PSE and control samples. Research revealed that 20% bee honey solution reduced the lowering rate of L* values in fresh cut pear slices during storage period (Lin et al., 2006). Inhibitory activity of bee honey on PPO and inhibition of enzymatic browning in fresh cut apple slices and grape juice was also reported by Oszmianski and Lee (1990). Honey peptides and amino acids may reduce PPO activity due to the metal chelating action of essential copper at the active site of PPO and formed a stable complex with Cu²⁺. Apple juice treated with bee honey inhibits browning intensity due to the inactivation of PPO by the action of reducing substances in bee honey (Gacche et al., 2009).

Figure 01: Superficial colors, L* (A), a* (B) and b* (C) of fresh-cut guava dipped in bee honey (BH), banana leaf extracts (BLE) and pumpkin seed extract (PSE) solutions during storage at 4°C for 6 days.
a* and b* values represent red color and yellow color of the guava slices, respectively. An overall increasing in a* values and decreasing in b* values were noticed with prolonging the storage time. It is noticeable that BLE and PSE treated guava slices showed slight decreasing trends of a* values after treatment while BH resulted in increasing but there was no significant difference (p>0.05). After 1st day of storage, a*(redness) values of control guava slices increased from -0.42 to 0.82, while the BLE, PSE and BH treatment decreased a* values from -0.49 to -0.73, -0.48 to -0.58 and -0.39 to -0.43 respectively. On 1st day, a* value of control sample was significantly higher (p<0.05) than other three samples. This decreasing trend suggested that initially the samples became greenish due to BLE, PSE and honey treatment. However, as the day progressed, the values were gradually increased in all treatments. On the 6th day, it is seen that the BH treated sample had the significantly (p<0.05) lower a* values compared to control and PSE, while BLE had a statistically equal ability with honey treatment to retain a* value of the guava slices. On the other hand, b* value (yellowness) increased for BH (from 18.33 to 20.68), BLE (from 17.5 to 20.00) and PSE (from 18.17 to 18.70) treated samples up to 1st storage day. However, after 1st day of storage, b* values of BH, BLE and PSE treated samples were decreasing, though b* values of control sample were decreasing from initial day and were significantly lowered (p<0.05) than others at each day of analysis. The result indicates that all these treatments inhibited the browning but bee honey worked better than other treatments in guava slices.

Figure 02 illustrates the total color changes (∆E*) of the guava slices treated with BH, BLE, PSE solution and untreated slices. Guava slices treated with 35% BH had the lowest color changes (p<0.05) than other treatments during storage period. However, PSE did not significantly (p>0.05) differ to BLE treatment. Total color difference (∆E*) of the control sample was higher (p<0.05) than BH, BLE, PSE. Among three treatments, PSE showed a moderate effect on total color differences. ∆E* values for PSE treatment were significantly (p<0.05) lower than control and higher than BLE and BH treated guava slices on and after 3rd day. The low ∆E* in the 35% BH, BLE and PSE treated slices could be clearly correlated to the inhibition of browning and high L* values (Figure 01). The result suggested that all three treatments might retain superficial color of fresh cut guava slices during storage period.

Effect of BH, BLE and PSE on browning intensity of fresh cut guava slices

Figure 03 illustrates the browning intensity in ∆OD_{420}/100 g fresh weight (FW) of fresh-cut guava slices. It is found that BH and BLE had significantly (p<0.05) better ability to retard enzymatic browning than control and PSE treated guava slices during storage periods. PSE treatment resulted in significantly (p<0.05) higher browning intensity than BLE and BH, and lower than the control sample. For 1st day of storage, browning intensity of control guava slices was significantly (p<0.05) higher than BH, BLE and PSE treated guava slices. Bee honey contained reducing compounds namely, ascorbic acid, riboflavin and antioxidants (White et al., 1961), which made honey as an excellent alternative for controlling oxidative browning (McEvily et al., 1992). Inhibitory activity of BH, BLE and PSE indicates inhibition of enzymatic browning in fresh cut guava slices. The reduction of PPO activity by BH, BLE and PSE might be due to the formation of oxidation reaction products derived from ascorbic acid, sulfites, sulfur containing amino acids, organic acids and phenolic acids (Eissa et al., 2014). Similar prevention capacity of different extracts in term of browning prevention intensity was reported by several authors (Eman et al., 2015; Deepthi et al., 2016; Kaur and Kapoor, 2000).
Table 02 shows the percent inhibition (% IV) of enzymatic browning in guava slices. The % IV values for each inhibitor were calculated using ∆L values obtained at 6 days of storage. The highest %IV (75.68±0.39%) was exhibited by BH treated slices followed by BLE (59.93±0.40%) and PSE (36.67±0.26%), respectively. This result suggested that BH and BLE could be an effective browning inhibitor under specific conditions (4°C) and time. The research also shows that %IV was less than 50% in PSE treated slices, indicating the fact that PSE fared poorly as a browning inhibitor of guava slices compared to BLE and BH.

Table 02: Browning inhibition capacity (%) of the bee honey, banana leaf extract and pumpkin seed extract

<table>
<thead>
<tr>
<th>Treatments</th>
<th>∆L control(final-initial)</th>
<th>∆L treatment(final-initial)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLE</td>
<td>18.79±0.13</td>
<td>7.53±0.08</td>
<td>59.93±0.40%</td>
</tr>
<tr>
<td>PSE</td>
<td>18.79±0.13</td>
<td>11.90±0.12</td>
<td>36.67±0.26%</td>
</tr>
<tr>
<td>BH</td>
<td>18.79±0.13</td>
<td>4.57±0.05</td>
<td>75.68±0.39%</td>
</tr>
</tbody>
</table>

Here, data are presented as mean ± standard deviation. Different superscripts in a column indicate significant difference among samples by LSD test at p<0.05. BLE = Banana leaf extract, PSE = Pumpkin seed extract, BH = Bee honey.
Effect of BH, BLE and PSE on vitamin C retention of fresh cut guava slices

Vitamin C content of fresh-cut guava slices in all the treated samples decreased as shown in Figure 04. Based on 1st order kinetic model, the vitamin-c degradation rate constants were higher in the control guava slice (0.0153) followed by PSE (0.0117), BLE (0.0047) and BH (0.0037) treated guava slices, respectively. This might be due to the autoxidation of the ascorbic acids. However, BH solution and BLE solution show less degradation of ascorbic acid than PSE treatment during 6 days of storage. This might be due to less autoxidation of ascorbic acid by protective layer of BH and BLE which control/reduce the permeability of oxygen (O₂) and carbon-di-oxide (CO₂) (Ayranci and Tunc, 2004). This result is in agreement with (Mohammed, 1999) who found that bee honey solution can retain the vitamin C of the stored mango slices.

Effect of BH, BLE and PSE on titratable acidity content of fresh cut guava slices

Figure 05 demonstrates the titratable acidity of guava slices for all three treatments and control. Titratable acidity was higher in all untreated fresh-cut guava slices. Treatment of fresh-cut guava slices by BH (0.63±0.0)³, BLE (0.64±0.04) and PSE (0.68±0.04) solution resulted in significantly (p<0.05) lowering the titratable acidity than control sample (0.82±0.04%), respectively. This might be due to soaking of fresh-cut guava in different solutions having low acidity. However, the total acidity (%) was decreased with increasing of storage period.
But this decreasing trend was higher in untreated guava slices. Based on initial day’s values, a decrease of 15.92, 6.22, 10.73 and 4.79% titratable acidity was noticed in control, BLE, PSE and BH treated guava slices on 6th storage day. Thus, it can be revealed that BH and BLE had the potential on retaining the acidity level during storage. The result is in agreement with Eman et al. (2015) who revealed that bee honey and citric acid treatments resulted in retaining higher titratable acidity of fruits and fresh-cut of guava during refrigerated storage.

**Effect of BH, BLE and PSE on microbial content of fresh cut guava slices**

Table 03 indicates the effect of BH, BLE and PSE on the microbial growth on fresh-cut guava slices at different days of refrigerated storage. It was noticeable that, BH and PSE remained most efficient in controlling microbial growth than other treatment up to 3rd storage day. On 6th day storage, the highest total viable count was observed in the control (7.40 x 10^4 CFU/g) and the lowest was observed for BH treated (2.00 x 10^3 CFU/g) samples, whereas the total viable count for BLE and PSE treated sample on 6th day was 4.74 x 10^3 and 6.50 x 10^3 CFU/g, respectively.

The results were in conformity with Eman et al. (2015), who found the similar potentiality of postharvest treatments (bee honey and citric acid) against microbial attack of fresh-cut guava slices. Most of the micro-organisms are in inactive forms and these organisms can hardly survive in honey due to its specific properties such as hygroscopicity, acidity, peroxide content, antibiotic activities and hyperosmolarity etc. (Olaitan et al., 2007).

**CONCLUSION**

Fresh-cut guava slices were treated with three different solutions like bee honey, banana leaf extract and pumpkin seed extract in order to prevent quality degradation during refrigerated storage. It was found that all three treatments had the potentiality against browning and degradation of vitamin-C, acidity and microbial parameters of the fresh-cut guava slices. The quality preservation capacity of the three treatments can be ranked as the bee honey>banana leaf extract>pumpkin seed extract. Finally, this study recommends that bee honey, banana leaf extract and pumpkin seed extract can be used to inhibit enzymatic browning and to retain the freshness of guava fruits, but the 35% bee honey can be used to get the best outcome. Further studies are suggested to find the optimum percentage of BH or BLE or PSE against quality changes of fresh-cut guava.

**Data Availability Statement**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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