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Food Preservation: Use of Silk Fibroin as an Edible Coating on Bananas

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ABSTRACT: Silk fibroin, the main structural protein of silk, is a biomaterial that has been extensively studied in textile, biomedical, and electronic applications. Due to its hydrophobic nature and its ability to assemble into antiparallel, beta-pleated sheets, the strength, flexibility, and conformability of fibroin have also led to its use as a coating to prolong produce. This study investigates whether the application of a 1% weight/volume aqueous silk fibroin suspension to bananas would prolong their shelf life; additionally, it attempts to determine whether increasing the beta-sheet content of the protein via water annealing (exposure of fibroin to water vapor in a vacuum at constant temperature) would be more effective than coating alone. Measured parameters of fruit spoilage assessed daily in the study were: visible signs of ripening, weight loss, maintenance of turgor, and mold/yeast growth. After the 8-day ripening period, bananas coated in fibroin and stored ambiently at room temperature exhibited less visible signs of spoilage, lost less weight, maintained more firmness/turgor, and grew fewer molds and yeast than did the control cohort. The effect of all measured parameters was slightly more pronounced in the group of bananas that were coated and annealed. Considering that one-third of the food produced in this country is never eaten, this research holds the promise of safely and effectively enhancing food preservation methods, with the hope of far-reaching societal impacts.

KEYWORDS: Biochemistry, Structural Biochemistry, Food Preservation, Silk Fibroin, Water-Annealing, Beta-Pleated Sheet.

Introduction

According to a recent study published by the US Environmental Protection Agency,[1] more than one-third of the food produced in this country is never eaten, wasting the resources used to produce the food and creating a variety of downstream environmental impacts. Additionally, regarding fruits and vegetables specifically, the Food and Agriculture Organization of the United Nations estimates a fifty percent loss of crops throughout the food supply chain, generally concentrated in the post-harvest, distribution, and end-user consumption stages.[2] Food waste contributes to food insecurity, reduced economic efficiency, and impaired efforts to address energy conservation and climate change. Improving the post-harvest shelf life of foods will not only lessen the need for new food production, but also reduce projected deforestation, biodiversity loss, greenhouse gas emissions, and water pollution and scarcity.

Many treatments to extend the shelf life of perishable produce have been explored, including cryopreservation, treatment with synthetic fungicides, modified atmospheric packaging, and osmotic and temperature treatments. Edible coating materials have also shown utility in food preservation, specifically those that demonstrate biocompatibility, biodegradability, antibacterial and antifungal properties, membrane-forming ability, and safety. Currently utilized coating materials include proteins, polysaccharides, resins, and lipids; however, each lacks some of the requisite physical and chemical characteristics of the ideal fruit and vegetable coating to prolong shelf life. Some desirable physical characteristics of an edible preservative coating include: barrier properties to control gas and moisture transfer; mechanical and tensile strength to resist cracking; and transparency to maintain the natural appearance of the fruit. Chemically, an ideal edible coating should have antimicrobial and/or antioxidant properties to impair or slow the growth of pathogens responsible for fruit spoilage, and should preferably be naturally occurring materials.

Silk fibroin is a biomaterial that has been extensively studied for its potential in textile, biomedical, and electronic applications.[2-4] It has been affirmed by the U.S. Food and Drug Administration (FDA) as a non-toxic substance and is classified as generally recognized as safe (GRAS) for its intended use as a surface-finishing agent on food. Silk fibroin is the main structural component of silk and is produced by the silkworm, *Bombyx mori (B. mori)*. The properties of silk fibroin are derived from its structure, which consists of hydrophobic blocks separated by hydrophilic acidic spacers.[2,4,5] In its natural state, silk fibroin forms antiparallel beta-pleated sheets as its secondary structure, providing strength and resilience to the protein. Additionally, the beta-pleated sheet structure of fibroin, combined with its inter- and intra-molecular hydrogen bonding, confers high flexibility and conformability of the protein. Notably, prior studies have shown that the beta-pleated sheet content of fibroin can be regulated and adjusted based on the time and temperature at which fibroin is exposed to water vapor or other polar solvents in vacuum conditions (annealing).[2,6]

Based on prior studies using silk fibroin as an edible coating for perishable food preservation, and considering that a 1% weight/volume fibroin solution was noted to be safe and efficacious in those studies, we sought to utilize the intrinsic properties of silk fibroin, such as its hydrophobicity and conformability, to design a 1% weight/volume water-based suspension of fibroin that assembles on the surface of miniature bananas upon coating. We also increased the beta-pleated

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sheet content of the fibroin via water annealing to determine whether increasing the content of beta-pleated sheets would confer additional benefit in prolonging the spoilage of the bananas. Six roughly identical miniature bananas were used in the study: two without fibroin added (control), two with fibroin solution coating, and two coated with the same concentration of fibroin and then water annealed for twelve hours. The bananas' physical appearance, weight loss, loss of turgidity, and presence of microbial growth were then recorded for all bananas as indicators of spoilage. This study is designed to contribute to the growing body of literature on safe, effective, and readily available preservative coatings to help address the critical issue of food spoilage.

Methods

Sample and Coating Preparation:

Six non-ripe miniature bananas were purchased, ensuring they were roughly the same sizes, shapes, and textures, and had similar degrees of mechanical damage. Similarities in color/ripeness, visible evidence of bruising, and texture/firmness to light pressure were subjectively assessed by the author. All bananas were weighed and photographed pre-experiment. Using a graduated cylinder, 1 g of silk fibroin powder (Advanced Biomatrix CytoSilk lypophilized silk fibroin) was dissolved in 100 mL of distilled water to generate a 1% weight/volume solution of silk fibroin in a clear plastic container. This was accomplished via gentle agitation and mixing until a homogeneous, clear, straw-colored solution was obtained. The bananas were labeled as follows: 2 bananas served as controls and were not treated with the fibroin or annealed ("Control #1" and "Control #2"); 2 bananas were coated with fibroin solution ("Dipped #1" and "Dipped #2"); 2 bananas were coated with fibroin solution, and then water annealed ("Dipped & Annealed #1" and "Dipped & Annealed #2").

All 4 were coated equally and uniformly with the fibroin solution by separately submerging each banana in the fibroin solution in a gallon-size Ziploc bag. Each banana was completely submerged in the solution for 60 seconds. All bananas were then placed on a drying rack at room temperature (approximately 21° C) for 4 hours.

Annealing:

After the 4-hour drying period, the 2 bananas labeled "Dipped & Annealed #1" and "Dipped & Annealed #2" were water annealed as follows. 100 mL of distilled water was poured into a vacuum-sealed bag. A small plastic container was inverted and placed at the bottom of the bag. The banana labeled "Dipped & Annealed #1" was placed on top of the container, with care being taken for the water not to contact the banana. The bag was then vacuum sealed using a food vacuum sealer. This process was then repeated for the banana labeled "Dipped & Annealed #2." Both vacuum-sealed bags were allowed to remain at room temperature (21° C) for 12 hours.

Storage and Sampling Cadence:

All bananas were stored at room temperature (21 °C) for the duration of the study period. Weights, in grams (taken on a digital scale) and digital photographs of each banana were obtained once daily for the duration of the study.

Weight Loss:

Weight loss of each banana, in grams, was calculated by subtracting the weight of each banana on day 8 of the study period from the initial weight of each. This data was recorded.

Turgidity and Resistance to Depression:

A 200 g weight was placed on each banana on day 8, and the degree of vertical depression by the weight, measured in millimeters, was photographed, measured, and recorded for each.

Fungal Plating and Enumeration:

On day 8, each of the bananas was swabbed, uniformly on all sides for 10 seconds, with sterile cotton-tipped applicators. Each sterile applicator was then used in a "back and forth" manner for a total of 5 seconds to plate material on labeled dichloran rose bengal chloramphenicol (DRBC) agar petri dishes, with care being taken to uniformly cover the surface of each plate. Microbial colony growth was manually counted and recorded for each petri dish after the 8-day period.

Results and Discussion

Results:

The four bananas treated with the 1% aqueous fibroin solution showed less visible signs of ripening and spoilage (i.e., less browning and bruising) than the two non-treated controls after the 8-day period. The two dipped and water annealed bananas also showed less visible signs of ripening than did their dipped, but non-water annealed counterparts **(Figure 1)**.

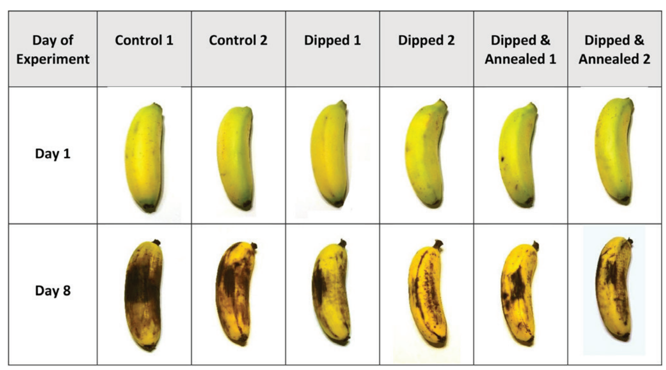

Figure 1: Digital photographs document the appearance of all 6 bananas between day 1 and day 8: 2 untreated controls, 2 dipped in 1% silk fibroin solution, and 2 dipped in 1% silk fibroin solution and subsequently water annealed. The most prominent visible signs of ripening occurred in the control group. The bananas that were dipped in silk fibroin and those that were dipped and water annealed maintained visible evidence of freshness longer, with less darkening, bruising, and shriveling seen.

Consistent with the volume loss that occurs during fruit ripening, all 6 bananas underwent some degree of weight loss during the study period. However, the most significant weight loss was exhibited in the untreated control cohort. Control bananas 1 and 2 showed a loss of 9.15 g and 7.34 g (or a 23.91% and 22.87% reduction in starting weight), respectively. Bananas 1 and 2 dipped in the silk fibroin solution lost 6.84 g and 7.26 g (18.86% and 19.66%), respectively. Bananas 1 and 2 that

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were dipped and then water annealed lost the least amount of weight at 6.54 g and 6.79 g (20.06% and 18.12%), respectively **(Table 1, Table 2, Figure 2, Figure 3)**. The average weight loss at the end of the study was 23.39% for controls, 19.26% for the dipped group, and 19.09% for the dipped and annealed cohort **(Table 3, Figure 4)**.

Table 1: Daily measured weights of all bananas on days 1 to 8 are shown. Calculated changes in weight between day 1 and day 8 are also displayed. Weight loss is a physical change associated with fruit ripening and spoilage. Consistent with the preservation effect of silk fibroin, those bananas dipped in silk fibroin and those dipped and subsequently annealed demonstrated less weight loss by the end of the study compared to untreated controls (8.25% vs. 7.05% average decrease in weight and 8.25% vs. 6.67%, respectively).

Banana Description	Day 1 (g)	Day 2 (g)	Day 3 (g)	Day 4 (g)	Day 5 (g)	Day 6 (g)	Day 7 (g)	Day 8 (g)	Δ in Weight (g)	Avg Δ in Weight (g)	% Δ in Weight	Avg % Δ in Weight
Control #1	38.27	36.64	35.19	34.25	33.18	31.95	30.43	29.12	-9.15	-8.25	-23.91%	-23.39%
Control #2	32.09	30.68	29.55	28.82	28.00	27.03	25.79	24.75	-7.34		-22.87%	
Dipped #1	36.27	35.06	34.00	33.27	32.44	31.53	30.40	29.43	-6.84	-7.05	-18.86%	-19.26%
Dipped #2	36.92	35.45	34.20	33.41	32.53	31.61	30.59	29.66	-7.26		-19.66%	
Dipped & Annealed #1	32.60	31.78	30.58	29.82	29.00	28.10	27.00	26.06	-6.54	-6.67	-20.06%	-19.09%
Dipped & Annealed #2	37.48	36.55	35.28	34.51	33.65	32.76	31.68	30.69	-6.79		-18.12%	

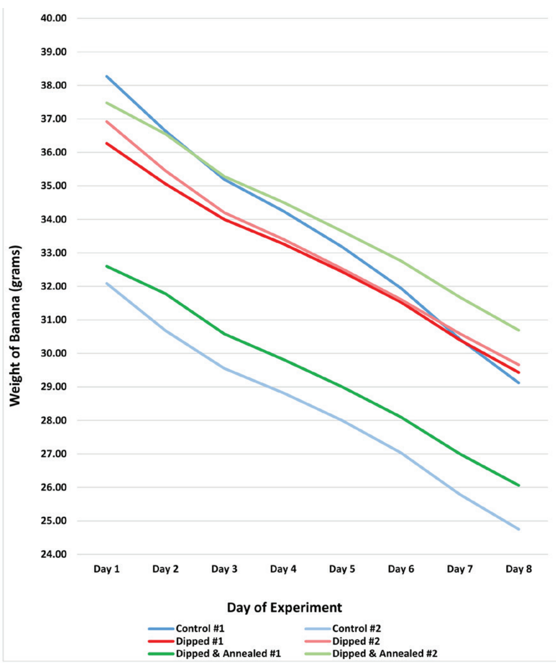

Figure 2: This graph displays the weight loss of each banana continuously from day 1 to day 8 of the study. Bananas in the experimental groups lost less weight (i.e. maintained freshness longer) than did the controls. This effect was most pronounced in the bananas that were dipped in silk fibroin solution and then water annealed.

Table 2: Reduction in weight of each banana after 8 days is shown. Untreated control bananas lost more weight by the end of the study than did either the dipped or the dipped and annealed fruit. The bananas that were dipped and subsequently water annealed lost the least amount of weight.

Banana Description	Loss in Weight (grams)
Control #1	9.15
Control #2	7.34
Dipped #1	6.84
Dipped #2	7.26
Dipped & Annealed #1	6.54
Dipped & Annealed #2	6.79

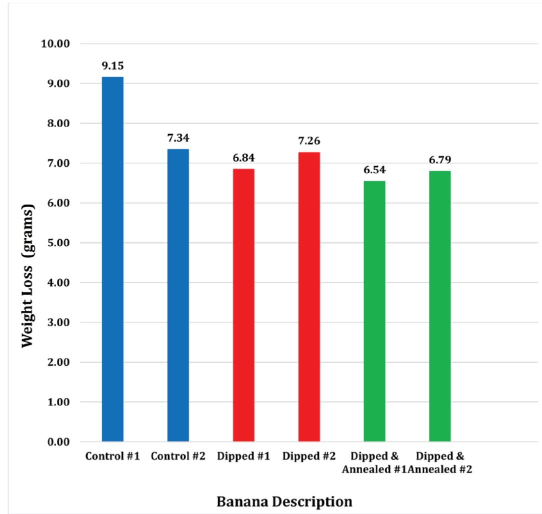

Figure 3: The bar graph depicts weight loss in each banana on day 8 of the study period. Weight loss, associated with fruit ripening and spoilage, was most pronounced in the control cohort. Bananas dipped in silk fibroin solution lost less weight than controls, and those dipped and subsequently annealed lost the least.

Table 3: Average percentage of weight loss in each cohort by day 8 is shown. Untreated control bananas lost an average 23.39% of their initial weight at the end of the study period, compared to 19.26% for those dipped in silk fibroin and 19.09% for the dipped and annealed cohort. Improved maintenance of weight corresponds to the preservation effect of the silk fibroin solution.

Bananas Description	Average Percent of Weight Loss (%)
Control #1 and #2	23.39%
Dipped #1 and #2	19.26%
Dipped & Annealed #1 and #2	19.09%

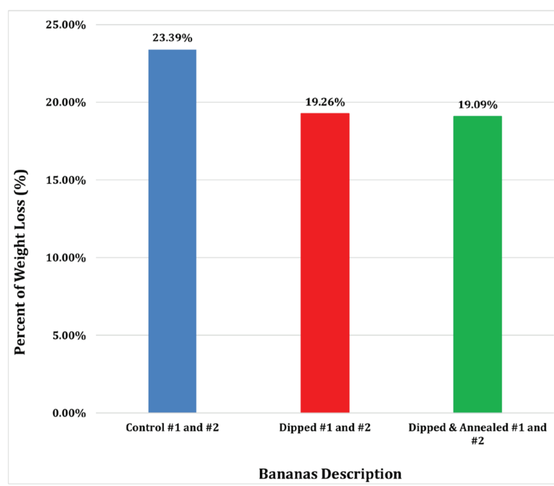

Figure 4: The bar graph depicts the average percentage of weight loss across all 3 cohorts (control, dipped, and dipped & annealed) after the 8 day study period. As expected, treated bananas maintained their initial weight better than untreated controls.

Turgidity of each banana was measured via the degree of depression, measured in millimeters, by the application of a 200 g weight. Given the volume loss of all bananas previously recorded, all experienced decreased turgidity after the study period, though in different amounts. The depth of depression by the weight for Control bananas 1 and 2 was 5 mm each; for Dipped bananas 1 and 2, the amount was 1.5 mm and 2 mm, respectively; and for Dipped & Annealed bananas 1 and

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2, the amount was 1 mm and 0.5 mm, respectively **(Table 4, Figure 5)**.

Table 4: The degree of vertical depression (as an indicator of turgor pressure) of each banana after 8 days via an applied 200 g weight is displayed. Turgidity was preserved in all 4 silk-fibroin treated bananas compared to controls, and this effect was most pronounced in the bananas dipped and then annealed. Untreated controls underwent ripening and softening sooner, resulting in an increased amount of depression with the applied weight.

Banana Description	Degree of Depression With 200 g Weight (mm)
Control #1	5.0
Control #2	5.0
Dipped #1	1.5
Dipped #2	2.0
Dipped & Annealed #1	1.0
Dipped & Annealed #2	0.5

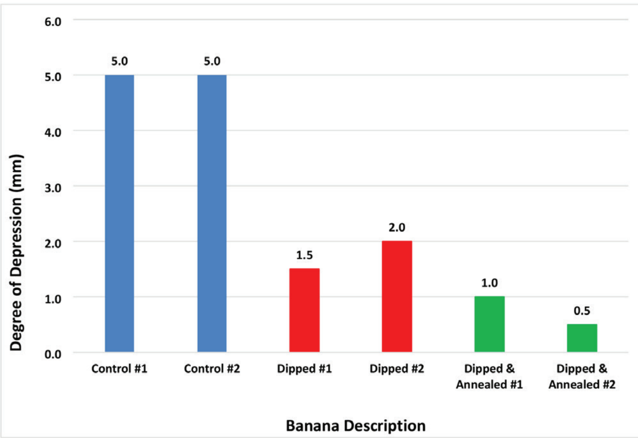

Figure 5: The graph demonstrates the degree of vertical depression of each banana on day 8 via an applied 200 g weight. The amount of depression was greatest in the control group, indicating loss of turgidity associated with ripening and spoilage.

To analyze the degree of microbial growth associated with fruit spoilage, mold/yeast colonies on agar plates were counted for each of the six bananas. Eight days after swabbing and plating, the agar for Control bananas 1 and 2 had 43 and 39 mold/yeast colonies, respectively, growing. Dipped bananas 1 and 2 showed 2 and 0 mold/yeast colonies, respectively. Dipped & Annealed bananas 1 and 2 showed 1 and 0 mold/yeast colonies, respectively. This data is presented in **Table 5, Figure 6**.

Table 5: Calculated mold and yeast colony counts grown on DRBC agar plates on days 1 to 8 of all bananas. Untreated controls showed the greatest degree of mold and yeast growth, associated with fruit spoilage. In comparison, silk fibroin dipped bananas grew significantly fewer organisms, and those dipped and annealed demonstrated the lowest number of colonies.

Banana Description	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control #1	0	0	0	2	7	18	32	43
Control #2	0	0	0	2	4	7	20	39
Dipped #1	0	0	0	0	0	2	2	2
Dipped #2	0	0	0	0	0	0	0	0
Dipped & Annealed #1	0	0	0	0	0	1	1	1
Dipped & Annealed #2	0	0	0	0	0	0	0	0

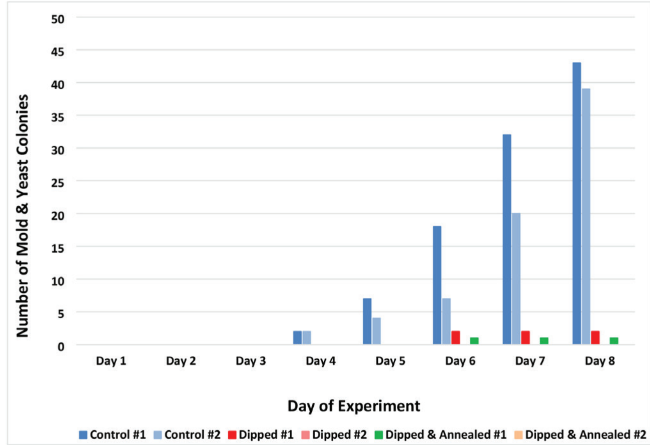

Figure 6: Mold and yeast colonies counted on DRBC agar for all bananas on days 1 to 8. Graphical representation of organism growth highlights the significant decrease in molds and yeast associated with fruit spoilage in the treated bananas compared to controls.

Discussion:

According to a 2021 report published by the U.S. Environmental Protection Agency,[1] approximately 35 to 36% of the U.S. food supply, or roughly 152 metric tons of food, is wasted along all the stages of the food supply chain. Notably, approximately 50% of this waste is experienced at the consumption portion of the supply chain. Fruits and vegetables, followed by dairy and eggs, are the most commonly wasted food items. Therefore, safe, effective, and readily available preservation methods for perishable foods are imperative.

Silk from *Bombyx mori* (silkworm) has been extensively studied in bioengineering due to its biocompatibility, robust mechanical performance, ease of processing, and ready supply.[5] Silk from silkworms is comprised of two primary proteins: sericin (25%) and fibroin (75%). Sericin is a glue-like, amorphous, and soluble protein that positions itself across the surface of two parallel fibroin fibers, binding them together and helping provide structural integrity of the fibers. Sericin can be removed from fibroin via a thermochemical process called degumming. Silk fibroin, in contrast, is a structural fibrous protein that adopts a semi-crystalline structure that imparts stiffness and strength. In its natural state, silk fibroin organizes into antiparallel beta-pleated sheets. A combination of beta-pleated sheets along with inter- and intra-molecular hydrogen bonding helps provide both flexibility and conformability.[2] These intrinsic properties of silk fibroin have allowed the protein to be used in a wide variety of biomedical applications, including drug delivery, biomaterial processing, wound healing, gene therapy, and bone regeneration.[2-5]

Notably, silk fibroin has also been studied in food processing as a way to preserve the post-harvest shelf life of perishable food. Silk fibroin has been investigated as a component in food packaging systems,[7,8] as well as an odorless and edible coating material on the foods themselves.[2,9] Prior studies have also demonstrated that the beta-pleated sheet content of the protein can be increased through a process called water annealing,[2,6] in which fruit coated with silk fibroin is exposed to water vapor in a vacuum-sealed environment and held at constant temperature for a period of time. Polar solvents favor the exposure of polar amino acid side chains to the solvent and bury nonpolar side chains within the core of the molecule, promoting folding of the protein. Additionally, beta-sheet structures are then stabilized by hydrogen bonding, increasing the formation and stability of this secondary structure. Increased beta-pleated sheet content correlated with improved food preservation.[2] This study sought to determine whether the application of a fibroin solution to fresh fruit would prolong its shelf life and reduce spoilage compared to an identical group of controls, and whether fibroin coating plus water annealing would be superior to coating alone.

Four parameters of fruit spoilage were utilized in our study: physical appearance, decrease in weight, loss of firmness/turgidity, and presence of microbial growth (specifically yeasts and molds). Digital photographs confirm decreased discoloration of the bananas treated with fibroin compared to controls, and the dipped and water annealed group looked fresher at the end of the period than did their dipped and non-annealed counterparts. After the 8-day banana ripening period, control bananas lost an average of 8.25 grams (or a 23.39% average decrease from their starting weight). Bananas that were dipped in fibroin lost less weight than controls: an average of 7.05 grams or 19.26% average decrease. The bananas that were both dipped and then water annealed for 12 hours lost the least amount of weight: 6.67 grams (19.07%). Loss of fruit firmness was documented by the amount of compression caused by application of a 200-gram weight, and a similar trend was seen: control bananas were least firm, with a compression height of 5 mm; dipped bananas compressed between 1.5 and 2 mm, and dipped & annealed bananas showed only 0.5 to 1 mm of compression. The most common organisms associated with fruit spoilage are molds and yeasts. Therefore, dichloran rose bengal chloramphenicol (DRBC) was utilized in the microbial analysis in this study because of the medium's superiority in enumerating foodborne yeasts and molds, specifically.[10] Although the bananas that were dipped and annealed showed modest improvements across all measured parameters compared to their dipped-only counterpoints, the small sample size in the study precludes the ability to say whether these differences reached statistical significance.

These results compare favorably to a prior study[2] that compared ripening of fresh strawberries and bananas that were fibroin dipped, dipped and water annealed, and non-dipped. The investigators noted statistically significant improvement in water loss, weight loss, and oxygen diffusion in dipped versus control strawberries; the effect was more pronounced in the annealed cohort. Similarly, treated bananas better maintained turgidity and firmness in a statistically significant fashion than did untreated controls.

Limitations of this study included a small sample size. A larger number of bananas used in the study would have added statistical power to the investigation and allowed for an analysis of whether measured differences between groups were statistically significant. Additionally, quantitative analysis using instrumentation to assess textural and color differences between bananas and quantifying ethylene gas influx as a marker for browning seen in ripe bananas would reduce some of the more subjective assessments that were made in the study. These represent areas of research for future studies.

Conclusion

A 1% weight/volume aqueous silk fibroin solution effectively extended the post-harvest shelf life of bananas in all of the parameters measured. This effect was even more pronounced in the fruit that was coated and water annealed. It is thought that the crystalline beta-pleated sheet coating of the hydrophobic fibroin prolongs the freshness of fruit by slowing fruit respiration, decreasing water loss and dehydration, and subsequently extending fruit firmness. Water annealing increases the degree of beta-pleated sheet content of the fibroin coating, thereby enhancing its preservative effect. The implication of this study, and that of hopeful future follow-up investigations, would be the addition of silk fibroin as a safe, effective, and readily available preservative to the armamentarium against the staggering effects of food waste.

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