**Enhancing the postharvest quality of Indian jujube (*Ziziphus mauritiana*) using aloe vera gel and ascorbic acid-based edible coatings**

Abstract

Indian jujube (*Ziziphus mauritiana*) is a highly perishable fruit with significant nutritional and economic value, yet its short shelf life and susceptibility to postharvest losses limit its market potential. Edible coatings have emerged as a promising solution to enhance fruit preservation by reducing moisture loss and maintaining quality during storage. This study aimed to develop aloe vera gel (AG) and ascorbic acid (AA)-based edible coatings and assess their effectiveness in preserving the postharvest quality of Indian jujube. Three coating formulations were prepared: diluted AG (1:3) with 0% AA, 3% AA, and 5% AA. The coated and uncoated fruits were stored for 15 days and evaluated for weight loss, total soluble solids (TSS), pH, titratable acidity (TA), ascorbic acid content, total phenolic content (TPC), and total antioxidant activity. The results indicated that AG+5% AA-coated fruits exhibited the least weight loss (14.69%) by day 15, whereas control samples showed the highest (27.35%). TSS values increased more slowly in coated samples, with AG+5% AA maintaining the lowest increase (11.13° Brix) compared to the control (15.16° Brix). Titratable acidity decreased at a slower rate in coated fruits, with AG+5% AA retaining a TA of 0.58%, while the control dropped to 0.52%. Additionally, coated samples better preserved ascorbic acid content and total phenolic content, with AG+5% AA demonstrating the highest retention. Total antioxidant activity remained significantly higher in coated fruits, further supporting the effectiveness of the coatings in delaying quality degradation. These findings suggest that aloe vera gel and ascorbic acid-based edible coatings effectively reduce postharvest deterioration by minimizing weight loss, preserving physicochemical properties, and enhancing antioxidant retention in Indian jujube. This natural preservation method offers a sustainable approach to extending the shelf life and improving the commercial viability of fresh fruits.

**Keywords:** Indian jujube, Aloe vera gel, Ascorbic acid, Edible coating, Postharvest quality, Antioxidant activities, Storage stability

**1. Introduction**

*Ziziphus mauritiana*, commonly known as Indian jujube, is a highly nutritious fruit cultivated in various regions worldwide under different names, including Chinese date, Chinese apple, apple ber, Indian plum, and dunks (Sarkar *et al.*, 2022). The fruit exhibits diverse shapes and sizes, ranging from oval to oblong and obovate, with lengths of 2.5–6.25 cm and an average weight of 9.6 g. It consists primarily of pulp (85.94%), mortar (15%), and juice (40%) by weight (Afroz *et al.*, 2014). When slightly underripe, the fruit is mildly succulent with a pleasant aroma, whereas the fully mature fruit develops a shiny red appearance, a juicy texture, and a sweet taste resembling apples.

Bangladesh is an agriculturally rich country where a vast quantity of fruits and vegetables is produced annually. In 2011–2012, the agricultural sector contributed 19.29% to the country's Gross Domestic Product (GDP) (Sikder & Islam, 2019). Indian jujube is a popular seasonal fruit in Bangladesh due to its high yield potential, nutritional value, and medicinal properties. It is a rich source of essential vitamins such as vitamin C, vitamin A, and B-complex vitamins, along with minerals including calcium (Ca), magnesium (Mg), potassium (K), bromine (Br), rubidium (Rb), and lanthanum (La) (Al-Reza *et al.*, 2010). The primary sugars present in the fruit include galactose, fructose, and glucose (Muchuweti *et al.*, 2005). Moreover, it contains several phenolic compounds, such as p-hydroxybenzoic acid, caffeine acid, ferulic acid, and p-coumaric acid, with vanillic acid being the least abundant at approximately 2.5 mg/kg. The Ziziphus species has been traditionally recognized in Asian countries, particularly in China and Taiwan, for its medicinal benefits in treating allergic reactions, gastrointestinal disorders, urinary dysfunction, respiratory problems, anxiety, depression, and liver diseases (Hua *et al.*, 2022; Popstoyanova *et al.*, 2024).

Despite its nutritional benefits and economic importance, post-harvest losses of fruits and vegetables in Bangladesh remain a major challenge. Indian jujube is highly perishable, with a storage life of less than ten days under ambient conditions. Post-harvest losses of fruits and vegetables in Bangladesh are estimated to range between 18% and 44%, amounting to an annual financial loss of approximately 3,392 crore Bangladeshi Taka (Hamim *et al.*, 2014; Hossain *et al.*, 2017). To mitigate these losses, the application of edible coatings has gained increasing attention as a promising post-harvest preservation strategy (Liu *et al.*, 2023; Bhadu *et al.*, 2024; Naveed *et al.*, 2024). Edible coatings have been utilized since the late 1940s for preserving fresh produce, including bananas, citrus fruits, pomegranates, apples, papayas, and cucumbers (Alam *et al.*, 2020; Li & Barth, 1998). Among various bio-preservatives, aloe vera gel (AG) has emerged as a natural and effective coating material due to its antimicrobial properties and ability to retain moisture, thereby extending the shelf life of fruits and vegetables (Rodríguez *et al.*, 2010).

The demand for natural antioxidants is rising due to growing concerns over the long-term health risks associated with synthetic preservatives (Sarkar, Ahmed, *et al.*, 2020; Sarkar, Rahman, *et al.*, 2020; Roy *et al.*, 2021). Antioxidant-rich foods, including fruits, vegetables and legumes, contribute to maintaining cellular health by neutralizing free radicals and preventing oxidative stress. These compounds also stabilize essential nutrients, such as vitamins A, C, B-complex, folic acid, vitamin E, and thiamine, ensuring their bioavailability in the human body (Roy *et al.*, 2022; Azam *et al.*, 2023; Sarkar *et al.*, 2024; Sarkar *et al.*, 2025). Ascorbic acid (AA) is a potent antioxidant widely used in food preservation, particularly in meat products, to prevent oxidation and maintain nutritional quality. When incorporated into edible coatings, AA helps preserve vitamin C content and enhances the overall antioxidant capacity of coated fruits.

Despite the known benefits of edible coatings, limited research has been conducted on their application in maintaining the post-harvest quality of Indian jujube. This study aims to develop and evaluate the effectiveness of aloe vera gel and ascorbic acid-based edible coatings in preserving the physicochemical properties of fresh Indian jujube during storage. The research focuses on assessing the impact of these coatings on fruit ripening, weight loss, acidity, total soluble solids, ascorbic acid content, antioxidant activity, and phenolic content over a storage period.

**2. Materials and methods**

2.1 Sample preparation

Fresh Indian jujube (*Ziziphus mauritiana*) fruits were collected from a local market in Sylhet, Bangladesh. Fruits of uniform size, shape, and color were selected for the study, while blemished or damaged fruits were discarded. A total of 24 fruits per treatment group were used, ensuring sufficient replicates for each analysis. The experiment included three treatment groups: (i) diluted aloe vera gel (AG) (1:3) without ascorbic acid (AA), (ii) AG with 3% AA, and (iii) AG with 5% AA, along with an untreated control group (Sogvar *et al.*, 2016).

Before treatment, both the fruits and aloe vera leaves were washed under running tap water. Before treatment, both the fruits and aloe vera leaves were washed under running tap water and air-dried at room temperature for an hour. Aloe vera gel was extracted from the leaves and blended, then filtered through a fine mesh sieve to remove fibers. The gel was diluted in a 1:3 ratio with distilled water and divided into three batches. Two of these batches were further mixed with 3% and 5% AA, respectively, to create edible coating solutions. The fruits were then dipped in their respective coating solutions for five minutes at room temperature, air-dried, and stored under ambient conditions (**25 ± 2 °C**) for further evaluation.

**2.2 Physicochemical properties**

**2.2.1 Weight loss**

The weight loss of the samples was measured following the method described by Martínez-Romero *et al.* (2006). To determine the weight loss, the initial weight of each sample was recorded before storage. The weight was then measured again after 3, 6, 9, 12, and 15 days of storage. The difference between the initial and final weight was used to calculate the percentage of weight loss over time.

**2.2.2 Total soluble solid (TSS)**

The total soluble solids (TSS) of the samples were measured following the method of Hasan *et al.* (2022). Juice was extracted from the jujube fruits using a juice extractor machine, and the liquid was then filtered through filter paper to remove any solid particles. A hand refractometer was used to determine the TSS of the filtered juice, with the results expressed in degrees Brix.

**2.2.3 pH**

The pH of the samples was measured using a pH meter calibrated with a standard buffer solution at pH 7.0. The temperature was maintained at 28°C during the measurement. Before inserting the glass electrode into the sample solution, it was standardized with the buffer solution and thoroughly rinsed with distilled water to ensure accuracy (Hasan *et al.*, 2025).

**2.2.4 Total acidity**

Titratable acidity was determined using the method described by Athmaselvi *et al.* (2013). First, 10 mL of extracted jujube juice was placed in a beaker and mixed with 25 mL of distilled water. The mixture was then titrated with 0.1 M sodium hydroxide (NaOH) solution using phenolphthalein as an indicator. The NaOH solution was added dropwise while continuously stirring until a persistent light pink color appeared, indicating the endpoint. The volume of NaOH used was recorded, and the total acidity was calculated based on the equivalent factor of the predominant acid in the fruit. The results were expressed as a percentage of citric acid.

**2.3 Antioxidant activities**

2.3.1 Ascorbic acid

Ascorbic acid content was determined by following the method mentioned in Vallespir *et al.* (2019). The equation used for the determination of vitamin C were followed:

mg of vitamin-C per 100 g sample$ =\frac{T×D×V1}{W×V2}×100$

where T represents the titre or volume of titrant used, D is the dye factor, V1 is the total volume of the prepared solution, V2 is the volume of the extract used for titration, and W is the weight of the sample in grams. The calibration curve was constructed using standard L-ascorbic acid, with the equation y = 9.94x + 0.2896 and an R² value of 0.9976.

2.3.2 Total phenolic content

Total phenolic content was determined by the method described by Sarkar *et al.* (2021) with slight modification. A 0.5 mL aliquot of the sample extract was mixed with 8.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 minutes. Subsequently, 1 mL of a 35% sodium carbonate solution was added, and the solution was thoroughly mixed. The reaction mixture was then left undisturbed at ambient temperature for 20 minutes before measuring the absorbance at 765 nm using a spectrophotometer. A blank sample was prepared by replacing the extract with distilled water and was used to calibrate the instrument. The total phenolic content was quantified by comparing the absorbance values with a standard curve generated using different concentrations of Gallic acid. The results were expressed in milligrams of Gallic acid equivalent per gram of dry extract, using the equation y = 0.009x - 0.010 with an R² value of 0.999.

2.3.3 Total antioxidant activity

The antioxidant activity of the samples was assessed using the DPPH radical-scavenging method as described by Saikia *et al.* (2015). A 2 mL aliquot of 0.2 mg methanolic DPPH solution was added to 2 mL of the sample extract at different concentrations. The mixture was vigorously shaken for 15 seconds to ensure proper mixing. The solutions were then incubated in a dark environment for 10 minutes to allow the reaction to occur. After the incubation period, absorbance was measured at 517 nm using a UV-Visible spectrophotometer against a blank sample. The DPPH radical-scavenging activity of each sample extract was calculated using the following equation:

DPPH radical-scavenging activity = $\frac{A0-A}{A0}×100$

where A is the absorbance of the sample-containing DPPH solution, and A0 is the absorbance of the control solution without the sample extract.

2.4 Statistical analysis

All measurements were conducted in triplicate, and the results are presented as Mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA), followed by Tukey’s multiple comparison test to assess significant differences among samples. The analysis was carried out using GraphPad Prism 8 software, with statistical significance set at p < 0.05.

**3. Results and discussion**

**3.1 Physicochemical properties**

The physicochemical properties of Indian jujube underwent significant changes during storage, with variations observed between coated and uncoated samples. The application of aloe vera gel (AG) and ascorbic acid (AA)-based coatings played a crucial role in reducing weight loss, maintaining total soluble solids (TSS), regulating pH, and preserving titratable acidity (TA).

Weight loss in all samples increased progressively over the 15-day storage period, with control samples exhibiting the highest moisture loss due to direct exposure to ambient conditions. In contrast, the AG+5% AA-coated fruits showed the lowest weight loss, demonstrating the moisture-retentive effect of the edible coatings. On day 3, the control samples lost 6.93% of their initial weight, while AG+5% AA-coated fruits exhibited significantly lower weight loss (3.47%). By day 15, weight loss reached 23.26% in the control group, whereas AG+5% AA-coated samples retained more moisture, limiting weight loss to 14.72% (Table 1). The significant reduction in weight loss among coated samples is likely due to the water barrier properties of aloe vera gel, which reduced transpiration and evaporation. Aloe vera gel’s hygroscopic nature enhances water retention by forming a semi-permeable coating, minimizing dehydration by slowing down moisture diffusion (Morillon & Lassalles, 2002). Furthermore, the incorporation of ascorbic acid in coatings may have contributed to reduced oxidative stress and membrane integrity preservation, further limiting weight loss.

Total soluble solids (TSS) represent the concentration of soluble sugars and organic acids, which typically increase as fruit ripens. However, in this study, coated samples exhibited a slower increase in TSS compared to uncoated samples, suggesting a potential delay in sugar metabolism due to the coatings’ role in respiration regulation. Total soluble solids (TSS) were consistently higher in the control samples compared to the coated ones. On day 15, the highest TSS value was observed in the control samples (15.16 °Brix), whereas the AG+5% AA-coated samples retained the lowest TSS levels (11.13 °Brix). There was a minor difference in TSS between AG+3% AA and AG+5% AA-coated samples (Table 1). The coated fruits exhibited lower TSS levels, which could be due to a reduced respiration rate, thereby slowing down sugar metabolism. During storage, TSS levels typically increase as a result of respiration, which converts complex carbohydrates into simple sugars (Ali *et al.*, 2011).A similar outcome was observed in aloe vera-coated nectarines, where respiration was significantly suppressed, preserving fruit quality for an extended period (Ahmed *et al.*, 2009).

**Table 1: Weight loss and total soluble solids (TSS) of coated and uncoated Indian jujube during storage.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Coating Condition** | **Day 0** | **Day 3** | **Day 6** | **Day 9** | **Day 12** | **Day 15** |
| Weight loss (%) | Control | 0 | 6.93±0.003a | 11.25±0.006a | 15.23±0.025a | 20.11±0.029a | 23.26±0.050a |
| AG | - | 6.61±0.023b | 9.41±0.230b | 13.30±0.176b | 16.28±0.126b | 18.80±0.138b |
| AG+3%AA | - | 4.84±0.006c | 8.82±0.050c | 10.55±0.0115c | 13.74±0.132c | 16.80±0.015c |
| AG+5%AA | - | 3.47±0.006d | 7.90±0.006d | 9.54±0.004d | 12.22±0.116d | 14.72±0.025d |
| TSS (oBrix) | Control | 9.02±0.190a | 10.03±0.152a | 11.4±0.010a | 13.60±0.100a | 14.46±0.015a | 15.16±0.057a |
| AG | - | 9.70±0.010b | 10.26±0.057b | 11.46±0.057b | 12.73±0.058b | 13.46±0.016b |
| AG+3%AA | - | 9.40±0.010c | 9.73±0.058c | 10.13±0.057c | 10.86±0.057c | 11.26±0.104c |
| AG+5%AA | - | 9.27±0.006c | 9.40±0.010d | 9.67±0.050d | 10.73±0.050c | 11.13±0.076c |

All the data is presented as a mean value with a standard deviation. Values in a column within each parameter with distinct letters are significantly different at p < 0.05.

The pH levels of both coated and uncoated samples exhibited significant differences throughout the storage period. By day 15, the control samples recorded the highest pH (5.05), indicating greater acid degradation, while the AG+5% AA-coated fruits retained the lowest pH (4.72), suggesting better preservation of acidity. Similarly, the pH values of AG+3% AA (4.84) and AG (4.89) remained significantly lower than that of the uncoated control (Table 2). The relatively stable pH in coated samples suggests that the edible coatings played a crucial role in maintaining acidity levels by delaying acid degradation. The gradual rise in pH over time is commonly associated with respiratory metabolism, where organic acids are consumed as substrates. However, the presence of ascorbic acid (AA) in the coatings may have further contributed to acidity retention, as AA is inherently acidic and can slow down pH fluctuations by acting as an antioxidant and stabilizing organic acids. The protective effect of aloe vera-based edible coatings is likely due to their semi-permeable nature, which modulates gas exchange and reduces oxygen diffusion. This regulation of O₂ and CO₂ concentrations within the fruit’s microenvironment could have played a role in slowing down respiration and oxidative degradation, ultimately delaying senescence (Padmaja & Bosco, 2014). Similar results have been reported in Aloe-pectin treated jujube, where edible coatings effectively reduced pH fluctuations and prolonged fruit freshness (Morillon *et al.*, 2002; Athmaselvi *et al.*, 2013).

**Table 2: pH and titratable acidity (TA) of of coated and uncoated Indian jujube during storage.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Coating Condition** | **Day 0** | **Day 3** | **Day 6** | **Day 9** | **Day 12** | **Day 15** |
| pH | Control | 4.31±0.025a | 4.62±0.025a | 4.71±0.016a | 4.75±0.006a | 4.88±0.040a | 5.05±0.005a |
| AG | - | 4.53±0.010b | 4.63±0.017b | 4.66±0.015b | 4.74±0.010b | 4.89±0.015b |
| AG+3%AA | - | 4.45±0.020c | 4.59±0.015c | 4.65±0.015bc | 4.71±0.006bc | 4.84±0.012c |
| AG+5%AA | - | 4.42±0.026c | 4.56±0.015c | 4.62±0.015c | 4.65±0.007c | 4.72±0.015d |
| TA (%) | Control | 0.65±0.005a | 0.61±0.0105a | 0.58±0.002a | 0.57±0.003a | 0.54±0.002a | 0.52±0.001a |
| AG | - | 0.63±0.007b | 0.59±0.002b | 0.58±0.004b | 0.56±0.004b | 0.53±0.002ab |
| AG+3%AA | - | 0.65±0.003bc | 0.62±0.003c | 0.61±0.001c | 0.58±0.005c | 0.55±0.001ab |
| AG+5%AA | - | 0.64±0.004c | 0.63±0.020d | 0.62±0.004d | 0.59±0.005d | 0.58±0.011b |

All the data is presented as a mean value with a standard deviation. Values in a column within each parameter with a distinct letter are significantly different at p < 0.05.

Titratable acidity (TA) exhibited a significant increase in coated samples, with AG+5% AA-coated fruits maintaining the highest acidity (0.58%), compared to 0.52% in the uncoated control samples by day 15 (Table 2). The higher acidity retention in coated samples aligns with findings in AG-coated table grapes, where edible coatings helped reduce acid degradation and prolong storage stability (Serrano *et al.*, 2006). The preservation of acidity in coated fruits is likely attributed to the oxygen barrier properties of the aloe vera gel, which slowed the oxidation of organic acids and reduced metabolic respiration rates. As organic acids serve as primary respiratory substrates, slower oxygen diffusion through the coating may have limited acid consumption, thereby maintaining a lower pH and higher TA levels (Guillén *et al.*, 2013). Additionally, ascorbic acid (AA) in the coating may have acted as a buffering agent, further stabilizing acidity by reducing oxidative degradation of fruit acids.

Overall, the application of aloe vera gel and ascorbic acid-based coatings had a significant impact on postharvest quality, influencing key parameters such as weight loss, TSS, pH, and titratable acidity. The findings highlight the potential of natural edible coatings as an eco-friendly and effective alternative for postharvest preservation, reducing losses and maintaining fruit marketability during storage.

**3.2 Antioxidant activities**

The total phenolic content (TPC) exhibited a progressive decline over the 15-day storage period, with a notable variation between coated and uncoated samples. On day 3, the highest TPC was recorded in AG+5% AA-treated samples (224.3 mg GAE/100g), while the lowest was observed in the uncoated control (212.33 mg GAE/100g). This trend persisted throughout the storage period, with coated fruits maintaining significantly higher phenolic content compared to untreated samples. By day 15, the control samples exhibited the most pronounced reduction in TPC, indicating higher susceptibility to oxidative degradation (Fig. 1).

**Fig. 1:** Total phenolic content of coated and uncoated Indian jujube during storage.

Different letters above each bar within the same period indicate significant differences at *p* < 0.05.

The observed decrease in TPC might be due to the oxidative and enzymatic degradation, which accelerates in the absence of protective barriers. Phenolic compounds play a crucial role in antioxidant defense, and their preservation is essential for maintaining fruit quality and nutritional value (Hossain *et al.*, 2021; Galib *et al.*, 2022; Biswas *et al.*, 2023). The application of aloe vera gel (AG) and ascorbic acid (AA)-based coatings effectively mitigated TPC loss, likely by reducing oxygen permeability, limiting polyphenol oxidase (PPO) activity, and minimizing exposure to oxidative stress. This aligns with findings from previous studies, where aloe vera and antioxidant-based coatings have been shown to significantly retard phenolic oxidation and enhance bioactive compound stability (Sogvar *et al.*, 2016). Moreover, the retention of phenolic compounds in AG+5% AA-coated fruits suggests that AA acted synergistically with AG, reinforcing the antioxidant barrier and further slowing down phenolic oxidation. This protective effect is crucial for prolonging the postharvest quality of Indian jujube, as phenolics contribute to free radical scavenging activity, microbial resistance, and overall fruit stability.

**Fig. 2:** Total antioxidant activity of coated and uncoated Indian jujube during storage.

Different letters above each bar within the same period indicate significant differences at *p* < 0.05.

The ascorbic acid content of treated and untreated samples was significantly different. The ascorbic acid value of the untreated ones diminished drastically while the ascorbic acid content of treated samples was reserved better. The control samples had the lowest level of ascorbic acid in all storage times, and the samples treated with AG+5% AA coating had the most enormous amount. On day 15, it was 5.29 mg/100g for AG+5% AA coated sample and 2.81 mg/100g for the control sample (Fig. 3).

**Fig. 3:** Ascorbic acid value of coated and uncoated Indian jujube during storage. Different letters above each bar within the same period indicate significant differences at *p* < 0.05.

The rapid degradation of ascorbic acid in untreated fruits is possibly due to oxidative degradation, which is exacerbated by increased exposure to oxygen and enzymatic activity during storage. Ascorbic acid is highly sensitive to oxidation, and its degradation is accelerated in the presence of oxygen, light, and elevated temperatures. The protective effect of aloe vera gel and ascorbic acid-based coatings can be explained by their low oxygen permeability, which creates a semi-permeable barrier, limiting oxygen diffusion and reducing oxidative stress. This aligns with previous findings where edible coatings have been reported to inhibit enzymatic oxidation, delay vitamin C degradation, and maintain fruit nutritional quality (Ayranci & Tunc, 2003). Furthermore, the incorporation of ascorbic acid within the coatings may have contributed to the enhanced retention in coated fruits. The AG+5% AA formulation, in particular, provided a higher external supply of ascorbic acid, which may have diffused into the fruit tissue, reinforcing the natural antioxidant system and further delaying degradation. This highlights the potential of AG and AA coatings as an effective postharvest preservation strategy, helping to maintain the nutritional and antioxidant properties of Indian jujube for an extended period.

**5. Conclusion**

This study demonstrates that aloe vera gel (AG) and ascorbic acid (AA)-based edible coatings effectively enhance the postharvest quality and extend the shelf life of Indian jujube (*Ziziphus mauritiana*). The application of these coatings significantly reduced weight loss and preserved key physicochemical properties and antioxidant activity throughout storage. The findings highlight that AG combined with 5% AA exhibited the best performance in maintaining fruit freshness, reducing moisture loss, and preserving ascorbic acid and phenolic content. The results suggest that aloe vera gel and ascorbic acid coatings provide a natural and sustainable method for postharvest preservation, reducing reliance on synthetic preservatives while ensuring food safety and nutritional retention. This approach holds great potential for minimizing postharvest losses, improving the commercial viability of Indian jujube, and promoting eco-friendly food preservation techniques. Future research should focus on optimizing the coating formulations, conducting microbiological assessments to determine antimicrobial effectiveness, and evaluating consumer acceptability through sensory analysis.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**Acknowledgements**

The authors sincerely appreciate the Department of Food Engineering and Tea Technology at Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh, for providing the necessary materials and laboratory support, which were instrumental in successfully conducting this research.

**Authors’ Contributions**

Animesh Sarkar designed and supervised the study, wrote the protocol and the manuscript. Nafisa Sadaf analyzed the samples of the study, wrote the manuscript and searched the literature. Mahabub Alam, Prantik Roy, Md Sumon Miah and Mahbuba Rahman managed the literature searches, performed the statistical analysis, and wrote the manuscript. All authors read and approved the final manuscript.

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